Unveiling the link between normal development and brain cancer formation

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OTKRIVANJE VEZE IZMEĐU NORMALNOG RAZVOJA I NASTANKA RAKA MOZGA

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ABSTRACT

Neural stem cells are a group of cells capable of self-renewal and differentiation into various subtypes. Proper regulation of these cells is essential for normal development and function of the central nervous system. When this regulation fails, it can result in the formation of tumors, such as gliomas.

In this thesis, we tried to clarify the relationship between neurogenesis and tumor formation, emphasizing the molecular and cellular mechanisms that potentially drive the process of tumorigenesis. By synthesizing current research on the expression of transcription factors and genes involved in both neurogenesis and tumorigenesis, this thesis aims to highlight critical pathways and factors in brain tumor development. Identifying the cell of origin for each tumor type can reveal lineage-specific therapeutic vulnerabilities and opportunities to detect early malignant or even pre-malignant abnormal cell states, as certain cells may be more susceptible to oncogenic attacks than others. Understanding these mechanisms may reveal new therapeutic targets and strategies for preventing and treating brain cancer.

Keywords: gliomas, neurogenesis, tumorigenesis, regulatory mechanisms

SAŽETAK

Neuralne matične stanice su skupina stanica koja ima sposobnost samoobnavljanja i diferencijacije u različite podtipove. Pravilna regulacija ovih stanica ključna je za normalan razvoj i funkcioniranje središnjeg živčanog sustava. Kada ova regulacija zakaže, to može rezultirati formiranjem tumora, kao što su gliomi.

U ovom radu, pokušali smo razjasniti odnos između neurogeneze i nastanka tumora, ističući molekularne i stanične mehanizme koji potencijalno pokreću proces tumorigeneze. Pregledom trenutnih istraživanja o ekspresiji transkripcijskih faktora i gena uključenih i u neurogenezu i u tumorigenezu, ovaj rad nastoji istaknuti ključne puteve i faktore u razvoju tumora mozga. Identificiranje podrijetla stanice za svaku vrstu tumora može otkriti specifične terapijske slabosti i mogućnost ranog otkrivanja malignih ili čak pre-malignih abnormalnih stanja stanica, budući da određene stanice mogu biti osjetljivije na onkogene napade od drugih. Razumijevanje ovih mehanizama može otkriti nove terapijske mete i strategije za prevenciju i liječenje raka mozga.

Ključne riječi: gliomi, neurogeneza, tumorigeneza, regulatorni mehanizmi

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1. Introduction

One of the biggest challenges in modern medicine is posed by brain cancers, particularly gliomas. Gliomas are the most common type of malignant primary brain tumor, responsible for 80.8% of all cases. They are characterized by high occurrence, so despite advances in medical research, the survival rate for patients suffering from gliomas is very low (1).

What characterizes gliomas is the fast rate at which glioma cells multiply and their high ability to infiltrate surrounding tissues. Because of this, surgical procedures cannot completely remove all glioma cells, causing their recurrence. This also lowers the efficiency of other treatment methods, such as radiation therapy and chemotherapy (2).

Cancer cells are believed to change the signaling pathways by which normal cells control their cell proliferation, differentiation, and survival. The process of glioma development is called gliomagenesis, and it shares several similarities with the process of forming new neurons, called neurogenesis (3).

Some of the most important pathways in balancing the proliferation and differentiation of NSCs are Wnt, TGF-ß, and Notch signaling pathways. A change in their regulation can cause abnormal cell behavior, leading to brain cancer development (4). Because of this, we decided to primarily focus on these pathways while exploring the connection between normal development and tumorigenesis in this thesis.

A significant amount of research suggests that gliomas derive from genetically aberrant cells with neuroglial stem cell-like properties by reactivation of different stem cell-like development gene programs, and this causes the cells to multiply uncontrollably and form tumors. Dysregulation of the normal cell cycle is possible due to the dysregulation of key signaling

pathways and factors that link normal development with tumor formation (3,4), which highlights the need to better understand these mechanisms.

Scientists have developed various experimental models in order to better understand how these key pathways function. Some of these models are used to deepen the already existing knowledge by gaining important information regarding the molecular mechanisms causing the formation of gliomas (5,6). Single-cell RNA sequencing enables the study of glioma heterogeneity at the level of individual cells. This allows researchers to pinpoint what exactly caused the formation of glioma cells and opens the possibility of developing treatments that target the exact cause of the disease (7).

2. Thesis objectives

This thesis aims to compare neurogenesis with tumorigenesis while focusing specifically on brain tumors known as gliomas. The core objective is to identify the abnormalities that occur during tumorigenesis compared to regular brain development.

