

# Relevant coexpression of *STMN1*, *MELK* and *FOXM1* in glioblastoma and review of the impact of *STMN1* in cancer biology

Fernanda de Oliveira Serachi<sup>1</sup>, Suely Kazue Nagahashi Marie<sup>1,II</sup>, Sueli Mieko Oba-Shinjo<sup>1</sup>

<sup>1</sup> Universidade de Sao Paulo, Faculdade de Medicina (FMUSP), Department of Neurology, Laboratory of Molecular and Cellular Biology (LIM 15), Sao Paulo, SP, Brazil

<sup>II</sup> Universidade de Sao Paulo, Center for Studies of Cellular and Molecular Therapy (NAP-NETCEM-NUCEL), Sao Paulo, Brazil

**OBJECTIVE:** To analyze the associated expression of *STMN1*, *MELK* and *FOXM1* in search of alternative drugable target in glioblastoma (GBM) and to review relevant functional roles of *STMN1* in cancer biology.

**METHOD:** *STMN1*, *MELK* and *FOXM1* expressions were studied by quantitative PCR and their coexpressions were analyzed in two independent glioblastoma cohorts. A review of articles in indexed journals that addressed the multiple functional aspects of *STMN1* was conducted, focusing on the most recent reports discussing its role in cancer, in chemoresistance and in upstream pathways involving *MELK* and *FOXM1*.

**RESULTS:** Significant associated expressions of *MELK* and *FOXM1* were observed with *STMN1* in GBM. Additionally, the literature review highlighted the relevance of *STMN1* in cancer progression.

**CONCLUSION:** *STMN1* is very important to induce events in cancer development and progression, as cellular proliferation, migration, and drug resistance. Therefore, *STMN1* can be an important therapeutic target for a large number of human cancers. In glioblastoma, the most aggressive brain tumor, the *MELK/FOXM1/STMN1* presented significant associated expressions, thus pointing *MELK* and *FOXM1* as alternative targets for therapy instead of *STMN1*, which is highly expressed in normal brain tissue. Continuous functional research to understand the *STMN1* signaling pathway is worthwhile to improve the therapeutic approaches in cancer.

**KEYWORDS:** Stathmin, cytoskeleton, microtubules, glioblastoma.

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E-mail: fernandaserachi@usp.br

## INTRODUCTION

Cell proliferation and migration are two relevant features in cancer biology determining tumor growth and invasion/metastasis. The subcellular cytoskeleton is essential to control these processes.<sup>1</sup> This includes the microtubule dynamic behavior involving rapid switches between periods of polymerization (growth) and depolymerization (shrinkage) at the microtubule extremity (named dynamic instability).

Currently, several proteins are known to be related to microtubule interaction with tubulin, and participate

in microtubule dynamics. The stathmin (STMN) family members are among those proteins that inhibit microtubule polymerization. Four members of evolutionarily conserved cytosolic proteins compose this family, namely STMN1 to 4. STMN1 and STMN3 are ubiquitously expressed in different cells, while STMN2 and STMN4 are more restricted to the nervous system.<sup>2</sup> These proteins share up to 70% of sequence homology in a highly conserved C-terminus within the tubulin-binding stathmin-like domain, and in the N-terminus region containing the phosphorylation sites, which also dictates their cellular localization.<sup>3,4</sup>

STMN1 is also known as Oncoprotein 18 (Op 18); it is an important microtubule dynamics regulator involved in cell proliferation, differentiation, cell cycle progression

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and migration. STMN1 has been described as associated to a wide range of malignancies and is a target for alternative therapy in cancer treatment.<sup>5</sup>

STMN2, also known as the superior cervical ganglion-10 protein (SCG10), has been reported as a neuron-specific growth-associated phosphoprotein, abundant in the growth cone of neurons. In particular, STMN2 is described as a neuronal marker at an early stage of neural development, playing a regulatory role in the control of neuronal differentiation.<sup>6</sup> Previous studies have also described its role in osteogenesis.<sup>7</sup> In liver tumorigenesis, it has been described as a target of  $\beta$ -catenin/TCF-mediated transcription in the Wnt dependent regulation of microtubule dynamics in hepatoma cells.<sup>8</sup> Moreover, STMN2 plays a role in promoting the invasive potential of gastric cancer cells.<sup>9</sup>

