Journal of Cellular Physiology

Tubulin Targets in the Pathobiology and Therapy of Glioblastoma Multiforme. II. γ-Tubulin

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Glioblastoma multiforme (GBM) is the most common and deadliest form of primary brain cancer in adults. Despite advances in molecular biology and genetics of cancer there is no currently available treatment for these tumors. Aberrant patterns of γ-tubulin expression and compartmentalization in GBM have been reported lending credence to the assertion that these changes might underlie perturbations in microtubule nucleation and mitosis associated with glioma tumorigenesis and tumor progression. This minireview focuses on the role of γ-tubulin in the pathobiology of GBM in the light of emerging concepts concerning the function of γ-tubulin and its potential role in tumorigenesis putting forward the concept that γ-tubulin might serve as a novel marker of anaplastic change in gliomas.
J. Cell. Physiol. 221: 514–520, 2009.

Gliomas are the most common primary brain tumors of all ages and constitute a genetically and phenotypically heterogeneous group of primary brain tumors arising from glia or their precursors in the central nervous system (CNS). Most gliomas are of astrocytic origin (astrocytomas) although oligodendrogliomas and mixed glial tumors are also common. The clinical behavior of astrocytomas is reflected in a four-tier histological grade system (grades I–IV) according to an ascending scale of malignancy. Glioblastoma multiforme (GBM) (grade IV) is the most prevalent and deadliest form of glioma and brain cancer in adults. Two forms of GBM are recognized, primary or de novo and secondary, which follows an evolutionary progression from grade II through grade IV lesions (von Deimling et al., 1993; Watanabe et al., 1996). Currently, GBM is not amenable to conventional surgical, radiotherapeutic, and/or chemotherapeutic interventions and as such, it carries a dismal prognosis. Despite a plethora of scientific publications reflecting advances in the molecular biology and genetics (including integrated genomic analysis) of brain tumors during that past decade (Parsons et al., 2008), no significant strides have been made in the treatment of GBM.

The authors of this review advocate a fresh approach to GBM pathobiology and development of a new class of diagnostic/prognostic brain tumor markers with potential therapeutic implications based on altered tubulin functions, which underlie malignant (anaplastic) transformation of gliomas and progression into GBMs. In recent years, we have shown overexpression and ectopic compartmentaization of γ-tubulin in GBMs (Katsetos et al., 2006, 2007). This review focuses on the intricate changes in the expression, distribution, and compartmentalization of γ-tubulin in cancer with emphasis on glial tumors and GBM tumorigenesis.

Centrosomes

The centrosomes are small non-membranous juxtanuclear cytoplasmic organelles composed of a pair of barrel-shaped centrioles surrounded by an amorphous pericentriolar matrix. Each centriole comprises nine sets of triplet microtubules while other protein structures are found both within and outside the centrioles. The pericentriolar material comprises a large repertoire of proteins including microtubule nucleating and docking proteins as well as numerous key regulators of

Abbreviations: CNS, central nervous system; γTuRC, γ-tubulin ring complex; GBM, glioblastoma multiforme; GCP, γ-tubulin complex protein; MTOCs, microtubule-organizing centers; SAC, spindle assembly checkpoint.

Christos D. Katsetos and Pavel Dráber contributed equally to this work.

Contract grant sponsor: Ministry of Education, Youth and Sports of the Czech Republic;
Contract grant number: LC545.
Contract grant sponsor: Institutional Research Support;
Contract grant number: AVOZ 50520514.
Contract grant sponsor: St. Christopher's Foundation for Children.

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Received 20 April 2009; Accepted 22 June 2009

Published online in Wiley InterScience (www.interscience.wiley.com.), 31 July 2009.
DOI: 10.1002/jcp.21884

cell-cycle progression (Stearns et al., 1991; Zheng et al., 1991; Stearns and Kirschner, 1994; Dictenberg et al., 1998; Young et al., 2000; Doxsey, 2001; Krämer et al., 2002, 2004; Nigg, 2002). Normal diploid somatic cells contain a single centrosome. Centrosomes undergo duplication precisely once before cell division and this process is linked to the cell division cycle via *cyclin-dependent kinase* (cdk) 2 activity that couples centriole duplication to the onset of DNA replication at the G(1)/S phase transition (Krämer et al., 2002).

