BIDGE Publications

Stem Cells and Cancer

Editor: Dr. Öğr. Üyesi Günsel Kirman

ISBN: 978-625-372-534-1

Page Layout: Gözde YÜCEL

1st Edition:

Publication Date: 25.12.2024

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

Content

Changes in the Microenvironment of Kras Mutant Cancers.....4
Nevruz ALIS SOYLEYİCİ 4
Sabire GULER 4
Taste Buds.....31
Seçil Nazife PARLAK 31
Use of in vivo and in vitro Models in Neorological Diseases and their Comparison 54
Mehmet ÖZSAN 54
Hasan Hüseyin DÖNMEZ.....54

CHAPTER I

Changes in the Microenvironment of Kras Mutant Cancers

Nevruz ALIS SOYLEYİCİ[1]
Sabire GULER[2]

Introduction

Current evidence indicates that cancer cells require a heterogeneous tumor microenvironment consisting of cellular and non-cellular components for carcinogenesis and metastasis (Downs-Canner et al., 2022). The interaction of cancer cells with these microenvironmental components provides them with features such as sustaining proliferative signals, apoptosis resistance, induction of angiogenesis, and immune escape. However, inflammation and hypoxic conditions in the tumor microenvironment significantly impact immunotherapy (Hanahan & Weinberg, 2011; Topcu, n.d.).

[1] PhD Student, Bursa Uludag University, Institute of Health Sciences, Veterinary Histology & Embriology, Bursa/Turkey, Orcid: 0000-0002-7692-5064
[2] Assoc. Prof. Dr., Bursa Uludag University, Department of Histology & Embryology, School of Veterinary Medicine, Uludag University, Bursa/Turkey

Oncogenic KRAS mutations have a high prevalence in various cancers, particularly in pancreatic ductal adenocarcinoma, colorectal carcinoma, and non-small cell lung cancers (Albitar et al., 2017; Fendrich et al., 2011; Salgia et al., 2021). It has been demonstrated that KRAS plays a role as both an anti-inflammatory and pro-inflammatory regulator in the tumor microenvironment, directing common metabolic programs that facilitate tumor survival, growth, and immune escape (Beatty & Gladney, 2015; Dias Carvalho et al., 2018; Stewart & Abrams, 2008).

In KRAS-mutated cancers, immune microenvironment elements such as T-reg, cancer-associated macrophages (CAMs), and cancer-associated fibroblasts (CAFs) are frequently targeted and signal transduction is rearranged. Since this facilitates immune escape, it directly affects tumor growth and development (Dias Carvalho et al., 2019).

The literature search for this review was conducted in Crossref, PubMed, ProQuest, and Google Scholar databases to investigate the effects of KRAS mutations on the microenvironment.

According to WHO data, cancer is a disease whose incidence and mortality rate continue to increase rapidly and is the second cause of death after cardiovascular diseases (Bray et al., 2021; World Health Organization, 2020). According to GLOBOCAN 2020 data, as of 2018, approximately 19.3 million new cancer cases, and 10 million cancer-related deaths have been reported worldwide (Sung et al., 2021). Thirty different mutations have been reported to play a role in human cancers(Buday & Downward, 2008; *Signatures of Mutational Processes in Human Cancer | Nature*, n.d.). Studies in the literature suggest that the KRAS-G12C mutation in lung

adenocarcinoma is linked to smoking-related mutations. Additionally, there are studies that report an association between the lack of DNA repair in gastric and endometrial cancers and the G12D and G13D mutations of KRAS (Westcott et al., 2015).

1. RAS Gene and mutations

RAS genes were initially defined as viral genes transferred from the rodent genome and responsible for the oncogenic properties of RNA tumor viruses. Ras proteins were considered as products of oncogenes capable of inducing cellular transformation until recently(Buday & Downward, 2008; Malumbres & Barbacid, 2003).

RAS proteins, belonging to the GTPase protein family, play a key role in activating signaling pathways that control cell proliferation, survival, and differentiation (Simanshu et al., 2017). It has a key role between inactive and active structures bound to GDP, and transmitting extracellular signals to the cell nucleus through growth factor receptors, primarily Epidermal Growth Factor Receptor (EGFR), as well as Mitogen-Activated Protein Kinase (MAPK), Phosphoinositide 3-Kinase (PI3K) and Stem Cell Factor (SCF) (Jiang et al., 2010). While Ras mutations are common in cancer, the frequency and patterns of mutations associated with each Ras gene can vary depending on the specific cancer type. Recent studies have confirmed that mutant RAS proteins are associated with many human cancer types, primarily pancreatic, colorectal, lung, and urogenital cancers. Mutations in the RAS gene are driving forces in tumor formation and development (Cox & Der, 2010; Timar & Kashofer, 2020). The incidence of RAS gene mutations varies from 10% to 30% in different cancer types. Studies have shown that pancreatic cancers have the highest incidence of these mutations,

accounting for approximately 90% of cases in the literature (Buscail et al., 2020; Scharpf et al., 2020). For other cancers have been reported ~50% in colorectal adenocarcinomas and thyroid tumors, and ~30% in lung adenocarcinomas and myeloid leukemias (Prior et al., 2020; Salgia et al., 2021).

RAS gene encodes three different RAS proteins in humans: Kirsten Rat Sarcoma (KRAS), Harvey Rat Sarcoma (HRAS) and Neuroblastoma Rat Sarcoma (NRAS). Among these, KRAS has two isoforms derived from RNA splicing, known as KRAS4A and KRAS4B. While the KRAS4B isoform is more dominant, it has been shown that the KRAS4A isoform is also significantly expressed in many tissues (Hobbs et al., 2016; Tsai et al., 2015).

KRAS mutation is the most common oncogenic alteration (%21,6) in the RAS family in human cancers (Salgia et al., 2021). The highest incidence of KRAS mutations are pancreatic, colorectal, and lung cancers respectively (Hamarsheh et al., 2020; Salgia et al., 2021). KRAS, a proto-oncogene belonging to the RAS family, encodes a low-molecular-weight small GTPase that plays key roles in the control of cellular growth and differentiation, transitioning with the regulation MAPK and PI3K pathways. Mutant KRAS remains persistently active and leads to aggressive cell growth and uncontrolled proliferation even in the absence of signal reception (Baines et al., 2011; Cazzanelli et al., 2018; Downward, 2003; Hamarsheh et al., 2020).

Figure-1 depicts the proinflammatory effects mediated by the activation of transcription factors, cytokine production, NOD-like Receptor Pyrin Domain-containing 3 (NLRP3) inflammatory activation, and the release of chemokines induced by oncogenic

KRAS activation and the immune cells in the tumor microenvironment affected by these signals. Additionally, KRAS can promote the remodeling of the stroma by exerting various effects on endothelial cells, fibroblasts, and the extracellular matrix, thereby inducing metastasis (Dias Carvalho et al., 2018).

Point mutations in the KRAS gene frequently target hotspots in codons 12 and 13. These mutations are commonly associated with various cancers and can significantly impact cell signaling and tumorigenesis. The most commonly observed changes among KRAS mutations are G12D and G12V, followed by G12R (Lu et al., 2016). These mutations in the oncogene either hinder the ability of KRAS to hydrolyze GTP or induce the conversion of GDP to GTP. This activation affects signaling pathways that regulate numerous fundamental cellular processes, including proliferation, growth, and survival, thereby promoting cancer progression (Haigis, 2017; Hobbs et al., 2016). The presence of a KRAS mutation is indeed often associated with poor prognosis and resistance to certain cancer treatments, particularly in various types of cancer. These mutations can affect treatment response and the overall outcome for individuals with cancer (Hames et al., 2016). Mutant KRAS causes resistance to anti-EGFR therapies, thus depriving patients of effective treatment options (Dias Carvalho et al., 2019; Duldulao et al., 2013; Hames et al., 2016).

2. The tumor microenvironment

It is accepted that cancer is characterized by distinct features such as resistance to cell death, genetic instability, and mutation presence, unlimited proliferation, evasion of growth suppressors, enhanced inflammation, altered metabolism, and angiogenesis, as

well as the ability to promote invasion, and metastasis (Hanahan & Weinberg, 2011; Mohla & Witz, 2010). However, scientific studies have revealed that tumor tissue is not solely composed of tumor cells but also includes stromal cells and non-cellular microenvironmental elements (Senthebane et al., 2017, 2018).

The key processes involved in tumorigenesis include tumor cell proliferation, evasion of growth suppression, resistance to cell death, induction of angiogenesis, initiation of invasion, dysregulation of cellular energy, evasion of immune destruction, and the presence of chronic inflammation. (Lu et al., 2016).

Hanahan and Weinberg have defined the acquired and essential fundamental biological capabilities that cancer cells have gained for their development, growth, and dissemination throughout the entire process, including distant metastasis, considering the fundamental steps of carcinogenesis. They have described these essential basic biological capabilities as follows (Hanahan & Weinberg, 2011).

- Sustained signaling for proliferation
- Resistance to cell death
- Induced angiogenesis
- Initiation of invasion
- Infiltration
- Reaching the lymphatic circulation (intravasation) and exiting the circulation to invade tissues (extravasation)

- Proliferation and progression in the metastatic site.

Tumor formation and metastasis require a heterogeneous microenvironment consisting of stromal cells derived from normal tissue. The tumor microenvironment is a heterogeneous and complex organization comprising tumor, stromal, and endothelial cells; crosstalk between the tumor and natural/adaptive immune cells (Downs-Canner et al., 2022). The environment includes both cellular and non-cellular components. The cellular components primarily consist of tumor cells, mesenchymal stem cells, stromal cells, fibroblasts, pericytes, type 2 macrophages (M2), lymphocytes (T and B), adipocytes, pericytes, and follicular dendritic cells (Figure 2). The non-cellular components of the microenvironment include cytokines, growth factors, DNA and RNA, and the extracellular matrix. In addition, the inflammatory and hypoxic conditions present in the microenvironment can reduce the effectiveness of the immune response and contribute to drug resistance in immunotherapy (Topcu, n.d.). Inflammatory cells present in the microenvironment include T lymphocytes, NK cells, and tumor-associated M2 macrophages. Granulocytes, mast cells, and macrophages are located around the tumor periphery, natural killer (NK) cells are found in the stroma, and T lymphocytes are situated at the border of the microenvironment and in lymph nodes (Senovilla et al., 2012). KRAS mutations have been closely associated with the modulation of tumor inflammation in various studies. This relationship has a key role in tumorigenesis, as affects immune response and the effectiveness of treatments (Tsai et al., 2015). Fu et al. (2021) linked the majority of cases of colorectal cancer with chronic inflammatory diseases specifically, which have a high prevalence of KRAS mutations. They also found a strong negative correlation between

KRAS mutations and infiltrating lymphocytes, inflammation, cytolytic activities, and Human Leucocyte Antigen (HLA) gene expression within the tumor (Fu et al., 2020).

Recent studies have demonstrated that KRAS mutations can lead to the secretion of anti-inflammatory cytokines, including Transforming Growth Factor-Beta (TGF-β), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), and IL-10 (Hamarsheh et al., 2020). These cytokines contribute to maintaining an immunosuppressive tumor microenvironment, thereby supporting tumor progression. Additionally, KRAS has been reported to interfere with the secretion of pro-inflammatory cytokines, such as Intercellular Adhesion Molecule-1 (ICAM-1), Tumor Necrosis Factor-Alpha (TNF-α), and IL-18, which possess anti-tumor properties (Hamarsheh et al., 2020; Lyssiotis & Kimmelman, 2017; Pereira et al., 2022).

The importance of the tumor microenvironment in cancer biology continues to grow because of its impact on modulating cancer cell activities that determine the success of tumor progression (Pereira et al., 2022).

During tumor progression, tumor cells employ several survival strategies to proliferate under adverse microenvironmental conditions and evade the impact of key regulators/effectors of immune response to bypass anti-tumor defenses. The involvement of the tumor microenvironment affects lymphocyte infiltration and the effectiveness of the anti-tumor immune response (Beatty & Gladney, 2015; Chen et al., 2019; Stewart & Abrams, 2008).

Figure 3 illustrates a scenario in which KRAS activation in KRAS-mutant cancer cells exerts a significant influence on various

components of the tumor microenvironment, thereby supporting cancer progression. KRAS-mutant cancer cells release molecules that play a role in attracting neutrophils and M1 macrophages. Additionally, they contribute to the accumulation of MDSCs, inhibit CD8+ cytotoxic T lymphocyte activation, and promote Treg differentiation, ultimately fostering a less reactive and more tolerogenic environment. Furthermore, Th17 recruitment, fibroblast activation, angiogenesis and endothelial cell recruitment, as well as ECM remodeling, are among the other microenvironmental changes orchestrated by mutant KRAS cells.

While KRAS mutant cancer cells may individually affect tumor microenvironment components, it's crucial to recognize that these components are interconnected and mutually regulate each other's characteristics and functions. Therefore, KRAS mutant cancer cells likely exert a collective impact on the entire microenvironment by influencing one component in a correlated manner .