STMN3, also known as SCLIP, is involved in the development of the central nervous system, including axonal branching and dendritic differentiation of Purkinje cells.<sup>10,11</sup> STMN3 is highly expressed in glioma samples, and has been associated to migration and invasion of glioma cells.<sup>12</sup> Additionally, STMN3 has been described as a modulator of the sensitivity of ovarian cancer cells to microtubule-targeting drugs by preventing the formation of the spindle and consequently promoting mitosis arrest.<sup>13</sup>

STMN4, also known as RB3, presents two splice variants, RB3' and RB3".<sup>14</sup> STMN4 has a putative role in neuronal morphogenesis and plasticity.<sup>15,16</sup>

Among the members of the stathmin family, STMN1 is the most studied member, and cumulative evidence singles out STMN1 as a candidate target for cancer therapy. However, STMN1 can hardly become an eligible drug for brain tumors, as its expression is high in brain tissue,<sup>17</sup> and consequently undesirable side effects would be expected. Therefore, the search for more suitable up or downstream targets in the STMN1 signaling pathway is an alternative strategy. On this rationale, we previously linked MELK upstream to STMN1 in glioma cells,<sup>17</sup> and have also demonstrated the importance of MELK in astrocytoma progression, mainly in GBM.<sup>18</sup> More recently, AKT/FOXM1/STMN1 pathway has been reported to confer multidrug resistance phenotype in non-small cell lung cancer.<sup>19</sup> FOXM1 is a transcription factor of the forkhead family that plays critical roles in cell cycle progression and cell fate decision.

In the present work, we analyzed the *STMN1*, *MELK* and *FOXM1* associated expressions in our GBM cohort, and validated our results in an expanded independent public cohort *in silico*. To highlight the relevance of this pathway in cancer biology, we also present a *review* focused on the role of STMN1 in tumor progression promoting proliferation and migration through regulation of microtubule dynamics.

## ■ MATERIALS AND METHODS

### Analysis of Cases and Gene Expression

Eighty-seven astrocytomas grade IV or GBM and 22 non-neoplastic (NN) brain anonymized tissues from epilepsy patients subjected to temporal lobectomy were obtained during therapeutic surgery from patients treated by the Neurosurgery Group of the Department of Neurology at Hospital das Clínicas of the Faculdade de Medicina da Universidade de São Paulo. Written informed consents were obtained from all patients in accordance with ethical guidelines. This project was approved by the Ethic Committee of Faculdade de Medicina da Universidade de Sao Paulo (case # 0263/07).

Samples were immediately snap-frozen in liquid nitrogen and necrotic and non-neoplastic areas were removed by microdissection from the tumoral blocks prior to RNA extraction. Total RNA extraction, reverse transcription and qRT-PCR (Sybr Green approach) were performed as previously described.<sup>17</sup> Quantitative data were normalized using the geometric mean of three reference genes suitable for the analysis: hypoxanthine phosphoribosyltransferase (HPRT), glucuronidase beta (GUSB) and TATA box-binding protein (TBP), as previously demonstrated by our group.<sup>20</sup> Primers of housekeeping genes, STMN1 and MELK are described in our previous report.<sup>17</sup> Primers for FOXM1 were synthesized by (Integrated DNA Technologies, IDT, Coralville, IA) as follows (5' to 3'): FOXM1 F: GAAGAACTC-CATCCGCCACA, FOXM1 R: TCAAGTAGCGGTTGGCACTG. All reactions were performed in duplicates and the  $2^{-\Delta Ct}$  method was applied to calculate gene expression levels, where  $\Delta Ct = [Ct \text{ target gene}] - [\text{geometric mean Ct of reference genes}]$  and Ct is the cycle threshold. The median values of gene expression were used to divide samples with high and low expression.

### Analysis of The Cancer Genome Atlas (TCGA) GBM gene expression database

*STMN1*, *MELK* and *FOXM1* gene expression levels were analyzed in an independent cohort at the cBio Portal for Cancer Genomics database (<http://www.cbioportal.org>).<sup>21</sup> RNAseq data set of 154 cases of GBM<sup>22</sup> was used to assess coexpression analysis of mRNA levels (z-score, RNA Seq V2 RSEM).

### Statistical analyses

Mann Whitney tests were performed to compare *STMN1*, *MELK* and *FOXM1* expression levels between GBM and NN samples. Correlations between gene expression values in different groups of tumors were assessed using the Spearman-rho correlation tests (non-parametric test).

## Literature review focused in STMN1

A literature search was conducted in the PubMed database using the following terms: “stathmin”, “cancer”. Only articles in English were selected, with a search ending in September 2017. We selected reviews and articles that described STMN1 and cancer. We focused specially in the most recent data of STMN1 role in cancer treatment and chemoresistance.