Studies in animals show that specific transcription factors and genes are expressed at certain development time points to facilitate the development of specific cell types. It has been hypothesized that similar processes occur in tumors, given that there is often a recognizable pattern in the cellular changes during tumorigenesis. By comparing the regulatory mechanisms involved in neurogenesis and tumorigenesis, we tried to pinpoint critical pathways and factors changed during gliomas' formation. This comparison could provide valuable insights into potential therapeutic targets, better prevention strategies, and better brain cancer treatment.

3. Stem cells and neurogenesis

Stem cells are a group of cells that can self-renew and differentiate into various types of cells (8,9) and are present in both embryo and adult tissues (10). Stem cells can either be totipotent, pluripotent, multipotent, oligopotent, or unipotent based on their ability to differentiate.

Cells with the lowest potential to differentiate are called unipotent stem cells. Unipotent stem cells can develop into only one type of cell (10). Oligopotent stem cells share some similarities with multipotent stem cells but are more restricted. They can specialize into a couple of closely related cell types. Next are multipotent stem cells that have a higher differentiation ability, and that allows them to develop into various types of cells inside a specific tissue. Pluripotent stem cells can develop into different types of cells found within ectoderm, mesoderm, and endoderm, but cannot develop into extraembryonic structures such as the placenta (11). Totipotent stem cells have the highest differentiation potential, allowing them to differentiate into any cell type within an organism (11).

3.1. Neurogenesis

To fully understand how our CNS functions, we need to better understand the mechanisms responsible for its development and regulation. The process by which new neurons are formed in the CNS is called neurogenesis.

The generation of neuronal diversity involves spatial and temporal patterning mechanisms, where a single progenitor sequentially produces multiple cell types (12).

In *Drosophila melanogaster* and vertebrates, neural stem cells undergo multiple rounds of asymmetric division to self-renew and produce intermediate progenitors and neurons of distinct fates. These cells can either divide symmetrically or asymmetrically (13).

Neural progenitor cells can either be classified as type 0, I, or II. Type 0 cells divide asymmetrically multiple times, generating one new neuron. Type I also divides asymmetrically, but they do not directly generate neurons, but a ganglion mother cell. Those cells later symmetrically divide into either two neurons or glial cells. Type II cells are a bit different, and they divide to self-renew and produce multiple intermediate neural progenitors. Those intermediate neural progenitors later divide asymmetrically to produce a few ganglion mother cells, hence they produce lineages with a larger number of neurons (12,13).

The process of temporal patterning was described for the first time in the ventral nerve cord of *Drosophila melanogaster*. In developing ventral nerve cord, type I neuroblasts undergo a series of temporal identity windows during which they express certain temporal transcription factors: Hunchback, Krüppel, POU domain proteins, Castor, and Grainy head, depending on how old they are (12,13). Each of these factors plays an important role in defining the fate of a particular cell, for example, Hunchback stimulates early-born, deep-layer neurons, while Castor plays a role in deciding fates of later-born, superficially located neurons (13).

The best evidence for a cascade of temporal transcription factors in vertebrates is the mammalian retina, specifically the mouse retina, where various temporal transcription factors have an important role in generating the retinal neurons. The Ikaros family zinc finger protein 1 (Ikzf1) is important for forming the retinal ganglion cells, amacrine, and horizontal cells. This protein is expressed in early retinal progenitor cells because it plays a significant role in early development. Transcription factors expressed during mid-neurogenesis are called Pou2f1/Pou2f2, and they have a role in controlling the production of cone photoreceptors and horizontal cells. Additionally, another temporal transcription factor is expressed in mid-neurogenesis. This temporal transcription factor of the

Fox domain is called FoxN4, and it has a role in the formation of amacrine, horizontal, cone, and rod cells (12).

Intrinsic temporal factors that are present in the neurogenesis of vertebrates are often homologous to those found in *Drosophila melanogaster*. A mammalian temporal transcription factor called Ikaros is an ortholog of the Hunchback factor found in *Drosophila melanogaster*. Additionally, another transcription factor found in mammals called Caz1 is an ortholog of *Drosophila melanogaster* transcription called Castor (13).

In both invertebrates and vertebrates, conserved mechanisms of temporal and spatial patterning enable neural progenitors to produce a wide variety of cell types in the CNS. With the evolution of the brain and its increasing complexity, new types of progenitors have emerged (13).

Understanding the temporal and spatial factors that determine neuronal diversity will be crucial for generating the desired neuronal types for cell replacement therapy (12).

3.2. Neural stem cells

Neural stem cells (NSCs), known as being multipotent, are very important for the normal functioning of the central nervous system (CNS). These cells primarily differentiate into neurons, oligodendrocytes, and astrocytes (8). Neurogenesis, a process of forming new neurons, is at its peak during prenatal development but occurs even during adulthood in restricted brain regions (10).