Centrosomes subserve diverse cellular functions. The centrosome is the site for microtubule nucleation both in the context of cell division (cytokinesis) and mitosis, ensuring balanced chromosome segregation (Brinkley and Goepfert, 1998; D'Assoro et al., 2002; Nigg, 2002), as well as in the growth and organization of cytoplasmic microtubules contributing to cell polarity and architecture (Brinkley, 1985). Centrosome-nucleated microtubules are organized into astral arrays in interphase and mitotic spindles in mitosis. The nucleation of microtubules occurs from within the pericentriolar material where the key microtubule nucleating protein, γ-tubulin is located (Joshi et al., 1992; Zheng et al., 1995). During normal mitosis, two centrosomes ensure the assembly of bipolar spindles and proper chromosomal segregation. Extra copies of centrosomes result in the formation of multipolar spindles resulting in chromosomal missegregation (Nigg, 2002; Krämer et al., 2004). While the traditional and best-characterized function of centrosomes is to mediate the strictly bipolar separation of chromosomes during mitosis, centrosomes are also involved in divergent regulatory events of the cell cycle including entry into mitosis, cytokinesis, G(1)/S transition, and monitoring of DNA damage as attested by a growing list of centrosome-associated regulatory proteins (Krämer et al., 2004). To this end, the centrosome is also involved in the initiation of the S phase and the regulation of cell-cycle progression (Brinkley, 1985; Khodjakov and Rieder, 1999; Doxsey, 2001; Hinchcliffe et al., 2001) and it is also involved in DNA damage response by way of integrating cell-cycle arrest and repair signals in response to genotoxic stress (Löffler et al., 2006). At the centrosome, γ-tubulin forms a protein complex with pericentrin which exhibits a lattice-like organization (Dictenberg et al., 1998; Young et al., 2000).

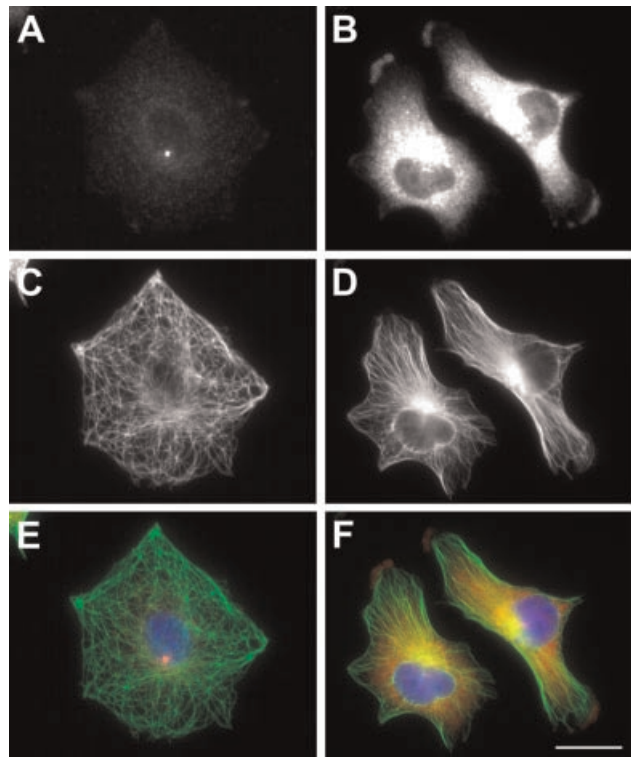

Fig. 1. Comparison of γ-tubulin distribution in fibroblast and glioblastoma cells. Mouse embryonal fibroblasts 3T3 (A,C,E) or human glioblastoma cells T98G (B,D,F) were fixed in methanol and double-label stained with mouse monoclonal antibody TU-31 (Nováková et al., 1996) to γ-tubulin (A,B) and rabbit polyclonal antibody to α-tubulin (C,D). Superposition of images are shown in E and F (γ-tubulin in red; α-tubulin in green; nuclei labelled with DNA-binding dye DAPI in blue). Fluorescence images were captured under identical conditions (200 msec exposure) and processed in exactly the same manner. Scale bar, 20 μm.

