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The RNA-binding protein Sam68 is a multifunctional player in human cancer

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Abstract

Src associated in mitosis, of 68 kDa (Sam68) is a KH domain RNA-binding protein that belongs to the signal transduction and activation of RNA family. Although ubiquitously expressed, Sam68 plays very specialized roles in different cellular environments. In most cells, Sam68 resides in the nucleus and is involved in several steps of mRNA processing, from transcription, to alternative splicing, to nuclear export. In addition, Sam68 translocates to the cytoplasm upon cell stimulation, cell cycle transitions or viral infections, where it takes part to signaling complexes and associates with the mRNA translation machinery. Recent evidence has linked Sam68 function to the onset and progression of endocrine tumors, such as prostate and breast carcinomas. Notably, all the biochemical activities reported for Sam68 seem to be implicated in carcinogenesis. Herein, we review the recent advancement in the knowledge of Sam68 function and regulation and discuss it in the frame of its participation to neoplastic transformation and tumor progression.

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Introduction

Src associated in mitosis, of 68 kDa (Sam68) was originally identified as the first mitotic substrate of the tyrosine kinase Src in fibroblasts (Fumagalli *et al.* 1994, Taylor & Shalloway 1994). Sam68 belongs to the signal transduction and activation of RNA (STAR) family of RNA-binding proteins (RBPs). This evolutionary conserved group of proteins plays roles in cell proliferation and differentiation, which affect key developmental processes (Vernet & Artzt 1997, Volk *et al.* 2008, Sette *et al.* 2010). STAR proteins owe their name to the presence of a structural domain for the binding of RNA, the GRP33/SAM68/GLD-1 (GSG) domain, flanked by regulatory regions containing motifs for protein–protein interactions and residues that are modified posttranslationally (Sette 2010). This multimodular structure allows STAR proteins to exert different functions in the cell (Vernet & Artzt 1997).

Sam68 can be considered the prototype of STAR proteins (Lukong & Richard 2003), because it undergoes multiple post-translational modifications that finely modulate its subcellular localization, interaction with signaling proteins, affinity for target RNAs and function (Lukong & Richard 2003, Sette 2010, Sette *et al.* 2010). Sam68 contains six proline-rich sequences, spanning both regulatory regions located at the N-terminus and C-terminus of the GSG domain, and a tyrosine-rich region at the C-terminus. These motifs form docking sites for signaling proteins containing Src homology 3 (SH3) and SH2 domains respectively (Fumagalli *et al.* 1994, Taylor & Shalloway 1994, Richard *et al.* 1995, Taylor *et al.* 1995). Notably, tyrosine phosphorylation of Sam68 strongly affects its activity (Sette 2010). Most STAR proteins homodimerize through their GSG domain, and tyrosine phosphorylation by Src-related kinases impairs their homodimerization (Chen *et al.* 1997). This post-translational modification also reduces the affinity of Sam68 for synthetic RNA *in vitro*. Since binding to RNA was also impaired by the interaction of SH3 domains with Sam68 (Taylor *et al.* 1995), it is likely that the association with signaling proteins uncouples Sam68 from its target RNAs in the cell. Additional post-translational modifications also influence the biochemical properties of Sam68. For instance,

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methylation by the arginine methyltransferase PRMT1 impairs its interaction with SH3 domains (Bedford *et al*. 2000) and is required for nuclear translocation of newly synthesized Sam68 (Côtè *et al*. 2003). Analysis of a panel of epithelial cell lines revealed that Sam68 can be acetylated *in vivo*, especially in breast cancer cells, and this modification increased the affinity of Sam68 for RNA *in vitro* (Babic *et al*. 2004). Lastly, Sam68 is also target of sumoylation by the SUMO E3 ligase PIAS1, which enhances its transcriptional repression activity (Babic *et al*. 2006). This complex network of interactions with signal transduction proteins has suggested that Sam68 is involved in several cellular processes.

The recent development of a mouse *Sam68* knockout model has unveiled the physiological processes in which Sam68 is involved. Sam68-deficient mice displayed higher mortality rates soon after birth. Nevertheless, mice that survived beyond weaning showed a normal lifespan, with adult females displaying defects in bone metabolism, likely due to aberrant differentiation of mesenchymal stem cells (Richard *et al*. 2005). Subsequent studies pointed to an important function of Sam68 also in endocrine organs. First, it was demonstrated that the development of sexual organs was strongly delayed in $Sam68^{-/-}$ females (Richard *et al*. 2008). Ductal outgrowth in the mammary gland was reduced in 6-week-old knockout mice, with partial recovery by 12 weeks of age. Similarly, the uterus in 6-week-old $Sam68^{-/-}$ females appeared atrophic and was less developed than in wild-type littermates, whereas the difference was attenuated in older mice (Richard *et al*. 2008). Furthermore, $Sam68^{-/-}$ females displayed a reduction in the number of developing ovarian follicles, an alteration of estrous cycles and their fertility was severely impaired (Bianchi *et al*. 2010). Similarly, spermatogenesis and fertility were impaired in *Sam68* knockout male mice, which likely involved both nuclear RNA processing events (Paronetto *et al*. 2011) and translational regulation of a subset of mRNAs during spermiogenesis (Paronetto *et al*. 2009).

