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Review

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

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Abstract

The high attrition rates in glioblastoma (GB) therapeutic development stem largely from preclinical models that fail to adequately recapitulate the dynamic tumor-host ecosystem. Unlike previous reviews that characterize glioma cell lines in isolation, this article integrates tumor biology with the distinct neuro-immune-endocrine landscapes of major laboratory rat strains. We critically evaluate standard rat malignant glioma cell lines (C6, F98, RG2, 9L) alongside transplantable tissue models (GB 101.8, GB 15/47), which offer enhanced translational relevance, demonstrating that the predictive value of any model is contingent upon the specific "glioma model and host strain" pairing and the individual physiological characteristics of the host. We provide evidence that strain-specific hypothalamic-pituitary-adrenal (HPA) axis reactivity (e.g., hyper-reactive Fischer 344 versus normo-reactive Wistar) acts as a decisive, yet often overlooked, modulator of the tumor microenvironment and therapeutic response. The review delineates the utility and limitations of these models, specifically addressing the MHC incompatibilities of the widely used C6 model in immunotherapy research, while contrasting it with the immune-evasive phenotypes of RG2 and the GB 101.8 tissue model. Furthermore, we highlight the superiority of tissue transplants in preserving cellular polyclonality and diffuse infiltration patterns compared to the circumscribed growth often observed in cell line-derived tumors. Consequently, we propose a strategic selection paradigm wherein immunogenic models serve as bioindicators of host immunocompetence, while invasive, non-immunogenic systems (F98, RG2, GB 101.8) are utilized to investigate therapeutic resistance and systemic host-tumor interactions.

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

1. Introduction

Glioblastoma (GB) remains one of the most aggressive and lethal malignant neoplasms, with recurrence occurring in almost all patients despite comprehensive treatment [1,2]. Therapeutic failure is primarily attributed to the tumor's diffuse infiltrative growth, which prevents complete surgical resection [3], its profound inter- and intratumoral heterogeneity [4], and its dynamic ability to adapt to therapeutic pressures and the microenvironment [5].

While tumor-intrinsic oncogenic pathways are well-established drivers of GB [6–10], a complete understanding of its pathogenesis requires a broader perspective. A defining feature of GB is its existence as part of a dynamic, bidirectional "tumor-organism" system, which elicits complex local and systemic reactions that can both suppress and support the tumor [11,12]. The organism's response includes negative effects such as tumor-induced immunosuppression [6,7,13–15], metabolic dysfunction like cachexia [16], and hormonal dysregulation [17–20]. Conversely, systemic physiological processes, particularly those mediated by neuro-immune-endocrine axes, can exert

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significant antitumor effects. One proposed mechanism is the induction of gene programs driven by physical and cognitive activity. These programs function as natural mechanisms of cellular reprogramming—evolutionarily conserved adaptive strategies. They facilitate the restoration of tissue function via key molecular regulators [21,22]. The HPA axis, a central stress-response pathway, is a critical mediator in this interplay. Its dysregulation, whether through the direct physical effects of the tumor or as a result of complex systemic interactions, can create a macroenvironment that either inhibits or promotes tumor progression [23–25].

This complex systems-level dialogue underscores the critical need for preclinical models that recapitulate not only the key histological and molecular features of human GB but also the intact systemic physiology of the host organism [26]. Advanced platforms like patient-derived xenografts in immunodeficient mice, while valuable for certain applications, fail to replicate the functional immune system and the integrated neuro-immune-endocrine circuitry essential for studying these interactions [27–30]. Consequently, immunocompetent syngeneic models in rats remain indispensable tools in experimental neuro-oncology [31].

The value of these rat models, however, is critically dependent on the informed selection of a syngeneic "glioma model – host strain" pair. Significant physiological, neurochemical, and genetic differences between various rat strains (e.g., Wistar, Fischer F344, Lewis) [32–42] fundamentally shape tumor engraftment, progression, and response to therapy. Despite the widespread use of these models, a comprehensive comparative analysis linking the distinct neurobiological profiles of rat strains to their impact on glioma biology is lacking.

The aim of this review is to systematize comparative data on rat strains and models of high-grade gliomas. We will consolidate existing evidence on how specific neuro-endocrine-immune characteristics of different host strains influence tumor progression, thereby providing a rational framework for selecting the most appropriate "host-model" pair to investigate specific aspects of GB biology and therapy.

2. Neurobiological Mechanisms in Rat Glioma Models: From Synaptic Integration to Immune Evasion

Rat glioma models have been instrumental in elucidating fundamental neurobiological mechanisms of tumor progression. For instance, C6 glioma implantation disrupts hippocampal neuroplasticity by impairing synaptic potentiation and elevating the Glu/GABA ratio, leading to neurocognitive deficits [43]. The formation of functional glutamatergic and GABAergic synapses between neurons and tumor cells, which are dependent on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, represents a key integration mechanism [44]. In RG2 rat gliomas, microdialysis revealed significantly elevated extracellular glutamate and glycine in peritumoral regions, potentially contributing to neuronal dysfunction [45]. Concurrently, 9L gliosarcoma progression correlates with sensory deficits and reactive synaptic plasticity in peri-tumoral areas [46].

Mitochondrial localization of P2Y receptors in C6 glioma cells and rat astrocytes suggests potential intercellular metabolic interactions [47]. The F98 rat glioma model demonstrates critical BBB heterogeneity, with infiltrative growth occurring behind an intact blood-brain barrier [48,49]. Tumors ensure nutrient supply through vessel co-option, a phenomenon first described using C6 glioma cells in rat striatum [50]. Furthermore, the lactate-to-bicarbonate ratio derived from hyperpolarized 13C-pyruvate MRSI predicts survival in C6 glioma rats, serving as a biomarker for metabolic reprogramming [51].

