The tumour suppressor function of the scaffolding protein spinophilin

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Abstract

Spinophilin is a scaffolding protein with modular domains that govern its interaction with a large number of cellular proteins. The Spinophilin gene locus is localized at chromosome 17q21, a chromosomal region frequently affected by genomic instability in different human tumours. The scaffolding protein interacts with the tumour-suppressor ARF which has suggested a role for Spinophilin in cell growth. More recently, in vitro and in vivo studies demonstrated that Spinophilin is a new tumour suppressor acting via the regulation of pRb. A clear downregulation of Spinophilin is found in several human cancer types. Moreover, Spinophilin loss is associated with a poor patient prognosis in carcinoma. Currently, there are controversial findings regarding a functional relationship between Spinophilin and p53 in cell cycle regulation and in carcinogenesis. Here we present the available data regarding Spinophilin function as a tumour suppressor.

Key words

CaSR, G protein-coupled receptor, signaling

1- Introduction

Protein phosphatase 1 (PP1) is a widespread expressed phosphoSerine/phosphoThreonine PP involved in many cellular processes (Ceulemans and Bollen, 2004). There are four isoforms of PP1 catalytic subunit (PP1c): PP1α, PP1β, PP1γ1 and PP1γ2, the latter two arising through alternative splicing (Sasaki et al., 1990). PP1c can form complexes with up to 50 regulatory subunits converting the enzyme into many different forms, which have distinct substrates specificities, restricted subcellular locations and diverse regulations (Cohen, 2002). In late 1990s, a novel PP1c binding protein that is a potent modulator of PP1 activity was characterized in rat brain and named spinophilin (Spn) (Allen et al., 1997). In the same time, two novel actin filament-binding proteins were purified from rat brain and named neurarabin 1 and neurarabin 2 (NEURal tissue-specific-Actin-Binding proteIN), and the latter was further identified as Spn (Nakanishi et al., 1997). Spn is expressed ubiquitously while neurarabin 1 is expressed almost exclusively in neuronal cells. Spn exhibits the characteristics of scaffolding proteins with multiple protein interaction domains (Allen et al., 1997; Sarrouilhe et al., 2006). Scaffolding proteins link signalling enzymes, substrates and potential effectors (such as channels, receptors) into a multiprotein signalling complex that may be anchored to the cytoskeleton. In the years after this discovery, the spectrum of Spn partners and functions has expanded but has remained mostly in the field of neurobiology (Sarrouilhe et al., 2006). Spn has been implicated in the pathophysiology of several central nervous system (CNS) diseases, among which are Parkinson's disease, schizophrenia and mood disorders (Law et al., 2004; Brown et al., 2005). Spn is highly enriched at the synaptic membrane in dendritic spines, the site of excitatory neurotransmission and thus may control PP1 functions during synaptic activity (Ouimet et al.,
2004). Spn regulates plasticity at the postsynaptic density (PSD) by targeting PP1c to α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors, promoting their down regulation by dephosphorylation and thus regulating the efficiency of post-synaptic glutamatergic neurotransmission. Spn and neurabin I play different roles in hippocampal and striatal synaptic plasticity. Spn is involved in long-term depression (LTD) but not in long-term potentiation (LTP) whereas neurabin I contributes selectively to LTP but not LTD (Feng et al., 2000; Allen et al., 2006; Wu et al., 2008). In the same way, the two scaffolding proteins form a functional pair of opposing regulators that reciprocally regulate signalling intensity by some seven-transmembrane domain receptors (Wang et al., 2007). Thus, an emerging notion is that Spn and neurabin I may differentially affect their target proteins and perform quite distinctive function in cell.