## RESULTS

We aimed to analyze the association of *STMN1*, *MELK* and *FOXM1* in two independent cohorts of GBM. Initially, we analyzed *STMN1*, *MELK* and *FOXM1* expression levels in 87 GBM samples compared to 22 non-neoplastic (NN) brain samples in our case database (Figure 1A, B and C).

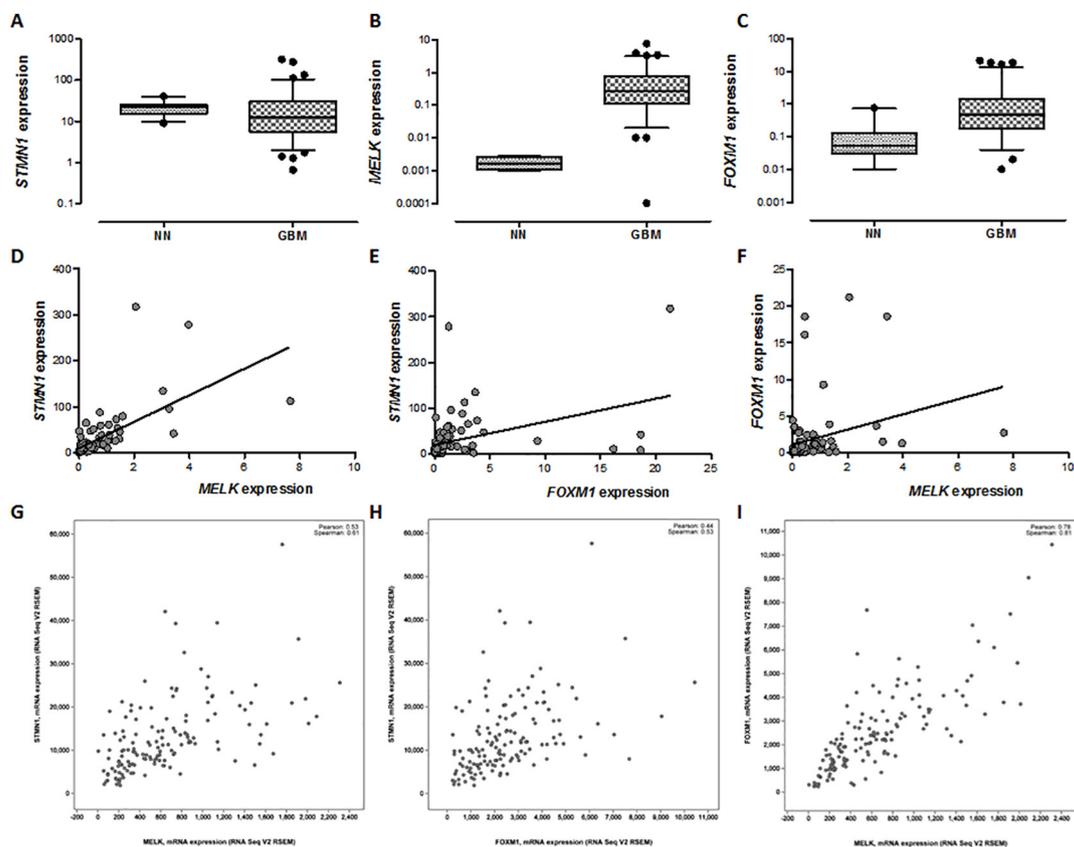
Coexpression analyses were significantly positive for *STMN1* and *MELK*, *STMN1* and *FOXM1* and *MELK* and *FOXM1* (Figure 1D, E and F). Our results were validated in a larger independent public database of TCGA corroborating the tight association among *MELK*, *FOXM1* and *STMN1* (Figure 1G, H and I).

Additionally, we divided the GBM cases of our cohort in low (44 cases) and high (43 cases) *STMN1* expression. In the first group, there were 11 cases (25%) that presented high *FOXM1* expression, while in the second group there were 32 cases (73%) with high *FOXM1* expression (Figure 2). Our data indicate that overexpression of *STMN1* correlates to an overexpression of *FOXM1*.