One of the abilities that NSCs possess is the ability to suppress tumors (8). For example, NSCs are able to inhibit tumor formation when they are transplanted with mouse glioma cells (14). In order to use the information gathered in stem cell research into practical therapies, researchers have to understand the molecular mechanisms that control the growth, specialization, and migration of neural stem cells (8).

3.3. Neurogenesis in the adult brain

Adult neurogenesis occurs throughout life in restricted brain regions (8). The adult brain's ability to regenerate some neurons is crucial for maintaining normal cognitive functions. Initially observed in songbirds and rodents (8), the discovery of adult neurogenesis was a significant breakthrough in neuroscience, and the later discovery of NSCs within the human brain significantly improved our understanding of neurogenesis.

In mammals, two primary germinal zones retain neurogenic activity in adulthood: the dentate gyrus and the subventricular zone (8,10). These two areas are well-documented areas where neurogenesis in adults occurs, but whether neurogenesis is possible in other brain regions in adults is still debatable.

Even though neurogenesis in the adult brain isn't as prevalent as during prenatal development, it is still important for daily life. For example, hippocampal neurogenesis is very important for processes such as learning, memory, and regulating the way we respond to stress (8). One way in which a person can enhance neurogenesis is through physical activity. This was shown in animal models, but research on human neurogenesis is increasing (10).

Regulation of adult neurogenesis in mammals involves both internal and external factors. Internal regulation includes transcription factors, epigenetic modifications, and metabolic pathways. External regulation includes various signaling molecules and signals from the bloodstream, as well as both mature and immature neurons. Both internal and external signals can influence one another (15).

Stem cell proliferation and differentiation is a strictly regulated process (8) because the changes in regulation can lead to tumor development.

4. Tumor biology

When tumor cells divide uncontrollably, they form a mass of mutated cells called a neoplasm. This process involves two main stages: tumor initiation and progression (4).Mutations primarily associated with genes that regulate the cell cycle and cell survival characterize tumor initiation, causing the abnormal proliferation of a single cell into multiple abnormal clones. As the number of mutations continues to increase, different types of cells start to develop, and the tumor continues to progress.

Stem cell-like cancer cells are a group of cells within a solid tumor. They share some characteristics with normal stem cells, such as the ability for self-renewal, which makes it particularly difficult to destroy them during patient treatments (2).

4.1. Brain tumors

Brain tumors are a result of abnormal division of cells found in different parts of the brain, including neurons, glial cells, and meninges. We can categorize these tumors as primary, secondary, malignant, or non-malignant (1).

One way to know whether a tumor is a primary or a secondary tumor is to know where and how it formed. Primary brain tumors develop from cells that can be located either in the brain or in its tight surroundings. On the other hand, secondary brain tumors form as a result of metastasis from other body parts. The formation of secondary brain tumors typically indicates malignant nature, while primary brain tumors may also manifest as benign growths.

Out of all the different types of brain tumors, gliomas are the most common type (16) and they are responsible for 80.8% of all cases (1). It is very common for a person who had a glioma in the past to develop it again despite finishing cancer treatment, so despite advances in medical

research, the survival rate for patients suffering from gliomas is very low. Gliomas usually occur in patients aged 50 to 70 (8).

4.2. Glioma classification

The World Health Organization (WHO) grades glioma from 1 to 4, based on how aggressive they are and how fast do their cells divide. Slower-growing gliomas, categorized as "low grade," include grades 1 and 2. On the other hand, "high grade" gliomas include grades 3 and 4. The most aggressive brain tumor, glioblastoma, is always classified as grade 4. It is one of the deadliest human cancers, with an average life expectancy of around one year after it is diagnosed (17,18).

The classification of CNS tumors was updated in 2021 and now includes the latest advancements in molecular profiling. After using the morphological characteristics system for decades, the classification system has shifted towards a molecular-based approach (18). This new classification now includes mutations in isocitrate dehydrogenase (IDH), whether there is codeletion of 1p/19q and specific molecular markers that impact tumor behavior (19).

One of the most clinically relevant information for treating gliomas is their subtype. In the context of mutations in the IDH gene, there are two main types of gliomas: those with IDH mutations and those without. Astrocytomas and oligodendrogliomas are specific types of glioma identified by mutations in the IDH1 or IDH2 genes. Gliomas that do not have these mutations, for example glioblastomas, are called IDH-wildtype gliomas.

In the context of the 1p/19q codeletion status, a certain type of glioma can either have this mutation or not have it. Oligodendrogliomas are a type of glioma that contains 1p/19q codeletion, while on the other hand astrocytomas typically do not have this codeletion.