The defects observed in *Sam68* knockout mice likely reflect the multiple activities attributed to Sam68 in signal transduction pathways and in RNA processing. Indeed, several observations support the notion that this versatile protein finely tunes cellular processes in response to external and internal cues (Busà & Sette 2010). Moreover, recent findings highlight how aberrant regulation of Sam68 function concurs to oncogenic transformation and/or cancer progression, thereby pointing to this STAR protein as a possible therapeutic target (Lukong & Richard 2007). Nevertheless, since Sam68 plays multiple functions in the cell, it has remained difficult to clearly define which of its activities is more relevant to tumorigenesis.

Role of Sam68 in signaling

Sam68 acts as a scaffold protein in response to activation of membrane-bound receptors such as the T-cell receptor (Andreotti *et al*. 1997, Fusaki *et al*. 1997, Jabado *et al*. 1997, Jauliac *et al*. 1998, Hawkins & Marcy 2001), leptin receptor (Martín-Romero & Sánchez-Margalet 2001, Sánchez-Margalet *et al*. 2003*b*), and insulin receptor (Sánchez-Margalet & Najib 1999, Sánchez-Margalet *et al*. 2003*a*). Since the role of Sam68 as signaling molecule has been recently reviewed (Najib *et al*. 2005, Lukong & Richard 2007), herein, we will describe only the more recent observations related to human cancer.

Sam68 was shown to favor the interaction between PLCγ1 and the Src-related kinase Fyn (Paronetto *et al*. 2003), leading to phosphorylation and activation of the phospholipase (Sette *et al*. 2002, Paronetto *et al*. 2003). Assembly of the Sam68/PLCγ1/Fyn complex was stimulated by expression of a truncated form of the tyrosine kinase receptor c-KIT. Notably, this truncated receptor is aberrantly expressed in a subgroup of prostate cancer (PCa) patients, and its expression correlates with enhanced activation of Src and tyrosine phosphorylation of Sam68 (Paronetto *et al*. 2004), suggesting that this pathway is involved in Src-mediated tumorigenesis in the prostate.

Tyrosine phosphorylation of Sam68 might also play a role in breast cancer cells. The breast tumor kinase BRK is a non-receptor tyrosine kinase overexpressed in human breast cancer cells (Barker *et al*. 1997), where it promotes proliferation and anchorage-independent growth (Ostrander *et al*. 2010). Sam68 was identified as one of the first substrates of BRK, which phosphorylates tyrosine residues overlapping the nuclear localization signal of Sam68. Upon mitogenic stimulation of breast cancer cells with epidermal growth factor (EGF), BRK-dependent phosphorylation induces transient subcellular relocalization of Sam68 (Lukong *et al*. 2005). Since tyrosine phosphorylation decreases the RNA-binding activity of Sam68 while it enhances its interaction with signaling proteins (Lukong & Richard 2003), it is likely that activation of BRK leads to a functional reprograming of Sam68 activity in breast cancer cells. Notably, both Sam68 and BRK are upregulated in breast cancer and support cell proliferation and invasiveness (Barker *et al*. 1997, Ostrander *et al*. 2010, Song *et al*. 2010). Cell migration

and polarized movements are promoted by Sam68 in an RNA-binding-independent manner (Huot *et al.* 2009*a*). Sam68 seems to be required for negative feedback inhibition of Src, as the activity of the kinase is constitutively high in *Sam68*$^{-/-}$ murine embryonic fibroblasts (Huot *et al.* 2009*a*). Thus, deregulated Src activity might underlie the defects in actin cytoskeleton and cell migration observed in Sam68-deficient fibroblasts. Cell migration was also impaired in HeLa cells depleted of endogenous Sam68, confirming this observation in cancer cells (Huot *et al.* 2009*b*). Since activation of BRK also promotes cell migration, while inducing transient relocalization of Sam68 in the cytoplasm, it is possible that these proteins are part of the same signaling complex that regulates growth factor-dependent cell migration.

The signal transduction properties of Sam68 in cancer cells have also been recently investigated by proteomic analyses (Huot *et al.* 2009*b*, Rajan *et al.* 2009). In line with its reported functions, many of the Sam68-interacting proteins identified in HeLa cells and PCa cells are RBPs, cytoskeletal proteins and signal transduction proteins. Sam68 was found in large macromolecular complexes in HeLa cells, which required cellular RNA integrity (Huot *et al.* 2009*b*). Moreover, treatments with phorbol 12-myristate 13-acetate or EGF induced a change in the size of the Sam68-containing complex (Huot *et al.* 2009*b*), suggesting that external cues modulate Sam68 function in cancer cells by affecting the nature of its protein–protein and protein–RNA interactions. Although not directly tested, it is likely that BRK or an Src-related kinase is involved in this process, as both kinases can be activated by engagement of the EGF receptor.