Morphological studies have established that Spn is enriched at plasma membrane of cells although the protein is also expressed widely throughout the cytoplasm (Smith et al., 1999; Richman et al., 2001; Tsukada et al., 2003). Spn, which is expressed partly in the nucleus in mammalian cells, interacts in vitro and in vivo with the tumor-suppressor ARF (Alternative Reading Frame). Moreover, a role for Spn in cell growth was suggested, and this effect was enhanced by the interaction between Spn and ARF (Vivo et al., 2001). More recent studies showed that Spn is a new tumour suppressor and that a clear downregulation of this protein is found in several cancer types (Carnero, 2012). Furthermore, Spn loss is associated with poor patient prognosis in carcinomas (Sarrouilhe, 2014). This review aims to outline the state of knowledge regarding Spn function in carcinogenesis.

2- Spinophilin structure

The primate (homo sapiens and Callithrix jacchus) Spn proteins contain 815 amino acids whereas the rodent Spn (rattus norvegicus and mus musculus) have 817 amino acids. These sequences are very similar, with few amino acids substitutions compared to the human sequence in C-terminus but the N-terminus is more variable even if the variability is weak (Figure 1). Consequently, few differences are observed when we compared these sequences to the human one: the rat and human Spn proteins share 96% sequence identity (Allen et al., 1997; Vivo et al., 2001). In Cricetulus griseus, the sequence is shorter than the others: 631 amino acids. Gene analysis and biochemical approaches have contributed to define in Spn a number of distinct modular domains. This 130 kDa protein contains one F-actin-, a receptor- and a PP1c- binding domains, a PSD95/DLG/zo-1 (PDZ) and three coiled-coil domains. Figure 2 provides a schematic diagram of the main Spn structural domains.

In the five species of the Figure 1, the coiled-coil region has high identity with only one variation detected in Cricetulus griseus. The PDZ domain, the pentapeptide motif of PP1c-binding domain and the sextapeptide allowing the binding selectivity of PP1c isoforms, present the same identity. Moreover, the phosphoSer are conserved except the Ser-177 which is only detected in rat. Being not detected in mouse (G as in primates), Ser-177 is not a consequence of the rodent-specific high substitution rate.

Spn has been isolated from rat brain as a protein interacting with F-actin (Satoh et al., 1998). Its F-actin-binding domain determined to be amino acids 1-154 is both necessary and sufficient to mediate actin polymers binding and cross-linking. Nuclear Magnetic Resonance (NMR) and circular dichroism (CD) spectroscopy studies showed that Spn F-actin-binding domain is intrinsically unstructured and that upon binding to F-actin it adopts a more ordered structure (a phenomenon also called folding-upon-binding). Another actin binding property, namely a F-actin pointed end capping activity was recently proposed for this domain (Schüler and Peti, 2007). Spn, PP1c and F-actin can form a trimeric complex in vitro.

A receptor-interacting domain, located between amino acids 151-444, interacts with the third intracellular loop (3i) of various seven transmembrane domain receptors (Smith et al., 1999; Richman et al., 2001) such as the dopamine D2 receptor (D2R), some subtypes of the α-adrenergic (AR) and muscarinic-acetylcholine (m-AChR) receptors.

The primary PP1c-binding domain is located within residues 417-494 of Spn and this domain contains a pentapeptide motif (R-K-I-H-F) between amino acids 447 and 451 that is conserved in other PP1c regulatory subunits. A domain C-terminal to this canonical PP1-binding motif, located within amino acids 464 and 470, is essential for PP1 isoform selectivity in vitro and for selective targeting in cells (Carmody et al., 2008). Recently, the 3-dimensional structure of the PP1/Spn holoenzyme was determined. Spn is an unstructured protein in its unbound state that undergoes a folding transition upon interaction with PP1c into a single, stable conformation. The scaffolding protein binds to PP1c and blocks some potential substrate binding sites without altering its active site, then didactically substrate specificity of the enzyme (Ragusa et al., 2010). A further study showed that the PP1/Spn holoenzyme is dynamic in solution.
Figure 1. Alignment of amino acid sequences of spinophilin in different species. Blast and Align programs via UniProt site were used.
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Figure 2. Schematic drawing of spinophilin structure. The canonical protein phosphatase 1-binding domain is located within amino acids 447 and 451 in spinophilin.

