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Review

Genetically engineered mice models and generating glioblastoma multiforme in the brain

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ABSTRACT

Glioblastoma multiforme (GBM) is a brain tumor that occurs in adults. It is one of the most common malignancies in the world and its incidence is increasing every year. Patients with GBM have a poor prognosis and treatment is largely based on the recurrence rate. In this review, we examine therapeutic modalities against GBM using genetically modified mouse models, which play an important role in cancer research.

Keywords: Cancer stem cells, central nervous system, genetically modified mouse models, glioblastoma multiforme, oncogenes.

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor in adults. These fast-growing tumors infiltrate brain tissue and can lead to death in a very short time if left untreated. Although standard treatments include surgery, radiotherapy, and chemotherapy, the median survival time is only 15 months and five-year survival is less than 5%.[1] Glioblastoma multiforme, the most aggressive brain tumor in adults, ranks first as the most malignant tumor in the central nervous system (CNS), surpassing even metastatic spread.[2] According to the latest classifications of the World Health Organization, GBM is considered one of the highest-grade (IV) brain tumors.

These tumors are composed of abnormally developing and rapidly proliferating cells from brain cells (astrocytes) known as star cells. A Stage IV neoplasm is a type of cancer that shows uncontrolled growth and has a high potential to spread to surrounding tissues. We can describe the late stage as a phase where the tumor has grown extensively, resulting in significant damage to the brain. The main reason why GBM requires a complex diagnosis and treatment plan is that these tumors are highly aggressive and tend to spread to critical areas that affect brain function. Therefore, developing more effective methods to treat GBM is of great importance to improve the survival and quality of life of patients.

GENETICALLY MODIFIED MOUSE MODELS

Mouse models play an indispensable role in understanding human cancers and developing treatments. These models mimic the onset, development, and spread of cancer, allowing researchers to understand the disease and test new drugs. Advances in genetic engineering have made it possible to more accurately model human cancers by making more precise adjustments to the mouse genome. For example, mouse models of melanoma cancer can closely mimic the genetic and molecular features of human melanomas.[3]

Genetically modified mouse models (GEMM) play an important role in cancer research, mimicking tumor formation in the environment with a natural immune system. These tumors have characteristics similar to human tumors

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and can metastasize spontaneously. The GEMM models are more realistic than cancer cell injection models and can be used to validate candidate cancer genes and drug targets, evaluate treatment efficacy, study the impact of the tumor microenvironment, and investigate mechanisms of drug resistance. Thanks to the latest technologies, it is possible to create GEMM models faster and closer to human cancers. With these advanced models, it is aimed to accelerate clinical trials of cancer treatments and facilitate the delivery of new treatment methods to patients.[4]

However, genetic, physiological, and immune system differences between mouse and human models should not be overlooked. These differences mean that mouse models may not always accurately reflect human disease. For example, mouse models are an important tool in melanoma research. Mouse models are useful for contributing to the biological understanding of melanoma and for testing new treatment strategies. These models can mirror some aspects of human melanoma and provide important clues for understanding the behavior of the disease. However, it is not always possible for mouse models to capture all the nuances of human disease. Therefore, researchers are constantly striving to develop better mouse models that are closer to human disease.[5]

Today, many different mouse models are available for various types of cancer, including melanoma. However, each of these models has its advantages and disadvantages. For example, some models target specific genetic mutations, while others focus on modeling environmental factors that cause cancer. Researchers must choose the most appropriate model to answer a specific question. The standard approach to treating GBM consists of surgical removal of the tumor followed by radiotherapy and chemotherapy.[6] Unfortunately, these treatments are unlikely to defeat the disease.