Role of Sam68 in transcription

Sam68 was suggested to link signaling pathways with gene transcription. Sam68 interacts with the transcriptional cofactor CBP and inhibits CBP-dependent gene transcription independently of its RNA-binding activity (Hong *et al.* 2002). The mechanism proposed is a competition between Sam68 and coregulators for the binding to CBP (Fig. 1). The inhibition of CBP activity by Sam68 could explain the repressive effects observed on the expression of cell cycle regulators upon Sam68 overexpression. Sam68 induces a G1 phase arrest in murine NIH-3T3 cells, which correlates with downregulation of *cyclin D1* and *cyclin E* transcripts (Taylor *et al.* 2004). These effects did not depend on its RNA-binding activity and could be enhanced by SUMOylation of Sam68 on lysine residues, mediated by the SUMO E3 ligase PIAS1 (Taylor *et al.* 2004, Babic *et al.* 2006). Recently, it has been proposed that Sam68 SUMOylation is favored by the interaction of PIAS1 with another splicing factor, SF2/ASF (Pelisch *et al.* 2010; Fig. 1). Thus, the interplay between these two splicing regulators might enhance transcriptional repression of *cyclin D1*.

The transcriptional role of Sam68 can also directly affect cancer cell biology. In PCa cells, Sam68 interacts with the androgen receptor (AR), thereby enhancing its transcriptional activity (Rajan *et al.* 2008). Interestingly, this interaction may alter the cyclin D1-mediated feedback control mechanism of AR activity (Knudsen 2006). Cyclin D1 interacts with the AR and is able to counteract its transcriptional activity, thereby exerting anti-proliferative effects (Burd *et al.* 2005). Thus, the upregulation of Sam68 observed in PCa cells (Busà *et al.* 2007, Rajan *et al.* 2008) could modulate AR activity both directly, by binding to AR and enhancing its activity, and indirectly by repressing *cyclin D1* transcription (Fig. 1). Notably, the positive function of cyclin D1 on cell cycle progression in PCa cells is preserved by the expression of an alternative splicing isoform, cyclin D1b, which is predictive of a poor outcome in patients (Comstock *et al.* 2009). Cyclin D1b is not able to inhibit AR and displays stronger oncogenic properties (Burd *et al.* 2006). Upregulation of Sam68 in PCa cells promotes the splicing of *cyclin D1b*, while repressing the canonical *cyclin D1a* isoform (Paronetto *et al.* 2010), suggesting that this versatile protein strongly promotes AR activity by multiple direct and indirect mechanisms.

Role of Sam68 in alternative splicing

Mounting evidence indicates a role for Sam68 in alternative splicing. This step in pre-mRNA processing affects the majority of human genes and represents a key mechanism to generate protein diversity in higher eukaryotes (Chen & Manley 2009, Hartmann & Valcárcel 2009). Usage of an alternatively spliced exon is dictated by sequences present at its boundaries as well as by enhancer and silencer sequences that can be located few hundred bases from the alternatively spliced exons (Black 2003). Together, with the spliceosome, many additional splicing factors, such as SR proteins and hnRNPs, can take part to exon definition in the pre-mRNA, hence contributing to the complex regulation of alternative splicing (Black 2003).

A possible role for Sam68 in splicing was initially suggested by its binding, together with general splicing factor U2AF65 and the polypyrimidine tract-binding

