From Molecular Symphony to Therapeutic Harmonies: Exploring Nutrigenomics, Enzymatic Frontiers, Flavonoid Therapeutics, and Autoimmune Skin Challenges

Editor
Metin Ertaş

BİDGE

BIDGE Publications

From Molecular Symphony to Therapeutic Harmonies: Exploring Nutrigenomics, Enzymatic Frontiers, Flavonoid Therapeutics, and Autoimmune Skin Challenges

Editor: Assist. Prof. Dr. Metin ERTAŞ

ISBN: 978-625-6707-59-7

Page Layout: Gözde YÜCEL

1st Edition:

Publication Date: 25.12.2023

BIDGE Publications,

Certificate No: 71374

www.bidgeyayinlari.com.tr - bidgeyayinlari@gmail.com

Krc Bilişim Ticaret ve Organizasyon Ltd. Şti.
Güzeltepe Mahallesi Abidin Daver Sokak Sefer Apartmanı No: 7/9 Çankaya / Ankara

PREFACE

In the vast realm of scientific inquiry, our quest for understanding extends beyond the boundaries of individual disciplines, weaving a tapestry that connects nutrition, genomics, and medical research. The titles before you encapsulate a journey into the depths of scientific exploration, each representing a distinct facet of our quest to unravel the mysteries of life, health, and the intricate interplay between our choices and our biology.

The first exploration takes us into the fascinating world of nutrigenomics, a frontier where the choices we make in our daily diets intricately intersect with the aging process. Through the lens of honey bees, a remarkable model organism, we peer into the genetic mechanisms that dictate longevity and well-being. The humble honey bee, a creature of ecological importance, serves as an unexpected yet illuminating guide through the labyrinth of molecular intricacies that underpin the relationship between diet and aging.

Next, we pivot to a groundbreaking chapter in enzymology, where two catalysts, TvTS and (MpMS), have emerged as veritable keystones, unlocking doors to an era of unprecedented scientific discovery. These enzymes, with their cryptic acronyms, beckon us into the world of molecular processes that have the potential to reshape our understanding of genetic pathways and their implications for human health and longevity.

As we traverse the scientific landscape, our attention turns to the therapeutic potential embedded in the natural compounds known as flavonoids. These compounds, found abundantly in our diets, reveal a compelling story of hope in the context of Alzheimer's Disease. Through meticulous investigation, we explore the therapeutic effects of flavonoids, envisioning a future where these natural agents may become vital tools in the arsenal against neurodegenerative afflictions.

Our journey then takes a turn toward the enigmatic realms of autoimmune skin disorders. First, we delve into the complexities of Psoriasis, a condition that challenges our understanding of immune

responses and inflammatory processes. Following this, we unravel the mysteries of Alopecia Areata, an autoimmune skin disease that disrupts the delicate balance of hair growth. In these chapters, we confront the intricate mechanisms that govern self-directed immune responses, aiming to illuminate pathways toward targeted therapeutic interventions for those grappling with these challenging conditions.

This compilation invites you to embark on a voyage of discovery, where the threads of nutrigenomics, enzymology, therapeutics, and autoimmune research are woven together into a narrative that transcends individual disciplines. As you traverse the chapters that follow, may you find inspiration in the ceaseless pursuit of knowledge and the collective endeavor to unlock the secrets of life's intricacies.

Editor
Assist. Prof. Dr. Metin ERTAŞ

Contents

PREFACE 3

Contents 5

Nutrigenomic Approach for Investigating The Associations of Diet and Aging: Honey Bees as a Model Organism 7

Gizem SONMEZ OSKAY 7

Devrim OSKAY 7

Enzymes that Opened a New Era for The Scientific Community: TvTS and MpMS 27

İdris ARSLAN 27

Therapeutic Effects of Flavonoids on Alzheimer's Disease 35

Esra Nur YEŞİLKENT 35

Medine Sibel KARAĞAÇ 35

Neslihan ÖZTÜRK 35

Hamid CEYLAN 35
Autoimmune Skin Disorder: Psoriasis 70
Esra PALABIYIK 70
Handan UĞUZ 70
Bahri AVCI 70
Ayşe Nurseli SULUMER 70
Hakan AŞKIN 70
Otoimmün Cilt Hastalığı: Alopesi Areata 91
Handan UĞUZ 91
Esra PALABIYIK 91
Ayşe Nurseli SULUMER[3] 91
Bahri AVCI[4] 91
Hakan AŞKIN[5] 91
The Integration of RNA Sequencing and Proteomics in Early Detection and Personalized Treatment in Cancer Research 111
Duygu KIRKIK 111
Sevgi KALKANLI TAS 111
Effects of Amifostine Against Gamma-radiation on HeLa Cells and Apoptotic Genes Expression 122
Gül ÖZCAN 122
Özlem DAĞDEVİREN 122
Uğur SERBES 122
İdil ÇETİN 122

CHAPTER I

Nutrigenomic Approach for Investigating The Associations of Diet and Aging: Honey Bees as a Model Organism

Gizem SONMEZ OSKAY[1]
Devrim OSKAY[2]

Introduction

The aging of populations worldwide is occurring at an accelerated rate compared to the past. Age 65+ is the fastest-growing demographic globally. The elderly population is estimated to have a significant increase, surpassing 1.5 billion people by the year 2050 (Fong & et al., 2021). It has been predicted that a significant proportion, around 80%, of health issues experienced during later adulthood might potentially be averted or postponed via the adoption of lifestyle modifications among those aged 55 to 65 years (Milte & McNaughton, 2016). Aging is a progressive and irreversible

[1] Gizem SONMEZ OSKAY, PhD., ORCID: 0000-0003-4724-9340. Corresponding author: sonmezgiz@gmail.com
[2] Devrim OSKAY, Asst. Prof., Department of Agricultural Biotechnology, Faculty of Agriculture, Namik Kemal University, Tekirdag, ORCID: 0000-0002-3410-2780.

pathophysiological phenomenon characterized by the decline of physiological integrity and functional capacity, ultimately resulting in increasing sensitivity to mortality (Cui & et al., 2012; Cai & et al., 2022; Guo & et al., 2022). The process of aging is associated with the progressive accumulation of biomolecular damage, which varies across individuals due to the interplay between genetic and environmental factors. Several mechanisms contribute to the complexity of this process, such as cellular senescence, genomic instability, telomere attrition, epigenetic, proteostasis loss, mitochondrial dysfunction, chronic inflammation, deregulated nutrient-sensing, and stem cell exhaustion (Zhang & et al., 2022; Keshavarz & et al., 2023; López-Otín & et al., 2023). Several of these mechanisms are interconnected with nutrient-sensing signaling pathways, indicating that nutrition plays a pivotal role in the aging process (Chen & et al., 2022). Nutrigenomics is a nascent scientific discipline that investigates the genetic alterations resulting from dietary factors, hence including the convergence of nutrition, wellness, and genomics. It has been shown to assist in evaluating the effects of nutrients on the processes of aging, longevity, and healthspan, as well as the underlying mechanisms involved in these events (Ye & et al., 2007). The use of nutrigenomic investigations and comprehension of diet-disease correlations may be employed to create personalized nutritional and pharmaceuticals (Evangelakou & et al., 2019).

Throughout human history, animal-based research has been used as the main driver for endeavors aimed at discovering remedies for diverse diseases and advancing pharmaceutical innovation. Preclinical research with diverse animal models owing to their advantages of short lifecycles, high fertility, simple anatomical structures, evolutionarily conserved genes, and cost-effectiveness provides the basis for understanding the pathophysiology of diseases human. Within this framework, several model organisms, such as mammals, yeast, nematodes, fish, and flies, have been utilized as tools for investigating complex mechanisms and potential therapies. (Maleszka, 2014; Andersen & Winter, 2017; Robinson & et al.,

2019; Mukherjee & et al., 2022; Lu & et al., 2022; Adhish & Manjubala, 2023). These models present a wide range of particular methodologies, enabling comprehensive genetic examination of several key biological processes using diverse techniques (Irion & Nüsslein-Volhard, 2022).

For more than a century, insects have been extensively used as model organisms. The use of insects as research models has several technical and ethical advantages when compared to vertebrate models (Brandt, 2020). In recent years, insects have gained considerable attention as ideal model organisms notably in the study of aging and disease in humans. As noted in previous studies, it is clear that sequencing insect genomes offers a unique opportunity to evaluate gene correlates of human diseases (Adams & et al., 2000; Fortini & et al., 2000).

As a social insect, honey bees (*Apis mellifera* L.) play a crucial role in supporting worldwide agriculture thanks to pollination services. Besides the agricultural contribution, the economic worth of honey bees lies in their ability to produce very valuable functional and healthy food, including honey, pollen, royal jelly, propolis, etc. (Romero & et al., 2019). The Honey bee Genome Project has contributed valuable insights into the genetic underpinnings of honey bee biology, the molecular mechanisms underlying social behavior, and the evolutionary aspect. So, this project has positioned the honey bee as a significant model organism for future scientific studies (Zheng & Hu, 2009). Several human genes exhibit homology within the honey bee genome, with particular emphasis on metabolic enzymes, components of insulin signaling, and the innate immune system (Wang & et al., 2018). The honey bee genome undergoes methylation in a manner similar to the mammalian genome. It has been shown that due to its smaller size compared to the human genome, the honey bee genome facilitates the measurement and interpretation procedures by having a relatively low proportion of methylated DNA (Ford, 2013). Honey bees are an especially ideal model organism for investigating the molecular basis of the aging mechanism (Keller & Jemielity, 2006). It is even significant in

comprehending the mechanisms that control the cognitive decline that comes with advancing age (Behrends & et al., 2007). As well, honey bees can be used as a suitable model organism for studying age-related alterations in brain function, since learning and memory processes can be readily assessed in controlled laboratory conditions (Behrends & Scheiner, 2010). Moreover, the difference in lifespan between a queen bee and worker bees in the colony, which is ascribed to variations in diet, has substantial importance for investigating the molecular mechanism that underlies the age-related impacts of nutrition.

Given the global population's fast aging, an increase in age-related diseases, and the challenges of doing clinical research on the connection between nutrition and human aging, model organisms have emerged as the main focus in dietary longevity research, as shown in Figure 1.

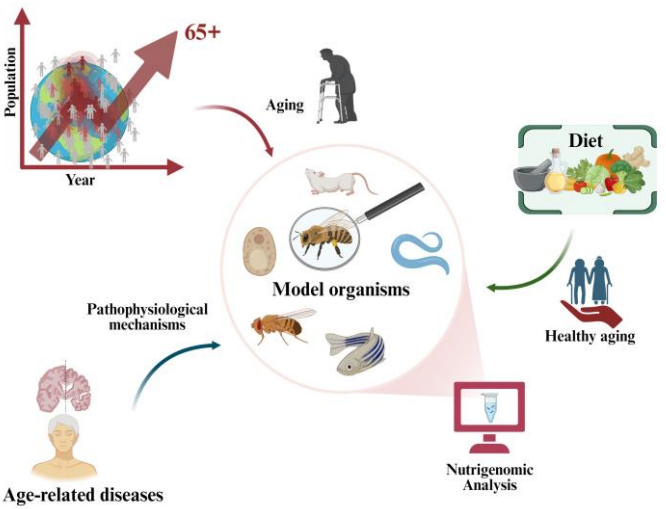


Figure 1. Insight into nutrigenomic research to explore the impact of nutrition on the aging process in honey bees.

The chapter concludes by integrating information derived from the molecular mechanism underlying the relationship between nutrition and aging in honey bees. Additionally, it explores the

potential of honey bees as a model organism in nutrigenomic studies for investigating the impact of diverse diets on longevity.

The molecular pathways involved diet and aging in honey bees

The honey bee colonies consist of workers, drones (seasonally), and a single queen. The average lifespan also differs considerably across different castes. The usual lifespan of a honey bee queen is between one and two years, but the lifespan of a worker honey bee is between 2 and 20 weeks. The lifespan of honey bees is greatly affected by nutrition, and whether the honey bees in the colony are workers or queens depends on the difference in feeding (Page & Peng, 2001; Remolina & et al., 2007). The queen bee assumes the role of reproduction in the colony, while worker bees undertake various responsibilities both within and outside the hive, including brood care, nectar collection, pollen gathering, and water collection, by the division of labor based on age-related requirements. The division of labor in honey bees is controlled by complex interactions involving vitellogenin (vg), juvenile hormone (JH), and the insulin/insulin-like growth signaling/target of rapamycin (IIS/TOR) pathway (Corona & et al., 2023). Many researchers have reported that the lifespan of honey bee workers with the same genotype in the colony is mostly regulated by environmental factors (Winston, 1980; Winston, 1987; Seeley, 1995; Behrends & Scheiner, 2010). Also, it has been explained in many research that aging in honey bee workers is plasticity depending on different tasks and social behaviors in the colony rather than chronological age. (Huang & Robinson, 1996; Giray & Robinson, 1994; Münch & et al., 2008; Rueppell & et al., 2008; Münch & Amdam, 2010; Amdam, 2011; Münch & et al., 2013; Rueppell & et al., 2016). The architecture of the honey bee genome depends on plasticity, including changes in gene expression and chromatin arrangement, and has implications not just for physiology and diseases in humans, but also for other instances of flexibility (Duncan & et al., 2020).

Vg is a phosphoglycoprotein that is produced by the fat body and has a role in the development of eggs in queen bees. Typically, research has shown a correlation between rising levels of JH and declining levels of vitellogenin Vg in the hemolymph during the shift from duties performed within the hive to those performed outside the hive. If the colony experiences the reverse situation, such as a shortage of young worker bees, forager bees may revert to their nursing responsibilities. According to reports, this process of reversal is accompanied by an augmentation in Vg synthesis and a reduction in JH level (Lourenço & et al., 2019). In animals, implementing a restricted diet or inhibiting genes related to dietary restriction often leads to an extension of longevity. Conversely, restricting dietary intake in honey bees reduces their longevity by hastening a process of behavioral development that ultimately leads to their departure from the nest as foragers. Namely, nutrition influences the behavioral transitions of worker bees during the aging process. The regulation of nutrient signaling, the honey bee caste determination process, dietary restriction, and senescence is principally governed by two pathways: IIS and TOR pathway (Wheeler & et al., 2013; Zhang & et al., 2021). These nutrient-signaling pathways exert their influence on honey bee caste formation via juvenile hormone (Mutti & et al., 2011). According to research when the insulin receptor substrate gene, which is linked to the IIS system, is turned off in honey bees, they become more mature behaviorally faster and live shorter lives (Ihle & et al., 2019). Another study has shown that vitellogenin inhibits insulin/insulin-like peptide signaling, which is recognized to accelerate aging and reduce longevity (Lee & et al., 2015). Besides, these pathways respond to nutritional changes by regulating downstream genes with antioxidant, antimicrobial, or metabolic functions, and can also regulate lifespan as a consequence. It is well recognized that nectar/honey serves as the primary energy source in the diet of honey bees. Pollen, on the other hand, provides the essential protein supply required for the growth of both larvae and adult bees. An imbalanced ratio of protein to carbohydrates in the diet may have negative effects on the survival and ovarian development of adult individuals, as well

as the growth of larvae (Bouchebti & et al., 2022). A recent research revealed that worker honey bees who were deprived of pollen during the first week of their adult life had indications of premature aging (Martelli & et al., 2022).

The potential role of epigenetic responses to diet in aging of honey bees

Honey bees are considered the most intriguing model for studying the impact of diet-mediated epigenetic modifications on longevity. The queen bee has a much longer lifespan compared to the worker honey bee, with an average duration that is twenty times greater. The difference in longevity could be explained by the variation in nutrients received during the larval period. While larvae fed with royal jelly turn into long-lived queen bees, it has been reported that royal jelly suppresses the expression of *de novo* methyltransferase (DNMT3) by causing changes in DNA methylation patterns (Ford & et al., 2011). Research has shown that the fatty acid (10-HDA), which is the main component of royal jelly, may effectively hinder the activity of histone deacetylase 3. This inhibition promotes the acetylation of histones, hence facilitating the transformation of larvae into queens (Spannhoff & et al., 2011; Polsinelli & Yu, 2018). A recent study has shown that the royal jelly diet includes biologically active chemicals that may inhibit the enzymes DNMT3A and histone deacetylase 3 (HDAC3), which function in tandem to facilitate the creation of a compacted chromatin structure by suppressing transcription (Alhosin, 2023). A new study has shown that the modification of H3K4me1 may play an act in creating and sustaining caste-specific transcriptional programs in honey bees (Zhang & et al., 2023). Kojić et al. (2023) found that administering sodium butyrate, a histone deacetylase inhibitor, as a supplement to honey bees resulted in extended longevity. This increase in lifespan was attributed to the upregulation of vitellogenin, HDAC1, and HDAC3 isoforms. Another investigation showed that p-coumaric acid, which is present in honey and bee bread, modifies gene expression by differentially

regulating genes associated with caste determination (Mao & et al., 2015). Research examining the impact of dietary phytochemicals on the longevity of honey bees found that the presence of casein in the diet eliminated the ability of p-coumaric acid to prolong honey bee lifespan when quercetin was not present (Liao & et al., 2017). Rasmussen et al. (2021), reported that curcumin effectively prevented or partly mitigated the reduction in longevity resulting from ethanol take-in, as well as the ethanol-induced alterations in DNA methylation. The developmental alterations in DNA 5-methylcytosine levels in queen bees are influenced differentially by their natural larval diet compared to workers and drones. Methylation levels exhibited an increase in the queens, whereas a decrease was seen in the drones and workers (Strachecka & et al., 2015). The impact of DNA methylation on the lifespan of worker honey bees, specifically concerning vitellogenin expression, has been shown. This effect is regardless of the role of the juvenile hormone (Cardoso-Júnior & et al., 2018). Various research has proven that RNA-based epigenetic processes, including caste-specific microRNAs and N6-methyladenosine mRNA modification, are also involved in caste differentiation and aging (Ashby & et al., 2016; Wang & et al., 2021; Lowe & et al., 2022).

The epigenetic regulation of lifespan is modulated by sirtuins. Sirtuins play a crucial role in slowing down cellular aging and increasing the lifespan of an organism by controlling a range of cellular activities. Additionally, it contributes to the postponement of age-related telomere degradation, the preservation of genomic stability, and the enhancement of DNA damage repair (Lee & et al., 2019). Sirt family genes and TOR are recognized components of the calorie restriction pathway, wherein both caloric restriction and TOR suppression result in heightened expression of the pivotal sirtuin regulator. Research has shown that sirtuin genes are linked to a long lifespan and have a significant impact on the relationship between diet and longevity. Paoli et al. (2014), in their research unequivocally demonstrated the impact of the amino acid/carbohydrate ratio in the diet on Sir2 expression. According to

Rascón et al. (2012), resveratrol activates a process that depends on calorie restriction to extend the lifespan of honey bees.

Nutrigenomics studies

Nutrigenomics is an emerging subject of study that is generating significant attention. Nutrigenomics is the study of how technologies like nutrigenetics (the study of DNA, including SNPs), transcriptomics (mRNA), proteomics, and metabolomics relate to nutrition (Lal & et al., 2022; Pandita & Pandita, 2022).

The rapidly growing area of nutrigenomics has seen a surge in research focused on understanding how different diets (protein and/or carbohydrate) impact the gene expression patterns of adult honey bees. In their native habitat, honey bees rely significantly on nectar and pollen sources for their nourishment. Nevertheless, in the natural environment, particularly during periods of scarcity, the inclusion of dietary supplements becomes obligatory in the nourishment of honey bees as a result of the inadequate supply of accessible plant resources. Several studies have investigated how honey bees respond to honey, pollen, and/or artificial diets. The studies examined the effects of pollen, microalgae, amino acids, lipids, and carbohydrate diets on metabolic processes, as well as their impact on lifespan and stress responses (DeGrandi-Hoffman & et al., 2018; Ricigliano, 2020). However, there are a restricted amount of nutrigenomic studies that specifically focus on the molecular impacts of nutrition. The study conducted by Alaux et al. (2011) on nutrigenomics in honey bees demonstrated that pollen stimulates nutritional sensing and metabolic pathways. It also has a beneficial influence on genes related to the synthesis of certain antimicrobial peptides and genes connected with lifespan. Rutter et al. (2019), discovered around 2000 transcripts that exhibit variations based on the quality of monofloral diets in honey bees. The majority of these genes are linked to nutritional signaling and immune responses. A study compared two varroa-resistant honey bees that were fed pollen and spirulina microalgae to see how genetic diversity affected how they responded to natural and artificial diets. The research

emphasized the presence of genotype-specific dietary responses in honey bees (Ricigliano & et al., 2021). The research examined the nutrigenomic effects of a diet containing mushroom extract on honey bees, focusing on their survival, food intake, and disease infection level. The findings indicated that the diet had a stimulating impact on four out of the five measured genes (Glavinic & et al., 2021). According to Camilli et al. (2022), zinc supplementation influences the expression of many genes and has a significant impact on the development of honey bees. Research on dietary supplements might potentially enhance future studies in the area of honey bee nutrigenomics by elucidating their impact on the regulation of several genes that are differently expressed, thereby highlighting their significant contribution to honey bee healthy longevity.

Conclusion

Honey bees play a vital part in the operation of ecology, agriculture, and human society. Their functions in domains including pollination, biodiversity preservation, food production, and economic contributions are crucial for a well-rounded and sustainable ecosystem. It has been used as a model organism in several scientific investigations to enhance the comprehension of intricate biological processes and behavior.

Honey bees are advantageous in the field of nutrigenomics because they have clear dietary requirements and may be used to study various genomic, transcriptomic, and proteomic sources. They are also useful for studying the impact of nutrition on aging and age-related diseases. In the future, using honey bees as a reflection of humans may uncover novel gene-longevity connections in response to diet. This might facilitate the investigation of underlying molecular mechanisms and pathways to uncover novel dietary targets for aging processes and age-related diseases.

References

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., ... & Saunders, R. D. (2000). The genome sequence of Drosophila melanogaster. Science, 287(5461), 2185-2195. *Doi: 10.1126/science.287.5461.2185*

Adhish, M., & Manjubala, I. (2023). Effectiveness of zebrafish models in understanding human diseases—A review of models. *Heliyon*. Doi: 10.1016/j.heliyon. 2023.e14557

Alhosin, M. (2023). Epigenetics Mechanisms of Honeybees: Secrets of Royal Jelly. *Epigenetics Insights*, *16*, 25168657231213717. Doi:10.1177/25168657231213

Alaux, C., Dantec, C., Parrinello, H., & Le Conte, Y. (2011). Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. *BMC genomics*, *12*(1), 1-14. Doi:10.1186/1471-2164-12-496

Andersen, M. L., & Winter, L. M. (2017). Animal models in biological and biomedical research-experimental and ethical concerns. *Anais da Academia Brasileira de Ciências*, *91*, e20170238. Doi:10.1590/0001-3765201720170238

Amdam, G. V. (2011). Social context, stress, and plasticity of aging. *Aging Cell*, *10*(1), 18-27. Doi:10.1111/j.1474-9726.2010.00647.x

Ashby, R., Forêt, S., Searle, I., & Maleszka, R. (2016). MicroRNAs in honey bee caste determination. *Scientific reports*, *6*(1), 18794. Doi:10.1038/srep18794

Behrends, A., & Scheiner, R. (2010). Learning at old age: a study on winter bees. *Frontiers in Behavioral Neuroscience*, *4*, 1563. Doi:10.3389/fnbeh.2010.00015

Behrends, A., Scheiner, R., Baker, N., & Amdam, G. V. (2007). Cognitive aging is linked to social role in honey bees (Apis

mellifera). *Experimental Gerontology*, *42*(12), 1146-1153. Doi: 10.1016/j.exger.2007.09.003

Bouchebti, S., Wright, G. A., & Shafir, S. (2022). Macronutrient balance has opposing effects on cognition and survival in honey bees. *Functional Ecology*, *36*(10), 2558-2568. Doi:10.1111/1365-2435.14143

Brandt, A. (2020). Insects as model organisms for basic and applied research. Justus-Liebig-Universität Gießen, Institut für Insektenbiotechnologie.

Cai, Y., Song, W., Li, J., Jing, Y., Liang, C., Zhang, L., ... & Liu, G. H. (2022). The landscape of aging. *Science China Life Sciences*, *65*(12), 2354-2454. Doi:10.1007/s11427-022-2161-3

Camilli, M. P., Kadri, S. M., Alvarez, M. V. N., Ribolla, P. E. M., & Orsi, R. O. (2022). Zinc supplementation modifies brain tissue transcriptome of Apis mellifera honeybees. *BMC genomics*, *23*(1), 282. Doi:10.1186/s12864-022-08464-1

Cardoso-Júnior, C. A., Guidugli-Lazzarini, K. R., & Hartfelder, K. (2018). DNA methylation affects the lifespan of honey bee (*Apis mellifera* L.) workers–evidence for a regulatory module that involves vitellogenin expression but is independent of juvenile hormone function. *Insect Biochemistry and Molecular Biology*, *92*, 21-29. Doi: 10.1016/j.ibmb.2017.11.005

Chen, Y., Hamidu, S., Yang, X., Yan, Y., Wang, Q., Li, L., ... & Li, Y. (2022). Dietary supplements and natural products: an update on their clinical effectiveness and molecular mechanisms of action during accelerated biological aging. *Frontiers in Genetics*, *13*, 880421. Doi:10.3389/fgene.2022.880421

Corona, M., Branchiccela, B., Alburaki, M., Palmer-Young, E. C., Madella, S., Chen, Y., & Evans, J. D. (2023). Decoupling the effects of nutrition, age, and behavioral caste on honey bee physiology, immunity, and colony health. *Frontiers in Physiology*, *14*, 1149840. Doi:10.3389/fphys.2023.1149840

Cui, H., Kong, Y., & Zhang, H. (2012). Oxidative stress, mitochondrial dysfunction, and aging. *Journal of Signal Transduction*. Doi:10.1155/2012/646354

DeGrandi-Hoffman, G., Gage, S. L., Corby-Harris, V., Carroll, M., Chambers, M., Graham, H., ... & Ziolkowski, N. (2018). Connecting the nutrient composition of seasonal pollens with changing nutritional needs of honey bee (Apis mellifera L.) colonies. *Journal of Insect Physiology*, *109*, 114-124. Doi:10.1016/j.jinsphys.2018.07.002

Duncan, E. J., Leask, M. P., & Dearden, P. K. (2020). Genome architecture facilitates phenotypic plasticity in the honeybee (Apis mellifera). *Molecular Biology and Evolution*, *37*(7), 1964-1978. Doi:10.1093/molbev/msaa057

Evangelakou, Z., Manola, M., Gumeni, S., & Trougakos, I. P. (2019). Nutrigenomics as a tool to study the impact of diet on aging and age-related diseases: the Drosophila approach. *Genes & Nutrition*, 14(1), 1-18. Doi:10.1186/s12263-019-0638-6

Fong, B. Y., Chiu, W. K., Chan, W. F., & Lam, T. Y. (2021). A review study of a green diet and healthy ageing. *International journal of environmental research and public health*, *18*(15), 8024. Doi:10.3390/ijerph18158024

Ford, D. (2013). Honeybees and cell lines as models of DNA methylation and aging in response to diet. *Experimental Gerontology*, *48*(7), 614-619. Doi: 10.1016/j.exger.2012.07.010

Ford, D., Ions, L. J., Alatawi, F., & Wakeling, L. A. (2011). The potential role of epigenetic responses to diet in ageing. *Proceedings of the Nutrition Society*, *70*(3), 374-384. Doi:10.1017/S0029665111000851

Fortini, M. E., Skupski, M. P., Boguski, M. S., & Hariharan, I. K. (2000). A survey of human disease gene counterparts in the Drosophila genome. *The Journal of Cell Biology*, *150*(2), F23-F30. Doi:10.1083/jcb.150.2.F23

Glavinic, U., Rajkovic, M., Vunduk, J., Vejnovic, B., Stevanovic, J., Milenkovic, I., & Stanimirovic, Z. (2021). Effects of Agaricus bisporus mushroom extract on honey bees infected with Nosema ceranae. *Insects*, *12*(10), 915. Doi**:**10.3390/insects12100915

Giray, T., & Robinson, G. E. (1994). Effects of intracolony variability in behavioral development on plasticity of division of labor in honey bee colonies. *Behavioral Ecology and Sociobiology*, *35*, 13-20. Doi:10.1007/BF00167054

Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., & Li, J. (2022). Aging and aging-related diseases: From molecular mechanisms to interventions and treatments. *Signal Transduction and Targeted Therapy*, *7*(1), 391. Doi:10.1038/s41392-022-01251-0

Huang, Z. Y., & Robinson, G. E. (1996). Regulation of honey bee division of labor by colony age demography. *Behavioral Ecology and Sociobiology*, *39*, 147-158. Doi.10.1007/s002650050276

Ihle, K. E., Mutti, N. S., Kaftanoglu, O., & Amdam, G. V. (2019). Insulin receptor substrate gene knockdown accelerates behavioural maturation and shortens lifespan in honeybee workers. *Insects*, *10*(11), 390. Doi:10.3390/insects10110390

Irion, U., & Nüsslein-Volhard, C. (2022). Developmental genetics with model organisms. *Proceedings of the National Academy of Sciences*, *119*(30), e2122148119. Doi:10.1073/pnas.2122148119

Keller, L., & Jemielity, S. (2006). Social insects as a model to study the molecular basis of ageing. *Experimental Gerontology*, *41*(6), 553-556. Doi: 10.1016/j.exger.2006.04.002

Keshavarz, M., Xie, K., Schaaf, K., Bano, D., & Ehninger, D. (2023). Targeting the "hallmarks of aging" to slow aging and treat age-related disease: fact or fiction?. *Molecular Psychiatry*, *28*(1), 242-255. Doi:10.1038/s41380-022-01680-x

Kojić, D., Čelić, T., Spremo, J., Orčić, S., Vukašinović, E., Đorđievski, S., ... & Purać, J. Beneficial Effect of Sodium Butyrate, a Histone Deacetylase Inhibitor, on Honey Bees Immune Response and Oxidative Status. *Available at SSRN 4525693*.

