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Review

Comparative Characterization of High-Grade Glioma Models in Rats: Importance for Neurobiology

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Abstract

Glioblastoma (GB) is a fatal disease with extremely low survival. Combined treatment is being developed to prolong life, alleviate physical and mental state, which combines standard therapy with cognitive-behavioral interventions. Rats are the most accessible models for the preclinical study of physiology, behavior, and cognitive functions. CNS tumor models vary; among the differences are the rat strain, the glioma cell line, the carcinogen used for its induction, the tumor growth pattern and the rate of penetration into the surrounding brain tissues, morphology, metabolism, key signaling pathways. In this review, we examine the properties of four main rat strains — Wistar, Long-Evans, Sprague-Dawley, Fischer 344, Lewis and Wistar-Kyoto — and the most commonly used malignant glioma models, including the C6, RG2, F98 and 9L cell lines, as well as the 101.8 and 15/47 tissues. We also discuss the pathophysiology of human glioblastoma growth as a benchmark for modern neuro-oncological research and emphasize the importance of distinguishing between spontaneous and transplantable tumors, as they can contribute to the discrepancy in the results of clinical and preclinical studies. The aim of this review is to analyze factors critical for experimental neuroscience by characterizing rat strains and malignant glioma models.

Keywords: rat glioma models; C6; RG2; F98; glioblastoma; physiology; rat strains; morphology; behavior; therapy

1. Introduction

Glioblastoma (GB) is the most aggressive type of brain tumor in adults, characterized by an extremely low survival rate. Both the tumor and its associated therapy cause neurological and behavioral impairments due to local and systemic changes in the body. Psychosomatic and physiotherapy interventions can improve the physical and psychological health of patients with brain tumors [1]. The survival and quality of life of GB patients are influenced by genetic, environmental, stress-related, social, and behavioral factors [2].

Modeling tumor growth in animals is crucial for studying the fundamental mechanisms of tumor initiation and progression, as well as for identifying promising diagnostic and therapeutic approaches for subsequent clinical research. The immune status of GB patients, along with features of model animals—such as hormonal regulation, sex, strain, and individual differences—significantly affects tumor incidence and survival. Developing effective anti-tumor and palliative therapies requires relevant models that accurately reproduce the morphological, molecular genetic, and physiological features of human GB. The main rat models of malignant gliomas include cell lines (C6, RG2, F98, 9L) and transplantable tissue models (101.8, 15/47, etc.) [3].

A key advantage of transplantable tissue models lies in their diffuse growth pattern, which is characteristic of human GB. In contrast, models based on cell lines predominantly exhibit limited growth, belonging to the restricted glioma subtype; moreover, their frequent extra-cranial and extra-

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cerebral growth complicates the formation of homogeneous experimental groups. Both diffuse human malignant gliomas and animal glioma models possess unique molecular biological parameters, including the production of neurotransmitters, hormones, and their receptors, which also influence survival [4]. Therefore, one potential strategy for combination therapy could involve stimulating or inhibiting the production of neurotransmitters and hormones. This could be achieved locally via inhibitors or systemically through interventions like cognitive-behavioral therapy targeting the reward and reinforcement mechanisms.

Various laboratory rat strains (Wistar, Sprague Dawley, Long-Evans, Fischer, etc.) exhibit physiological differences in anatomy and immune and endocrine systems, as well as behavioral differences in physical activity and stress responses [5]. These and other features of glioma models and rat strains are detailed in our review and must be considered when planning neuro-oncology research. Studies of various physiological and pathological models have demonstrated differences between rat strains, colonies from different commercial suppliers [6], and individual variations [7–9]. These differences are attributed to genetic drift within colonies [7], and genetic and early environmental factors [10].

Rats vary in: Spontaneous tumor incidence [11]; Cerebrovascular anatomy, cerebral blood flow, body weight, and thymus weight [12]; Levels of neurotrophic factors and neurotransmitters, their receptor expression [13], and responses to them; Genetic predisposition to aggression [9]; Amygdala gene expression and glutamate-mediated neuronal excitability [14]; Hypoxic responses [15]; Susceptibility to autoimmune diseases [16]; Noradrenergic spinal cord innervation; Neuropathic pain behavior; Metabolic and endocrine responses, including differences in stress reactivity [17], corticosteroid-binding globulin, and adrenocorticotropic hormone (ACTH) levels [7,10]; Responses to novelty and stress-coping strategies [17].

These differences extend to critical components of the hypothalamic-pituitary-adrenal (HPA) axis, hippocampus-dependent learning, and depression-like behavior, which in turn regulate the immune system and inflammation [4,16]. These factors also influence the aforementioned processes and may affect tumor initiation and growth. A unified approach to the study of GB, combining neurobiology and oncology, is a promising avenue for therapy development. The aim of this review is to analyze factors critical for experimental neuroscience by providing a comparative characterization of rat strains and malignant glioma models.

2. Neurobiology of Glioblastoma, Current Research Directions: From Synaptic Integration to Systemic Immune Evasion

Glioblastoma exhibits the capacity to induce widespread neural plasticity, leveraging the brain's unique ability for functional integration by co-opting its normal physiological processes for its own purposes [18]. Key mechanisms of this integration include the formation of pseudo-synaptic connections with neurons using neurotransmitters (GABA, glutamate, acetylcholine), which redirects neuronal activity to stimulate tumor cell proliferation [19]; the seizure of astrocyte mitochondria to obtain energy and metabolic precursors [20]; and the co-option of blood vessels to ensure nutrient supply [21]. Thus, tumor cells use the usual cellular biological processes, significantly redistributing them between the cells of the tumor and its microenvironment (TME), including between it and neuro-glial complexes [22,23].

Furthermore, the phenotypic plasticity of tumor cells—including their capacity for dedifferentiation, differentiation, and transdifferentiation—allows them to enter a stem-like state under unfavorable conditions and to facilitate relapse and even form vessel-like structures (vasculogenic mimicry) under favorable conditions [24]. This plasticity underscores the potential for systemic interventions to modulate tumor growth. Cancer stem cells play a particularly important role, as their intrinsic calcium oscillatory activity allows them to act as pacemakers, imposing a pathological rhythm on both the networked tumor cells and surrounding neurons [25]. Additional communication pathways include signal transmission through gap junctions and the extrasynaptic

 doi:10.20944/preprints202511.0135.v1

space [26]. Furthermore, the acidic environment created by the tumor via lactate production enhances immunosuppression and supports metabolic reprogramming of the TME [27].

Glioblastoma evades immune surveillance through systemic T-lymphocyte depletion (CD4+/CD8+) and the establishment of a physical barrier that mimics the blood-brain barrier. This enables local tumor signals to override systemic regulation. Compounding this, stress hormones (norepinephrine, cortisol) not only promote angiogenesis and tumor progression but, in the context of chronic stress and glucocorticoid resistance, can also lead to inflammation and a breakdown in immune regulation [28]. Thus, GB actively reconfigures the brain's microenvironment, establishing a pathological signaling hierarchy in which its own "commands" dominate over normal physiology.

This process is facilitated by several interconnected mechanisms. First, the tumor directly suppresses the ability of neighboring cells to respond to external stimuli. Research indicates that GB cells secrete various factors, such as pro-inflammatory cytokines (e.g., TGF-β) and metabolites (notably lactate), which can reduce the expression and sensitivity of key receptors on neurons and glial cells [29,30]. For instance, exposure to the TME can suppress adrenergic receptors, rendering neurons less responsive to systemic signals like norepinephrine.