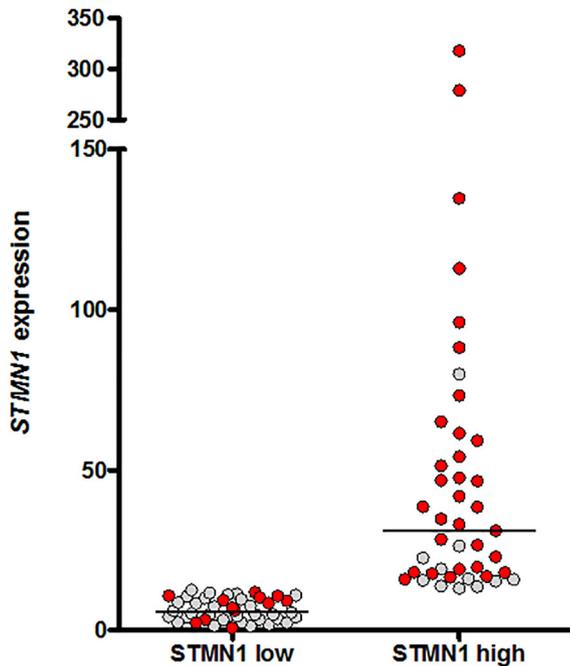
## DISCUSSION

Altogether, our data demonstrate that *MELK* and *FOXM1* expressions are significantly associated to *STMN1* expression, pointing them as promising alternative targets in the STMN1 signaling pathway for glioma therapy.

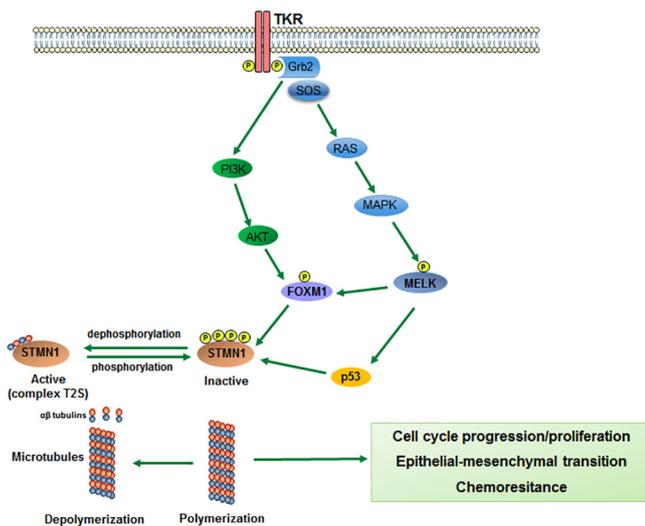
A schematic illustration of the signaling pathway involving STMN1, MELK and FOXM1 is proposed in Figure 3, implementing the interaction network recently published.<sup>19</sup> STMN1 plays a central role in the regulation of cell cycle, proliferation, epithelial mesenchymal transition and chemoresistance, crucial processes in cancer progression. The details of these STMN1 roles are reviewed and presented below.



**Figure 1.** *STMN1*, *MELK* and *FOXM1* expression levels in glioblastoma (GBM) and non-neoplastic brain tissue samples (NN). A, B, C: Box and whiskers plots of *STMN1*, *MELK* and *FOXM1* gene expressions in GBM and NN groups analyzed by real time PCR. The top and the bottom of boxes represent the first and third quartiles, respectively, and the lines in the middle the median expression in the groups. The error bars show the range of 5-95th percentiles (whiskers). D, E, F: Coexpressions between *STMN1* and *MELK*, *STMN1* and *FOXM1* and *FOXM1* and *MELK* expression levels in our GBM series (Spearman-rho test). G, H, I: Coexpressions between *STMN1* and *MELK*, *STMN1* and *FOXM1* and *FOXM1* and *MELK* expression levels analyzed in RNAseq data of 157 cases from a GBM-TCGA cohort by z-Score (of RSEM).



**Figure 2.** *STMN1* and *FOXM1* expression analysis of our GBM cohort. We divided the GBM samples according to *STMN1* expression (low and high, based on median gene expression values). The horizontal bars represent the median expression of each group. Red dots represent cases with high *FOXM1* expression levels (cases above median of *FOXM1* expression level).



