Overexpression of human γ-tubulin small complex proteins GCP2 and GCP3 in glioblastomas and human glioblastoma cell lines*

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Abstract

One of the key components required for microtubule nucleation and stabilization is γ-tubulin, a minor member of the tubulin superfamily, concentrated in interphase cells at the centrosomes, the conventional microtubule organizing centers (MTOC). Nucleation-competent γ-tubulin forms complexes with other proteins. The human γ-tubulin small complex (γTuSC) comprises two molecules of γ-tubulin and one molecule each of GCP (γ-tubulin complex proteins) 2 and 3. We have previously demonstrated that γ-tubulin is overexpressed in glioblastomas (J Neuropathol Exp Neurol 2006; 65: 465-77, Neurochem Res 2007; 32: 1387-98) and in medulloblastomas (J Cell Physiol 2010; 223: 519-29). The expression and distribution of the microtubule nucleating proteins GCP2 and GCP3 was studied in four human glioblastoma cell lines (U87MG, U118MG, U138MG, and T98G) and in clinical tissue samples representing all grades of diffuse astrocytic gliomas (n=45). Polyclonal and monoclonal antibodies to GCP2 and GCP3 were used for immunohistochemistry, immunofluorescence and immunoblotting. In the normal adult central nervous system, GCP2 and GCP3 were distributed predominantly in neurons, subpopulations of glia, and brain endothelial cells. As compared to normal brain tissues, GCP2 and GCP3 immunoreactivity was significantly increased in diffuse astrocytic gliomas, especially in glioblastomas (P<0.005). A strong linear relationship was also determined between the percentages of γ-tubulin and GCP2/GCP3-labeled tumor cells (P<0.005). Three overlapping patterns of localization were identified in clinical tumor samples: (a) diffuse cytoplasmic, (b) multi-punctate cytoplasmic exhibiting a proclivity for the periphery of cells (GCP3), and (c) nuclear. In addition to neoplastic glial cells, prominent GCP2 and GCP3 expression was observed in areas of vascular proliferation and hypertrophy (tumor angiogenesis). In interphase glioblastoma cell lines, staining for GCPs was mainly concentrated in the centrosomes, but punctate and diffuse staining was also observed in the cytosol and nucleoli. Nucleolar localization was fixation-dependent. In mitotic cells, GCPs were found on poles of mitotic spindles. By immunoblotting, increased levels of GCP2 were detected in glioblastoma cells as compared to normal human astrocytes. Reciprocal immunoprecipitation experiments unveiled that GCPs and γ-tubulin formed complexes in soluble cytoplasmic pools, where substantial amounts of these proteins were located. Quantitative real-time PCR revealed a significant increase in the expression of GCP2 and GCP3 transcripts in glioblastoma cells as compared to normal human astrocytes (P<0.01 to <0.005). Our results indicate that overexpression of GCP2 and GCP3 in glioblastomas may be linked either to an increased and ectopic microtubule nucleation or to other unknown functions associated with a malignant phenotype. The enrichment of GCP3 in the leading edges of glioblastoma cells might suggest involvement of GCPs in tumor cell migration and invasion, calling for future functional studies. Microtubule nucleating proteins may potentially serve as therapeutic targets in gliomas.

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