Second, the tumor's local signals create a powerful pathological signaling field that dominates the TME. The most striking example is the "glutamate storm"—a massive release of glutamate by GB cells via the cysteine-glutamate antiporter xc-system [31]. This leads to persistent overactivation of neuronal N-methyl-D-aspartate receptors, resulting in excitotoxicity and functional exhaustion [32]. A neuron in such a constant state of overstimulation loses its ability to adequately process more subtle physiological signals from the external environment.

Consequently, a clear signaling hierarchy is established within the TME. The tumor's own signals (e.g., glutamate, electrical impulses, and growth factors) are the strongest and most prioritized, acting locally at high concentrations. Signals from neighboring neurons are subordinated to the pathological rhythm imposed by the tumor. Systemic signals from the blood (e.g., hormones, cytokines) are the most disadvantaged, being weakened by physical barriers, encountering suppressed receptors, and targeting cells that are preoccupied with processing tumor-derived stimuli.

In conclusion, GB can be characterized not as a passive accumulation of cells, but as a highly integrated pathological "parasitic organ" that actively remodels the neuronal network, subordinating its function to serve the tumor's own interests [33]. Despite the predominance of local tumor signals, existing evidence indicates that systemic hormonal regulation—such as thyroid hormones, stress-related hormones (e.g., cortisol), the somatotropic system (GH/IGF-1), and sex hormones—influences both immune system activity and the onset and progression of tumors [28,34–36]. This raises a central question: can systemic bodily reactions exert a meaningful influence on tumor growth?

If, yes, cognitive behavioral therapy, physical activity can be applied as a non-pharmacological intervention in the combined treatment of brain tumors [37,38]. Its therapeutic effects are mediated through the modulation of the immune and endocrine systems. The combination of cognitive behavioral therapy with therapies aimed at damaging tumor cells and inhibiting their crosstalk on the TME could lead to enhanced effects. Physical activity, both in cancer patients and laboratory animals, is one such factor linked to improved survival. The mechanism is partly attributed to the acute increase in IL-6 production by skeletal muscles during contraction. This exercise-induced IL-6 can bind to receptors on immune cells like NK-cells, thereby potentially stimulating an antitumor immune response [1,39]. A promising therapeutic approach is, therefore, to combine strategies aimed at suppressing these specific mechanisms of tumor survival with standard treatments, which may enhance therapeutic efficacy and improve patient quality of life.

The diagnosis of malignant glioma is typically established at late stages due to the tumor's biological features and the brain's compensatory capabilities [40]. The news of the disease causes severe psychological trauma, leading to a sharp increase in stress and anxiety, regardless of the patient's personal characteristics [41]. Furthermore, individuals with initially low anxiety levels, who lack experience in coping with severe stress, may be less adapted to it. Stress is not only subjectively

 doi:10.20944/preprints202511.0135.v1

heavy but also exerts a physiological influence on the disease through neuro-immune mechanisms, affecting the function of immune cells within the TME [42]. A personalized approach that considers both the biological parameters of the tumor and the psychophysiological state of the patient becomes the optimal strategy for comprehensive care [43].

3. Characteristics of Commonly Used Laboratory Rat Strains

Laboratory rat strains are characterized by their extensive use in research, with records of neurophysiological studies dating back to the 17th century. Their rapid reproduction, fertility, low cost, over 90% genomic similarity to humans, and larger brain size relative to mice have established them as a primary model organism [44]. Rats are capable of learning complex behavioral tasks, including those investigating reinforcement learning and behavioral inhibition [45].

To reduce genetic variability and enhance experimental precision and reproducibility, specific laboratory rat lines (strains) have been developed, which require regular genetic monitoring [46]. There are approximately 1,000 rat lines, categorized as outbred, inbred, transgenic, and knockout. Among these, the Wistar, Sprague Dawley (SD), Long-Evans (LE), Lewis, Wistar Kyoto (WKY), and Fischer (F344) strains are the most widely used. The Wistar strain, in particular, has given rise to many other outbred and inbred lines.

The term “line” (or stock/strain) typically refers to closed populations bred by any method except strict inbreeding. Outbreeding crosses unrelated individuals, including from different lines or species, while inbreeding involves mating close relatives. Outbred strains conceal recessive traits through heterozygosity; only first-generation hybrids (F1) ensure high reproducibility [47]. Consequently, data from outbred rats must be interpreted cautiously, as they may not represent all individuals within the strain due to significant heterogeneity, including behavioral variation [10]. “Sickness behavior” in rodents often coincides with altered depression-like behavior, resembling human conditions [48]. Blood transcriptome studies across strains reveal a shared stress response, with differences potentially linked to fear memory and genetic programming. Population variations exist in HPA axis function, showing high, medium, or low stressor sensitivity. For instance, elevated corticotrophin-releasing hormone expression in the central amygdala correlates with more emotional and anxious phenotypes, exacerbating conditioned fear and visceral nociception. Unique strain-specific transcriptomic responses to chronic stress may illustrate individual vulnerability or resilience [17].

3.1. Spontaneous Tumors in Laboratory Rats

The prevalence of spontaneous tumors, including those of the nervous system (NS), varies among species. NS tumors are most common in laboratory rats but are extremely rare in monkeys, mice, and hamsters. Rat strains also show specific differences in NS tumor incidence: F344 rats exhibit the lowest frequency. Wistar and SD rats show glioma rates of ~1.5% in males and 0.7–0.8% in females [49], substantially higher than the human GB incidence of ~0.003%–0.006% [50]. Other data suggests the incidence of CNS tumors is generally comparable between Wistar, SD, and Han-Wistar strains (2.33%, 2.54%, and 2.89%, respectively) [51]. Spontaneous tumor frequency varies by strain, sex, and age [52]. In SD rats the incidence of tumors was 22% in females and 5% in males; in LE rats the incidence of tumors was 28% in females and 10% in males. F1 hybrids (SD × LE) exhibit rates of 67% in females and 32% in males—approximately double that of the parental strains [53]. High inbreeding also increases tumor frequency.

Morphological, sensory, and motor differences exist among the six primary studied rat strains [54].

3.2. Wistar Strain Rats

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The outbred Wistar strain (albino, Rattus norvegicus), established in 1906 at the Wistar Institute (USA), is used in behavioral studies, geriatrics, infectious diseases, therapy safety/efficacy, and surgical models (Table. 1). These rats are more active and trainable than many other strains.

Wistar show robust humoral and cellular immune responses to ovalbumin sensitization [55]. Wistar rats (the parent strain) are less susceptible to inflammation due to their pronounced humoral immune response compared to their daughter line of Lewis rats. They are normoreactive along the HPA axis, and a more stress-sensitive system relative to the daughter line provides more adequate regulation of the immune system, which reduces their susceptibility to rampant inflammation and autoimmune diseases [56].

They exhibit reduced avoidance behavior, an enhanced startle response (without hyperarousal), increased expression of immune-related genes, higher baseline neuroimmune and pro-inflammatory microglial gene expression in the prelimbic cortex and central amygdala, greater peripheral inflammation, and higher locomotion regardless of sex. Acute stress elevates auditory thresholds in Wistars [56]. Social fear behavior is more pronounced in Wistars than in SD rats [57].

In apomorphine-induced stereotypy tests, ~50% of Wistars display intense gnawing, while others show no response [10]. Wistars selected for high or low anxiety in the Elevated Plus Maze show no differences in basal or stress-induced ACTH and corticosterone levels [10]. Orexinergic neurons in the hypothalamic perifornical area express serotonin receptors in Wistar rats [58]. In males, various stressors cause differentiated reactions of steroid hormones [59].