Lal, M. K., Sharma, E., Tiwari, R. K., Devi, R., Mishra, U. N., Thakur, R., ... & Sahu, S. K. (2022). Nutrient-mediated perception and signalling in human metabolism: a perspective of nutrigenomics. *International Journal of Molecular Sciences*, *23*(19), 11305. Doi.org/10.3390/ijms231911305

Lee, H. Y., Lee, S. H., & Min, K. J. (2015). Insects as a model system for aging studies. *Entomological research*, *45*(1), 1-8. Doi:10.1111/1748-5967.12088

Lee, S. H., Lee, J. H., Lee, H. Y., & Min, K. J. (2019). Sirtuin signaling in cellular senescence and aging. *BMB reports*, *52*(1), 24. Doi:10.5483/BMBRep.2019.52.1.290

Liao, L. H., Wu, W. Y., & Berenbaum, M. R. (2017). Impacts of dietary phytochemicals in the presence and absence of pesticides on longevity of honey bees (Apis mellifera). *Insects*, *8*(1), 22. Doi:10.3390/insects8010022

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2023). Hallmarks of aging: An expanding universe. *Cell*. Doi: 10.1016/j.cell.2022.11.001

Lourenço, A. P., Martins, J. R., Torres, F. A. S., Mackert, A., Aguiar, L. R., Hartfelder, K., ... & Simões, Z. L. P. (2019). Immunosenescence in honey bees (Apis mellifera L.) is caused by intrinsic senescence and behavioral physiology. *Experimental Gerontology*, *119*, 174-183. Doi: 10.1016/j.exger.2019.02.005

Lowe, R., Wojciechowski, M., Ellis, N., & Hurd, P. J. (2022). Chromatin accessibility-based characterisation of brain gene regulatory networks in three distinct honey bee polyphenisms. *Nucleic Acids Research*, *50*(20), 11550-11562. Doi:10.1093/nar/gkac992

Lu, H., Ma, L., Zhang, Y., Feng, Y., Zhang, J., & Wang, S. (2022). Current animal model systems for ovarian aging research. *Aging and Disease*, *13*(4), 1183. Doi:10.14336/AD.2021.1209

Maleszka, R. (2014). The social honey bee in biomedical research: realities and expectations. *Drug Discovery Today: Disease Models*, *12*, 7-13. Doi: 10.1016/j.ddmod.2014.06.001

Mao, W., Schuler, M. A., & Berenbaum, M. R. (2015). A dietary phytochemical alters caste-associated gene expression in honey bees. *Science advances*, *1*(7), e1500795. Doi: 10.1126/sciadv.1500795

Martelli, F., Falcon, T., Pinheiro, D. G., Simões, Z. L., & Nunes, F. M. (2022). Worker bees (*Apis mellifera*) deprived of pollen in the first week of adulthood exhibit signs of premature aging. *Insect Biochemistry and Molecular Biology*, *146*, 103774. Doi: 10.1016/j.ibmb.2022.103774

Milte, C. M., & McNaughton, S. A. (2016). Dietary patterns and successful ageing: a systematic review. *European Journal of Nutrition*, *55*, 423-450. Doi:10.1007/s00394-015-1123-7

Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 18. Doi:10.1186/s42826-022-00128-1

Mutti, N. S., Dolezal, A. G., Wolschin, F., Mutti, J. S., Gill, K. S., & Amdam, G. V. (2011). IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. *Journal of Experimental Biology*, *214*(23), 3977-3984. Doi:10.1242/jeb.061499

Münch, D., & Amdam, G. V. (2010). The curious case of aging plasticity in honey bees. *FEBS Letters*, *584*(12), 2496-2503. Doi: 10.1016/j.febslet.2010.04.007

Münch, D., Amdam, G. V., & Wolschin, F. (2008). Ageing in a eusocial insect: molecular and physiological characteristics of life

span plasticity in the honey bee. *Functional ecology*, *22*(3), 407-421. Doi:10.1111/j.1365-2435.2008.01419.x

Münch, D., Kreibich, C. D., & Amdam, G. V. (2013). Aging and its modulation in a long-lived worker caste of the honey bee. *Journal of Experimental Biology*, *216*(9), 1638-1649. Doi.org/10.1242/jeb.078915

Pandita, D., & Pandita, A. (2022). Omics Technology for the Promotion of Nutraceuticals and Functional Foods. *Frontiers in physiology*, *13*, 817247. Doi:10.3389/fphys.2022.817247

Page Jr, R. E., & Peng, C. Y. S. (2001). Aging and development in social insects with emphasis on the honey bee, Apis mellifera L. *Experimental Gerontology*, *36*(4-6), 695-711. Doi:10.1016/S0531-5565(00)00236-9

Paoli, P. P., Wakeling, L. A., Wright, G. A., & Ford, D. (2014). The dietary proportion of essential amino acids and Sir2 influence lifespan in the honeybee. *Age*, *36*, 1239-1247. Doi:10.1007/s11357-014-9649-9

Polsinelli, G. A., & Yu, H. D. (2018). Regulation of histone deacetylase 3 by metal cations and 10-hydroxy-2E-decenoic acid: Possible epigenetic mechanisms of queen-worker bee differentiation. *Plos one*, *13*(12), e0204538. Doi:10.1371/journal.pone.0204538

Rascón, B., Hubbard, B. P., Sinclair, D. A., & Amdam, G. V. (2012). The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to caloric restriction. *Aging (Albany NY)*, *4*(7), 499. Doi:10.18632/aging.100474

Rasmussen, E. M., Seier, K. L., Pedersen, I. K., Kreibich, C., Amdam, G. V., Münch, D., & Dahl, J. A. (2021). Screening bioactive food compounds in honey bees suggests curcumin blocks alcohol-induced damage to longevity and DNA methylation. *Scientific Reports*, *11*(1), 19156. Doi:10.1038/s41598-021-98614-4

Remolina, S. C., Hafez, D. M., Robinson, G. E., & Hughes, K. A. (2007). Senescence in the worker honey bee Apis mellifera. *Journal of Insect Physiology*, *53*(10), 1027-1033. Doi: 10.1016/j.jinsphys.2007.05.015

Ricigliano, V. A. (2020). Microalgae as a promising and sustainable nutrition source for managed honey bees. *Archives of Insect Biochemistry and Physiology*, *104*(1), e21658. Doi:10.1002/arch.21658

Ricigliano, V. A., Ihle, K. E., & Williams, S. T. (2021). Nutrigenetic comparison of two Varroa-resistant honey bee stocks fed pollen and spirulina microalgae. *Apidologie*, *52*(4), 873-886. Doi:10.1007/s13592-021-00877-3

Robinson, N. B., Krieger, K., Khan, F. M., Huffman, W., Chang, M., Naik, A., ... & Gaudino, M. (2019). The current state of animal models in research: A review. *International Journal of Surgery*, *72*, 9-13. Doi: 10.1016/j.ijsu.2019.10.015

Romero, S., Nastasa, A., Chapman, A., Kwong, W. K., & Foster, L. J. (2019). The honey bee gut microbiota: strategies for study and characterization. *Insect molecular biology*, *28*(4), 455-472. Doi:10.1111/imb.12567

Rueppell, O., Aumer, D., & Moritz, R. F. (2016). Ties between ageing plasticity and reproductive physiology in honey bees (Apis mellifera) reveal a positive relation between fecundity and longevity as consequence of advanced social evolution. *Current Opinion in Insect Science*, *16*, 64-68. Doi: 10.1016/j.cois.2016.05.009

Rueppell, O., Linford, R., Gardner, P., Coleman, J., & Fine, K. (2008). Aging and demographic plasticity in response to experimental age structures in honeybees (Apis mellifera L). *Behavioral Ecology and Sociobiology*, *62*, 1621-1631. Doi:10.1007/s00265-008-0591-7

Rutter, L., Carrillo-Tripp, J., Bonning, B. C., Cook, D., Toth, A. L., & Dolezal, A. G. (2019). Transcriptomic responses to diet

quality and viral infection in Apis mellifera. *BMC genomics*, *20*, 1-20. Doi:10.1186/s12864-019-5767-1

Seeley, T. D. (1995). *The Wisdom of the Hive*. Cambridge, MA: Harvard University Press.

Spannhoff, A., Kim, Y. K., Raynal, N. J. M., Gharibyan, V., Su, M. B., Zhou, Y. Y., ... & Bedford, M. T. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO reports*, *12*(3), 238-243. Doi:10.1038/embor.2011.9

Strachecka, A., Olszewski, K., Bajda, M., & Demetraki-Paleolog, J. (2015). Natural larval diet differently influences the pattern of developmental changes in DNA 5-methylcytosine levels in Apis mellifera queens as compared with workers and drones. *Biochemistry (Moscow)*, *80*, 1019-1025. Doi:10.1134/S0006297915080076

Wang, M., Xiao, Y., Li, Y., Wang, X., Qi, S., Wang, Y., ... & Wu, L. (2021). RNA m6A modification functions in larval development and caste differentiation in honeybee (Apis mellifera). *Cell Reports*, *34*(1). Doi:10.1016/j.celrep.2020.108580

Wang, X., Zhang, X., Zhang, Z., Lang, H., & Zheng, H. (2018). Honey bee as a model organism to study gut microbiota and diseases. *Drug Discovery Today: Disease Models*, *28*, 35-42. Doi: 10.1016/j.ddmod.2019.08.010

Wheeler, D. E., Buck, N. A., & Evans, J. D. (2014). Expression of insulin/insulin-like signalling and TOR pathway genes in honey bee caste determination. *Insect Molecular Biology*, *23*(1), 113-121. Doi: 10.1111/imb.12065

Winston, M. L. (1980). Seasonal patterns of brood rearing and worker longevity in colonies of the Africanized honey bee (Hymenoptera: Apidae) in South America. *Journal of the Kansas Entomological Society*, 157-165.

Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.

Ye, J., Cui, X., Loraine, A., Bynum, K., Kim, N. C., White, G., ... & Ruden, D. M. (2007). Methods for nutrigenomics and longevity studies in Drosophila: effects of diets high in sucrose, palmitic acid, soy, or beef. *Biological Aging: Methods and Protocols*, 111-141. Doi:10.1007/978-1-59745-361-5_10

Zhang, Y., Li, Z., He, X., Wang, Z., & Zeng, Z. (2023). H3K4me1 Modification Functions in Caste Differentiation in Honey Bees. *International Journal of Molecular Sciences*, *24*(7), 6217. Doi:10.3390/ijms24076217

Zhang, L., Pitcher, L. E., Yousefzadeh, M. J., Niedernhofer, L. J., Robbins, P. D., & Zhu, Y. (2022). Cellular senescence: A key therapeutic target in aging and diseases. *Journal of Clinical Investigation*, *132*(15), e158450. Doi:10.1172/JCI158450

Zhang, J. J., Xi, G. S., & Zhao, J. (2021). Vitellogenin regulates estrogen-related receptor expression by crosstalk with the JH and IIS-TOR signaling pathway in Polyrhachis vicina Roger (Hymenoptera, Formicidae). *General and Comparative Endocrinology*, *310*, 113836. Doi: 10.1016/j.ygcen.2021.113836

Zheng, H., & Hu, F. (2009). Honeybee: a newly emerged model organism. *Acta Entomologica Sinica*, *52*(2), 210-215.

CHAPTER II

Enzymes that Opened a New Era for The Scientific Community: TvTS and MpMS

İdris ARSLAN[1]

Introduction

The term 'Terpene' describes the natural products derived from five carbon isoprene units with a 30C polycyclic structure produced by the evolutionary optimisation of nature and endowed with versatile biological activities. Additionally terpenoids are the largest group of natural products synthesized by living sources, representing 60% of the known natural products. They are ubiquitous; thus, they are present in almost all classess of living organisms, but more common in terestrial higher plants (Zhang et al. 2021).

According to their chemical skeleton, terpenes or terpenoids are further classified as monoterpenoids (C10), hemiterpenoids (C5),

[1] Zonguldak Bülent Ecevit University, Faculty of Science, Molecular Biology and Genetics, Zonguldak, Türkiye

sesquiterpenoids (15C), diterpenoids (20C), sesterterpenoids (C25), triterpenoids (C30), and tetraterpenoids/carotenoids (C40). Moreover, derivatives with more C atoms are called 'Homo-derivatives', while those with fewer C atoms are classified as 'Nor-derivatives' of the terpenoids. Triterpenes can be also divided into subgroups, such as acyclic, mono-, bi-, tri-, tetra- and penta- (cyclic) triterpenoids according to their C skeletal backbone (Nguyen et al. 2015).

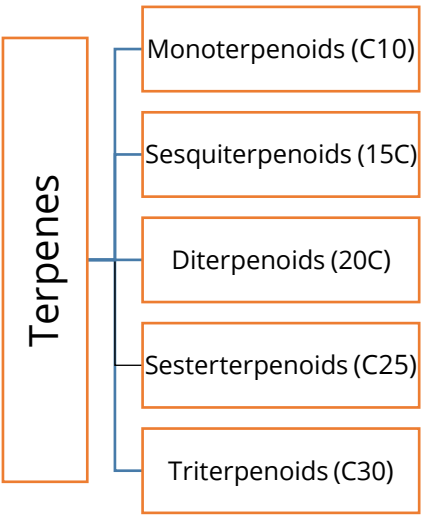


Figure 1. Classification of terpenoids based on carbon skeleton backbone

Additionally, the term meroterpenoid states natural products of mixed biochemical origin which partially originated from terpenoid pathways (Cornforth 1968). Triterpenoids have been reported to be characterized approximately 200 distinct skeletons with more than 30.000 substances and 100 unique cyclical triterpenoid frames uptill now (Deng et al. 2020). Plant-derived triterpenoids were reported to play an active role in the cellular processes such as permeability of the membranes, cell respiration, photosynthetic reactions and *up*- or *down*- regulation of growth and/or development (Tholl 2015). Terpenoids possess fascinating

pharmacological activity including, anticancer, anti-inflammatory, anti-allergic and enzyme inhibitory activity (Arslan, 2023).

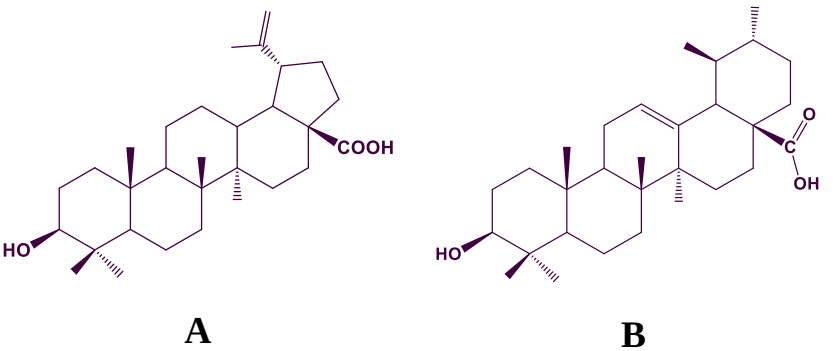


Figure 2. A common naturally occurring triterpenoids betulinic acid (A) ursolic acid (B)

Synthesis of triterpenoid compounds

Most recently, triterpenes were known to be produced by triterpene synthases from acyclic squalene and/or (*S*)-2,3-epoxysqualene (Abe 2007).

So far, terpenoids were known to be naturally only synthesized from C5 units, isopentenyl pyrophosphate (IPP), also known as isopentenyl diphosphate (IDP) an intermediate in the cytosolic mevalonate pathway (MVA) and plasticidal methylerythritol phosphate/deoxyxylulose 5-phosphate pathway (MEP/DOXP) (Shi et al. 2010).

2,3-oxidosqualene a precursor unit of triterpenoids is synthesized via the mevalonic pathway and then diversified structurally through 2,3-oxidosqualene cyclases and several scaffold-regulating enzymes including cytochrome P450 monooxygenases (P450s or CYP), UDP-glycosyltransferases (UGTs) and acyltransferases. Moreover, the most of bioactive triterpenoids are purified from natural sources by often conventional techniques of extraction and sometimes semi-synthesized. It was

reported to be these methods of supply are time- and energy consuming and do not generally align with sustainability purposes. Recent progressess in genetic and metabolic engineering fields have revealed expectations for the distinct approaches of triterpenoid pathway in heterologous sources such as *Escherichia coli*, *Saccharomyces cerevisiae* and *Nicotiana benthamiana*, which seem to be reasonably promising alternatives (Dinday and Ghosh 2023).

There are numerous articles supporting triterpenoids are produced solely via squalene precursor. The most recent study has provided sufficient evidence to challenge a long-established idea. It is now known that triterpenoids are not synthesized solely via squalene (Tao et al, 2022).

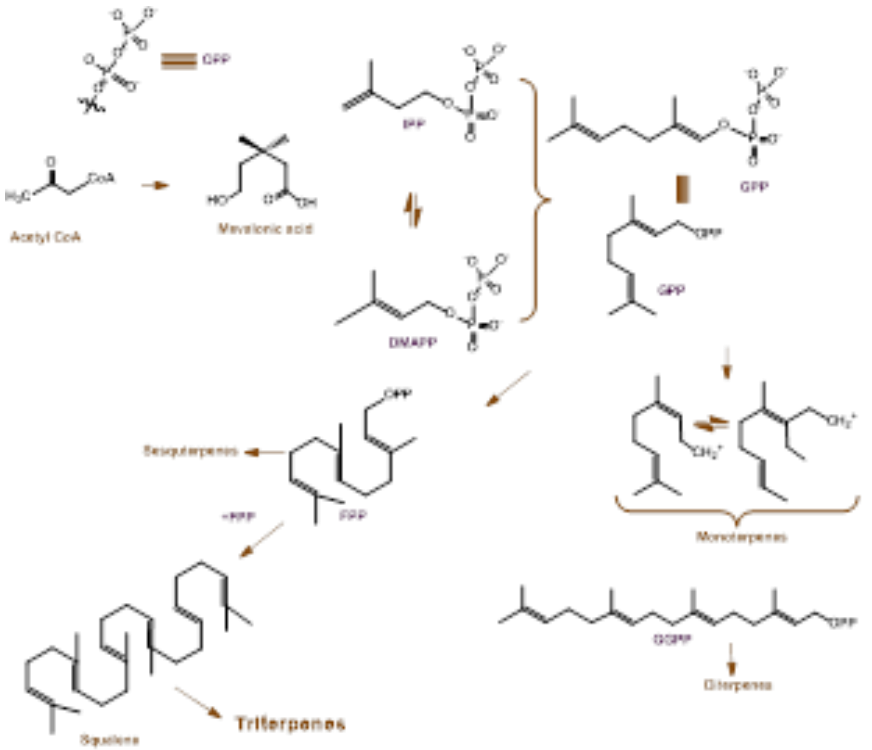


Figure 3. Biosynthesis pathways of terpenoid compounds in natural sources

Recently, non-squalene triterpenes have been discovered and two fungal chimeric class I triterpene synthases, *Talaromyces verruculosus* talaropentaene synthase and *Macrophomina phaseolina* macrophomene synthase were characterized by Tao and colleagues (2022).

Both enzymes were found to accept dimethylallyl diphosphate and isopentenyl diphosphate or hexaprenyl diphosphate as substrates, representing the first examples of non-squalene-dependent triterpene biosynthesis. Although the classical pathway for triterpenes proceeds through squalene, non-squalene triterpenes are generated by bifunctional terpene synthases which convert dimethylallyl diphosphate and isopentenyl diphosphate through hexaprenyl diphosphate into the triterpenes.

After oxidation to (*S*)-2,3-epoxysqualene, lanosterol synthase catalyses the biosynthesis of lanosterol, the precursor to steroids and saponins. These enzymes are classified as class II terpene synthases with a conserved DXDD motif for substrate activation through protonation.

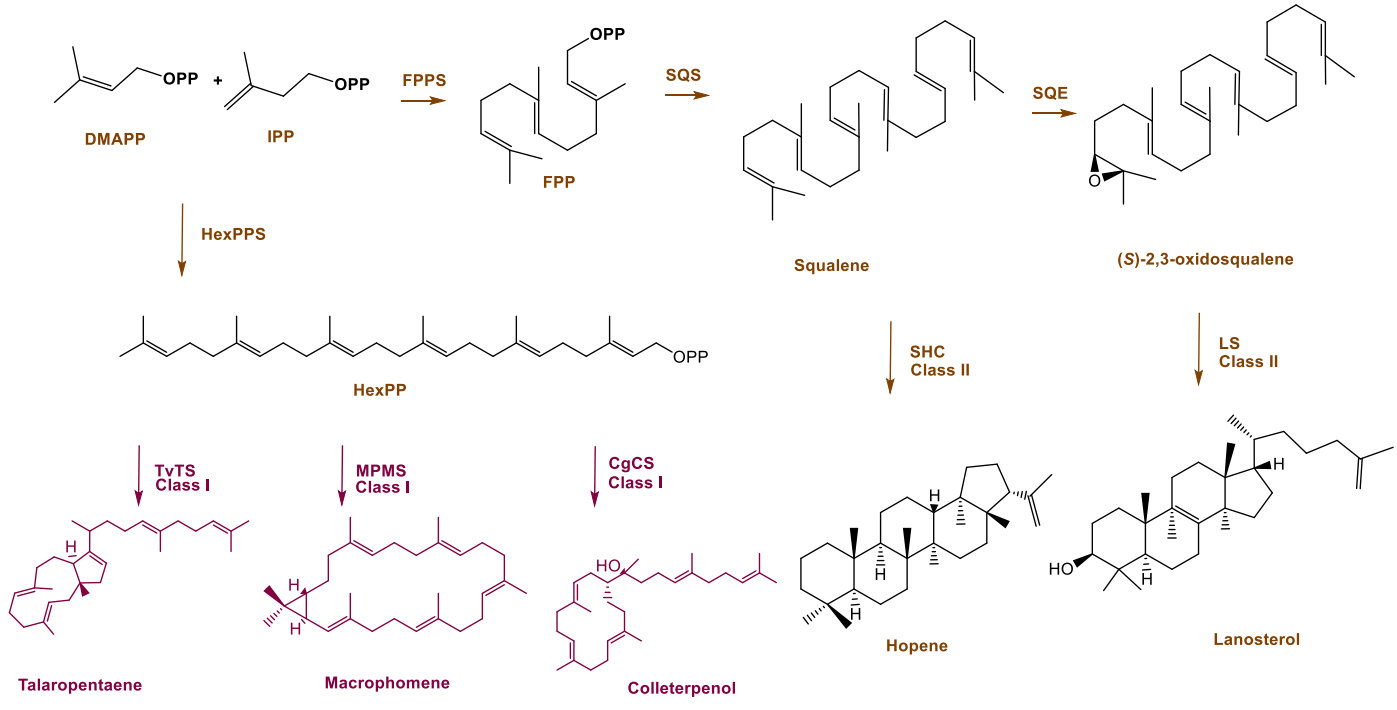


Figure 4. Biosynthesis pathways of terpenoid compounds in natural sources

Conclusion

The synthesis of the same compound through different biochemical pathways shows that there may be shunt mechanisms between the pathways. However, the structure and enzyme activity of enzymes that play an active role in the synthesis pathway are important in the development of new approaches for the production of relevant compounds.

Undoubtedly, this new discovery regarding triterpenoid biosynthesis will profoundly affect the overview of triterpenoid synthesis. There is now strong evidence to consider new approaches for the synthesis of terpene compounds used in industrial practices. The extraordinary pharmacological and biological activities of triterpenoid compounds will highly motivate researchers to test the effectiveness of these newly discovered pathways and uncover their advantages.

REFERENCES

Abe, I. (2007) Enzymatic synthesis of cyclic triterpenes. *Nat. Prod. Rep.* 24, 1311–1331.

Arslan, I. (2023) Natural PAK1 inhibitors: potent anti-inflammatory effectors for prevention of pulmonary fibrosis in COVID-19 therapy. Natural Product Research 10:1-13. doi: 10.1080/14786419.2023.2254454.

Cornforth, J. W. (1968) Terpenoid biosynthesis, Chem Br, 4 (3), 102-106,

Deng, J, Xiao, J. & H. Yang, H. (2020) Handbook of Dietary Phytochemicals, Springer Singapore, Singapore, 2020, pp. 1–53.

Dinday S, Ghosh S. (2023). Recent advances in triterpenoid pathway elucidation and engineering. Biotechnol Adv. 68:108214.

Nguyen VT, Tung NT, Cuong TD et al (2015) Cytotoxic and antiangiogenic effects of lanostane triterpenoids from Ganoderma lucidum. Phytochem Lett 12:69–74.

Tao H, Lauterbach L, Bian G, Chen R, Hou A, Mori T, Cheng S, Hu B, Lu L, Mu X, Li M, Adachi N, Kawasaki M, Moriya T, Senda T, Wang X, Deng Z, Abe I, Dickschat JS, Liu T. Discovery of non-squalene triterpenes. Nature. 2022 Jun;606(7913):414-419.

Shi L, Ren A, Mu D, Zhao M (2010) Current progress in the study on biosynthesis and regulation of ganoderic acids. Appl Microbiol Biotechnol 88:1243–1251.

Tholl D (2015) Biosynthesis and biological functions of terpenoids in plants. 63–106. 10.1007/10-2014_295.

Zhang X, Zhang S, Zhao S, Wang X, Liu B, Xu H (2021) Click chemistry in natural product modification. Frontiers in Chemistry. 9:774977.

CHAPTER III

Therapeutic Effects of Flavonoids on Alzheimer's Disease

Esra Nur YEŞİLKENT[1]
Medine Sibel KARAĞAÇ[2]
Neslihan ÖZTÜRK[3]
Hamid CEYLAN[4]

Introduction

It is known that many different factors such as inflammation and oxidative stress, as well as major hallmarks such as amyloid β

[1] PhD Student, Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Türkiye
[2] PhD Student, Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Türkiye
[3] PhD Student, Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Türkiye
[4] Assoc. Prof. Dr., Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Türkiye

accumulation and tau protein hyperphosphorylation, play a role in the development and progression of Alzheimer's Disease (AD). This situation is considered to be the reason why today's strategies in the fight against AD fail. Therefore, therapeutics and new strategies with multifaceted prevention properties against the disease are needed. Many different in vitro and in vivo studies conducted in recent years have shown that flavonoids with phytochemical properties are versatile alternative and natural compounds that have the potential to be effective in combating AD. This review aims to guide the use of flavonoids, which are thought to have significant potential, in AD therapy based on the existing literature.

Alzheimer's Disease

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive memory impairment and loss of cognitive functions caused by neuronal cell death [1]. Alzheimer's, a type of dementia, accounts for about two-thirds of dementia patients over the age of 65. It is one of the biggest health problems of the 21st century and has been recognized as a global health problem by the World Health Organization [2]. The fact that the disease lasts a lifetime, reduces cognitive ability, interferes with daily activities, and causes people to forget even their own identity, imposes an emotional and financial burden on society. It is known that head trauma, depression, high parental age, family history of dementia, cardiovascular activity, cerebrovascular disease, and smoking increase the risk of AD, especially with advancing age [3]. Depending on the degree of the disease, these symptoms begin with impairment in functions such as memory, comprehension, and speech, and in later stages, language disorder, psychosis, dyspraxia, sleep disorder, anxiety, and finally dependence on the caregiver occur [4].

Epidemiology of AD

AD is the most common neurodegenerative disorder worldwide, accounting for approximately 70% of all dementia cases. The incidence of AD increases with age, doubling every five to ten years [5]. The World Health Organization predicts that the number of patients, which is over 55 million today, will increase four times by 2050. Considering that advanced age is the most important risk factor for AD and that the average life expectancy is increasing while the birth rate is decreasing worldwide, it seems that this increase in the prevalence of the disease is inevitable [6, 7]. In addition to the human burden of the disease, its financial burden is also becoming a major problem. AD cost $604 billion worldwide in 2010 alone, with the annual total cost per person estimated at $590 for mild dementia and $25,510 for severe dementia. With AD being accompanied by other diseases, the annual cost per person increases considerably [7].

Risk Factors for AD

In addition to the age factor, various risk factors for AD have been identified. The presence of a family history of dementia increases the risk of AD by 1% to 30% [8]. In addition, factors such as nutrition, diabetes, obesity, genetic factors, gender, head trauma, depression, high parental age, physical and mental inactivity, low education level, environmental factors, and cardiovascular diseases play a role in dementia. It is thought that 30% of dementia can be prevented by eliminating these risk factors [9]. Genetic risk factors for AD include inherited mutations in APP, PSEN1, and PSEN2, and more common genetic variations such as APOE (knopman). However, genetic predispositions pose a lower risk for AD than other factors [10].

Stages of AD

AD consists of three stages: mild cognitive impairment (MLD), moderate-stage dementia, and advanced-stage dementia. MCI is the name given to the transition period between aging and

dementia, and patients in this stage have impairments in memory or non-memory areas such as speech [11]. It is known that cases without dementia or with very mild cognitive difficulties have a 10% probability of progressing to higher dementia severity [12].