3. The effects of KRAS mutant cancer cells on the extracellular matrix

The extracellular matrix, the most abundant component of the tumor microenvironment, is composed of complex macromolecular networks that form three-dimensional supramolecular structures with diverse biomechanical and biochemical properties. It regulates cell growth, migration, survival and differentiation by binding to specific receptors (*The Extracellular Matrix: Not Just Pretty Fibrils | Science*, n.d.). While primarily defined as the scaffold that organizes tissues, it also plays a crucial role in regulating tissue development and homeostasis. Any

disruption in its homogeneity can create a favorable environment for neoplasia (Mammoto & Ingber, 2010; Pickup et al., 2014).

In the early stages of cancer, the extracellular matrix collaborates with stromal cells, playing an anti-tumorigenic role. However, more recently, it tends to shift towards a pro-tumorigenic role, actively participating in tumorigenesis and contributing to the acquisition of specific cancer characteristics (Hanahan & Weinberg, 2011; Senthebane et al., 2018).

Fibroblast activation protein (FAP), a cell surface serine protease, is selectively expressed on cancer-associated fibroblasts and pericytes in epithelial tumors (*Regulation of Fibroblast Activation Protein-α Expression: Focus on Intracellular Protein Interactions | Journal of Medicinal Chemistry*, n.d.). Santos et al. demonstrated that genetic deletion and pharmacological inhibition of FAP in an immunocompetent syngeneic mouse model with K-rasG12D-driven lung cancer inhibited tumor growth. Consequently, they found that FAP depletion indirectly inhibited tumor cell proliferation, increased collagen accumulation, reduced myofibroblast content, and decreased vascular density in tumors (Santos et al., 2009).

Tape et al. conducted a study employing a cell-specific proteome tagging technique alongside multivariate phosphoproteomics to explore mutant KRAS signaling in pancreatic adenocarcinoma cells. Their investigation unveiled that the Hedgehog (Hh) signaling pathway, emanating from cancer cells, induces changes in the fibroblast proteome, prompting the production of extracellular matrix components such as collagen and MMPs. Moreover, cancer-derived Hh fosters the expression of

growth factors such as IGF1 and GAS6 by fibroblasts, leading to a non-autonomous cell signaling to cancer cells (Tape et al., 2016).

Considering all these findings, it can be concluded that both systemic and local KRAS-mediated effects on the extracellular matrix significantly influence tumor cell motility, invasive behavior, and metastatic capacity.

4. Cancer-Associated Fibroblasts (CAFs)

CAFs play a very important role in acquiring and sustaining various cancer-related characteristics. These include immune regulation and therapy response, as well as involvement in epithelial-mesenchymal transition, tumor growth, angiogenesis, cell migration, invasion, metastasis, and remodeling of the extracellular matrix (Quail & Joyce, 2013; Ziani et al., 2018). Studies conducted on pancreatic cancer models have shown the role of KRAS in mediating fibroblast activation through the Hh signaling pathway, and it has been reported that the Hh pathway reduces stroma in pancreatic ductal carcinoma (Fendrich et al., 2011). In another study by Ji et al., they showed that the activation of the Hh pathway by oncogenic KRAS promotes tumorigenesis in a pancreatic cancer model (Ji et al., 2007).

5. Immunity and KRAS

One of the immune evasion mechanisms associated with KRAS-mutant cancer cells, which is the decreased expression of major histocompatibility complex class I (MHCI) leading to impaired antigen presentation capacity (Atkins et al., 2004). Additionally, the up-regulation of Programmed Death Ligand 1 (PD-L1), inhibits T-cell recognition, contributing to immune evasion.

These cells also exhibit increased expression and secretion of various inflammatory cytokines.

An inflammatory cytokine, Chemokine CXC Ligand-3 (CXCL3), interacts with the Chemokine CXC receptor-2 (CXCR2) on myeloid-derived suppressor cells (MDSCs), which leads to the preservation and buildup of these immunosuppressive cells (Purohit et al., 2016). The accumulation of MDSCs in the tumor microenvironment is linked to granulocyte-macrophage colony-stimulating factor (GM-CSF).

Moreover, the increased expression of TGFβ1 and IL-10 contributes to immunosuppression by promoting the conversion of CD25−CD4+T cells into CTLA4+/FOXP3+/CD122+Tregs (Figure 4).

Recent research in lung cancer has shown a correlation between PD-L1 expression, KRAS mutations, a history of smoking, and wild-type EGFR (Huynh et al., 2016). The up-regulation of PD-L1 can be linked to the activation of the KRAS-mediated ERK signaling pathway. However, it's worth emphasizing that the association between KRAS and PD-L1 expression levels could be influenced by other gene mutations commonly associated with lung cancer, such as STK11/ LKB1 and P53/ (Dong et al., 2017; Koyama et al., 2016; Skoulidis et al., 2015).

In a 2018 study conducted by Falk et al., which included 219 patients with lung adenocarcinoma, the relationship between specific mutant KRAS proteins and immunity was examined. The study revealed that there was a relatively lower incidence of PD-L1 expression (24%) in KRAS-mutant lung adenocarcinoma compared to the reported PD-L1 expression rate in the general population of

lung adenocarcinoma (50%). However, they did note a higher incidence of PD-L1 expression in patients exposed to tobacco compared to those who had not previously used tobacco (Falk et al., 2018).

In their research on mesenchymal KRAS-p53 mutant lung cancer, Konen et al. found that neurotrophic receptor tyrosine kinase-1 (NTRK1) expression is increased in tumors treated with PD-1 inhibitors. Their study revealed that NTRK1 regulates the JAK/STAT signaling pathway, leading to the upregulation of PD-L1 expression on tumor cells (Konen et al., 2019). Consequently, this leads to the exhaustion of CD8+ T cells in the tumor microenvironment.

Although data is limited, the relationship between PD-L1 and PD-1 expression with KRAS activation has been explored in two other cancer models, namely pancreatic and colorectal cancers, where KRAS mutations are highly prevalent. Studies have indicated that in pancreatic cancer, KRAS activation is associated with increased PD-1 expression (*PD-1/PD-L1 Expression and Regorafenib Clinical Efficacy on Refractory Pancreatic Cancer Patient.* | *Journal of Clinical Oncology*, n.d.). In contrast to lung and pancreatic cancers, the current data suggests that KRAS mutations in colorectal cancers are predictive of low PD-L1 expression and weak immune infiltration (Albitar et al., 2017).

The positive correlations observed between KRAS mutations and the expression of the PD-1/PD-L1 immunosuppressive axis in lung and pancreatic cancers suggest a high likelihood of disrupting the progression of KRAS-mutant tumors with these therapeutic strategies. These findings provide an instructive model for targeting

this specific tumor group by disrupting their interactions with microenvironment components.

However, in colorectal cancer, the presence of KRAS mutations may indicate the limited effectiveness of anti-PD-1/PD-L1 therapy. These results emphasize the potential of blocking the PD-1/PD-L1 axis as a promising treatment strategy to restore an active immune response in a subset of patients with lung adenocarcinoma. This group faces limited treatment options and may harbor KRAS mutations or lack widespread molecular alterations.

6. Conclusion

In conclusion, the studies mentioned above demonstrate the impact of KRAS mutations on constructing a favorable immune microenvironment that supports evasion from immune surveillance and promotes disease progression. From a clinical perspective, these observations are of critical importance as they pave the way for a better understanding of how KRAS activation influences the response to immunotherapeutic approaches. These findings offer valuable insights into how to target specific tumor groups by disrupting their interactions with microenvironment components.

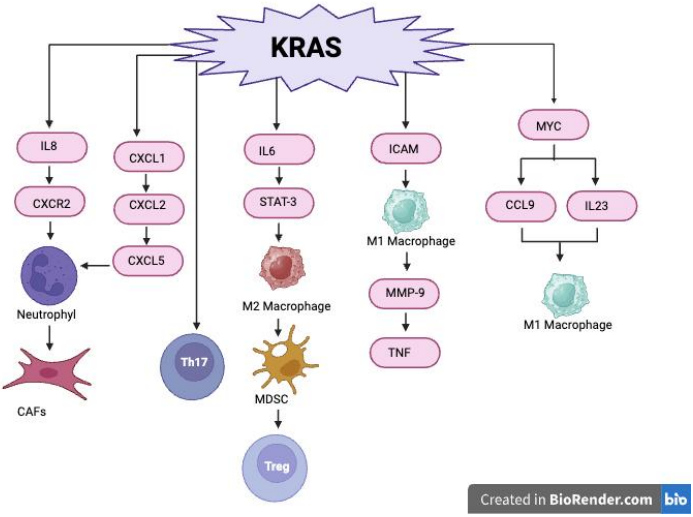

Figure 1: Proinflammatory effects of KRAS-induced inflammation in cancer.

**Adapted from Dias-Carvalho et al., 2018*

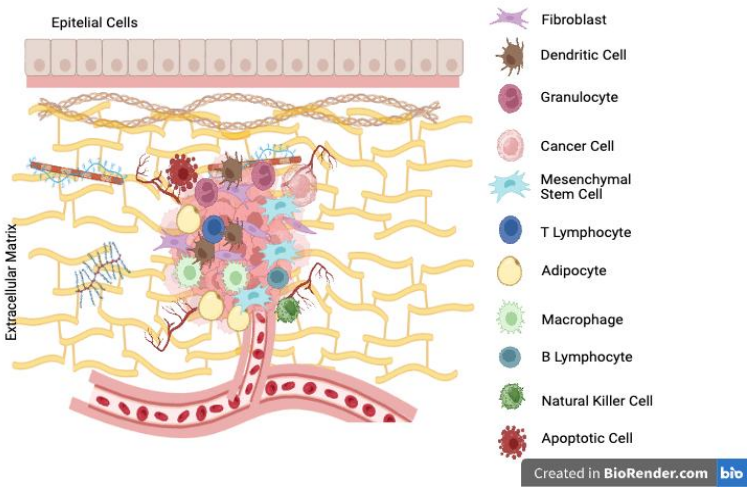

Figure 2: Components of the tumor microenvironment.

**Adapted from Dzobo et al., 2023*

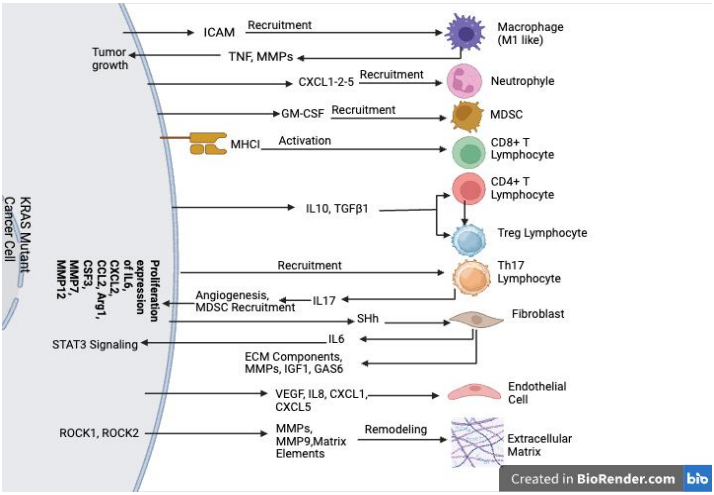

Figure 3: Paracrine effects of KRAS-mutant cancer cells.

**Adapted from Dias-Carvalho et al., 2018*

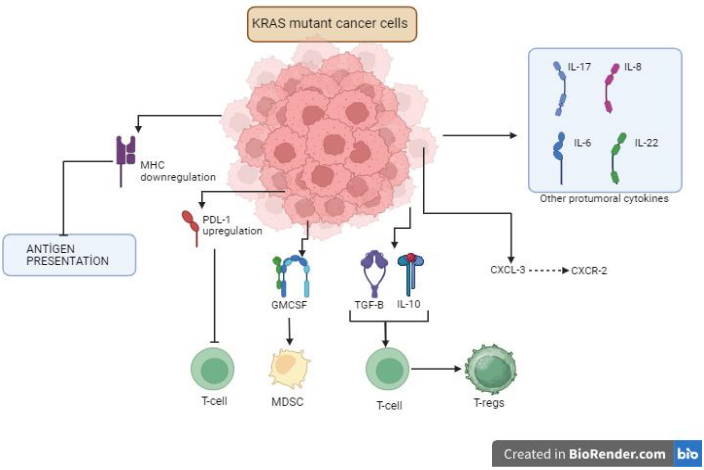

Figure 4: KRAS-driven immunosuppressive tumor microenvironment.