Low-dose cycloheximide induces cAMP-dependent differentiation and cell cycle arrest in C6 glioma cells [51], while endothelial progenitor cell integration into C6 glioma vasculature represents a new mosaic pattern in glioma vascularization [52].

Finally, GB establishes immune evasion through systemic T-lymphocyte depletion (CD4+/CD8+) and the formation of physical barriers that mimic the BBB [53]. Simultaneously, stress hormones promote angiogenesis and tumor progression [17]. Together, these processes create complex tumor-

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host interactions where systemic hormonal regulation influences tumor progression via neuro-immune mechanisms [14,17,40].

The accumulated evidence from rat models demonstrates that GB progression involves coordinated synaptic integration, metabolic reprogramming, and immune evasion mechanisms. However, the interpretation of these findings is critically dependent on the specific rat glioma model-host used, as their genetic background, immune, and growth patterns vary substantially, influencing the observed neurobiological and immunological phenotypes.

3. Systemic Host Factors and Therapeutic Implications

This body of evidence raises a central question: can systemic physiological reactions exert a meaningful influence on tumor growth, and if so, can this influence be harnessed for therapeutic purposes? Emerging evidence from both clinical observations and preclinical models suggests an affirmative answer [12,54,55].

A primary pathway for this systemic influence is the neuro-immune-endocrine regulatory axis, particularly the HPA axis and the sympathetic nervous system (SNS). It is known that HPA regulatory pathways are often disrupted in cancer patients, both due to the direct physical impact of the tumor or therapy and as a result of complex systemic biological interactions [23,24]. Furthermore, new research in the field of cancer neuroscience suggests that tumors can actively "hijack" normal communication systems between the brain and the body, including the HPA axis, to create a macroenvironment that supports their survival and progression [12].

The influence of systemic factors is particularly evident in stress response pathways. In contrast to the potential benefits of acute stress [56–58], chronic stress, by activating the HPA axis and SNS, promotes tumor progression [56]. This is supported by data indicating that chronic psychological stress is an independent negative prognostic factor in GB patients [59,60]. It has been experimentally established that chronic psychosocial stress, mediated by β-adrenergic signaling, stimulates angiogenesis, invasion, and tumor growth [61,62]. On a molecular level, chronic stress triggers the release of glucocorticoids, which activates neutrophils and the formation of neutrophil extracellular traps, creating a favorable environment for metastasis [63]. Concurrently, cellular stress from therapy induces epigenetic instability in glioma cells, increasing their adaptability and resistance to treatment [64]. The importance of the microenvironment for aggressive tumors is further supported by data on angiosarcoma [65]. This justifies the therapeutic potential of targeting such neural targets as β-adrenoceptors and acetylcholine receptors [58], a concept confirmed by studies demonstrating the role of vagal regulation in pancreatic cancer prognosis [66], the association of acetylcholinesterase inhibitors with colorectal cancer risk [67], and the fundamental role of innervation in tumor progression [68].

Conversely, behavioral and lifestyle interventions aimed at reducing chronic stress levels can counteract these pro-tumor mechanisms and exert a positive influence. These effects are realized through the modulation of the neuro-immune-endocrine regulatory axis. For instance, regular physical activity is associated with anti-stress effects, suppression of pro-inflammatory signals (IL-6), and activation of cytotoxic immunity (NK and CD8+ T cells) [8,9]. Behavioral interventions based on positive reinforcement: environmental enrichment [69], social support [70], meditation [71] are mediated by reduced activity of the HPA axis and SNS. This leads to decreased levels of cortisol and catecholamines, which, in turn, reduces the production of pro-inflammatory cytokines (IL-6, TNF-α) and enhances the activity of natural killer (NK) cells and cytotoxic T-lymphocytes. Dietary interventions (e.g., fasting-mimicking diets) reduce chemotherapy toxicity, improve hematological and metabolic parameters (glucose, IGF-1), and suppress systemic inflammation (hs-CRP) [72].

Thus, non-pharmacological interventions are increasingly recognized as valuable components in combined brain tumor treatment. Cognitive-behavioral and physical activity demonstrate therapeutic effects mediated through modulation of both immune and endocrine systems [73–75]. Physical activity, in particular, shows a consistent association with improved survival in cancer patients and experimental models [8,9]. The mechanism involves exercise-induced IL-6 production

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by skeletal muscles, which can bind to receptors on immune cells like NK-cells, potentially stimulating antitumor immune responses [76]. Furthermore, preserved functionally active brain areas within GB-affected regions are positively correlated with patient survival [77], suggesting that patients with greater functional preservation may derive enhanced benefit from such interventions. This integrated approach represents a shift toward comprehensive care that addresses both local tumor dynamics and systemic host factors [12,78].

The critical impact of these systemic pathways is directly demonstrable in preclinical models, where the distinct neuro-endocrine-immune makeup of different rat strains exerts a direct influence on tumor graft uptake, progression, and therapy response. For example, Fischer (F344) rats exhibit a hyperreactive HPA axis and a Th2 immune profile, while Lewis rats demonstrate a hypoactive HPA axis and a predominant Th1 response [35,37,41,79–83]. This fundamental variability underscores that the host's physiological state is not a mere background variable but a decisive factor in glioma biology.