Another subtype of gliomas is H3-mutant gliomas, characterized by changes in the histone H3 gene. Diffuse midline gliomas, particularly those that

contain the H3 K27M mutation, are known as being among the most common H3-mutant gliomas (18).

4.3. Gliomagenesis

Malignant glioma, known for its infiltrative nature and high lethality, is the most invasive and aggressive primary brain tumor found in adult patients. These tumors develop and progress through gliomagenesis, a process that is characterized by changes in signaling pathways that drive tumor formation. Notably, pathways such as Wnt, Notch, and TGF-ß, crucial for normal neural development and tumorigenesis, are also important in this process (3).

4.3.1. The Wingless/Int1 (Wnt) signaling pathway

The Wnt signaling pathway has a crucial role in controlling the ability of NSCs to self-renew, differentiate, and proliferate (3,20). It is active during various CNS development stages (20), engaging through either the canonical or non-canonical pathways. The canonical Wnt pathway relies on β-catenin, which is critical for gene transcription regulation and cell fate determination. In contrast, the non-canonical pathway operates independently of β-catenin, often influencing cellular processes such as movement and polarity (3,20).

The Wnt pathway inhibits ubiquitylation and degradation of transcription factors, which makes them active (4). When Wnt proteins bind to a complex composed of Frizzled and LRP receptors (21) they disrupt the destruction complex formed by Dvl, AXIN, APC, and GSK-3ß. The breakdown of this complex initiates a signaling cascade that causes the stabilization of β-catenin. Within the Wnt signaling pathway, ß-catenin is a protein that functions as a transcriptional activator (4).

When Wnt signaling is absent, β-catenin undergoes phosphorylation by GSK-3ß located on a destruction complex, leading to its degradation (Figure 1.A). However, when Wnt binding is present, the destruction complex gets

disrupted, and that prevents β-catenin from degrading. The lack of degradation leads to the translocation of β-catenin to the cell nucleus. In the nucleus, ß-catenin associates with Tcf transcription factors (21) and activates target genes involved in Wnt cell signaling and determining cell fate (4) (Figure 1.B).

Mutations that block the process of β-catenin breakdown when Wnt signaling is inactive lead to abnormal activation and regulation of the Wnt pathway (Figure 1.C), and that causes uncontrolled cell proliferation leading to tumor growth (3).