Cancer stem cells

Cancer stem cells (CSCs) are a subset of tumor cells that exhibit self-renewal ability, high tumorigenicity, and resistance to standard cancer treatments such as chemotherapy and radiotherapy.[7] These cells contribute significantly to tumor initiation, progression, metastasis, and recurrence. Cancer stem cells are found in different regions of the tumor microenvironment (TME), where they can adapt to various stress factors such as hypoxia, leading to increased aggressiveness and resistance to therapy.[8]

The concept of CSCs was first described in lymphoma. Although the specific characteristics of CSCs in lymphoma differ from those in other cancers, the general principles of CSC biology apply. Research on CSCs in various cancers, including GBM, has highlighted their importance for treatment resistance and relapse. Targeting CSCs with innovative approaches holds promise for improving outcomes in cancer treatment.[9]

Advantages of genetically modified mouse models

Glioblastoma multiforme is a molecularly complex and aggressive cancer, and accurate animal models are needed for the development of novel therapeutic approaches. GEMMs have become a promising tool in this field as they mimic the genetic characteristics of human GBM. Compared to conventional models, GEMMs can be generated quickly and cost-effectively (6-12 months *vs.* 2-3 years), have genetically homogeneous populations, and have more similar characteristics to human GBM. Thus, the results obtained are more accurate, reliable, and clinically relevant.[10]

Effective GEMM in glioblastoma multiforme formation

Genetically modified mouse models have been instrumental in the study of GBM, a type of brain cancer. Several effective GEMM models have been developed to study GBM formation. One notable model is the replication competent avian-like sarcoma (RCAS) virus/tv-a system, which allows targeted expression of oncogenes in specific cell types in the brain, mimicking the genetic changes seen in human GBM.

RCAS/tv-a model

The RCAS/tv-a model is one of the most widely used GEMMs for modeling GBM.[11] This system allows for the targeted expression of oncogenes in specific cell types in the brain, mimicking the genetic changes seen in human GBM. By introducing oncogenes such as epidermal growth factor receptor (EGFR), and platelet-derived growth factor (PDGF) into specific cell populations

in the brain, researchers can trigger the formation of GBM-like tumors in mice.[12] An oncogene is a gene with the potential to cause cancer. When mutated or expressed at high levels, oncogenes can promote abnormal cell growth and division, leading to cancer development. These genes can encode proteins involved in cell signaling, cell cycle regulation, and other cellular processes that, when dysregulated, contribute to tumor formation. Examples of oncogenes include EGFR, MYC, and RAS, as in the example just given. Understanding oncogenes and their role in cancer development is crucial for developing targeted therapies to treat various types of cancer.

Newer tv-a strains (e.g., Pax3-tv-a, Ctv-a, Btv-a) driven by promoters active in specific cell populations in the brain at various stages of neurodevelopment have helped to study the origin and biology of different CNS tumors, such as pediatric gliomas and ependymomas.[13] As the understanding of the processes underlying neurobiology and gliomagenesis improves, new tv-a rodent strains may be created for use with the growing diversity of RCAS vectors.

Neurodevelopment is a complex process in which different cell populations in the brain are driven by promoters that are activated at specific times. In recent years, tv-a strains, a new type of mouse model used to study various stages of neurodevelopment, have become an important research tool. Examples such as Pax3-tv-a, Ctv-a, and Btv-a have been created using promoters that are active in specific cell populations, making it possible to understand the details of neurodevelopment through these models. Furthermore, tv-a mouse models are used to understand the origin and biology of different CNS tumors, especially pediatric gliomas, and ependymomas.[14] These tumors can occur in the brain and spine. Tv-a mouse models have been considered as an effective tool to investigate the development process of such tumors and the molecular mechanisms involved.

Glioblastoma multiforme is actually linked to the CNS as it is a type of brain tumor that originates from glial cells in the brain. It is the most common and aggressive primary brain tumor in adults and directly affects the CNS. Studies have highlighted the link between malignancy and stemness in GBM and have shown that specific molecular mechanisms are involved in the CNS that contribute to the pathogenesis of this type of brain cancer.[15] Furthermore, research has shown that glioma cells take advantage of pathways active in normal CNS cells, including those involved in neurotransmitter signaling, to develop and progress in the brain.[16]

Researchers hope that with increasing knowledge in neurobiology and gliomagenesis, they will be able to generate new strains of tv-a mice and use them together with RCAS vectors. These new models could contribute to a better understanding of CNS tumors and allow the development of more effective treatments for these diseases.

