EXPERT OPINION

Stathmin 1: a protein with many tasks. New biomarker and potential target in cancer

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Stathmin 1 (STMN1) is a critical protein involved in microtubule polymerization and is necessary for survival of cancer cells. This editorial describes the role of targeted therapeutics which disrupt STMN1 modulation and such effect on cancer survival.

1. Introduction

The 'many tasks' of Stathmin 1 (STMN1) were recently described by Belletti and Baldassarre [1]. The name stathmin is derived from the term 'stathmos', the Greek word for 'relay'. It represents stathmin's role as a critical intermediate during signal transduction in modulation and control of microtubule polymerization. STMN1 is a protein composed of 149 amino acids organized into four domains (I – IV) as defined by limited proteolysis. The core region (amino acids 42 – 126) is the minimum fragment required for tubulin interaction with the additional requirement of either an N- or C-terminal extension [2]. There are four phosphorylation domains, designated as Ser 16, 25, 38, and 63. Of the four phosphorylation sites, only Ser 16 is conserved throughout the STMN1 family.

2. STMN1 function

Modulation of STMN1 [3] can result in mitotic arrest, thereby exhibiting a critical role in microtubule dynamics. Microtubules are protein polymers comprising α/β tubulin heterodimers, which contribute to and are essential for the structure and function of the cell. These functions include intracellular transport, cell motility, and polarity. Dynamics of microtubule function can best be described as an alternating pattern of stabilization and destabilization. Stathmin-mediated destabilization can result from either tubulin sequestration or 'catastrophe'. The latter results from microtubule depolymerization and is counterbalanced by 'rescue', which is effected by polymerization. The transition between the two phases during the various portions of the cell cycle is regulated by microtubule-stabilizing and microtubule-destabilizing proteins [4]. The process of mitotic spindle formation is a coordinated, balanced interaction between the stabilizing activities of microtubule-associated proteins (XMAP215, EB1), motor proteins (predominantly kinesin, e.g., Eg-5), and plus-end depolymerases, including XKCM1, MCAK and STMN1 [5]. A tightly regulated sequenced pattern of STMN1 phosphorylation and de-phosphorylation is necessary for entry into prophase and, terminally, into cytokinesis, respectively [6,7]. As recently pointed out by Belletti and Baldassarre [1], these functions are critical for malignant cell survival.

It is also postulated that during the metaphase to anaphase transition, stathmin effects poleward kinetochore spindle movement by increasing minus-end catastrophe frequency [8]. Moreover, it has been shown that exogenous STMN1 affects metaphase-to-anaphase transition by its role in kinetochore-associated microtubule detachment during anaphase, thereby resulting in chromosomal instability with

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over a 100-fold increase in micronucleus formation [9]. To exit mitosis and allow for cytokinesis, the microtubules undergo depolymerization, which requires the dephosphorylation of stathmin reactivating its tubulin-binding property.

3. Relevance of STMN1 to cancer

Decreased expression of p27 has been linked to prognosis in a number of tumor types. Besides being a CDK inhibitor of Cyclin D1, p27 has been shown to play a role in cell motility [10]. Baldassare recently showed that stathmin is a p27-binding partner, and on the basis of his data postulates that p27 interferes with stathmin binding and sequestration of tubulin, consequently inhibiting cell motility and microtubule depolymerization. Furthermore, p27 is downregulated in transformed cells. Low p27 and high STMN1 correlate with metastatic behavior of sarcoma cells *in vivo* [10]. Similar results have been shown in other cancers [11,12].

Based on differential proteogenomic signaling assessments of patients from our program [13,14], as well as published literature by others, there is a sound rationale for the therapeutic targeting of STMN1 in cancer patients [15]. Stathmin is highly expressed in a variety of human malignancies [16,17] and has been shown to be upregulated in multiple cancers. Moreover, upregulated stathmin has been shown to be extensively correlated with poor survival. In one particular analysis involving 1,076 endometrial cancer specimens, stathmin overexpression was significantly associated with poor survival of the patients and with nodal metastasis [18].

