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Wiki review

Class III β-tubulin in normal and cancer tissues

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ABSTRACT

Microtubules are polymeric structures composed of tubulin subunits. Each subunit consists of a heterodimer of α- and β-tubulin. At least seven β-tubulin isotypes, or classes, have been identified in human cells, and constitutive isotype expression appears to be tissue specific. Class III β-tubulin (βIII-tubulin) expression is normally confined to testes and tissues derived from neural cristae. However, its expression can be induced in other tissues, both normal and neoplastic, subjected to a toxic microenvironment characterized by hypoxia and poor nutrient supply. In this review, we will summarize the mechanisms underlying βIII-tubulin constitutive and induced expression. We will also illustrate its capacity to serve as a biomarker of neural commitment in normal tissues and as a pure prognostic biomarker in cancer patients.

1. βIII-Tubulin and the microtubules

Microtubules are filamentous, cytoskeletal polymers found in all eukaryotes and are involved in critical cellular processes such as mitosis, cell motility, and intracellular transport. The core cylinders of microtubules are composed of basic building blocks, α- and β-tubulin monomers, that spontaneously assemble to form functional subunits called heterodimers. These α/β heterodimers polymerize in a linear fashion to form "head to tail" polar protofilaments with β-tubulin facing headwards and α-tubulin oriented toward the tails. Along with the head to tail interaction, heterodimers interact also laterally to form the cylinder that is the microtubule. Although the term "cytoskeletal" may imply stasis and permanence, microtubules are in fact highly dynamic and ethereal. The vibrant kinetics of microtubule assembly and disassembly are self-regulated at least in part by β-tubulins, which are not only structural proteins but GTPases as well. As critical yet ever-changing components of cell function, microtubules are often targeted when designing novel therapeutics and pharmaceuticals (Jordan and Kamath, 2007).

Multiple genes encode α- and β-tubulin isotypes. These genes show high homology (Ferlini et al., 2007) and conservation across vertebrate species (Tuszynski et al., 2006) indicating origins from a common ancestral gene. Both α- and β-tubulin isotypes, like cytokeratin isotypes, demonstrate differential tissue expression patterns during embryogenesis, suggesting diversity in functionality and a specific role in the differentiation processes (Sullivan and Cleveland, 1986). At least seven β-tubulin isotypes have been identified in the human genome (Ferlini et al., 2007). These vary in the final 15 C-terminus residues which are exposed for the interaction with microtubule associated proteins (MAPs). They also differ in the composition of the 3′-UTR flanking region implicated in transcriptional and translational regulation. These modifications confer properties which match the metabolic and structural needs of a given tissue.

βIII-Tubulin is encoded by the *TUBB3* gene which firstly appeared in fish, both osteoichthyes and chondrichthyes (Tuszynski et al., 2006; Joe et al., 2008), thus coinciding with the time of vertebrate evolution and exposure to the higher tension of oxygen (Towe, 1970). βIII-Tubulin is constitutively expressed in the central and peripheral nervous systems and in the testes, specifically in Sertoli cells.

In *in vitro* cultured cancer cells, constitutive *TUBB3* expression seems regulated in a cell-cycle dependent way, with maximal expression at the G_2/M phase of the cell cycle (Shibazaki et al., 2012). Such constitutive expression is linked to the expression levels of the RE1-silencing transcription factor (REST). REST binds to the first *TUBB3* exon and suppresses the constitutive *TUBB3* expression (Gao et al., 2012). In other tissues, both normal and neoplastic, the expression of βIII-tubulin is induced by exposure to a toxic microenvironment featured by hypoxia and poor nutrient supply.

In this review, we will describe the regulation of *TUBB3* in humans and its functional role in normal and diseased tissues. We will also

Abbreviations: βIII-Tubulin, class III β-tubulin; MAP, microtubule associated proteins; ABC, ATP binding cassette; UTR, untranslated region; CFEOM3, congenital fibrosis of extraocular muscles 3; GBP-1, guanylate-binding protein 1; PIM-1, proviral integration site 1; HIF-1α, hypoxia inducible factor 1α; HIF-2α, hypoxia inducible factor 2α.

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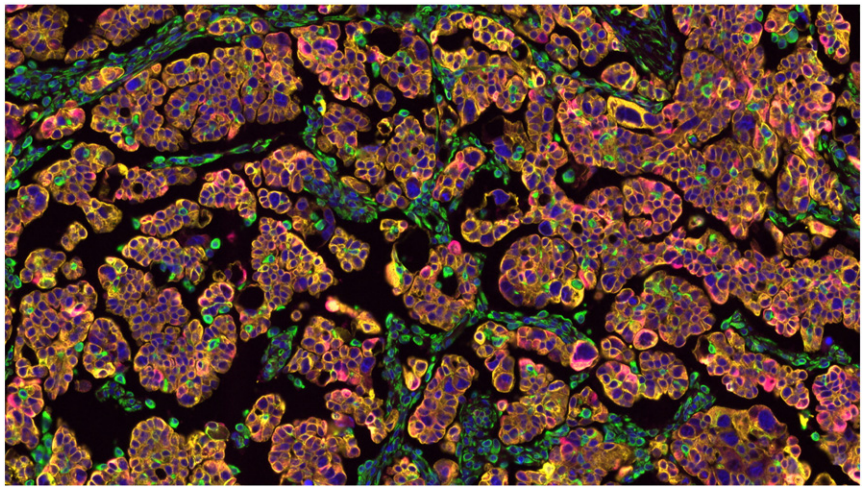

Fig. 1. Representative multiplexed fluorescent immunohistochemistry of a high grade serous ovarian cancer case stained with anti-*TUBB3*. In blue — cell nuclei stained with DAPI; in green — stromal cells stained with anti-vimentin; in yellow — epithelial cancer cells stained with anti-cytokeratin; in pink — *TUBB3* expression stained with anti-*TUBB3* (clone TUJ1). The TUJ1 pattern of staining is cytoplasmic and as in this case is often heterogeneous within the cancer cell population in high grade serous ovarian.

provide a context for understanding why βIII-tubulin has been extensively documented as a biomarker of poor outcome in a panel of solid malignancies.