4. Characteristics of Commonly Used Immunocompetent Laboratory Rat Strains

Laboratory rats are essential models in biomedical research due to their genomic similarity to humans (>90%), rapid reproduction, and cost-effectiveness [38,84]. Their capacity for learning complex behavioral tasks [38] makes them valuable for neuro-oncology studies. Approximately 1,000 rat lines exist, categorized as outbred, inbred, transgenic, and knockout, with Wistar, SD, LE, Lewis, Wistar Kyoto and F344 being most widely used [85].

However, a complete understanding of pathogenesis requires looking beyond the tumor itself. A defining feature of GB is its existence as part of a dynamic "tumor-organism" system. One proposed mechanism of host resilience is the induction of gene programs driven by physical and cognitive activity. These programs function as natural mechanisms of cellular reprogramming—evolutionarily conserved adaptive strategies that facilitate the restoration of tissue function via key molecular regulators.

Consequently, the individual neuro-immune-endocrine status of the host organism, encompassing stress resilience and anxiety levels, acts as a direct modulator of tumor engraftment and development. The critical distinction between inbred (genetically homogeneous) and outbred (maintaining heterozygosity) strains affects experimental interpretation, as outbred rats show substantial individual variation in these behavioral phenotypes and stress responses [41,86,87]. This genetic diversity underpins variations in HPA axis function across strains [41], making strain selection crucial for experimental design.

4.1. Spontaneous Tumors in Laboratory Rats

Nervous system tumors are more common in laboratory rats than in other species. Strain-specific differences exist, with F344 rats showing the lowest frequency, while Wistar and SD rats exhibit glioma rates of ~1.5% in males and 0.7-0.8% in females [88]—a substantially higher incidence than the human GB incidence of ~0.003-0.006% [89]. Overall CNS tumor incidence ranges from 2.33% to 2.89% across Wistar, SD, and Han-Wistar strains [88]. Tumor frequency is influenced by sex, age, and the degree of inbreeding [41,88], with notable differences between strains: SD rats show 22% incidence in females and 5% in males, while LE rats show 28% in females and 10% in males. Strikingly, F1 hybrids (SD × LE) exhibit dramatically higher rates of 67% in females and 32% in males [90].

4.2. Wistar Strain Rats

The outbred Wistar strain (albino, *Rattus norvegicus*), established in 1906 at the Wistar Institute, is used in behavioral studies, geriatrics, infectious diseases, therapy safety/efficacy, and surgical models (Table 1). This strain exhibits robust humoral and cellular immune responses [53] with a normoreactive HPA axis contributing to adequate immune regulation [35]. Their intermediate anxiety phenotype, positioned between high-anxiety F344 and low-anxiety Lewis strains [35,41], makes them suitable for behavioral studies.

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Table 1. Characteristics of Major Laboratory Rat Strains and Lines.

Strain	Genetic Identity	HPA Axis/ Stress Status	Spontaneous CNS Tumor Rate	Spontaneous CNS Tumor Rate
Wistar [52,88,91]	Outbred	Normoreactive; Intermediate anxiety	Robust humoral/cellular immunity	High
Long-Evans [43,90]	Outbred	Hyperreactive; High basal ACTH/Cort; Low stress resistance	Lower thymus weight (immune implication)	High
Sprague-Dawley [41,88]	Outbred	Normoreactive; Moderate stress resistance	Prone to obesity/metabolic shifts; high incidence of malignant glioma	High
Fischer [49,86,92]	Inbred	Hyperreactive; High anxiety	Anti-inflammatory cytokine bias; resistant to carcinogens.	Low
Lewis [48,51,53]	Inbred	Hyporeactive	Th1-type bias (pro-inflammatory/autoimmune prone); Host for CNS-1 model.	Low
Wistar Kyoto [20,93,94]	Inbred	Hyperreactive; Depressive phenotype	High glucocorticoid background; low background pathology.	Very Low

Behaviorally, they display reduced avoidance behavior and an enhanced startle response [35], along with higher anxiety levels compared to SD rats and specific sexual behaviors linked to hypothalamic glycinergic neurotransmission [92]. Cognitively, they show a stronger reaction to cholinergic blockade than LE rats, and chronic stress induces social behavior changes [95].

4.3. Long-Evans Strain Rats

The outbred Long-Evans (LE, hooded) strain (*Rattus norvegicus*), created in 1915 by crossing Wistar females with wild grey male rats, is used in neurology, toxicology, ophthalmology, behavior, obesity, and alcoholism research [43]. They have different morphology, sensorimotor and locomotor abilities compared to Fischer, Lewis, Sprague-Dawley and Wistar rats [96]. LE rats are a model of marked HPA axis hyperactivity [43]. They exhibit greater HPA sensitivity to stressors, with higher basal ACTH and corticosterone levels during the light phase compared to Wistar, SD, and Fischer rats [43]. Male LE rats show a large ACTH response to stressors, and the expression of corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus is higher than in other strains [43]. They have a lower thymus weight than SD rats, which affects immunity [43]. Chronically stressed high-anxiety LE rats show depression-like symptoms [97]. LE rats have superior vision to albino strains and perform well in visual-cue tasks [96]. In a model of auditory sensitivity, the highest prevalence of tinnitus was in LE rats (75%), compared to SD (50%) and Wistar (33%) [98].

4.4. Sprague Dawley Strain Rats

The outbred Sprague Dawley (SD) strain (albino, *Rattus norvegicus*), derived from the Wistar strain in 1925, 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 rats show lower baseline anxiety than Wistar, LE, and F344 strains, with moderate stress reactivity [41]. They exhibit high and variable tumor growth rates, with malignant glioma (<1%) being the most common CNS tumor in carcinogenicity studies [41,88,90]. Their preference for fat/sucrose-rich diets [99] and variable behavioral responses make them a common model in neuro-oncology.