γ-Tubulin and Microtubule Nucleation

γ-Tubulin has a central role in microtubule nucleation. It was first discovered in filamentous fungus *Aspergillus nidulans* as a product of the *mipA* gene during genetic screening for proteins that interact with β-tubulin (Oakley et al., 1990). Experimental depletion of γ-tubulin led to a depletion of microtubules and to growth arrest. Immunolocalization of γ-tubulin revealed an enrichment of this protein in microtubule-organizing centers (MTOCs). A typical localization of γ-tubulin in mouse embryonal 3T3 cells is shown in Figure 1A. Up to now, γ-tubulin cDNAs and genes have been cloned and sequenced from a huge variety of organisms, suggesting that that γ-tubulin is present in all eukaryotes. In mammalian cells, two γ-tubulin genes TUBG1 and TUBG2 exist, encoding two closely related isotypes (Wise et al., 2000). TUBG1 is ubiquitously expressed in all cell types, whereas TUBG2 has been found mainly in the brain (Yuba-Kubo et al., 2005).

Multiple charge variants were detected by two-dimensional electrophoresis in brain (Détraves et al., 1997; Sulimenko et al., 2002), chicken erythrocytes (Linhartová et al., 2002) as well as mammalian cell lines (Moudjou et al., 1996; Kukharskyy et al., 2004). These findings indicate that γ-tubulin, like the α- and β-tubulin counterparts, could be subject to posttranslational modifications. Phosphorylation of the mouse (Kukharskyy et al., 2004) and budding yeast γ-tubulin has been reported (Vogel et al., 2001). In *Saccharomyces cerevisiae*, γ-tubulin is phosphorylated in G1 phase of the cell cycle, and dephosphorylated in mitosis. A highly conserved tyrosine residue 455 near the carboxy terminus is required for phosphorylation (Vogel et al., 2001). Deletion of this tyrosine-containing motif results in localization defects of microtubule plus-end-interacting proteins (+TIPS) (Cuschieri et al., 2006). In *Drosophila* γ-tubulin is phosphorylated by Wee1 kinase (Stumpff et al., 2005). Interestingly, complexes of γ-tubulin with protein tyrosine kinases of Src family have been demonstrated in mammalian cells (Dráberová et al., 1999; Kukharskyy et al., 2004; Sulimenko et al., 2006; Macurek et al., 2008). Moreover, complexes containing γ-tubulin and polo-like kinase (Feng et al., 1999), MARK 4 (microtubule affinity-regulating kinase 4) (Trinczek et al., 2004) or regulating subunit p85α of phosphoinositide 3-kinase (Kapeller et al., 1995) have been described. In the latter case, direct binding of γ-tubulin to C-terminal SH (Src homology) 2 domain of p85α was reported (Macurek et al., 2008). γ-Tubulin also forms complexes with phosphorylated LIM kinases during early stages of mitosis (Chakrabarti et al., 2007). Collectively taken, these data strongly suggest that kinases might be involved in the regulation of γ-tubulin interactions. Human γ-tubulin is also mono-ubiquitinated at lysines 48 and 344 by the ubiquitin–ligase complex BRCA1/BARD1 (Starita et al., 2004).