The E2F sites in the stathmin promoter also appear involved in the regulation of stathmin via activity of c-JUN and FoxM1 [19]. Negative regulators of stathmin involved in cancer have also been identified. For instance, induction of WT p53 results in decreased stathmin expression via direct transcriptional repression [20], possibly involving p21^{WAF1} and EGR-1 [21].

Stathmin expression and function have also been shown to be controlled at the post-transcriptional level with involvement of cancer-related proteins. In colorectal cancer cells, overexpression of PUMA, a p53-induced effector of apoptosis [22], results in stathmin degradation via a proteasome-dependent pathway. This finding is further supported by the fact that in some tumor samples the expression levels of stathmin mRNA and protein are uncoupled [23], suggesting that this mechanism may be important in some pathological conditions. More widely accepted, the modulation of stathmin activity can be achieved by protein sequestration. The CDK inhibitor p27^{Kip1} [24] and the transcription factor STAT3 [25] are both able to bind stathmin, therefore preventing its ability to sequester free αβ-tubulin heterodimers.

Several studies have demonstrated that stathmin depletion results in cell cycle arrest and induction of apoptosis, thereby playing a significant role in malignant cell death. This is further supported by the observation that stathmin is a target of ASK1-p38 and JNK kinases, signals involved in response to cellular stress. Stathmin has also shown to be protective of arsenic- [26] and paclitaxel-induced apoptosis [19,27]. It is also interesting to note that in human cancer stathmin expression may influence sensitivity/resistance to treatment with selected cytotoxic drugs. Several studies *in vitro* indicate that tumor-derived cell lines with high stathmin expression negatively influence the response to microtubule targeting drugs [19,28].

4. Potential STMN1-targeting therapeutics

A variety of target-specific anti-stathmin effectors, including ribozymes [29] and si-RNA [16,30,] have been used to silence stathmin *in vitro* as singlets [16,29,30] and in combination with chemotherapeutic agents where additive synergistic interactions have been demonstrated (i.e., taxanes) [31-33]. Both ribozyme and siRNA inhibition of stathmin mRNA result in an increase in G2M phase cell population, an inhibition of clonogenicity, and a marked increase in apoptosis [16,30,34]. The latter may be due to the effect of modulation of microtubule network mobility on the proportion of Bax/Bcl-2 and Bax/Bcl-xL heterodimers [35,36]. More recently, we have demonstrated marked (93%) knockdown of STMN1 using a novel bifunctional RNA interference technology [37]. We have shown a 5-log dosing improvement in growth inhibition involving CCL-247 colon cancer cells comparing bi-shRNAi STMN1 to siRNAi STMN1 targeting the identical mRNA sequence and in coordination with cleavage product quantitation. These results justified efficacy testing in murine models, which demonstrated response and survival advantage in multiple tumor models following systemic treatment with bi-shRNAi STMN1 LP [38]. These results, combined with safety demonstrated by toxicity and biodistribution studies in STMN1 biorelevant pigs, enabled Phase I clinical investigation to be opened with a novel bi-shRNAi STMN1 LP therapeutic.

5. Conclusion

STMN1 is extensively involved in signal transduction of malignant cells. Experimental therapeutic modalities, such as bi-shRNAi STMN1 LP, may one day contribute to cancer management as a novel STMN1 down-modulator. A Phase I clinical trial has recently been initiated to test the effect of STMN1 knockdown on cancer response. Chemotherapy modalities, such as taxanes, have broad clinical reach, commonly involving breast cancer, lung cancer, ovarian cancer, prostate cancer, sarcoma, and gastric cancer to name a few. The mechanism of action by which taxanes mediate antitumor activity involves microtubule polymerization. It is likely that initial clinical opportunities with STMN1 knockdown will involve combination with taxanes.

Declaration of interest

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