4.5. Fischer Strain Rats

The inbred Fischer 344 strain (F344, albino, *Rattus norvegicus*), developed in 1920 at the Crocker Institute, is widely used in modeling aging, oncology, nutrition, and surgical procedures. F344 rats exhibit high HPA axis reactivity under prolonged stress [49] and high innate anxiety [41]. Their unique immunological profile includes a propensity for an anti-inflammatory cytokine profile [48],

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contributing to lower overall tumor incidence and resistance to chemical carcinogens [42]. This immune signature makes them valuable for immunotherapy studies.

4.6. Lewis Strain Rats

The Lewis rat is an inbred strain (albino, *Rattus norvegicus*) that originated from Wistar stock in the early 1950s. It features a hyporeactive HPA axis [51] and a Th1-skewed immune response [48], which underlies its high susceptibility to autoimmune diseases. The CNS-1 glioma model, developed in this strain [50], is weakly immunogenic and exhibits an infiltrative growth pattern, making it valuable for neuro-oncology studies.

4.7. Wistar Kyoto Strain Rats

The Wistar Kyoto (WKY, albino, *Rattus norvegicus*) inbred strain was originally developed from outbred Wistar stock at Kyoto University as the normotensive control for the spontaneously hypertensive rat. WKY rats exhibit a hyperreactive HPA axis with elevated basal ACTH/corticosterone and prolonged stress responses [93]. Their depressive-like phenotype includes increased immobility in forced swim tests, reduced exploration, and anhedonia [93]. Distinct substrains model different depression sub-groups with genetic variations relevant to human studies [94].

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

Pilot studies of mutant IDH1 inhibitors [100,101] and drugs targeting EGFR [102] have demonstrated limited efficacy in high-grade glioma. These failures underscore the biological complexity of GB and the inadequacy of simplified preclinical models. Human GB is not a static entity but exists as a dynamic, evolving ecosystem defined by profound intra- and intertumoral heterogeneity driven by selective pressures from the microenvironment and therapy [3]. Unlike cell lines with a limited number of subclones, human GB consists of multiple cellular subclones, each possessing unique and adaptable pathways for survival and proliferation [103,104]. Consequently, the utility of rat models depends not merely on their histological resemblance to GB, but on how accurately they recapitulate these core biological behaviors: diffuse invasion, molecular heterogeneity, and interaction with the host immune system.

In this broader context, standard glioma models in immunocompetent animals remain highly relevant [31]. Unlike genetically engineered mouse models that often reproduce only specific pathway abnormalities, rat models are categorized into two distinct biological groups: cell line-based models (C6, RG2, F98, 9L) and transplantable tissue models (GB 101.8, GB 15/47, and others) [32–34]. The fundamental distinction lies in plasticity: models based on established cell lines represent selected subclones adapted to in vitro growth. These models often demonstrate limited infiltration, representing a more circumscribed glioma subtype, and their potential for extracerebral spread can hinder the formation of homogeneous experimental groups [31,32]. In contrast, transplantable tissue models retain cellular polyclonality and the diffuse growth pattern—distinctive features of human GB [3]—while critically lacking the artifact of extracerebral growth [33,34].

5.1. Spontaneous Glioma Model

Spontaneous malignant gliomas in rats serve as a phenotypic benchmark for experimental models. They are characterized by extensive, highly cellular, and often multicentric or diffuse growth that spreads across multiple CNS regions without clear boundaries, typically infiltrating adjacent structures via Virchow-Robin spaces [105]. Histopathological examination reveals feature characteristic of high-grade malignancy, including perineural satellitosis, hemorrhages, and pseudopalisading necrosis [105].

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A key diagnostic distinction from human pathology lies in the immunohistochemical profile. While human GB is defined by GFAP positivity, aggressive spontaneous and chemically induced rat gliomas are typically GFAP-negative and Vimentin-positive (often also Leu-7-negative and S-100-positive) [105,106]. The GFAP signal observed in these rat tumors usually originates from entrapped reactive astrocytes rather than neoplastic cells. Furthermore, unlike human GB, spontaneous rat gliomas often lack multinucleated giant cells and robust vascular endothelial proliferation [105]. This phenotypic drift suggests that many high-grade rat models represent a more undifferentiated or mesenchymal subtype of glioma.

In the context of the 2021 WHO Classification of CNS Tumors, which emphasizes integrating molecular data with histology [107], these tumors typically present as *IDH*-wildtype, providing a relevant context for modeling GB. Tumor incidence exhibits strain-dependent variation, with F344 and SD rats generally demonstrating higher rates of spontaneous neural neoplasms compared to Wistar rats [42,88,90].

