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Mini-review

Potential role of tubulin tyrosine ligase-like enzymes in tumorigenesis and cancer cell resistance

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ABSTRACT

Polyglutamylation of tubulin and other non-tubulin substrates is a reversible posttranslational modification brought about by tubulin tyrosine-like ligases. Altered polyglutamylation is linked to tumorigenesis and resistance to chemotherapeutic drugs that target the microtubule, and therefore is a potential pharmacological target in cancer therapy. Despite the large amount of research focused on the development of anticancer agents, only a small number of well-characterized inhibitors of polyglutamylases have been identified, including the phosphinic acid-based inhibitors of *Ttll7*. In this minireview, we summarize the role of polyglutamylation in cancer, and draw attention to the largely unexplored area of polyglutamylase inhibition in the treatment of cancer.

Introduction

Microtubules (MTs) are an important cellular target in cancer therapy. Among different regulators of MTs, posttranslational modifications (PTMs) of tubulin have significant effects on MT function and behavior [1]. Fourteen different PTMs of tubulin have been identified, with acetylation/deacetylation, tyrosination/detyrosination, polyglycylation, and polyglutamylation being the most extensively studied tubulin PTMs [1]. Increased polyglutamylation and deregulated tyrosination/detyrosination are increasingly being recognized as significant mediators of cancer progression in patients [2,3]. Herein, we present our perspectives on polyglutamylation as a promising new target in cancer therapy and discuss current trends in the development of inhibitors of polyglutamylases as potential new chemotherapeutics.

Polyglutamylation

Background and significance

First observed in α-tubulin from mammalian brain [4], polyglutamylation is a complex, reversible PTM of the C-terminal tail of tubulin that harbors the binding sites for many MT-associated proteins (MAPs) and molecular motors [5] (Fig. 1). By regulating the interaction of MAPs with MTs, polyglutamylation may have major effects on MT-based cellular processes [6,7]. It is also possible, however, as suggested by Backer et al. [8] that polyglutamylation acts as a signal for a selective group of MAPs to associate with MTs for specific cellular functions (Fig. 1). Backer et al. [8] clearly show selective association of the centriole and spindle-associated protein (CSAP) with polyglutamylated tubulin.

Polyglutamylation is carried out by nine tubulin tyrosine ligase-like (*Ttll*) enzymes [9] and is reversed by four cytosolic carboxypeptidases (CCPs) [10,11]. Anywhere from one to twenty glutamate residue-long chain may be added to the C-terminal of α- or β-tubulin (Fig. 1). Although α- and β-tubulin polyglutamylation have been well characterized in neuronal [12] and ciliated cells [13], the extent to which tubulin is polyglutamylated in cancer cells remains poorly characterized. Given that MT properties are modulated by polyglutamylation, the use of specific PTM inhibitors that have more subtle downstream effects on MT

Abbreviations: CSAP, centriole and spindle-associated protein; MAP, microtubule-associated protein; MSA, microtubule-stabilizing agent; MT, microtubule; PTM, posttranslational modification; Ttll, tubulin tyrosination ligase-like.

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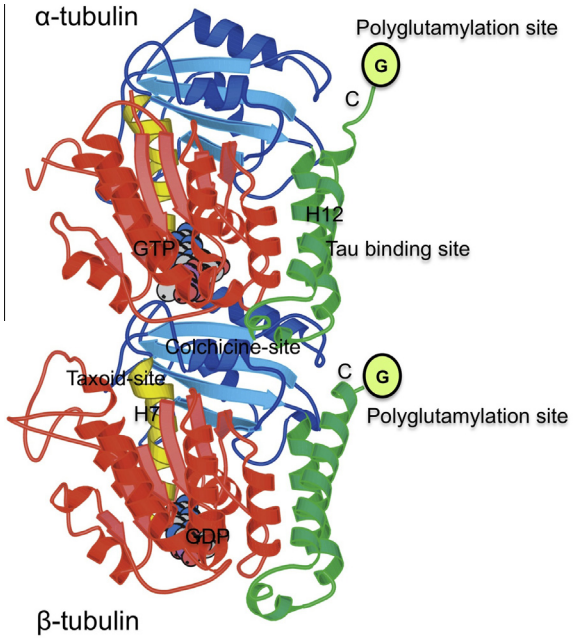

Fig. 1. Structure of α- and β-tubulin containing GTP and GDP, respectively. C-terminal tubulin tails (labelled C) are sites for polyglutamylation where one to twenty glutamyl units can be added. Preceding the C-terminal tail is helix 12 (H12) of α- and β-tubulin, which represents the major tau and MAP binding region. Lengths of the glutamyl side chains at the C-termini significantly regulate the interaction of MAPs, and other MT-interacting proteins, like CSAP, to the MT. By regulating such interactions, polyglutamylation can affect MT stability and the responses to MT-targeting drugs that bind to either the taxoid-site or the colchicine-site. The ribbon structure of α- and β-tubulin is reproduced with permission from the Medical Research Council, UK.

behavior may prove advantageous in controlling cancer cell proliferation, compared to MT-targeting drugs that more grossly affect MT structure and function.