**Adapted from https://www.science.org/doi/abs/10.1126/science.1176009*

REFERENCES

Albitar, M., Sudarsanam, S., Ma, W., Jiang, S., Chen, W., Funari, V. A., & Agersborg, S. (2017). Expression of PD-L1 in colorectal cancer that lack mutations in RAS or TP53 genes. Journal of Clinical Oncology, 35(15_suppl), e14500–e14500. https://doi.org/10.1200/JCO.2017.35.15_suppl.e14500

Atkins, D., Breuckmann, A., Schmahl, G. E., Binner, P., Ferrone, S., Krummenauer, F., Störkel, S., & Seliger, B. (2004). MHC class I antigen processing pathway defects, ras mutations and disease stage in colorectal carcinoma. International Journal of Cancer, 109(2), 265–273. https://doi.org/10.1002/ijc.11681

Baines, A. T., Xu, D., & Der, C. J. (2011). Inhibition of Ras for cancer treatment: The search continues. Future Medicinal Chemistry, 3(14), 1787–1808. https://doi.org/10.4155/fmc.11.121

Beatty, G. L., & Gladney, W. L. (2015). Immune Escape Mechanisms as a Guide for Cancer Immunotherapy. Clinical Cancer Research, 21(4), 687–692. https://doi.org/10.1158/1078-0432.CCR-14-1860

Bray, F., Laversanne, M., Weiderpass, E., & Soerjomataram, I. (2021). The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer, 127(16), 3029–3030. https://doi.org/10.1002/cncr.33587

Buday, L., & Downward, J. (2008). Many faces of Ras activation. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1786(2), 178–187. https://doi.org/10.1016/j.bbcan.2008.05.001

Buscail, L., Bournet, B., & Cordelier, P. (2020). Role of oncogenic KRAS in the diagnosis, prognosis and treatment of

pancreatic cancer. Nature Reviews Gastroenterology & Hepatology, 17(3), Article 3. https://doi.org/10.1038/s41575-019-0245-4

Cazzanelli, G., Pereira, F., Alves, S., Francisco, R., Azevedo, L., Dias Carvalho, P., Almeida, A., Côrte-Real, M., Oliveira, M., Lucas, C., Sousa, M., & Preto, A. (2018). The Yeast Saccharomyces cerevisiae as a Model for Understanding RAS Proteins and their Role in Human Tumorigenesis. Cells, 7(2), 14. https://doi.org/10.3390/cells7020014

Chen, Z., Yang, Y., Liu, L. L., & Lundqvist, A. (2019). Strategies to Augment Natural Killer (NK) Cell Activity against Solid Tumors. Cancers, 11(7), 1040. https://doi.org/10.3390/cancers11071040

Cox, A. D., & Der, C. J. (2010). Ras history. Small GTPases, 1(1), 2–27. https://doi.org/10.4161/sgtp.1.1.12178

Dias Carvalho, P., Guimarães, C. F., Cardoso, A. P., Mendonça, S., Costa, Â. M., Oliveira, M. J., & Velho, S. (2018). KRAS Oncogenic Signaling Extends beyond Cancer Cells to Orchestrate the Microenvironment. Cancer Research, 78(1), 7–14. https://doi.org/10.1158/0008-5472.CAN-17-2084

Dias Carvalho, P., Machado, A. L., Martins, F., Seruca, R., & Velho, S. (2019). Targeting the Tumor Microenvironment: An Unexplored Strategy for Mutant KRAS Tumors. Cancers, 11(12), Article 12. https://doi.org/10.3390/cancers11122010

Dong, Z.-Y., Zhong, W.-Z., Zhang, X.-C., Su, J., Xie, Z., Liu, S.-Y., Tu, H.-Y., Chen, H.-J., Sun, Y.-L., Zhou, Q., Yang, J.-J., Yang, X.-N., Lin, J.-X., Yan, H.-H., Zhai, H.-R., Yan, L.-X., Liao, R.-Q., Wu, S.-P., & Wu, Y.-L. (2017). Potential Predictive Value of

TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. Clinical Cancer Research, 23(12), 3012–3024. https://doi.org/10.1158/1078-0432.CCR-16-2554

Downs-Canner, S. M., Meier, J., Vincent, B. G., & Serody, J. S. (2022). B Cell Function in the Tumor Microenvironment. Annual Review of Immunology, 40(1), 169–193. https://doi.org/10.1146/annurev-immunol-101220-015603

Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. Nature Reviews Cancer, 3(1), 11–22. https://doi.org/10.1038/nrc969

Duldulao, M. P., Lee, W., Nelson, R. A., Li, W., Chen, Z., Kim, J., & Garcia-Aguilar, J. (2013). Mutations in Specific Codons of the KRAS Oncogene are Associated with Variable Resistance to Neoadjuvant Chemoradiation Therapy in Patients with Rectal Adenocarcinoma. Annals of Surgical Oncology, 20(7), 2166–2171. https://doi.org/10.1245/s10434-013-2910-0

Falk, A. T., Yazbeck, N., Guibert, N., Chamorey, E., Paquet, A., Ribeyre, L., Bence, C., Zahaf, K., Leroy, S., Marquette, C.-H., Cohen, C., Mograbi, B., Mazières, J., Hofman, V., Brest, P., Hofman, P., & Ilié, M. (2018). Effect of mutant variants of the KRAS gene on PD-L1 expression and on the immune microenvironment and association with clinical outcome in lung adenocarcinoma patients. Lung Cancer, 121, 70–75. https://doi.org/10.1016/j.lungcan.2018.05.009

Fendrich, V., Oh, E., Bang, S., Karikari, C., Ottenhof, N., Bisht, S., Lauth, M., Brossart, P., Katsanis, N., Maitra, A., &

Feldmann, G. (2011). Ectopic Overexpression of Sonic Hedgehog (Shh) Induces Stromal Expansion and Metaplasia in the Adult Murine Pancreas. Neoplasia, 13(10), 923-IN18. https://doi.org/10.1593/neo.11088

Fu, X., Wang, X., Duanmu, J., Li, T., & Jiang, Q. (2020). KRAS mutations are negatively correlated with immunity in colon cancer. Aging (Albany NY), 13(1), 750–768. https://doi.org/10.18632/aging.202182

Haigis, K. M. (2017). KRAS Alleles: The Devil Is in the Detail. Trends in Cancer, 3(10), 686–697. https://doi.org/10.1016/j.trecan.2017.08.006

Hamarsheh, S., Groß, O., Brummer, T., & Zeiser, R. (2020). Immune modulatory effects of oncogenic KRAS in cancer. Nature Communications, 11(1), Article 1. https://doi.org/10.1038/s41467-020-19288-6

Hames, M. L., Chen, H., Iams, W., Aston, J., Lovly, C. M., & Horn, L. (2016). Correlation between KRAS mutation status and response to chemotherapy in patients with advanced non-small cell lung cancer☆. Lung Cancer, 92, 29–34. https://doi.org/10.1016/j.lungcan.2015.11.004

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. Cell, 144(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hobbs, G. A., Der, C. J., & Rossman, K. L. (2016). RAS isoforms and mutations in cancer at a glance. Journal of Cell Science, 129(7), 1287–1292. https://doi.org/10.1242/jcs.182873

Huynh, T. G., Morales-Oyarvide, V., Campo, M. J., Gainor, J. F., Bozkurtlar, E., Uruga, H., Zhao, L., Gomez-Caraballo, M., Hata, A. N., Mark, E. J., Lanuti, M., Engelman, J. A., & Mino-Kenudson, M. (2016). Programmed Cell Death Ligand 1 Expression in Resected Lung Adenocarcinomas: Association with Immune Microenvironment. Journal of Thoracic Oncology, 11(11), 1869–1878. https://doi.org/10.1016/j.jtho.2016.08.134

Ji, Z., Mei, F. C., Xie, J., & Cheng, X. (2007). Oncogenic KRAS Activates Hedgehog Signaling Pathway in Pancreatic Cancer Cells *. Journal of Biological Chemistry, 282(19), 14048–14055. https://doi.org/10.1074/jbc.M611089200

Jiang, Y., Mackley, H., Cheng, H., & Ajani, J. A. (2010). Use of K-Ras as a predictive biomarker for selecting anti-EGF receptor/pathway treatment. Biomarkers in Medicine, 4(4), 535–541. https://doi.org/10.2217/bmm.10.74

Konen, J. M., Rodriguez, B. L., Fradette, J. J., Gibson, L., Davis, D., Minelli, R., Peoples, M. D., Kovacs, J., Carugo, A., Bristow, C., Heffernan, T., & Gibbons, D. L. (2019). Ntrk1 Promotes Resistance to PD-1 Checkpoint Blockade in Mesenchymal Kras/p53 Mutant Lung Cancer. Cancers, 11(4), Article 4. https://doi.org/10.3390/cancers11040462

Koyama, S., Akbay, E. A., Li, Y. Y., Aref, A. R., Skoulidis, F., Herter-Sprie, G. S., Buczkowski, K. A., Liu, Y., Awad, M. M., Denning, W. L., Diao, L., Wang, J., Parra-Cuentas, E. R., Wistuba, I. I., Soucheray, M., Thai, T., Asahina, H., Kitajima, S., Altabef, A., … Wong, K.-K. (2016). STK11/LKB1 Deficiency Promotes Neutrophil Recruitment and Proinflammatory Cytokine Production to Suppress T-cell Activity in the Lung Tumor Microenvironment.

Cancer Research, 76(5), 999–1008. https://doi.org/10.1158/0008-5472.CAN-15-1439

Lu, S., Jang, H., Nussinov, R., & Zhang, J. (2016). The Structural Basis of Oncogenic Mutations G12, G13 and Q61 in Small GTPase K-Ras4B. Scientific Reports, 6(1), Article 1. https://doi.org/10.1038/srep21949

Lyssiotis, C. A., & Kimmelman, A. C. (2017). Metabolic Interactions in the Tumor Microenvironment. Trends in Cell Biology, 27(11), 863–875. https://doi.org/10.1016/j.tcb.2017.06.003

Malumbres, M., & Barbacid, M. (2003). RAS oncogenes: The first 30 years. Nature Reviews Cancer, 3(6), 459–465. https://doi.org/10.1038/nrc1097

Mammoto, T., & Ingber, D. E. (2010). Mechanical control of tissue and organ development. Development, 137(9), 1407–1420. https://doi.org/10.1242/dev.024166

Mohla, S., & Witz, I. P. (2010). The 5th International Conference on Tumor Microenvironment: Progression, Therapy and Prevention Versailles, France, October 20–24, 2009. Cancer Microenvironment, 3(1), 1–5. https://doi.org/10.1007/s12307-010-0039-2

PD-1/PD-L1 expression and regorafenib clinical efficacy on refractory pancreatic cancer patient. | Journal of Clinical Oncology. (n.d.). Retrieved May 11, 2023, from https://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.e15684

Pereira, F., Ferreira, A., Reis, C. A., Sousa, M. J., Oliveira, M. J., & Preto, A. (2022). KRAS as a Modulator of the Inflammatory

Tumor Microenvironment: Therapeutic Implications. Cells, 11(3), Article 3. https://doi.org/10.3390/cells11030398

Pickup, M. W., Mouw, J. K., & Weaver, V. M. (2014). The extracellular matrix modulates the hallmarks of cancer. EMBO Reports, 15(12), 1243–1253. https://doi.org/10.15252/embr.201439246

Prior, I. A., Hood, F. E., & Hartley, J. L. (2020). The frequency of Ras mutations in cancer. Cancer Research, 80(14), 2969–2974. https://doi.org/10.1158/0008-5472.CAN-19-3682

Purohit, A., Varney, M., Rachagani, S., Ouellette, M. M., Batra, S. K., & Singh, R. K. (2016). CXCR2 signaling regulates KRAS(G12D)-induced autocrine growth of pancreatic cancer. Oncotarget, 7(6), 7280–7296. https://doi.org/10.18632/oncotarget.6906

Quail, D. F., & Joyce, J. A. (2013). Microenvironmental regulation of tumor progression and metastasis. Nature Medicine, 19(11), 1423–1437. https://doi.org/10.1038/nm.3394

Regulation of Fibroblast Activation Protein-α Expression: Focus on Intracellular Protein Interactions | Journal of Medicinal Chemistry. (n.d.). Retrieved October 26, 2023, from https://pubs.acs.org/doi/abs/10.1021/acs.jmedchem.1c01010

Salgia, R., Pharaon, R., Mambetsariev, I., Nam, A., & Sattler, M. (2021). The improbable targeted therapy: KRAS as an emerging target in non-small cell lung cancer (NSCLC). Cell Reports Medicine, 2(1), 100186. https://doi.org/10.1016/j.xcrm.2020.100186

Santos, A. M., Jung, J., Aziz, N., Kissil, J. L., & Puré, E. (2009). Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. The Journal of Clinical Investigation, 119(12), 3613–3625. https://doi.org/10.1172/JCI38988

Scharpf, R., Riely, G., Awad, M., Lenoue-Newton, M., Ricciuti, B., Rudolph, J., Raskin, L., Park, A., Lee, J., Lovly, C., & Anagnostou, V. (2020). Abstract 1095: Comprehensive pan-cancer analyses of RAS genomic diversity. Cancer Research, 80(16_Supplement), 1095. https://doi.org/10.1158/1538-7445.AM2020-1095

Senovilla, L., Vacchelli, E., Galon, J., Adjemian, S., Eggermont, A., Fridman, W. H., Sautès-Fridman, C., Ma, Y., Tartour, E., Zitvogel, L., Kroemer, G., & Galluzzi, L. (2012). Trial watch. OncoImmunology, 1(8), 1323–1343. https://doi.org/10.4161/onci.22009