**Figure 3.** *STMN1*, *MELK* and *FOXM1* signaling. Activation of a tyrosine kinase receptor (TKR) activates PI3K/AKT and RAS/MAPK signaling pathways. Both pathways are activated through formation of the complex GRB/SOS (growth factor receptor-bound protein 2/ Son of Sevenless homologs), which binds to phosphorylated TKR. Both PI3K and RAS/MAPK pathways result in *STMN1* phosphorylation, through *MELK* and *FOXM1*. Phosphorylation inactivates *STMN1* and allows the association of tubulin dimers and polymerization of microtubules. *STMN1* dephosphorylation activates the protein, causing in the sequestration of tubulin. This dynamics of *STMN1* activation/inactivation results in depolymerization/polymerization of microtubules and consequently cell cycle progression/proliferation, epithelial-mesenchymal transition and chemoresistance. (Figure adapted from Marie et al., 2016)

### STMN1 and microtubule dynamics

Tight regulation of cytoskeletal microtubule dynamics in living cells is essential for many cellular functions. Microtubules are a network of filaments comprising heterodimer  $\alpha/\beta$ -tubulin subunits that play a key role during cell events such as proliferation, migration and differentiation. The dynamic reorganization of microtubules in cells is regulated by proteins that promote their assembly (stabilizers) or disassembly (destabilizers). Microtubules (dis)assembly is partially determined by the concentration of free tubulin heterodimers in the cytoplasm, where it determines the growth rate of microtubule by incorporation of tubulin at its ends. *STMN1* is one of the most prominent and rapid microtubule regulators in response to cell needs. *STMN1* downregulation increases the concentration of microtubule polymers and decreases the concentration of free tubulin heterodimers.<sup>23</sup>

### STMN1 (de)phosphorylation and cell cycle

The dynamic regulation of the tubulin assembly by *STMN1* is performed by its four extremely conserved phosphorylation sites within the N-terminal domain: Ser16, Ser25, Ser38 and Ser63.<sup>24,25</sup> The dephosphorylated (active) *STMN1* promotes the depolymerization of microtubules by sequestering tubulin heterodimers into a ternary complex T2S where one *STMN1* molecule interacts with two molecules of  $\alpha,\beta$ -tubulin through the stathmin-like domain.<sup>26</sup> On the other hand, the phosphorylated (inactive) *STMN1* impacts negatively on *STMN1*-tubulin association, and therefore promotes microtubule stabilization and formation of mitotic spindle.<sup>27</sup> This post-translational phosphorylation of *STMN1* by multiple kinases is largely dependent on specific stimulus especially during cell cycle progression, and migration.<sup>28</sup>

In mitotic cells, sequential phosphorylation of the four residues of *STMN1* blocks tubulin binding to T2S and terminates depolymerization activity, consequently allowing the spindle formation.<sup>29</sup> Initially, a moderate *STMN1* inactivation is achieved by Ser25 and Ser38 phosphorylation by MAPK and p34<sup>cdk2</sup> during G1/S phase. Next, for the metaphase initiation, these two residues are phosphorylated by CDK1, a master regulator of M phase progression, and also by CDK2 and CDK5.<sup>30,31</sup>

*STMN1* total inactivation occurs by sequential phosphorylation of Ser16 and Ser63 residues.<sup>32,33</sup> by protein kinase A (PKA),<sup>24,34</sup> Aurora B kinase,<sup>35</sup> p65PAK,<sup>36</sup> or Ca<sup>2+</sup>/calmodulin-dependent kinases isoforms CaMKII and IV at the final step of M phase.<sup>37,38</sup> Such a condition allows the mitotic spindle to be properly organized.<sup>32,39</sup> When chromosome segregation is completed, the spindle must be disassembled to allow proper exit from mitosis, and enter to anaphase and telophase.<sup>40</sup> The microtubule-depolymerizing

activity of STMN1 is restored by dephosphorylation to disassemble the mitotic spindle. At this point, different serine/threonine protein phosphatases, as protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A) and protein phosphatase 2B (PP2B), dephosphorylate STMN1.<sup>41,42</sup>

Additionally, STMN1 phosphorylation at Ser28 and Ser38 residues is also mediated by c-Jun N-terminal kinase (JNK).<sup>43,44</sup> The JNKs are stress-activated serine/threonine kinases that regulate both cell death and cell proliferation, and they are also regulators of critical processes such as inflammation and metabolism. c-JUN overexpression also stimulates STMN1 transcription via direct activation of its promoter by the activating transcription factor (ATF)-like or by indirect activation of the E2F activity.<sup>45</sup> Extracellular signal-regulated kinases (ERK), also known as mitogen-activated kinase (MAPK) act as an integration point for multiple biochemical signals, and also phosphorylate STMN1 of unknown function, that is frequently up-regulated in transformed cells. Stimulation of various cell-surface receptors results in extensive phosphorylation of Op18 and this protein has, therefore, previously been implicated in intracellular signaling. In the present study, by expression of specific Op18 cDNA mutant constructs and phosphopeptide mapping, we have identified *in vivo* phosphorylation sites. In conjunction with *in vitro* phosphorylation experiments, using purified wild-type and mutant Op18 proteins in combination with a series of kinases, these results have identified two distinct proline-directed kinase families that phosphorylate Op18 with overlapping but distinct site preference. These two kinase families, mitogen activated protein (MAPK). Also in the MAPK cascade, apoptosis signal-regulating kinase (ASK1) activates the JNK and p38 MAPK cascades through STMN1 phosphorylation and ASK1 is involved in a broad range of activities including cell differentiation and stress-induced apoptosis.<sup>45-48</sup> Moreover, ribosomal protein S6 kinase A3 (RPS6KA3, RSK2) can reduce microtubule depolymerization by phosphorylation of STMN1 specifically at Ser16 residue.<sup>49</sup>