The versatility and efficiency of the RCAS/tv-a system are demonstrated by validating computational and mathematical analyses of human GBM data in mouse models to link the three GBM transcriptional molecular subtypes (classical, proneural, and mesenchymal) in an evolutionary framework. Ozawa *et al.*[17] reported that the more primitive tumors were of "proneural" character. In both Ntv-a and Gtv-a mice, overexpression of PDGF-A, predicted by computational analyses as the strongest initial driving event in glioma evolution, was one of the only transformations sufficient to drive gliomagenesis *in vivo*.

The versatility and efficiency of the RCAS/tv-a system were confirmed by computational and mathematical analyses of human GBM data in mouse models, revealing evolutionary links between GBM transcriptional molecular subtypes. Glioblastoma multiforme transcriptional molecular subtypes include classical, proneural, and mesenchymal. In the context of GBM, the terms "classical", "proneural" and "mesenchymal" refer to different molecular subtypes of the disease. These subtypes are defined by the expression of specific genes and proteins associated with different clinical outcomes and therapeutic responses. Classic GBM is characterized by the expression of genes associated with cell proliferation and angiogenesis, such as EGFR and PDGF receptors. Classical GBM is usually associated with a more aggressive clinical course and poor prognosis.

Proneural GBM is characterized by the expression of genes associated with neuronal differentiation and development, such as oligodendrocyte transcription factor 2 and nestin. It is generally associated with a better prognosis and may be more sensitive to certain therapies. Mesenchymal GBM is characterized by the expression of genes associated with mesenchymal differentiation and extracellular matrix remodeling, such as CD44 and Mer tyrosine kinase. It is usually associated with a more aggressive clinical course and treatment resistance.[18-21]

Researchers have reported that the more primitive tumors have a "proneural" character.[22] Computational analyses in the Ntv-a and Gtv-a mouse models predicted that the strongest initial driving event in glioma evolution was overexpression of PDGF-A. This finding suggests that PDGF-A overexpression may play a critical role in the initiation of glioma development in these mouse models. Understanding the impact of PDGF-A overexpression on glioma evolution may provide valuable insights into the molecular mechanisms underlying glioma formation and progression, potentially leading to the identification of novel therapeutic targets for this type of brain cancer. The Ntv-a and Gtv-a models are GEMM widely used in GBM studies. These models are based on the RCAS/tv-a system, which allows for targeted expression of oncogenes in specific cell populations in the brain, mimicking the genetic changes observed in human GBM. In the Ntv-a model, oncogenes are delivered to specific cell populations in the brain using the RCAS/tv-a system. This model allows researchers to trigger the formation of GBM-like tumors in mice by delivering oncogenes such as EGFRvIII or PDGF to targeted cells. The Ntv-a model has been valuable in studying GBM pathogenesis and testing potential therapeutic strategies.[23] The Gtv-a model is another GEMM that uses the RCAS/tv-a system to introduce oncogenes into specific cell populations in the brain. By targeting cells with oncogenes associated with GBM, researchers can replicate key aspects of GBM formation and progression in mice. The Gtv-a model, along with other GEMMs, contributes to our understanding of the molecular mechanisms underlying GBM.[24] Both the Ntv-a and Gtv-a models play an important role in advancing research on GBM by providing insights into the genetic alterations and pathways involved in this aggressive brain cancer.

Targeting tumor suppressors

These models focus on inactivating genes such as p53, PTEN, or Rb, which normally act as brakes on cell growth.[25] By eliminating these protectors, researchers can study how uncontrolled proliferation contributes to GBM.