5.2. Glioma Model Immunogenicity: A Spectrum of Tumor Rejection and Immune Evasion.

The most critical variable confounding preclinical studies is the host immune response, with models generally falling into two distinct categories: those triggering allogeneic rejection and those exhibiting true immune evasion. The C6 model, although widely used, represents a potential "false positive" for immunotherapy studies, particularly in Wistar or SD rats. This is due to the expression of the *Rt1u* haplotype (MHC-I ortholog), which is allogeneic to the *Rt1l* haplotype of Wistar hosts, triggering a potent humoral and cellular rejection response [108,109]. Consequently, tumor regression in this model often reflects allo-rejection characterized by heavy cytotoxic T-cell infiltration rather than therapeutic efficacy [107]. Similarly, the 9L gliosarcoma expresses native ortholog of human MHC-I molecules and remains highly immunogenic even in syngeneic F344 hosts, where its rapid growth allows it to outpace rather than evade the immune response [110]. In stark contrast, the F98 and RG2 models mimic the "cold" immune microenvironment characteristic of human GB. These models exhibit low immunogenicity not through mismatch, but via active suppression mechanisms, such as the downregulation of ortholog of human MHC-II and co-stimulatory molecules (e.g., B7.1/CD80) [50,111]. This absence of T-cell co-stimulation enables invasive growth without triggering significant lymphocytic infiltration. Furthermore, the GB 101.8 tissue model demonstrates a sophisticated evasion mechanism that closely parallels human biology. Our data reveal a post-translational defect in Beta-2-microglobulin (*B2m*), preventing the proper assembly of the ortholog of human MHC-I complex (*Rt1.A*) [32]. Crucially, these cells overexpress *Cd80* in the absence of MHC-I; without the necessary antigen presentation signal, CD80 may preferentially bind to the inhibitory receptor CTLA-4 rather than CD28. This mechanism effectively converts the tumor into an "immune trap," promoting T-cell anergy or exhaustion [32].

5.3. Invasion Patterns and Molecular Drivers

The capacity for brain invasion serves as a fundamental differentiator between "sarcoma-like" and "true glioma" models. The 9L and C6 models typically exhibit circumscribed growth; for instance, the 9L model forms sarcomatous masses with sharp borders and minimal invasion [50], while C6 tumors often grow as compact masses in many strains (Table 2). Consequently, these models are generally poor predictors for therapies specifically targeting invasion. In contrast, the F98 and RG2 lines, and particularly the GB 101.8 tissue model, accurately recapitulate the diffuse infiltration characteristic of human GB. In these models, individual tumor cells migrate far from the main mass, utilizing perivascular spaces and white matter tracts [33,54]. The molecular drivers of this phenotype vary: in F98 cells, invasion is linked to *Mct4* expression and hypoxia-driven pathways [8], whereas in the GB 101.8 model, the invasive phenotype correlates with a distinct molecular signature comprising *Pdgfra* overexpression and *Tp53inp2* upregulation (a p53 target), despite an *Idh1/2*-wildtype status [32]. A critical therapeutic implication of these differences is that while cell line models like F98 and

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RG2 pose a risk of extra-axial spread in 12–23% of cases [61], which can confound survival data, the GB 101.8 model is unique in its lack of extracranial growth [33].

5.4. Molecular Heterogeneity and Receptor Status

Model selection based on key molecular characteristics requires careful consideration of interspecies variations (Tables 2 and 3). These phenotypic differences are rooted in distinct molecular mechanisms. Invasion pathways in models like RG2 and C6 are often driven by well-characterized signaling cascades such as PDGFR [49,53,112]. Similarly, immunogenic profiles directly correlate with the expression of the ortholog of human MHC-I: the highly immunogenic C6 and 9L models exhibit robust antigen presentation, whereas the low immunogenicity of F98 and RG2 is associated with poor expression of key immune molecules like the ortholog of human MHC-II and B7.1 (CD80), fostering an immunosuppressive tumor microenvironment (TME) [50,54,111].

Molecular profiling reveals significant differences between models, highlighting that while human GB is typically characterized by *IDH1*/2-wildtype status and frequent p53 mutations, rat models demonstrate distinct molecular patterns. The 9L and F98 models harbor *Tp53* mutations, whereas RG2 and C6 maintain wildtype *Tp53*—though all rat models consistently show *Idh1*/2-wildtype status, mirroring the human disease [113]. *Egfr* and *Pdgfr* expression patterns further differentiate these models. Although *EGFR* and *PDGFR* overexpression represents a hallmark of human GB [113], the 9L model uniquely shows decreased expression of both receptors [50]. Conversely, the 101.8 GB model demonstrates a distinct molecular signature characterized by *Egfr* downregulation, *Idh1*/2-wildtype status, and *Pdgfra* overexpression [32]. These molecular distinctions are critical for interpreting therapeutic responses in preclinical trials.

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Table 2. Key Characteristics of Malignant Glioma Models.

Model / Origin	Host Strain (In-/Outbred)	Induction/ Cell Dose	Immuno-genicity	Latency (months)	Growth/BBB	Key Molecular Markers	Hormones/ Receptors	Metabolic Profile/Neurotransmitters
HUMAN								
Glioblastoma [1,4,7,100–102,114]	-	- -	Spontaneous/Immu-nosuppressive TME	-	Infiltrative/ partially disrupted or altered	*EGFR-amp/mut, PTEN-, TERT+, IDH1*-wt, +7/-10; ↑*PDGFR, IGF-1*	IGF, INS, EPO, GH, Test, E2, T3; BKR, ER	↑Gln/Glu, AcyICn, NADPH, BET/ ACh; Glu, GABA; DA, 5-HT, NE; SP, NPY; ATP; NO
RAT MODELS								
High grade glioma cell line								
C6 [50,54,107,108]	Wistar-Furth	MNU 10^4-10^5	High	1	Circumscribed/ partially disrupted	*Trp53*-wt (p53 status variable (Wild-type origin, prone to mutation during passaging), ↑*Pdgfra, Igf1, Egfr, Erbb3; Gfap-Idh1*/2-wt	GR, Test; 5-HT2A; DA; AR, ER	↓tCr, Tau, hTau, Ala, Ace, GSH, Gln, NAA, Asp; ↑Gly, Gln, Lip1.3
9L [50,110]	Fischer 344	MNU 10^4	High	0,5	Circumscribed/ Markedly disrupted	*Idh1*/2-wt, Mut*Trp53*, ↑*Egfr;* ↓*Pten, Fgfr-1, Pdgfrβ*	5-HT2A	↑Gln, ↓mIns
F98 [50,53,61,111]	Fischer 344	ENU 10^2- 10^5	Low	0,5-1	Infiltrative/Markedly disrupted	Mut *Trp53;* ↑*Pdgfrb, Ras, Egfr;* Gfap+, Vim+	ER	↑Gln, Gly, tCho/tCr, Lac; ↓tCr, NAA, Gua, mIns, Glu, GABA/ SP/NPY alterations
RG2 [50]	Fischer 344	ENU 10^2- 10^5	Non	0,5-1	Infiltrative/Markedly disrupted	*Trp53*-wt,↑*Pdgfrb, Igf2, Erbb3, Idh1*/2-wt	-	↑PCho, GPCho; ↓NAA, Glu, Gln, tCr