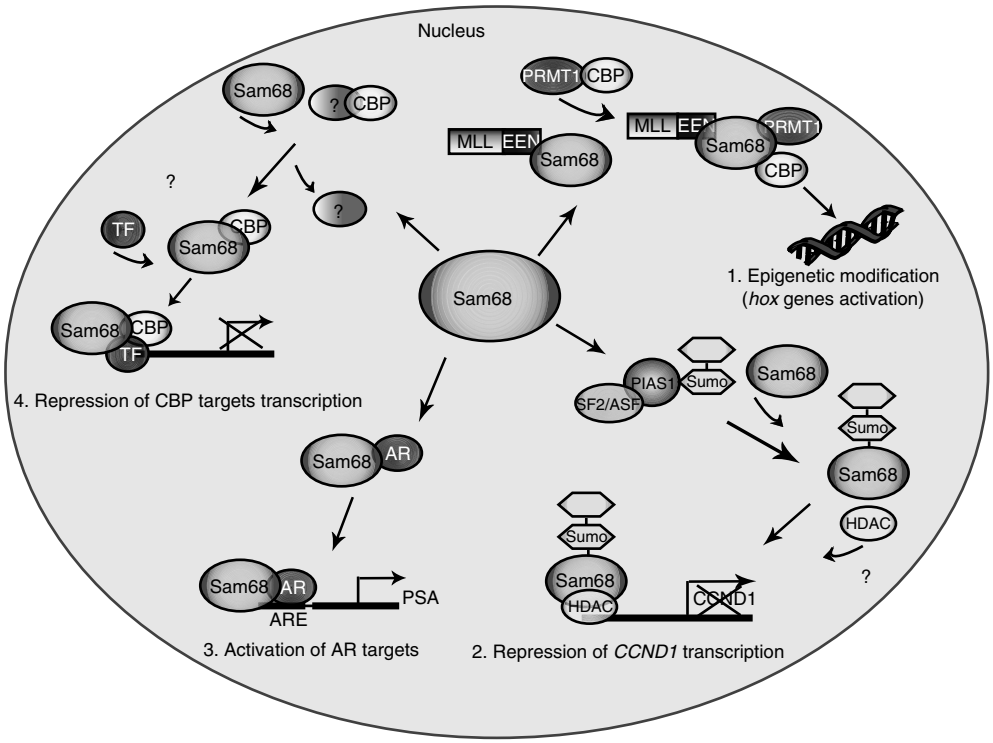

Figure 1 Schematic models of the multiple roles played by Sam68 in transcriptional regulation. The interaction of Sam68 with the oncogenic fusion protein MLL-EEN allows recruitment of the histone methyltransferase PMRT1 and the acetyltransferase CBP to MLL responsive sequences (model 1), inducing epigenetic modifications and activating MLL downstream targets (i.e. *HOX* genes). The PIAS1-SF2/ASF complex SUMOylates Sam68, which hypothetically interacts with a histone-deacetylase (HDAC), and represses *cyclin D1* (*CCDN1*) transcription (model 2), thus enhancing androgen receptor (AR) activity. Sam68 also directly interacts with the AR and binds to androgen responsive elements (AREs) leading to AR targets activation (i.e. *PSA* gene) (model 3). Sam68 interacts with CBP, competing with other unknown cofactors for the binding or displacing other cofactors bound to CBP. Sam68 binds to CBP and hypothetically bridges it to a transcriptional repressor factor (TF), thus negatively regulating CBP targets transcription (model 4).

protein, to an intronic regulatory element located between the polypyrimidine tract and the 3′ splice site of the *β-tropomyosin* pre-mRNA (Grossman *et al*. 1998). Moreover, SLM2, a close homolog of Sam68 (Di Fruscio *et al*. 1999, Venables *et al*. 1999) was shown to interact with splicing factors and to modulate splicing of reporter minigenes (Stoss *et al*. 2001). A direct involvement of Sam68 in the regulation of alternative splicing was provided a year later (Matter *et al*. 2002), when it was demonstrated that this RBP promoted the inclusion of the variable exon v5 in the *CD44* pre-mRNA. Importantly, *CD44* encodes a cell surface molecule involved in cell adhesion, proliferation, and migration of cancer cells (Naor *et al*. 2002). *CD44* encodes more than 20 alternative transcripts through incorporation of nine variable exons, whose inclusion in the mature transcript correlates with both tumorigenesis and metastasis (Wielenga *et al*. 2000, Helliwell 2001). Since depletion of endogenous Sam68 strongly reduced inclusion of several variable exons in *CD44* (Cheng & Sharp 2006), the splicing activity of Sam68 seems directly related to its oncogenic properties and might explain its upregulation in many human cancers (Busà & Sette 2010, Elliott & Rajan 2010). Another key point of the original work of König *et al*. was the identification of Sam68 as a downstream target of the RAS/mitogen-activated protein kinases (MAPK) ERK1 and 2 (ERK1/2) pathway (Matter *et al*. 2002), which is frequently activated in cancer. Stimulation of this signaling pathway by growth factors leads to serine/threonine phosphorylation of Sam68 and enhancement of its splicing activity (Fig. 2). These results proved for the first time a role of Sam68 in linking signaling pathways with RNA processing events.

Subsequent studies have illustrated in more detail the function of Sam68 in alternative splicing. Sam68 binds to U2AF65, a component of the U2 snRNP, and its phosphorylation by ERK1/2 was proposed to fine-tune occupancy of the 3′ splice site by U2AF65 (Tisserant & König 2008). Importantly, binding of Sam68 to exon v5 of the *CD44* pre-mRNA is increased