Senthebane, D. A., Jonker, T., Rowe, A., Thomford, N. E., Munro, D., Dandara, C., Wonkam, A., Govender, D., Calder, B., Soares, N. C., Blackburn, J. M., Parker, M. I., & Dzobo, K. (2018). The Role of Tumor Microenvironment in Chemoresistance: 3D Extracellular Matrices as Accomplices. International Journal of Molecular Sciences, 19(10), Article 10. https://doi.org/10.3390/ijms19102861

Senthebane, D. A., Rowe, A., Thomford, N. E., Shipanga, H., Munro, D., Mazeedi, M. A. M. A., Almazyadi, H. A. M., Kallmeyer, K., Dandara, C., Pepper, M. S., Parker, M. I., & Dzobo, K. (2017). The Role of Tumor Microenvironment in Chemoresistance: To Survive, Keep Your Enemies Closer. International Journal of

Molecular Sciences, 18(7), Article 7. https://doi.org/10.3390/ijms18071586

Signatures of mutational processes in human cancer | Nature. (n.d.). Retrieved October 16, 2023, from https://www.nature.com/articles/nature12477

Simanshu, D. K., Nissley, D. V., & McCormick, F. (2017). RAS Proteins and Their Regulators in Human Disease. Cell, 170(1), 17–33. https://doi.org/10.1016/j.cell.2017.06.009

Skoulidis, F., Byers, L. A., Diao, L., Papadimitrakopoulou, V. A., Tong, P., Izzo, J., Behrens, C., Kadara, H., Parra, E. R., Canales, J. R., Zhang, J., Giri, U., Gudikote, J., Cortez, M. A., Yang, C., Fan, Y., Peyton, M., Girard, L., Coombes, K. R., … Heymach, J. V. (2015). Co-occurring Genomic Alterations Define Major Subsets of KRAS-Mutant Lung Adenocarcinoma with Distinct Biology, Immune Profiles, and Therapeutic Vulnerabilities. Cancer Discovery, 5(8), 860–877. https://doi.org/10.1158/2159-8290.CD-14-1236

Stewart, T. J., & Abrams, S. I. (2008). How tumours escape mass destruction. Oncogene, 27(45), Article 45. https://doi.org/10.1038/onc.2008.268

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

Tape, C. J., Ling, S., Dimitriadi, M., McMahon, K. M., Worboys, J. D., Leong, H. S., Norrie, I. C., Miller, C. J.,

Poulogiannis, G., Lauffenburger, D. A., & Jørgensen, C. (2016). Oncogenic KRAS Regulates Tumor Cell Signaling via Stromal Reciprocation. Cell, 165(4), 910–920. https://doi.org/10.1016/j.cell.2016.03.029

The Extracellular Matrix: Not Just Pretty Fibrils | Science. (n.d.). Retrieved May 11, 2023, from https://www.science.org/doi/abs/10.1126/science.1176009

Timar, J., & Kashofer, K. (2020). Molecular epidemiology and diagnostics of KRAS mutations in human cancer. Cancer and Metastasis Reviews, 39(4), 1029–1038. https://doi.org/10.1007/s10555-020-09915-5

Topcu, K. S. B. (n.d.). Tümör İlerlemesinde Tümör Mikroçevrenin Rolü.

Tsai, F. D., Lopes, M. S., Zhou, M., Court, H., Ponce, O., Fiordalisi, J. J., Gierut, J. J., Cox, A. D., Haigis, K. M., & Philips, M. R. (2015). K-Ras4A splice variant is widely expressed in cancer and uses a hybrid membrane-targeting motif. Proceedings of the National Academy of Sciences, 112(3), 779–784. https://doi.org/10.1073/pnas.1412811112

Westcott, P. M. K., Halliwill, K. D., To, M. D., Rashid, M., Rust, A. G., Keane, T. M., Delrosario, R., Jen, K.-Y., Gurley, K. E., Kemp, C. J., Fredlund, E., Quigley, D. A., Adams, D. J., & Balmain, A. (2015). The mutational landscapes of genetic and chemical models of Kras-driven lung cancer. Nature, 517(7535), Article 7535. https://doi.org/10.1038/nature13898

World Health Organization. (2020). World Health Statistics 2020. Security Research Hub Reports. https://digitalcommons.fiu.edu/srhreports/health/health/28

Ziani, L., Chouaib, S., & Thiery, J. (2018). Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts. Frontiers in Immunology, 9. https://www.frontiersin.org/articles/10.3389/fimmu.2018.00414

CHAPTER II

Taste Buds

Seçil Nazife PARLAK[1]

Introduction

Taste is a sensory system that begins in the mouth and involves taste buds which contain chemoreceptors. These receptors react with saliva, transmitting sensory information to the brain where taste perception is processed. The tongue, cheeks, and other parts of the mouth have papillae housing the taste buds, with the tongue being the most sensitive organ. There are four types of papillae: filiform, fungiform, foliate, and circumvallate. Some of these play a role in the sense of taste and contain taste buds.

Taste buds consist of four distinct types of cells: type I supportive cells, type II receptor cells, type III presynaptic cells, and taste cell precursors. Type I cells support and regulate the ionic environment, type II cells detect sweet, umami, and bitter tastes

[1] Dr. Öğr. Üyesi, Ağrı İbrahim Çeçen Üniversitesi Tıp Fakültesi, Temel Tıp Bilimleri Bölümü, Histoloji ve Embriyoloji Ana Bilim Dalı, Ağrı/Türkiye, Orcid : 0000-0001-9577-986X, E-mail: seeparlak@gmail.com

through specific receptors, and type III cells, which are least common, form synapses with nerve fibers and detect sour tastes. Taste cell precursors differentiate into the three main cell types within the taste buds.

Recent studies show that taste receptors are also found in non-taste organs like the digestive tract, pancreas, heart, brain, and respiratory system.

The sense of taste

Taste is a sensory system that begins in the mouth and extends to the brain, where taste sensations are perceived (Trivedi, 2012). Throughout the process of food consumption, this system functions when ingesting substances through the mouth and experiencing taste perception. Substances that are ingested undergo a chemical reaction with saliva whereby the substances stimulate the cells acting as receptors in specialized structures called taste buds. Their stimulation is achieved with chemoreceptors located in the cell membranes that allow for taste sensation (Sizer, 2012). From the ingested food and beverages, sensory information from the sense of taste, smell, and trigeminal nerve stimulation are transferred to the taste cortex in the brain. After this information is processed, the taste of the substance ingested and the pleasure it gives are identified. Taste is sensed from the main and most dense part of the tongue, from the inside of the cheeks, the upper part of the waist, and the small tongue. On the tongue, papillae contain up to 80% of the taste buds, 10% are on the soft palate, and 10% scattered on other intraoral organs.

Histological evaluation of the tongue and taste sensors

Histological evaluation of the tongue, the most sensitive taste organ, showed that it is covered with thousands of small folds and projections. There are four types of these structures, which can be seen with the naked eye and are called papillae (Chiego Jr, 2013). These include filiform (filamentous), fungiform (mushroom-shaped), circumvallate (V-shaped grooved), and foliate (leafy) papillae. Filiform papillae are thread-shaped, small, long, cone-shaped, numerous, and dispersed throughout the tongue's surface. These structures are usually keratinized and lack taste receptors (Gartner, 2020). Fungiform papillae are mushroom-shaped papillae that have a smooth and expanded top surface on the upper side and narrow stalks on the lower side of the tongue. They are irregularly distributed between the filiform papillae and the first two thirds of the tongue include fungiform papillae. Human foliate papillae are not well developed, form parallel ridges and grooves on the posterior surface, and are home to numerous taste buds on the tongue's lateral and upper regions. Circumvallate papillae are large and circular with smooth surfaces and extend over the other papillae (Yıldız & Özdamar, 2009). The circumvallate papillae are situated on a V-shaped area on the back third of the tongue. There is a deep groove covering the basal portion of the tongue and the epithelium is not keratinized. Taste buds are abundantly located at the top of the fungiform papillae and in small numbers on the sides of the circumvallate and foliate papillae (Rhoades & Bell, 2012). In total, there are about 10000 taste buds in these papillae. The papillae are specialized to sense sweet tastes at the tip of the tongue, sour and salty tastes on the sides, bitter tastes at the back, and umami taste on

the front two-thirds. The tastes are sensed by receptor cells in the taste buds.

Cell morphology in taste buds

A single taste bud is an average of 70 μm in height, 40 μm in diameter, and is an onion-like spherical structure consisting of up to 50 cells (Rhoades & Bell, 2012). A flavor hole, or pore, is located at the tip of the apex of the taste buds, which are exposed to the epithelial surface. Four different types of cells are found inside taste buds: precursor cells for taste cells, type I supporting (glial-like) cells, type II receptor cells, and type III synaptic cells. Within the taste buds, there are other cells apart from taste cell progenitors. These cells extend from the taste buds' pore to the basal lamina. The shape of their apical ends is conical, and they have microvilli reaching the taste hole, greatly increasing the surface area. From the lateral sides of their apical surface, the cells are connected by tight junctional complexes. Type I supporting cells have histological features resembling the two aforementioned two cell types, although these cells do not have synaptic connections. These three cell types have a lifespan of approximately 10 days and are regenerated by differentiation of taste cell precursor cells at the base of the taste buds into basal cells. The relocation of taste cells that synapse with the growing basal cell causes the synapses to break and new ones to form. Some of these cells are found at the basal ends of the taste buds' synapse with cranial VII (facial), IX (glossopharyngeal), and X (vagus) nerves. These nerves provide information to the solitary nucleus, thalamus, insula, cingulate cortex, and limbic system (Bradley & Grabauskas, 1998; Smith, LI, & Davis, 1998). It is thought that these connecting cells are secondary receptors used as

hair cells in the ear as an example and therefore, are anatomically different from afferent sensory nerves. Approximately 50 afferent nerve fibers enter the taste bud and branch and synapse with multiple such cells.

Type I supportive (glial-like) cells

The supporting framework of taste buds is made up of type I supportive (glial-like) cells, which account for the majority of cell types present and are believed to function in clearing neurotransmitters (Perea-Martinez, Nagai, & Chaudhari, 2013). Type I supporting cells have distinctive electrophysiologic characteristics—they do not have voltage-gated Ca^{2+} currents but accommodate tiny inward and outward voltage-gated K^{+} and Na^{2+} currents (Spector, Travers, & Norgren, 1993). These cells include amiloride-sensitive sodium channel subunit α (α-ENaC), which is thought to be the primary mediator of low salt perception (Chandrashekar, 2010; Vandenbeuch, Clapp, & Kinnamon, 2008). In mice, the deletion of α-ENaC in taste bud cells led to a total loss of behavioral salt attraction (Chandrashekar, 2010). However, the precise signaling pathways that are triggered when a low-salt substance enters type I promoter cells and how these cells interact with nerve fibers remain a mystery. In addition to expressing α-ENaC, these cells also have a membrane-bound ATPase expressed on their surface, which inhibits the release of ATP from nearby cells. Within the taste bud, type I cells form a lamellar configuration that encloses and sits between the other cell types. These cells are hypothesized to regulate the distribution of cell signaling molecules along the taste bud, maintain the ionic environment, and isolate ion fluctuations to certain parts of the taste bud (Finger, 2005; Pumplin,

Yu, & Smith, 1997). Type I supporting cells that detect salty taste and are tightly wrapped around other cells inside the taste bud are localized in the membranes where NTPDase2 (+) expression is found (Miura, Scott, Harada, & Barlow, 2014).

Type II receptor cells

Type II receptor cells are the second most common type of sensory cell of the tongue. They express receptors for umami, bitter, and sweet taste sensations (DeFazio, 2006; Tomchik, 2007; Yoshida, 2006). TAS1Rs are heterodimeric GPCRs that are made up of three receptor types that sense sweet and umami flavors: taste receptor type 1 receptor 1 (TAS1R1), TAS1R2, and TAS1R3. While sweet flavors (such as fructose, sucrose, glucose, plus artificial sweeteners like sucralose etc) activate the heterodimeric receptors TAS1R2 and TAS1R3 (Jiang, 2004; Max, 2001; Nelson, 2001; Xu, 2004), umami flavors (such as meat, meat broth, glutamate, mushrooms, and L-amino acids) do the same for the heterodimeric receptors TAS1R1 and TAS1R3 (Li, 2002; Nelson, 2002; Xu, 2004). Caffeine, denatonium benzoate, and quinine are examples of bitter tastes that are detected by GPCRs belonging to the taste receptor type 2 (TAS2R) family, which has around 30 members (Behrens, 2007; Chandrashekar, 2000; Meyerhof, 2010). Type II receptor cells only react to sweet, umami, or bitter gustatory chemicals because each receptor cell expresses a specific member of the TAS1R or TAS2R family (each cell that senses bitter taste can express TAS2Rs). To sense sweet, umami and bitter tastes, type II cells express GPCRs and downstream effectors that help mediate inositol-mediated Ca^{2+} signaling (Perea-Martinez, 2013). However, the cytoplasm of these cells contains PLCβ2 and the protein that is removed from the nuclei

of taste cells that contain PLCβ2 (+). Conventional synapses of afferent nerve fibers are not formed by type II cells. Conversely, these cells can release ATP through semi channels, which can subsequently trigger the activation of purinergic receptors (P2N2 and P2X3) present on the nerve fibers of the cranial nerves that supply each taste bud.