For example, genetic alterations in human GBM, such as loss of tumor suppressor genes like PTEN, TP53, and CDKN2A, or activation of oncogenic signaling pathways like p21-RAS, PI3K, EGFR, can be modeled in GEMMs. In particular, loss of PTEN can lead to uncontrolled growth and proliferation of cells. Loss of PTEN is quite common in GBM and is known to negatively affect the prognosis of patients.[26] Or in another example, the TP53 gene is a tumor suppressor gene that protects cells against DNA damage. TP53 mutations can cause cells to become unable to repair DNA damage and become cancerous. TP53 mutations are quite common in GBM and are known to negatively affect the prognosis of patients.[27] Different GEMMs may represent various subtypes of GBM or different stages of the disease process. Therefore, it is important to choose the GEMM model that best fits the research question.[28]

Transplantation of human GBM cells into immunodeficient mice is based on research showing the existence of a tumor hierarchy and that cells at the top of this hierarchy have specific properties. In these studies, the gradual dilution of cells resulted in only a certain subset of cells being able to regenerate the tumor, supporting a hierarchical process of tumor progression. However, there has been a lack of suitable tools to directly isolate and characterize this subset of cells. Therefore, cells known as glioma stem cells were identified based on their ability to form spheres *in vitro* and tumors *in vivo*. However, it is still unclear how these cells identified in culture behave in the actual GBM tumor. Since GBM is a highly heterogeneous and invasive tumor, it interacts extensively with the TME in the CNS. This may be one of the factors contributing to the ineffectiveness of conventional chemotherapy in GBM. Genetically engineered spontaneous GBM,

GEMMs support this hypothesis. However, similar evidence is not yet sufficiently available in human GBM studies. Single-cell sequencing technology examining intracellular heterogeneity and transcriptional plasticity in human GBM suggests that the tumor cell population is composed of plastic or alternatively proliferating cancer stem cells. Slowly proliferating or quiescent CSCs play an important role in treatment resistance and disease recurrence in several cancer types, including non-small cell lung cancer, glioblastoma, breast cancer, and colorectal carcinoma. These slow-proliferating CSCs are often more resistant to anti-cancer therapies and can initiate new tumor growth. Studies have shown that targeting these quiescent CSCs may be beneficial in cancer treatment.[29-32]

In colorectal carcinoma, for example, while conventional therapies primarily target rapidly dividing cancer cells, CSCs, which are mostly quiescent and have the ability to self-renew, may escape these therapies, leading to treatment resistance and disease relapse. Several signaling pathways such as Wnt/β-catenin, Notch, Hedgehog, Hippo/YAP, and PI3K/Akt contribute to the self-renewal and therapy-resistance properties of CSCs in colorectal carcinoma.[33]

In conclusion, GEMMs play an important role in cancer research. These models are used to contribute to the understanding of human cancers and the development of treatments. GEMM models are an important tool for understanding the molecular mechanisms and biology of GBM. These models are critical for developing more effective methods to treat GBM. GEMMs are an important tool to understand the molecular and biological mechanisms of GBM and to improve its treatment. GEMMs designed to mimic the different subtypes of GBM, of which there are three main molecular subtypes: classical, proneural, and mesenchymal, can be used to investigate the role of CHDs and the efficacy of novel therapies. Although GEMMs cannot fully mimic human GBM, they will continue to play an important role in GBM research and help us develop more effective methods of treating GBM. In particular, tv-a models form a subset of GEMMs and are used specifically in the study of GBM. These models are used to better understand the molecular underpinnings and subtypes of the disease, evaluate treatment strategies, and identify potential therapeutic targets. However, despite the advantages of models, it is important to consider the genetic and physiological differences between mice and humans. Mouse models may not accurately reflect human diseases, so the direct applicability of the findings to humans should be questioned. TV-a models are a valuable tool for understanding the molecular subtypes and evolution of Glioblastoma. By mimicking the development of GBM, these models are used to understand the fundamental biological processes of the disease. This understanding is important for developing new treatment strategies and finding more effective methods to fight GBM.

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