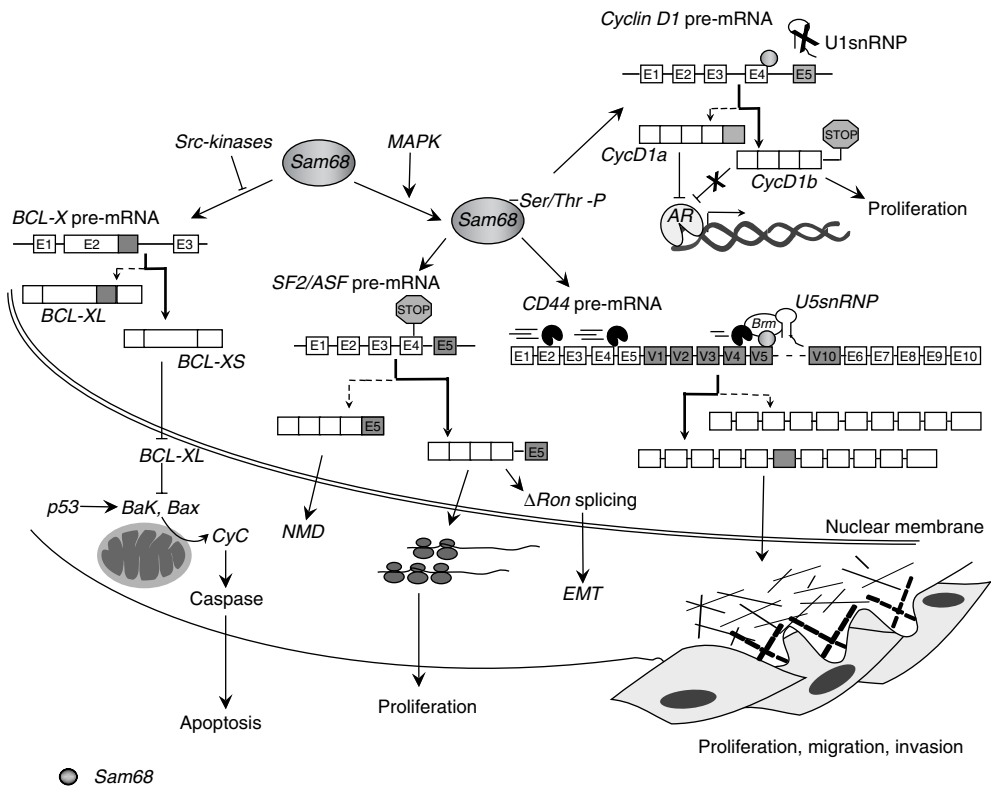

Figure 2 Schematic representation of alternative splicing events regulated by Sam68 in cancer cells. Sam68 promotes the alternative splicing of *BCL-X*. This activity can be modulated through its tyrosine phosphorylation by Src family kinases, thereby switching the role of Sam68 from pro-apoptotic to anti-apoptotic and allowing cells to differentially react to external cues. Sam68 also promotes splicing events that regulate cell proliferation. Binding of Sam68 to intron 4 interferes with the recruitment of the U1 snRNP at the intron 4/exon 4 junction and enhances retention of intron 4 in the *cyclin D1b* variant. The expression of cyclin D1b interrupts a negative feedback in the regulation of androgen receptor (AR) transcriptional activity, thereby promoting cell proliferation. Inclusion of variable exons in the *CD44* pre-mRNA is specific to cancer cells. Sam68 promotes the inclusion of variable exon v5. Sam68 interacts with U5 snRNP and the chromatin remodeling protein Brm, thereby favoring the inclusion of the alternative exons in *CD44*, which correlate with cancer progression and invasiveness. The alternative splicing of an intron in the 3′ untranslated region (UTR) of *SF2/ASF* regulates its degradation by nonsense-mediated decay (NMD) of the mRNA. Sam68 promotes retention of this intron in the *SF2/ASF* pre-mRNA, thereby stabilizing it. Accumulation of SF2/ASF in turn favors the splicing of *ΔRon*, an oncogenic variant of *RON*, which triggers epithelial–mesenchymal transition (EMT).

upon MAPK activation triggered by treatments of cells with phorbol esters (Batsché *et al*. 2006). This stimulation also enhanced the interaction of Sam68 with the U5 snRNP, and recruitment of the chromatin remodeling protein Brm, which was suggested to slow down the RNA polymerase II and to allow inclusion of the *CD44* alternative exons (Batsché *et al*. 2006). The functional interaction between the MAPK pathway and the activity of the Brm/Sam68 complex is also involved in the splicing of the human papillomavirus (HPV) polycistronic pre-mRNA. It was shown that EGF stimulated splicing of the E6 variant of *HPV16*, through a mechanism depending on activation of ERK1/2 and Brm/Sam68 (Rosenberger *et al*. 2010). This observation suggests the involvement of Sam68 in the regulation of the life cycle of HPV and neoplastic transformation in cervical cancer.