In the middle stage, the symptoms of the disease become clearer. Patients cannot go out of the house alone and have difficulty meeting their daily needs, such as eating, dressing, toileting, and bathroom use [13]. In the more advanced stage of dementia, symptoms such as severe memory impairment, language use difficulties, psychosis, dyspraxia, dystonia, olfactory disorder, sleep disorder, urinary incontinence, hallucinations, skepticism, and anxiety, which require the patient's complete dependence on the caregiver, can occur [2, 14].

Pathology, Symptoms and Diagnosis of AD

The most common and distinctive lesions found in the Alzheimer's brain are extracellular amyloid plaques composed of Aβ protein and intracellular neurofibrillary tangles formed by tau proteins [15]. Although synaptic degeneration, hippocampal neuronal loss, and aneuploidy are some of the later described pathological features, only plaques and tangles are currently considered for Alzheimer's pathological criteria [16]. The most important evidence that Aβ and tau are the main causes of the disease comes from studies of cases with APP, PSEN1, and PSEN2 mutations [17]. APP is the precursor to Aβ peptides, and APP mutations also affect Aβ cleavage and aggregation. In particular, neurotic plaques are important as the main criterion for the diagnosis of AD at autopsy. Because there is a proven link between neuronal loss and disease severity in AD and senile plaques [18, 19].

The most important symptoms defined for AD are memory disorders in the MCI stage, speech disorders, and abnormalities in the dementia stage, such as advanced memory impairment, dyspraxia, psychosis, and anxiety. Apart from these, common symptoms include headache, gait abnormalities, paralysis, major

psychosis symptoms seen in the final stages of the disease, and being confined to bed [20, 21].

The best-identified imaging techniques for AD are magnetic resonance imaging (MRI) and amyloid positron emission tomography (amyloid PET). Amyloid PET imaging is particularly useful in the differential diagnosis of early-onset AD, as it enables the diagnosis of moderate amyloid deposition and brain synaptic density [22]. There are also techniques other than MRI and amyloid PET. Techniques such as PET, fMRI, and single-photon emission computed tomography (SPECT) to map dysfunctions of smaller portions of the medial, temporal, and perietal lobes, Computed Tomography (CT) and Magnetic Resonance imaging (MRI) for analysis of cerebrospinal fluid (CSF) Aβ and tau are used to identify behavioral changes [22]. MRI is also recommended by experts as the first step after clinical evaluation [23]. AD is difficult to diagnose at an early stage because some patients show symptoms that are too severe to affect daily activities and do not meet the criteria for dementia. The most effective tests that can be used to identify MCIs in the early stages of the disease are neuropsychological tests [24].

Treatment of AD

No effective treatment has yet been found to stop the progression of AD or prevent the disease, but there are two categories of drugs approved for the treatment of Alzheimer's. These are acetylcholinesterase inhibitors (AChEIs) and N-methyl D-aspartate (NMDA) antagonists [25].

Acetylcholine (ACh) is a very important chemical for memory and cognitive functions. Loss of cholinergic function has been found to be closely associated with cognitive dysfunction. The activity of AChE must be limited in order to maintain the amount of ACh. The best treatment strategy developed for this purpose is acetylcholinesterase inhibitors, and there are three drugs in this category. These drugs are Dopenzil, rivastigmine, and Galantamine. Of these, dopenzil is used in all stages of Alzheimer's. Rivastigmine

and Galantamine are used for treatments in MCI and dementia [26-28]. Additionally, tacrine was the first drug approved for the treatment of Alzheimer's and was banned due to safety concerns [29]. A drug called memantine is used to block NMDA receptors. Memantine is used for the treatment of moderate Alzheimer's and can be taken with cholinesterase inhibitors [30]. These drugs, all approved by the FDA, are known to stabilize cognitive decline for up to 3–6 months. Although these drugs are used for the treatment of Alzheimer's, their efficacy is quite limited, and they have side effects such as nausea, vomiting, diarrhea, dizziness, sleep disturbances, body aches, and constipation [31]. For these reasons, plants are especially used in the treatment of psychiatric and neurological diseases [32].

Many medicinal plants contain various phytochemicals that inhibit the development of neurodegenerative diseases [33]. Flavonoids are very common phytochemicals in nature and are found in almost every plant. Foods rich in flavonoids are known to have protective and therapeutic effects for various diseases, including AD [34]. Certain flavonoids have also been directly associated with the progression of AD. Herbal flavonoids are thought to be potential therapeutics for various neurodegenerative diseases due to their lower cost, easy availability, and fewer side effects [35].

Flavonoids

Flavonoids are secondary metabolites found in nearly all plant cells [36, 37]. These bioactive phytochemical components, which are found in almost all of the plant, are the source of the medicinal value of the plant [38]. It is known that more than 10,000 species of flavonoids have been isolated and described since their discovery [39]. Flavonoids are generally used as natural therapeutic agents [40]. They use the phenylpropanoid pathway and are naturally synthesized due to their ability to be absorbed [41].

Flavonoids can be used in general non-synthetic dyes [42, 43], the basis of cosmetic products [44], and anti-wrinkle skin agents, and many more [45]. In addition, additives that strengthen the preservation and quality of foods used as antioxidants, coloring and flavoring can be obtained through flavonoids [46]. Recently, intense interest and curiosity about medicinal plants with known therapeutic potential is due to flavonoids [47]. Flavonoids have been used for many years, with the belief in their benefits being passed down from generation to generation. They have valuable biological properties that support health by helping to eliminate symptoms, which can be used in the early diagnosis of various diseases.

Biological Roles and Effects of Flavonoids

It is known that the absorption of flavonoids varies depending on many physicochemical properties in dietary intake. The absorption of flavonoids from the small intestine depends on whether they are glycosides or aglycones. The absorption of flavonoids in aglycon form is easily accomplished by the cells of the small intestine. However, glycosides must be converted to flavonoids in aglycon form for absorption [48]. According to one study, an alternative mechanism is thought to be through the breakdown of flavonoids in the form of glycosides by the enzyme lactase fluoroidzin hydrolase (LPH), a group of β-glucosidases found in the membrane structure of the small intestine. Thus, the absorption of these flavonoids, which are converted to aglycone form, occurs more easily in the small intestine [49]. The presence of specific substrate expected in the LPH enzyme may vary depending on the glycoside type of flavonoids (glucosides, galactosides, arabinosides, xylosides and rhamnocytes) [50]. Flavonoids, which do not have LPH-specific substrate and exist in glycoside form, are transported to the colon. Here, the hydrolysis process is carried out by bacteria. In addition, released aglycones are also degraded in this column [51]. Considering that the absorption power of the small intestine cells is higher than the colon, glycosides are expected to be absorbed in lesser amounts in the colon.

Following absorption, events of glucuronidation, sulfation, or methylation are expected for flavonoids to be conjugated in the liver. If these events do not occur, they are metabolized to yield smaller phenolic compounds [52]. The conjugation reactions that take place prevent the free presence of aglycon-formed flavonoids other than catechins in the urine or plasma [53]. Flavonoid molecules that cannot be absorbed are transferred to the large intestine, where they are broken down with the help of microorganisms, and the ring structure of flavonoids is destroyed. At the beginning of digestion, flavonoids can be broken down into monomer and dimer structures in the stomach, an organ with a high acidic pH. Molecules in polymeric structure are transmitted to the colon and degradation is carried out by bacteria. The sugar molecule in the structure of flavonoids is a preferred determinant in terms of bioavailability. In addition, studies have shown that the dimerization process greatly reduces the bioavailability. Absorbed flavonoids exhibit a variety of therapeutic effects in the body, including antioxidant, anticarcinogenic, antidiabetic, antimicrobial, and antiviral.

Antioxidant Activity

The arrangement of functional subgroups in the structure of flavonoids according to the nuclear structure is effective in shaping their antioxidant activities. For example, the presence of hydroxyl groups in flavonoids, their configuration, the total number of these groups, radical scavenging ability, and metal ion chelation are greatly influenced by various mechanisms of antioxidant activity [54]. The hydroxyl configuration of the B-ring structure is one of the most important determinants of the reduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Thanks to this configuration, a relatively stable flavonoid radical is formed by donating hydrogen and an electron to peroxyl, hydroxyl and peroxynitrite radical molecules [55, 56].

Antioxidant action mechanisms have been observed in 3 different ways: disruption of ROS formation mechanism by inhibiting chelating elements or enzymes involved in free radical

formation, removal of ROS, up-regulation or protection of antioxidant defenses [57]. Finally, flavonoids are known to inhibit some microsomal enzymes involved in ROS generation. These enzymes include mitochondrial succinoxidase, monooxygenase, nicotinamide adenine dinucleotide oxidase, and glutathione S-transferase [58].

Anticarcinogenic Activity

It is known that fruits and vegetables with intense flavonoids are chemopreventive agents. From the beginning to the end of the carcinogenicity process, flavonoids have been observed to act through a variety of mechanisms, including developmental and hormonal activities [59]. To explain the basic working principle of flavonoids at the molecular level, we can mention the inhibition of tyrosine kinase, cell cycle arrest, estrogen receptor binding capacity, downregulation of mutant p53 protein, inhibition of the expression of Ras proteins and inhibition of heat shock proteins [60].

P53 mutations are among the most common genetic abnormalities in all types of cancer. Inhibition of p53 expression in cancer cells can cause cycle arrest in the G2-M phase during the cell cycle [61]. In some studies in cell lines derived from breast cancer patients, flavonoids have been observed to reduce the measured expression for the mutant p53 protein to almost undetectable levels [62]. Tyrosine kinase enzymes are a family of proteins found among membrane proteins in the cell, and their task is to capture growth factor signals and direct them to the cell nucleus. According to studies, it is thought that these enzymes disrupt growth control and take part in oncogenesis in this way. It is known that drugs used to reduce tyrosine kinase activity have antitumor effects, but do not cause many of the side effects observed in chemotherapy. Quercetin is known to be the first tyrosine kinase inhibitor examined during phase I studies [63, 64]. It forms a complex with the interaction between heat shock proteins and mutant p53 molecule, allowing tumor cells to inhibit cell cycle arrest mechanisms. They also allow advanced, proliferating and disseminated cancer cells to survive

even under different challenging conditions. Flavonoids restrict the production of heat shock proteins in different cells in tissues of different cancer types [65].

Antidiabetic Activity

Various flavonoid types such as quercetin, rutin, apigenin, catechin, venoruton, and naringenin have been observed to have strong hepatoprotective effects in studies [66]. During common chronic diseases such as diabetes, the likelihood of hepatic clinical manifestations increases. In the liver of a person with diabetes, there is a significant decrease in the amount of ROS, the expression levels of glutathione and glutamate-cysteine ligase. Anthocyanidins are gaining increasing attention for their preventive effects on various diseases [67]. Anthocyanidin 3-O-β-glucoside (C3G) increases cAMP levels, then activates protein kinase A (PKA) and increases hepatic Gclc expression. As a result of this biochemical process, phosphorylation of cAMP response element binding protein (CREB) is regulated, promoting CREB DNA binding and increasing Gclc transcription. In addition, increasing Gclc expression results in decreased hepatic ROS levels and proapoptotic signaling. In addition, C3G therapy suppresses the release of proinflammatory cytokines and protects against adipose tissue formation by reducing lipid peroxidation in the liver [68, 69].

Various clinical studies have demonstrated the advantages of flavonoids in the treatment of digestive-related problems such as anorexia, nausea, abdominal pain and hepatobiliary dysfunction. It has been reported that the flavonoids hirustrin and avicularin, as well as Equisetum arvense flavonoids isolated from many other sources, may be countermeasures against hepatotoxicity induced by chemical processes in HepG2 cells [70, 71].

Antimicrobial Activity

It is known that plants synthesize flavonoids in response to infections of microbial origin. Therefore, they are considered suitable as antimicrobial agents effective against various

microorganisms in vitro. Studies have shown that plant extracts containing flavonoids have antibacterial properties [72-74]. Flavonoids with antibacterial properties can show activity in more than one cellular target after consumption. These interact with proteins to form complexes through hydrophobic effects and non-specific forces such as hydrogen bonding. They are also known to form covalent bonds. Therefore, the antimicrobial properties of flavonoids can be affected by their ability to inactivate cell membrane transport proteins, enzymes, and microbial adhesins. In addition, lipophilic flavonoids can damage the microbial membrane structure and cause disruption [75, 76]. Catechins, one of the subgroups of flavonoids and a kind of secondary metabolite, is a compound whose antimicrobial activities have been extensively investigated and it has been found to have intense protective activity. These compounds have been proven to show antibacterial activity in vitro against many bacteria such as *Vibrio cholerae, Streptococcus mutans, Shigella dysenteriae* [77, 78].

Antiviral Activity

Flavonoids are an important resource for the discovery and over time development of new antiviral drugs, due to their abundance in nature and the known low probability of side effects [79]. Most research on antiviral compounds focuses on inhibiting various enzymes possessed by viral organisms. Viruses are organisms with a DNA or RNA genome model. They rely on the metabolism of the host organism and its environment to continue to reproduce and maintain their vitality. Once they have taken over the host's cellular machinery, they multiply and spread [80].

There are various mechanisms by which viruses are inhibited by flavonoids. By preventing viruses from contacting cells and penetrating the cell membrane, they can interrupt different stages of viral DNA's replication, translation and protein processing processes. They can also prevent the spread of viruses to other healthy host cells [81]. In a study, it was observed that flavonoids can attach themselves to the surface proteins of viruses and prevent

the virus from entering the host cells. According to this study, some flavonoids affect the replication process by acting as inhibitor molecules during the transcription stage. Others inhibit the late stages of microviral assembly, packaging and release processes. Flavonoids can also regulate the immune system and reduce viral load [82].

Chemistry of Flavonoids

Flavonoids were first discovered in orange cells in 1930. When it was first isolated, it was thought to belong to a new vitamin class and was called vitamin P. Later, it was understood that this substance was a flavonoid and it is known that there are over 4000 isolated flavonoid species today [83].

Flavonoid characterization is usually achieved using nuclear magnetic resonance (NMR) and mass spectrometry (MS) technologies. Chemically, flavonoids have a fifteen-carbon backbone consisting of a heterocyclic pyran ring and two benzene ring structures attached to it. These carbon atoms are arranged in 3 rings (A, B and C) in the literature. The flavonoid classes differ from each other in the saturation level of the C ring. Each compound within a class differs in the configuration pattern of the A and B rings, which affects the antioxidant properties and phenoxy radical stability of the substances [84]. According to these differences, flavonoids can be in glycoside form, aglycone form and methylated derivative form [85]. The six-membered ring attached to the benzene ring forming the backbone in the flavonoid structure may be an α-pyron or its dihydro derivative [86]. The flavonoid class is divided into flavanones (2-position) and isoflavonoids (3-position) depending on the location of the benzenoid subgroups. The free radical scavenging potential of flavonoids, which are natural polyphenolic compounds, varies depending on the presence and location of free −OH groups on the skeleton. Flavonoids are usually hydroxylated at 2, 3, 3', 4', 5, 5', 7 positions [87]. In addition, flavonoids containing more than one hydroxyl group are more effective antioxidants than those with a single hydroxyl group [88].

Bioavailability of Flavonoids

Bioavailability is defined as "the rate at which a drug or active substance enters the circulation systemically or the degree to which it becomes fully usable at biological targets". Although significant progress has been made in understanding the bioavailability mechanism of flavonoids, their efficacy is still the subject of research [89, 90]. Therefore, flavonoids, an alternative therapeutic target with cost-effective, safe and less toxic effects, are often recommended in the treatment of many diseases [91]. Current therapies are often inadequate for chronic and non-chronic diseases such as cardiovascular, autoimmune, neurodegenerative, diabetes and cancer [92]. The complex structures and high molecular weights of flavonoids cause poor bioavailability [93]. Poor bioavailability, a major limitation, is seen as a major barrier to the efficacy of flavonoids [92, 94]. Accordingly, the focus should be on finding factors that inhibit the in vivo bioavailability of dietary flavonoids. It has been shown that the bioavailability mechanism of flavonoids depends on their biological activities, functional groups, metabolism, composition and number of hydroxyl groups [93]. Flavonoids generally have low plasma concentrations [95]. Therefore, plant flavonoids are considered as combined therapy in both in vitro and in vivo models. [94]. The bioavailability levels of flavonoids vary according to dietary sources, and these compounds interact with other nutrients and may reduce glucose absorption in the body [96]. Consumption of oil together with flavonoids increases the absorption of these compounds in the gut and, in direct proportion, their bioavailability. [97]. However, the data obtained shows that flavonoids' bioavailability decreases when consumed with proteins [98]. In addition, the microbiome population present in the gut is of great importance for the absorption and metabolism of flavonoids. [99]. Immediately after consumption, it is hydrolyzed in the intestine and subsequently absorbed by intestinal epithelial cells. After absorption, flavonoids are metabolized in the small intestine, liver, and kidney, respectively. The flavonoids that then enter the circulation go through a series of biochemical processes before

reaching the tissues. This series of biochemical events includes processes such as glucuronidation, methylation and sulfation, which are factors that affect the biological activities of flavonoids [100]. It appears that flavonoids reach high concentrations in the blood plasma approximately two hours after consuming foods rich in flavonoids. However, their concentrations differ according to the type of flavonoids. In studies, it was found that anthocyanins showed low concentrations in plasma, while quercetin, gallic acid, catechin, glucosides and isoflavones showed high concentrations. [101].

Classification of Flavonoids

Flavonoids generally accumulate as glycosides in organelles called vacuoles in plant cells [102]. Differences in the structure of flavonoid compounds are the main factor in their classification [103]. Flavonoids are classified into six main groups: flavanones, flavonols, anthocyanins, flavones, isoflavones, and flavanols [103, 104].

Flavonones are found in all parts of the plant, especially depending on the type [48]. Hesperetin and naringenin, which are important flavanone compounds that attract attention with their high amounts in foods, are found in lemon, orange, grapefruit and citrus [105]. It has been proven that flavanones exhibit various pharmacological activities, including anti-inflammatory, anticancer, and antiviral [106, 107]. In addition, it has been found that the compound naringenin can reduce the production of nitrates and nitrites, which are indicators of intestinal edema formation during intestinal inflammation [108].

Flavonols (epicatechin, catechin, myricetin, quercetin, kaempferol gallocatechin) are known as the condensed form of tannins and have a very complex structure [109]. Flavanols are abundant in a wide variety of plant derivatives such as tea, onions, broccoli, cocoa, kiwi, apples, lentils and broad beans. It is commonly known to be found in plant seeds or in the skins of fruits and vegetables [110]. In many studies, it has been determined that

flavonols play an important role in defense against pathogens [111, 112]. Catechins have been found to be able to modulate cell proliferation and apoptosis as well as have anti-carcinogenic activity [113].

Anthocyanins are a group of phytochemicals that include red, purple, blue and orange pigments present in many fruits and vegetables [109]. It contains malvidin, delphinidin, cyanidin and pelargonidin anthocyanidins, commonly found in tea, honey, cocoa, hazelnuts, olive oil and grains [103]. Anthocyanins have been shown to play an important role in cardiovascular diseases, cholesterol, antioxidant activity and cytotoxic conditions [114]. In addition, this group of flavonoids has been found to preserve DNA integrity and antioxidant levels in tissues [115].

Flavones are one of the largest classes of flavonoids and are glycosides abundant in celery, capsicum, orange and tea. One of the two main flavones that can be consumed is apigenin and the other is luteolin. It has been determined that apigenin protects cells against free radicals. In a study, it was determined that this compound plays a role as a regulator of the antioxidant defense system in pancreatic cells [116]. It has also been found that apigenin effectively reduces inflammation and neuroinflammation and prevents the loss of antioxidant enzyme activity in cells [92, 117].

Isoflavones are an important subclass of flavonoids. It has been found that it is found in fruits, vegetables and nuts as well as in legumes in general. Soybeans contain isoflavones such as genistein and daidzein [118]. It has been determined that isoflavones have effective antioxidant properties and as a result, they reduce the risk of cancer by preventing DNA damage [103]. Isoflavone compounds are known to have positive effects on heart health and menopause [119]. Isoflavones have an important place among natural products that can support cardiovascular health in the postmenopausal period [120]. A diet rich in isoflavones and containing soy protein has been proven to have a powerful effect on regulating hormonal status and the menstrual cycle [121]. Epidemiological studies have also shown

that consuming high amounts of soy isoflavones reduces the risk of breast cancer [122].

Unlike other flavonoid groups, flavanols, also called dihydroflavones, are obtained from a limited number of plants [123]. Long-term consumption of foods rich in flavanols is thought to have powerful effects on improving endothelial function and preventing cardiovascular diseases [103]. The basic building block of tannins, catechin, is one of the most important compounds of the flavanol group. In addition, it has been determined that catechins play an effective role in cell proliferation, apoptosis mechanism, and Nuclear Factor kappa B (NFKB) pathway [113].

Flavonoids in Dıet

Flavonoids represent the most common group of natural polyphenolic compounds [124]. The potential relationship between dietary flavonoids and health is a popular area of study. These bioactive compounds make up about 70% of the diet [95]. In epidemiological studies, it has been shown that consumption of foods rich in flavonoids such as fruits, vegetables, grains, cocoa, and tea, reduces the risk of developing certain diseases [125]. In addition to the various pharmacological effects of flavonoids, it has been found that they act as free radical scavengers, metal chelators, and antioxidant agents [126]. It has been determined that flavonoid-based diets play an important protective role in life-threatening diseases such as coronary heart disease and cancer [127]. Fruits and vegetables are seen as the main sources of dietary antioxidants. The daily intake of flavonoids has been found to range from approximately 25 mg to 1 g [128, 129]. In vitro studies on the antioxidant activities of plant flavonoids in foods and their protective effects on biological systems have revealed the potential effects of flavonoids in the diet [39, 130]. Dietary studies using foods rich in flavonoid compounds have shown that these compounds positively affect memory and learning in animal models of disease [131, 132]. In addition, it has been established that flavonoids inhibit the

activities of microglial cells, are involved in inflammatory processes in the nervous system, and have strong antidepressant effects [133].

Neuroprotective Effects of Flavonoids in AD

It is known that favonoids are able to cross the blood-brain barrier (BBB) and are therefore potentially useful agents in the prevention of neurodegenerative disorders [134]. The biological activities, potential mechanisms of action, and targets of natural flavonoids in AD have been the subject of many studies. In particular, data has been obtained indicating that certain flavonoid-rich foods have a definite protective effect on Alzheimer's.

Cocoa, tea, and wine are flavonoid-containing and widely consumed food items. One study examined the relationship between the intake of these foods and cognitive performance [135]. At the end of the study, it was determined that the subjects who consumed chocolate, tea, and wine had better cognitive performance than the others. Although the best effect was observed with wine consumption, excessive alcohol intake was identified as the cause of Alzheimer's. This is because the relationship between these foodstuffs and cognitive performance is dose-dependent [136].

A high soy diet has also been found to improve memory and frontal lobe function. In a study of 33 postmenopausal female subjects, the subjects were supplemented with soy extract for 12 weeks, and it was found that subjects who took isoflavone supplements showed better improvement in memory and attention experiments [137].

Tannic acid is a flavonoid found in various legumes, most commonly green tea, bananas, dates, raspberries, and wine. It has antioxidant, antiviral, antibacterial, anticarcinogenic, and anti-inflammatory effects [138]. In addition to all these, tannic acid has also been found to have neuroprotective effects against AD. Tannic acid has been shown to be a natural inhibitor of β-secretase (BACE1) activity, which is responsible for Aβ production. In addition, it is

known that tannic acid inhibits the in vitro aggregation of tau peptide, which is the main component of NFTs.

Catechin and epicatechin are also pharmaceuticals used in the prevention of AD. These flavonoids have been found to reduce amyloid deposits and neurofibrillary tangles by reducing Aβ accumulation and tau pathology. In addition, it was observed that these compounds showed neuroprotective effects in aged mice with cognitive problems by reducing oxidative stress. Therefore, catechin and epicatechin are recognized as flavonoids that increase cognition and alleviate AD [139].

Quercetin is one of the plant-derived flavonoids with the strongest antioxidant activity [140]. It is abundant in fruits and vegetables that are widely used in daily diets. Quercetin has been found to have neuroprotective effects, improving learning, cognitive functions, and memory in AD [141]. It is known that it protects neurons from oxidative damage by reducing lipid peroxidation, and amyloid beta proteins prevent fibril formation and cell fragmentation. It increases the level of acetylcholine by inhibiting AChE, thereby improving the cognitive symptoms of AD [142]. Despite all these results, there is a lack of knowledge about the precise mechanism by which flavonoids improve cognitive function, which reduces the risk of AD.

Conclusion

Flavonoids are commonly found in natural foods. It is considered an alternative way of treating AD with natural compounds taken through diet or supplementation. In vivo and in vitro studies using flavonoids have proven to have healing properties in the mechanisms of AD. However, new flavonoid-based dietary practices to reduce the risk of AD are thought to require further study, including the specific mechanisms by which flavonoids exert potential neuroprotective effects. Understanding the mechanisms underlying flavonoid-protein interactions in AD may be a promising

step in developing new neuroprotective strategies for neurodegenerative diseases.

REFERENCES

1. McGeer, P.L. and E.G. McGeer, The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Research Reviews, 1995. 21(2): p. 195-218.
2. Lane, C.A., J. Hardy, and J.M. Schott, Alzheimer's disease. European journal of neurology, 2018. 25(1): p. 59-70.
3. Santos, C.Y., et al., Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: a review and synthesis. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 2017. 7: p. 69-87.
4. Pinyopornpanish, K., et al., Impact of behavioral and psychological symptoms of Alzheimer's disease on caregiver outcomes. Scientific Reports, 2022. 12(1): p. 1-9.
5. Niu, H., et al., Prevalence and incidence of Alzheimer's disease in Europe: A meta-analysis. Neurología (English Edition), 2017. 32(8): p. 523-532.
6. Nandi, A., et al., Global and regional projections of the economic burden of Alzheimer's disease and related dementias from 2019 to 2050: A value of statistical life approach. EClinicalMedicine, 2022. 51: p. 101580.
7. Dodel, R., et al., Determinants of societal costs in Alzheimer's disease: GERAS study baseline results. Alzheimer's & Dementia, 2015. 11(8): p. 933-945.
8. Scarabino, D., et al., Influence of family history of dementia in the development and progression of late-onset Alzheimer's disease. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 2016. 171(2): p. 250-256.

9. Crous-Bou, M., et al., Alzheimer's disease prevention: from risk factors to early intervention. Alzheimer's research & therapy, 2017. 9: p. 1-9.
10. Bekris, L.M., et al., Genetics of Alzheimer disease. Journal of geriatric psychiatry and neurology, 2010. 23(4): p. 213-227.
11. Ardanaz, C.G., et al., JNK3 overexpression in the entorhinal cortex disseminates to the hippocampus and induces cognitive deficiencies and Tau misfolding. 2022.
12. Albert, M.S., et al., The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia, 2011. 7(3): p. 270-279.
13. Adams, K.B. and S. Sanders, Alzheimer's caregiver differences in experience of loss, grief reactions and depressive symptoms across stage of disease: a mixed-method analysis. Dementia, 2004. 3(2): p. 195-210.
14. Gauthier, S., P. Scheltens, and J. Cummings, Alzheimer's Disease and Related Disorders. 2005: CRC Press.
15. Hardy, J., Has the amyloid cascade hypothesis for Alzheimer's disease been proved? Current Alzheimer Research, 2006. 3(1): p. 71-73.
16. Fillenbaum, G.G., et al., Consortium to Establish a Registry for Alzheimer's Disease (CERAD): the first twenty years. Alzheimer's & dementia, 2008. 4(2): p. 96-109.
17. Cruchaga, C., et al., Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PloS one, 2012. 7(2): p. e31039.

18. Ly, P.T., F. Cai, and W. Song, Detection of neuritic plaques in Alzheimer's disease mouse model. JoVE (Journal of Visualized Experiments), 2011(53): p. e2831.

19. Dickson, D., Neuropathological diagnosis of Alzheimer's disease: a perspective from longitudinal clinicopathological studies. Neurobiology of aging, 1997. 18(4): p. S21-S26.

20. Merriam, A.E., et al., The psychiatric symptoms of Alzheimer's disease. Journal of the American Geriatrics Society, 1988. 36(1): p. 7-22.

21. Joe, E. and J.M. Ringman, Cognitive symptoms of Alzheimer's disease: clinical management and prevention. BMJ, 2019. 367.

22. Scheltens, P., Imaging in Alzheimer's disease. Dialogues in clinical neuroscience, 2022.

23. Johnson, K.A., et al., Brain imaging in Alzheimer disease. Cold Spring Harbor perspectives in medicine, 2012. 2(4): p. a006213.

24. Bondi, M.W., et al., Neuropsychological contributions to the early identification of Alzheimer's disease. Neuropsychology review, 2008. 18: p. 73-90.

25. Breijyeh, Z. and R. Karaman, Comprehensive review on Alzheimer's disease: causes and treatment. Molecules, 2020. 25(24): p. 5789.

26. Cacabelos, R., Donepezil in Alzheimer's disease: From conventional trials to pharmacogenetics. Neuropsychiatric disease and treatment, 2007. 3(3): p. 303-333.

27. Annicchiarico, R., et al., Rivastigmine in Alzheimer's disease: Cognitive function and quality of life. Therapeutics and Clinical Risk Management, 2007. 3(6): p. 1113-1123.