Additionally, peptide hormones as gonadotropin-releasing hormone (LHRH) secreted from hypothalamic neurons, regulators of LH and FSH synthesis and release, have also been described to induce STMN1 phosphorylation in a PKC-dependent pathway.<sup>50</sup> More recently, thyroid hormone receptor (THR) has been reported as a transcription regulator of STMN1 in hepatocellular carcinoma. Thyroxine (T<sub>3</sub>) binds to nuclear TRHs to exert numerous physiological processes, including ontogenesis, cell growth, cellular differentiation and metabolism. Clinical and experimental observations suggest that T<sub>3</sub> might regulate microtubule network assembly through repression of STMN1 expression.<sup>51</sup>

In addition to hormones and growth factors, ion channels can also activate STMN1. Activation of ion channels initiate a Ca<sup>+2</sup> response in parallel with activation of several

protein kinase families, particularly Ca<sup>+2</sup>/Calmodulin-dependent protein kinase type Gr which is mainly expressed at high levels in neural cells and CD4-positive T lymphocytes. And, as consequence STMN1 is phosphorylated.<sup>37,38</sup>

Therefore, STMN1 is able to integrate multiple extracellular inputs through hormone peptides, ion channels, and growth factor receptors to intracellular molecular networks, regulating multiple cellular activities and signaling pathways.

### STMN1 and cell migration

Cell migration is a complex cellular behavior that results from the coordinated changes in the regulation of microtubule dynamics.<sup>52</sup> Therefore, STMN1, a master microtubule regulator, is also involved in cell migration, with crucial role in cytoskeletal rearrangements for formation and dispersal of adhesion sites between cells and extracellular matrix. Intrinsically, STMN1 is involved in extension and retraction of leading edges which depend on polymerization of actin microfilaments, and microtubule assembly (stability) and disassembly (instability).<sup>53,54</sup>

Microtubules may participate in cell migration in a Rac1- and p21-activated kinase-dependent manner. In the advancing cell edge of the migrating cells, there is a Rac1 mediated microtubule net growth dependent on Pak kinase activity. Pak1 can directly phosphorylate STMN1 at S16 residue, in EGF-stimulated cells. This leads to downregulation of the STMN1 inhibitory activity on bulk tubulin polymerization with consequent microtubule growth.<sup>55</sup>

Moreover, STMN1 may interact with p27 and Cdk2/Cdk5, leading to enhanced protein phosphorylation and consequent tubulin stabilization and inhibition of cell migration.<sup>56</sup>

Another recent study has demonstrated that STMN1 phosphorylation at Ser25 and Ser38 is required to maintain the migration properties of breast cancer cells through interaction with glucose-regulated protein of molecular mass 78 (GRP78). Furthermore, this interaction is regulated by MEK kinase-dependent phosphorylation of STMN1, which has an important role in cell proliferation, differentiation, migration and invasion of breast cancer cells with impact on tumor recurrence and metastasis.<sup>57</sup> Similarly, STMN1 was described as playing a fundamental role in neuroblastoma cells by regulating the invasion and transendothelial migration by RhoA/ROCK signaling, in a microtubule-independent manner.<sup>58</sup> Association of STMN1 expression with metastasis has also been reported in other types of tumor, indicating STMN1 as a molecular biomarker for the risk of metastasis.<sup>5</sup>