Although γ-tubulin is localized on MTOCs, a larger amount of γ-tubulin is in soluble form. γ-Tubulin appears in two main complexes: the large γ-tubulin ring complex (γTuRC; around

2.2 MDa) and the γ-tubulin small complex (γ-TuSC; around 280 kDa). The human γ-TuSC comprises two molecules of γ-tubulin and one molecule each of GCP2 and GCP3 (γ-tubulin complex proteins) (Murphy et al., 1998). The γTuRC derives from the 5-7 γ-TuSC by condensation and association with proteins GCP4, GCP5, GCP6 (Murphy et al., 2001), and GCP-WD/NEDD1 (Lüders et al., 2006). Electron microscopic tomography indicates that associated proteins, not involved in γ-TuSC, form the cap of the ring structure (Moritz et al., 2000). Hundreds of γTuRC-like rings were found in pericentriolar material of *Drosophila* centrosomes. The existence of these rings correlated with the ability of centrosomes to nucleate microtubules (Schnackenberg and Palazzo, 2001). There is evidence that γ-tubulin may associate with the microtubule wall (Leguy et al., 2000; Linhartová et al., 2002). γ-Tubulin has also been found to bind to membraneous components of the cell from which can nucleate non-centrosomal microtubules (Chabin-Brion et al., 2001; Dryková et al., 2003; Ríos et al., 2004; Macurek et al., 2008).

γ-Tubulin Functions Independent of Microtubule Nucleation in Non-Transformed Cells

A centrosome-independent role for γ-TuRC proteins in the *spindle assembly checkpoint* (SAC) has been proposed (Müller et al., 2006). The observation that depletion of the γ-TuRC components using RNA interference (RNAi) activates SAC has led some authors to suggest that γ-TuRC proteins play molecular roles in SAC activation (Müller et al., 2006). However, others have asserted that this conclusion is premature because depletion of γ-TuRC components leads to pleiotropic spindle defects, which are known to activate kinetochore-derived checkpoint signaling (Taylor et al., 2007).

γ-Tubulin complexes may also be involved in regulating microtubule dynamics. Recent findings indicate that the dynamics of microtubule plus ends are altered, depending on the expression of γ-tubulin complex proteins (reviewed in Raynaud-Messina and Merdes, 2007). Overlapping role of γ-tubulin with C-terminal kinesin-like protein Pkl1 in bipolar spindle formation has been reported (Paluh et al., 2000; Rodriguez et al., 2008).

In mammalian cell nuclei γ-tubulin is co-localized with Rad51, a protein that plays an essential role in recombination repair of DNA double-strand breaks and DNA crosslinking. This recruitment of Rad51 and γ-tubulin in the same nuclear complex is increased in the presence of DNA damage produced by genotoxic treatments either during S phase or in exponentially growing cells but is not affected by microtubule poisons such as taxol or colcemid, which do not have direct interactions with DNA (Lesca et al., 2005).

Centrosome Amplification in Cancer

Numerical, structural, and functional abnormalities of centrosomes are known to lead to mitotic spindle abnormalities, including multipolar or monopolar spindles (Brinkley and Goepfert, 1998; Doxsey, 2001). These abnormalities result in aneuploidy and chromosomal breaks in daughter cells (Doxsey, 2001). The chromosome missegregation in malignant tumors could result from defects in centrosome function (Brinkley and Goepfert, 1998; D'Assoro et al., 2002; Nigg, 2002; Salisbury et al., 2004; Emdad et al., 2005). This process is commonly known as "centrosome amplification" and entails alterations in the expression of a multitude of centrosome-associated molecules.

The presence of numerous key regulators of cell-cycle progression at the centrosome raises the conjecture that the centrosome itself provides an important structural context for coordinating cell-cycle regulation (Doxsey, 2001; Nigg, 2002). Genes involved in different signal transduction pathways have been implicated in centrosome amplification. These include genes of the p53 pathway (*p53*, *WAF-1*, *Gadd45*, *Mdm2*) and the DNA-repair pathway (*ATR*, *BRCA-1*, *BRCA-2*, *XRCC2/3*), as well as genes involved in ubiquitin-related protein degradation (*Tsg101*, *Skp2*, *RAD6*) and mitosis (*Aurora-A*) (reviewed in Zhou et al., 1998; Goepfert et al., 2002; Nigg, 2002; Stenoien et al., 2003).