28. Scott, L.J. and K.L. Goa, Galantamine: a review of its use in Alzheimer's disease. Drugs, 2000. 60: p. 1095-1122.

29. Crismon, M.L., Tacrine: first drug approved for Alzheimer's disease. Annals of Pharmacotherapy, 1994. 28(6): p. 744-751.

30. Liu, J., et al., The role of NMDA receptors in Alzheimer's disease. Frontiers in neuroscience, 2019. 13: p. 43.

31. Bezerra da Silva, C., et al., Effect of donepezil, tacrine, galantamine and rivastigmine on acetylcholinesterase inhibition in Dugesia tigrina. Molecules, 2016. 21(1): p. 53.

32. Singhal, A.K., V. Naithani, and O.P. Bangar, Medicinal plants with a potential to treat Alzheimer and associated symptoms. International Journal of Nutrition, Pharmacology, Neurological Diseases, 2012. 2(2): p. 84-91.

33. Kim, M.H., S.-H. Kim, and W.M. Yang, Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. Planta medica, 2014. 80(15): p. 1249-1258.

34. Bakhtiari, M., et al., Protective effects of flavonoids against Alzheimer's disease-related neural dysfunctions. Biomedicine & Pharmacotherapy, 2017. 93: p. 218-229.

35. Faydaoğlu, E. and M.S. Sürücüoğlu, Geçmişten günümüze tıbbi ve aromatik bitkilerin kullanılması ve ekonomik önemi. Kastamonu University Journal of Forestry Faculty, 2011. 11(1): p. 52-67.

36. Shan, X., et al., Comparison of lipoxygenase, cyclooxygenase, xanthine oxidase inhibitory effects and cytotoxic activities of selected flavonoids. DEStech Trans. Environ. Energy Earth Sci, 2017.

37. Feliciano, R.P., et al., Flavonoid intake and cardiovascular disease risk. Current Opinion in Food Science, 2015. 2: p. 92-99.

38. Thilakarathna, S.H. and H.V. Rupasinghe, Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients, 2013. 5(9): p. 3367-3387.

39. Kozłowska, A. and D. Szostak-Węgierek, Flavonoids–food sources, health benefits, and mechanisms involved. Bioactive molecules in food, 2019: p. 53-78.

40. Shkondrov, A., et al., Determination of flavonoids in related Astragalus species (Sect. Incani) occurring in Bulgaria. Comptes rendus de l'Académie Bulg. des Sci, 2017. 70: p. 363-366.

41. Pandey, K.B. and S.I. Rizvi, Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity, 2009. 2: p. 270-278.

42. Villela, A., et al., Photo-stability of a flavonoid dye in presence of aluminium ions. Dyes and Pigments, 2019. 162: p. 222-231.

43. Paramita, V., et al., Application of Flavonoid and Anthocyanin Contents from Rambutan (Nephelium lappaceum) Peel as Natural Dyes on Cotton Fabric. Advanced Science Letters, 2018. 24(12): p. 9853-9855.

44. Danihelová, M., J. Viskupicová, and E. Sturdik, Lipophilization of flavonoids for their food, therapeutic and cosmetic applications. Acta Chimica Slovaca, 2012. 5(1): p. 59.

45. Chuarienthong, P., N. Lourith, and P. Leelapornpisid, Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. International journal of cosmetic science, 2010. 32(2): p. 99-106.

46. Craig, W.J., Health-promoting properties of common herbs. The American journal of clinical nutrition, 1999. 70(3): p. 491s-499s.

47. Kumar, S., et al., Protective efficacy of Solanum xanthocarpum root extracts against free radical damage: phytochemical analysis and antioxidant effect. Cellular and Molecular Biology, 2012. 58(1): p. 174-181.

48. Panche, A.N., A.D. Diwan, and S.R. Chandra, Flavonoids: an overview. Journal of nutritional science, 2016. 5: p. e47.

49. Pourmorad, F., S. Hosseinimehr, and N. Shahabimajd, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African journal of biotechnology, 2006. 5(11).

50. Kumar, S. and A. Pandey, Antioxidant, lipo-protective and antibacterial activities of phytoconstituents present in Solanum xanthocarpum root. International Review of Biophysical Chemistry, 2012. 3(3): p. 42-47.

51. Hollman, P.C., et al., The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. Free radical research, 1999. 31(6): p. 569-573.

52. Day, A.J., et al., Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS letters, 2000. 468(2-3): p. 166-170.

53. Walle, T., Absorption and metabolism of flavonoids. Free radical biology and medicine, 2004. 36(7): p. 829-837.

54. Scheline, R.R., Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacological reviews, 1973. 25(4): p. 451-523.

55. Bravo, L., Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews, 1998. 56(11): p. 317-333.

56. Hollman, P.C., Absorption, bioavailability, and metabolism of flavonoids. Pharmaceutical biology, 2004. 42(sup1): p. 74-83.

57. Hollman, P.C., et al., Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS letters, 1997. 418(1-2): p. 152-156.

58. Spencer, J.P., et al., Decomposition of cocoa procyanidins in the gastric milieu. Biochemical and biophysical research communications, 2000. 272(1): p. 236-241.

59. Benzie, I., et al., Consumption of green tea causes rapid increase in plasma antioxidant power in humans. Nutrition and cancer, 1999. 34(1): p. 83-87.

60. Pandey, A., A. Mishra, and A. Mishra, Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant Cinnamomum tamala. Cellular and Molecular Biology, 2012. 58(1): p. 142-147.

61. Cao, G., E. Sofic, and R.L. Prior, Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free radical biology and medicine, 1997. 22(5): p. 749-760.

62. Halliwell, B. and J.M. Gutteridge, Free radicals in biology and medicine. 2015: Oxford university press, USA.

63. Mishra, A., S. Kumar, and A.K. Pandey, Scientific validation of the medicinal efficacy of Tinospora cordifolia. The Scientific World Journal, 2013. 2013.

64. Brown, E.J., et al., Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. Biochemical Journal, 1998. 330(3): p. 1173-1178.

65. Mishra, A., et al., Bauhinia variegata leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. BioMed Research International, 2013. 2013.

66. Ho, C.-T., Food phytochemicals for cancer prevention II. 1994: American Chemical Society.

67. Brusselmans, K., et al., Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. Journal of Biological Chemistry, 2005. 280(7): p. 5636-5645.

68. Block, G., B. Patterson, and A. Subar, Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutrition and cancer, 1992. 18(1): p. 1-29.

69. Duthie, G.G., S.J. Duthie, and J.A. Kyle, Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition research reviews, 2000. 13(1): p. 79-106.

70. Davis, W., et al., Antioxidants and cancer III: quercetin. Altern Med Rev, 2000. 5(3): p. 196-208.

71. Swinnen, J.V., et al., Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. International journal of cancer, 2002. 98(1): p. 19-22.

72. Brusselmans, K., et al., Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. International Journal of Cancer, 2003. 106(6): p. 856-862.

73. Tapas, A.R., D. Sakarkar, and R. Kakde, Flavonoids as nutraceuticals: a review. Tropical journal of Pharmaceutical research, 2008. 7(3): p. 1089-1099.

74. Zhu, W., et al., The anthocyanin cyanidin-3-O-β-glucoside, a flavonoid, increases hepatic glutathione synthesis and protects hepatocytes against reactive oxygen species during hyperglycemia: Involvement of a cAMP–PKA-dependent signaling pathway. Free Radical Biology and Medicine, 2012. 52(2): p. 314-327.

75. Sonnenbichler, J. and I. Zetl, Progress in Clinical Biological Research. 1986, Alan R. Liss New York.

76. Spencer, J.P., D. Vauzour, and C. Rendeiro, Flavonoids and cognition: the molecular mechanisms underlying their behavioural effects. Archives of biochemistry and biophysics, 2009. 492(1-2): p. 1-9.

77. Wu, Y., et al., Hepatoprotective effect of total flavonoids from Laggera alata against carbon tetrachloride-induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. Journal of Biomedical Science, 2006. 13(4): p. 569-578.

78. Kim, S.M., et al., Hepatoprotective effect of flavonoid glycosides from Lespedeza cuneata against oxidative stress induced by tert-butyl hyperoxide. Phytotherapy Research, 2011. 25(7): p. 1011-1017.

79. Mishra, A., et al., Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants. Cellular and Molecular Biology, 2011. 57(1): p. 16-25.

80. Moerman, D.E., An analysis of the food plants and drug plants of native North America. Journal of ethnopharmacology, 1996. 52(1): p. 1-22.

81. Gerdin, B. and E. Svensjö, Inhibitory effect of the flavonoid O-(beta-hydroxyethyl)-rutoside on increased microvascular permeability induced by various agents in rat skin. International journal of microcirculation, clinical and experimental, 1983. 2(1): p. 39-46.

82. Cushnie, T.T. and A.J. Lamb, Antimicrobial activity of flavonoids. International journal of antimicrobial agents, 2005. 26(5): p. 343-356.

83. Cowan, M.M., Plant products as antimicrobial agents. Clinical microbiology reviews, 1999. 12(4): p. 564-582.

84. Yao, L.H., et al., Flavonoids in food and their health benefits. Plant foods for human nutrition, 2004. 59: p. 113-122.

85. Mishra, A.K., et al., Inhibitory activity of Indian spice plant Cinnamomum zeylanicum extracts against Alternaria solani and Curvularia lunata, the pathogenic dematiaceous moulds. Annals of clinical microbiology and antimicrobials, 2009. 8(1): p. 1-7.

86. Borris, R.P., Natural products research: perspectives from a major pharmaceutical company. Journal of ethnopharmacology, 1996. 51(1-3): p. 29-38.

87. Zandi, K., et al., Antiviral activity of four types of bioflavonoid against dengue virus type-2. Virology journal, 2011. 8(1): p. 1-11.

88. Middleton Jr, E., Effect of plant flavonoids on immune and inflammatory cell function. Flavonoids in the living system, 1998: p. 175-182.

89. Hu, M., B. Wu, and Z. Liu, Bioavailability of polyphenols and flavonoids in the era of precision medicine. 2017, ACS Publications. p. 2861-2863.

90. Ben-Shabat, S., et al., Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. Drug delivery and translational research, 2020. 10: p. 354-367.

91. D'Amelia, V., et al., The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding. Phytochemistry Reviews, 2018. 17: p. 611-625.

92. Ginwala, R., et al., Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants, 2019. 8(2): p. 35.

93. Mondal, S. and S. Rahaman, Flavonoids: A vital resource in healthcare and medicine. Pharm. Pharmacol. Int. J, 2020. 8(2): p. 91-104.

94. Vazhappilly, C.G., et al., Current methodologies to refine bioavailability, delivery, and therapeutic efficacy of plant flavonoids in cancer treatment. The Journal of Nutritional Biochemistry, 2021. 94: p. 108623.

95. Scalbert, A. and G. Williamson, Dietary intake and bioavailability of polyphenols. The Journal of nutrition, 2000. 130(8): p. 2073S-2085S.

96. Jakobek, L., Interactions of polyphenols with carbohydrates, lipids and proteins. Food chemistry, 2015. 175: p. 556-567.

97. Gonzales, G.B., et al., Flavonoid interactions during digestion, absorption, distribution and metabolism: a sequential structure–activity/property relationship-based approach in the study of bioavailability and bioactivity. Drug metabolism reviews, 2015. 47(2): p. 175-190.

98. Świeca, M., et al., The influence of protein–flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. Food Chemistry, 2013. 141(1): p. 451-458.

99. Cassidy, A. and A.-M. Minihane, The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. The American journal of clinical nutrition, 2017. 105(1): p. 10-22.

100. Kopustinskiene, D.M., et al., Flavonoids as anticancer agents. Nutrients, 2020. 12(2): p. 457.

101. Rodríguez-García, C., C. Sánchez-Quesada, and J.J. Gaforio, Dietary flavonoids as cancer chemopreventive agents: An updated review of human studies. Antioxidants, 2019. 8(5): p. 137.

102. Shen, N., et al., Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chemistry, 2022: p. 132531.

103. Brodowska, K.M., Natural flavonoids: classification, potential role, and application of flavonoid analogues. European Journal of Biological Research, 2017. 7(2): p. 108-123.

104. Singla, R.K., et al., Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. 2019, Oxford University Press. p. 1397-1400.

105. Khan, M.K. and O. Dangles, A comprehensive review on flavanones, the major citrus polyphenols. Journal of Food Composition and Analysis, 2014. 33(1): p. 85-104.

106. Kumar, S. and A.K. Pandey, Chemistry and biological activities of flavonoids: an overview. The scientific world journal, 2013. 2013.

107. Burmańczuk, A., et al., The Effect of Hesperidin, Chrysin, and Naringenin on Somatic Cell Count in Mastitis Dairy Cows After Multiple Intramammary Administration. Annals of Animal Science, 2022. 22(1): p. 155-172.

108. Karim, N., et al., A recent review of citrus flavanone naringenin on metabolic diseases and its potential sources for high yield-production. Trends in Food Science & Technology, 2018. 79: p. 35-54.

109. de Pascual-Teresa, S., D.A. Moreno, and C. García-Viguera, Flavanols and anthocyanins in cardiovascular health: a review of current evidence. International journal of molecular sciences, 2010. 11(4): p. 1679-1703.

110. Määttä-Riihinen, K.R., et al., Catechins and procyanidins in berries of Vaccinium species and their antioxidant activity. Journal of Agricultural and Food Chemistry, 2005. 53(22): p. 8485-8491.

111. Punyasiri, P., et al., Flavonoid biosynthesis in the tea plant Camellia sinensis: properties of enzymes of the

prominent epicatechin and catechin pathways. Archives of Biochemistry and Biophysics, 2004. 431(1): p. 22-30.

112. Samanta, A., G. Das, and S.K. Das, Roles of flavonoids in plants. Carbon, 2011. 100(6): p. 12-35.

113. Gramza, A., J. Korczak, and R. Amarowicz, Tea polyphenols-their antioxidant properties and biological activity-a review. Polish Journal of food and nutrition sciences, 2005. 14(3): p. 219.

114. Reis, J.F., et al., Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. Journal of translational medicine, 2016. 14(1): p. 1-16.

115. Khoo, H.E., et al., Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & nutrition research, 2017. 61(1): p. 1361779.

116. Wang, S., et al., The flavonoid-rich fraction from rhizomes of Smilax glabra Roxb. ameliorates renal oxidative stress and inflammation in uric acid nephropathy rats through promoting uric acid excretion. Biomedicine & Pharmacotherapy, 2019. 111: p. 162-168.

117. Ahmad, A., P. Kumari, and M. Ahmad, Apigenin attenuates edifenphos-induced toxicity by modulating ROS-mediated oxidative stress, mitochondrial dysfunction and caspase signal pathway in rat liver and kidney. Pesticide biochemistry and physiology, 2019. 159: p. 163-172.

118. Kim, M.A. and M.J. Kim, Isoflavone profiles and antioxidant properties in different parts of soybean sprout. Journal of food science, 2020. 85(3): p. 689-695.

119. Gómez-Zorita, S., et al., Scientific evidence supporting the beneficial effects of isoflavones on human health. Nutrients, 2020. 12(12): p. 3853.

120. Cano, A., M.Á. García-Pérez, and J.J. Tarín, Isoflavones and cardiovascular disease. Maturitas, 2010. 67(3): p. 219-226.

121. Zhang, C., et al., Soy product and isoflavone intake and breast cancer risk defined by hormone receptor status. Cancer science, 2010. 101(2): p. 501-507.

122. Wood, C.E., et al., Dietary soy isoflavones inhibit estrogen effects in the postmenopausal breast. Cancer Research, 2006. 66(2): p. 1241-1249.

123. Sir Elkhatim, K.A., R.A. Elagib, and A.B. Hassan, Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food science & nutrition, 2018. 6(5): p. 1214-1219.

124. Prior, R.L. and G. Cao, Flavonoids: diet and health relationships. Nutrition in clinical care, 2000. 3(5): p. 279-288.

125. Egert, S. and G. Rimbach, Which sources of flavonoids: complex diets or dietary supplements? Advances in Nutrition, 2011. 2(1): p. 8-14.

126. Uddin, M.S., et al., Molecular insight into the therapeutic promise of flavonoids against Alzheimer's disease. Molecules, 2020. 25(6): p. 1267.

127. Hills Jr, R.D., et al., Gut microbiome: profound implications for diet and disease. Nutrients, 2019. 11(7): p. 1613.

128. Davis, J.M., E.A. Murphy, and M.D. Carmichael, Effects of the dietary flavonoid quercetin upon performance and health. Current sports medicine reports, 2009. 8(4): p. 206-213.

129. Kuhnau, J., Flavonoids. A class of semi-essential food components: Their role in human nutrition. World review of nutrition and dietetics, 1976.

130. Hodgson, J.M. and K.D. Croft, Dietary flavonoids: effects on endothelial function and blood pressure. Journal of the Science of Food and Agriculture, 2006. 86(15): p. 2492-2498.

131. Haque, A.M., et al., Long-term administration of green tea catechins improves spatial cognition learning ability in rats. The Journal of nutrition, 2006. 136(4): p. 1043-1047.

132. Sokolov, A.N., et al., Chocolate and the brain: neurobiological impact of cocoa flavanols on cognition and behavior. Neuroscience & Biobehavioral Reviews, 2013. 37(10): p. 2445-2453.

133. Hwang, J., et al., Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. Neuropharmacology, 2008. 55(5): p. 826-834.

134. Gao, X., et al., Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology, 2012. 78(15): p. 1138-1145.

135. Nurk, E., et al., Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. The Journal of nutrition, 2009. 139(1): p. 120-127.

136. de Lorimier, A.A., Alcohol, wine, and health. The American Journal of Surgery, 2000. 180(5): p. 357-361.

137. Duffy, R., H. Wiseman, and S.E. File, Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. Pharmacology Biochemistry and Behavior, 2003. 75(3): p. 721-729.

138. Brglez Mojzer, E., et al., Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules, 2016. 21(7): p. 901.

139. Zhao, S., et al., Procyanidins and Alzheimer's disease. Molecular Neurobiology, 2019. 56: p. 5556-5567.

140. Brüll, V., et al., Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-) hypertension: a randomised double-blinded placebo-controlled cross-over trial. British journal of nutrition, 2015. 114(8): p. 1263-1277.

141. Singh, S.K., et al., Overview of Alzheimer's disease and some therapeutic approaches targeting Aβ by using several synthetic and herbal compounds. Oxidative medicine and cellular longevity, 2016. 2016.

142. Khan, H., et al., Neuroprotective effects of quercetin in Alzheimer's disease. Biomolecules, 2019. 10(1): p. 59.

CHAPTER IV

Autoimmune Skin Disorder: Psoriasis

Esra PALABIYIK[1]
Handan UĞUZ[2]
Bahri AVCI[3]
Ayşe Nurseli SULUMER[4]
Hakan AŞKIN[5]

Abstract

Psoriasis, which is suffering from approximately 125 million people worldwide, is known as a common inflammatory skin disease caused by the interaction between genetic and environmental risk factors. It is mostly seen in joint areas and skin, as well as associated

[1] Dr, Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, esraozdemir.tr@gmail.com, **ORCID**: 0000-0002-3066-1921
[2] RA, Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, Turkey, handanuguz@atauni.edu.tr, **ORCID**: 0000-0001-5444-1459
[3] Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, bahri.avci90@erzurum.edu.tr, ORCID: 0000-0001-8451-5463
[4] Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, nurselim288@gmail.com, ORCID: 0000-0002-2001-2186
[5] Prof. Dr, Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, hakanbiyolog@gmail.com, ORCID: 0000-0003-3248-759X

with cardiovascular diseases, mental problems and inflammatory arthritis. Topical treatments (retinoids and immunomodulatory agents) are recommended for mild psoriasis, and the use of biological products (tumor necrosis factor a (TNF-a) and IL-17, IL-12 and IL-13 inhibitors) for moderate and severe diseases. Psoriasis vulgaris, also known as plaque psoriasis, is the most common type of psoriasis with various dermatological symptoms.

What is Psoriasis?

Psoriasis is portrayed as a chronic inflammatory skin disease that includes environmental factors and disease-causing environmental factors, that is, innate and adaptive immune processes. This disease occurs as a result of abnormal differentiation of keratinocytes, epidermal proliferation and infiltration of effective leukocytes in the epidermis-dermis (Albanesi et al., 2007; Romanelli et al., 2018). It affects approximately 2-3% of the world's population, varying by region (Michalek et al., 2017).

Epidemiology

The prevalence of psoriasis, which has approximately 125 million people worldwide, varies in different regions. This change was equally distributed among men and women (Parisi et al., 2013). While genetic factors have a very important place in the formation and progression of psoriasis, environmental factors may be a factor in increasing the severity of this disease (Nair et al., 2006).

Classification

The clinical and dermatological symptoms of psoriasis vary according to the type of psoriasis. Among the most common variants are Guttate psoriasis, Erythrodermic psoriasis, Pustular psoriasis and Inverse psoriasis, especially Plaque psoriasis.

1. Plaque psoriasis (Psoriasis vulgaris)

It contains about 80-90% of all the factors that cause psoriasis to occur. This disease manifests itself as scaly patches with clear borders, erythema or plaque. These plates coalesce to cover the large skin surface. It is most commonly seen on the outer surfaces of the limbs, trunk and scalp. Developing moderate to severe plaque psoriasis can cause strong itching (Armstrong & Read, 2020).

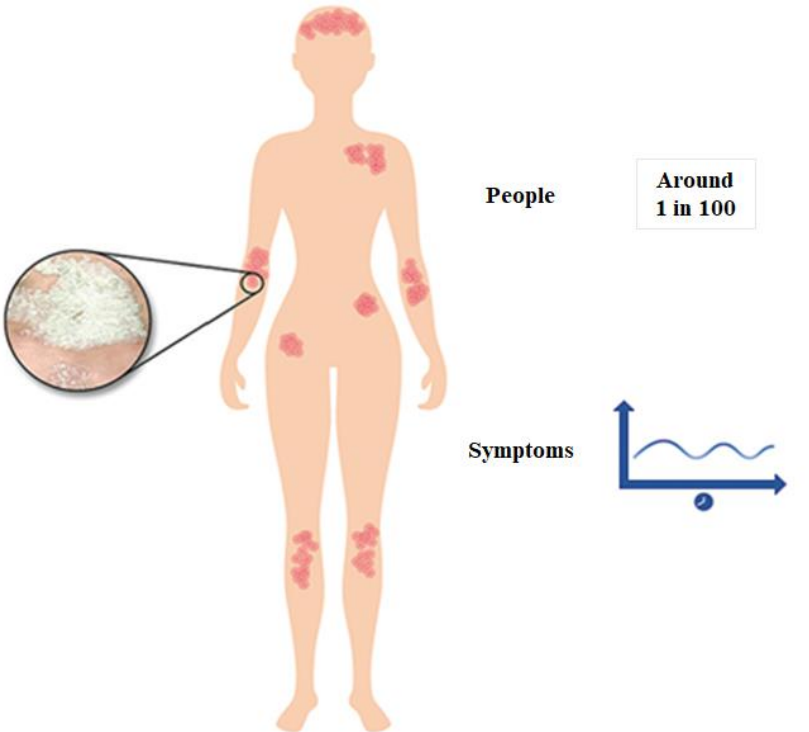


Figure 1. *Locations of Plaque psoriasis in the body (Bachelez et al., 2022).*

Histologically, the most prominent features are thickened epidermis, hyperproliferation/abnormal keratinocyte differentiation and parakeratosis. Diseases accompanying plaque psoriasis are Psoriatic arthritis, Inflammatory bowel disease, Diabetes and Cardiovascular disease (Bachelez et al., 2022).

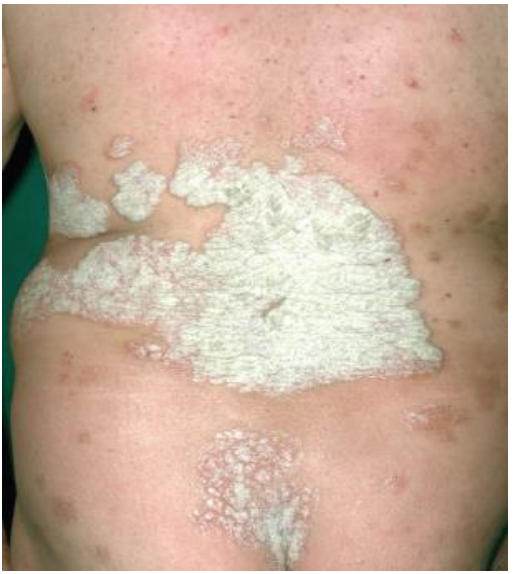


Figure 2. *Dermatological appearance of Plaque psoriasis, characterized by the presence of scaly plaques (Bachelez et al., 2022).*

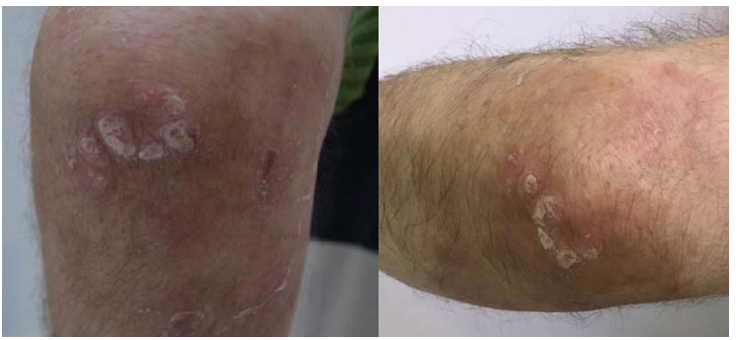


Figure 3. *Appearance of Plaque psoriasis on the left elbow (Ardeleanu et al., 2020).*

2. Guttate psoriasis

It manifests as a red scaly lesion about 3-5 mm in diameter. It spreads in a population of 2%, mostly children and adolescents. It is triggered by group A streptococcal infections in the tonsils. Plaque psoriasis is likely to occur in 33% of patients with guttate psoriasis (Cotton et al., 2011; Rendon & Schäkel, 2019).

Guttate psoriasis, which is clinically described as a subclass of cutaneous psoriasis, has a droplet-like appearance that usually spreads to the trunk and limbs. It is characterized as the onset of monomorphic, erythematous and squamous macules or papules (Maruani et al., 2019).

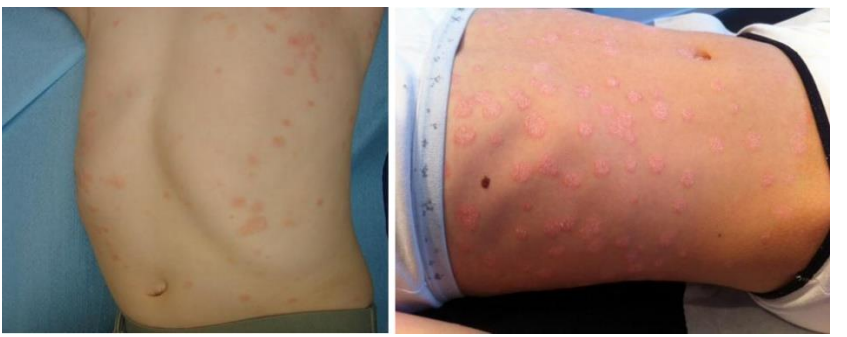


Figure 4. *Appearance of guttate psoriasis on the trunk (Maruani et al., 2019)*

3. Erythrodermic psoriasis

Erythrodermic psoriasis is a variant that covers most of the cutaneous surface (%80-90), triggering scaling or developing exfoliation. Although it is rare, it is a life-threatening dermatological emergency, causing fever and shock due to temperature control and fluid loss (Armstrong & Read, 2020; Cotton et al., 2011). Compared to patients with plaque psoriasis, the Th2 immune response is characterized by high serum immunoglobulin E level, serum level of IL-13, IL-4 and IL-10 levels, and low Th1/Th2 ratio. The most distinctive features of Erythrodermic psoriasis are that it appears as

lesions that are thinner, resemble a dusty surface, vary in size, and do not have sharp borders (Shao et al., 2020).

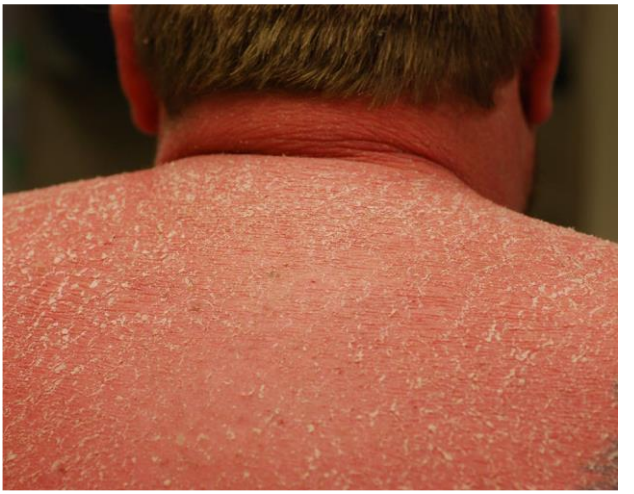


Figure 5. *Appearance of erythrodermic psoriasis on the cutaneous surface (Shao et al., 2020).*

4. Pustular psoriasis

Pustular psoriasis can be affected by factors such as infection, sudden decrease in steroids, pregnancy, and a decrease in calcium below 8.5 mg. In addition, interleukin 36 receptor antagonist (IL36RN) interacts with sequence variation. Its scope is limited to the hands (palms) and feet (fingertips and nail apparatus). Two phenotypes have been described: Hallopeau acrodermatitis continuum and psoriasis pustulosa palmoplantaris (Tsoi et al., 2017; Navarini et al., 2017).