Other splicing targets of Sam68 also support its involvement in tumor progression. As mentioned above, the *cyclin D1* proto-oncogene encodes for two splicing variants, the full-length D1a variant and a shorter isoform, named D1b, arising from retention of intron 4 and premature termination of translation (Knudsen 2006). Cyclin D1b is more oncogenic than the D1a variant, although the mechanism involved in its higher transforming activity is scarcely understood. Sam68 binds sequences in intron 4 and promotes its retention in the pre-mRNA, likely by competing with recruitment of the U1 snRNP (Paronetto *et al*. 2010; Fig. 2). As shown for *CD44* and *HPV*, Sam68-mediated *cyclin D1* splicing is stimulated by expression of an oncogenic form of RAS and activation of the MAPK pathway in PCa cells (Paronetto *et al*. 2010). A similar mechanism may also apply to the

newly proposed role of Sam68 in epithelial-to-mesenchymal transition (EMT; Valacca *et al*. 2010), a key step in the progression of cancer cells to a metastatic stage (Thiery *et al*. 2009). Phosphorylation of Sam68 by the MAPK pathway promotes the retention of an intron in the 3′ UTR of the proto-oncogene *SF2/ASF*. This splicing event stabilizes SF2/ASF by preventing nonsense-mediated decay of its transcript (Valacca *et al*. 2010). In turn, accumulation of SF2/ASF favors the splicing of the proto-oncogene ΔRon, which triggers EMT in colon cancer cells (Ghigna *et al*. 2005, Valacca *et al*. 2010; Fig. 2).

A more complicated picture of the role of Sam68-mediated splicing in cancer cells is offered by regulation of the apoptotic gene *BCL-X* (Paronetto *et al*. 2007). This gene encodes two splicing variants that exert opposite effects on cell survival. *BCL-X* exon 2 contains two alternative 5′ splice sites; choice of the proximal site at the end of the exon leads to splicing of the anti-apoptotic BCL-X_L, whereas selection of the distal site within the exon causes expression of the pro-apoptotic BCL-X_S (Boise *et al*. 1993). Notably, the choice of the alternative splice site in *BCL-X* reflects the sensitivity of the cell toward agents that induce apoptosis. Overexpression of Sam68 in HEK293 cells induces the pro-apoptotic BCL-X_S and cell death in a dose-dependent manner (Paronetto *et al*. 2007). This effect requires the RNA-binding activity of Sam68 as well as its interaction with hnRNPA1 through the C-terminal domain. These observations would suggest that upregulation of Sam68 is detrimental for cell survival. However, independent studies indicate that both Sam68 and the anti-apoptotic BCL-X_L variant are upregulated in PCa patients (Mercatante *et al*. 2002, Busà *et al*. 2007, Rajan *et al*. 2008). Moreover, we found that depletion of the endogenous Sam68 in PCa cells caused downregulation of BCL-X_L at both RNA and protein levels (Busà *et al*. 2007). These apparently conflicting results can be reconciled by the observation that tyrosine phosphorylation of Sam68 by Src-like kinases reverts *BCL-X* splicing and promotes the anti-apoptotic BCL-X_L variant (Paronetto *et al*. 2007), thereby inhibiting cell death (Brignatz *et al*. 2009). Interestingly, Src activity is often increased in cancer cells (Irby & Yeatman 2000), and it correlates with Sam68 phosphorylation in prostate and breast carcinomas (Paronetto *et al*. 2004, Lukong *et al*. 2005). Therefore, tyrosine phosphorylation of Sam68 in cancer cells may represent a mechanism to protect them from apoptosis by altering the BCL-X_S/BCL-X_L ratio (Paronetto *et al*. 2007; Fig. 2).

An extensive analysis of Sam68 pre-mRNA targets came from its knockdown in mouse neuroblastoma cells, coupled with analysis of splicing sensitive microarrays. Twenty-four novel exons regulated by Sam68 in neuronal cells were identified (Chawla *et al*. 2009). A number of these novel targets are known to play a role in neurogenesis, and their altered splicing may underlie the defects in motor coordination observed in *Sam68*$^{-/-}$ mice (Lukong & Richard 2008). Moreover, the splicing activity of Sam68 has been recently implicated in the onset of two human neurodegenerative diseases. In cells of patients affected by spinal muscular atrophy, Sam68 was shown to promote skipping of exon 7 in the *SMN2* gene (Pedrotti *et al*. 2010), which leads to a non-functional SMN protein (Pedrotti & Sette 2010). Interference with its activity by retroviral infection of dominant-negative Sam68 mutants restored exon 7 splicing and accumulation of the SMN protein product (Pedrotti *et al*. 2010). On the contrary, sequestration of Sam68 in RNA aggregates in cells of patients affected by fragile X-associated tremor/ataxia syndrome acted as a nucleation center for the recruitment of splicing factors. This mechanism was proposed to cause splicing defects that may affect the pathology (Sellier *et al*. 2010). Thus, regulation of the splicing activity of Sam68 might be relevant to other human diseases beside cancer.