Previous studies conducted more than a decade ago independently in the laboratories of Stephen Doxsey and Jeffrey Salisbury have established the presence of centrosome derangements in a variety of common human epithelial cancers (carcinomas) including those of the breast and the prostate (Pihan et al., 1998; Lingle et al., 2002). Abnormal centrosomes were identified in precancerous lesions and specifically in those lesions that were aneuploid (Pihan et al., 2003). In addition, a significant increase in the expression of pericentrin in human carcinomas of the prostate was demonstrated (Pihan et al., 2001).

Whereas the mechanisms of centrosome amplification are not fully elucidated, several models have been proposed in this regard (reviewed in Brinkley and Goepfert, 1998; Nigg, 2002; Salisbury et al., 2004; Emdad et al., 2005). Pihan et al. (2001) have provided strong experimental evidence in support of the idea that primary centrosome dysfunction may be pivotal to tumorigenesis, having demonstrated that overexpression of the centrosomal protein pericentrin, in primary prostate epithelial cell transfectants gives rise to cells with tumor-like phenotypic features. Centrosome dysfunction reflected by abnormalities in the expression and sorting of centrosomal proteins may precede changes of DNA content. Several lines of evidence, to date, point to aneuploidy as a cause rather than an effect of anaplastic transformation (Sen, 2000). Even though it is unclear whether abnormalities in centrosomal proteins constitute the primary cause of chromosomal instability and aneuploidy, or whether they represent epiphenomena secondary to cell-cycle deregulation, centrosomal abnormalities are important correlates of tumorigenesis and tumor progression (Nigg, 2002; Krämer, 2005). Centrosome abnormalities have been reported in a wide range of common epithelial cancers (Pihan et al., 1998, 2001, 2003; Sato et al., 1999; Kuo et al., 2000; Setoguchi et al., 2001; Salisbury et al., 2004), in mesenchymal solid tumors (Al-Romaih et al., 2003), and in hematological malignancies (Krämer et al., 2005).

In addition to their role in chromosomal instability and aneuploidy, structurally and/or functionally aberrant centrosomes may potentially contribute to malignant transformation by altering tumor cell architecture and motility (Nigg, 2002). Because centrosomes govern and coordinate all microtubule-related functions, by virtue of controlling the number, polarity, and distribution of microtubules (Nigg, 2002), and also considering that centrosomes determine the cell cleavage planes and symmetry of cytoplasmic division, the impact of centrosomal abnormalities on tumor architecture can provide important insights into the cytoskeletal changes in cancer cells (Pihan et al., 2001; Lingle et al., 2002; Nigg, 2002).

Increased or otherwise altered expression of centrosomal proteins are among the signature features and potential sequels of centrosome amplification, including γ-tubulin and pericentrin (Pihan et al., 1998, 2001; Sato et al., 1999; Kuo et al., 2000; Setoguchi et al., 2001), as well as increased protein phosphorylation and enhanced and/or impaired microtubule nucleating capacity (reviewed in D'Assoro et al., 2002; Nigg, 2002; Salisbury et al., 2004).

γ-Tubulin in Cancer

Although it is believed that centrosome amplification can lead to the formation of multipolar mitotic spindles and increase the

risk of generating aneuploid cells (Nigg, 2002), the molecular mechanisms responsible for this remain largely unknown. Molecular profiling has revealed changes in the expression of both TUBG1 and TUBG2 genes in breast cancer cells (Orsetti et al., 2004), prostate cancer cells (Li et al., 2005) as well as in thyroid carcinoma (Montero-Conde et al., 2007), gliomas (Rickman et al., 2001) and in pediatric pilocytic astrocytomas (Potter et al., 2008). Interestingly, increased γ-tubulin expression occurs in preinvasive lesions and carcinomas of the breast (Liu et al., 2009; Niu et al., 2009). The latter indicates that alterations of γ-tubulin expression and subcellular sorting may also play a role in early tumorigenesis prior to the development of neoplastic invasion, tumor progression, or metastasis. However, both systematic preclinical studies and experimental functional work examining the relationship between altered expression, localization, and mutation of γ-tubulins in cells and the risk of malignant transformation are still lacking.