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Model / Origin	Host Strain (In-/Outbred)	Induction/ Cell Dose	Immuno-genicity	Latency (months)	Growth/BBB	Key Molecular Markers	Hormones/ Receptors	Metabolic Profile/Neurotransmitters
High grade glioma tissue								
GB 101.8 [32–34]	Wistar	DMBA 10^5-10^6	Low/ Non	0,5	Infiltrative/ Markedly disrupted	↑*Cdkn2b, Pdgfra, Gja1, Vim, Ncam1;* ↓*Pten, Egfr, Gfap-; Idh1*/2-wt	TfR, ER, GR	
GB 15/47 (unpublished data)	Wistar	ENU 10^5-10^6	Low/ Non	1	Infiltrative/ Markedly disrupted	*CD133-; Olig2+ VEGF+ Vim+,* ↑*Cdkn2a, Pik3ca, Trp53, Vegfa, Hif1a, Pdgfra*	GR-	

Key to Abbreviations: DMBA (7,12-Dimethylbenz[a]anthracene); ENU (N-Ethyl-N-nitrosourea); MNU (Methylnitrosourea); TME (Tumor Microenvironment); AMP (Amplification); MUT (Mutation); ↑ (overexpression); ↓ (downregulation); 5-HT2A (serotonin receptor 2A); AR (Adrenergic receptor); ER (Estrogen receptor); GR (Glucocorticoid receptor); TfR (Transferrin receptor); BET (Bromodomain and Extra-Terminal motif proteins); 5-HT (Serotonin); ACh (Acetylcholine); ATP (Adenosine triphosphate); DA (Dopamine); GABA (Gamma-aminobutyric acid); Glu (Glutamate); NE (Norepinephrine); NO (Nitric oxide); NPY (Neuropeptide Y); SP (Substance P); BKR (Bradykinin receptor); E2 (Estradiol); EPO (Erythropoietin); GH (Growth hormone); IGF (Insulin-like growth factor); INS (Insulin); T3 (Triiodothyronine); Test (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); NADPH (Nicotinamide adenine dinucleotide phosphate).

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5.5. Transplantable Tissue Glioblastoma Models (GB 101.8 and GB 15/47): Recapitulating Organotypic Heterogeneity and the BBB

While cell line-based models are convenient, they often fail to reproduce the organotypic architecture of human tumors due to selective pressure in vitro. The transplantable tissue models GB 101.8 and GB 15/47, maintained exclusively in vivo, offer a biologically superior alternative that preserves the structural and molecular complexity of human GB.

Unlike the circumscribed growth of 9L or the variable encapsulation of C6, the GB 101.8 and GB 15/47 models exhibit the hallmark histopathological features of human high-grade glioma: high cellularity, nuclear pleomorphism, robust microvascular proliferation, and pathognomonic pseudopalisading necrosis [32,34]. Crucially, they replicate the specific invasive patterns of human GB, spreading along white matter tracts and perivascular spaces (Scherer's structures) far beyond the main tumor mass [33].

This invasive pattern creates a heterogeneous BBB profile that mimics the clinical challenge. While the tumor core exhibits a disrupted, leaky BBB (permeable to contrast agents), the invasive margin resides behind an intact or semi-intact BBB [33]. This contrasts with many cell-line models that form a uniformly leaky "mass," making tissue models indispensable for evaluating the true CNS penetration of therapeutic agents.

Molecular profiling positions the GB 101.8 model as a distinct analog of the mesenchymal subtype of human GB. It is characterized by the upregulation of mesenchymal markers (vimentin, fibronectin) and overexpression of *Pdgfra* and *Tp53inp2*, concomitant with the downregulation of *Egfr* [32]. This PDGFRα-driven signature, occurring in the absence of *Idh1*/2 mutations, provides a specific platform for testing therapies targeting the mesenchymal transition—a key driver of treatment resistance in humans.

This model is particularly significant for immunotherapy research due to a defined mechanism of immune escape. Our data reveal a post-translational defect in Beta-2-microglobulin (*B2m*), leading to the loss of surface MHC-I expression. Paradoxically, these cells overexpress the co-stimulatory molecule CD80 (unpublished data). In the absence of MHC-I signal 1, CD80 likely engages the inhibitory receptor CTLA-4 on T-cells rather than CD28, effectively functioning as an "immune trap" [32]. This makes GB 101.8 an ideal model for testing strategies to overcome "cold" TME.