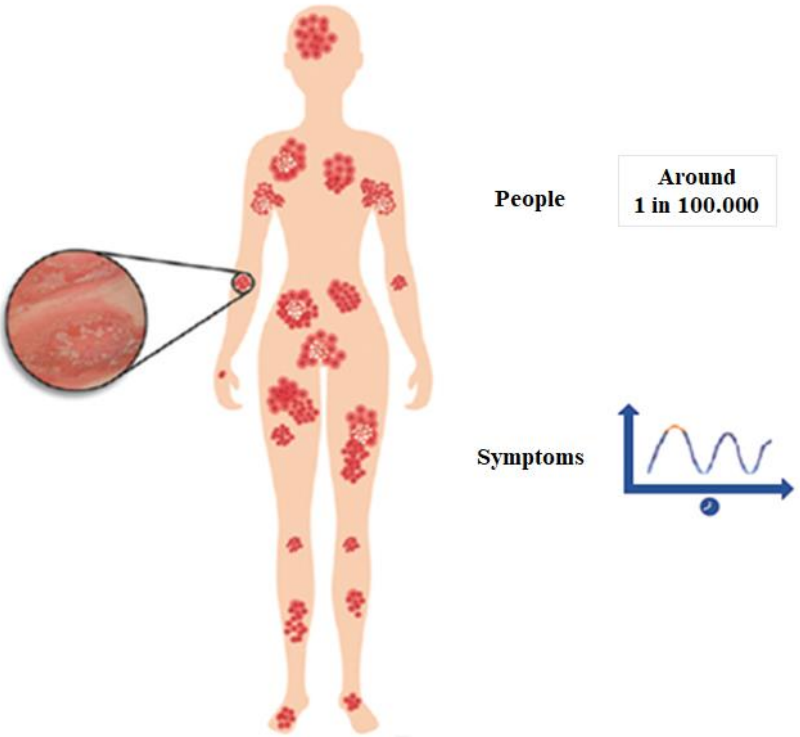


Figure 6. *Locations of Pustular psoriasis in the body (Bachelez et al., 2022).*

Pustular psoriasis presents with a histology characterized by spongy pustules, subcorneal pustules and neutrophil infiltrates. Extracutaneous symptoms (cholestasis, cholangitis, interstitial pneumonia, acute renal failure) have been associated with diseases such as obesity, hypertension, hyperlipidemia and diabetes (Bachelez et al., 2022).

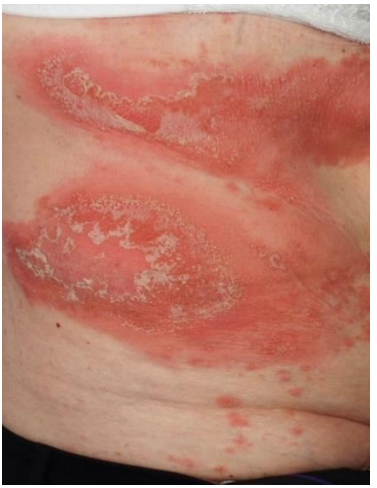


Figure 7. Dermatological presentation of pustular psoriasis, dominated by numerous confluent white/yellowish pustules.

5. Inverse psoriasis

Inverse psoriasis, also known as flexural psoriasis, is manifested by red, sharp-edged and thinner patches in the intertriginous, underarm and inguinal regions (Armstrong & Read, 2020; Rendon & Schäkel, 2019).

The prevalence of inverse psoriasis varies between 3% and 36%. It shows typical features in children, mostly in young babies. Sudden occurrence in adults has been considered as a marker of HIV infection. Although it remains in a limited area on the body surface, it greatly affects the quality of life (Micali et al., 2019).

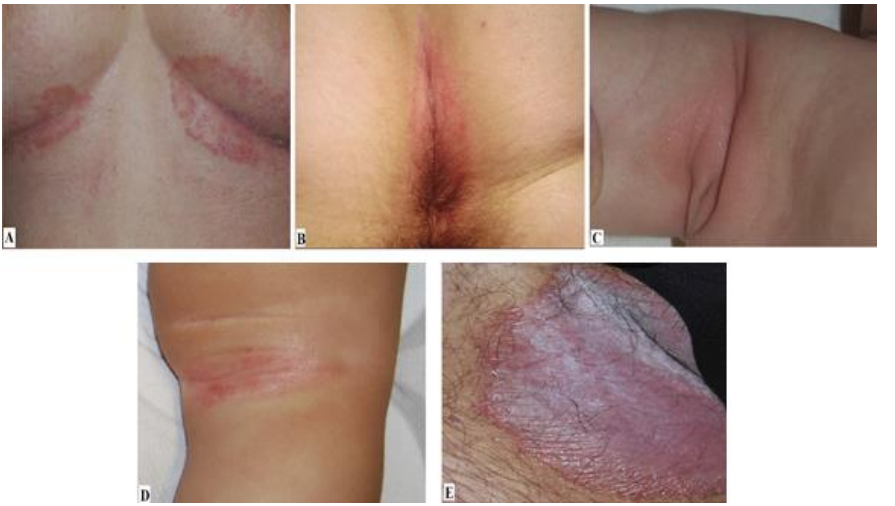
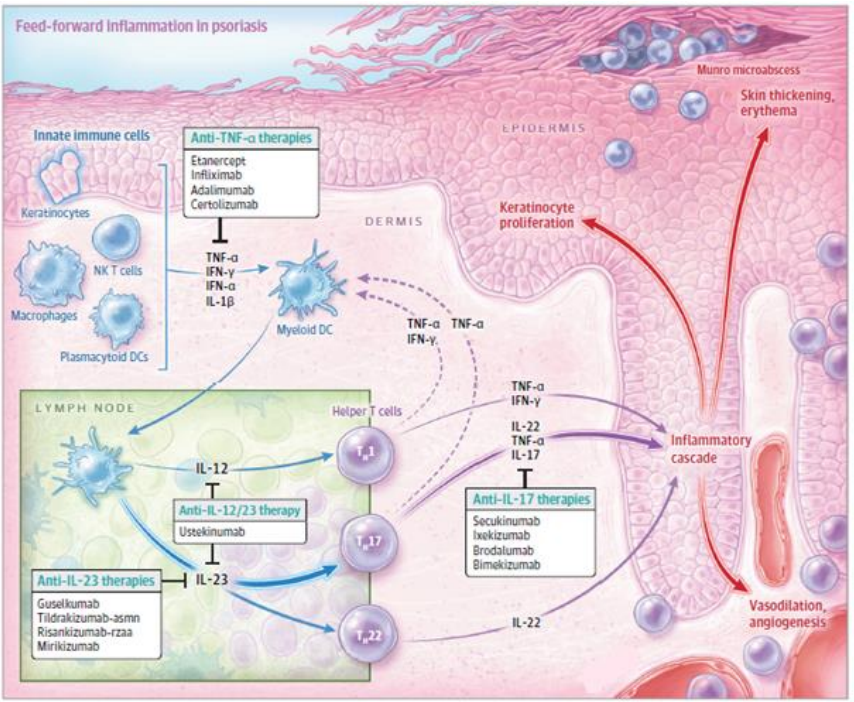


Figure 8. *Appearance of inverse psoriasis in different areas of the body (A): 38 years old, female, under breast, (B): 54 years old, female, perianal region, (C): Baby, right armpit, (D): 5 years old, child, right popliteal fossa, (E) : 46 years old, male, right groin (Micali et al., 2019).*

Pathogenesis

Although the pathogenesis of psoriasis has not been fully explained, it is known to have a complex structure. In the middle of this structure, there is an increase in the activation of the members of the adaptive immune system. In other words, various cells, including plasmacytoid dendritic cells, T cells, macrophages, neutrophils and keratinocytes, increase the release of cytokines and activate myeloid dendritic cells (Rendon & Schäkel, 2019). DNA-LL37 stimulates plasmacytoid dendritic cells to secrete interferon alpha (IFN-a), and myeloid dendritic cells become active. Accordingly, IL-12 and IL-23 release occurs. While IL-12 ensures the differentiation of T cells into TH1 cells, IL-23 ensures the survival and proliferation of TH17 and TH22 cells. Interferongamma(IFN-y) and TNF-a are secreted by TH-1, IL-17, IL-22 and TNF-a by TH17, and IL-22 by TH22. These secreted cytokines cause the downregulation of keratinocytes and the entry of

immune cells into the lesioned skin. The most striking feature of this disease is the irregular proliferation of keratinocytes and unwanted differentiation due to inflammation (Fig 9) (Frank et al., 2009; Alwan & Nestle, 2015).



*****DC, indicates dendritic cell; IFN, interferon; NK, natural killer.

Figure 9*. Pathophysiology of Psoriasis.*

Autoimmunity

It is thought that psoriasis has an autoimmune pathogenesis. Because psoriasis is defined as a common inflammatory skin disease that occurs through T cells that are specific to autoantigen and contribute to the development, general structure and chronic state of the disease (Rendon & Schäkel, 2019; Arakawa et al., 2015). The human leukocyte antigen (HLA) class I allele, HLA-C*06:02, is an important risk gene in psoriasis. LL37 is the well-known T cell

autoantigen in this disease. LL37-specific epidermal CD4+ and CD8+ T cells are found in moderate to severe plaque psoriasis. In addition, T cells specific for LL37 produce IFN-γ, while CD4+T cells are responsible for producing IL-17, IL-21 and IL-22 (Lande et al., 2014). LL37-specific T cells contribute to the development of psoriasis by being found especially in the skin and blood where lesions occur. CD8+T cells mediate epidermotropism, autoantigen recognition, and increased secretion of Th17 cytokines. ADAMTSL5, defined as a melanocytic autoantigen, is limited to HLA-C*06:02, which is recognized by the CD8+T cell TCR (T cell receptor). In other words, in psoriasis lesions, CD8+T cells that are activated against ADAMTSL5 expressed by melanocytes are abundant. This shows that melanocytes are clear autoimmune target cells. It has become clear that CD8+T is a psoriatic autoantigen by inducing IL-17A in CD8+T cells as a result of stimulation of ADAMTSL5 (Arakawa et al., 2015).

Treatment

Since psoriasis recurs chronically, it requires a long-term treatment process (Rendon & Schäkel, 2019). In order to determine the treatment conditions, first of all, it is very important to define the degree of the disease and the diseases it triggers (Mrowietz et al., 2011). Inflammatory, infectious and neoplastic conditions such as atopic dermatitis, seborrheic dermatitis, pityriasis rosea, syphilis and cutaneous T-cell lymphoma are important distinguishing features for the definition of psoriasis. Such conditions cause itching and characteristic morphologies. However, in cases that cannot be evaluated, a skin biopsy may be required (Singh et al., 2018). Psoriasis-related diseases are generally examined under three groups as mild, moderate and severe psoriasis. This grouping; It is formed according to the percentage of surface area affected, the measurement of quality of life, and the clinical severity of the lesions that ocur (Mrowietz et al., 2011).

Topical corticosteroids, vitamin D analogues, calcineurin inhibitors, keratolytics, and targeted phototherapy or their

combinations are considered as treatment options in mild to moderate psoriasis covering 3-5% of the body surface (Rendon & Schäkel, 2019; Diluvio et al., 2006). In moderate and severe psoriasis, which covers 3-10% of the body surface, systemic treatment (biological drugs, oral agents and phototherapy) is often used. The use of biological drugs is used more effectively in treatment than others. In addition to these, topical treatments can also be preferred as adjunctive treatment, not alone (Armstrong et al., 2020). Drugs used in the treatment of psoriasis are shown in Table 1 (Rendon & Schäkel, 2019).

Table1. *Drugs considered suitable for use in the treatment of psoriasis*

Drug	Mechanism	Application
Methotrexate	Dihydrofolate reductase inhibition blocks purine biosynthesis; induction of lymphocyte apoptosis	s.c./oral
Cyclosporin	Calcineurin inhibition leading to reduced IL-2	oral
Acitretin	Normalization of keratinocyte proliferation/differentiation through retinoid receptor binding	oral
Fumarate	ntracellular glutathione, modulation of Nrf2, NF-κB, and HIF-1α; promoting a shift from a pro-inflammatory Th1/Th17 response to an anti-inflammatory/regulatory Th2 response.	oral
Apremilast	DE4 inhibitor increases in tracellular cAMP levels in immune and non-immune cell types modulating inflammation	oral
Etanercept	Dimeric human fusion protein mimicking TNF-αR	s.c
Infliximab	Chimeric IgG1κ monoclonal antibody that binds to soluble and transmembrane forms of TNF-α	i.v.
Adalimumab	Human monoclonal antibody against TNF-α	s.c
Certolizumab	Fab portion of humanized monoclonal antibody against TNF-α conjugated to polyethylene glycol	s.c
Ustekinumab	uman IgG1k monoclonal antibody that binds with specificity to the p40 protein subunit used by both the interleukin (IL)-12 and IL-23 cytokines IL-12/IL-23 p40	s.c
Tildrakizumab	umanized IgG1κ, which selectively blocks IL-23 by binding to its p19 subunit	s.c
Guselkumab	Human immunoglobulin G1 lambda (IgG1λ) monoclonal antibody that selectively blocks IL-23 by binding to its p19 subunit	s.c
Risankizumab	umanized IgG1 monoclonal antibody that inhibits interleukin-23 by specifically targeting the p19 subunit	s.c
Secukinumab	Human IgG1κ monoclonal antibody against IL-17A	s.c
Ixekizumab	Humanized, immunoglobulin G4κ monoclonal antibody selectively binds and neutralizes IL-17A	s.c
Brodalumab	Human monoclonal IgG2 antibody directed at the IL-17RA	s.c

Comorbidities

Although psoriasis is known to affect the skin area, studies have shown that it affects joints and different organ locations and causes the development of some diseases. In the light of these data, it is thought that psoriasis is not only a dermatological disease, but also a unique condition (Sommer et al., 2006; Gerdes et al., 2016). About 33% of psoriasis patients develop psoriatic arthritis during their lifetime (Mease & Goffe, 2005). However, diseases such as hyperlipidemia, Cardiometabolic diseases (myocardial infarction, stroke, and peripheral vascular disease), Type 2 diabetes, Psychological diseases (depression, anxiety), Inflammatory bowel diseases (Crohn disease, ulcerative colitis), and hypertension, such as psoriasis in a normal individual It has been supported by studies that it is seen twice as often in individuals with the disease (Sommer et al., 2006; Gerdes et al., 2016; Elmets et al., 2019).

Risk Factors

Behavioral and environmental factors, especially genetic factors, contribute greatly to the development of psoriasis (Tsoi et al., 2017). Loci (PSORS1-9, PSORSASI) and alleles (HLA Cw6, HLADQ*02:01, CCHCR1, and CYP1A1) are indicated as genetic risk factors for the development of psoriasis (Tsoi et al., 2017; Liu et al., 2008). In individuals with high sensitivity, environmental factors such as skin trauma due to Streptococcal infections, consumption of addictive substances, drugs (such as lithium and interferon) and stress factors trigger psoriasis (Armstrong, 2014). In other words, risk factors affecting psoriasis can be grouped as internal and external factors. Its schematized form is shown in Figure 10 (Kamiya et al., 2019).

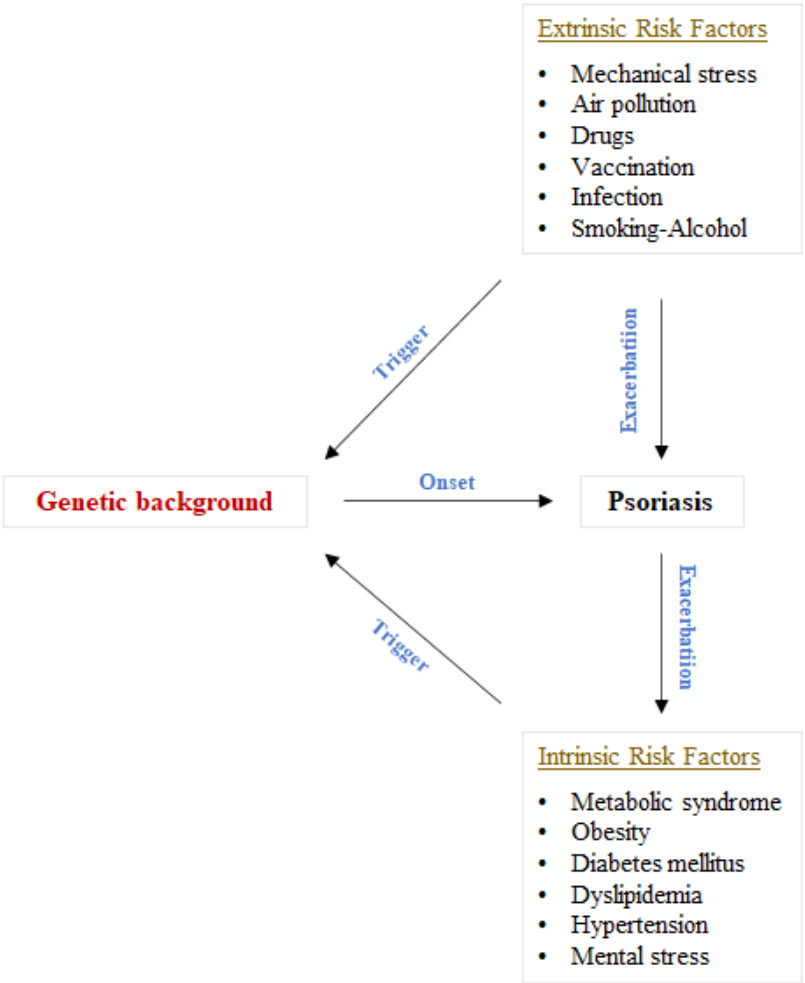


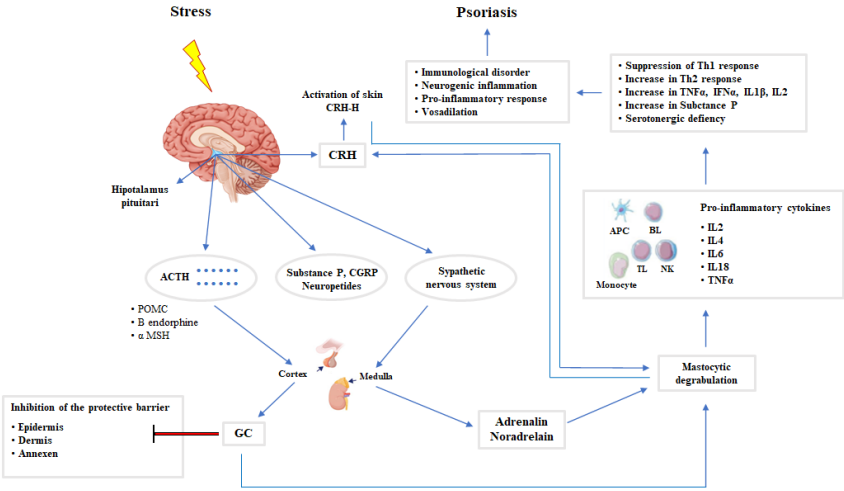
Figure 10. *Internal and external risk factors that initiate and exacerbate psoriasis.*

Stress

According to the data obtained from studies on the relationship between stress and psoriasis, it has been observed that 26-88% of individuals suffering from psoriasis have stress-related complaints (Rousset & Halioua, 2018). The secretion of glucocorticoids (Arnetz

et al., 1985) begins to decrease with the development of psoriasis and there is a concomitant increase in catecholamines (Buske-Kirschbaum et al., 2006). This affects the hypothalamic–pituitary axis and stimulates the secretion of corticotropin-releasing hormone. When stress physiology is examined, it has been reported that degranulation of mast cells is stimulated and proinflammatory cytokines are released due to stress due to these oscillations. This interaction is also thought to cause an increase in the degree of psoriasis (Rousset & Halioua, 2018). Because, it has been shown that the expression of Corticotropin-releasing hormone receptor 1 increases in psoriasis plaques, and cytokines such as TNF-alpha, interferon-alpha, interleukin 1 and 6 are secreted during psoriasis and increase CRH secretion by causing inhibition of serotonin secretion. In this case, the tendency to stress causes an increase in the rate or an increase in stress (Cevirgen et al., 2012).

In psoriasis patients due to stress factors; It has been observed that evening cortisol is directly proportional to the PASI score (Brunoni et al., 2014), the permeability of the epidermal barrier changes, the healing process of wounds on the skin is prolonged, the inhibition of epidermal lipid synthesis, which is effective in keratinocyte proliferation (Garg et al., 2001), and the production of substance P (Fortune et al., 2005), which plays an active role in the increase of inflammation, accelerates. The mechanism between stress and psoriasis is clearly illustrated in Figure 11. (Rousset & Halioua, 2018).



***** CRH-R, corticotropin-releasing hormone receptor; ACTH, adrenocorticotropic hormone; POMC, proopiomelanocortin; a MSH, a Melanocyte stimulating hormone; GC, Glucocorticoid; CGRP, Calcitonine gene-related peptide, IL, interleukin; TNF-α, tumor necrosis factor-alpha.

Figure 11. *The physiology of the link between stress and psoriasis.*

Conclusions

Psoriasis is a common inflammatory skin disease associated with numerous diseases: medical, psychological and social. It can often be determined genetically. It causes a serious decrease in the quality of life of patients. However, a clear understanding of its pathophysiology and effective immune mechanism can be seen as an important step to improve this condition. Because it will help increase the diversity in the treatment methods to be applied.

REFERENCE

Albanesi, C., De Pità, O., & Girolomoni, G. (2007). Resident skin cells in psoriasis: a special look at the pathogenetic functions of keratinocytes. *Clinics in dermatology*, *25*(6), 581-588. doi:10.1016/j.clindermatol.2007.08.013

Alwan, W., & Nestle, F. O. (2015). Pathogenesis and treatment of psoriasis: exploiting pathophysiological pathways for precision medicine. *Clin Exp Rheumatol*, *33*(5 Suppl 93), S2-6.

Arakawa, A., Siewert, K., Stöhr, J., Besgen, P., Kim, S. M., Rühl, G., ... & Prinz, J. C. (2015). Melanocyte antigen triggers autoimmunity in human psoriasis. *Journal of Experimental Medicine*, *212*(13), 2203-2212. doi:10.1084/jem.20151093

Ardeleanu, V., Radaschin, D. S., & Tatu, A. L. (2020). Excimer laser for psoriasis treatment: A case report and short review. *Experimental and therapeutic medicine*, *20*(1), 52-55. doi:10.3892/etm.2020.8529

Armstrong AW, Puig L, Joshi A, et al. Comparison of biologics and oral treatments for plaque psoriasis: ameta-analysis. *JAMA Dermatol*. Published online February 5, 2020. doi:10.1001/ jamadermatol.2019.4029. doi:10.1001/jamadermatol.2019.4029

Armstrong AW. Psoriasis provoked or exacerbated by medications: identifying culprit drugs. *JAMA Dermatol*. 2014;150(9):963. doi:10.1001/jamadermatol.2014.1019

Armstrong, A. W., & Read, C. (2020). Pathophysiology, clinical presentation, and treatment of psoriasis: a review. *Jama*, *323*(19), 1945-1960. doi:10.1001/jama.2020.4006

Arnetz BB, Fjellner B, Eneroth P, et al. Stress and psoriasis: psychoendocrine and metabolic reactions in psoriatic patients during standardized stressor exposure. Psychosom Med 1985; 47: 528–541.

Bachelez, H., Barker, J., Burden, A. D., Navarini, A. A., & Krueger, J. G. (2022). Generalized pustular psoriasis is a disease distinct from psoriasis vulgaris: Evidence and expert opinion. *Expert*

Review of Clinical Immunology, *18*(10), 1033-1047. doi:10.1080/1744666X.2022.2116003

Brunoni AR, Santos IS, Sabbag C, et al. Psoriasis severity and hypothalamic-pituitary-adrenal axis function: results from the CALIPSO study. Braz J Med Biol Res 2014; 47: 1102–1106. doi:10.1590/1414-431X20143762

Buske-Kirschbaum A, Ebrecht M, Kern S, et al. Endocrine stress responses in TH1-mediated chronic inflammatory skin disease (psoriasis vulgaris)–do they parallel stress-induced endocrine changes in TH2-mediated inflammatory dermatoses (atopic dermatitis)? Psychoneuroendocrinology 2006; 31: 439– 446. doi:10.1016/j.psyneuen.2005.10.006

Cevirgen Cemil B, Canpolat F, Yılmazer D, et al. The association of PASI scores with CRH-R1 expression in patients with psoriasis. Arch Dermatol Res 2012; 304: 127–132. doi:10.1007/s00403-012-1218-4

Cotton, D., Taıchman, D., Wıllıams, S., Patel, R. V., & Lebwohl, M. (2011). In the Clinic Psoriasis. *Annals of internal medicine*, *155*(3). doi:10.7326/0003-4819-155-3-201108020-01002

Diluvio, L.; Vollmer, S.; Besgen, P.; Ellwart, J.W.; Chimenti, S.; Prinz, J.C. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. J. Immunol. 2006, 176, 7104–7111. doi:10.4049/jimmunol.176.11.7104

Elmets CA, Lim HW, Stoff B, et al. Joint American Academy of Dermatology-National Psoriasis Foundation guidelines of care for the management and treatment of psoriasis with phototherapy. *J Am Acad Dermatol*. 2019;81(3):775-804. doi.org/10.1016/j.jaad.2019.04.042

Fortune DG, Richards HL, Griffiths CE. Psychologic factors in psoriasis: consequences, mechanisms, and interventions. Dermatol Clin 2005; 23: 681–694. doi:10.1016/j.det.2005.05.022

Frank, O., Nestle, M., Daniel, H., Kaplan, M., & Barker, J. (2009). Mechanisms of disease: Psoriasis. *N Engl J Med*, *361*, 496-509.

Garg A, Chren MM, Sands LP, et al. Psychological stress perturbs epidermal permeability barrier homeostasis: implications for the pathogenesis of stress-associated skin disorders. Arch Dermatol 2001; 137: 53–59. doi:10.1001/archderm.137.1.53

Gerdes, S.; Mrowietz, U.; Boehncke,W.H. Comorbidity in psoriasis. Hautarzt 2016, 67, 438–444. doi:10.1007/s00105-016-3805-3

Kamiya, K., Kishimoto, M., Sugai, J., Komine, M., & Ohtsuki, M. (2019). Risk factors for the development of psoriasis. *International Journal of Molecular Sciences*, *20*(18), 4347. doi:10.3390/ijms20184347

Lande, R., Botti, E., Jandus, C., Dojcinovic, D., Fanelli, G., Conrad, C., ... & Frasca, L. (2014). The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nature communications*, *5*(1), 5621. doi:10.1038/ncomms6621

Liu Y, Helms C, LiaoW, et al. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet*. 2008;4 (3):e1000041. doi:10.1371/journal.pgen.1000041

Maruani, A., Samimi, M., Stembridge, N., Hay, R. A., Tavernier, E., Hughes, C., & Le Cleach, L. (2019). Non-antistreptococcal interventions for acute guttate psoriasis or an acute guttate flare of chronic psoriasis. *Cochrane Database of Systematic Reviews*, (4). doi:10.1002/14651858.CD011541.pub2

Mease P, Goffe BS. Diagnosis and treatment of psoriatic arthritis. *J AmAcad Dermatol*. 2005;52(1): 1-19. doi:10.1016/j.jaad.2004.06.013

Micali, G., Verzì, A. E., Giuffrida, G., Panebianco, E., Musumeci, M. L., & Lacarrubba, F. (2019). Inverse psoriasis: from

diagnosis to current treatment options. *Clinical, cosmetic and investigational dermatology*, 953-959. doi:10.2147/CCID.S189000

Michalek, I. M., Loring, B., & John, S. M. (2017). A systematic review of worldwide epidemiology of psoriasis. *Journal of the European Academy of Dermatology and Venereology*, *31*(2), 205-212. doi:10.1111/jdv.13854

Mrowietz, U.; Kragballe, K.; Reich, K.; Spuls, P.; Griffiths, C.E.; Nast, A.; Franke, J.; Antoniou, C.; Arenberger, P.; Balieva, F.; et al. Definition of treatment goals for moderate to severe psoriasis: A European consensus. Arch. Dermatol. Res. 2011, 303, 1–10. doi: 10.1007/s00403-010-1080-1

Nair, R. P., Stuart, P. E., Nistor, I., Hiremagalore, R., Chia, N. V., Jenisch, S., ... & Elder, J. T. (2006). Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *The American Journal of Human Genetics*, *78*(5), 827-851.