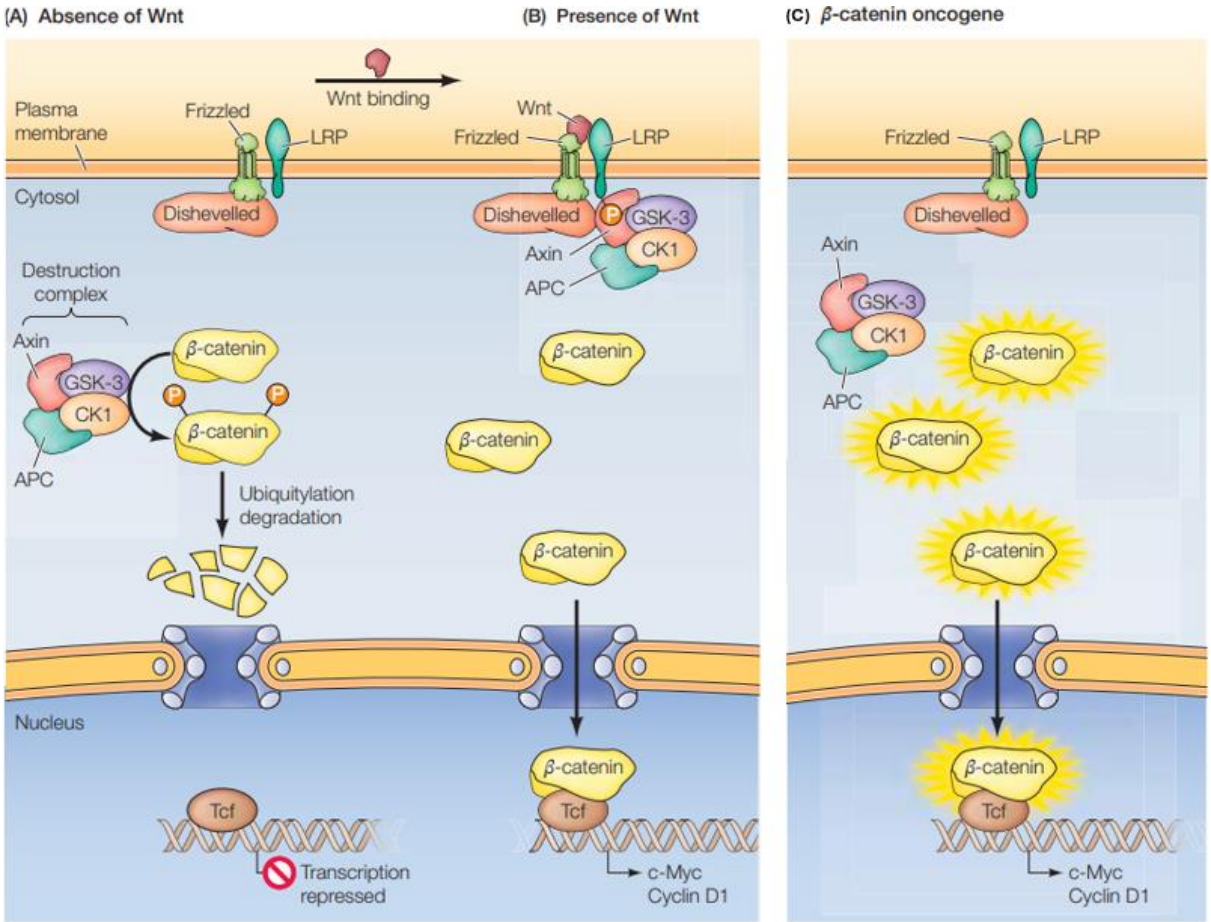

Figure 1. ***The mechanism of Wnt pathway and oncogenic activity of ß-catenin.*** *(A) When Wnt is absent in the normal cell, β-catenin is broken down. (B) When Wnt binds to receptors, it stabilizes β-catenin, allowing it to enter the nucleus and affect gene activity. (C) When mutations disrupt the Wnt pathway and stop ß-catenin from degrading, it changes into an*

oncogene (4)*. The figure is adapted from Cooper GM. The Cel:l A Molecular Approach. 8th ed., 2018.*

The non-canonical Wnt pathway, specifically a ligand that activates this pathway called Wnt-5a (22) is crucial for the invasion regulation of human glioma stem cells (GSCs) *in vivo* (3). Wnt-5a promotes the invasive migration of tumor cells by controlling the activity of matrix metalloproteinase, which breaks down the extracellular matrix (ECM). However, since it can inhibit tumor proliferation and metastasis, Wnt-5a also acts as a tumor suppressor (21). These opposing functions show that further tests are needed to better understand the roles of this non-canonical Wnt ligand, and that can cause an improvement in current therapeutic approaches (22).

4.3.2. The TGF-ß/Smad pathway

TGF-ß, short for transforming growth factor ß, is a polypeptide with an important role in controlling cell proliferation and differentiation, the process of apoptosis (23), and the migration of normal and tumor cells (3,4,20). This pathway ensures a balance between the differentiation and proliferation of neurons and glial cells, contributing to proper brain development and function.

Within the human body, we can find at least 30 types of TGF-β proteins, and each of them is able to induce unique and important response in target cells. This diversity is the consequence of interactions between different type I and type II receptors, which activates different Smad family members (4). The TGF-β/Smad pathway involves a receptor-linked protein kinase that phosphorylates and triggers the activation of a transcription factor (23). Specifically, TGF-β receptors are serine/threonine kinases (4).

The TGF-β receptors consist of two different polypeptides known as type I and II. (23). After the ligand binds, the type II receptor encourages the type I receptor to undergo phosphorylation, which then causes the phosphorylation of Smad transcription factors. When Smads are

phosphorylated, they create complexes that migrate to the nucleus, making the expression of specific genes easier (4)(Figure 2).

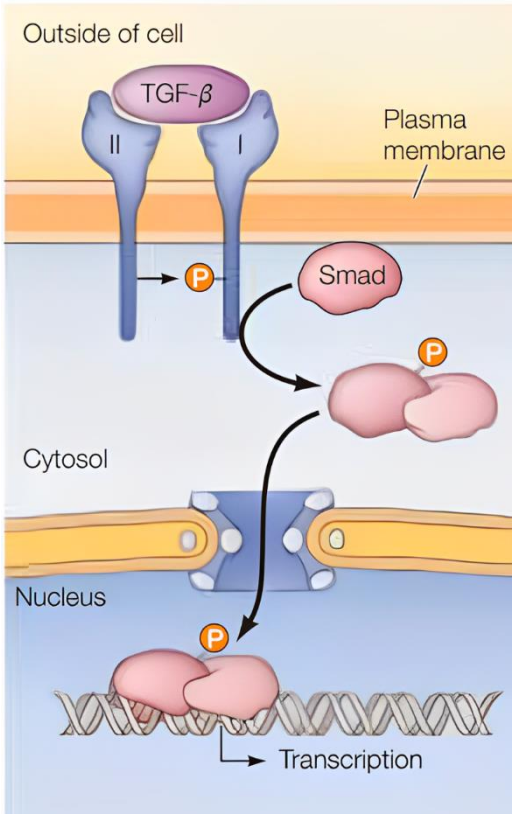

Figure 2. ***The mechanism of TGF-ß/Smad pathway.*** *TGF-β signaling involves type I and II receptors dimerizing. The type II receptor activates type I, which then phosphorylates Smad proteins. These Smads form complexes and move to the nucleus to activate target gene transcription* (4)*. The figure is taken from Cooper GM. The Cell: A Molecular Approach. 8th ed., 2018.*