The GB 15/47 model offers a unique physiological control. Unlike most glioma models (including GB 101.8 and C6) that express Glucocorticoid Receptors (GR), the GB 15/47 tumor is GR-negative (unpublished data). This rare characteristic allows researchers to disentangle the direct effects of stress hormones on tumor cells from their systemic effects on the host immune system and TME, providing a precise tool for neuro-endocrine-oncology studies.

6. A Practical Framework for Selecting Rat Glioma Models

6.1. Strategic Selection Guidelines

No single model perfects recapitulate human GB. Selection must be driven by the specific biological question, balancing the trade-offs between immunogenicity, invasiveness, and logistic feasibility (Table 3).

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Table 3. Comparative Model Profiles: Strain Dependency and Key Characteristics.

Model	Syngeneic Host	Immunogenicity / MHC Expression	Growth Pattern	Recommended Application
C6	Wistar-Furth [50,54]	High. Allogeneic RT1u (ortholog of human MHC-I); triggers strong humoral/cellular immunity [107,108]. Microglia/macrophages in the TME can express ortholog of human MHC-II, but their function is suppressed in vivo [115].	Strain-dependent: Focal invasion in Wistar rats; circumscribed in SD/LE rats [116]. Extra-cranial or extra-axial growth.	Metabolic/Imaging studies. Caution: Avoid for immunotherapy or survival studies due to rejection artifacts.
9L	Fischer 344 [50,54]	Intermediate/High. Immunogenic in syngeneic hosts with consistent ortholog of human MHC-I expression [54,110].	Minimally invasive. Sharp borders, sarcomatous morphology [54]. Extra-cranial or extra-axial growth.	Reproducibility control. Good for preliminary drug toxicity screening; poor for invasion/immunology.
F98	Fischer 344 [50,54]	Low. Weakly or non-immunogenic; low ortholog of human MHC-I expression [54]. It expresses tumor antigens, but ortholog of human MHC-I expression is poorly characterized and may decrease during invasion; the model is known for its immunosuppressive TME [111].	Highly invasive with satellite islands, perivascular clustering, and vascular co-option [54]. Extra-cranial or extra-axial growth.	Therapy Resistance. Modeling radio-resistance and invasion-targeting drugs.
RG2	Fischer 344 [50]	Low. It lacks expression of ortholog of human MHC-II and B7.1, leading to minimal lymphocytic infiltration [50].	Highly invasive. It displays clear borders but extensive local spread [50]. Extra-cranial or extra-axial growth.	Immuno-oncology. Best cell line for studying "cold" tumors and BBB permeability.
GB 101.8	Wistar [32–34]	Low (Mechanistic Evasion). Defective *B2m* assembly (ortholog of human MHC-I loss) + *CD80* overexpression ("Immune Trap").	Diffusely Infiltrative. Mimics human GB dissemination; No extracranial growth [32,34].	Translational Neuro-oncology. Ideal for studying invasion, TME interactions, and heterogeneity without cell-line artifacts.

6.2. Strategic Model Selection Guidelines

Based on the comparative analysis presented, the selection of a rat glioma model must be strategically aligned with specific research objectives. For investigations into immunotherapy and immuno-oncology, the use of models with low intrinsic immunogenicity is paramount to evaluate novel agents without the confounding influence of alloreactive effects. In this context, the RG2 and F98 models, maintained in syngeneic F344 hosts, represent the preferred choices. Their proficient mechanisms of immune evasion—characterized by low expression of the human MHC-I ortholog and a lack of co-stimulatory molecules—create a realistic barrier for immune activation [50,54,111]. Conversely, the C6 and 9L models are generally unsuitable for such precision immuno-oncology studies due to their high and intermediate immunogenicity, respectively, which can result in therapy-independent tumor control.

When the research focus shifts to invasion mechanisms and therapy resistance, selecting highly invasive models becomes essential to recapitulate the diffuse infiltrative growth that defines human GB. Both the F98 and RG2 cell lines exhibit robust invasive patterns, featuring satellite islands and perivascular clustering [54]. However, the GB 101.8 transplantable tissue model is particularly advantageous for this research direction. It demonstrates marked diffuse infiltration with the

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migration of individual cells far from the main tumor mass and pronounced diversity of tumor cell subclones [32,34]. Crucially, unlike invasive cell-line models, the GB 101.8 model lacks the confounding factor of extracranial growth.

Finally, for general therapeutic screening of non-immune modalities, well-characterized models retain significant utility. The C6 model, supported by an extensive historical database, remains suitable for the initial, broad-spectrum screening of cytotoxic agents or radiation, provided that its allogeneic nature is acknowledged and results concerning immune-mediated effects are interpreted with caution [107]. Similarly, the 9L model serves a valuable role for historical comparisons or in experimental designs where a highly immunogenic, circumscribed tumor morphology is acceptable [50].

6.2. Conclusion: Towards a Rational Selection Paradigm

In conclusion, no single rat glioma model perfectly recapitulates the entirety of human GB biology. Researchers must adopt a strategic approach, prioritizing their primary research question. The fundamental choice often lies between focusing on invasive behavior (favoring F98, RG2, GB 101.8) or immune interactions (favoring RG2, F98). A thorough understanding of each model's strain-specific requirements and inherent limitations, as outlined in this framework, is the cornerstone of generating rigorous, reproducible, and ultimately translatable preclinical data.

7. Conclusions

Failures in therapy in glioblastoma (GB) treatment underscore the critical need for preclinical models that transcend simplified disease representations. This review highlights that meaningful progress requires the informed selection of biologically complex, syngeneic experimental systems, recognizing the host organism not as a passive recipient but as an active participant in gliomagenesis. The host's integrated neuro-immune-endocrine status, encompassing stress resilience, anxiety levels, and individual immune profile, plays a decisive role in tumor engraftment, progression, and microenvironment formation.