The complex adopts a significant more extended conformation in solution than in the crystal structure. This is the result of a flexible linker (amino acids 490-494) between the PP1c-binding and the PDZ domains. The four residue flexibility is likely important for Spn biological role (Ragusa et al., 2011).

Spn also contains a single consensus sequence in PDZ, amino acids 494-585 (Allen et al., 1997). The structure of the Spn PDZ domain has been recently solved by NMR spectroscopy. The PDZ domain directly binds to carboxy-terminal peptides derived from glutamatergic AMPA and NMDA receptors (Kelker et al., 2007).

Sequence analysis predicted that the carboxy-terminal region of Spn (amino acids 664-814) forms 3 coiled-coil domains. Neurabins were observed as multimeric forms in vitro and in vivo. Spn and neurabin 1 homo- and hetero-dimerize via their carboxy-terminal coiled-coil domains (MacMillan et al., 1999; Oliver et al., 2002).

Consensus sequences for phosphorylation by several protein kinases (PK), including cAMP-dependent PK (PKA), Ca²⁺/calmodulin-dependent PK II (CaMKII), cyclin-dependent PK5 (Cdk5), extracellular-signal regulated PK (ERK) and protein tyrosine kinases were observed in Spn. Two major sites of phosphorylation for PKA (Ser-177 not conserved in human, and Ser-94) and two others sites for CaMKII phosphorylation (Ser-100 and Ser-116) were located within and near the F-actin-binding domain of Spn. The protein is phosphorylated in intact cells by PKA at Ser-94 and Ser-177 and by CaMKII at Ser-100 and Ser-116 (Hsieh-Wilson et al., 2003; Grossman et al., 2004). Moreover, neurabins can be phosphorylated in vitro and in intact cells by Cdk5 on Ser-17 and ERK2 (MAPK1) on Ser-15 and Ser-205, phosphoSer-17 being abundant in neuronal cells (Futter et al., 2005). Several potential tyrosine phosphorylation sites lie within the coiled-coil regions, within a region adjacent to the PDZ domain and within the receptor-interacting domain.

3- The Spinophilin interactome

Spn interactome includes cytoskeletal molecules (F-actin, doublecortin, neurabin 1, Spn), enzymes (like PP1 and CaMKII), regulator of G-protein signalling protein (like RGS8), guanine nucleotide exchange factors (like kalirin 7), membrane receptors [like the α-ARs, m-AChRs, D2R, δ- and µ-opioid receptors (OR) and cholecystokinin (CCK) receptors], and other proteins like ions channels [The transient receptor potential canonical (TRPC), the type 2 ryanodine receptor (RYR2)], TGN38 and ARF.

Shortly after the cloning of Spn as a novel PP1c-binding protein, another laboratory cloned this protein based on its ability to bind to F-actin (Satoh et al., 1998).

Recombinant Spn and neurabin 1 interacted with each other when co-expressed in cells. On the other hand, recombinant Spn was shown to form homodimers, trimers or tetramers by interaction between coiled-coil domains. Spn homomorphic complexes are thought to contribute to its actin-cross-linking activity (Satoh et al., 1998).

Doublecortin (DCX) is a microtubule-associated protein that can induce microtubule polymerization and stabilize microtubules filaments. Immunoprecipitation experiments with brain extracts showed that Spn and DCX interact in cultured cells (Tsukada et al., 2003). In vitro assays showed that DCX also binds to and bundles F-actin, suggesting that the protein cross-links microtubules and F-actin.

The distribution of DCX between the two cytoskeletons can be regulated by Spn and by phosphorylation of DCX and it was proposed that Spn could localize and enhance the binding of phosphorylated DCX to F-actin (Tsukada et al., 2005).