Compared to the SD line, Wistar rats have a higher level of anxiety and more specific sexual behavior, which is associated with differences in glycinergic neurotransmission in the neural networks of the medial preoptic region of the hypothalamus [60]. Wistar typically exhibit an intermediate anxiety phenotype, often rated between the high-anxiety F344 and the low-anxiety Lewis strains. In cognitive tests of active avoidance requiring coordination, Wistar rats reacted more strongly to cholinergic blockade than LE rats, although no differences were detected in navigation tests like the Morris water maze. LE rats showed the best baseline results in tests for active place avoidance, indicative of their high innate cognitive abilities, and in the visible platform test (a Morris water maze variant), suggesting superior eyesight [61].

Chronic stress from social isolation in Wistar females increased sociality while decreasing preference for “social novelty,” risk assessment, and exploratory activity [62]. Wistar rats are highly active and show the largest gradient between activity in the light and dark phases, with ~223% higher locomotor activity during dark periods. Although female Wistar rats are the most active group, the maximum movement speed of female LE animals is the highest among all groups [63].

Table 1. Characteristics of Major Laboratory Rat Strains and Lines [17,60,64–70].

Strain/Line	Genetic Identity	Physiology	Research Models	Spontaneous Tumors, Incidence (%)
Wistar	Outbred	A normoreactive HPA axis, Active; Moderately stress-resistant	Osteoarthritis; Obesity; Metabolism; Oncology; Toxicology; Teratology; Hematology; Geriatrics; Physiological studies; Anxiety-related sexual behavior	High
Long-Evans	Outbred	A hyperreactive HPA axis, Good vision; Pronounced HPA-axis reactivity to stressors; High basal and stress-induced ACTH levels; Low stress resistance	Behavior; Neurological studies; Toxicology; Physiological studies; Alcohol exposure effects	High

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Strain/Line	Genetic Identity	Physiology	Research Models	Spontaneous Tumors, Incidence (%)
Sprague-Dawley	Outbred	A normoreactive HPA axis, Less active; Rapid body weight gain; Moderately stress-resistant	Osteoarthritis (pronounced joint damage); Obesity; Oncology; Toxicology; Non-anxiety-related sexual behavior	High
Fischer	Inbred	A hyperreactive HPA axis, Moderately stress-resistant	Not specified	Low
Lewis	Inbred	A HPA axis hyporeactive, A Th1-type response	Autoimmune diseases, Inflammation, Transplantation studies, Metabolic diseases	Low
Wistar Kyoto	Inbred	A hyperreactive HPA axis, One of the more robust and stable strains with low background pathology	Cardiovascular research (as a normotensive control), Behavioral studies (genetic model of depression, therapy-resistant, and anxiety disorders)	Very Low

3.3. Long-Evans Strain Rats

The outbred Long-Evans strain (LE, Rattus norvegicus), created in 1915 by crossing Wistar females with wild grey male rats, is resistant to respiratory diseases and preferred for surgical studies with inhalational anesthetics. The LE strain typically demonstrates a moderate-to-high anxiety phenotype compared to the more common SD and Wistar strains. LE rats are known for their heightened reactivity and can be more challenging to handle, reflecting a greater degree of neophobia. It is used in neurology, toxicology, ophthalmology, behavior, obesity, alcoholism, and models of HPA axis hyperactivity [12].

Key differences from other strains likely reside in brain function [12]. Chronically stressed high-anxiety LE rats show depression-like symptoms, elevated c-Fos and 5-HT1A receptor levels in the hypothalamic PVN [71]. LE rats exhibit a reduced trophic response to ACTH and lower body and thymus weight than SD rats, which affects immunity. Their activity is higher than in SD rats, but anxiety-related behavioral differences are absent. LE rats show novelty-seeking hyperactivity with high exploration motivation. Corticotrophin-releasing hormone levels are higher in LE than in Wistar rats. Chronic stress impairs brain reward systems in LE, evidenced by anhedonia and despair phenotypes [71].

LE rats display greater HPA sensitivity to stressors, with higher basal ACTH and corticosterone than Wistar, SD, and Fischer rats during the light phase (16:00/19:00 h), and lower corticosterone at night (02:00 h)—a pattern typical for lab animals. SD and LE rats show minimal ACTH at early light (10:00 h) and peaks before dark (19:00 h). Both exhibit higher corticosteroid-binding globulin at 19:00 h than at 10:00 h. LE rats are studied for maternal behavior, the long-term effects of early-life experience on stress reactivity, and visual-cue tasks (having superior vision to albino strains). They perform spatial memory tasks similarly to wild rats, outperforming other lab strains.

Acute stress elevates ACTH more in LE than in SD rats, though basal levels are similar. Acute forced swim stress alters hypothalamic/amygdalar corticosteroid-binding globulin neither mRNA nor mineralocorticoid receptor levels in SD or LE rats [12]. Hypothalamic glucocorticoid receptor mRNA remains unchanged post-stress in both. The hippocampal CA1 region shows lower basal glucocorticoid receptor mRNA in LE than in SD; acute stress has no effect. Under basal conditions, LE rats exhibit lower levels of glucocorticoid receptor mRNA in the dentate gyrus compared to SD

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rats. Furthermore, following stress, this expression shows a further significant decrease specifically in the LE strain.

Resting HPA hormones (ACTH/corticosterone) are elevated at light onset in LE vs. Fischer and Wistar rats; post-stress; only ACTH increases in LE [12]. Circulating glucocorticoids bind to corticosteroid-binding globulin (transcortin), potentially acting as a reservoir that limits availability during stress. Exogenous corticosterone alters hippocampal and prefrontal serotonin release in rats, possibly via 5-HT1A receptor desensitization, and increases dopamine synthesis in the VTA and mPFC. LE rats show stronger ACTH responses to novel environments but not to forced swim [12].

In a model of auditory sensitivity and fright reaction, the highest prevalence of tinnitus was in LE rats (75%), compared to SD (50%) and Wistar (33%) [72]. Male LE rats are a good model of pronounced hypothalamic-pituitary-adrenal hyperactivity, showing a large ACTH response to stressors. The expression of corticotrophin-releasing hormone in the paraventricular nucleus of the hypothalamus is higher in LE rats than in other strains. They have a lower thymus weight than SD rats and showed higher levels of ACTH at rest and under stress than Wistar and Fischer rats [12].

3.4. Sprague Dawley Strain Rats

The outbred Sprague Dawley (SD) strain (albino, Rattus norvegicus), derived from the Wistar strain in 1925 (USA), is reproductively active, docile, and easily handled. It is used in neuro-oncology, time-controlled-pregnancy models, safety/efficacy studies, nutrition research, geriatrics, obesity, and surgical models.

SD generally show lower baseline anxiety than Wistar, LE, and F344 rats, making them a common choice for studies where a moderate, baseline anxiety level is desired. In carcinogenicity studies of the SD rat NC, malignant brain glioma (<1%) was the most common, followed by granular cell tumor, meningioma, mixed glioma, schwannoma, and meningeal sarcoma; benign tumors included ependymoma and glioma. Spinal cord tumors included benign and malignant glioma and malignant schwannoma.