Type III presynaptic cells

Type III cells that most closely resemble neurons are called presynaptic cells and are the least common of this cell type. Type III cells are unique among taste bud cells in that they form traditional neural synapses via afferent sensory nerve fibers. Similar to neurons, these cells have voltage-gated Ca^{2+} channels and when they depolarize, they release norepinephrine, γ-aminobutyric acid (GABA), serotonin, and vesicular acetylcholine (Dvoryanchikov, 2011). Additionally, they express channels for polycystic kidney disease 1-like 3 (PKD1L3) and 2-like 1 (PKD2L1) protein, which are both involved in the perception of sour (acidic) taste (LopezJimenez, 2006). Mice lacking PKD2L1-expressing type III cells either showed no response at all or showed reduced sensitivity to acidic substances (ie, citric acid) (Horio, 2011; Huang, 2006). Increased salt concentrations allow type II cells to sense bitterness and type III cells to sense sourness (Miura, Kusakabe, & Harada, 2006). NCAM expression is seen on the surface of type III cells when responding to sour stimuli, and NCAM signals some of the nerve fibers that extend to the buds.

Taste cell precursors

A tiny, diverse collection of cells known as taste cell progenitors are found around the base of the taste bud. It is no longer

believed that this group of cells is only found at the base of the taste bud despite being originally thought to represent particular progenitor cells for types of differentiated taste bud cells (Castillo, 2014; Liu, 2013; Oka, 2013; Perea-Martinez, 2013). Taste cell progenitors are lengthy, postmitotic cells that proliferate outside of taste buds and originate from K5+/K14+/Gli1+ keratinocytes. Following their last division, cells with their fate determined as taste cells enter the taste buds and mature into taste bud precursors, which are oval cells in the basal compartment of the taste bud comprising sonic hedgehog protein (SHH). After mitosis, SHH+ cells can differentiate into all three types of taste cells and then become non-tasting epithelial cells (Barlow & Klein, 2015). Taste bud cell differentiation is regulated by SHH. Within taste buds, SHH-expressing cells communicate with SHH-responsive cells outside the taste bud, which express zinc finger protein (GLI1) and patched 1 (PTCH1). The taste buds on the adult mouse tongue are surrounded by several fields of SHH-responsive cells (Li, 2002). Cells expressing SHH in taste buds are progenitors to three other cell types, as shown by lineage-tracing experiments (Castillo, 2014). The lineage of taste bud cells were confirmed to be outside the taste bud in another investigation where relatively few (<10%) cells were shown to grow in taste buds (Perea-Martinez, 2013). Therefore, the small cells at the base of the taste buds are not progenitor cells, but are immature precursor cells and taste cells.

Cells containing taste receptors external to taste buds

Taste receptors have been found in various organs that lack taste in addition to taste bud cells. Recent studies have shown ectopic localization of these taste receptors and some non-taste related

physiological functions have been identified. There are two types of ectopic expressions of taste receptors: those expressed in taste bud-like cells outside the taste buds and expression in non-taste related cells, such as in the brain, urinary bladder, pancreas, heart and so on (genuine-ectopically expressed taste receptors).

Taste receptor expression in taste bud-like cells

Tufts are a unique epithelial cell population that has a specific tubulovesicular system characterized by a narrowed apical surface and blunt, elongated microvilli protruding from this surface (O'Leary, Schneider, & Locksley, 2019). They have been observed to be distributed throughout the epithelial mucosa, digestive tract, upper and lower respiratory tract, urinary tract, and have even been observed in the thymus (Haber, 2017; Howitt, 2016; Lee, 2014; Miller, 2018; Plasschaert, 2018; Saunders, 2014). Tuft cells are also known as brush cells or solitary chemosensory cells (SCCs), depending on where they are found (Deckmann, 2014; Hollenhorst, 2022; Saunders, 2014; Tizzano, 2011; Zheng, 2019). Tuft cells are divided into two categories (Haber, 2017; Montoro, 2018). Tuft-1 cells and tastebud type II receptor cells share several transcriptome characteristics. Conversely, Tuft-2 cells show immune-related gene enrichment (Haber, 2017; Xiong, 2022). Tuft-1 cells are the primary source of expression for taste receptors and their subclassifications. This categorization holds true for many organs, such as the digestive, respiratory, and urinary systems, and also the thymus (Haber, 2017; Miller, 2018; Montoro, 2018). Tuft cells resemble taste buds given their similar shape, gene expression, and signalling pathways (Gerbe, 2016; Matsumoto, 2011; Yamashita, 2017). These

subclassifications require further investigation of tuft cells in other organs.

Genuine-ectopically expressed taste receptors

Sensing a range of intestinal luminal nutrients, intestinal epithelial enteroendocrine cells (EECs) secrete neuropeptides and hormones, such as glucagon-like peptide-1 (GLP-1), serotonin, and cholecystokinin (CCK) to produce interoceptive hunger-satiety signals and regulate metabolic responses. Recently, results of single-cell RNA transcriptomics experiments have proposed that enteroendocrine cells could be sweet taste receptors. Experiments including the sorting of CCK-GFP-labeled cells and single-cell qRT-PCR revealed that approximately 20% of CCK-positive cells produced substantial quantities of Tas1r3 (Buchanan, 2022).

Pancreatic β-cells release insulin in response to foods (eg, fructose), artificial sweeteners (eg, saccharin), and changing blood glucose levels (Zorlu, 2018). In mouse pancreatic islets, fructose triggers the release of insulin. Mice injected with fructose stimulate insulin secretion, either in the presence or in the absence of glucose. Remarkably, Tas1r2 or Tas1r3 genetic ablation alters glucose sensitivity and insulin release.

Some glucose-sensing neurons also express sweet taste receptors, however, the majority of the chemical components that these neurons use to detect glucose in the brain are comparable to those found in pancreatic β-cells (Ashford, Boden, & Treherne, 1990). The precise cell types expressing sweet taste receptors in the brain remain unknown, despite RT-PCR studies supporting their expression (Kohno, 2016). According to a study by Jang et al., (2021), Tas1r2 expression was found in neurons expressing

proopiomelanocortin (POMC) in the hypothalamus, which senses excess body energy input. Tas1r2 expression was also found in neurons expressing agouti related peptide (AgRP), which opposes the function of POMC neurons and indicates body energy depletion. Beyond these hypothalamic neurons, a neuronal and non-neuronal population of cells throughout the entire brain exhibited increased Tas1r2 expression. This population included circumventricular organs, such as the organum vasculosum lamina terminalis, subcommisural organ, subfornical organ, area postrema, and median eminence (ME) (Jang, 2021). Similarly, it was shown that several brain regions expressed other taste receptors, including Tas1r3 and Tas2rs, which binds to Tas1r2 (Herrera Moro Chao, 2016; Singh, 2011).

Both human and mouse Tas1r2 and Tas1r3 expression has been reported in bladder urothelial layer umbrella cells. Artificial sweeteners, such saccharin, have been shown to increase smooth muscle contraction in murine bladder, and detrusor smooth muscle cells have been observed to express bitter taste receptors (Elliott, Kapoor, & Tincello, 2011).

Umami receptor subunits were found to be somewhat expressed in cardiac fibroblasts and bitter taste receptor expression was found in cardiomyocytes (Foster, 2013). Tas1r1 and Tas1r3 (that contain the umami receptor) and distinct subsets of type 2 taste receptors (TAS2/Tas2) were found to be expressed in rodent and human heart tissues as shown by RT-qPCR (Foster, 2013). Taste receptors are thought to act as metabolite and nutrient sensors in the heart.

Studies have shown that several bitter taste receptors (TAS2Rs) are highly expressed in human airway smooth muscle. Bitter-tasting compounds have shown greater potency in inducing airway smooth muscle relaxation in vitro and tracheal tension in vivo. In mouse models of asthma, the administration of bitter chemicals in aerosol form reduced bronchial hyperresponsiveness and allergic airway inflammation. Conversely, the L-type Ca2+ channel that mediates bronchoconstriction was attenuated by the canonical taste receptor signalling pathway (Deshpande, 2010; Deshpande, 2011).

In vascular smooth muscle cells, few bitter taste receptors also express TAS2R, and TAS2R agonists have been shown to have profound effects on vascular smooth muscle (Manson, 2014). Since TAS2R expression levels are similar to those of the α1A adrenoceptor (essential for smooth muscle contraction) and because they evoked muscle relaxation in an antagonistic manner against the receptor, it is suggested that bitter taste receptors play a role in smooth muscle cells and that bitter tastes have an endothelium-unrelated effect on smooth muscle that induces relaxation (Manson, 2014).

Conclucion

This widespread presence of taste receptors suggests that the sense of taste is integral not only to food perception but also to broader physiological processes. It highlights the adaptability of sensory systems in performing diverse functions across different tissues and organs.

Overall, the sense of taste is a multifaceted sensory system involving intricate cellular and molecular interactions. The

discovery of taste receptors in non-taste organs opens new avenues for understanding how taste perception influences various bodily functions and contributes to overall health and well-being. This expanding knowledge underscores the importance of taste receptors beyond their traditional role in the gustatory system.

References

Ashford, M. L., Boden, P. R., & Treherne, J. M. (1990). Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. Pflügers Archiv, 415, 479-483. doi: 10.1007/BF00373626.

Barlow, L. A., & Klein, O. D. (2015). Developing and regenerating a sense of taste. Current topics in developmental biology, 111, 401-419. doi: 10.1016/bs.ctdb.2014.11.012.

Behrens, M., Foerster, S., Staehler, F., Raguse, J.-D., & Meyerhof, W. (2007). Gustatory expression pattern of the human TAS2R bitter receptor gene family reveals a heterogenous population of bitter responsive taste receptor cells. Journal of Neuroscience, 27(46), 12630-12640. doi: 10.1523/JNEUROSCI.1168-07.2007.

Bradley, R. M., & Grabauskas, G. (1998). Neural Circuits for Taste: Excitation, Inhibition, and Synaptic Plasticity in the Rostral Gustatory Zone of the Nucleus of the Solitary Tract a. Annals of the New York Academy of Sciences, 855(1), 467-474. https://doi.org/10.1111/j.1749-6632.1998.tb10607.x

Buchanan, K. L., Rupprecht, L. E., Kaelberer, M. M., Sahasrabudhe, A., Klein, M. E., Villalobos, J. A., . . . Park, S. (2022). The preference for sugar over sweetener depends on a gut sensor cell. Nature neuroscience, 25(2), 191-200. doi: 10.1038/s41593-021-00982-7.

Castillo, D., Seidel, K., Salcedo, E., Ahn, C., de Sauvage, F. J., Klein, O. D., & Barlow, L. A. (2014). Induction of ectopic taste buds by SHH reveals the competency and plasticity of adult lingual

epithelium. Development, 141(15), 2993-3002. doi: 10.1242/dev.107631

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J., & Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. Nature, 464(7286), 297-301. doi: 10.1038/nature08783.

Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., . . . Ryba, N. J. (2000). T2Rs function as bitter taste receptors. Cell, 100(6), 703-711. doi: 10.1016/s0092-8674(00)80706-0.

Chiego Jr, D. J. (2013). Essentials of Oral Histology and Embryology-E-Book: A Clinical Approach: Elsevier Health Sciences. ABD; Elsevier.

Deckmann, K., Filipski, K., Krasteva-Christ, G., Fronius, M., Althaus, M., Rafiq, A., . . . Wessels, L. (2014). Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. Proceedings of the National Academy of Sciences, 111(22), 8287-8292. doi: 10.1073/pnas.1402436111.

DeFazio, R. A., Dvoryanchikov, G., Maruyama, Y., Kim, J. W., Pereira, E., Roper, S. D., & Chaudhari, N. (2006). Separate populations of receptor cells and presynaptic cells in mouse taste buds. Journal of Neuroscience, 26(15), 3971-3980. doi: 10.1523/JNEUROSCI.0515-06.2006.

Deshpande, D. A., Robinett, K. S., Wang, W. C., Sham, J. S., An, S. S., & Liggett, S. B. (2011). Bronchodilator activity of bitter tastants in human tissue. Nature medicine, 17(7), 776-778. doi: 10.1038/nm0711-776b.

Deshpande, D. A., Wang, W. C., McIlmoyle, E. L., Robinett, K. S., Schillinger, R. M., An, S. S., . . . Liggett, S. B. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nature medicine, 16(11), 1299-1304. doi: 10.1038/nm.2237.

Dvoryanchikov, G., Huang, Y. A., Barro-Soria, R., Chaudhari, N., & Roper, S. D. (2011). GABA, its receptors, and GABAergic inhibition in mouse taste buds. Journal of Neuroscience, 31(15), 5782-5791. doi: 10.1523/JNEUROSCI.5559-10.2011

Elliott, R. A., Kapoor, S., & Tincello, D. G. (2011). Expression and distribution of the sweet taste receptor isoforms T1R2 and T1R3 in human and rat bladders. The Journal of urology, 186(6), 2455-2462. doi: 10.1016/j.juro.2011.07.083.