2. Gene structure of human *TUBB3*

The human *TUBB3* gene is located on chromosome 16q24.3 and consists of 4 exons that transcribe a protein of 450 aa. A shorter isoform of 378 aa derived from the alternative splicing of exon 1 is devoid of a part of the N-terminus and may be responsible for mitochondrial expression (Andre et al., 2000; Cicchillitti et al., 2008), whose functional significance remains unknown. Similar to other β-tubulin isotypes, βIII-tubulin has a GTPase domain which is essential in regulating microtubule dynamics (Katsetos and Draber, 2012). Class I (the most represented and constitutively expressed isotype) and βIII-tubulin differ by just 13 aa within the 1–429 aa region, whereas region 430–450 aa is completely divergent. These variations in the primary structure affect the paclitaxel binding domain (Ferlini et al., 2005, 2007; Magnani et al., 2006) on βIII-tubulin and may account for the ability of this isotype to confer resistance to paclitaxel initiated apoptosis (Ferlini et al., 2009). Since paclitaxel is a Nur-77 mimic, βIII-tubulin may represent the inherent survival pathway to Nur-77 induced cell death (Ferlini et al., 2009). In fact, Nur-77 translocation from the nucleus to mitochondria activates a cell death program (Lin et al., 2004), which is inhibited by βIII-tubulin expression (Ferlini et al., 2009).

3. Expression and function of βIII-tubulin

A recent PUBMED search using keywords "class III β-tubulin" or "*TUBB3*" retrieved 610 references. Of these, 51% (309/610) relate to its role in neural differentiation. The knowledge of *TUBB3* as a neuronal marker dates back to 1989 when the first monoclonal anti-class III β-tubulin antibody (TUJ1) became available (Caccamo et al., 1989). The expression of βIII-tubulin by medulloepithelial rosettes suggested that this isotype was one of the earliest markers to signal neuronal commitment in primitive human neuroepithelium. This hypothesis was later confirmed in other species (Lee et al., 1990; Linhartova et al., 1992). The critical importance of the *TUBB3* gene in neural development has also been confirmed by the study of *TUBB3* mutations in congenital syndromes. A panel of at least eight heterozygous missense mutations was shown to produce congenital fibrosis of extraocular muscles 3 (CFEOM3), a group of eye movement disorders caused by the dysfunction of the oculomotor nerve (Tischfield et al., 2010). The classical CFEOM3 symptoms, ptosis and restricted eye movements, are observed at birth. These symptoms are associated with additional nervous system disorders. CFEOM3 patients exhibit peripheral axonal neuropathy, facial paralysis, and often intellectual and behavioral impairments. Conventional neuroimaging reveals a spectrum of abnormalities including hypoplasia of oculomotor nerves along with dysgenesis of the corpus callosum, anterior commissure, and corticospinal tracts. The commonest *TUBB3* mutation causing CFEOM3 results in a R262C amino acid substitution. A *TUBB3R262C* knock-in mouse model reveals axon guidance defects of the oculomotor nerve and central axon tracts without evidence of cortical cell migration abnormalities (Tischfield et al., 2010). By contrast, six other diverse missense mutations in the *TUBB3* gene were observed in patients with cortical disorganization and axonal abnormalities associated with pontocerebellar hypoplasia but without ocular motility defects typical of CFEOM3 (Poirier et al., 2010). The diverse spectra of phenotypic changes related to congenital *TUBB3* mutations highlights the pivotal role this protein plays in neuronal development. As further evidence of its specificity, *TUBB3* inactivation impairs neural progenitor proliferation which cannot be rescued or restored by other β-tubulins (Saillour et al., 2014).

TUBB3 expression is also constitutively expressed in the testis where it is regulated by androgen exposure during ontogenesis in mouse and rat Sertoli cells (De Gendt et al., 2011). Hormonal influence of *TUBB3* expression has also been identified in neoplasia. Breast carcinoma cells, for example, show *TUBB3* under the control of estradiol *via* the estrogen receptor (Saussede-Aim et al., 2009). In prostate cancer, βIII-tubulin expression is strongly associated with both TMPRSS2:ERG rearrangement, ERG expression and PTEN deletions, three key oncogenetic features of aggressive prostate cancer (Tsourlakis et al., 2014). Also, colorectal cancer is more aggressive in young male patients in whom testosterone elevation and activation of *TUBB3* are connected with poor outcome (Shahabi et al., 2013; Orsted et al., 2014).

4. βIII-Tubulin and taxane-resistance: a need for critical revision?

The primary interest in βIII-tubulin in oncology relates to its putative role in taxane-resistance. Large metanalyses (Reiman et al., 2012) and a number of additional smaller studies (Ferrandina et al., 2007; Urano et al., 2006; Ohishi et al., 2007; Umezu et al., 2008; Terry et al., 2009; Ishida et al., 2009; Hayashi et al., 2009; Azuma et al., 2009; Yoon et al., 2010; Ploussard et al., 2010; Miyamoto et al., 2010; Koh et al., 2010; Mariani et al., 2012; Leskela et al., 2011; Hirai et al., 2011; Levallet et al., 2012; Zhang et al., 2012; Vilmar et al., 2012; Roque et al., 2014) have clearly indicated that βIII-tubulin is linked to poor outcome (See Fig. 1.). Taxanes inhibit tubulin depolymerization thereby increasing