Like the Wnt pathway ligand, TGF-β has opposing roles. TGF-ß has a tumor-suppressing role during tumor development by restricting glial cell growth (20,23). On the other hand, TGF-ß also functions as an oncogene during tumor invasion and migration in various cancers, including glioblastoma (3,20,23).

4.3.3. The Notch pathway

The Notch pathway is very important for early neurodevelopment and maintaining the NSC population (20), while also having an important role in the process of organ formation and tissue repair (24).

It involves direct interactions between cells that are close to one another, called neighboring cells. In this process, the Notch protein has a role as a receptor for signals that originate from transmembrane proteins, such as Delta, which are placed on nearby cells (4). When a ligand binds to Notch, it triggers a process where Notch is cleaved by γ-secretase. This causes the intracellular domain of Notch to be released and move into the nucleus. In the nucleus, the intracellular domain binds with a transcription factor called CSL (Figure 3). This causes the function of CSL to shift, and CSL becomes a gene activator instead of being a gene suppressor. These genes activated by CSL are involved in producing other regulatory proteins that influence transcription and determine cell fate (4).

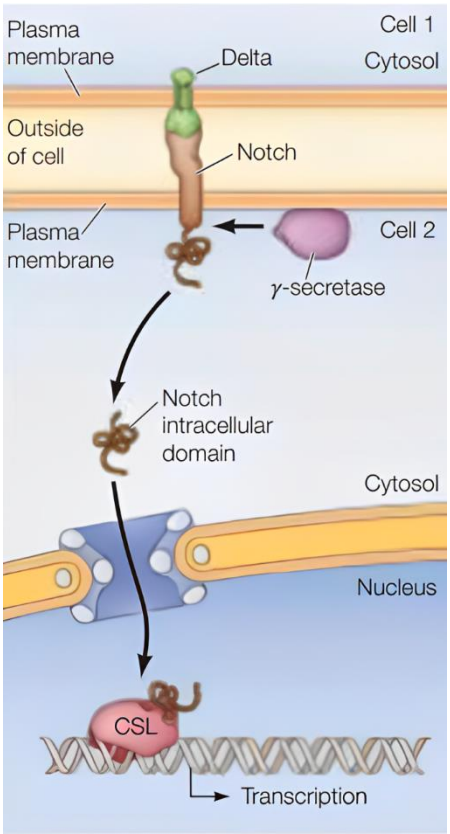

Figure 3. ***The mechanism of the Notch pathway.*** *Notch receives signals from adjacent cells through transmembrane proteins like Delta. Binding triggers Notch cleavage by γ-secretase, releasing its intracellular domain to the nucleus to stimulate gene expression via the CSL transcription factor* (4)*. The figure is taken from Cooper GM. The Cell: A Molecular Approach. 8th ed., 2018.*

Initially, researchers believed that the Notch signaling pathway promoted cancer growth in glioma by maintaining cancer stem cells. However, blocking Notch signaling has decreased tumor development in glioma models. One of the Notch receptors called Notch 1 (24) promotes cancer, particularly when in combination with other mutations such as p53 loss. It has a role in maintaining the ability of self-renewal and increasing the invasion of glioma stem cells. As opposing to that, in certain types of gliomas, Notch may also function as a tumor suppressor. This dual role of Notch in glioma further emphasizes the need for additional research (24).

4.4. Glioblastoma heterogeneity

Glioblastoma (GBM) is classified as grade 4 glioma by WHO (19) which means that it is one of the most aggressive and deadly forms of CNS tumors (25,26). It shows significant intratumoral heterogeneity (7,27), which significantly impacts the advancement of the disease and its prognosis. When we say that a certain tumor exhibits intratumoral diversity, that means that different cell populations can be found within that tumor (27). It is hypothesized that the reason which causes cellular heterogeneity could be the transition of tumor cells into a stem-cell like state. We can divide glioblastoma into different subtypes that vary within the same tumor (25), and this diversity contributes to their ability to adapt and resist conventional treatments, causing significant problems for successful therapy.

4.4.1. Inights from single-cell RNA sequencing

In the last ten years, single-cell transcriptomics has significantly improved the molecular profiling of GBM cells, by improving the understanding of the

genetic and molecular features that contribute to the development and advancements of tumors (7).