Consequently, the compatibility of the "glioma model – rat strain" pair constitutes the cornerstone of experimental validity. A rational framework for model selection must prioritize immunogenicity, invasive capacity, and growth reproducibility. Weakly immunogenic models (F98, RG2, GB 101.8) are indispensable for evaluating the systemic effects of physical and cognitive activity, as well as immunotherapy efficacy under conditions of both intact and suppressed immunity. Conversely, highly immunogenic models (C6, 9L) retain significant utility as sensitive bioindicators for assessing the host's immune competence and investigating the mechanisms of anti-tumor immune responses.

Furthermore, model selection must ensure the recapitulation of human GB growth patterns. The RG2, F98, and particularly the GB 101.8 tissue model best reproduce the heterogeneity of tumor subclones, molecular invasion pathways, and diffuse infiltration, offering distinct advantages over circumscribed models such as 9L.

Selection must also be aligned with molecular heterogeneity. While standard rat models mimic the *Idh*-wildtype status, like human GB, they diverge in *Tp53* mutation status and *Egfr*/*Pdgfr* expression patterns. Thus, the specific molecular landscape of the model must strictly correspond to the research objectives.

Looking forward, overcoming GB resistance will require combinatorial strategies targeting multiple vulnerabilities—from oncogenic signaling to metabolic reprogramming—evaluated in phenotypically matched hosts. By integrating the principles of cancer neuroscience and ensuring comprehensive biological compatibility between the model and the host, researchers can generate reproducible, clinically relevant data essential for accelerating the development of effective therapies.

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Abbreviations

The following abbreviations are used in this manuscript:

5-HT	Serotonin
5-HT2A	Serotonin receptor 2A
Ace	Angiotensin-Converting Enzyme
ACh	Acetylcholine
ACTH	Adrenocorticotropic Hormone
AcylCn	Acyl carnitines
Ala	Alanine
AR	Adrenergic/Androgen receptor
Asp	Aspartate
ATP	Adenosine triphosphate
B2m	Beta-2-microglobulin
B7.1	CD80
BBB	Blood brain barrier
BET	Bromodomain and Extra-Terminal motif proteins
BKR	Bradykinin receptor
CD133	Prominin-1 (Stem cell marker)
Cdkn2a/b	Cyclin-Dependent Kinase Inhibitor 2A/B
CNS	Central Nervous System
CRH	Corticotropin-releasing hormone
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
DA	Dopamine
DMBA	7,12-Dimethylbenz[a]anthracene
E2	Estradiol
Egfr/EGFR	Epidermal Growth Factor Receptor
ENU	N-Ethyl-N-nitrosourea
EPO	Erythropoietin
ER	Estrogen receptor
Erbb3	Receptor Tyrosine-Protein Kinase ErbB-3
F344	Fischer 344 (rat strain)
GABA	Gamma-aminobutyric acid
GB	Glioblastoma
GFAP	Glial Fibrillary Acidic Protein
GH	Growth hormone
Gja1	Gap junction alpha-1 protein
Gln	Glutamine
Glu	Glutamate
GPCho	Glycerophosphocholine
GR	Glucocorticoid receptor
GSH	Glutathione
Gua	Guanosine
Hif1a	Hypoxia Inducible Factor 1 Alpha
HPA	Hypothalamic-Pituitary-Adrenal axis
hs-CRP	high-sensitivity C-reactive protein
IDH	Isocitrate Dehydrogenase
IGF-1	Insulin-like Growth Factor 1
Igf1/2	Insulin-like Growth Factor 1/2 (gene)

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IL-6	Interleukin-6
Ins	Insulin
Lac	Lactate
LE	Long-Evans (rat strain)
Lip1.3	Lipid peak at 1.3 ppm
Mct4	Monocarboxylate transporter 4
mIns	myo-Inositol
MHC	Major Histocompatibility Complex
MNU	N-Methyl-N-nitrosourea
Mut	Mutation
NAA	N-acetylaspartate
NADPH	Nicotinamide adenine dinucleotide phosphate
Ncam1	Neural Cell Adhesion Molecule 1
NE	Norepinephrine
NK-cells	Natural Killer Cells
NO	Nitric oxide
NPY	Neuropeptide Y
Olig2	Oligodendrocyte Transcription Factor 2
PCho	Phosphocholine
Pdgfr/PDGFR	Platelet-Derived Growth Factor Receptor
Pik3ca	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PTAH	Phosphotungstic Acid Hematoxylin
Pten/PTEN	Phosphatase and TENsin homolog
Ras	Rat Sarcoma virus oncogene
RT1	Rat Major Histocompatibility Complex
S100	S100 Calcium Binding Protein
SD	Sprague Dawley (rat strain)
SP	Substance P
T3	Triiodothyronine
Tau	Taurine
TERT	Telomerase Reverse Transcriptase
Test	Testosterone
TfR	Transferrin receptor
Th1/Th2	T-helper type 1 / 2 immune response
TME	Tumor Microenvironment
TNF-α	Tumor Necrosis Factor Alpha
Tp53/Trp53	Tumor Protein P53
Tp53inp2	Tumor Protein P53 Inducible Nuclear Protein 2
VEGF	Vascular Endothelial Growth Factor
Vim	Vimentin
WHO	World Health Organization
WKY	Wistar Kyoto (rat strain)

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