Chronic inflammatory pain (Freund's complete adjuvant model) induces thermal hypersensitivity in SD rats without pain avoidance, anxiety-like behavior, social deficits, or spatial memory impairments in three-chamber sociability or T-maze object recognition tests; however, novel object exploration time decreases [73]. Tumors grow at high and variable rates in this strain [74]. SD rats demonstrate lower baseline sensitivity to mechanical pain than F344 rats [75].

Immobilization stress categorizes SD rats into slow and fast recovery groups with differing corticosterone levels [10]. Comprehensive behavioral analysis shows housing conditions affect cognitive test outcomes in SD and LE rats [76]. Male SD rats show greater behavioral variability than Wistar rats [77]. Furthermore, SD rats have been shown to have a higher preference for diets rich in fat and sucrose compared to Wistar rats [78].

The SD rat strain exhibits a moderate stress reactivity, which is lower than that of LE rats, and a capacity for superior spontaneous recovery of motor function after NC injury. For instance, in a study of spinal cord injury, SD rats demonstrated more rapid spontaneous recovery of locomotor activity compared to Wistar, Lewis, and F344 rats. This enhanced recovery has been correlated with better preservation of neural pathways.

3.5. Fischer Strain Rats

The inbred Fischer 344 strain (F344, albino, Rattus norvegicus), developed in 1920 at the Crocker Institute (USA), is widely used in modeling aging, oncology, nutrition, and surgical procedures. Fischer rats exhibit high HPA axis reactivity under prolonged acute stress, making them a valuable model for studying its genetic basis [12]. Behaviorally, this strain is characterized by high innate anxiety and fearfulness. They show little exploratory drive in novel environments, such as the center of an open field, and demonstrate a pronounced expression of recent and recovered fear memory compared to low-stress lines, regardless of their immediate stress status [17].

 doi:10.20944/preprints202511.0135.v1

These profound phenotypical differences are likely linked to the strain's unique immunological profile. F344 rats are notably resistant to various challenges, a trait attributed to factors such as an increased number of regulatory T-cells (Treg) and a propensity for an anti-inflammatory cytokine profile compared to other rat strains [16,54].

This distinct immune signature may contribute to their observed resistance to the tumor-forming effects of chemicals like N-ethyl-N-nitrosourea (ENU), to which LE rats are highly susceptible. F344 rats appear to be less sensitive than for detecting unknown neuro-oncogenic chemicals SD rats [79].

3.6. Lewis Strain Rats

The Lewis rat is an inbred strain (albino, Rattus norvegicus) with a well-documented history, originating from Wistar stock in the early 1950s by Dr. M.Lewis. The Lewis rat strain is characterized by HPA axis hyporeactivity.

This strain is particularly renowned for its unique immunological profile, characterized by strong cellular immune responses that are predominantly skewed towards a Th1-type response. This response is mediated by cytokines such as IL-2 and IFN-γ. This immunophenotype is a key factor in the strain's high susceptibility to both cell-mediated immunopathology and inducible Th1-mediated autoimmune diseases [64]. Lewis rats are also used as models for studying metabolic diseases, as they are sensitive to diet-induced obesity and diabetes, as well as to streptozotocin-induced diabetes.

The standard, widely accepted, and syngeneic glioma model for the Lewis rat is the CNS-1 glioma. This model was developed by administering methylnitrosourea (MNU) to a Lewis rat. It is weakly immunogenic and exhibits an infiltrative growth pattern, making it a particularly valuable tool for experimental neuro-oncology studies that require the Lewis rat's specific immunology [65].

Behavioral and neuroendocrine studies have revealed significant variations between different substrains of Lewis rats. These findings indicate that substrains of this inbred rat can exhibit marked behavioral differences, which may only partially correlate with differences in the HPA system [66].

3.7. Wistar Kyoto Strain Rats

The Wistar Kyoto (WKY, albino, Rattus norvegicus) rat inbred strain was originally developed from outbred Wistar stock at Kyoto University (1960, Japan) as the normotensive control for the Spontaneously Hypertensive Rat in cardiovascular research.

The WKY strain has been established as a prominent and well-validated genetic model of depression, therapy-resistant, and anxiety disorders. These rats exhibit a behavioral profile that aligns with core features of human depression, including increased immobility in the forced swim test, interpreted as "behavioral despair," and reduced locomotion and exploration in novel environments like the open field test, indicating high levels of anxiety-like behavior and a passive coping strategy. A key feature of this depressive-like phenotype is anhedonia, demonstrated by a reduced preference for sucrose, which is further exacerbated by exposure to chronic mild stress [67].

Crucially, the strain is characterized by a hyperreactive HPA axis. Compared to other rat strains, WKY rats show elevated basal levels of ACTH and corticosterone, and they mount an exaggerated and prolonged hormonal response to both acute and chronic stressors. This impaired stress response is coupled with alterations in central neurotransmitter systems, including dopaminergic and noradrenergic pathways, as well as changes in thyroid-stimulating hormone. Furthermore, studies have documented circadian rhythm abnormalities in these rats, such as reduced nighttime activity [68,69].

The validity of the depression model is further strengthened by the existence of distinct WKY substrains, which are nearly isogenic but display clear behavioral differences in depressive-like behavior. These substrains are thought to model different subgroups of human depression and share genetic variations affecting genes implicated in human depression studies [70].

4. Morphological and Molecular-Biological Characteristics of Major High-Grade Rat Glioma Models

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4.1. Spontaneous Glioma Model

According to the International nomenclature and diagnostic criteria for pathologies in rats and mice, spontaneous malignant rat gliomas consist of binucleated granular cells positive for lysozyme, phosphotungstic acid hematoxylin (PTAH), and vimentin. The intracellular granules stain with periodic acid-schiff and PTAH both before and after diastase digestion. These tumors are extensive, highly cellular, and often multicentric or diffuse, spreading across multiple CNS regions without clear boundaries.

Characteristic histopathological features include perineural satellitosis, pseudopalisading necrosis, and spread along Virchow-Robin spaces. Infiltration of the meninges and ependyma is common. The tumor cells are polymorphic and poorly differentiated, with varying nuclear shapes and indistinct boundaries, and may show protoplasmic or fibrillary differentiation. Glial fibrillary acidic protein (GFAP)-positive reactive astrocytes are present, alongside hemorrhages and necrosis.

A key distinction from human GB is that spontaneous rodent gliomas are typically negative for GFAP and PTAH, whereas human and domestic animal GBs are usually positive. Chemically induced ENU gliomas in rats are also negative for GFAP and Leu-7 but positive for S-100 and vimentin, though they contain GFAP-positive reactive astrocytes. Unlike human GB, spontaneous rat gliomas do not feature multinucleated giant cells or vascular endothelial proliferation [51].

Tumor incidence varies by strain; the F344 and SD lines generally have the highest rates, exceeding those in Wistar rats. SD rats show a higher incidence of pituitary adenomas and astrocytomas. The reported frequency of neural neoplasms depends on the strain, gender, and the number of histological sections examined [49]. The granulocytic cell tumor is the most common spontaneous CNS tumor in rats.

4.2. C6 Cell Glioma Model

The C6 glioma cell line was developed in the late 1960s by repeated administration of MNU to adult Wistar-Furth inbred strain rats (Table. 2). Although of Wistar -Furth origin, it can be implanted into outbred SD and LE rats without immediate rejection. Orthotopic implantation into the frontoparietal lobe (typically 1×10^5 cells in 5 μl) is standard, though tumor take rates are variable.

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Table 2. Key Characteristics of Malignant Glioma Models [44–46,80–93].