Several studies have shown that Spn preferentially binds to PP1γ and PP1α isoforms in brain extracts (MacMillan et al., 1999; Terry-Lorenzo et al., 2002; Carmody et al., 2004).

GST-Spn fusion proteins containing the PP1c-binding domain potently inhibit PP1 enzymatic activity in vitro (Allen et al., 1997; Colbran et al., 2003).

However, it was recently shown that instead of inhibiting PP1c directly, Spn regulated enzymatic activity by directing its substrate specificity (Ragusa et al., 2010).

Spn can associate with the tyrosine phosphatase SHP-1 and the complex modulates platelet
activation by sequestering RGS10 and RGS18. The sequence surrounding the phosphorylation site Y398 in Spn fits a consensus ITIM sequence (I/V/L/SxY(p)xIx/I/L) and forms a binding site for SHP1 (Ma et al., 2012). p70S6K is a mitogen-activated PK that regulates cell survival and growth. p70S6K interaction with neurexin 1 (Burnett et al., 1998) and Spn was demonstrated (Allen and Greengard, unpublished observation). The interaction implicates the PDZ domain of neureamins and the carboxy-terminal five amino acids of the PK, CaMKII directly and indirectly associates with N- and C-terminal domains of Spn. Thus, Spn can target CaMKII to F-actin as well as target PP1 to CaMKII (Baucum et al., 2012).

Regulator of G-protein signalling (RGS) proteins play a crucial role in the shutting off process of G-protein-mediated responses (Ishii and Kurachi, 2003). Spn binds to different members of the RGS family (Wang et al., 2005; Wang et al., 2007). For example, Spn binds to the 391-545 amino acids of the scaffolding protein and the 6-9 amino acids of the N-terminus of RGS8 (Fujii et al., 2008).

Guanine nucleotide exchange factors (GEF) activate small G protein through the exchange of bound GDP for GTP. Several GEF were shown to interact with Spn. For example, Spn, through its carboxy-terminus containing the PDZ and coiled-coil domains interacts with kalirin-7, the neuronal GEF for Rac1 (Penzes et al., 2001).

Spn interacts with some receptors that belong to the superfamily of GPCRs, mainly in the CNS. Using the receptor binding domain of the family (Wang et al., 2005). In the cerebellum, Spn can bind to the M1-m-AChR using the receptor binding domain of the scaffolding protein (Fujii et al., 2008). Spn can also interact with the M2- and M3-m-AChRs but the binding ability to the M3-m-AChR seems to be weaker than those to the M1- and M2-m-AChR (Wang et al., 2007; Kurogi et al., 2009). Moreover, Spn binds to the 3i loop of CCKA and CCKB receptors (Wang et al., 2007). The receptor binding domain of Spn also associates with the 3i loop and a conserved region of the C-terminal tails of β- and µ-OR (Fourra et al., 2012). Spn also interacts with the ionotropic NMDA and AMPA-type glutamate receptors. PDZ domain directly binds to GluR2-, GluR3- (AMPA receptor) and NR1C2-, NR2A/B- and NR2C/D- (NMDA receptor) derived peptides (Kelker et al., 2007).

TRPC ion channels are Ca²⁺ /cation selective channels that are highly expressed in the central nervous system. Spn was identified with other dendritic spines proteins as a protein partner of TRPC5 and TRPC6 channels (Goel et al., 2005). In cardiomyocytes, Spn targets PP1 to RYR2 via binding to a leucine zipper (LZ) motif of RYR2 and a LZ motif on Spn (amino acids 300-634) causing dephosphorylation and modulation of the channel activity (Marx et al., 2001).

TGN38 is an integral membrane protein that constitutively cycles between the trans-Golgi network (TGN) and plasma membrane via endosomal intermediates. TGN38 directly interacts with the coiled-coil region of Spn, preferentially with the dimerized proteins (Stephens and Banting, 1999). Spn has been shown to interact with the nuclear protein ARF in mammalian cells. The amino acids sequence 605-726, of the coiled-coil region of Spn, seems to be involved and an intact ARF N-terminal region (amino acids 1-65) is necessary for this interaction (Vivo et al., 2001).

