Secreted Frizzled Related Proteins (Sfrps) are a family of secreted proteins that can bind both to Wnt ligands and Frizzled receptors, thereby modulating the Wnt signalling cascades. Recent studies have shown that Sfrps can also interact with Wnt unrelated molecules such as RANKL, a member of the tumor necrosis factor family, Tolloid metalloproteinases or integrin-fibronectin complexes. Alterations in the levels of Sfrp expression have been recently associated with different pathological conditions, including tumor formation and bone and myocardial disorders. Here, we summarise the evidence that relates Sfrps with these diseases and discuss how the proposed multiple Sfrp interactions with Wnt related and unrelated pathways may explain their implication in such diverse pathologies.

**Keywords**: Secreted Frizzled Related Protein; Wnt signalling; cancer; bone repair; myocardium

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Correspondence: Pilar Esteve and Paola Bovolenta, Departamento de Neurobiología Molecular, Celular y del Desarrollo, Instituto Cajal (CSIC) and CIBER de Enfermedades Raras (CIBERER), Avda. Dr. Arce 37, Madrid 28002, Spain.
e-mail: PilarEsteve@cajal.csic.es or bovolenta@cajal.csic.es

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that form three disulfide bridges. This motif characterises a number of unrelated proteins including Netrin-1, tissue inhibitors of metalloproteinases (TIMPs), complement proteins and TypeI procollagen C-proteinase enhancer proteins (PCOLCEs) (Banyai and Patthy 1999).

Although it was initially shown that the CRD domain of Sfrps is necessary and sufficient for Wnt binding (Lin et al. 1997; Bafico et al. 1999), subsequent studies demonstrated that the CRD domain also interact with Fz and Tolloid-like metalloproteases (Lee et al. 2006; Lopez-Rios et al. 2008; Kobayashi et al. 2009) while the NTR domain can tightly bind to Wnt ligands independently of the presence of the CRD domain (Uren et al. 2000; Lopez-Rios et al. 2008), supporting the context dependent functions observed for Sfrps.

Below we discuss how this variety of possible Sfrps interactions may explain their implication in unrelated pathological processes such as cancer, bone or myocardial diseases. We will focus on Sfrp1 and Sfrp2, since these are the family members, which received most attention.

**Sfrp1 and Sfrp2 generally protect from carcinogenesis**

The suppression or down-regulation of Sfrp1 and Sfrp2 expression seems to be a key event in the onset of tumour formation. In addition to somatic mutations, epigenetic DNA alterations play crucial roles in tumorigenesis (Baylin and Herman 2000; Feinberg and Tycko 2004). Methylation of gene promoter regions enriched in CpG dinucleotides (CpG islands) is among the epigenetic modifications that controls the levels of gene transcription. Hypermethylation of CpG islands causes gene silencing and is considered one of the most frequent epigenetic alterations associated with cancer in humans (Esteller 2005a, 2005b). Numerous genes became hypermethylated and silenced in different type of cancers but Sfrp1 and Sfrp2 hypermethylation seems to occur from the very beginning in basically all tumour types, including breast (Zhou et al. 1998; Turashvili et al. 2006; Suzuki et al. 2008), gastric (To et al. 2001), cervix (Ko et al. 2002), hepatocellular (Huang et al. 2007), pancreas (Bu et al. 2008), lung (Fukui et al. 2005), ovary (Yakada et al. 2004), renal (Dahl et al. 2007; Gunz et al. 2007), prostate (Lodygin et al. 2005) and colon (Suzuki et al. 2002; Caldwell et al. 2004; Suzuki et al. 2004) carcinomas, as well as in non-solid tumours like myeloid leukaemias (Jost et al., 2008) or myelomas (Jost et al. 2009). The correlation between cancer and loss of Sfrp1 expression is so strong that, at least for bladder carcinomas (Stoehr et al. 2004; Urakami et al. 2006), it has been proposed that the status of Sfrp1 promoter methylation could serve as an epigenetic biomarker for cancer detection and progression, where hypermethylation will be associated with unfavourable prognosis (Lee et al. 2004; Veeck et al. 2006).

In line with these epigenetic studies, a large-scale analysis in tumours derived from a total of 36 organs demonstrated a complete loss of Sfrp1 expression in 82% of the analysed samples (Dahl et al. 2007). Deletion of the chromosomal region 8p12-p11.1 comprising the SFRP1 locus has been also reported in a few cancer cases (Ugolini et al. 1999; Stoehr et al. 2004; Dahl et al. 2007). A tempting speculation from these studies is that Sfrp1, and possibly the related Sfrp2, act as tumour suppressor genes (Lee et al. 2004).
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2004; Veeck et al. 2006) and their silencing may predispose to neoplastic progression (Wong et al. 2002). Supporting this possibility, forced Sfrp1 re-expression in breast (Suzuki et al. 2008; Matsuda et al. 2009), hepatocellular (Shih et al. 2007; Jiang et al. 2009), prostate (Lodygin et al. 2005) and cervical tumours (Chung et al. 2009), where Sfrp1 had been silenced, induces apoptosis, suppresses cell proliferation, transformation and invasion both in vitro and in vivo. Despite these observations, the incidence of spontaneous tumour formation is not increased in Sfrp1 null mice (Trevant et al. 2008), suggesting that additional genetic alterations are necessary for the tumour onset and/or that Sfrp1 suppression is relevant for tumour progression more than for its genesis.