Finger, T. E., Danilova, V., Barrows, J., Bartel, D. L., Vigers, A. J., Stone, L., . . . Kinnamon, S. C. (2005). ATP signaling is crucial for communication from taste buds to gustatory nerves. Science, 310(5753), 1495-1499. doi: 10.1126/science.1118435.

Foster, S. R., Porrello, E. R., Purdue, B., Chan, H.-W., Voigt, A., Frenzel, S., . . . Molenaar, P. (2013). Expression, regulation and putative nutrient-sensing function of taste GPCRs in the heart. PloS one, 8(5), e64579. doi: 10.1371/journal.pone.0064579

Gartner, L. P. (2020). Textbook of Histology E-Book. 5th Edition, Elsevier Health Sciences.

Gerbe, F., Sidot, E., Smyth, D. J., Ohmoto, M., Matsumoto, I., Dardalhon, V., . . . Brulin, B. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature, 529(7585), 226-230. doi: 10.1038/nature16527.

Haber, A. L., Biton, M., Rogel, N., Herbst, R. H., Shekhar, K., Smillie, C., . . . Katz, Y. (2017). A single-cell survey of the small intestinal epithelium. Nature, 551(7680), 333-339. doi: 10.1038/nature24489.

Herrera Moro Chao, D., Argmann, C., Van Eijk, M., Boot, R., Ottenhoff, R., Van Roomen, C., . . . Kalsbeek, A. (2016). Impact of obesity on taste receptor expression in extra-oral tissues: emphasis on hypothalamus and brainstem. Scientific reports, 6(1), 29094. doi: 10.1038/srep29094

Hollenhorst, M. I., Nandigama, R., Evers, S. B., Gamayun, I., Wadood, N. A., Salah, A., . . . Gebhardt, A. (2022). Bitter taste signaling in tracheal epithelial brush cells elicits innate immune responses to bacterial infection. The Journal of clinical investigation, 132(13). doi: 10.1172/JCI150951.

Horio, N., Yoshida, R., Yasumatsu, K., Yanagawa, Y., Ishimaru, Y., Matsunami, H., & Ninomiya, Y. (2011). Sour taste responses in mice lacking PKD channels. PloS one, 6(5), e20007. doi: 10.1371/journal.pone.0020007.

Howitt, M. R., Lavoie, S., Michaud, M., Blum, A. M., Tran, S. V., Weinstock, J. V., . . . Osborne, L. C. (2016). Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science, 351(6279), 1329-1333. doi: 10.1126/science.aaf1648.

Huang, A. L., Chen, X., Hoon, M. A., Chandrashekar, J., Guo, W., Tränkner, D., . . . Zuker, C. S. (2006). The cells and logic for mammalian sour taste detection. Nature, 442(7105), 934-938. doi: 10.1038/nature05084.

Jang, J. H., Kim, H. K., Seo, D. W., Ki, S. Y., Park, S., Choi, S.-H., . . . Jeong, Y. T. (2021). Whole-brain mapping of the expression pattern of T1R2, a subunit specific to the sweet taste receptor. Frontiers in Neuroanatomy, 15, 751839. doi: 10.3389/fnana.2021.751839.

Jiang, P., Ji, Q., Liu, Z., Snyder, L. A., Benard, L. M., Margolskee, R. F., & Max, M. (2004). The cysteine-rich region of T1R3 determines responses to intensely sweet proteins. Journal of Biological Chemistry, 279(43), 45068-45075. doi: 10.1074/jbc.M406779200.

Kohno, D., Koike, M., Ninomiya, Y., Kojima, I., Kitamura, T., & Yada, T. (2016). Sweet taste receptor serves to activate glucose-and leptin-responsive neurons in the hypothalamic arcuate nucleus and participates in glucose responsiveness. Frontiers in neuroscience, 10, 502. doi: 10.3389/fnins.2016.00502.

Lee, R. J., Kofonow, J. M., Rosen, P. L., Siebert, A. P., Chen, B., Doghramji, L., . . . Kennedy, D. W. (2014). Bitter and sweet taste receptors regulate human upper respiratory innate immunity. The Journal of clinical investigation, 124(3), 1393-1405. doi: 10.1172/JCI72094.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. Proceedings of the National Academy of Sciences, 99(7), 4692-4696. doi: 10.1073/pnas.072090199.

Liu, H. X., Ermilov, A., Grachtchouk, M., Li, L., Gumucio, D. L., Dlugosz, A. A., & Mistretta, C. M. (2013). Multiple Shh signaling centers participate in fungiform papilla and taste bud

formation and maintenance. Developmental biology, 382(1), 82-97. doi: 10.1016/j.ydbio.2013.07.022.

LopezJimenez, N. D., Cavenagh, M. M., Sainz, E., Cruz-Ithier, M. A., Battey, J. F., & Sullivan, S. L. (2006). Two members of the TRPP family of ion channels, Pkd1l3 and Pkd2l1, are co-expressed in a subset of taste receptor cells. Journal of neurochemistry, 98(1), 68-77. doi: 10.1111/j.1471-4159.2006.03842.x.

Manson, M. L., Säfholm, J., Al-Ameri, M., Bergman, P., Orre, A.-C., Swärd, K., . . . Adner, M. (2014). Bitter taste receptor agonists mediate relaxation of human and rodent vascular smooth muscle. European journal of pharmacology, 740, 302-311. doi: 10.1016/j.ejphar.2014.07.005.

Matsumoto, I., Ohmoto, M., Narukawa, M., Yoshihara, Y., & Abe, K. (2011). Skn-1a (Pou2f3) specifies taste receptor cell lineage. Nature neuroscience, 14(6), 685-687. doi: 10.1038/nn.2820

Max, M., Shanker, Y. G., Huang, L., Rong, M., Liu, Z., Campagne, F., . . . Margolskee, R. F. (2001). Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. Nature genetics, 28(1), 58-63. doi: 10.1038/ng0501-58.

Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., . . . Behrens, M. (2010). The molecular receptive ranges of human TAS2R bitter taste receptors. Chemical senses, 35(2), 157-170. doi: 10.1093/chemse/bjp092.

Miller, C. N., Proekt, I., von Moltke, J., Wells, K. L., Rajpurkar, A. R., Wang, H., . . . Pollack, J. L. (2018). Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte

development. Nature, 559(7715), 627-631. doi: 10.1038/s41586-018-0345-2.

Miura, H., Kusakabe, Y., & Harada, S. (2006). Cell lineage and differentiation in taste buds. Archives of histology and cytology, 69(4), 209-225. doi: 10.1679/aohc.69.209

Miura, H., Scott, J. K., Harada, S., & Barlow, L. A. (2014). Sonic hedgehog–expressing basal cells are general post-mitotic precursors of functional taste receptor cells. Developmental Dynamics, 243(10), 1286-1297. doi: 10.1002/dvdy.24121.

Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birket, S. E., . . . Villoria, J. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. Nature, 560(7718), 319-324. doi: 10.1038/s41586-018-0393-7.

Nelson, G., Chandrashekar, J., Hoon, M. A., Feng, L., Zhao, G., Ryba, N. J., & Zuker, C. S. (2002). An amino-acid taste receptor. Nature, 416(6877), 199-202. doi: 10.1038/nature726.

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J., & Zuker, C. S. (2001). Mammalian sweet taste receptors. Cell, 106(3), 381-390. doi: 10.1016/s0092-8674(01)00451-2.

O'Leary, C. E., Schneider, C., & Locksley, R. M. (2019). Tuft cells—systemically dispersed sensory epithelia integrating immune and neural circuitry. Annual review of immunology, 37, 47-72. doi: 10.1146/annurev-immunol-042718-041505.

Oka, Y., Butnaru, M., von Buchholtz, L., Ryba, N. J., & Zuker, C. S. (2013). High salt recruits aversive taste pathways. Nature, 494(7438), 472-475.

Perea-Martinez, I., Nagai, T., & Chaudhari, N. (2013). Functional cell types in taste buds have distinct longevities. PloS one, 8(1), e53399. doi: 10.1371/journal.pone.0053399

Plasschaert, L. W., Žilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., . . . Jaffe, A. B. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature, 560(7718), 377-381. doi: 10.1038/s41586-018-0394-6.

Pumplin, D. W., Yu, C., & Smith, D. V. (1997). Light and dark cells of rat vallate taste buds are morphologically distinct cell types. Journal of Comparative Neurology, 378(3), 389-410. doi: 10.1002/(sici)1096-9861(19970217)378:3<389::aid-cne7>3.0.co;2-#.

Rhoades, R. and Bell, D.R. (2013) Medical physiology: principles for clinical medicine. 4th Edition, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia.

Saunders, C. J., Christensen, M., Finger, T. E., & Tizzano, M. (2014). Cholinergic neurotransmission links solitary chemosensory cells to nasal inflammation. Proceedings of the National Academy of Sciences, 111(16), 6075-6080. doi: 10.1073/pnas.1402251111.

Singh, N., Vrontakis, M., Parkinson, F., & Chelikani, P. (2011). Functional bitter taste receptors are expressed in brain cells. Biochemical and biophysical research communications, 406(1), 146-151. doi: 10.1016/j.bbrc.2011.02.016.

Sizer, L. (2012). How pleasure works: The new science of why we like what we like by Bloom, Paul. The Journal of Aesthetics and Art Criticism, 70(4), 394-397. doi:

10.1111/jaac.2012.70.sayı-4

Smith, D. V., LI, C. S., & Davis, B. J. (1998). Excitatory and Inhibitory Modulation of Taste Responses in the Hamster Brainstem a. Annals of the New York Academy of Sciences, 855(1), 450-456. doi: 10.1111/j.1749-6632.1998.tb10605.x.

Spector, A. C., Travers, S. P., & Norgren, R. (1993). Taste receptors on the anterior tongue and nasoincisor ducts of rats contribute synergistically to behavioral responses to sucrose. Behavioral neuroscience, 107(4), 694. https://doi.org/10.1037/0735-7044.107.4.694

Tizzano, M., Cristofoletti, M., Sbarbati, A., & Finger, T. E. (2011). Expression of taste receptors in solitary chemosensory cells of rodent airways. BMC pulmonary medicine, 11, 1-12. doi: 10.1186/1471-2466-11-3.

Tomchik, S. M., Berg, S., Kim, J. W., Chaudhari, N., & Roper, S. D. (2007). Breadth of tuning and taste coding in mammalian taste buds. Journal of Neuroscience, 27(40), 10840-10848. doi: 10.1523/JNEUROSCI.1863-07.2007

Trivedi, B. P. (2012). Gustatory system: The finer points of taste. Nature, 486(7403), S2-S3. doi: 10.1038/486S2a.

Vandenbeuch, A., Clapp, T. R., & Kinnamon, S. C. (2008). Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC neuroscience, 9, 1-13. doi: 10.1186/1471-2202-9-1.

Xiong, Z., Zhu, X., Geng, J., Xu, Y., Wu, R., Li, C., . . . Tian, Y. (2022). Intestinal Tuft-2 cells exert antimicrobial immunity via sensing bacterial metabolite N-undecanoylglycine. Immunity, 55(4),

686-700. e687. doi: 10.1016/j.immuni.2022.03.001. Epub 2022 Mar 22.

Xu, H., Staszewski, L., Tang, H., Adler, E., Zoller, M., & Li, X. (2004). Different functional roles of T1R subunits in the heteromeric taste receptors. Proceedings of the National Academy of Sciences, 101(39), 14258-14263. doi: 10.1073/pnas.0404384101

Yamashita, J., Ohmoto, M., Yamaguchi, T., Matsumoto, I., & Hirota, J. (2017). Skn-1a/Pou2f3 functions as a master regulator to generate Trpm5-expressing chemosensory cells in mice. PloS one, 12(12), e0189340. https://doi.org/10.1371/journal.pone.0189340

Yıldız, H. T., & Özdamar, S. (2009). İnsan fetuslarinda dil papillalarinin gelişiminin taramali elektron mikroskobunda incelenmesi. Sağlık Bilimleri Dergisi, 18(3), 129-137.

Yoshida, R., Shigemura, N., Sanematsu, K., Yasumatsu, K., Ishizuka, S., & Ninomiya, Y. (2006). Taste responsiveness of fungiform taste cells with action potentials. Journal of neurophysiology, 96(6), 3088-3095. doi: 10.1152/jn.00409.2006

Zheng, X., Tizzano, M., Redding, K., He, J., Peng, X., Jiang, P., . . . Margolskee, R. F. (2019). Gingival solitary chemosensory cells are immune sentinels for periodontitis. Nature communications, 10(1), 4496. doi: 10.1038/s41467-019-12505-x.

Zorlu, G. (2018). Yüksek fruktozlu mısır şurubu tüketen sıçanlarda olası davranış değişikliklerinin görüntü işleme teknikleriyle analizi. T.C. Firat University Institute of Health Sciences, Department of Biophysics, Master's Thesis, Elazığ.