4- Spinophilin as a tumour suppressor

The Spn gene locus is located on chromosome 17 at position 17q21.33, a cytogenetic area frequently associated with microsatellite instability and loss of heterozygosity (LOH) observed in different human tumours. This region contains a relatively high density of known (such as BRCA1), putative as well as several yet-unidentified candidate tumour suppressor genes located distal to BRCA1 locus. Thus, several studies in breast and ovarian carcinomas have suggested the presence of an unknown tumour suppressor gene in the area that includes the Spn locus. However, despite these preliminary genetic correlations, no in-depth analysis of the role of Spn as a tumour suppressor has been made.

The Amancio Carnero laboratory from the Instituto de Biomedicina de Sevilla, in Spain, have addressed this possibility in vitro and in vivo, in three articles published in 2011. In the first study, immunohistochemical analysis of 35 human lung tumours at different stages and of different histopathological grades showed that Spn protein is absent in 20% and reduced in another 37% of tumours, compared to normal lung tissue (Molina-Pinelo et al., 2011). The loss of Spn expression correlated with a less differentiated phenotype, higher grade and poor prognosis. Lower or null levels of Spn also correlated with nuclear accumulation of p53, and so to mutated p53 or loss of its wild-type activity. Moreover, loss of Spn increased the tumorigenic properties of p53 deleted- or p53 mutated-lung tumour cells. The data of this study showed that Spn down-regulation in lung tumours contributes to carcinogenesis in the absence of p53. There are several mechanisms that might contribute to Spn down-regulation in
tumours, including miRNAs overexpression. miRNA106*, targeting Spn, are overexpressed in a small subset of patients with decreased Spn levels. Overexpression of miRNA106* significantly increased the tumorigenic properties of lung cancer cell lines. The results suggested that miRNA106* overexpression is found in subsets of lung tumours that contribute to tumorigenesis through Spn down-regulation in the absence of p53. In a second study, tumour suppression by Spn was explored in an in vivo model using genetically modified mice (Ferrer et al., 2011b). Spn-null (-/-) mice displayed increased survival, the number of premalignant lesions in tissues such as the mammary ducts and early appearance of spontaneous tumours, such as lymphoma, when compared to WT littermates. In another series of experiments, the presence of mutant p53 activity (p53R172H) in the mammary glands was evaluated on a Spn heterozygous (+/-) or homozygous (-/-) background in mice. An increased number of premalignant lesions and of mammary carcinomas were observed in Spn heterozygous (+/-) or homozygous (-/-) mice when compared to WT littermates. The results confirmed the functional relationship between Spn and p53 in tumorigenicity and showed that Spn loss contributes tumour progression rather than the tumour initiation. In a third study using mouse embryonic fibroblasts (MEFs), it was suggested that Spn acts as a tumour suppressor by the regulation of the stability of PP1alpha, thereby regulating its activity on pRb (the phosphorylated form of the Retinoblastoma protein). This function of PP1alpha has been associated with the growth arrest response; the hypophosphorylated form of Rb protein being the most abundant when cells are delayed in their growth (Ceulemans and Bollen, 2004). The ectopic overexpression of Spn in immortalized MEFs greatly reduced tumour cell growth. Moreover, the absence of Spn (Spn(-/-) MEF) down-regulated PP1alpha activity resulting in a high level of pRb (Ferrer et al., 2011a). High level of proliferative phosphorylated Rb leads to e2F activation, a compensatory ARF transcription, and consequently p53 activation. As they regulate the cell cycle, p53 and ARF are both tumour suppressors, which are themselves regulated by MDM2 (Mouse double minute 2) protein shuttle between the nucleus and cytoplasm (Kamijo et al., 1998; Pomerantz et al., 1998). Moreover, Sherr et al. (2005) suggested for the first time a p53-independent pathway via the ARF sumoylation. Ha et al. (2007) described ARF as a melanoma tumour suppressor by inducing p53-independent senescence. Moreover, Du et al. (2011) demonstrated the functional roles of ERK and p21 for ARF in p53-independent tumour suppression. Furthermore, in a p53-independent pathway, the over-expression of wild-type c-myc obviously up-regulates the expression of p14 (ARF) (Liu et al., 2012). Some members of the family of e2F transcription factors are also involved in cell cycle regulation; in particular E2F1 which expressions increase induces augmentation of ARF which can bind MDM2 and stabilize p53. In p53 (-/-) MEF, reduced levels of Spn enhanced tumorigenic potential of the cells. Indeed, inhibition of e2F by Rb being lifted, this results in cell proliferation no longer controlled by p53. Moreover, the absence of Spn contributes to genetic alterations during MEF immortalization, particularly p53 mutations. These results extend the observations made by the authors using a Spn-null mice model (Ferrer et al., 2011b). In summary, the results suggested that Spn is a new tumour suppressor acting via the regulation of pRb and which function is revealed in the absence of a functional p53 (Sarrouilhe and Ladeveze, 2012). This is, therefore, suggestive of partially redundant functions in their tumour suppression properties (Santamaría and Malumbres, 2011). The results also suggest that the specific outcome can be context-dependent. Spn loss may be beneficial by potentiating p53 in response to acute stress, and in contrast it can be deleterious under sustained mitogenic stress (Palmero, 2011). This feature is reminiscent of NIAM (Nucleolar Interaction of ARF and MDM2 protein) which acts through the same partners p53 and ARF (Tompkins et al., 2007).