### STMN1 and cancer

STMN1 modifications have been frequently reported in cancer. In 2010, Jeon et al. first reported positive correlation of STMN1 overexpression with lymph node metastasis,

migration foci and vascular invasion, with negative impact in recurrence free survival of diffuse type of gastric carcinoma. The same group demonstrated the oncogenic role of STMN1 by the decrease of proliferation rate, migration and invasion of gastric cancer cells *in vitro* through STMN1 inhibition.<sup>59</sup> Henceforth, STMN1 has been considered as a mitotic regulator oncoprotein that modulates microtubule stability.<sup>60</sup>

STMN1 expression is also upregulated in various human malignancies, including colorectal,<sup>61</sup> ovarian,<sup>62,63</sup> hepatocellular,<sup>64,65</sup> gastric,<sup>66,67</sup> cutaneous,<sup>68</sup> prostate,<sup>69</sup> breast,<sup>70,71</sup> cervical,<sup>72</sup> lung,<sup>73-75</sup> bladder,<sup>76</sup> colorectal,<sup>61,77</sup> pancreas,<sup>78,79</sup> nasopharyngeal,<sup>80</sup> esophageal,<sup>81,82</sup> oral squamous cell,<sup>83</sup> gallbladder,<sup>84</sup> endometrial cancer,<sup>85,86</sup> choleangiocarcinoma,<sup>87</sup> GBM,<sup>17</sup> medulloblastoma,<sup>88,89</sup> meningiomas<sup>90,91</sup> and acute leukemia.<sup>92</sup> Upregulated STMN1 expression and/or activity (phosphorylation status) have been correlated with tumor grade, tumor progression, invasion/ metastasis, poor survival and drug resistance in several types of malignancies firstly identified as the downstream target of many signal transduction pathways. Several studies then indicated that stathmin is overexpressed in many types of human malignancies, thus deserving the name of Oncoprotein 18 (Op18,<sup>5,28</sup> highlighting the central role of STMN1 in tumor onset and progression. Accordingly, cumulative evidences have demonstrated reduction of important features of tumor, such as cell proliferation, motility, and metastasis by STMN1 downregulation.

In cancer, the most common and studied mechanism of STMN1 activation is mediated by phosphorylation by several intracellular signaling kinases, as mentioned above, but it can be also mediated by protein sequestration. The p27<sup>Kip1</sup>, a cyclin-dependent kinase (CDK) inhibitor,<sup>55,93,94</sup> and STAT3, a transcription factor signal transducer and activator transcription 3,<sup>95,96</sup> are both able to bind to STMN1, and consequently preventing its ability to sequester free tubulin heterodimers. FOXM1 is another transcription factor able to activate STMN1, as demonstrated recently in non-small cell lung cancer.<sup>19</sup> Recently, we identified STMN1 as one of the proteins downstream the maternal embryonic leucine zipper kinase (MELK) pathway in GBM cell lines.<sup>17</sup> Similar to STMN1, MELK is involved in tumor cell cycle, proliferation and differentiation in several human cancers.<sup>97</sup> MELK silencing has led to the decrease of *STMN1* expression.<sup>17</sup> And, MELK directly binds to FOXM1 and regulates its phosphorylation,<sup>98</sup> and consequently FOXM1 modulates STMN1 expression.<sup>17</sup>

On the other hand, STMN1 downregulation can be modulated by *TP53*, a transcription factor that represses *STMN1* transcription and regulates cell cycle arrest at the G2/M and G1/S checkpoints.<sup>99,100</sup> There are cumulative evidences that STMN1 is the key downstream target of p53, mainly in cells harboring mutant p53 protein, as in hepatocellular carcinoma patients. And such condition is

associated to a poorer prognosis.<sup>101</sup> Corroborating these observations, *STMN1* inhibition in cancer cells harboring TP53 mutation has decreased cell proliferation and viability, increased apoptosis and suppressed tumorigenicity, suggesting that STMN1 is required for the survival of p53-mutant cells.<sup>102-105</sup> Recently, it was suggested that overexpressed STMN1 interacts with p53 and contributes to the gain-of-function of p53.<sup>105</sup> Altogether, these data suggest that targeting STMN1 may be an interesting approach to treat different types of cancers with aberrant p53 function.

Additionally, small non-coding RNAs, micro-RNAs (miRNAs) also modulate STMN1 expression. STMN1 is negatively regulated by the oncogene miR-221 during epithelial-mesenchymal transition (EMT) in bladder cancer cells<sup>106</sup> and by miR-34a in osteosarcoma.<sup>107</sup> Downregulation of miR-223 has been described to increase STMN1 expression in liver cancer, stimulating tumor cell growth and mobility.<sup>108</sup>

These cumulative evidences corroborating the oncoprotein properties of STMN1 turn it a potential therapeutic target.