Single-cell RNA sequencing (scRNA-seq) allows us to analyze genes expressed at the single-cell level by isolating thousands of individual cells from a tumor sample, sequencing their RNA, and analyzing the data to identify different cell populations and their gene expression profiles.

Recently scRNA-seq methods have greatly improved, primarily because of the development of microfluidic devices that make cell barcoding easier. These devices enable parallel sequencing of thousands of cells. The process of droplet-based scRNA-seq typically involves the following steps: creating a suspension of single cells from a tissue, encapsulating the cells with a uniquely barcoded microparticle in a droplet, lysing cells after their encapsulation, capturing a cell's mRNAs on its corresponding microparticle, performing reverse transcription, amplification, and RNA sequencing of thousands of cells in a single reaction, and using the barcodes to determine the cell of origin for each of the transcripts (28).

This technique has revealed the presence of glioma stem cells. These cells are able to self-renew and adapt into different cell types within the tumor using the same mechanisms which are used by normal stem cells (24) and that increases their growth and ability to withstand treatment. A significant number of studies have linked this small subpopulation within GBM to intratumoral heterogeneity, treatment resistance, and relapse (19,29).

While significant advancements in scRNA-seq have been achieved, it is essential to acknowledge the challenges that still need to be taken care of, such as high costs (7). In the future, focus should be on developing predictive models which incorporate different bioinformatical, statistical and machine learning tools. Using these techniques would allow us to predict various future biological outcomes. The main goal in developing these models is to enable them to predict specific cellular behaviors, response to treatment, and disease progression by analyzing the data collected by

analyses of cells at the single-cell level. This has the potential to significantly increase our understanding of disease mechanisms and improve therapeutic strategies (7,19,27).

5. Experimental models in studying brain tumors

Brain tumors usually have a very high mortality rate and can impact people of all age groups. The primary types of brain tumors include gliomas, meningiomas, ependymomas, and medulloblastomas (30). Researchers have developed different types of models to study brain tumors, but many of the existing models lack reliability because of factors such as tumor heterogeneity. Some of the existing *in vivo* glioma models are mouse models, *Drosophila melanogaster* models, and zebrafish models (5,6). Accurate and trustworthy models are very important for better understanding gliomas and improving patient treatment (6). Each model type has its own strengths and weaknesses, which should be considered in research and during therapy development.

5.1. *In vivo* models

5.1.1. Mouse models

In Xenograft models, cells derived from patients or established cell lines (e.g., U251, U87) are injected into mouse brains. These models include Patient-Derived Xenografts (PDX) (6), which maintain the characteristics of the original tumor, but they lack an intact immune system (30).

Carcinogen-induced models are developed by inducing tumors in mice using carcinogens like ethyl-nitrosourea (6) or methylcholantrene. These models allow scientists to study the interactions that happen in the immune system of tumors. Still, it is important to not forget that these models do not entirely replicate human gliomas (30).

Genetically Engineered Mouse Models (GEMMs) are created by introducing specific genetic modifications that are targeted in order to investigate the initiation and progression of tumors (31). Common genetic modifications include PDGFA/B amplification and mutations in KRAS, EGFR, and TP53. These models also allow scientists to study the interactions within the tumor immune system and how different mutations affect tumor behavior.

However, these models lack intra-tumor heterogeneity and have differences in the blood-brain barrier when compared to humans (30).

5.1.2. *Drosophila melanogaster* models

Drosophila melanogaster is a good model for glioma research because of extensive genetic tools and conserved signaling pathways found in this animal. Key pathways involved in glioma, such as EGFR and PI3K (5) are conserved in *Drosophila,* and that makes it a relevant model for genetic screens and pharmacological tests (30).

One of the *Drosophila* glioma models involves perturbing EGFR and PI3K signaling (5), leading to diffuse glial neoplasia and helping identify genes like dRIOK1 and dRIOK2, which encode proteins that are involved in the regulation of the cell cycle. Another type of *Drosophila* model helps to understand the effect that GSCs have on a tumor by showing how transcription factors like FOXD1 and ASCL1 can affect tumor processes such as growth and cell differentiation (30).

However, *Drosophila* models do not have an adaptive immune system, and that limits the research of tumor immune interactions. These models are also not able to completely replicate human brain tumor heterogeneity. Despite these limitations, but due to powerful genetic tools, *Drosophila* remains a valuable model for genetic and pharmacological studies, significantly aiding glioma research (30).

5.1.3. Zebrafish models

Zebrafish (*Danio rerio*) is a model that is frequently used for glioma research because of the rapid embryonic development, small size, and transparency of zebrafish, which makes it easier to test various treatments (6). Induction of gliomas in transgenic zebrafish often involves the activation of pathways such as EGFR/RAS/ERK/AKT, and that results in the formation of tumors that resemble mesenchymal GBMs (30).