Glioma Model	Host Strain	Induct. Method	Cell Inoc. (Cells)	Immuno-genicity	Avg. Latency (months)	Growth Pattern / BBB Integrity	Molecular Markers	Hormones/ Receptors	Neurotransmitters	Metabolic Profile
HUMAN										
Glioblastoma	-	-	-	TME Immune-suppression	3	Infiltrative/ partially disrupted or altered	*RB; PDGFA/B, IGF-1, EGFR, ERBB3/HER3; TERT, PTEN; PIK3CA, CDKN2A/B; IDH1/2-wt, TP53-wt; RAS, RB; EGFR-amp/mut; +7/-10; JAK-STAT, NF-KB, SHH*	IGF, Ins, EPO, GH, Testo, E2, T3; BK, ER	ACh; Glu, GABA; DA, 5-HT, NE; SP, NPY; ATP; NO	↑Gln/Glu, AcyICn, NADPH, Bet
RAT MODELS										
High grade glioma cell line										
C6	Wistar - Furth	MNU	10^4	High	1	Circumscribed/ partially disrupted	*Mut Trp53, Cdkn2a/b-, Pik3ca+; ↑Pdgfβ, Igf-1, Egfr, Erbb3/Her3 S100b, Gfap, Bace1, Pen-2, Rb, Egf, Vegf, Nicastrin, Gsk-3β, Aβ, Tau/pTau, Ros, Glut1, Cx43, Idh1/2-wt*	GC, Testo; 5-HT2A; DA; AdrR, ER	-	↓tCr, Tau, hTau, Ala, Ace, GSH, Gln, NAA, Asp; ↑Gly, Gln, Lip1.3
9L	Fischer	MNU	10^4	High	0,5	Circumscribed/ Markedly disrupted	*MutTrp53; Cdkn2a-; ↑Tgfa, Egfr; ↓Fgf2, Fgf9, Fgfr1, Pdgfrb*	5-HT2AR	-	↑Gln, ↓mIns

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Glioma Model	Host Strain	Induct. Method	Cell Inoc. (Cells)	Immuno-genicity	Avg. Latency (months)	Growth Pattern / BBB Integrity	Molecular Markers	Hormones/ Receptors	Neurotransmitters	Metabolic Profile
F98	Fischer	ENU	102-105	Low	0,5-1	Infiltrative/Markedly disrupted	*MutTrp53; ↑Pdgfb, Ras, Egfr, Ccnd1/2, Gja1, Rb1; ↓Brca1* Gfap+, Vim+, CD3+ T-cell infiltration ↓	ER	SP/NPY alterations	↑Gln, Gly, tCho/tCr, Lac; ↓tCr, NAA, Gua, mIns, Glu, GABA
RG2	Fischer	ENU	102-105	Non-immunogenic	0,5-1	Infiltrative/ Markedly disrupted	*TP53-wt ↑Pdgfb, Igf2, Ras, Erbb3, Ccnd2; Cdkn2a-*	-	-	↑PCho, GPCho; ↓NAA, Glu, Gln, tCr
High grade glioma tissue										
101.8	Wistar	DMBA	10^5-106	Immunogenic	0,5	Infiltrative/ Markedly disrupted	*↑Egfr, Gja1, Bsat1, Vim+, Ncam1, Vegfa, Chi3l1, Sox2, Cdkn2a, Trp53, Gfap- ↓S100b, Melk, Epas1, Prom1, Pten, Mki67, Pik3ca, Hif1a, Olig2, Fn1, Nos1, Igfbp3, Idh1/2-wt*	TfR, ER, GR	-	
15/47	Wistar	ENU	10^5-106	Low	1	Infiltrative/ Markedly disrupted	*CD133-; Olig2+ VEGF+ Vim+ ↑Cdkn2a, Pik3ca, Trp53, Vegfa, Hif1a, Prom1, Sox2, Melk, Pdgfra, Gdnf, Mgmt, Abcb1b* CD3+ T-cells: 15.2%	GR-	-	

Key to Abbreviations: DMBA (7,12-Dimethylbenz[a]anthracene); ENU (N-Ethyl-N-nitrosourea); MNU (Methylnitrosourea); IV (Intravenous); TME (Tumor Microenvironment); AMP (Amplification); MUT (Mutation); ↑ (overexpression); ↓ (downregulation); 5-HT2A (serotonin receptor 2A); AdrR (Adrenaline receptors); ER (Estrogen receptor); GR (Glucocorticoid receptor); TfR (Transferrin receptor); Bet (Bromodomain and Extra-Terminal motif proteins); Cx43 (Connexin 43); 5-HT (Serotonin); ACh (Acetylcholine); ATP (Adenosine triphosphate); DA (Dopamine);

 doi:10.20944/preprints202511.0135.v1

GABA (Gamma-aminobutyric acid); Glu (Glutamate); NE (Norepinephrine); NO (Nitric oxide); NPY (Neuropeptide Y); SP (Substance P); BK (Bradykinin); E2 (Estradiol); EPO (Erythropoietin); GC (Glucocorticoid); GH (Growth hormone); IGF (Insulin-like growth factor); Ins (Insulin); T3 (Triiodothyronine); Testo (Testosterone); AcyICn (Acyl carnitines); Ala (Alanine); Asp (Aspartate); Glc (Glucose); Gln (Glutamine); Gua (Guanosine); Lac (Lactate); mIns (myo-Inositol); NAA (N-acetylaspartate); PCho (Phosphocholine); GPCho (Glycerophosphocholine); Tau (Taurine); tCho (total Choline); tCr (total Creatine); COX (Cyclooxygenases); LOX (Lipoxygenases); HETEs (Hydroxyeicosatetraenoic Acids); EETs (Epoxyeicosatrienoic Acids); NADPH (Nicotinamide adenine dinucleotide phosphate).

 doi:10.20944/preprints202511.0135.v1

This method facilitates growth measurements and avoids the limitations of subcutaneous models. Histologically, C6 gliomas are pleomorphic with round-to-elongated nuclei, exhibiting a "fir-tree" growth pattern and focal invasion in Wistar rats. Scattered necrotic foci with pseudopalisading tumor cells and high peripheral mitotic activity are observed [81]. These cells share markers with human GB, such as S100B and EGFR, but lack GFAP and show variable vimentin expression [93].

Genetically, C6 frequently harbors p16/CDKN2A/NK4A locus mutations but retains wild-type p53 and shows minimal PTEN expression—a profile that diverges from human GB, where p53 mutations are common [88]. While its extensive characterization makes it valuable for genetic studies, its significant immunogenicity is a critical limitation.

It triggers strong antibody responses even in Wistar rats, loses invasiveness in non-Wistar strains (growing in an encapsulated manner), and is unsuitable for immunotherapy or survival studies due to the lack of a true syngeneic host [94]. The C6-Wistar model shows similarities in permeability and metabolism to human GB but lacks consistent invasive growth. Its variable tumor growth patterns suggest a high dependence on the host microenvironment [85].

4.3. 101.8 and 15/47 Transplantable Tissue Glioma Models

These GB tissue lines, chemically induced (101.8 by 7,12-dimethyl-5,6-benzo[a]anthracene and 15/47 by ENU), are maintained via serial transplantation. Both exhibit high cellularity, vascularization, pleomorphism, pathological mitotic activity, hemorrhagic foci, and pseudopalisading necrosis.