Another Spn-interacting molecule is DCX, an actin-binding and microtubule-binding protein that seems to be a tumour suppressor of glioma. When DCX is ectopically expressed into the DCX-deficient U87 glioma cells, there is a marked suppression of the transformed phenotype. The cells manifest a reduced rate of growth in vitro and are arrested in the G2 phase of the cell cycle. Moreover, DCX-transfected U87 glioma cells do not generate tumours in immunocompromised nude rats. In DCX-transfected U87 cells, phosphorylated DCX binds specifically to Spn and this interaction inhibits proliferation and anchorage-independent growth in glioma cells. In contrast, DCX-mediated growth repression is lost in glioma cells treated with siRNA to Spn and in HEK 293 (human embryonic kidney) Spn null cell line (Santra et al., 2006). DCX, Spn and PP1alpha were found in the same protein complex from mouse brain extracts (Shmueli et al., 2006). DCX-mediated growth arrest in glioma cells may be through inactivation of PP1alpha activity by Spn/DCX interaction in the cytosol. Inhibition of PP1alpha activity is involved in two mechanistic links of reduction of glioma tumour-associated progressions: firstly, catastrophe in mitotic microtubule spindle that blocks mitosis; secondly, depolymerization of actin that inhibits glioma cell invasion (Santra et al., 2009).
Moreover, double transfection with DCX and Spn reduced self-renewal in brain tumour stem cells via incomplete cell cycle endomitosis (Santra et al., 2011). But, is there relevance for Spn as a prognostic marker in patients with cancer? Spn is absent in 20% and reduced in another 37% of human lung tumors (Molina-Pinelo et al., 2011).