### STMN1 as potential therapeutic target

STMN1 expression may be modulated interfering in the several mechanisms enumerated above, and also through NF- $\kappa$ B in pancreatic cancer, where *STMN1* silencing reduced cell viability and promoted cell cycle arrest at G2/M phase.<sup>78</sup> Or, by inhibiting of *HIF-1 $\alpha$*  and *VEGF* mRNA levels through the decrease of AKT phosphorylation in the PI3K/AKT/mTOR signaling pathway, as in ovarian cancer.<sup>109</sup>

Of note, STMN1 modulation may be of interest to approach multidrug resistance. Chemoresistance of several solid cancers, including non-small cell lung (NSCLC), esophageal, breast, gastric, endometrial, bladder, retinoblastoma, glioma, osteosarcoma and colorectal cancers has been related to overexpression of *STMN1*. This association was described especially for microtubule-destabilizing drugs, as taxol, paclitaxel and docetaxel, but also for platinum, temozolamide, doxorubicin, arsenic acid, gefitinib and zoledronic acid.<sup>110-112</sup> More recently, upregulation of *STMN1* expression by FOXM1 has been described in NSCLC. STMN1 overexpression was related to EMT and conferred multidrug tyrosine kinase inhibitors (TKIs) resistance on these cells. Mechanistically TKIs, the first group of target-based compounds used as therapy for large numbers of cancer, activate AKT/FOXM1/STMN1 pathway that has conferred multidrug resistance phenotype.<sup>19</sup>

## ■ SUMMARY

Altogether, the results reviewed above suggest that expression of the STMN1 is very important to induce events in cancer development and progression, as cellular proliferation, migration, and drug resistance. Therefore, STMN1

can be an important candidate target for a large number of human cancers. In GBM, the most aggressive brain tumor, the MELK/FOXM1/STMN1 presented significant associated expressions, thus pointing MELK and FOXM1 as alternative targets for therapy instead of STMN1, which is highly expressed in normal brain tissue. In conclusion, continuous research to better elucidate the interacting mechanism with STMN1 looking for new therapeutic strategies is worthwhile.

## ■ ACKNOWLEDGEMENTS

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## ■ AUTHOR CONTRIBUTION

F.O.S, S.K.N.M. and S.M.O.S. conceived and wrote the manuscript.

## ■ CONFLICT OF INTEREST

All the authors declare that have no conflicts of interest with respect to this manuscript.

## COEXPRESSION RELEVANTE DE *STMN1*, *MELK* E *FOXM1* EM GLIOBLASTOMA E REVISÃO DO IMPACTO DE *STMN1* NA BIOLOGIA DO CÂNCER

**OBJETIVO:** Analisar as expressões associadas de *STMN1*, *MELK* e *FOXM1* na procura de alvos alternativos de tratamento em glioblastoma (GBM) e revisar os papéis funcionais relevantes de *STMN1* na biologia do câncer.

**MÉTODO:** As expressões de *STMN1*, *MELK* e *FOXM1* foram estudadas por PCR quantitativo e suas coexpressões foram analisadas em dois coortes independentes de GBM. A revisão dos artigos publicados em revistas indexadas na procura dos aspectos funcionais múltiplos de *STMN1* foi conduzida focando-se nos estudos mais recentes discutindo o seu papel em câncer, quimiorresistência e vias de sinalização envolvendo *MELK* e *FOXM1*.

**RESULTADOS:** Observou-se expressões associadas significantes de *MELK* e *FOXM1* com *STMN1*. Adicionalmente, a revisão da literatura salientou a relevância do *STMN1* na progressão do câncer.

**CONCLUSÃO:** *STMN1* é muito importante nos eventos relacionados ao desenvolvimento e progressão do câncer, como proliferação celular, migração e resistência ao tratamento. Desta forma, *STMN1* pode ser um forte alvo terapêutico em um grande número de cânceres humanos.

Em GBM, o tumor cerebral mais agressivo, MELK/FOXM1/STMN1 apresentaram significativa associação em suas expressões gênicas, indicando, portanto, MELK e FOXM1 como alvos alternativos para terapia em substituição ao STMN1, que apresenta alta expressão no tecido cerebral normal. Perseverar nos estudos funcionais para o entendimento da via de sinalização do STMN1 é relevante para melhorar os esquemas terapêuticos para câncer.

**PALAVRAS-CHAVE:** Stathmin, citoesqueleto, microtúbulos, glioblastoma

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