Navarini, A. A., Burden, A. D., Capon, F., Mrowietz, U., Puig, L., Köks, S., ... & Warren, R. (2017). European consensus statement on phenotypes of pustular psoriasis. *Journal of the European Academy of Dermatology and Venereology*, *31*(11), 1792-1799. doi:10.1111/jdv.14386

Parisi, R., Symmons, D. P., Griffiths, C. E., & Ashcroft, D. M. (2013). Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *Journal of Investigative Dermatology*, *133*(2), 377-385. doi:10.1038/jid.2012.339

Rendon, A., & Schäkel, K. (2019). Psoriasis pathogenesis and treatment. *International journal of molecular sciences*, *20*(6), 1475. doi:10.3390/ijms20061475

Romanelli, P., Volpe, E., Borsellino, G., Romanelli, M., & Chiricozzi, A. (2018). Scanning the immunopathogenesis of psoriasis. *International Journal of Molecular Sciences*, *19*(10.3390). doi:10.3390/ijms19010179

Rousset, L., & Halioua, B. (2018). Stress and psoriasis. *International journal of dermatology*, *57*(10), 1165-1172. doi:10.1111/ijd.14032

Shao, S., Wang, G., Maverakis, E., & Gudjonsson, J. E. (2020). Targeted treatment for erythrodermic psoriasis: rationale and recent advances. *Drugs*, *80*(6), 525-534. doi:10.1007/s40265-020-01283-2

Singh JA, Guyatt G, Ogdie A, et al. Special article: 2018 American College of Rheumatology/National Psoriasis Foundation guideline for the treatment of psoriatic arthritis. *Arthritis Rheumatol*. 2019;71(1):5-32. doi:10.1002/ art.40726

Sommer, D.M.; Jenisch, S.; Suchan, M.; Christophers, E.; Weichenthal, M. Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. Arch. Dermatol. Res. 2006, 298, 321–328. doi:10.1007/s00403-006-0703-z

Tsoi LC, Stuart PE, Tian C, et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nat Commun*. 2017;8:15382. doi: 10.1038/ncomms15382

Tsoi, L. C., Stuart, P. E., Tian, C., Gudjonsson, J. E., Das, S., Zawistowski, M., ... & Elder, J. T. (2017). Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nature communications*, *8*(1), 15382. doi: 10.1038/ncomms15382

CHAPTER V

Otoimmün Cilt Hastalığı: Alopesi Areata

Handan UĞUZ[1]
Esra PALABIYIK [2]
Ayşe Nurseli SULUMER[3]
Bahri AVCI[3]
Hakan AŞKIN[4]

Alopesi areata (AA), kafa derisinde ve/veya vücutta iz bırakmayan, otoimmün, inflamatuar saç dökülmesidir (Satyaprakash, 2014). Bu hastalığın bilinen alt grupları arasında terminal kafa derisi kıllarının tamamen yok olduğu (alopesi totalis veya AT) ve terminal kafa derisiyle birlikte (alopesi universalis veya AU) yer almaktadır (Sehgal, 2003; Lee et al., 2018). AA ile ilgili

[1] RA, Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, Turkey, handanuguz@atauni.edu.tr, **ORCID**: 0000-0001-5444-1459
[2] Dr, Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, esraozdemir.tr@gmail.com, **ORCID**: 0000-0002-3066-1921
[3] Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, nurselim288@gmail.com, **ORCID**: 0000-0002-2001-2186
[3] Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, bahri.avci90@erzurum.edu.tr, ORCID: 0000-0001-8451-5463
[4] Prof. Dr, Department of Molecular Biology and Genetics, Ataturk University, Erzurum, Turkey, hakanbiyolog@gmail.com, ORCID: 0000-0003-3248-759X

insidans ve prevalans ile ilgili az sayıda çalışma olmasın rağmen, AA'nın insidansının %0,1-0,2 olduğu ve yaşam boyu riskinin %1,7 olduğu, erkek ve kadınların eşit olarak etkilendiği bildirilmiştir (Naik et al., 2021).

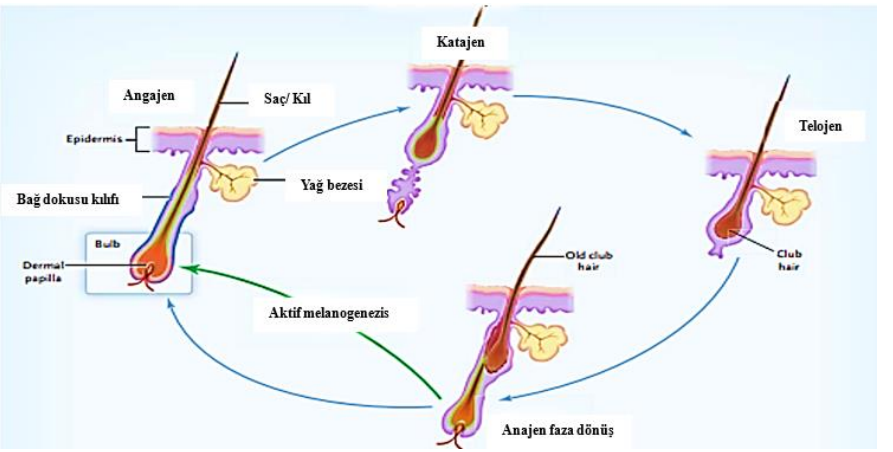
Epidemiyolojisi

AA en yaygın görülen inflamatuvar saç dökülmesi ve Amerika Birleşik Devletleri'nde yaklaşık 4,5 milyon kişiyi etkilemektedir (Wang, 2014). Etnik kökene ve bireyin yaşadığı bölgeye bağlı kalmaksızın, AA prevalansı %0,1 ila %0,2 arasındadır ve yaşam boyu risk %2 olarak hesaplanmıştır (Naik et al. 2021). Bozukluk 3 yaşın altındaki çocukları nadiren etkilese de hastaların çoğu nispeten gençtir: %66'sı 30 yaşın altında ve sadece %20'si 40 yaşın üzerindedir. Hastalık her iki cinsiyeti de eşit düzeyde etkilemekte ve cinsiyet ayrımı yoktur, ancak 21 ila 30 yaş arasındaki bir grup insan üzerinde yapılan bir çalışmada daha fazla erkeğin etkilendiği bulunmuştur (Kyriakis, 2009).

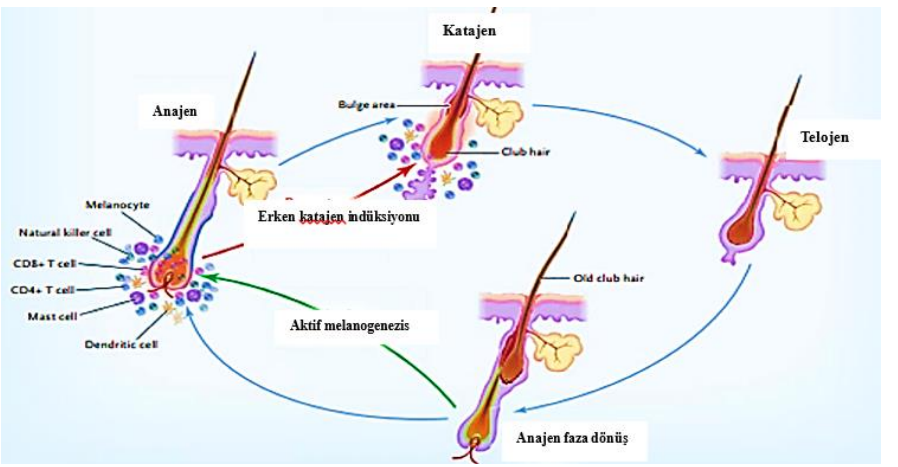
Normal Saç Büyümesi

Alopesi areata'nın klinik özellikleri ve teşhis sürecini daha iyi anlamak için, bu durumun neden olduğu değişiklikleri ve saç folikülünün normal immünobiyolojik mekanizmalarını daha iyi anlamak gerekmektedir. Saç folikülleri, insan vücudunda kapsamlı bir şekilde bulunan, ömür boyu süren, döngüsel dönüşüm geçiren tek organdır (Oh et al., 2016). Çok hızlı bir büyüme, pigmentasyon ve saç teli üretimi döneminden (anajen, aktif büyüme fazı, evre I'den VI'ya kadar değişen sınıflandırma ile) kısa, apoptoza dayalı bir organ involüsyonu evresine (katajen) geçerler. Katajen evresinden sonra, saç folikülleri anajen evresine yeniden girmeden önce telojen (göreceli bir durgunluk) dönemine girer (Şekil 1). Bu rejeneratif döngü (Şekil 2), çoğunlukla şişkinlik olarak adlandırılan bölgede bulunan keratinosit ve melanosit kök hücrelerinin sayısındaki artış ile mümkün olmaktadır (Gilhar et al., 2012). Saç folikülü döngüsü ve rejenerasyonu kök hücreye bağlı olsa da saç teli üretimi ve pigmentasyonu bu kök hücrelerin farklılaşmalarıyla gerçekleştirilir. Hızla çoğalan bu keratinositler ve pigment üreten melanositler

anajen saç matriksinde (Şekil 1) bulunurlar. Bu da AA inflamatuar saldırının ana hedefidir (Rajabi et al., 2018).



Şekil 1. Sağlıklı saç siklusu (Gilhar et al., 2012)

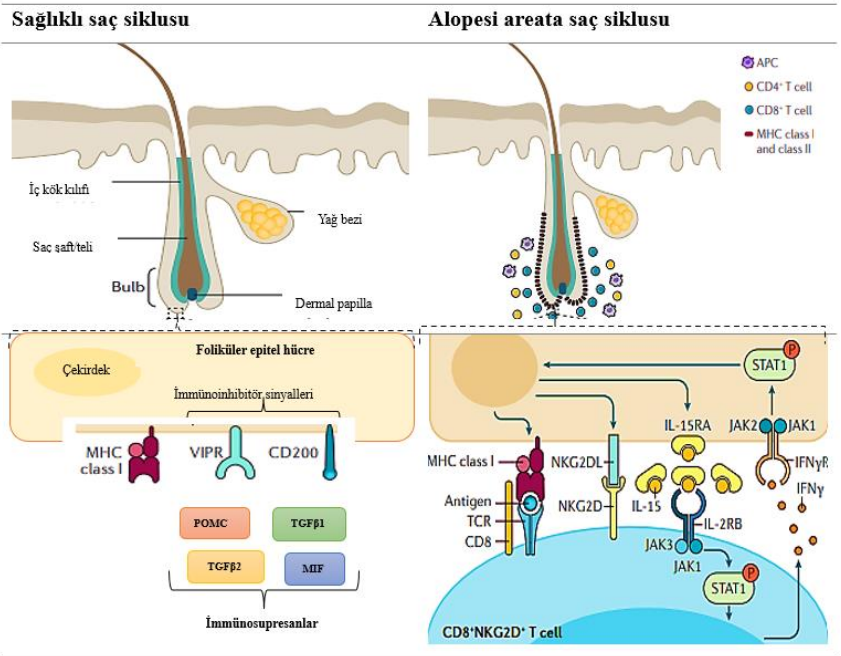


Şekil 2: *Alopesi areata saç siklusu (Gilhar et al., 2012)*

Saç Folikülü İmmünobiyolojisi

Saç folikülü, immünolojik bir özelliğe sahiptir. Genellikle intrafoliküler olarak ifade edilen otoantijenlere (vücudun kendi

hücrelerine karşı tepki veren moleküller) karşı otoimmün bir saldırıyı engelleyen bir tür immün ayrıcalık ortamı oluşturmaktadır. Bu bağışıklık, vücudun kendi hücrelerine karşı bağışıklık sisteminin aşırı tepki vermesini önleyen bir mekanizmadır (Paus et al., 2018). İmmun ayrıcalık, saç folükülündeki hücrelere karşı otoimmün reaksiyonların inhibe edilmesini sağlayan birtakım mekanizmalar (MHC sınıf Ia stabilize edici β2 mikroglobulin ile majör histokompatibilite kompleksi [MHC] sınıf Ia antijenler) içerir. Bu mekanizmalar, $CD8^+$ T lenfositlerinin saç folikülü hücrelerine aşırı reaksiyon vermesini inhibe eder (Ito et al., 2004; Paus et al., 2018). Saç foliküllerine ilişkin immün ayrıcalığın fizyolojik işlevi tam olarak bilinmese pigment üretimiyle ilişkili birkaç (vitiligo ve halo nevüsler vb) otoantijenin yüksek oranda immünojenik olduğunu bilinmektedir. Bu nedenle, aktif saç teli pigmentasyonu sırasında üretilen melanogenez ile ilişkili otoantijenlerin ve belki de diğer anajen ile ilişkili saç folikülü otoantijenlerinin, halihazırda mevcut olan otoreaktif $CD8^+$ T hücrelerini çekme konusunda yapısal bir risk oluşturduğudur (Gilhar et al., 2012). Klasik bağışıklık ayrıcalığı ile korunan diğer dokularda olduğu gibi (örneğin, gözün ön odası, merkezi sinir sistemi ve fetal trofoblast), MHC sınıf I moleküllerinin aşağı regülasyonu, folikülle ilişkili otoantijenlerin CD8+ T hücrelerine sunulma riskini azaltmaya yardımcı olabilir (Christoph et al., 2000; Paus e al., 2005; Carson et al., 2006).



Şekil 3: *Sağlıklı ve AA saç siklusu immünolojisi (Pratt et al. 2017)*

Bununla birlikte, MHC sınıf I moleküllerinin bu aşağı regülasyonu, saç folikülünün doğal öldürücü (NK) hücreler tarafından saldırıya uğraması riskini beraberinde getirir, NK hücreleri tanıma için hazırlandığından ve MHC sınıf I-negatif hücreleri ortadan kaldırır (Höglund and Brodin et al., 2010). Bu riski azaltmak için, sağlıklı saç foliküllerinin NK-hücre reseptörlerinin (NKG2D) aktivasyonunu uyaran ligandların ekspresyonunu azalttığı ve dönüştürücü büyüme faktörleri β1 (TGF- β1) ve β2, α melanosit uyarıcı hormon ve makrofaj göçünü engelleyici faktörü gibi NK-hücre ve T-hücre işlevlerini engelleyen molekülleri salgıladığı görülmektedir (Gilhar et al., 2012).

Alopesi Areata Mekanizması

1. Genetik

AA genetik temeli için yapılan çalışmalarda, aday gen ilişkilendirme, transkripsiyonel profil analizi ve Genom çapında bileşik analizler (GWAS) gibi çeşitli teknikler kullanıldı. Başlangıçta, otoimmün hastalıklarda rol oynayan genlere odaklanıldı ve bu genlerin AA yanı sıra başka hastalıklarda da (iltihabi bağırsak hastalığı, sedef, tip 1 diyabet, multpli skleroz gibi) rol oynadığı bulundu (Petukhova and Christiano et al., 2013). Özellikle, İnsan Lökosit Antijeni (HLA) bölgesi, AA fenotipine önemli bir katkı sağlayan bir bölge olarak belirlendi. Son GWAS analizlerinde HLA sinyalinin genellikle HLA-DRB1 bölgesine odaklanılmıştır, bu bölgedeki varyasyonların AA duyarlılığı artırabileceğini göstermiştir. Genetik çalışmalar, AA ile ilişkilendirilen genler arasındaki karmaşık bir yapıya sahip olduğunu vurgulamaktadır (Colombe et al., 1999; Kavak et al., 2000; Hordinsky 2013).

Daha sonraki büyük ölçekli genetik çalışmalar, AA ile ilişkili belirlenmiş ve HLA bölgesi genlerinin rolünü daha da doğrulamıştır. Bu genler Tablo 1'de gösterilmiştir (Pratt et al., 2017).

Tablo 1. *Alopesi Areata Patogenezinde Rol Oynayan Genler*

Genom ile ilişkili çalışmalar (GWAS) ile belirlenen genler	*COXL, ATXN2, BCL2L11 (BIM olarak da bilinir), BTNL2, C6orf10, CD28, CIITA, CLEC16A (KIAA0350 olarak da bilinir),), CTLA4, EMSY (C11orf30 olarak da bilinir), ERBB3, ICOS, IKZF4, IL2, IL2RA, IL4, IL13, IL15RA, IL21, LRRC32 (GARP olarak da bilinir), MICA, NOTCH4, NR4A3, PRDX5, PTPN22, RAET1L (ULBP6 olarak da bilinir), SH2B3 (LNK olarak da bilinir), SOCS1, SPATA5, STX17 ve ULBP3.*
Kantitatif özellik dokusu (QTL) ile belirlenen genler	*ADAMTS20, CASP3, CD3E, CRISP1, CRTAM, HLA kompleksi, IL2RB, IL10RA, IL12RB1, IL15, JAK3, LTA, LTB, LY6D, NCAM1, PLEC, SOX10, THY1, TNF, TRHR ve TRPS1*
QTL ve GWAS ile belirlenen genler	*HLA-DQA1, HLA-DQA2, HLA-DQB2 ve HLA-DRA*
Kopya sayısı varyasyon analiziyle belirlenen genler	*MCHR2 ve MCHR2-AS1*
Transkriptom ifadesine göre belirlenen genler	*CXCL9, CXCL10, CXCL11 and CXCR3*

Yapılan çalışmalara bakıldığında, çeşitli GWAS analizle sonucunda AA ile ilişkili 14 genetik lokus belirlemiştir. Bunların çoğunun immün sistemde görev aldığı bilinmektedir (Petukhova et al., 2010; Hordisky 2013; Petukhova and Christiano, 2016). Özellikle, doğal öldürücü (NK) hücre reseptörü D (NKG2D;

KLRK1 tarafından kodlanır) ligandları NKG2DL3 (ULBP3 tarafından kodlanır) ve retinoik asit erken transkript 1L proteinini (RAET1L tarafından kodlanır; ULBP6 olarak da bilinir) kodlayan genleri barındıran bir lokus, diğer otoimmün hastalıklarda değil, AA da benzersiz bir şekilde rol alır. Bu da patogenezde önemli bir rolü olduğunun göstergesidir. Aslında bu durum, CD8+NKG2D+ T hücrelerinin AA patogenezinin ana efektörleri olduğunu gösteren fonksiyonel immünolojik çalışmalardan kaynaklanmıştır. CD8+NKG2D+ T hücrelerinin hayatta kalmaları için IL-15 sinyaline bağlı olmaları, yeni terapötik ilaçların geliştirilmesinde Janus kinaz (JAK) inhibitörlerinin kullanılmasının önünü açmıştır.

Ayrıca, genetik çalışmaları doğrulamak ve etkilenen bölgelerdeki gen ifadesi değişikliklerini değerlendirmek için gen ekspresyonu çalışmaları kullanılmıştır. Bu çalışmalarda hastalığın ilerlemesinde gen ekspresyon düzeyi değişen daha fazla geni tanımlamıştır (Carroll et al., 2002; Sundberg et al., 2003; Sundberg et al., 2004; McPhee et al., 2012). Gen ifadesi profilleme çalışmaları, interferon-γ (IFNγ) yolağının ve ilgili sitokinlerin baskın ifadelerinin yanı sıra, her ikisine de JAK (Janus kinaz) tarafından aracılık edilen sitotoksik T hücreleri için baskın bir profil ortaya koymuştur. Bu profillerin tespiti, JAK inhibitörleri kullanılarak odaklanmayı daha da rafine etmiştir. Son dönemde, AA ile ilişkilendirilen genetik kopya sayısı varyasyonları (**Tab 1**), genom çapında yapılan taramalar sonucunda belirlenmiştir (Fischer et al. 2016).

Hem hayvanlarda hem de insanlarda yapılan genetik çalışmaların çoğu AA otoimmün yönlerine daha fazla odaklanılmasına rağmen saç dökülmesi büyük ölçüde saç telinin kırılganlığı ve kopmasından kaynaklanmaktadır. AA fare modelinde sisteince zengin salgı proteini 1 (Crisp1), QTL, shotgun proteomik ve in suti hibridizasyon kombinasyonu kullanılarak *Alaa1* geni aday gen olarak tanımlanmıştır (Rice et al., 2009; Rice et al., 2012; Sundberg et al., 2014). Otoimmün temelli inflamasyon kıl gövdesinin gelişimini ve büyümesini düzensizleştirebilse de spontan AA en ciddi şekilde etkilenen soy olan C3H/HeJ farelerinde CRISP1'in bulunmaması, CRISP1'in fare kılının kıl gövdesini

hastalıklara yatkın hale getiren önemli bir yapısal bileşeni olabileceğini düşündürmüştür. Ancak bu proteinin insanlardaki AA bir rolünün olup olmadığı henüz belirlenmemiştir. Bu çalışmalar doğrultusunda, genetik faktörlerin sadece hastalığın başlangıcında etkili olmadığı aynı zamanda, hastalığın klinik belirtilerinin çok çeşitli ve değişken (pleomorfik klinik sunum) olmasına da neden olur.

2. Saç dökülmesinin patodinamiği

AA bireylerde saç büyümesinin yapısı ve mekanizması değişmiş hatta bozulmuştur (şekil 1 ve 2). Saç dökülmesinin başlangıç evresiyle ilgili yapılan çalışmalarda telojen saçların ve anormal saç şaftlarının oranında büyük bir artış olduğu ve sağlıklı bireylerdeki saça kıyasla şaftın kırılganlığının artmasına (distrofik saç) neden olduğu bulunmuştur (Eckert et al., 1968). Normalde çoğu kıl anajen fazdadır ve bu faz altı aşamaya (anajen I-VI) ayrılır, distrofik kıllar kılın bir aşaması değildir. Aslında AA bireylerdeki ilk olay, saç folikülünün anajen fazdan, katajen ve telojen fazlara hızlı bir şekilde ilerlemesi olarak görünmektedir. Daha az etkilenmiş olan saç folikülleri anajen fazda kalır ancak hastalığın seyrine ve şiddetine bağlı olarak sonunda telojen faza geçerek distrofik saç telleri üretmiştir (Messenger et al., 1986). Etkilenen saç folikülleri yeniden anajen faza girer. Yamalı alopesi görülen bir çalışmada saç foliküllerinin neredeyse yarısından fazlası anajen fazdadır (Pratt et al., 2017).

Ünlem işareti kılları (bir tür distrofik kıl) AA'nın temel bir özelliğidir ve normalde sağlıklı kontrollerde görülmez (Messenger et al., 1986). Ünlem işaretli saçlar normal telojen fazındaki saçlar gibi iyi şekillendirilmiş bir çomak köke sahip olsa da kök genellikle daralır ve çomak saçlar normalden daha kolay dökülür. Saç folikülündeki bu değişiklikler, saçın folikül içinde hatalı bir şekilde tutunduğunu göstermektedir.

Peribulber inflamasyon, genellikle fokal lezyona komşu olan anajen evresinde bulunan foliküllerin çevresinde bulunur (Messenger et al., 1986). Erken lezyonlar, yağ bezinin üstündeki

bölümün ve bezin kendisinin korunmasıyla birlikte, yağ bezi seviyesinin altındaki folikül boyutunda bir azalma gösterir. Uzun süre devam eden hastalık sürecinde folikülün tamamı daha küçüktür. Küçük olan anajen folikül mitotik olarak aktiftir ve normal bir iç kök kılıfı üretir. Ancak kıl gövdesi korteksi tam olarak keratinize olmamıştır. Bu anomaliler saç folikülü gelişiminin anajen IV fazında durduğunu gösterir (Chase et al., 1951).

AT ve AU hastalarının kel bölgelerinin merkezinden alınan yatay biyopsilerde, anajen III/IV fazına geçememiş anajen foliküller bulunmuştur. Bu aşamada iç kök kılıfı konik şeklinde keratinize bir yapıdır, saç korteksi bunun altında farklılaşmaya başlamıştır. Saç folikülleri, anajen III/IV fazından telejon fazla kısa sürede geçerler. Bu da tekrarlanmış kısaltılmış döngülerden geçiyor gibi görünmektedir. AA hastalığında, hastalığın seyri azaldıkça, saç folikülleri anajen faza geçer.

3. Bağışıklık sistemi tepkisi

3. a. Hedef hücre

AA'nın histopatolojik özelliği, anajen fazındaki kıl foliküllerinin bulber bölgesinde ve çevresinde yoğunlaşan inflamatuar hücre infiltratıdır. Erken kortikal farklılaşma sürecindeki saç folikülü matriks epiteli, AA ile yapılan çalışmalar doğrultusunda saç foliküllerine yönelik immün saldırının birincil hedefi olarak görünmektedir. İlk olarak, etkilenen anajen foliküllerde matriks hücreleri vakuolar dejenerasyon (küçük, sitoplazmik vakuollerle karakterize edilen ölümcül olmayan bir hücre hasarı türü) gösterir ve bu da ünlem işareti saç gövdesinin oluşumunun sebebini açıklar (Messenger and Bleehen, 1984; Thies, 1966). Saç folikülündeki bu dejeneratif değişiklikler, saç telinde lokalize zayıflamaya sebep olur, bu da saç telinin deri yüzeyindeki ostiumdan çıkarken kırılmasıyla sonuçlanır. İkinci olarak, etkilenen saç folikülleri kortikal farklılaşmanın gerçekleşmediği telojen faza geri döner. Foliküller anajen faza normal olarak yeniden girer ancak kortikal farklılaşmanın başladığı nokta olan anajen III/IV fazının ötesine geçemez. Son olarak, inflamatuar hücre infiltratlarının

lokalize olduğu pre-kortikal bölgede anormal MHC sınıf I ve sınıf II ekspresyonu meydana gelir (Paus et al., 1993).

3. b. İmmün sistemin bozulması

Saç folikülü, saç folikülünde ifade edilen otoantijenlere karşı otoimmün tepkileri inhibe eden bağışıklık açısından ayrıcalıklı bir bölgedir (Tharumanathan 2015). Normal fizyolojik koşullar altında, saç folikülü içinde ve çevresinde, CD8+ T lenfositleri de dahil olmak üzere NK hücrelerine otoantijenlerin sunulması için gerekli yüzey moleküllerini baskılayan lokal bir immünoinhibitör sinyal ortamı oluşur. Saç foliküllerinin bu bağışıklık ayrıcalığının işlevi (veya işlevleri) kanıtlanmamış olsa da melanositlerde pigment üretimi ile ilgili yapılan çeşitli çalışmalar doğrultusunda bazı otoantijenlerin immünojenik olduğuna ve uygun koşullar altında immün ayrıcalığın kaybına neden olabileceği gösterilmiştir (Paus et al., 2005; Meyer et al., 2008; Ito et al., 2008; Trautman et al., 2009; Paus and Bertolini, 2013).

Saç folikülünün bağışıklık ayrıcalığının bozulması, AA başlıca nedenlerinden biri olarak düşünülmektedir. MHC sınıf I ve sınıf II moleküllerinin düşük ekspresyonu ve bir NK hücre inhibitörü olan makrofaj migrasyon inhibitör faktörünün (MIF) yüksek ekspresyonu, sağlıklı bireylerin saç folikülünden bir T lenfosit alt kümesinin- CD56+NKG2D+ NK hücreleri- infiltrasyonunu önler, ancak CD56+ NKG2D+ NK hücreleri AA hastalarının saç folikülleri etrafında bulunur. NK hücre aktive edici reseptörleri (NKG2D ve NKG2C gibi) ifade eden CD56+ NK hücrelerinin sayısı, AA hastaların periferik kan hücrelerinde sağlıklı kontrollere göre daha yüksekken, öldürücü hücre immünoglobulin benzeri reseptörler 2D2 veya 2D3 daha düşüktür (Ito et al., 2008). GWAS ile yapılan çalışmalar doğrultusunda önceki çalışmalar doğrulanmış ve ULBP gen kümesindeki (NKG2D ligandlarını kodlayan) polimorfizmleri AA yatkınlıkla ilişkilendirmiş ve fonksiyonel çalışmalar AA hastaların lezyonlu kıl foliküllerinde bu genlerin aşırı ekspresyonunu göstermiştir (Petukhova et al., 2010; Betz et al., 2015). Son olarak, saç folikülü epitelinde bulunan vazoaktif intestinal peptid (VIPR1 ve

VIPR2) reseptörlerinin ifadesi, alopesi areata hastalarının saç soğanlarında kontrollere kıyasla aşağı doğru düzenlenmiştir, ancak sinir liflerinde VIP ligand ifadesi normaldir, bu da hastaların VIPR aracılı sinyallemede kusurları olduğunu düşündürmektedir (Bertoni et al., 2016).

3. c. Otoantijen epitopları

Otoantijenin katılımı alopesi areata başlangıcındaki epitoplar varsayılmıştır. Melanin, melaninle ilişkili proteinler ve keratinosit kaynaklı antijenlerin alopesi areata bağlamında, özellikle alopesi areata döneminde beyaz saçların yeniden çıkmasının ve sonraki nüksetmelerde genellikle korunmasının bir ilişki önerdiğine dair gözlemlere işaret ediyor (Messenger and Bleehen, 1985; Paus et al., 1993; Gilhar et al., 2001; Fin et al., 2011; Erb et al., 2013). Trikohyalin (yapısal bir protein) ve tirozinaz ile ilişkili protein 2'den (günümüzde pigmentasyonda rol oynayan dopakrom tautomeraz olarak bilinmektedir) türetilen sentetik epitopların uygulanması, alopesi areata hastalarında normal kontrol hastalarından alınan benzer şekilde uyarılmış periferik kan mononükleer hücrelerine kıyasla önemli ölçüde daha yüksek sitotoksik T lenfosit yanıtlarına neden olmuştur (Wang et al., 2016).

4. Diğer faktörler

4.a. Oksidatif stres

Oksidatif stresin AA ve diğer cilt hastalıklarında rolü vardır (Alzoliban, 2014; Kalkan et al., 2015). AA hastalarının kanında sağlıklı kontrollere kıyasla önemli ölçüde daha yüksek malondialdehit (MDA) seviyeleri ve süperoksit dismutazın (SOD) antioksidan aktivitesi bulunmuştur (Fattah et al., 2011). Diğer bir çalışmada, AA bireylerin %32'sinde Reaktif oksijen türlerinin (ROS) hasar verdiği SOD'a karşı antikorlar bulunurken, sağlıklı bireylerde anlamlı antikor değerleri bulunmamıştır (Alzolibani, 2014). Yapılan çalışmalar sonucunda, oksidatif stresin ve SOD hasarının AA indüksiyonunda rol oynadığı görülmüştür (Acharya and Marthur, 2020). Ancak yapılan sınırlı genetik çalışmalar

doğrultusunda, AA ile SOD2 veya GPX1'deki polimorfizmler arasında bir ilişki bulunamamıştır (Kalkan et al. 2015).

