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Emerging Microtubule Targets in Glioma Therapy

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Major advances in the genomics and epigenomics of diffuse gliomas and glioblastoma to date have not been translated into effective therapy, necessitating pursuit of alternative treatment approaches for these therapeutically challenging tumors. Current knowledge of microtubules in cancer and the development of new microtubule-based treatment strategies for high-grade gliomas are the topic in this review article. Discussed are cellular, molecular, and pharmacologic aspects of the microtubule cytoskeleton underlying mitosis and interactions with other cellular partners involved in cell cycle progression, directional cell migration, and tumor invasion. Special focus is placed on (1) the aberrant overexpression of βIII-tubulin, a survival factor associated with hypoxic tumor microenvironment and dynamic instability of microtubules; (2) the ectopic overexpression of γ-tubulin, which in addition to its conventional role as a microtubule-nucleating protein has recently emerged as a transcription factor interacting with oncogenes and kinases; (3) the microtubule-severing ATPase spastin and its emerging role in cell motility of glioblastoma cells; and (4) the modulating role of posttranslational modifications of tubulin in the context of interaction of microtubules with motor proteins. Specific antineoplastic strategies discussed include downregulation of targeted molecules aimed at achieving a sensitization effect on currently used mainstay therapies. The potential role of new classes of tubulin-binding agents and ATPase inhibitors is also examined. Understanding the cellular and molecular mechanisms underpinning the distinct behaviors of microtubules in glioma tumorigenesis and drug resistance is key to the discovery of novel molecular targets that will fundamentally change the prognostic outlook of patients with diffuse high-grade gliomas.
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Introduction

Brain tumors constitute the most common type of solid tumors in children. Gliomas, which account for most primary tumors of the central nervous system (CNS) in children and adults, are broadly divided into low grade (World Health Organization [WHO] grades I and II) and high grade (WHO grades III and IV). Depending on their

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anatomical location, circumscribed gliomas (WHO grade I) can be amenable to gross total or subtotal surgical resection and hence potential cure. In contrast, diffuse gliomas, which span the histologic spectrum of grades II-IV tumors, are therapeutically challenging given their highly infiltrative nature and potential to undergo anaplastic transformation to become high-grade gliomas. Glioblastoma (WHO grade IV) is the most frequent and most malignant form of primary brain cancer in adults, whereas pilocytic astrocytoma, a low-grade tumor (WHO grade I), is the most common type of glioma in children. That said, high-grade gliomas, including glioblastoma, can also be encountered in children. In the pediatric setting, deep-seated diffuse thalamic gliomas and diffuse intrinsic pontine gliomas pose a formidable therapeutic challenge given their infiltrative growth pattern and propensity for anaplastic change. Overall, the prognosis of glioblastoma remains dismal for all age groups, with most patients dying within 1 year after diagnosis. In adults, primary and secondary glioblastomas constitute clinically and genetically distinct disease subtypes.[1] Primary glioblastomas develop rapidly de novo, affect mainly the elderly, and are genetically characterized by loss of heterozygosity, epidermal growth factor receptor (EGFR) amplification, p16INK4a deletion, and *PTEN* mutations.[1] Secondary glioblastomas manifest usually in younger patients, frequently exhibit *P53* mutations, and develop through tumor progression from low-grade diffuse astrocytoma or anaplastic astrocytoma.[1] Genetically, pediatric gliomas differ from adult gliomas.[2] In recent years, emphasis has been placed on stratifying brain tumors by molecular subtype based on the presence of specific mutations.[3,4] This approach is gaining increasingly clinical acceptance in the classification and typing of gliomas and medulloblastomas.[4] Although significant strides have been made in the genomics and epigenomics of brain tumors during the past decade, these discoveries have not been translated at the therapeutic level.

Currently, the standard approach to the treatment of glioblastoma combines surgical resection, chemotherapy, and radiotherapy. Beyond initial surgery aimed at reducing the tumor burden, the mainstay of therapy is based on the use of concurrent and adjuvant temozolomide, a DNA-binding agent, in conjunction with radiotherapy (radiochemotherapy).[5,6] Combined radiotherapy and chemotherapy with temozolomide have increased median survival time of 9-15 months, compared with radiosurgery alone.[5] However, increases in survival have been negligible (ie, 6-9 months for median progression-free survival and 14.6 months for overall survival times).[7] Hypermethylation (and thus functional inactivation) of the *O^6-methylguanin-DNA-methyltransferase (MGMT)* gene enhances chemosensitivity to temozolomide in this clinical setting.[8] Currently temozolomide treatment is administered regardless of *MGMT* methylation status. Treatment challenges are multifold and may be linked to, and compounded by, drug resistance and suboptimal drug delivery owing to hindrance from the blood-brain barrier.[9] Moreover, significant variations regarding responsiveness to treatment, or lack thereof, are observed among patients with tumors of the same histologic type and tumor grade. This necessitates the elucidation of new molecular targets that are biologically linked to cancer behaviors in gliomas and an urgent mandate for alternative innovative approaches to glioma therapy.

This review offers a critical appraisal of the current knowledge on the subject of the microtubule cytoskeleton in cancer stemming from an interdisciplinary effort undertaken by basic and clinical researchers to develop new treatment strategies in high-grade gliomas based on a rigorous interrogation of altered microtubule behavior in brain cancer cells.

Microtubules: Time-Honored Targets in Cancer Chemotherapy

One of the core strategies used in cancer pharmacology is to disrupt the integrity of microtubules in the mitotic spindle thus blocking and restraining mitotic division.[10-15] Microtubules in cancer cells have also emerged as a target aimed at countering tumor cell motility and invasion given the involvement of interphase microtubules in directional tumor cell migration and metastasis acting in concert with the actin cytoskeleton.[16-19] To that end, recent studies have raised awareness about control of Rho family GTPases by microtubules, which bears critical importance in the regulation of the actin cytoskeleton during cell migration, and microtubule dynamics.[19,20] In view of the fact that microtubules constitute the main target of numerous agents used in cancer chemotherapy, new insights into the mechanisms of dysregulation of microtubules in cancer cells would be crucial in the development of new molecularly targeted approaches for the rational treatment of diffuse gliomas and, in particular, the highly aggressive and devastating glioblastoma.

Microtubules, which are composed of αβ-tubulin heterodimers, are the targets of some of the most widely used and time-honored anticancer natural-product small molecule inhibitors, collectively referred to as *tubulin-binding agents* (TBAs).[10-13] TBAs are broadly classified as *microtubule-destabilizing* drugs and *microtubule-stabilizing drugs*.[10,13,14] The former are further subdivided into *vinca domain–binding agents* (vinca alkaloids and dolostatins) and *colchicine domain–binding agents* (colchicine and analogues) but also encompass other microtubule-depolymerizing compounds such as estramustine, noscapine, and certain psychoactive drugs (phenytoin).[13] The microtubule-polymerizing drugs are principally represented by the taxanes and epothilones (Box 1).[10,13,14]

TBAs are also commonly referred to as antimitotic drugs because they cause mitotic arrest and produce cell death.[10] TBA-induced tumoricidal action occurs either at the G1 phase of the cell cycle or after mitotic arrest; however, such action may escape by virtue of "mitotic slippage."[10] In addition to anticancer effects, TBAs exert antitumor angiogenic effects.[21] A major hurdle accounting for treatment