BRCA1, the breast and ovarian cancer-specific tumor suppressor, inhibits centrosomal microtubule nucleation via its ubiquitin ligase activity, and one of the known BRCA1 substrates is γ-tubulin. By controlling γ-TuRC localization, BRCA1 inhibits centrosome function, and loss of BRCA1 results in centrosome hyperactivity, supernumerary centrosomes and, possibly, aneuploidy (Sankaran et al., 2007).

There is strong evidence that perturbations of the γ-tubulin complex may play an important role in irregular cell division and mitosis. The γ-tubulin complex physically interacts with ELAC2 protein, which is encoded by a novel candidate cancer susceptibility gene located on chromosome 17p. Overexpression of ELAC2 protein in prostate cancer cells causes a delay in G2-M progression characterized by accumulation of cyclin B levels (Korver et al., 2003).

Expression of a high-risk human papillomavirus type 16 (HPV16) E7 oncoprotein is sufficient to induce aberrant centrosome duplication in primary human cells, that is linked to the development of aneuploidy. It has been reported that HPV16 E7 associates with the γ-tubulin and that the recruitment of γ-tubulin to the centrosome is altered in HPV16 E7-expressing cells (Nguyen et al., 2007).

We have recently shown that γ-tubulin amplification may be a pivotal mechanism underlying tumorigenesis in gliomas.

Overexpression of γ-Tubulin in Astrocytic Gliomas

Tumor progression in diffuse gliomas is an intricate multistep process characterized by accumulation of genetic defects and aneuploidy.

In an oligonucleotide microarray analysis of high- versus low-grade gliomas, Rickman et al. (2001) previously observed that genes encoding for a number of cytoskeletal and cytoskeleton-associated proteins including γ-tubulin and βIV-tubulin were highly expressed in GBM.

We have studied the expression and distribution of γ-tubulin in 56 primary diffuse astrocytic gliomas (grades II–IV) and in four human GBM cell lines (U87MG, U118MG, U138MG, and T98G) using panel of anti-peptide antibodies. In primary tumors, varying degrees of localization were detected in all tumor grades, but immunoreactivity was significantly increased in high-grade anaplastic astrocytomas and GBM as compared to low-grade diffuse astrocytomas ($P = 0.0001$) (Katsetos et al., 2006). The differential distribution of γ-tubulin on tissue sections from non-neoplastic glia and GBM is shown in Figures 2 and 3, respectively. By immunocytochemical staining two overlapping patterns of ectopic cellular localization were identified in both primary tumors and GBM cell lines: A punctate pattern, in which γ-tubulin was partially co-distributed with pericentrin in the pericentriolar region, and a diffuse pattern, independent of pericentrin staining, denoting a soluble pool of γ-tubulin. In addition, coalescent punctate

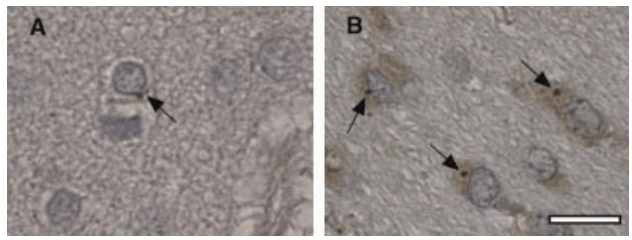

Fig. 2. Distribution of γ-tubulin on tissue sections from non-neoplastic glia. A: Adult human brain. B: Newborn piglet brain. Single or double dot-like juxtanuclear staining (arrows) is consistent with centrosomal/pericentriolar localization. Avidin–biotin complex (ABC) peroxidase with hematoxylin counterstain. Scale bar, 10 μm. Reproduced with permission from the Journal of Neuropathology and Experimental Neurology (Katsetos et al., 2006); Copyright © 2006 American Association of Neuropathologists, Inc.