A further analysis of Spn in human tumours shows that Spn mRNA is lost in a percentage of renal carcinomas and lung adenocarcinomas. A clear down-regulation of Spn was found in tumoral samples of the CNS (oligodendrogliomas, anaplastic astrocytomas, glioblastomas) when compared to normal nervous samples. Furthermore, lower levels of Spn mRNA correlate with higher grade of ovarian carcinoma and chronic myelogenous leukemia (Carnero, 2012). Two articles published in spring 2013 associated Spn loss with poor patient prognosis in patients with carcinoma (Sarrouilhe, 2014). The 17q chromosomal region is commonly impaired in hepatocellular carcinoma (Furge et al., 2005). In the first study, complete loss of Spn immunoreactivity was found in 42.3% hepatocellular carcinoma and reduced levels were found in additional 35.6% cases. Quantitative RT-PCR analysis confirmed in 70% cases a significant reduced Spn mRNA expression in tumour tissue compared with the corresponding non-neoplastic tissue. miRNA106a, targeting Spn in lung tumours, could not be detected in any of the hepatocellular carcinoma samples. Moreover, no correlations could be found for the number of Spn-positive tumour cells and p53 or ARF staining. These results suggested a p53-independent tumorigenic role of Spn in hepatocellular carcinoma. Disease recurrence was diagnosed after the 10-year follow-up in 85.2% cases with Spn low expression and 60.9% with Spn high expression. Death occurred in 76.5% cases with Spn low expression and in 56.5% cases with Spn high expression. Overall, low Spn expression is a factor for poor prognosis in hepatocellular carcinoma. In vitro experiments (human hepatoma cell line HepG2) and in vivo observations (Ki67-positive tumour cells) showed that reduced Spn expression significantly correlated with a higher proliferation of liver cancer cells (Aigelsreiter et al., 2013).

In the second study, the role of Spn was explored in colorectal carcinoma, in which a number of chromosomal regions are altered (Fearon, 2011). Among them, the 17q21 is lost in a high percentage of this carcinoma (Garcia-Patiño et al., 1998). Quantitative RT-PCR analysis showed that approximately 25% of colorectal carcinoma tumours had a greater than 50% decrease in Spn mRNA levels compared with normal colonic tissue. A tissue array of human colorectal carcinomas was generated to confirm this result by exploring the presence of Spn protein. 70% of colorectal carcinomas displayed high Spn levels (similar to the values observed in normal tissue), 20% showed intermediate levels and 10% showed no expression of Spn. Moreover, Spn down-regulation correlated with a more aggressive histologic phenotype (higher Ki67-positive tumour cells) and was associated with faster relapse and poorer survival in patients with advanced stages of colorectal carcinoma. The data also suggested that Spn loss induced a chemoresistance in patients with advanced stages of colorectal carcinoma that had received adjuvant fluoropyrimidine chemotherapy.
following surgical resection. Therefore, the identification of the levels of Spn in advanced stages of colorectal biopsies has prognostic and predictive value and might contribute to select patients who could or could not benefit from current chemotherapy. In vitro and in vivo experiments showed no functional relationship between Spn levels and the presence or absence of mutated p53 in colon cancer. The authors proposed that this correlation is dependent on the molecular context of the tumour cell (Estevez-Garcia et al., 2013).

5. Discussion and perspectives
We are still only at the early stage in unravelling the function of Spn in cell cycle regulation. Overall, the different studies on the tumour suppressor function of Spn show two pathways of cell cycle regulation by Spn. The first model is a pathway dependent of p53 and ARF. This pathway was previously described in several articles where Spn interacts with different partners localized in the nucleus (Figure 3A). The second is a pathway independent of both molecules. As Spn is ubiquitously expressed in the cell, the first model highlights the nuclear localization of Spn and its interaction with other nuclear proteins. The second model, more hypothetical, underlines the possibility that Spn could interact with cytoplasmic partners. The studies made on colorectal carcinomas show that Spn could play a role in a pathway independent of p53/ARF. One hypothesis is that the Ras/Raf pathway and more precisely K-Ras/B-Raf is implicated. This pathway, via Mek (tyrosine/threonine kinase) and Mapk (mitogen activated protein kinase) induces transcription factors and proliferation survival (Figure 3B).

Further studies are needed to elucidate the underlying mechanisms linking Spn to carcinomas and expand the prognostic and predictive value of the Spn expression level to other types of cancer.

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