Knockout models, such as nf1a/nf1b, help researchers understand the role of specific genes in tumor formation. In knockout models, scientists can disable certain genes, which allows them to examine the changes that will follow in the organism. This allows researchers to better understand the roles certain genes play in the development of cancer.

Additionally, xenotransplantation of human glioma cells into zebrafish allows live imaging of how tumors grow and spread (30).

Zebrafish, which lack an early-stage adaptive immune system, offer advantages over other animal models for this type of research because of the previously mentioned transparency, small size, and fast embryonic development. However, studying drug behavior in zebrafish remains challenging due to physiological differences when compared to humans (6,30).

5.2. Organoid models

Organoids are 3D models of human cell cultures. They are obtained from pluripotent stem cells and are able to imitate certain aspects of the development of the human brain, and that allows researchers to study neurodevelopment *in vitro* (32).

Methods such as 3D culturing, 3D printing, and bioengineering are used in these types of models and are a significant improvement in glioma research (32). They closely mimic the 3D architecture, cellular diversity, and functional complexity of human organs. These models do not have a cardiovascular network and a correct tumor microenvironment, but they are often used for the drug screening process and genetic studies. Researchers are working on finding a way to potentially fix the mentioned limitations in order to increase the usefulness of these models (30). The further development of organoid models is important because they allow researchers to perform experiments that are challenging to perform with animal models or humans. These models provided valuable information

about the stages of neurodevelopment that were previously impossible to obtain, providing researchers with valuable information that can be potentially used in improving existing patient treatments (32).

5.2.1. Patient-Derived Organoids

Patient-derived organoids are the first glioblastoma organoids created from patient biopsies. These organoids, cultivated in Matrigel-based 3D cultures, replicate several GBM characteristics, such as hypoxia. In 2020, researchers developed a method to derive and preserve patient-derived glioblastoma organoids (PDOs) without needing growth factors. PDOs maintain certain traits of the original tumor, making them a valuable tool for studying individual treatment responses and tumor-immune interactions (30).

5.2.2. Genetically Engineered Cerebral Organoids

Using 3D human organoids for disease modeling has the potential to connect conventional animal models with human studies (32). Genetically engineered organoids are made by using genome-editing techniques, for example CRISPR-Cas9, in order to cause specific oncogenic mutations in human cerebral organoids (33).

NeoCORs (neoplastic cerebral organoids) are a type of genetically engineered cerebral organoids that was developed in 2018. These specific models share certain characteristics, such as the magnitude of expansion and invasion *in vivo*, with cancer cells. NeoCORs allow scientists to study how genetic abnormalities affect cell function in a controlled environment where the genetic background is consistent. This allows researchers to understand the effect of specific genome changes without the variations caused by different genetic backgrounds (33).

In organoids which are derived from patient-induced pluripotent stem cells, neoCORs can be used to see how different individuals react to specific genetic mutations that can drive diseases such as cancer (33).

6. Conclusion

In conclusion, this thesis aims to unveil the link between normal development and brain cancer formation while focusing primarily on gliomas. Through a literature review, we tried to clarify some of the signaling pathways crucial for cell proliferation and differentiation, such as TGF-ß, Notch, and Wnt, that connect normal development with tumorigenesis.

Brain tumors such as gliomas, specifically glioblastomas, are characterized by tumor heterogeneity, which makes them one of the most aggressive forms of brain tumors and resistant to treatments. The use of various experimental models, such as animal models, organoids, and single-cell RNA sequencing, highlights the importance of glioma heterogeneity in understanding tumor behavior and resistance to treatment.

In this thesis, we highlighted the potential mechanisms that connect neurogenesis and brain cancer development. If tumor cells change those pathways, oncogenic pathways are activated, followed by the inactivation of tumor-suppressing mechanisms. Despite the progress made in recent years regarding normal development and tumorigenesis, further research is still necessary to develop therapies that target the source of glioma origin, which can improve patient treatments or even stop glioma from forming. Additionally, researchers still need to develop more advanced and clinically relevant models because existing experimental models do not fully replicate human brain tumors.

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INTERNSHIP

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Internship at Research and Development Laboratory of PharmaS Company

Engagement in capsule encapsulation;
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LANGUAGE SKILLS

Mother tongue(s): **CROATIAN**

Other language(s):

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken production	Spoken interaction	
ITALIAN	B2	B1	B1	B1	B1
ENGLISH	C1	C1	C1	C1	C1

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VOLUNTEERING

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Project "Putujući znanstvenici"

Demonstration and explanation of various experiments to kindergarteners.

PROJECTS

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STEM Games coordinator

Managed the coordination of the STEM Games project;
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