localizations especially in the U118MG cells were detected. A typical localization of γ-tubulin in glioblastoma cell line T98G is shown in Figure 1B. These immunofluorescence localizations do not appear to correspond to intact "supernumerary centrosomes" but may represent abnormal protein assemblies either in the form of "acentriolar bodies," aberrant accumulations of ectopic pericentriolar material and/or fragmented centrosomes (see Brinkley and Goepfert, 1998;

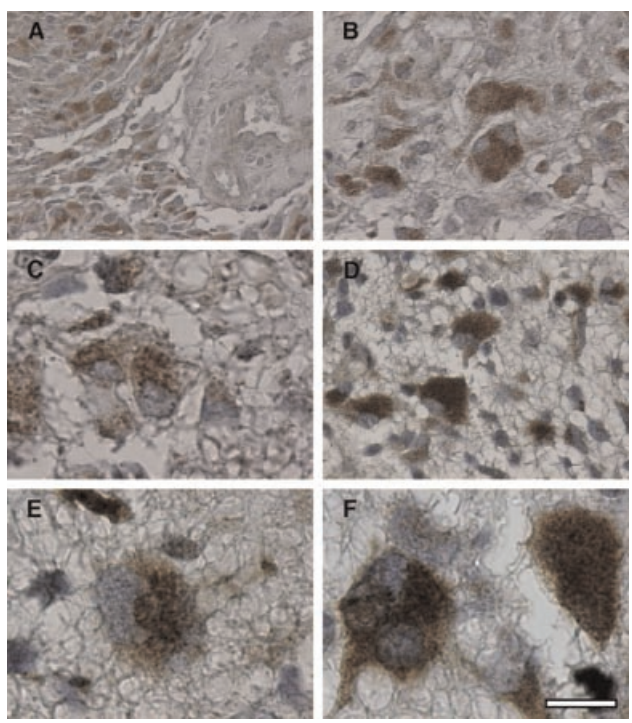

Fig. 3. Distribution of γ-tubulin on tissue sections from glioblastoma multiforme. Robust staining is present in a large number of tumor cells (A–D). Pleomorphic and multinucleated tumor cells exhibit innumerable, finely punctate and confluent cytoplasmic localizations merging into a diffuse staining pattern (B–F). ABC peroxidase with hematoxylin counterstain. Scale bars: (A) 50 μm; (B,D) 20 μm; (C,E,F) 10 μm. Reproduced with permission from the Journal of Neuropathology and Experimental Neurology (Katsetos et al., 2006); Copyright © 2006 American Association of Neuropathologists, Inc.

Nigg, 2002). It is noteworthy that a substantial proportion of cellular γ-tubulin has been detected in the detergent and nocodazole-resistant fraction of GBM cell line extracts (Katsetos et al., 2006, 2007). Quantitative real-time PCR studies have revealed significant increase in the expression of TUBG1 and TUBG2 transcripts in GBM cell lines as compared to human fetal astrocytes as well as human medulloblastoma and osteosarcoma cell lines (P. Dráber, E. Dráberová, C.D. Katsetos, et al., unpublished work). Divergent localizations of γ-tubulin and pericentrin were detected suggesting a differential distribution of these two centrosome-associated proteins in GBM cell lines (Katsetos et al., 2006). These findings suggest that centrosome amplification does not necessarily need to be accompanied by structurally intact supernumerary centrosomes, but rather it may be characterized by altered expression or modifications of centrosomal proteins, including γ-tubulin.

Interestingly, γ-tubulin is co-expressed with the class III β-tubulin isotype in GBM cells where they exhibit distinct patterns of compartmentalization and subcellular sorting (Katsetos et al., 2007). In T98G cells, βIII-tubulin was associated with microtubules whereas γ-tubulin exhibited striking diffuse cytoplasmic staining in addition to its expected centrosome-associated pericentriolar distribution. Treatment with different anti-microtubule drugs revealed that βIII-tubulin was not associated with insoluble γ-tubulin aggregates. On the other hand, immunoprecipitation experiments demonstrated that both tubulins formed complexes in soluble cytoplasmic pools. Collectively taken, these findings indicate that aberrant expression of βIII-tubulin and γ-tubulin may be linked to malignant changes in glioma cells (Katsetos et al., 2007).