The tumor exhibits a markedly infiltrative growth pattern with individual tumor cells migrating far beyond the main mass, closely resembling the behavior of human GB. GB 101.8 expresses GFAP, N-CAM, VEGF, vimentin, fibronectin, and receptors for EGFR, transferrin, and glucocorticoids. In contrast, GB 15/47 lacks glucocorticoid receptors, creating a model for studying phenotypic variability in glucocorticoid sensitivity.

GB 101.8 is considered superior to the C6 model in mimicking human GB due to its diffuse peripheral infiltration and differential gene expression between the tumor core and periphery. Etiologically, it represents concurrent malignant transformation of multiple glial components, with a high engraftment rate. Single-cell sequencing reveals significant cellular heterogeneity and a TME comprising ~50% recipient-derived cells, suggesting a complex, immune-modulatory role [95].

4.4. 9L Cell Gliosarcoma Model

This historically prominent model for chemotherapy and radiation studies was induced by MNU. Implantation into the striatum (1×10^5 cells/5 µl) yields high take rates. Histology shows spindle-shaped sarcomatous cells in whorled patterns with sharp borders and minimal brain invasion, Less vascularization; Smaller necrotic cores [81].

While $S100B^+$ and $EGFR^+$ like GB, it lacks GFAP and diffuse invasiveness. In Wistar rats, the tumor recruits $ED1^+$ microglia/macrophages and $CD4^+/CD8^+$ T-cells by the second week without effective growth suppression. Genetically, it harbors mutant p53 and EGFR overexpression but lacks PTEN, p16, FGFR-1, and PDGFRβ expression, diverging from the common genetic profile of human GB.

Although it is a promising model for studying p53-mutant interactions, its classification as a WHO Grade 4 gliosarcoma with unique histogenetic features limits its utility as a general GB model. High immunogenicity and encapsulated growth further restrict its use for studying invasive behavior.

4.5. F98 Cell Glioma Model

F98 gliomas consist of mixed spindle-shaped and polygonal cells that form whorled patterns, exhibit extensive brain invasion with satellite tumor islands, and show perivascular clustering, central necrosis, and edema. Syngeneic in Fischer rats, they exhibit low immunogenicity compared

 doi:10.20944/preprints202511.0135.v1

to the 9L model, making them suitable for studying heterogeneous, infiltrative tumors that resemble human GB.

Unlike the angiogenic RG2 model, the F98 glioma co-opts pre-existing blood vessels. F98 is a p53 mutant, while RG2 is p53 wild type [96]. F98 tumors resist standard therapies, mimicking treatment resistance in human GB. A significant limitation is extracranial/extra-axial growth in 12–23% of cases, likely due to cell reflux during injection or skull suture invasion, which often precludes long-term survival studies.

Implanted rats show behavioral deficits including reduced grooming, hypoactivity, balance impairment, hunched posture, seizures, weight loss, and rare aggression [97]. The widely used C6 and 9L models face critical constraints: C6 triggers immune rejection, while 9L shows poor brain infiltration.

Comparative metabolomic studies have revealed that the same cell line implanted in different strains, or different lines in the same strain, can yield distinct metabolic profiles [85]. This highlights the complex interaction between cell line genetics and host factors in determining the tumor phenotype. Although valuable for in vitro metabolic and genetic studies [85], the translational utility of these models for assessing therapeutics and dynamic growth is compromised.

A major consideration is that the microenvironment in these rodent models does not fully recapitulate the profoundly immunosuppressive nature of human GB. Therefore, encouraging therapeutic results obtained using these models must be interpreted with caution when extrapolating to human disease.

4.6. RG2 Cell Glioma Model

The RG2 glioma model, induced by ENU, is widely used due to its highly invasive phenotype, making it valuable for studies on local tumor spread and therapies targeting invasion [98,99]. However, the tumor mass itself may maintain relatively clear borders.

RG2, like many gliomas, expresses S100B and GFAP-negative, indicating its less differentiated status [100]. A key feature of the RG2 model is its low immunogenicity. The tumor elicits only minimal lymphocytic infiltration (e.g., low numbers of $CD4^+$/$CD8^+$ T-cells) and effectively evades immune surveillance, which contributes to its rapid progression.

The model is characterized by the overexpression of growth receptors typical of GB, such as EGFR and PDGFRβ [92]. It also frequently exhibits a functional loss of wild-type p53, placing it in a molecular subtype dependent on disruptions in the receptor tyrosine kinase pathway.

The RG2 model is the preferred choice for research focused on invasion mechanisms, anti-migration therapy, and overcoming the blood-brain barrier [101]. Its invasive nature and low immunogenicity make it more relevant for modeling the aggressive behavior of human GB, although the lack of GFAP expression and some genetic differences remain its limitations.

5. The Neurobiology of Gliomas: Pathophysiology of Experimental Models

5.1. Key Signaling Pathways and Hormonal Influences

A complex signaling network drives tumor progression. Bradykinin increases vascular permeability, stimulates vasodilation, glioma cell migration, and neovascularization, thereby delivering essential nutrients and growth factors to the tumor [83].

Neurotransmitters, released by both the NC and tumor cells, play a critical role [102]. Norepinephrine increases the viability of C6 glioma cells [81], and TNF-alpha-induced proliferation is mediated by the induction of the beta-2-adrenergic receptor [103]. Furthermore, norepinephrine affects circadian rhythmicity in C6 cells by inducing the Per1 clock gene [104].

Hormonal pathways are also implicated. Thyroid hormone stimulates mitogenic growth factor secretion from astrocytes and C6 cells [105]. Clinically, hypothyroidism correlates with a more favorable survival prognosis in patients with brain tumors [106], and targeting glioma stem cells through thyroid hormone suppression is being investigated in clinical trials [107].

 doi:10.20944/preprints202511.0135.v1

Similarly, conditions with elevated growth hormone, such as acromegaly, are associated with a higher risk of brain tumors [83].

5.2. Metabolic and Molecular Mimicry

Rat gliomas exhibit molecular mimicry of neuronal processes. Elevated expression of the calcium-regulated enzyme calpain in both C6 and RG2 models suggests a neuronal origin or a mechanism to utilize neuronal resources. The production of various lipid mediators (e.g., hydroxyeicosatetraenoic and epoxyeicosatrienoic acids) by RG2 glioma activates signaling pathways that promote angiogenesis, inflammation, and growth. A key metabolic shift involves arachidonic acid metabolism. While the lipoxygenase (LOX) pathway predominates in the healthy brain, the cyclooxygenase (COX) pathway is dominant in GB [108].

The COX-2 pathway promotes prostaglandin E_2 excess, angiogenesis, immunosuppression, and tumor invasiveness, and its inhibition can prevent radiation-enhanced F98 cell infiltration [109]. The LOX pathway regulates cerebral blood flow, synaptic plasticity, and neuronal excitability.

5.3. Neurotransmitter Systems in Glioma Pathophysiology

Gliomas actively manipulate neurotransmitter systems: glutamate, GABA, serotonin, dopamine. Glioma cells exhibit high expression of glutamate receptors [110] and secrete excessive glutamate, promoting proliferation, migration, and excitotoxicity [111,112].

The role of GABA and GABAergic neurons is complex, with evidence for both growth promoting [113] and inhibitory effects on glioma cells [114].

Activation of 5-HT2A receptors increases the proliferation and migration of C6 glioma cells and enhances the release of glial cell line-derived neurotrophic factor [102]. Serotonin also modulates tumor-associated macrophage (TAM) polarization [115]. This neurotransmitter exhibits antitumor and anti-angiogenic activity in C6 glioma models and can enhance the efficacy of chemo- and immunotherapy [116].