In addition to tubulin, nucleosome assembly proteins (NAPs) and MT binding protein, like the end binding protein 1 (EB1) also undergo modifications following polyglutamylation [14,15]. This raises the possibility that by directly acting on NAPs and EB1, polyglutamylation may regulate diverse cellular functions through pathways not immediately linked to tubulin [15]. Tubulin polyglutamylation, in particular the length of the glutamate side chain, activates the MT-severing proteins, spastin and katanin, leading to disassembly of MTs [16], further suggesting a possible role of polyglutamylation in regulating MT dynamics. Increased levels of spastin have been reported in glioblastomas, implicating spastin to tumor cell motility, migration and invasion [17]. Since polyglutamylases are expressed at high levels in the brain, *Ttll*s that elongate glutamate side chains and activate spastin may serve as potential drug targets. Interestingly, recent findings also implicate tubulin polyglutamylation-induced spastin activation in the loss of MTs in Alzheimer's disease [18].

Polyglutamylation and cancer progression

Tubulin tyrosine ligase-like enzymes in cancer progression

The tubulin tyrosine ligase-like enzyme *Ttll*12 is one of the most abundant antigens in cancer patients [19], with markedly increased expression in the prostate gland as the disease progresses from a benign to a metastatic stage [20]. Brants et al. [21] showed that overexpression of *Ttll*12 can delay cell cycle progression from G_2/M phase to G_1. It is plausible that such alterations in mitotic timing could reduce the efficacy of antimitotic drugs, although it has been suggested that functions of MT-targeting drugs other than mitotic block, such as inhibition of intracellular transport, are responsible for their actual mode of action in solid tumors [22,23]. There is evidence as well that polyglutamylation is required for dynein binding to the microtubule [7].

*Ttll*12 is an unusual member of the *Ttll* family of polyglutamylases, containing phylogenetically conserved SET and TTL domains that are involved in histone and tubulin modifications, respectively [20]. Although high levels of polyglutamylated tubulin and *Ttll*12 are detected in epithelial cells of prostate cancer patients [20], there is no direct evidence confirming *Ttll*12 as a tubulin polyglutamylase. *Ttll*12 has been shown to be involved in tubulin tyrosination and methylation of histone proteins [20,21], and it preferentially modifies the C-terminal of α-tubulin [21]. Similar to *Ttll*12, *Ttll*4, another polyglutamylase with links to cancer, is a β-tubulin modifier [24]. Increased activation of *Ttll*4 causes polyglutamylation of a non-tubulin substrate, proline-, glutamate- and leucine-rich protein 1 and also remodels chromatin in pancreatic ductal adenocarcinoma [25]. With diverse substrates, *Ttll*12 and *Ttll*4 are presumably two potentially important future therapeutic targets in the treatment of prostate and pancreatic cancer, respectively.

Cilia polyglutamylation and altered cell signaling in cancer

Polyglutamylation is particularly prevalent in ciliary microtubules [26]. The functional contribution of these modified microtubules in cilia is becoming better understood with further research. Decreased polyglutamylation by the disruption of specific polyglutamylation-conjugating enzymes reduces cilia beating in *Tetrahymena* [27], *Chlamydomonas reinhardtii* [7], and zebrafish [28]. In contrast, overexpression of β-tubulin-specific polyglutamylase results in inhibition of ciliary beating due to excessive polyglutamylation of ciliary MTs [9]. These findings clearly indicate that polyglutamylation has a direct effect on the functioning of cilia. In vertebrates, primary cilia are assembled and disassembled during the cell cycle, and defects in this process might in the long-term potentially result in the development of cancer [29]. Further, cilia participate in hedgehog signaling pathways [30], and abnormal hedgehog pathway function has been reported in diverse cancers, including basal cell carcinomas, medulloblastomas, rhabdomyosarcomas, glioblastomas, and breast and prostate cancers [30]. There are a number of anticancer drugs under development that target various steps of the hedgehog pathway [31]. Whether targeting ciliary functions in cancer cells is a good therapeutic option is still unknown; however, inhibition of cilia by disruption of polyglutamylation may prove to be an effective approach for treatment of some cancers [32].

Polyglutamylation and drug resistance in chemotherapy

Resistance to MT-stabilizing agents (MSAs) is linked to multifactorial cell-intrinsic mechanisms [33]; however, links have also been identified between tubulin PTMs and acquired resistance [34,35]. The role of polyglutamylated tubulin in drug resistance has been specifically implicated in advanced-stage prostate cancer [36]. For example, estramustine, a clinically used antimitotic drug, induces MT depolymerization in sensitive cells; however, cells that have increased tubulin polyglutamylation are 7-fold more resistant to estramustine [36]. Furthermore, paclitaxel-resistant breast cancer cells show an increase in levels of polyglutamylated tubulins [37]. It has also been shown that highly stabilized MTs following abnormal elongation of glutamyl side chains are less susceptible to nocadazole treatment [26].

Alexander et al. [38] showed that polyglutamylation contributes to the C-terminal negative charge that is required on neuron-specific βIII-tubulin during neuronal differentiation. It is now obvious that increased abundance of βIII-tubulin leads to both

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