Given the variety of Sfrps' functions described at the beginning, the obvious question is what are the molecular mechanisms by which Sfrp1 inhibits tumorigenesis. Ablation of Wnt signalling, due either to genetic mutations or to the altered expression of its pathway components, is a common event in the onset and progression of cancer (Nusse 2005; Polakis 2007). As mentioned above, Sfrps are thought to act as Wnt signalling inhibitors. From the initial description of a direct link between the epigenetic inactivation of Sfrp1 (Suzuki et al. 2002) and the upregulation of Wnt/β-catenin cascade in colon cancer (Suzuki et al. 2004), it has been generally accepted that Wnt/β-catenin repression is the mechanism by which Sfrp1 inhibits tumour cell growth and prevents metastatic invasion. This mechanism was proven in some cases when re-expression of Sfrp1 inhibited Wnt/β-catenin activation (Suzuki et al. 2004; Nojima et al. 2007; Shih et al. 2007). However, there are many examples of Wnt/β-catenin-independent carcinogenesis. In these tumour cells, Sfrp1 is similarly silenced and its restoration is sufficient to counteract their phenotype, indicating that the loss of Sfrp1 expression contributes to activate other oncogenic signalling pathways (Fukui et al. 2005; Nojima et al. 2007; Chung et al. 2009) as proposed for prostate cancer (Lodygin et al. 2005; Kawano et al. 2009). Among the alternative pathways it is worth considering those linked to metalloproteases, which are heavily involved in metastatic events. Indeed, members of the Sfrp family seem to control the activity of Tolloid-like metalloproteases (Lee et al. 2006; Muraoka et al. 2006; Kobayashi et al. 2009).

In contrast to the anti-tumorigenic and anti-metastatic effects described for Sfrp1, there are a number of reports suggesting that Sfrp1 can also act as an oncogene that confers metastatic properties to renal carcinoma (Saini et al. 2009) and promotes prostate carcinogenesis (Joesting et al. 2005). This dual function-as an oncogene or a tumour suppressor gene- occasionally observed even in the same type of cancer (Joesting et al. 2005; Kawano et al. 2009), suggests that the cellular context is an important determinant of Sfrp1 activity. This, again, should not be a surprise given the various forms in which Sfrp1 can influence Wnt or other signalling pathways. Therefore, Sfrp1 concentration together with the local availability of Fz, Wnts and downstream signalling components- including those of the non canonical pathway (Katoh 2005)- should be major determinants in the onset and progression of carcinogenesis (Uren et al. 2000; Bovolenta et al. 2008; Mii and Taira 2009). It still needs to be determined whether other molecular pathways important for carcinogenesis are directly affected by the changing Sfrp expression levels.

**Sfrp1 prevents bone repair**

Sfrp1 activity affects many aspects of bone and cartilage formation and consequently alterations in its expression or function cause bone and cartilage disorders. Sfrp1 is expressed in cartilage (Hoang et al. 1996) and in osteoblasts and osteoclasts. In vitro assays demonstrated that Sfrp1 inhibits osteoclast formation (Hausler et al. 2004) while genetic inactivation of Sfrp1 accelerates chondrocyte differentiation and endochondral ossification (Gaur et al. 2006) as well as trabecular bone formation in aged animals (Bodine et al. 2004, 2005). This last defect is the result of an increased osteoblast proliferation and differentiation associated with a slower lost of bone mass caused by a decrease of the physiological apoptosis of osteoblasts and osteocytos (Bodine et al. 2004, 2005).

Consistent with these results, Sfrp1 has been found to be over-expressed in bones after prolonged glucocorticoids treatment, which is known to induce osteoporosis likely through Sfrp1 activity (Wang et al. 2005). Similarly, Sfrp1 expression level may be an etiological factor of post-menopausal (Sims et al. 2008) and senile osteoporosis (Ohnaka et al. 2009) because different polymorphisms in the 3' untranslated region of Sfrp1 have been associated with variations in bone mineral density in women. On the contrary, genetic inactivation of Sfrp1 in mice improves bone fracture repair promoting osteogenesis and matrix mineralization (Gaur et al. 2009), two critical steps of bone repair.