The expression of neurotransmitter receptors on non-neuronal cells, including astrocytes and tumor cells, allows the tumor to respond to and manipulate these signaling networks [15–17].

5.4. The p53 and Norepinephrine Axis

The tumor suppressor p53 appears to influence the tumor's neural environment. Adrenergic neurons surrounding p53-mutant tumors differ from those near p53-wild-type tumors. The use of an adrenergic receptor blocker slowed tumor growth, suggesting that p53-mutant tumors stimulate a switch to norepinephrine signaling within the brain [117]. This indicates that norepinephrine's tumor-promoting action may be a local phenomenon, independent of the body's systemic stress status. This makes specific models particularly valuable: F98 and 9L gliomas are p53 mutants, 101.8 exhibits increased p53 activity, and C6 is p53 wild type.

5.5. Consideration of Sex as a Biological Variable

In many animal species, females are more active than males, a trait attributed to the regulation of estrogen and testosterone pathways. While this effect is consistent across rat strains, it is important to note that even when the estrous cycle is not controlled for, data from female rats are no more variable than data from males [118], supporting their reliable use in neurobiological experiments.

6. The Host Immune Response and Tumor Microenvironment Are Critical Determinants in Modeling Glioma Biology and Therapeutic Response

The tumor microenvironment (TME) constitutes a complex ecosystem comprising cancer cells, immune cells, endothelial cells, cancer-associated fibroblasts, and various acellular components. Within the TME, tumor immune surveillance critically depends on the function of major histocompatibility complex (MHC) molecules. MHC-I plays a pivotal role in initiating tumor immune

 doi:10.20944/preprints202511.0135.v1

responses by presenting specific antigens to CD8+ T-cells, thereby activating the adaptive immune system. However, MHC-I overexpression can transmit inhibitory signals to CD8+ T-cells, NK-cells, and TAMs, resulting in immunosuppressive effects and promoting tumor survival. Tumors can also present antigens via MHC-II for recognition by CD4+ T-cells, and this pathway may be similarly regulated to facilitate immune evasion. Emerging evidence in neuroimmunology indicates that elevated levels of MHC components, particularly MHC class I and II isoforms, can orchestrate immunoregulatory signaling within the TME. Mechanistic studies demonstrate that these molecules functionally modulate cytotoxic T-lymphocyte (CTL) activation thresholds and macrophage polarization states—critical determinants of the immune evasion niche characteristic of GB pathogenesis. Such immunological alterations correlate with enhanced tumor escape mechanisms and disease progression [120].

Successful tumor modeling necessitates careful consideration of genetic compatibility between donor and recipient to prevent immunogenicity and transplant rejection. Syngeneic transplantation, where the MHC is identical, prevents rejection. In contrast, allogeneic transplantation (MHC mismatch), as utilized in experimental tumor models, triggers immune responses through two primary pathways: direct recognition of donor MHC molecules by recipient T-cells, and indirect recognition of processed donor alloantigens presented by recipient antigen-presenting cells. This recognition activates CD4+ and CD8+ T-cells, stimulating production of pro-inflammatory cytokines (e.g., IL-2, IFN-γ, TNF-α) and effector mechanisms including CTL activity, macrophage infiltration, and antibody-mediated rejection. The balance between pro-inflammatory T-helper cells (Th1/Th17) and Tregs critically determines the outcome; inflammatory cytokines such as IL-6 can inhibit Treg function, shifting the balance toward rejection. Within the CNS, a graft-versus-host-like reaction may manifest as T-cell infiltration, upregulated TNF in microglia, and activation of NF-κB/p38 MAPK signaling pathways, thereby exacerbating neuroinflammation [119].

Effective elimination of cancer cells requires the presence of either CD8+ or CD4+ T-cells and direct contact between GB cells and immune cells [121]. During initial tumor formation stages, a paradoxical phenomenon occurs where a developing pro-inflammatory TME promotes successful tumor engraftment, consistent with Virchow's classical principle linking inflammation and oncogenesis. Subsequently, the transplant's fate is determined by complex interactions between tumor cells and the recipient organism. Tumor cells employ various immune evasion strategies, including downregulation of MHC expression, recruitment of T-regs, expression of immune checkpoint inhibitors (PD-1, CTLA-4), and active secretion of immunosuppressive cytokines (TGF-β, IL-10, VEGFA). These mechanisms contribute to forming an immunologically "cold" TME, enabling the tumor to evade immune surveillance and progress within the recipient organism [95]. Research has demonstrated that following intracerebral transplantation of GB 101.8 tissue with TME, gradual replacement of donor stroma by recipient cells occurs. This process is initiated by immune reactions due to histocompatibility differences and ischemia-reperfusion injury during transplantation [95].

The C6 glioma model exhibits complex MHC expression dynamics. While tumor cells themselves show low MHC expression, surrounding microglia and macrophages can express MHC class II molecules, particularly in the presence of infiltrating T-cells. Studies present conflicting data, showing correlation between MHC expression in microglia and increased tumor-infiltrating lymphocytes in C6 tumors, while also indicating that C6 glioma cells can downregulate MHC expression as an immune evasion strategy [122]. The rat F98 glioma exhibits weak immunogenicity. Although it expresses tumor antigens, its MHC class I and II expression remain inadequately characterized, though some studies suggest decreased expression during invasion. Other rat glioma lines like 9L gliosarcoma demonstrate consistent MHC I expression, whereas C6 glioma triggers strong allogeneic immune response. Generally, gliomas can downregulate MHC to avoid immune surveillance, and therapeutic approaches for F98 may focus on strategies to enhance MHC expression or overcome the tumor's immunosuppressive TME [123].

Microglia and macrophages (5-12% of total cellular composition) display differential immunological activity across glioma models. C6 and 9L tumors showed expression of B7.1 and

 doi:10.20944/preprints202511.0135.v1

MHC class II molecules, while RG2 gliomas lacked these markers. Notably, B7.1 and MHC class II expression correlated with increased tumor lymphocytic infiltration in C6 and 9L models. Basal B7.2 expression in the tumor environment did not substantially differ from normal brain tissue. However, after isolating microglia from C6 tumors and short-term in vitro culture, increased expression of all three surface antigens was observed. These findings indicate that microglial immune function is suppressed in gliomas, with the suppression degree directly correlating with the immunogenicity of specific experimental brain tumor models [124]. Attempts to enhance immunogenicity through B7.1 costimulatory molecule transfection in the weakly immunogenic F98 glioma model proved unsuccessful. Although stable transfection (F98/B7.1) confirmed surface expression of B7.1 and MHC class I antigens, no significant survival time differences were observed following intracerebral implantation. Vaccination strategies using mitomycin C-treated F98/B7.1 cells also failed to demonstrate enhanced protection against subsequent tumor challenge, indicating that B7.1 transfection alone is insufficient to augment F98 glioma immunogenicity [123].

Analysis of glioma models reveals MHC molecules as central regulators of anti-tumor immunity, demonstrating a dual role in both activating and suppressing immune responses. Gliomas employ multiple evasion strategies including MHC downregulation, T-reg recruitment, immune checkpoint expression, and immunosuppressive cytokine secretion. The immune function of microglia and macrophages varies significantly across models, often showing suppression that correlates with tumor immunogenicity. The failure of B7.1 transfection to enhance immunogenicity in F98 models highlights the complexity of tumor-immune interactions. These findings underscore that effective glioma modeling must account for the intricate tumor-microenvironment-immune network; with MHC-mediated mechanisms being particularly crucial for developing immunotherapeutic strategies and improving preclinical research predictive value.