Additional evidence of centrosome amplification in astrocytomas may be deduced from the increased human polo-like kinase-1 expression in these tumors (Dietzmann et al., 2001). Increased polo-like kinase expression has also been reported in three GBM cell lines (U87MG, U118MG, U138MG) and primary explants from patients with GBM (Dietzmann et al., 2001). In addition, c-Jun N-terminal kinase (JNK), a stress-activated protein kinase, which is associated with the centrosome (MacCorkle-Chosnek et al., 2001), is thought to play a role in glioma tumorigenesis (Tsuiki et al., 2003). Moreover, co-localization of γ-tubulin with nerve growth factor has been demonstrated at the centrosomes or the spindle poles throughout the cell cycle in the human glioblastoma cell line U251 MG (Zhang et al., 2005).

It is well established that deletion or mutational/functional inactivation of p53 leads to centrosome amplification (Tarapore and Fukasawa, 2002). Because *TP53* gene mutations are genotypic hallmarks of "secondary" GBMs, which arise as a consequence of malignant change in pre-existing diffuse low-grade astrocytomas (Watanabe et al., 1996), mutational inactivation of p53 may potentially account—in part—for the γ-tubulin overexpression encountered in these tumors.

In summary, our results indicate that overexpression and ectopic cellular distribution of γ-tubulin in astrocytic gliomas may be significant in the context of centrosome protein amplification and may be linked to tumor progression and anaplastic potential.

Potential Significance of γ-Tubulin Abnormalities in Tumor Cells

Cancer cells, particularly the highly malignant or anaplastic tumor phenotypes, may exhibit aberrant microtubule nucleation resulting in modified microtubule properties as reflected by the altered expression and aberrant phosphorylation of microtubule proteins, including tubulin isotypes (Katsetos et al., 2003). Thus, the microtubule nucleation capacity of aberrant centrosomes may either be reduced or enhanced, depending on the identity and modification of the overexpressed components of the pericentriolar material (Nigg, 2002). Such altered MTOCs will affect the synthesis and isotype composition of tubulin, rendering dynamically unstable microtubules thus contributing to abnormalities of cell shape, polarity, adhesion, and motility, including invasion (Katsetos et al., 2006). Khodjakov and Rieder (1999) have shown that at the onset of mitosis, the centrosome suddenly gains the ability to bind several times the amount of γ-tubulin than during interphase. Mitotically active tumor cells could thus require increased amounts of γ-tubulin in the context of spindle formation and cycle progression (Zhou et al., 2002).

We postulate that there are potentially two distinct, albeit not mutually exclusive, types of γ-tubulin abnormalities in cancer. One is centered on the presence of supernumerary centrosomes and ensuing centrosome dysfunction (what is conventionally referred to as "centrosome amplification") while the other has to do with abnormally increased and compartmentalized pools of γ-tubulin in malignant tumor cells, which may also exist in an ectopically aggregated form in the cytosol. Our observations on GBM cell lines indicate that increased γ-tubulin expression is not associated with supernumerary centrosomes but with aberrant accumulation of its cytoplasmic form (Katsetos et al., 2006). In support of this observation, we have demonstrated overt decoupling of γ-tubulin and pericentrin in GBM cell lines (Katsetos et al., 2006).

We propose that γ-tubulin perturbations in diffuse astrocytic gliomas (which may precede alterations of genomic stability) may be linked to tumor progression where it may potentially serve as a novel marker of anaplastic change. That being said, it remains to be determined, in a significantly larger cohort of patients, whether derangements in the expression and subcellular sorting of γ-tubulin may lay the foundation for a novel approach to molecular stratification and potential therapeutic strategies in gliomas.

Acknowledgments

This work was supported in part by Grant LC545 from Ministry of Education, Youth and Sports of the Czech Republic, by Institutional Research Support (AVOZ 50520514) and by a grant from the St. Christopher's Foundation for Children.

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