4. b. Çevresel etmenler

Vakaların çocuğunda AA atağının başlangıcı için belirgin bir açıklama bulunamaz, ancak yas, yaralanma sonrası duygusal veya fiziksel stres gibi etmenler ileri sürülmektedir (Caro, 2022). Aynı zamanda aşılar, ateşli hastalıklar ve ilaç gibi etmenler sebep olmaktadır. Japon ensefalit virüsü (Chu et al. 2016), hepatit B virüsü (Richardson et al., 2018), Clostridium tetani (Chen, 2022), herpes zoster virüsü (El Haydari et al., 2013) ve papillomavirüs (Geier and Geier, 2015 dahil olmak üzere çeşitli insan patojenlerine karşı yapılan aşılardan kısa bir süre sonra düşük sıklıkta bireylerde AA ortaya çıktığı bildirilmiştir.

KAYNAKÇA

Abdel Fattah, N. S. A., Ebrahim, A. A., & El Okda, E. S. (2011). Lipid peroxidation/antioxidant activity in patients with alopecia areata. *Journal of the European Academy of Dermatology and Venereology*, *25*(4), 403-408.

Acharya, P., & Mathur, M. C. (2020). Oxidative stress in alopecia areata: a systematic review and meta-analysis. *International journal of dermatology*, *59*(4), 434-440.

Alzolibani, A. A. (2014). Preferential recognition of hydroxyl radical-modified superoxide dismutase by circulating autoantibodies in patients with alopecia areata. *Annals of dermatology*, *26*(5), 576-583.

Bertolini, M., Pretzlaff, M., Sulk, M., Bähr, M., Gherardini, J., Uchida, Y., ... & Paus, R. (2016). Vasoactive intestinal peptide, whose receptor-mediated signalling may be defective in alopecia areata, provides protection from hair follicle immune privilege collapse. *British Journal of Dermatology*, *175*(3), 531-541.

Betz, R. C., Petukhova, L., Ripke, S., Huang, H., Menelaou, A., Redler, S., ... & Christiano, A. M. (2015). Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. *Nature communications*, *6*(1), 5966.

Caro, S. R. (2022). Alopecia Areata: Exploring the Literature. *Journal of the Dermatology Nurses' Association*, *14*(5), 214-219.

Carroll, J. M., McElwee, K. J., King Jr, L. E., Byrne, M. C., & Sundberg, J. P. (2012). Gene Array Profiling and Immunomodulation Studies De® ne a Cell-Mediated Immune Response Underlying the Pathogenesis of Alopecia Areata in a Mouse Model and Humans.

Carson, M. J., Doose, J. M., Melchior, B., Schmid, C. D., & Ploix, C. C. (2006). CNS immune privilege: hiding in plain sight. *Immunological reviews*, *213*(1), 48-65.

Chase, H. B., Rauch, H., & Smith, V. W. (1951). Critical stages of hair development and pigmentation in the mouse. *Physiological Zoology*, *24*(1), 1-8.

Christoph, T., Müller-Röver, S., Audring, H., Tobin, D. J., Hermes, B., Cotsarelis, G., ... & Paus, R. (2000). The human hair follicle immune system: cellular composition and immune privilege. *British Journal of Dermatology*, *142*(5), 862-873.

Colombe, B. W., Lou, C. D., & Price, V. H. (1999, December). The genetic basis of alopecia areata: HLA associations with patchy alopecia areata versus alopecia totalis and alopecia universalis. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 4, No. 3, pp. 216-219).

Eckert, J., Church, R. E., & Ebling, F. J. (1968). The pathogenesis of alopecia areata. *British Journal of Dermatology*, *80*(4), 203-210.

El Hayderi, L., Nikkels-Tassoudji, N., & Nikkels, A. F. (2013). Hair loss after varicella zoster virus infection. *Case reports in dermatology*, *5*(1), 43-47.

Erb, U., Freyschmidt-Paul, P., & Zöller, M. (2013). Tolerance induction by hair-specific keratins in murine alopecia areata. *Journal of leukocyte biology*, *94*(4), 845-857.

Gilhar, A., Etzioni, A., & Paus, R. (2012). Alopecia areata. *New England Journal of Medicine*, *366*(16), 1515-1525.

Finner, A. M. (2011). Alopecia areata: clinical presentation, diagnosis, and unusual cases. *Dermatologic therapy*, *24*(3), 348-354.

Fischer, J., Degenhardt, F., Hofmann, A., Redler, S., Basmanav, F. B., Heilmann-Heimbach, S., ... & Betz, R. C. (2017). Genomewide analysis of copy number variants in alopecia areata in a C entral E uropean cohort reveals association with MCHR 2. *Experimental Dermatology*, *26*(6), 536-541.

Geier, D. A., & Geier, M. R. (2015). A case–control study of quadrivalent human papillomavirus vaccine-associated autoimmune adverse events. *Clinical Rheumatology*, *34*(7), 1225-1231.

Gilhar, A., Etzioni, A., & Paus, R. (2012). Alopecia areata. *New England Journal of Medicine*, *366*(16), 1515-1525.

Gilhar, A., Assy, B., Shalaginov, R., Serafimovich, S., Landau, M., & Kalish, R. S. (2001). Melanocyte-associated T cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on Prkdcscid mice. *Journal of investigative dermatology*, *117*(6), 1357-1362.

Hordinsky, M. K. (2013, December). Overview of alopecia areata. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 16, No. 1, pp. S13-S15).

Höglund, P., & Brodin, P. (2010). Current perspectives of natural killer cell education by MHC class I molecules. *Nature reviews immunology*, *10*(10), 724-734.

Ito, T., Ito, N., Bettermann, A., Tokura, Y., Takigawa, M., & Paus, R. (2004). Collapse and restoration of MHC class-I-dependent immune privilege: exploiting the human hair follicle as a model. *The American journal of pathology*, *164*(2), 623-634.

Ito, T., Ito, N., Saatoff, M., Hashizume, H., Fukamizu, H., Nickoloff, B. J., ... & Paus, R. (2008). Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *Journal of Investigative Dermatology*, *128*(5), 1196-1206.

Ito, T., Meyer, K. C., Ito, N., & Paus, R. (2008). Immune privilege and the skin. *Dermatologic Immunity*, *10*, 27-52.

Kalkan, G., Seçkin, H. Y., Benli, İ., Akbaş, A., Baş, Y., Karakus, N., ... & Özyurt, H. (2015). Relationship between manganese superoxide dismutase (MnSODAla-9Val) and glutathione peroxidase (GPx1 Pro 197 Leu) gene polymorphisms and alopecia areata. *International journal of clinical and experimental medicine*, *8*(11), 21533.

Kavak, A., Baykal, C., Özarmağan, G., & Akar, U. (2000). HLA in alopecia areata. *International journal of dermatology*, *39*(8), 589-592.

Kyriakis, K. P., Paltatzidou, K., Kosma, E., Sofouri, E., Tadros, A., & Rachioti, E. (2009). Alopecia areata prevalence by gender and age. *Journal of the European Academy of Dermatology and Venereology*, *23*(5), 572-573.

Lee, S., Kim, B. J., Lee, Y. B., & Lee, W. S. (2018). Hair regrowth outcomes of contact immunotherapy for patients with alopecia areata: a systematic review and meta-analysis. *JAMA dermatology*, *154*(10), 1145-1151.

McPhee, C. G., Duncan, F. J., Silva, K. A., King Jr, L. E., HogenEsch, H., Roopenian, D. C., ... & Sundberg, J. P. (2012). Increased expression of Cxcr3 and its ligands, Cxcl9, and Cxcl10, during the development of alopecia areata in the mouse. *The Journal of investigative dermatology*, *132*(6), 1736.

Meyer, K. C., Klatte, J. E., Dinh, H. V., Harries, M. J., Reithmayer, K., Meyer, W., ... & Paus, R. (2008). Evidence that the bulge region is a site of relative immune privilege in human hair follicles. *British Journal of Dermatology*, *159*(5), 1077-1085.

Messenger, A. G., & Bleehen, S. S. (1985). Expression of HLA-DR by anagen hair follicles in alopecia areata. *Journal of investigative dermatology*, *85*(6), 569-572.

Messenger, A. G., Slater, D. N., & Bleehen, S. S. (1986). Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology. *British Journal of Dermatology*, *114*(3), 337-347.

Messenger, A. G., & Bleehen, S. S. (1984). Alopecia areata: light and electron microscopic pathology of the regrowing white hair. *British Journal of Dermatology*, *110*(2), 155-162.

Naik, P. P., & Farrukh, S. N. (2021). Association between alopecia areata and thyroid dysfunction. *Postgraduate Medicine*, *133*(8), 895-898.

Oh, J. W., Kloepper, J., Langan, E. A., Kim, Y., Yeo, J., Kim, M. J., ... & Plikus, M. V. (2016). A guide to studying human hair follicle cycling in vivo. *Journal of Investigative Dermatology*, *136*(1), 34-44.

Paus, R., Nickoloff, B. J., & Ito, T. (2005). A 'hairy'privilege. *Trends in immunology*, *26*(1), 32-40.

Paus, R., Slominski, A., & Czarnetzki, B. (1993). Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb?. *The Yale journal of biology and medicine*, *66*(6), 541.

Paus, R., Bulfone-Paus, S., & Bertolini, M. (2018, January). Hair follicle immune privilege revisited: the key to alopecia areata management. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 19, No. 1, pp. S12-S17).

Paus, R., Slominski, A., & Czarnetzki, B. (1993). Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb?. *The Yale journal of biology and medicine*, *66*(6), 541.

Paus, R., & Bertolini, M. (2013, December). The role of hair follicle immune privilege collapse in alopecia areata: status and perspectives. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 16, No. 1, pp. S25-S27). Elsevier.

Pratt, C. H., King, L. E., Messenger, A. G., Christiano, A. M., & Sundberg, J. P. (2017). Alopecia areata. *Nature reviews Disease primers*, *3*(1), 1-17.

Petukhova, L., Duvic, M., Hordinsky, M., Norris, D., Price, V., Shimomura, Y., ... & Christiano, A. M. (2010). Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature*, *466*(7302), 113-117.

Petukhova, L., & Christiano, A. M. (2013, December). The genetic architecture of alopecia areata. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 16, No. 1, pp. S16-S22).

Rajabi, F., Drake, L. A., Senna, M. M., & Rezaei, N. (2018). Alopecia areata: a review of disease pathogenesis. *British Journal of Dermatology*, *179*(5), 1033-1048.

Richardson, C. T., Hayden, M. S., Gilmore, E. S., & Poligone, B. (2018). Evaluation of the relationship between alopecia areata and viral antigen exposure. *American journal of clinical dermatology*, *19*, 119-126.

Rice, R. H., Rocke, D. M., Tsai, H. S., Silva, K. A., Lee, Y. J., & Sundberg, J. P. (2009). Distinguishing mouse strains by proteomic analysis of pelage hair. *Journal of investigative dermatology*, *129*(9), 2120-2125.

Rice, R. H., Bradshaw, K. M., Durbin-Johnson, B. P., Rocke, D. M., Eigenheer, R. A., Phinney, B. S., & Sundberg, J. P. (2012). Differentiating inbred mouse strains from each other and those with single gene mutations using hair proteomics. *PLoS One*, *7*(12), e51956.

Satyaprakash, K. (2014). Alopecia Areata: An Update. *Recent Advances in Dermatology-Volume 3*, *3*, 72.

Sehgal, V. N., & Jain, S. (2003). Alopecia areata: clinical perspective and an insight into pathogenesis. *The Journal of Dermatology*, *30*(4), 271-289.

Sundberg, J. P., Boggess, D., Silva, K. A., McElwee, K. J., King, L. E., Li, R., ... & Cox, G. A. (2003). Major locus on mouse chromosome 17 and minor locus on chromosome 9 are linked with alopecia areata in C3H/HeJ mice. *Journal of investigative dermatology*, *120*(5), 771-775.

Sundberg, J. P., Silva, K. A., Li, R., Cox, G. A., & King, L. E. (2004). Adult-onset alopecia areata is a complex polygenic trait in

the C3H/HeJ mouse model. *Journal of investigative dermatology*, *123*(2), 294-297.

Sundberg, J. P., Awgulewitsch, A., Pruett, N. D., Potter, C. S., Silva, K. A., Stearns, T. M., ... & Rice, R. H. (2014). Crisp1 and alopecia areata in C3H/HeJ mice. *Experimental and molecular pathology*, *97*(3), 525-528.

Trautman, S., Thompson, M., Roberts, J., & Thompson, C. T. (2009). Melanocytes: a possible autoimmune target in alopecia areata. *Journal of the American Academy of Dermatology*, *61*(3), 529-530.

Trautman, S., Thompson, M., Roberts, J., & Thompson, C. T. (2009). Melanocytes: a possible autoimmune target in alopecia areata. *Journal of the American Academy of Dermatology*, *61*(3), 529-530.

Tharumanathan, S. (2015). Understanding the biological mechanism of alopecia areata. *Am J Dermatol Venereol*, *4*(1), 1-4.

Thies, W. (1966). Comparative histologic studies in alopecia areata and scar-atrophy alopecia. *Archiv fur Klinische und Experimentelle Dermatologie*, *227*(1), 541-549.

Wang, E. H. C. (2014). *The pathogenesis of alopecia areata* (Doctoral dissertation, University of British Columbia).

Wang, E. H., Yu, M., Breitkopf, T., Akhoundsadegh, N., Wang, X., Shi, F. T., ... & McElwee, K. J. (2016). Identification of autoantigen epitopes in alopecia areata. *Journal of Investigative Dermatology*, *136*(8), 1617-1626.

CHAPTER VI

The Integration of RNA Sequencing and Proteomics in Early Detection and Personalized Treatment in Cancer Research

Duygu KIRKIK[1]
Sevgi KALKANLI TAS[2]

Introduction

The completion of the Human Genome Project marked the beginning of a new era in genetic research. During this period, RNA sequencing studies, especially on yeast, mice, and Arabidopsis, presented remarkable findings in the scientific community (Lister et al., 2008; Nagalakshmi et al., 2008; Mortazavi et al., 2008). The results of the project led scientists to investigate the non-coding regions of the genome and to develop RNA sequencing techniques, due to the realization that the number of genes in the human genome

[1] Dr. Öğr. Üyesi, Sağlık Bilimleri Üniversitesi Tıp Fakültesi
[2] Prof. Dr. Sağlık Bilimleri Üniversitesi Tıp Fakültesi

is fewer than anticipated and a large portion of DNA does not encode proteins. Today, studies like the ENCODE project, conducted across the entire genome, emphasize the significance of RNA in cellular functions and organism development (The ENCODE Project Consortium, 2012; Akkaya & Dinçer, 2013).

Once, the analysis of gene expression was possible only through limited methodologies such as real-time PCR and microarrays, but this situation has undergone a radical change in the last decade (Wolf, 2013). Next-generation sequencing (NGS) technologies have provided extensive information about intracellular biomolecules, enabling the low-cost, rapid, and efficient analysis of structures such as DNA, RNA, miRNA, and Piwi RNA. The rapid development of these technologies and bioinformatics tools has expanded the boundaries of genetic studies and brought RNA sequencing to the forefront of genetic research (Wolf, 2013).

Globally, the risk of developing cancer and cancer-related mortality rates are on the rise. According to global cancer statistics in 2023, approximately 1,958,310 new cancer cases and about 609,820 cancer-related deaths were estimated (Siegel et al., 2023). These figures indicate that cancer is one of the most serious health problems today, emphasizing that one in four people who die each day loses their life to cancer. The importance of early detection and diagnosis of cancer is increasingly recognized for reducing cancer-related mortality rates and prolonging patients' survival times. To develop specific treatment strategies for cancer cells and diagnose early-stage cancer, it is crucial to precisely identify changes in the protein mechanisms resulting from the metastasis of the relevant cancer and detect protein biomarkers. Understanding the pathophysiology of cancer at the protein level is of great significance for early diagnosis and detection, monitoring disease prognosis, administering effective treatment, and improving post-treatment survival rates. In this context, diagnosing cancer using specific markers in biological samples has become a common practice today. The detection of changes in the concentrations of these markers is used for screening high-risk individuals prone to cancer

development, early diagnosis, prognosis prediction, and monitoring treatment response (Levitsky & Dembitsky, 2015; Tagne et al., 2014).

This review aims to explore the pivotal role of RNA sequencing in advancing our understanding of cancer biology, with a focus on the identification and characterization of protein biomarkers for early cancer detection, diagnosis, and treatment monitoring.

Omics Technologies

The term proteome refers to the entire set of proteins produced by the genetic code of an organism (Wasinger et al., 1995). While an organism's genetic makeup remains constant, the proteome is dynamic and subject to various changes. The proteome arises as a result of gene expression and includes the diversity of different protein variants encoded by the same gene and post-translational modifications (PTMs). Fields, in 2001, described the field of proteomics as "not limited to the identification and quantification of proteins but also encompassing the determination of their locations, modifications, interactions with other proteins and macromolecules, activities, and ultimately their functions" (Fields, 2001). Proteomics involves both qualitative and quantitative analyses of the protein and protein diversity resulting from biological processes in cells or tissues. Proteomic technology includes principles based on the separation, identification, and analysis of proteins from samples. Proteomic technologies are interrelated and interconnected, divided into three main categories: comparative proteomics, structural proteomics, and functional proteomics. Comparative proteomics is the qualitative and quantitative analysis of protein expression under different conditions, examining proteins that change under disease states, drug effects, or various stimuli. For example, comparative proteomics can be used to compare protein expressions in samples from healthy and diseased individuals. Proteins specific to a disease or absent in normal cells can be identified and analyzed. In personalized medicine, comparative proteomics is a vital tool for determining the effectiveness of specific drug treatments. Structural

proteomics focuses on the three-dimensional structures of proteins, investigating their functions and interactions with other proteins. This field facilitates understanding of proteins' primary, secondary, tertiary, and quaternary structures and provides information about critical interactions, such as enzyme-substrate interactions. With advancing technology, computer-aided drug design is also evolving, and structural proteomics plays a significant role in the discovery and design of new drugs (Jung & Lee, 2004; Renfrey & Featherstone, 2002). Functional proteomics examines the functions of a protein, either alone or in conjunction with other proteins. Protein-protein interactions and the locations of modified proteins are determined through protein network maps. The effects of environmental factors, drugs, and endogenous chemicals on protein modifications are studied with functional proteomics (Monti et al., 2009; Bíliková et al., 2009). The qualitative and quantitative analysis of protein mixtures is a popular research topic in many advanced laboratories today. Proteomic research includes various strategies such as 'top-down', 'bottom-up', and the recently developed 'middle-down' approaches. These strategies are fundamentally based on analyzing proteins in samples either with or without breaking them down into amino acids.

Non-Coding RNAs in the Human Genome: Roles and Implications in Cancer

The majority of the human genome (over 75%) is actively transcribed, but only a small portion of these transcripts eventually become proteins (Djebali et al., 2012). The RNA molecules that are copied from the genome but do not code for proteins are known as non-coding RNA (ncRNA). These ncRNAs are divided into two main categories based on their size: Small non-coding RNAs (sncRNA), which are shorter than 200 nucleotides and typically include microRNAs (miRNA) and small nucleolar RNAs (snRNA); and long non-coding RNAs (lncRNA), which are longer than 200 nucleotides and comprise various RNA molecules. Recent studies have revealed that the human genome encodes approximately 15,000 different lncRNAs (Deniz & Erman, 2016). Like mRNAs, lncRNAs

are transcribed by RNA polymerase II and are usually subjected to the same processes as mRNAs (cap addition, splicing, polyadenylation). The first discovered mammalian lncRNA was H19, followed by the discovery of Xist lncRNA, which plays a role in X chromosome inactivation (Katayama et al., 2005; Cabili et al., 2011).

The classification of these non-coding RNAs has evolved over time, with some sources referring to them as "lincRNA" (long intergenic non-coding RNA), while others simply use the term "lncRNA" (long non-coding RNA). The term "intergenic" indicates that these RNAs are derived from genomic regions that do not code for proteins. These regions, once termed "junk DNA," overlap with areas now understood to code for RNA transcripts (Groux et al., 1989; Brown & Ballabio, 1991; Briggs & Pera, 2014; Laurent et al., 2015).

LncRNAs can be divided into five groups based on their genetic structures and locations: Intergenic lncRNAs (lincRNA), Intronic lncRNAs, Overlapping lncRNAs, Antisense lncRNAs, and Processed lncRNAs. Evolutionarily, lncRNA exons are more conserved than repetitive sequences but less so than exons of protein-coding genes. The different regions of lncRNA genes, especially the promoter and exon regions, are considered the most conserved parts of the gene (Derrien et al., 2012).

The significant connection between cancer and lncRNAs emerged from studies investigating the role of lncRNAs in development and cellular differentiation (Cogill & Wang, 2014). Numerous lncRNAs involved in cancer-related biological processes have been identified in the human genome. Consequently, it is believed that mutations, dysregulation, or abnormal expression of lncRNAs can contribute to cancer development. Genome-wide studies have revealed that many single nucleotide polymorphisms (SNPs) in the human genome are located in intergenic or intronic regions, potentially impacting lncRNA function (Hindorff et al., 2009). Similar to protein-coding genes, lncRNAs can function as

oncogenes or tumor suppressor genes, influencing tumorigenesis. To investigate the relationship between a specific lncRNA and a certain type of cancer, it is necessary to compare the expression levels of lncRNA in normal and cancerous cell lines, as well as in adjacent normal tissues and cancer tissues, using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (Zhang et al., 2016). A well-studied lncRNA associated with cancer is HOX antisense intergenic RNA (HOTAIR). In breast cancer cells, deregulation of HOTAIR results in the re-targeting of PRC2 gene expression, leading to a new pattern of gene expression similar to embryonic fibroblasts (Gupta et al., 2010). This redirection can exacerbate breast cancer progression, invasion, and metastasis

Advancements in Tissue Microarray Technology for Proteomic Analysis in Cancer Research

Tissue microarrays, also known as tissue microarray technology, are a proteomic method that enables the rapid identification of thousands of proteins. This technique involves using microarrays created by transferring 'donor' tissue cores to a 'recipient' block and is especially used in cancer research to identify cancer-specific proteins as prognostic or predictive biological markers. However, there are some limitations of this technology in accurately selecting and validating candidate protein biomarkers from various tumor samples (Wu, Hu, & Kavanagh, 2002). With today's advanced technology, these microarrays can be used not only to identify cancer-specific biomarkers, but also to create cancer-specific tissue array blocks for various situations, such as detecting individual cancer cases, preneoplastic and metastatic lesions, concurrent or time-varying cancer cases, and familial factors (Kim, 2001).

Conclusion

Despite researchers having made significant strides in understanding the molecular foundations of cancer, there are still gaps in both comprehending the mechanisms of the disease and in developing effective methods for early diagnosis and treatment.

Understanding the pathophysiology of the disease in its advanced stages is a critical element for the success of patient treatment. Today, diagnosing through markers via molecular analyses in biological samples is a common method. Changes in the concentrations of these markers are important for identifying individuals at risk, early diagnosis, prognosis assessment, and monitoring the response to treatment. The primary purpose of these markers used in the clinic is to reduce death and disease risks. Biomarkers are usually protein molecules detected or measured with monoclonal antibodies, but many molecules used or evaluated as potential markers are not ideal on their own. Theoretically, every disease can be identified and characterized by a unique biomarker. These markers could be either a single molecule or a panel of proteins that undergo changes in post-translational modifications in the disease state. The potential of proteins as diagnostic tools and advances in proteomic technologies have increased interest in biomarker research. Proteomics is used in cancer research, particularly in studies related to tumor development and progression. A significant portion of these studies focuses on the disruption of apoptotic mechanisms thought to play a key role in tumor growth and development.

REFERENCES

Lister, R., O'Malley, R.C., Tonti-Filippini, J., Gregory, B.D., Berry, C.C., Millar, A.H., Ecker, J.R. (2008). Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell, 133(3), 523-536.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., Snyder, M. (2008). The transcriptional landscape of the yeast genome defined by RNA sequencing. Science, 320, 1344-1349.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods, 7, 621-628.

The ENCODE Project Consortium. (2012). An integrated encyclopedia of DNA elements in the human genome. Nature, 489, 57-74.

Akkaya, Z.Y., Dinçer, P. (2013). Derleme: Tedavi yaklaşımlarında yeni bir dönem: Kodlamayan RNA'lar ve hastalıklar. Marmara Medical Journal, 26, 5-10.

Wolf, J.B. (2013). Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. Mol Ecol Resour, 13(4), 559-72.

Siegel, R.L., Miller, K.D., Wagle, N.S., Jemal, A. (2023). Cancer statistics, 2023. CA Cancer J Clin, 73(1), 17-48. Doi: 10.3322/caac.21763.

Levitsky, D.O., Dembitsky, V.M. (2015). Anti-breast Cancer Agents Derived from Plants. Natural Products & Bioprospecting, 5, 1–16.

Tagne, R.S., Telefo, B.P., Nyemb, J.N., Yemele, D.M., Njina, S.N., Goka, S.M.C., Farooq, A.D. (2014). Anticancer and antioxidant activities of methanol extracts and fractions of some

Cameroonian medicinal plants. Asian Pacific Journal of Tropical Medicine, 7(1), 442-447.

Wasinger, V.C., Cordwell, S.J., Cerpa-Poljak, A., Yan, J.X., Gooley, A.A., Wilkins, M.R., Duncan, M.W., Harris, R., Williams, K.L., Humphery-Smith, I. (1995). Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium. Electrophoresis, 16(1), 1090-1094.

Fields, S. (2001). Proteomics in genomeland. Science, 291(5507), 1221-1224.

Jung, J-W., Lee, W. (2004). Structure-based functional discovery of proteins: structural proteomics. Journal of biochemistry and molecular biology, 37(1), 28-34.

Renfrey, S., Featherstone, J. (2002). Structural proteomics. Nature Publishing Group.

Monti, M., Cozzolino, M., Cozzolino, F., Vitiello, G., Tedesco, R., Flagiello, A., Pucci, P. (2009). Puzzle of protein complexes in vivo: a present and future challenge for functional proteomics. Expert review of proteomics, 6(2), 159-169.

Bíliková, K., Mirgorodskaya, E., Bukovská, G., Gobom, J., Lehrach, H., Šimúth, J. (2009). Towards functional proteomics of minority component of honeybee royal jelly: The effect of posttranslational modifications on the antimicrobial activity of apalbumin2. Proteomics, 9(8), 2131-2138.

Djebali, S., et al. (2012). Landscape of transcription in human cells. Nature, 489(7414), 101-108.

Deniz, E., Erman, B. (2016). Long noncoding RNA (lincRNA), a new paradigm in gene expression control. Functional & integrative genomics, 1-9.

Katayama, S., et al. (2005). Antisense transcription in the mammalian transcriptome. Science, 309(5740), 1564-1566.

Cabili, M.N., et al. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & development, 25(18), 1915-1927.

Groux, H., et al. (1989). A 19-kDa human erythrocyte molecule H19 is involved in rosettes, present on nucleated cells, and required for T cell activation. Comparison of the roles of H19 and LFA-3 molecules in T cell activation. The Journal of Immunology, 142(9), 3013-3020.

Brown, C.J., Ballabio, A. (1991). A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature, 349(6304), 38.

Briggs, S.F., Pera, R.A.R. (2014). X chromosome inactivation: recent advances and a look forward. Current opinion in genetics & development, 28, 78-82.

Laurent, G.S., Wahlestedt, C., Kapranov, P. (2015). The Landscape of long noncoding RNA classification. Trends in Genetics, 31(5), 239-251.

Derrien, T., et al. (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome research, 22(9), 1775-1789.

Cogill, S.B., Wang, L. (2014). Co-expression network analysis of human lncRNAs and cancer genes. Cancer informatics, (Suppl. 5), 49.

Hindorff, L.A., et al. (2009). Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences, 106(23), 9362-9367.

Zhang, R., et al. (2016). LncRNAs and cancer (Review). Oncology Letters, 12(2), 1233-1239.

Gupta, R.A., et al. (2010). Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature, 464(7291), 1071-1076.

Wu, W., Hu, W., Kavanagh, J.J. (2002). Proteomics in cancer research. International Journal of Gynecological Cancer, 12(5), 409-423.

Kim, W.H. (2001). Tissue array technology for translational research. Experimental & molecular medicine, 33(1 Suppl), 135-148.

CHAPTER VII

Effects of Amifostine Against Gamma-radiation on HeLa Cells and Apoptotic Genes Expression

Gül ÖZCAN[1]
Özlem DAĞDEVİREN[2]
Uğur SERBES[3]
İdil ÇETİN[4]

Introduction

Cancer is a group of disorders which are characterized by uncontrolled cell proliferation and continue to live of cells that normally to die (Baloglu, 2001; Wang, Wang & Soong, 2000; Cardoso et al., 2017).