Role of Sam68 in cancer

Since its identification as a substrate of the oncogenic Src kinase, a role for Sam68 in cancer was hypothesized (Fumagalli *et al*. 1994, Taylor & Shalloway 1994). Initial studies using non-transformed murine fibroblasts pointed to a tumor suppressor function for Sam68. A retroviral-based antisense RNA screening identified Sam68 as a gene that promoted cell transformation when it was knocked out (Liu *et al*. 2000). Reduction of Sam68 expression in NIH-3T3 fibroblasts was associated with anchorage-independent growth and development of metastatic tumors in nude mice. However, reintroduction of Sam68 in the transformed cells did not revert the phenotype (Liu *et al*. 2000), suggesting that additional factors were sustaining cell transformation. Consistent with a role as tumor suppressor was also the observation that overexpression of Sam68 in NIH-3T3 fibroblasts caused cell cycle arrest in G1 and apoptosis (Taylor *et al*. 2004). Notably, Sam68-dependent cell death required its RNA-binding activity, whereas repression of cyclin D1 and cyclin E expression and inhibition of cell cycle progression were RNA-binding independent.

Although these studies supported a tumor suppressor role, direct investigation of Sam68 expression and function in cancer cells rather suggest a pro-oncogenic role of the protein. First, unlike other tumor suppressor genes, knockout of *Sam68* in mice does not sensitize to tumor formation *in vivo* (Richard *et al*. 2005). By contrast, *Sam68* haploinsufficiency delayed the onset of mammary tumors and reduced dissemination of metastasis (Richard *et al*. 2008). Furthermore, as reported in the previous sections, a number of experimental observations suggest a direct involvement of Sam68 in oncogenesis, through regulation of signal transduction pathways and modulation of gene expression, especially in endocrine-related cancers. For instance, Src activation by a truncated c-Kit receptor stimulates Sam68 phosphorylation in cultured cells and in PCa patients (Paronetto *et al*. 2003, 2004). Since tyrosine phosphorylation by Src-related kinases fine-tunes Sam68-mediated splicing and apoptosis (Paronetto *et al*. 2007, Brignatz *et al*. 2009), and Src is often activated in human cancers (Biscardi *et al*. 2000, Irby & Yeatman 2000), this mechanism might promote survival of neoplastic cells. In line with a role in PCa, immunohistochemical analyses indicated that Sam68 is frequently upregulated in patients (Busà *et al*. 2007, Rajan *et al*. 2008). Sam68 expression supported proliferation of PCa cells and protected them from genotoxic agents (Busà *et al*. 2007). The exact function(s) of Sam68 that contributes to PCa is still unknown. However, as described above, it is likely that its ability to modulate cancer-relevant splicing events (i.e. *BCL-X*, *CD44*, *cyclin D1* and *SF2/ASF*; Matter *et al*. 2002, Paronetto *et al*. 2007, 2010, Valacca *et al*. 2010), the transcriptional activity of AR (Rajan *et al*. 2008), and coupling between AR-dependent transcription and splicing (Rajan *et al*. 2008) all take part to prostate oncogenesis. Interestingly, treatment with mitoxantrone, a topoisomerase II inhibitor used in chemotherapy, triggered the relocalization of Sam68 to nuclear foci of active transcription together with the phosphorylated form of the RNA polymerase II and other splicing factors (Busà *et al*. 2010). This stress response might be implicated in the protective action of Sam68 in PCa cells, as it modulates splicing of oncogenic *CD44* variants and might contribute to cell survival (Busà & Sette 2010, Busà *et al*. 2010).

The positive function of Sam68 in neoplastic transformation has been recently confirmed in other human cancers. Sam68 is upregulated in breast cancer, where it correlates with shorter survival rates (Song *et al*. 2010). Similar to PCa cells, knockdown of the endogenous Sam68 in breast cancer cell lines reduced their proliferation rate and tumorigenicity, likely by affecting cell cycle progression. Silencing of endogenous Sam68 caused upregulation of cyclin-dependent kinase inhibitors, such as p21 and p27, and reduced phosphorylation of the retinoblastoma protein, which may account for the delayed cell cycle progression. Moreover, Sam68 depletion reduced phosphorylation of AKT and caused concomitant activation of the FOXO family of transcription factors (Song *et al*. 2010). Although the mechanism by which this regulation is exerted is still unknown, these results are in line with previous observations on the role of Sam68 in mammary tumorigenesis *in vivo*. Sam68 haploinsufficiency protects mice from mammary tumors triggered by the polyoma middle T antigen (PyMT) in breast cells (Richard *et al*. 2008). The rationale of this experiment stemmed from the knowledge that PyMT signaling induces breast tumors through Src. Since Sam68 is a substrate of Src, it was hypothesized that it could participate to activation of the PyMT pathway in breast cells. The reduction in Sam68 levels correlated with increased activity of Src and constitutive activation of its pathway (Richard *et al*. 2008), suggesting that Sam68 is part of a negative feedback control of Src in breast cells. *Sam68* heterozygote mice showed delayed onset of breast tumors and reduced number of lung metastases (Richard *et al*. 2008). These results suggest that high levels of Sam68 are required for cell transformation *in vivo* and support a role for Sam68 as proto-oncogene.