CHAPTER III

Use of in vivo and in vitro Models in Neorological Diseases and their Comparison

Mehmet ÖZSAN[1]
Hasan Hüseyin DÖNMEZ[2]

Introduction

Neurological diseases encompass various disorders that affect the central and peripheral nervous systems, leading to severe health issues. Neurological conditions like Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) affect different components of the nervous system, reducing individuals' quality of life and limiting daily activities. Diagnosis and treatment processes for these diseases are highly complex due to their distinct etiologies,

[1] Asst. Prof. Dr., Niğde Ömer Halisdemir University, Faculty of Medicine, Basic Medical Sciences, Niğde, Türkiye. Orcid: 0000-0001-9546-3478, Mehmet_ozsan@hotmail.com
[2] Prof. Dr., Selçuk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Konya, Türkiye. Orcid: 0000-0003-4664-8489, hdonmez@selcuk.edu.tr

pathophysiologies, and clinical symptoms (Ropper & Samuels, 2009).

The importance of neurological diseases extends beyond their direct effects on health and quality of life, as they also impose significant societal and economic burdens. According to the World Health Organization (WHO), neurological disorders are among the leading causes of disability worldwide, placing substantial financial strain on healthcare systems (World Health Organization, 2006). For instance, degenerative neurological diseases like Alzheimer's not only demand long-term care but also increase healthcare expenses, significantly affecting both patients' families and healthcare systems (Prince et al., 2015).

The treatment and management of neurological diseases require a multidisciplinary approach, involving fields such as neuroscience, genetics, pharmacology, and rehabilitation. Advances in neuroscience research have provided deeper insights into the functioning of the nervous system and the mechanisms of neurological diseases. These developments have enabled significant progress in creating new treatment strategies and improving patients' quality of life (Kandel et al., 2013).

Neurological Diseases: Definition and Importance

Neurological diseases encompass a range of disorders affecting the central and peripheral nervous systems. They impact various components of the nervous system, including the brain, spinal cord, nerves, and muscles. Common neurological disorders include Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). Each disease has unique etiologies, pathophysiologies, and clinical

symptoms, complicating the diagnosis and treatment processes (Ropper & Samuels, 2009).

The significance of neurological diseases can be understood not only through their direct effects on individuals' health and quality of life but also through their societal and economic impacts. According to the World Health Organization (WHO), neurological disorders are among the leading causes of disability globally, placing considerable financial demands on healthcare systems (World Health Organization, 2006). For example, degenerative neurological diseases like Alzheimer's significantly impact both caregivers and healthcare systems due to long-term care requirements and increased healthcare expenditures. The implications of these diseases on public health make the development of early diagnosis and effective treatment methods critical (Prince et al., 2015).

The treatment and management of neurological diseases necessitate a multidisciplinary approach involving fields such as neuroscience, genetics, pharmacology, and rehabilitation. Advances in neuroscience research have deepened our understanding of nervous system functioning and the mechanisms underlying neurological diseases. These advancements have enabled significant steps toward developing new therapeutic strategies and improving patients' quality of life (Kandel et al., 2013; Kısadere et al., 2022). Innovations in neuroprotective treatments (Kısadere et al., 2019, Kısadere et al., 2021; Hatipoğlu et al., 2024) and regenerative medicine, in particular, promise transformative changes in the future management of neurological diseases.

The Importance of Research Models

Research models play a critical role in understanding fundamental biological processes and disease mechanisms in scientific studies. These models provide tools to investigate biological events that cannot be directly observed in humans. In vivo and in vitro models allow for detailed exploration of these processes, enabling the development of new treatments and the improvement of existing therapies (Hughes, 2008). Exosomes, as highlighted by Kanan et al. (2022), have been increasingly recognized as critical biomarkers and mediators in the progression of neurodegenerative diseases, providing a novel perspective on the interplay between cellular mechanisms and disease pathophysiology.

In Vivo Models

In vivo models involve experiments conducted on living organisms, enabling researchers to study the holistic responses of an organism. In neurological diseases, animal models are frequently used to understand the complex structure and functions of the nervous system. These models can realistically simulate disease progression and treatment responses, providing researchers with in-depth insights into the etiology and pathophysiology of diseases (Van der Staay, 2006).

In Vitro Models

In vitro models involve experiments conducted in controlled laboratory environments, such as cell cultures. These models enable the detailed study of specific cellular and molecular mechanisms. When conducted using human cells, in vitro models yield results more closely aligned with human biology, facilitating a better understanding of cellular interactions and biochemical processes

(Breslin & O'Driscoll, 2013). Additionally, in vitro models offer ethical and cost advantages, as they do not require live animals and are highly reproducible.

The importance of research models is evident in the discovery of new therapeutic approaches and the optimization of existing treatments. For instance, research on Alzheimer's disease using both in vivo and in vitro models has enhanced understanding of the disease's pathological characteristics and potential treatment targets (Karran & Hardy, 2014). These models have facilitated the development of neuroprotective agents and other therapeutic strategies to slow disease progression.

In Vivo Models

Definition and Overview

In vivo models refer to scientific research conducted on living organisms. These models are used to understand disease pathophysiology, test new treatments, and examine biological processes. In vivo studies provide researchers with the opportunity to observe an organism's overall responses and complex biological interactions. These models are considered a critical component of preclinical research and are used to evaluate the safety and efficacy of new drugs (Hughes, 2008). Moreover, in vivo models offer a unique platform to study the complex interactions of genetic, environmental, and pharmacological factors. For instance, they are used to investigate the effects of environmental toxins on neurological diseases, helping to identify environmental risk factors (Perlman, 2016).

Commonly Used In Vivo Models

Rodent Models

Rodents, such as mice and rats, are widely used in neurological research due to their genetic similarity to humans and short reproductive cycles. These models are employed to study the mechanisms of neurological diseases like Alzheimer's, Parkinson's, epilepsy, and multiple sclerosis. For example, the 5xFAD mouse model is commonly used to investigate the pathological features and cognitive impairments associated with Alzheimer's disease (Oakley et al., 2006). Rodent models' ability to be genetically manipulated provides a significant advantage in studying the roles of specific genes in disease processes. Additionally, behavioral tests enable the assessment of cognitive and motor functions, which is critical for examining the functional effects of diseases (Eichenbaum, 2016).

Primate Models

Primates, such as macaques, play a vital role in neurological research due to their close genetic and physiological similarities to humans. These models are particularly valuable for studying complex brain functions and behaviors. For example, primate models have been instrumental in testing innovative therapies like deep brain stimulation for Parkinson's disease (Emborg, 2007). Primate models provide accurate representations of human social behaviors and cognitive processes, offering significant advantages in studying psychiatric and neurodegenerative diseases. However, their use is limited due to high costs and ethical concerns (Phillips et al., 2014).

Other Animal Models

In addition to rodents and primates, other animals such as zebrafish, rabbits, and pigs are used in neurological research. Zebrafish are particularly favored for developmental neurobiology and genetic studies, while pigs and rabbits are used for their larger brain structures and complex behaviors. For example, zebrafish models are widely used to study neurotoxicity and neurogenesis (Kalueff et al., 2014). Pig models, due to their anatomical and physiological similarities to the human brain, are utilized in the development of neurosurgical techniques and brain imaging studies. Rabbit models are often employed in neuropathology and testing neuroprotective agents (Swindle et al., 2012).

Advantages and Disadvantages

The primary advantage of in vivo models is their ability to examine an organism's holistic biological responses and complex system interactions. This is critical for understanding the natural progression of diseases and responses to treatments. However, in vivo models also have disadvantages, including ethical concerns, high costs, and limited ability to fully replicate human diseases. Furthermore, results obtained from animal models are not always directly translatable to humans due to genetic and physiological differences, which can make predicting clinical challenges more difficult (Bailey et al., 2014).

Example Studies

One example is the use of the 5xFAD mouse model in Alzheimer's research. This transgenic model carries mutations in human APP and presenilin genes, leading to early accumulation of amyloid-beta plaques and cognitive impairments, making it ideal for

studying the pathological processes of Alzheimer's disease (Oakley et al., 2006). Another example is the use of primate models in Parkinson's disease research, where MPTP-induced Parkinson's models replicate motor symptoms and neurodegeneration observed in humans, providing critical insights for testing new therapeutic strategies (Emborg, 2007). Additionally, studies using zebrafish models have provided valuable insights into neurogenesis and the regenerative capacity of neural cells, aiding the development of neuroprotective agents (Kalueff et al., 2014).

In Vitro Models

In vitro models refer to experiments conducted on cells or biological molecules in controlled laboratory environments. These models are used to investigate the mechanisms of biological processes and diseases in detail. In in vitro studies, cells, tissue slices, or biochemical components are manipulated under laboratory conditions to perform various experiments. This approach allows researchers to isolate and closely examine specific cellular and molecular mechanisms (Breslin & O'Driscoll, 2013). Additionally, in vitro models provide high-throughput screening methods for drug development, enabling the rapid and efficient evaluation of new therapeutic agents (Pampaloni et al., 2007).

Commonly Used In Vitro Models

Cell Culture Models

Cell culture models are among the most commonly used in vitro systems in biological research. In these models, cells are grown in appropriate culture media under laboratory conditions, allowing researchers to directly observe cellular activities, gene expression, and drug effects. Studies using neural cells (neurons, glial cells) are

employed to investigate the mechanisms of neurodegenerative diseases and potential therapeutic targets. For example, the SH-SY5Y human neuroblastoma cell line is frequently used as a model in Parkinson's disease research (Xicoy et al., 2017). Cell culture models are also utilized to study the effects of environmental stress factors (e.g., hypoxia or toxins) on cell health, contributing to a better understanding of disease pathophysiology (Gstraunthaler, 2003).

Organotypic Cultures

Organotypic cultures involve culturing organ or tissue slices in laboratory settings, providing models with in vivo-like three-dimensional structures and cellular organization. These models enable the realistic study of tissue-level interactions and functions. Brain slice cultures, in particular, are used to investigate the structure and functions of neural networks. Hippocampal slice cultures are an essential tool for studying conditions such as epilepsy and neurodegenerative diseases (Stoppini et al., 1991). These models allow for detailed examination of processes such as synaptic plasticity, cell death mechanisms, and the efficacy of neuroprotective agents, playing a critical role in developing therapeutic strategies (Müller et al., 2003).

Induced Pluripotent Stem Cells (iPSCs)

Induced pluripotent stem cells (iPSCs) are derived by genetically reprogramming adult somatic cells to a pluripotent state. iPSCs can differentiate into various cell types and allow for the creation of patient-specific cell models. This makes them a powerful tool for studying cellular mechanisms of diseases and developing personalized medicine applications. In neurological disease research, iPSCs are used to model conditions such as Alzheimer’s

disease, Parkinson's disease, and ALS (Takahashi & Yamanaka, 2006). Additionally, iPSCs can be employed to investigate the pathological processes and potential therapeutic targets of genetic disorders. They enable the study of patient-specific genetic variations and the recreation of disease-specific phenotypes in laboratory settings (Yamanaka, 2020).

Advantages and Disadvantages

The primary advantage of in vitro models is their ability to provide controlled and reproducible experimental conditions. These models allow for the detailed study of isolated cellular and molecular processes. In vitro studies are also generally less expensive and more ethically acceptable than in vivo models. However, the disadvantages include the artificial nature of the cellular environment, which differs from the natural biological context, and the inability to replicate systemic interactions (Antoni et al., 2015). Furthermore, the lack of biological complexity and whole-organism responses in in vitro models can limit their clinical relevance. Consequently, in vitro findings often need validation through in vivo models (Hartung, 2007).

Example Studies

Alzheimer's Disease and iPSCs

One example involves using iPSCs in Alzheimer's disease research. iPSCs derived from human fibroblasts have been differentiated into neuronal cells to study Alzheimer's-specific pathological features. These studies have provided critical insights into the cellular-level effects of amyloid-beta plaques and tau protein accumulation (Israel et al., 2012). Such findings play a significant

role in early diagnosis and the development of therapeutic strategies for Alzheimer's disease.

Epilepsy Research and Hippocampal Slice Cultures

Another example is the use of hippocampal slice cultures in epilepsy research. This model has been used to study seizure activity and evaluate the effects of neuroprotective agents (Stoppini et al., 1991). Hippocampal slice cultures have proven to be an important tool for understanding seizure mechanisms and assessing the efficacy of neuroprotective therapies (Müller et al., 2003).

Comparison of In Vivo and In Vitro Models

Comparison Criteria

Comparing in vivo and in vitro models is essential to understand how each model addresses specific research needs. Key criteria for comparison include complexity, realism, application areas, data generation speed, cost, and ethical considerations. These criteria help researchers determine which model is most suitable for a particular study (Perlman, 2016). Studies indicate that careful evaluation of these criteria is crucial, as both models have distinct advantages and limitations. Such evaluations enable the production of more accurate and reliable scientific results.

Model Complexity and Realism

In vivo models, being conducted on living organisms, best reflect the complexity and realism of biological processes and diseases. They provide opportunities to observe holistic responses and systemic interactions within an organism. For example, using mouse models to study the effects of a neurological disease on the brain allows researchers to realistically track disease progression and

behavioral changes (Van der Staay, 2006). Additionally, in vivo models are ideal for assessing complex biological processes such as immune responses and drug metabolic pathways.