7. Conclusions

Therefore, the path to developing effective treatments for glioblastoma relies on utilizing biologically complex and translationally relevant preclinical models. Our analysis underscores that glioblastoma is not merely an accumulation of proliferating cells, but a highly integrated pathological entity that actively remodels the brain's neural circuits as well as metabolic and immune landscape. It promotes its own growth through synaptic hijacking, metabolic co-option, and the creation of a profoundly immunosuppressive microenvironment. Experimental neuro-oncology demonstrates that no single model can fully capture the heterogeneity of the human disease. The selection of both the tumor model (e.g., C6, F98, 9L, 101.8) and the host strain (e.g., Wistar, Fischer, and Sprague-Dawley) is not a minor technical detail, but a fundamental determinant of experimental outcomes. Each combination presents a unique profile in terms of growth patterns, immunogenicity, and neuro-immune interactions, with MHC playing a central and dual role in regulating anti-tumor immunity. Consequently, a simplified approach is insufficient. A comprehensive strategy is required that consciously selects the optimal "tumor model–host strain" pair while accounting for the dynamic interaction between genetic predisposition, environmental factors, and the intricate tumor-microenvironment-immune network. Only by embracing this complexity, can we enhance the predictive power of preclinical research and develop the multifaceted therapies needed to target not only glioma cells but also the resilient pathological ecosystem they generate.

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Abbreviations

The following abbreviations are used in this manuscript:

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5-HT	Serotonin
5-HT2A	serotonin receptor 2A
Aβ	Amyloid Beta
ACE	Angiotensin-Converting Enzyme
ACh	Acetylcholine
ACTH	Adrenocorticotropic Hormone
AdrR	Adrenaline receptors
Ala	Alanine
AMP	Amplification
Asp	Aspartate
ATP	Adenosine triphosphate
AcyICn	Acyl carnitines
Bace1	Beta-Secretase 1
Bet	Bromodomain and Extra-Terminal motif proteins
BK	Bradykinin
Brca1	Brca1 gene/protein
Bsat1	Brain Specific Angiogenesis Inhibitor 1
Ccnd1/2	Ccnd1/2 genes/proteins
Ccnd2	Ccnd2 gene/protein
CD3	CD3 marker (T-cells)
CD133	CD133 marker
CDKN2A/B	Cyclin-Dependent Kinase Inhibitor 2A/B
CDKN2A/NK4A	Protein p16 (encoded by CDKN2A/INK4a genes)
Chi3l1	Chitinase-3-Like Protein 1
CNS	Central Nervous System
COX	Cyclooxygenases
CTL	Cytotoxic T-Lymphocyte
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
Cx43	Connexin 43
DA	Dopamine
DMBA	7,12-Dimethylbenz[a]anthracene
E2	Estradiol
EETs	Epoxyeicosatrienoic Acids
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
EGFR-amp/mut	EGFR amplification/mutation
ENU	N-Ethyl-N-nitrosourea
EPO	Erythropoietin
ER	Estrogen receptor
ERBB3/HER3	Receptor Tyrosine-Protein Kinase ErbB-3
F344	Fischer 344 (rat strain)
F98/B7.1	F98 rat glioma cell line stably transfected with B7.1

 doi:10.20944/preprints202511.0135.v1

Fgf2	Fgf2 gene/protein
Fgf9	Fgf9 gene/protein
Fgfr1	Fgfr1 gene/protein
Fn1	Fibronectin 1
GABA	Gamma-aminobutyric acid
GB	Glioblastoma
GC	Glucocorticoid
Gdnf	Glial Cell Derived Neurotrophic Factor
GFAP	Glial Fibrillary Acidic Protein
GH	Growth hormone
GH/IGF-1	Growth Hormone / Insulin-like Growth Factor 1
Gja1	Gja1 gene/protein
Glc	Glucose
Gln	Glutamine
Glu	Glutamate
Glut1	Glucose Transporter 1
GPCho	Glycerophosphocholine
GR	Glucocorticoid receptor
Gsk-3β	Glycogen Synthase Kinase 3 Beta
Gua	Guanosine
HETEs	Hydroxyeicosatetraenoic Acids
Hif1a	Hypoxia Inducible Factor 1 Alpha
HPA	Hypothalamic-Pituitary-Adrenal axis
IDH1/2-wt	Isocitrate Dehydrogenase 1/2 wild-type
IFN-γ	Interferon Gamma
IGF	Insulin-like growth factor
Igf-1	Igf-1 gene/protein
Igf2	Igf2 gene/protein
Igfbp3	Insulin-Like Growth Factor Binding Protein 3
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
Ins	Insulin
IV	Intravenous
JAK-STAT	Janus Kinase - Signal Transducer and Activator of Transcription pathway
Lac	Lactate
LE	Long-Evans (rat strain)
LOX	Lipoxygenases
mIns	myo-Inositol
mPFC	Medial Prefrontal Cortex
MNU	Methylnitrosourea

 doi:10.20944/preprints202511.0135.v1

Mut	Mutation
Mut Trp53	Mutated Trp53 gene
NAA	N-acetylaspartate
NADPH	Nicotinamide adenine dinucleotide phosphate
NCAM1	Neural Cell Adhesion Molecule 1
NE	Norepinephrine
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells pathway
NK-cells	Natural Killer Cells
NO	Nitric oxide
Nos1	Nitric Oxide Synthase 1
NPY	Neuropeptide Y
NS	Nervous System
Olig2	Oligodendrocyte Transcription Factor 2
PCho	Phosphocholine
PD-1	Programmed Cell Death Protein 1
PDGFA/B	Platelet-Derived Growth Factor A/B
Pdgfb	Pdgfb gene/protein
Pdgfrb	Pdgfrb gene/protein
Pdgfra	Pdgfra gene/protein
Pen-2	Presenilin Enhancer 2
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
Pik3ca	Pik3ca gene
Prom1	Prominin 1
PTEN	Phosphatase and TENsin homolog
PTAH	Phosphotungstic Acid Hematoxylin (stain)
PVN	Paraventricular Nucleus of the hypothalamus
RAS	Rat Sarcoma virus oncogene/pathway
RB	Retinoblastoma protein/pathway
Rb1	Rb1 gene/protein
Ros	Reactive Oxygen Species
S100b	S100 Calcium Binding Protein B
SD	Sprague Dawley (rat strain)
SHH	Sonic Hedgehog pathway
SOX2	SRY-Box Transcription Factor 2
SP	Substance P
T3	Triiodothyronine
Tau/pTau	Tau / hyperphosphorylated Tau
TERT	Telomerase Reverse Transcriptase
Testo	Testosterone
TfR	Transferrin receptor

 doi:10.20944/preprints202511.0135.v1

TGF-β	Transforming Growth Factor Beta
Th1-type	T-helper 1 type immune response
Th1/Th17	T-helper 1 / T-helper 17 cells
TME	Tumor Microenvironment
TNF-α	Tumor Necrosis Factor Alpha
TP53-wt	Tumor Protein P53 wild-type
Tregs	Regulatory T-cells
Trp53	Trp53 gene
VEGFA	Vascular Endothelial Growth Factor A
VEGF	Vascular Endothelial Growth Factor
Vegf	Vegf gene/protein
Vim	Vimentin
VTA	Ventral Tegmental Area
WKY	Wistar Kyoto (rat strain)
+7/-10	Chromosome 7 gain / Chromosome 10 loss

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