Increased expression of Sam68 correlated with poor prognosis also in patients affected by renal carcinoma (Zhang *et al*. 2009). Notably, in advanced stages of both breast (Song *et al*. 2010) and renal (Zhang *et al*. 2009) tumors, Sam68 was localized also in the cytoplasm. In both cases, cytoplasmic localization of the protein represented an independent factor of poor prognosis. This observation suggests a possible additional function of Sam68 in highly malignant cancer cells. Indeed, it was previously shown that Sam68 localizes in the cytoplasm of male germ cells (Paronetto *et al*. 2006) and neurons (Grange *et al*. 2004), where it associates with the translation initiation complex (Paronetto *et al*. 2009) and with polysomes (Grange *et al*. 2004, Paronetto *et al*. 2006), thereby regulating translation of selected mRNAs (Grange *et al*. 2009, Paronetto *et al*. 2009). Importantly, the translational activity of Sam68 in male germ cells was also stimulated by the RAS/MAPK pathway (Paronetto *et al*. 2006, 2009). Thus, in addition to its well documented ability to modulate cancer-specific splicing events (Matter *et al*. 2002, Paronetto *et al*. 2007, 2010, Busà *et al*. 2010), it is plausible to speculate that

cytoplasmic localization of Sam68 in advanced stages of tumors might contribute to neoplastic transformation by enhancing translation of specific mRNAs.

The ability of Sam68 to take part to protein–protein interactions may also promote oncogenesis. For instance, Sam68 is recruited by RET/PTC2 and RETMEN2B (Gorla *et al*. 2006), two oncogenes implicated in thyroid cancers. Tyrosine phosphorylation of Sam68 was increased in RET-transfected cells and thyroid tumors. Sam68 and RET oncoproteins also interacted with other regulators of splicing, however, the physiological relevance of this process in thyroid cancer was not directly investigated (Gorla *et al*. 2006). The signaling function of Sam68 contributes to the activity of two other oncogenes: Vav1 and mixed lineage leukemia (MLL)-EEN. A proteomic approach identified Sam68 as one of the proteins associating with the C-terminal SH3 domain of Vav1, which is necessary for cell transformation. Notably, mutations in Vav1 that disrupted the interaction with Sam68 also abolished the transforming potential of this oncogene, whereas coexpression of Vav1 and Sam68 in NIH-3T3 fibroblasts enhanced transformation (Lazer *et al*. 2007). These observations strongly suggested that recruitment of Sam68 by the SH3 domain of Vav1 is a critical step in cell transformation. Interaction of Sam68 with an SH3-containing oncogenic protein is also observed in leukemia. Chimeric fusion proteins of MLL are associated with development of acute myeloid leukemia (So *et al*. 1997). One of the first fusion proteins identified is MLL-EEN (So *et al*. 1997). Interestingly, the SH3 domain of EEN, which is necessary and sufficient for oncogenic activation by MLL-EEN, was shown to interact with the fourth proline-rich region of Sam68 (Cheung *et al*. 2007). In turn, Sam68 recruits both the histone methyltransferase PRMT1 and the histone acetyltransferase CBP to MLL responsive promoters (Fig. 1). Notably, CBP can also associate with wild-type MLL, but this interaction is not sufficient for transformation. Conversely, the Sam68-mediated recruitment of PRMT1 occurs only by the oncogenic MLL-EEN fusion protein and it is essential for neoplastic transformation. Depletion of the endogenous Sam68 suppressed MLL-EEN-mediated cell transformation, confirming the critical role played by Sam68. Once recruited by Sam68, PRMT1 induces epigenetic modifications that lead to deregulation of transcription of MLL-ENN downstream targets (Fig. 1), such as *Hox* genes, which are critical for the onset of leukemia (Cheung *et al*. 2007).

Thus, these studies strongly suggest that the ability of Sam68 to favor the formation of multimolecular complexes and to modulate various steps of RNA processing can enhance oncogenic transformation in different neoplastic settings.

Concluding remarks

Sam68 is a versatile protein involved in many biological processes. The presence of protein–protein interaction motifs allows it to function as a scaffold in signal transduction pathways and in transcription complexes. In addition, its RNA-binding activity confers to Sam68 the ability to modulate alternative splicing and translation of target mRNAs. Numerous observations link Sam68 to carcinogenesis. Notably, all the biochemical activities of Sam68 reported above have been implicated in its role in cancer. Sam68 is an attractive target for cancer therapy because it is not an essential gene (Richard *et al*. 2005). However, a better understanding of its function(s) in cancer cells is required to develop tools to interfere with its activity. Future studies are warranted to understand whether synthetic molecules targeting its protein–protein interaction motifs or its RNA-binding domain can be produced and are effective in reducing cancer cell proliferation and survival in live organisms.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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