In contrast, in vitro models are conducted in simpler and more controlled environments. Cell cultures and organotypic cultures enable isolated examination of specific cellular and molecular processes but cannot replicate systemic effects (Breslin & O'Driscoll, 2013). Therefore, in vitro models are particularly suitable for mechanistic studies and high-throughput screenings but often require validation using in vivo models.

Application Areas

In vivo models are widely used to study disease pathophysiology, treatment responses, and drug effects. These models are particularly suited for pharmacokinetic and pharmacodynamic studies (Hughes, 2008). In vivo studies provide detailed insights into processes such as drug distribution, metabolism, and elimination within the body.

In vitro models, on the other hand, are ideal for investigating cellular-level mechanisms, gene expression, and protein interactions. Cell cultures, biochemical analyses, and drug screening are common applications. For example, cancer cell lines are used to test the efficacy of new anticancer drugs (Antoni et al., 2015). In vitro models also have extensive applications in toxicology and pharmacology, as they deliver rapid and reproducible results.

Data Generation Speed and Cost

In vitro models typically produce data faster and are more cost-effective. Cell culture experiments can yield results in a short

time and enable large-scale screenings. Additionally, in vitro experiments require fewer resources and are easier to replicate under laboratory conditions (Breslin & O'Driscoll, 2013). This accelerates research processes and reduces costs.

Conversely, in vivo models take longer to produce results and are generally more expensive. Animal care, ethical approvals, and long-term experimental procedures increase the cost of in vivo studies (Perlman, 2016). In addition, in vivo studies are often more complex and time-consuming, as experimental procedures require extensive preparation and attention.

Ethical Considerations

The use of in vivo models is controversial due to animal welfare and ethical concerns. Animal experiments are subject to strict ethical guidelines and regulations to ensure animals do not endure unnecessary suffering. Attention to living conditions, ethical approval of experimental protocols, and measures to enhance animal welfare are critical in addressing the ethical dimension of research (Hughes, 2008).

In contrast, in vitro models are more ethically acceptable because they do not involve live animals and are conducted at the cellular level. As a result, they are often preferred by researchers and ethical review boards. However, the potential inability of in vitro models to fully represent human biology must also be considered.

Combined Use of Both Models

Hybrid Approaches

The combined use of in vivo and in vitro models can enhance the accuracy and reliability of research findings. Hybrid approaches

allow for the validation of findings from cellular-level studies in living organisms. For instance, a drug's efficacy discovered in cell culture studies can later be tested in animal models to confirm its effects. This approach accelerates the transition from laboratory to clinical stages and enables the generation of more comprehensive results (Breslin & O'Driscoll, 2013). Additionally, hybrid approaches overcome the limitations of individual model systems, enabling the production of more universally applicable and reliable findings. For example, toxic effects identified in in vitro screenings can be validated in in vivo models to ensure clinical relevance.

Advantages of Using Multiple Models

Using multiple models allows researchers to leverage the advantages of different systems to obtain more comprehensive and generalizable results. Examining a research question from different perspectives helps overcome model-specific limitations. For example, in neurological disease research, molecular mechanisms can be studied using cell cultures, followed by investigating systemic effects and behavioral outcomes using animal models (Van der Staay, 2006). This approach ensures that research findings are more reliable and clinically meaningful. Furthermore, comparing results from different models helps validate research findings and increases their acceptance within the scientific community.

Future Perspectives

Impact of Technological Advances on Models

Technological advancements play a crucial role in enhancing the precision and accuracy of biomedical research models. Tools like CRISPR-Cas9 gene-editing technology facilitate genetic modifications in both in vivo and in vitro models, enabling a deeper

exploration of disease mechanisms. Using CRISPR, specific gene mutations can be introduced in mouse and cell culture models, offering significant advantages in identifying genetic underpinnings and therapeutic targets of diseases (Hsu et al., 2014).

Moreover, cutting-edge technologies such as organ-on-a-chip and 3D bioprinting enhance the realism and complexity of in vitro models. Organ-on-a-chip technology replicates human tissues and organs on microfluidic chips, allowing researchers to better understand cell behavior in a more natural environment. This technology holds great potential in drug screening and toxicology testing as it provides a more physiological setting compared to traditional cell cultures (Bhatia & Ingber, 2014).

Emerging Model Approaches

Emerging model approaches aim to overcome the limitations of current research models. For instance, human brain organoids—three-dimensional structures derived from stem cells that mimic brain tissue—are being utilized to study the complex pathologies of neurological diseases and accelerate drug development. Brain organoids have played a pivotal role in understanding the effects of the Zika virus on human brain development (Lancaster et al., 2013).

Another innovative approach is the application of artificial intelligence (AI) and machine learning (ML) algorithms in biomedical research. These technologies analyze large datasets, aiding in the early diagnosis of diseases and the development of personalized treatment strategies. By processing complex data such as gene expression profiles and patient records, AI can help identify disease biomarkers and novel therapeutic targets (Esteva et al., 2019).

Personalized Medicine and the Role of Models

Personalized medicine aims to develop tailored treatment plans based on patients' genetic, environmental, and lifestyle factors. This approach enables more effective and targeted treatment of diseases. In personalized medicine research, in vitro and in vivo models play a significant role. Studies using patient-specific iPSCs are particularly important for understanding how diseases develop at an individual level and identifying the most effective treatment methods (Takahashi & Yamanaka, 2006).

Additionally, pharmacogenomic research helps determine how genetic profiles influence individual drug responses. This is critical for improving treatment efficacy and minimizing side effects. For example, targeted cancer therapies are often chosen based on patients' genetic mutations, significantly enhancing treatment effectiveness. These approaches underscore the future role and potential of personalized medicine (Collins & Varmus, 2015).

Conclusion

Summary and General Evaluation

In vivo and in vitro models are critical tools for understanding the pathophysiology of neurological diseases, developing new treatment methods, and improving existing therapies. In vivo models provide the most comprehensive representation of the complexity and realism of biological processes and diseases, allowing researchers to observe systemic responses and interactions in living organisms (Van der Staay, 2006). Conversely, in vitro models enable detailed studies at the cellular and molecular levels, often delivering faster and more cost-effective results (Breslin & O'Driscoll, 2013).

Both models have their advantages and limitations, and combining them enhances the accuracy and validity of research findings. Hybrid approaches and the use of multiple models allow for the validation of cellular-level findings in living organisms, leading to more comprehensive outcomes (Hughes, 2008). In the future, technological advancements and new model approaches will expand the capabilities and applications of these models.

Future Research Directions

Future research will continue to explore the impact of technological advancements and new modeling approaches on biomedical research. The expanded use of CRISPR-Cas9 and other gene-editing technologies will enable precise genetic modifications of disease models, facilitating a deeper understanding of disease mechanisms (Hsu et al., 2014). Additionally, technologies like organ-on-a-chip and 3D bioprinting will drive the development of more realistic and complex in vitro models, accelerating drug discovery processes (Bhatia & Ingber, 2014).

The rise of personalized medicine will promote the development of patient-specific treatment strategies. iPSCs and other stem cell technologies will enable the creation of customized disease models based on patients' genetic and biological characteristics. This will allow for more effective treatment of diseases and optimization of therapeutic responses on an individual level (Takahashi & Yamanaka, 2006). Furthermore, artificial intelligence and machine learning will support the analysis of large datasets, facilitating early disease diagnosis and personalized treatment approaches (Esteva et al., 2019).

References

Antoni, D., Burckel, H., Josset, E., & Noel, G. (2015). Three-dimensional cell culture: A breakthrough *in vivo*. *International Journal of Molecular Sciences*, 16(3), 5517-5527.

Bailey, J., Thew, M., & Balls, M. (2014). An analysis of the use of animal models in predicting human toxicology and drug safety. *Alternatives to Laboratory Animals*, 42(3), 181-199.

Bhatia, S. N., & Ingber, D. E. (2014). Microfluidic organs-on-chips. *Nature Biotechnology*, 32(8), 760-772.

Breslin, S., & O'Driscoll, L. (2013). Three-dimensional cell culture: the missing link in drug discovery. *Drug Discovery Today*, 18(5-6), 240-249.

Collins, F. S., & Varmus, H. (2015). A new initiative on precision medicine. *New England Journal of Medicine*, 372(9), 793-795.

Eichenbaum, H. (2016). Still searching for the engram. *Learning & Behavior*, 44(3), 209-222.

Emborg, M. E. (2007). Nonhuman primate models of Parkinson's disease. *ILAR Journal*, 48(4), 339-355.

Esteva, A., Robicquet, A., Ramsundar, B., Kuleshov, V., DePristo, M., Chou, K., ... & Dean, J. (2019). A guide to deep learning in healthcare. *Nature Medicine*, 25(1), 24-29.

Gstraunthaler, G. (2003). Alternatives to the use of fetal bovine serum: serum-free cell culture. *Altex*, 20(4), 275-281.

Hartung, T. (2007). Food for thought ... on cell culture. *Altex*, 24(3), 143-147.

Hatipoğlu, D, Ateş, BM, Şentürk G, Bulut A. (2024). Metabolomic modelling and neuroprotective effects of carvacrol against acrylamide toxicity in rat's brain and sciatic nerve. Clinical and Experimental Pharmacology and Physiology, 51(3), e13841.

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157(6), 1262-1278.

Hughes, I. F. (2008). The role of animal models in understanding human disease. *Journal of Applied Animal Research*, 33(1), 1-12.

Israel, M. A., Yuan, S. H., Bardy, C., Reyna, S. M., Mu, Y., Herrera, C., ... & Goldstein, L. S. (2012). Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature*, 482(7384), 216-220.

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, 35(2), 63-75.

Kanan, D. D., Güney, Ö., & Aksu, F. (2022). Exosomes and their relation to neurodegenerative diseases. Sağlık Bilimleri Dergisi. DOI:10.34108/eujhs.861542.

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. J. (2013). *Principles of Neural Science*. McGraw-Hill.

Karran, E., & Hardy, J. (2014). A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Annals of Neurology*, 76(2), 185-205.

Kısadere, İ., Aydın, M. F., Usta, M., & Dönmez, N., (2021). Protective effects of oral melatonin against cadmiuminduced neurotoxicity in Wistar rats. *Arhiv za higijenu rada i toksikologiju,* 72, 157-163.

Kısadere, İ., Dönmez, N., & Dönmez, H. H., (2019). The effects of quercetin on antioxidant and cytokine levels in rat hippocampus exposed to acute cadmium toxicity. *Journal of Cellular Neuroscience and Oxidative Stress,* Supp 1, 10.

Kısadere, İ., Karaman, M., Aydın, M. F., Dönmez, N., & Usta, M. (2022). The protective effects of chitosan oligosaccharide (COS) on cadmium induced neurotoxicity in Wistar rats. *Archives of Environmental and Occupational Health,* 77(9), 755-763.

Lancaster, M. A., Renner, M., Martin, C. A., Wenzel, D., Bicknell, L. S., Hurles, M. E., ... & Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. *Nature*, 501(7467), 373-379.

Müller, C. M., Buchs, P. A., & Stoppini, L. (2003). Organotypic culture of rat hippocampus. *Nature Protocols*, 3(4), 519-526.

Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., ... & Vassar, R. (2006). Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *Journal of Neuroscience*, 26(40), 10129-10140.

Pampaloni, F., Reynaud, E. G., & Stelzer, E. H. (2007). The third dimension bridges the gap between cell culture and live tissue. *Nature Reviews Molecular Cell Biology*, 8(10), 839-845.

Perlman, R. L. (2016). Mouse models of human disease: An evolutionary perspective. *Evolution, Medicine, and Public Health*, 2016(1), 170-176.

Phillips, K. A., Bales, K. L., Capitanio, J. P., Conley, A., Czoty, P. W., t Hart, B. A., ... & Voytko, M. L. (2014). Why primate models matter. *American Journal of Primatology*, 76(9), 801-827.

Prince, M., Wimo, A., Guerchet, M., Ali, G. C., Wu, Y. T., & Prina, M. (2015). *World Alzheimer Report 2015: The Global Impact of Dementia*. Alzheimer's Disease International.

Ropper, A. H., & Samuels, M. A. (2009). *Adams and Victor's Principles of Neurology*. McGraw-Hill Medical.

Stoppini, L., Buchs, P. A., & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *Journal of Neuroscience Methods*, 37(2), 173-182.

Swindle, M. M., Makin, A., Herron, A. J., Clubb Jr, F. J., & Frazier, K. S. (2012). Swine as models in biomedical research and toxicology testing. *Veterinary Pathology*, 49(2), 344-356.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126(4), 663-676.

Van der Staay, F. J. (2006). Animal models of behavioral dysfunctions: basic concepts and classification, and an evaluation strategy. *Brain Research Reviews*, 52(1), 131-159.

World Health Organization. (2006). *Neurological Disorders: Public Health Challenges*. World Health Organization.

Xicoy, H., Wieringa, B., & Martens, G. J. (2017). The SH-SY5Y cell line in Parkinson's disease research: a systematic review. *Molecular Neurodegeneration*, 12(1), 1-11.

Yamanaka, S. (2020). Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell*, 27(4), 523-531.