[1]Prof., Istanbul University, Faculty of Science, Biology Department
[2] Dr., Istanbul University, Institute of Graduate Studies in Science and Engineering, Department of Radiobiology
[3] M.Sc., Istanbul University, Institute of Graduate Studies in Science and Engineering, Department of Radiobiology
[4] Assoc. Prof., Istanbul University, Faculty of Science, Biology Department

The primary objective of cancer chemotherapy is to eradicate cancerous cells to the greatest extent possible while minimizing harm to healthy cells and preventing the formation of metastases. The use of chemotherapeutic agents conjunction with radiotherapy revive progress in the treatment of cancer (Montoro et. al., 2023; Ullah et. al., 2022; Gurka et al., 2017; Vici et al., 2014). Currently laboratory and clinical trials showed that Amifostine (AMI) is capable of protecting normal tissues against the toxic effects of radiotherapy and chemotherapy (Grochova & Smardova, 2007; Arican Ozcan, 2005). In recent years research on the life and death of the cell attract attention on the molecular mechanisms of apoptosis (Arican Ozcan et al., 2012).

Apoptosis or programmed cell death play an important role in many developmental and physiological processes and in keeping the cell number under control (Maino, 2017; Ioannidis & Psillos, 2017; Elmore, 2007; Hu & Kavanagh, 2003).

All cytotoxic drugs and radiotherapy programs initiate apoptosis in tumor cells and the resistance to apoptosis brings treatment failure (Tatar et. al., 2023; Arican Ozcan et al., 2012; Elmore, 2007; Hu & Kavanagh, 2003). Moreover, these treatments initiate apoptosis in normal cells and also show negative side effects on the bone marrow. However, new therapeutic aproaches like AMI treatment are promising about protecting normal cells from side effects but not the cancer cells (Arican Ozcan, 2005).

This study aimed to explore the antiproliferative and apoptotic effects following treatment with various radiation doses in HeLa cells. Additionally, it aims to ascertain the extent to which the cytoprotective effect of AMI diminishes and to identify any differences in comparison with the apoptotic effect. This study seeks to serve as an introductory exploration, fostering a more profound understanding and facilitating the development of innovative treatment methods grounded in the molecular mechanisms of cytoprotective agents.

Materials and Methods

Cell line

In our experiments, we used HeLa (CCL-2) cells, which are derived from cervical carcinoma. These cells were cultured in Minimum Essential Medium (MEM, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Gibco Lab.), 100 IU/ml penicillin (Pronapen, Pfizer), and 100 µg/ml streptomycin (streptomycin sulfate, İ.E. Ulagay). This standard cell culture medium was used to maintain the cells. The cells were passaged twice a week to ensure their growth and viability.

Passage process of cells

The passage process in our experiments started by growing the cells in culture dishes until they reached a sufficient density. Cells that did not adhere to the surface were collected in a centrifuge tube, while the cells that relatively adhered to the surface were washed with Hank's balanced salt solution (HBSS) and pipetted. The detached cells were then transferred to the same centrifuge tube and suspended. After centrifugation at 1500 rpm for 3 minutes, the supernatant was discarded, and the pellet was diluted with a culture medium specific to the cell type being used.

Determination of drug and radiation doses

The dose of AMI and radiation used in our experiments were determined based on dose levels in clinic. AMI injectable sterile stock solution of 1 mg/1 ml was kept at +4°C. 1 µg/ml dose of this stock solution reconstituted with culture medium that is specific to cells was applied to cells for 1 hour both alone and combination with 1 Gy, 2 Gy, 4 Gy and 8 Gy radiation doses. Experimental groups were established in this way.

Gamma irradiation unit

Cs-137 source in blood irradiation unit in Faculty of Medicine at İstanbul University, Foundation for Children with Leukemia was used irradiation of cells for all the experiments.

Mitochondrial Dehydrogenase Enzyme Activity

In our experiments MTT (3-(4,5-dimetiltiyazol-2-il)-2,5-difenil tetrazolyum bromid) method which determine cell viability was used as cytotoxicity test. MTT solution which dissolved in PBS were added as 40 µl/well. Cells were incubated in a 37˚C incubator for 4 hours. Formazan crystals were dissolved in 160 µl DMSO for 1 hour at 37˚C. After this period absorbance values of the experimental groups were measured at 570 nm against 690 nm reference wavelength using a Elisa reader (µQuant, Bio-Tek Instruments Inc). Cytotoxicity assessment was performed in this way (Denizot & Lang 1986; Mosmann, 1983).

Apoptotic index

At the end of the test application, a 1 ml cell suspension from the experimental group was collected in Eppendorf tubes and centrifuged at 4 °C, 1500 rpm for 5 minutes. The supernatant was discarded, and 200 µl of methanol: PBS (1:1) solution was added. The mixture was centrifuged again for 5 minutes at 1500 rpm, and the supernatant was discarded. The pellet was resuspended in 200 µl of pure methanol and kept at +4 °C. Slides were cleaned using an ethanol: HCl (1:1) mixture. After soaking the slides in this mixture for 24 hours, they were rinsed under flowing water for another 24 hours. The slides were then washed with distilled water at +4 °C and prepared for use. For sample preparation, the cells stored in Eppendorf tubes at +4 °C were centrifuged again at 1500 rpm for 5 minutes. The cells were suspended in methanol, dropped onto clean slides, and allowed to dry. The prepared slides were then stained with DAPI and placed in a light-tight container at 37 °C for 20 minutes on a shaker. After rinsing in PBS for 20 minutes, a coverslip was

placed on the slides, and they were examined using a fluorescence microscope. The apoptotic index values were determined by scoring the normal and apoptotic nuclei in the prepared slides according to the experimental groups.

Total RNA Isolation

In order to identify the effect at the molecular level, at first isolation of total RNA from cells in the control and experimental groups was performed using RNA isolation kit (Total RNA Kit, invitrogen).

RT-PCR of Bcl-2 gene family

In this study, the highest values of the apoptotic index expression levels of Bcl-2 genes involved in the molecular mechanism of apoptosis were investigated in both the control and experimental groups. To accomplish this, 7 genes (mcl-1, bfl-1, bax-α, bcl-2, bak, bik, and bcl-x) belonging to the Bcl-2 gene family were reproduced using the Bcl-2 kit (ApoPrimer Set Bcl-2 family, TAKARA) and RT-PCR (RT-PCR Kit, Promega) with isolated total RNAs. This allowed for the determination of gene expressions that play a role in apoptosis at the molecular level.

Statistical Analysis

ANOVA and Dunnett's test was applied to all experimental groups. Statistically $p<0.05$ significance level was based on evaluation of the results.

Results

According to the cytotoxicity results presented in Table 1, there were statistically significant decreases in the proliferation rates of the cells when treated with AMI alone and in combination with 1 Gy, 2 Gy, 4 Gy, and 8 Gy radiation doses compared to the control group ($p<0.05$). These findings indicate that the applied radiation

doses led to a significant reduction in cell proliferation, both in the presence of AMI and when used alone.

Table 1: *Measured Absorbance values of HeLa cells treated with AMI alone and combination with various radiation doses (±SD) (AMI:Amifostine, 1Gy:1Gray, 1Gy+AMI:1Gray+Amifostine, 8Gy:8Gray, 8Gy+AMI:8Gray+Amifostine). p<0,05:1 (significance according to control), 2 (significance according to the group applied AMI alone).*

Groups	Absorbance Values (570 – 690 nm)	±SD
Control	$813{,}5 \times 10^{-3}$	0,017
AMI	$854{,}5 \times 10^{-3}$	0,013
1 Gy	$618{,}1 \times 10^{-3}$ (1)(2)	0,009
1 Gy+AMI	$605{,}7 \times 10^{-3}$ (1)(2)	0,006
2 Gy	$533{,}2 \times 10^{-3}$ (1)(2)	0,008
2 Gy+AMI	$655{,}0 \times 10^{-3}$ (1)(2)	0,008
4 Gy	$462{,}4 \times 10^{-3}$ (1)(2)	0,009
4 Gy+AMI	$583{,}6 \times 10^{-3}$ (1)(2)	0,011
8 Gy	$442{,}3 \times 10^{-3}$ (1)(2)	0,009
8 Gy+AMI	$539{,}2 \times 10^{-3}$ (1)(2)	0,007

Figure 1 in the study displays the viability values obtained from the experimental series where four different radiation doses were applied to HeLa cells, either alone or in combination with AMI. The figure shows a significant decrease in cell proliferation rate in a dose-dependent manner for the experimental groups treated with radiation alone and the combination of AMI and radiation, compared to the control and the experimental group treated with AMI alone ($p<0.05$). This indicates that the application of radiation, either alone or in combination with AMI, resulted in a notable reduction in cell viability.

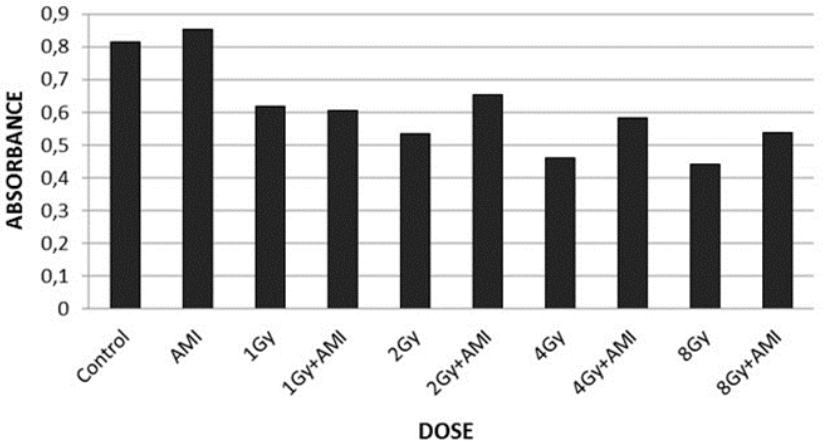


Figure 1. *Measured cytotoxic activity values of HeLa cells treated with AMI alone and combination with various radiation doses (±SD) (AMI:Amifostine, 1Gy:1Gray, 1Gy+AMI:1Gray+Amifostine, 8Gy:8Gray, 8Gy+AMI:8Gray+Amifostine).*

In our experiments, the viability percentages were determined and compared to the control group, which was considered 100%. These results were presented in Figure 2. It can be observed from the figure that the cytotoxicity caused by the radiation doses increased in a dose-dependent manner. The viability percentages for the radiation doses were determined as follows: 76%, 65%, 56%, and 54%. In the groups that were combined with AMI, the viability percentages were 74%, 80%, 72%, and 66%. To assess the extent of cytotoxicity caused by apoptosis, apoptotic index values were determined and presented in Figure 3.

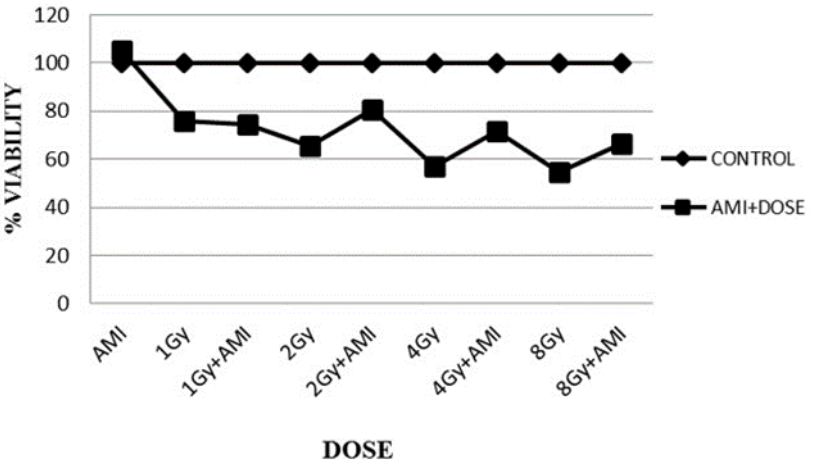


Figure 2. *Determined vitality values % of HeLa cells treated with AMI alone and combination with various radiation doses (±SD) (AMI:Amifostine, 1Gy:1Gray, 1Gy+AMI:1Gray+Amifostine, 8Gy:8Gray, 8Gy+AMI:8Gray+Amifostine).*

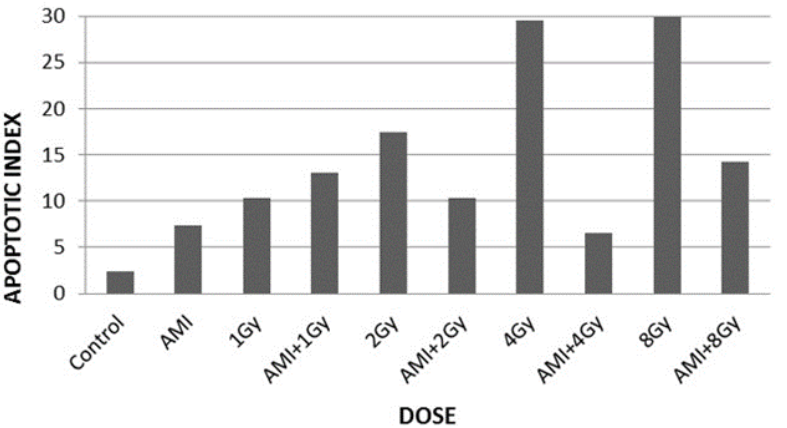


Figure 3. *Determined Apoptotic Index (AI) values of HeLa cells treated with AMI alone and combination with various radiation doses (±SD) (AMI:Amifostine, 1Gy:1Gray, 1Gy+AMI:1Gray+Amifostine, 8Gy:8Gray, 8Gy+AMI:8Gray+Amifostine).*

In our experiments, significant cytotoxic effects were observed in all experimental groups where AMI was administered, either alone or in combination with radiation, compared to the control group ($p < 0.05$). The apoptotic index values, as shown in the figure, were 2.4% in the control group, and 10.3%, 17.5%, 29.5%, and 30% in the groups treated with radiation doses of 1, 2, 4, and 8 Gy, respectively. After administering AMI for 1 hour, the apoptotic index values were 7.4% in the group treated with AMI alone, and 13.1%, 10.3%, 6.6%, and 14.3% in the groups treated with a combination of AMI and radiation. In the experimental groups where the maximum apoptotic index values were observed (1 Gy and 8 Gy radiation doses alone and in combination with AMI), the expression of 7 genes belonging to the Bcl-2 gene family was represented using RT-PCR, as shown in Figure 4.

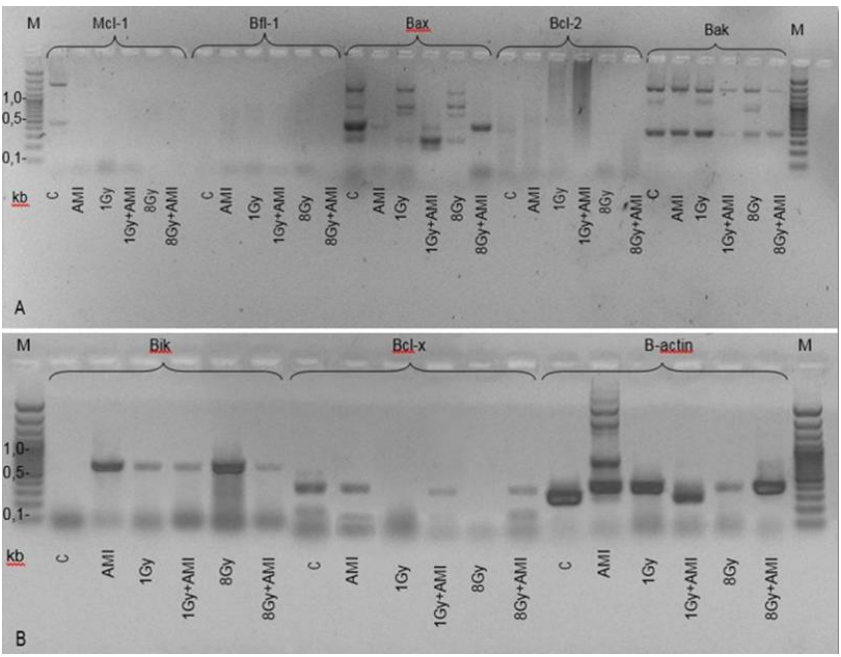


Figure 4. *Image of Agarose gel electrophoresis of A: Mcl-1, Bfl-1, Bax, Bcl-2, Bak genes and B:Bik, Bcl-x, β-aktin genes which are bcl-2 gene family members expression of HeLa cells treated with*

AMI alone and combination with various radiation doses M:1,5 kb Marker, C:Control, AMI:Amifostine, 1Gy:1Gray, 1Gy+AMI:1Gray+Amifostine, 8Gy:8Gray, 8Gy+AMI: 8Gray+Amifostine.

Both of the control and experimental groups, the presence of Mcl-1, Bfl-1, and Bcl-2 genes was identified. In the experimental group subjected to a radiation dose of 8 Gy and combined with AMI, it was observed that the expression of bax, a proapoptotic gene, exceeded that of bak. Furthermore, the experimental group subjected only to a radiation dose of 8 Gy displayed the highest level of expression for bik, another proapoptotic gene. Conversely, aside from the control group and the group solely treated with AMI, no significant expression level of the antiapoptotic Bcl-x gene was detected.

Acknowledgement

The study received financial assistance from the Research Fund at The University of Istanbul under Project number 2671, with reference UDP-37341.

Discussion and Conclusion

Chemotherapy, a potent anti-cancer treatment, has been widely recognized as the most impactful method for combatting malignant neoplasms (Sawada et al., 2003). A crucial aspect of effective therapeutic intervention and discovering new drugs lies in understanding the apoptotic process. By developing innovative strategies for cancer therapy, the goal is to restore the ability of tumor cells to undergo apoptosis, which they have lost (Elmore, 2007).

The molecular process of programmed cell death is not fully understood, but it is acknowledged that a self-destruct program, embedded in the cellular genetic memory, is triggered and initiated by various signals, pathophysiological conditions, and events like oxidative stress (Dorn, 2013; Elmore, 2007). Additionally, the mechanism of apoptosis differs depending on the specific cell type

and the stimuli it encounters. Intracellular signals that impact apoptosis generally fall into three categories: growth factors, oncogenes, and tumor suppressor genes (Kontomanolis et al., 2020; Fernandes et al., 2018). Furthermore, factors that induce necrosis, such as high temperature, radiation, cytotoxic anticancer drugs, and oxygen deprivation, result in a limited dosage of apoptotic response (Topcul & Cetin, 2013).

Insufficient understanding of the molecular alterations that contribute to the progression and spread of cancer hinders our ability to determine the exact treatment approach. To address this challenge, the agent known as amifostine offers potential by protecting against the adverse effects of anticancer agents and radiation (Ji et al., 2023; Arican Ozcan, 2005). This study aims to assess the cytoprotective properties of amifostine on cellular death due to radiation exposure across different cell lines, employing diverse cell kinetics and molecular biology techniques.

The role of the Bcl-2 gene is crucial in regulating apoptosis, which is the programmed cell death process. This gene produces an integral membrane protein called Bcl-2, which actively prevents apoptosis from occurring (Czabotar et al., 2023; Adams & Cory, 2018; Arican Ozcan et al., 2012). When the bcl-2 gene becomes active and starts to be overexpressed, the increased amount of Bcl-2 protein hinders apoptosis and promotes cell proliferation. Numerous studies have investigated the expression of bcl-2 in both normal and tumor tissues (Czabotar et al., 2023; Opferman & Kothari, 2018; Sawada et al., 2003).

The findings of this study revealed that the levels of expression of 7 genes play crucial roles in regulating apoptosis. It was observed that the expression of the proapoptotic gene, bak, was higher in the group exposed to a radiation dose of 1 Gy. Similarly, the expression of another proapoptotic gene, Bik, was higher in the group exposed to a radiation dose of 8 Gy.

Thus, according to the results determined by our experiments apoptosis is induced by low doses of radiation through bak gene and

by high doses of radiation through Bik gene in HeLa cells exposed to different radiation doses.

Cellular cycle cessation and cellular demise are crucial elements in the treatment of cancer (Wang, Bode & Zhang, 2023; Bodoor et al., 2014; Akman 2013), as well as in the induction of programmed cell death caused by radiation therapy. This study aims to explore the molecular mechanism underlying the cytoprotective effect of AMI. By examining the intersection of the cytoprotective effect and the mechanism of radiation-induced apoptosis, we can potentially uncover groundbreaking advancements in cancer chemotherapy, leading to the development of novel therapeutic approaches.

In our experiments increasing expression of proapoptotic Bax gene in the groups applied AMI combination with both 1 Gy and 8 Gy radiation dose according to the groups applied radiation alone will make important contributions the literature. To elucidate the molecular mechanism of radiation-induced apoptosis fully, analysis of a large number of genes and enzymes belong to internal and external pathways needs to research further.

References

Adams, JM, Cory, S. (2018). BCL-2 family: role in apoptosis and cancer therapy. Cell Death and Differentiation, 25, 27-36.

Akman, G. (2013). Evaluation of cyclin dependent kinase genes expression in HeLa cells under gamma radiation and hypobaric stress conditions, Thesis of Master Science. Institute of Science and Istanbul University, Istanbul.

Andrade, LM, Ribeiro De Campos TP, Leite MF, Goes AM. (2005). *In vitro* response of the human breast cancer cell line MDAMB-231 and human peripheral blood mononuclear cells exposed to ^{60}Co at single fraction. Brazilian archives of biology and technology, 48, 205-213.

Arican Ozcan, G. (2005). Cytoprotective effects of amifostine and cysteamine on cultured normal and tumor cells treated with paclitaxel in terms of mitotic index and ^{3}H-thymidine labeling index. Cancer Chemother Pharmacol, 56, 221-229.

Arican Ozcan, G, Cakir, O, Arican, E et al. (2012). Effects of geven root extract on proliferation of HeLa cells and bcl-2 gene expressions. African Journal of Biotechnology, 11, 4296-4304.

Baloglu, E. (2001). Synthesis and biological evaluation of paclitaxel analogs. Thesis of philosophy in chemistry, Virgina Polytechnic Institute and State University, Virgina.

Bodoor, K, Haddad, Y, Alkhateeb, A et al. (2014). DNA hypermethylation of cell cycle (p15 and p16) and apoptotic (p14, p53, dapk and tms1) genes in peripheral blood of leukemia patients. APJCP, 15(1), 75-84.

Cardoso, MFS, Castelletti, CHM, Lima-Filho, JL, Martins, DBG, Teixeira, JAC. (2017). Putative biomarkers for cervical cancer: SNVs, methylation and expression profiles. Mutation Research, 773, 161-173.

Czabotar, PE, Garcia-Saez, AJ. (2023). Mechanism of BCL-2 Family Proteins in Mitochondrial Apoptosis. Nature Reviews Molecular Cell Biology, 24, 732-748.

Denizot, F, Lang, R. (1986). Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods, 89, 271-277.

Dorn, GW. (2013). Molecular mechanisms that differentiate apoptosis from programmed necrosis. Toxicologic Pathology, 41, 227-234.

Elmore, S. (2007). Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol, 35, 495-516.

Fernandes, E, Silva, E, Figueira, FDS, Lettnin, AP, Carrett-Dias, M, Filgueira, DMVB, Kalil, S, Trindade, GS, Votto, APS. (2018). C-Phycocyanin: Cellular targets, mechanisms of action and multi drug resistance in cancer. Pharmacological Reports, 70, 75-80.

Grochova, D, Smardova, J. (2007). The antimutagenic and cytoprotective effects of amifostine: the role of p53. J Applied Biomedicine, 5, 171-178.

Gurka, MK, Kim, C, He, AR, Charabaty, A, Haddad, N, Turocy, J, Johnson, L, Jackson, P, Weiner, LM, Marshall, JL, Collins, SP, Pishvaian, MJ, Unger, K. (2017). Stereotactic body radiation therapy (SBRT) combined with chemotherapy for unresected pancreatic adenocarcinoma. Am J Clin Oncol, 40, 152-157,

Hu, W, Kavanagh, J. (2003), Anticancer therapy targeting the apoptotic pathway. Lancet Oncol, 4, 721-729.

Ioannidis, S, Psillos, S. (2017). In defense of methodological mechanism: The case of apoptosis. Axiomathes, 27 (6), 601-619.

Ji, L, Cui, P, Zhou, S, Qiu, L, Huang, H, Wang, C, Wang, J. (2023), Advances of Amifostine in Radiation Protection:

Administration and Delivery. Mol. Pharmaceutics, 20(11), 5383-5395.

Kontomanolis, EN, Koutras, A, Syllaios, A, Schizas, D, Mastoraki A, Garmpis, N, Diakosavvas, M, Angelou, K, Tsatsaris, G, Pagkalos, A, Ntounis, T, Fasoulakis, Z. (2020), Role of Oncogenes and Tumor-suppressor Genes in Carcinogenesis: A Review. Anticancer Research, 40, 6009-6015.

Maino, B, Paparone, S, Severini, C, Ciotti, MT, D'agata, V, Calissano, P, Cavallaro, S. (2017), Drug target identification at the crossroad of neuronal apoptosis and survival. Expert Opin Drug Discov, 12, 249-259.

Montoro, A, Obrador, E, Mistry, D, Forte, GI, Bravatà, V, Minafra, L, Calvaruso, M, Cammarata, FP, Falk, M, Schettino, G, Ahire, V, Daems, N, Boterberg, T, Dainiak, N, Chaudhary, P, Baatout, S, and Mishra KP. (2023). Radioprotectors, Radiomitigators, and Radiosensitizers. Sarah Baatout (Ed.), Radiobiology Textbook (pp. 571-628) Switzerland: Springer Nature.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65, 55-63.

Opferman, JT, Kothari, A. (2018). Anti-apoptotic BCL-2 family members in development. Cell Death and Differentiation, 25, 37-45.

Sawada, S, Mese, H, Sasaki, A, Yoshioka, N, Matsumura, T. (2003). Combination chemotherapy of paclitaxel and cisplatin induces apoptosis with bcl-2 phosphorylation in ac cisplatin-resistant human epidermoid carcinoma cell line. Cancer Chemother Pharmacol, 51, 505-511.

Tatar, C, Binay Avci, C., Acikgoz, E, Oktem, G. (2023). Doxorubicin –induced Senescence Promotes Resistance to Cell Death by Modulating Genes Associated with Apoptotic and Necrotic Pathways in Prostate Cancer DU145 $CD133^{+}/CD44^{+}$ Cells.

Biochemical and Biophysical Research Communications, 680, 194-210.

Topcul, M, Cetin, I. (2013). An innovative therapeutic approch in oncology: hyperthermia. European International Journal of Science and Technolog, 2, 73-80.

Ullah, A, Ullah, N, Nawaz, T, Aziz, T. (2022). Molecular Mechanisms of Sanguinarine in Cancer Prevention and Treatment. Anti-Cancer Agents in Medicinal Chemistry, 22, 1-7.

Vici, P, Mariani, L, Pizzuti, L et al. (2014). Emerging biological treatments for uterine cervical carcinoma. J Cancer, 5, 86-97.

Wang, TH, Wang, HS, Soong, YK. (2000). Paclitaxel-induced cell death. Cancer, 88, 2619-2628.

Wang, Q, Bode, AM, Zhang, T. (2023). Targeting CDK1 in Cancer: Mechanisms and İmplications. npj Precision Oncology, 7:58.

From Molecular Symphony to Therapeutic Harmonies: Exploring Nutrigenomics, Enzymatic Frontiers, Flavonoid Therapeutics, and Autoimmune Skin Challenges

In the vast realm of scientific inquiry, our quest for understanding extends beyond the boundaries of individual disciplines, weaving a tapestry that connects nutrition, genomics, and medical research. The titles before you encapsulate a journey into the depths of scientific exploration, each representing a distinct facet of our quest to unravel the mysteries of life, health, and the intricate interplay between our choices and our biology.

The first exploration takes us into the fascinating world of nutrigenomics, a frontier where the choices we make in our daily diets intricately intersect with the aging process. Through the lens of honey bees, a remarkable model organism, we peer into the genetic mechanisms that dictate longevity and well-being. The humble honey bee, a creature of ecological importance, serves as an unexpected yet illuminating guide through the labyrinth of molecular intricacies that underpin the relationship between diet and aging.

Next, we pivot to a groundbreaking chapter in enzymology, where two catalysts, TvTS and (MpMS), have emerged as veritable keystones, unlocking doors to an era of unprecedented scientific discovery. These enzymes, with their cryptic acronyms, beckon us into the world of molecular processes that have the potential to reshape our understanding of genetic pathways and their implications for human health and longevity.

As we traverse the scientific landscape, our attention turns to the therapeutic potential embedded in the natural compounds known as flavonoids. These compounds, found abundantly in our diets, reveal a compelling story of hope in the context of Alzheimer's Disease. Through meticulous investigation, we explore the therapeutic effects of flavonoids, envisioning a future where these natural agents may become vital tools in the arsenal against neurodegenerative afflictions.

Our journey then takes a turn toward the enigmatic realms of autoimmune skin disorders. First, we delve into the complexities of Psoriasis, a condition that challenges our understanding of immune responses and inflammatory processes. Following this, we unravel the mysteries of Alopecia Areata, an autoimmune skin disease that disrupts the delicate balance of hair growth. In these chapters, we confront the intricate mechanisms that govern self-directed immune responses, aiming to illuminate pathways toward targeted therapeutic interventions for those grappling with these challenging conditions.

This compilation invites you to embark on a voyage of discovery, where the threads of nutrigenomics, enzymology, therapeutics, and autoimmune research are woven together into a narrative that transcends individual disciplines. As you traverse the chapters that follow, may you find inspiration in the ceaseless pursuit of knowledge and the collective endeavor to unlock the secrets of life's intricacies.

BİDGE