In conclusion, Sfrp1 is involved in metabolic bone disorders, osteoporosis and aging. What the molecular consequences of Sfrp1 dysfunction in bone diseases are is still unclear but they may involve both the antagonism of Wnt/β-catenin pathway (Bodine et al. 2004) and the binding and inhibition of RANKL, which together with M-CSF stimulates osteoclast formation (Hausler et al. 2004). An additional mechanism may involve the control of Bone Morphogenetic Protein (BMP) signaling, which is a potent inducer of bone morphogenesis. Indeed, a non mammalian Sfrp family member, Sizzled, binds to BMP1/Tolloid, a metalloprotease that normally degrades the BMP antagonist, chordin thereby indirectly inhibiting BMP signaling (Lee et al. 2006; Muraoka et al. 2006). A similar role in inhibition of Bmp signaling has been reported for Sfrp1 and Sfrp2 during caudal neural tube closure in mice (Misra and Matise 2010).

Whether Sfrp1 independently affects all these pathways or there is cross talk among them still needs to be
determined. Notably, it has been recently demonstrated that Wnt signaling, through GSK3 activity, enhances BMP signaling (Fuentet al. 2007).

Independently of the mechanism of actions it is obvious that inhibitors of Sfrp1 could be useful pharmacological agents in fracture repair as well as to alleviate osteoporosis. Two classes of molecules have been so far tested. The first is a diphenylsulfone sulphonamide, which binds specifically to Sfrp1, suppresses osteocyte apoptosis and stimulates ex vivo murine bone formation in an organ culture assay (Bodine et al. 2009; Moore et al. 2009). The second one is a minooxothiazolidine, which also binds to Sfrp1. Its addition in an ex vivo mouse calvaria assay increases total bone area and the number of osteoblasts (Shi et al. 2009), clearly suggesting that Sfrp1 is a promising therapeutic target in bone degenerative diseases.

**Dual function of Sfrp2 in heart recovery**

The heart has a limited regenerative capacity and the development of novel regenerative therapies is one of the goals of current cardiovascular research. Stem cell based approaches are quite promising especially in cases of myocardial infarction (Rameshwar et al. 2010). In fact, transplantation of bone marrow-derived stem cells following heart ischemia promotes local recovery and improves the general function of the heart (Abdel-Latif et al. 2007). This recovery seems in large part due to paracrine factors secreted by transplanted stem cells (Gnecchi et al. 2005, 2008). Recent studies have demonstrated that Sfrp2 is one of the most relevant of these factors. Intra-cardiac transplantation of mesenchymal stem cells genetically engineered to over-express the serine/threonine kinase AKT led to a dramatic reduction of the infarction size and to the recovery of cardiac function in injured rodent hearts (Mangi et al. 2003). Microarray analysis revealed that Sfrp2 is among the upregulated proteins in Akt-mesechymal cells. Sfrp2 seems to be responsible for most of the beneficial effects of the transplanted stem cells during early stages of infarction. It promotes myocardial survival and repair, reduces the infarcted size (Mirotsou et al. 2007; Alfaro et al. 2008) and favor neovascularisation (Blankesteijn et al. 2001). The beneficial effects of cardiac repair of Sfrp2 are shared by Sfrp1. In transgenic mice overexpressing Sfrp1, infarct size was reduced and cardiac function preserved (Barandon et al. 2003). Although still unclear, these effects are probably related to the control of Wnt/ß-catenin signalling (Zhang et al. 2009).

After myocardial infarction, dead myocytes are removed and replaced by a fibrotic scar formed by collagen deposits. This cardiac fibrosis reduces elasticity and proportionally decreases the contractile function of the heart. Somewhat in contrast with the idea that Sfrp2 has a beneficial effect on heart recovery, a recent study demonstrated that collagen deposition and fibrosis were reduced in healed cardiac tissue of Sfrp2 null mice subjected to coronary artery ligation. As a consequence, their systolic function was improved as compared to that of wild type animals. This result is explained by the observations that normally the appearance of collagen fibres in the injured area of the heart coincides with the onset of Sfrp2 expression, suggesting that Sfrp2 favours collagen deposition and fibrosis (Kobayashi et al. 2009). Most notably, Sfrp2 simultaneously binds the metalloprotease Tolloid/BMP1 and the procollagen, enhancing BMP1 proteinase activity, thereby accelerating the processing of the procollagen precursor in mature collagen (Kobayashi et al. 2009)

Thus, Sfrp2 seem to have a dual effect on myocardial repair likely mediated by two unrelated mechanisms of action. In the initial stages of recovery from infarction, Sfrp2 reduces the size of the damaged tissue, likely through the inhibition of Wnt/canonical signalling. Soon after however, it adopts the function of a Type1 procollagen C-proteinase enhancer protein, leading to fibrosis. Thus, although Sfrp2 has been proposed as a useful agent for heart repair (Barandon et al. 2003; Mirotsou et al. 2007), we need a better understanding of its mechanisms of action to design therapeutic strategies that can separate its beneficial from its harmful effects on damaged myocardial tissue.

**Conclusions**

Here we have focused on the roles of Sfrp1 and 2 in tumour formation, bone and myocardial disorders. Nevertheless, there are several other pathological conditions in which these molecules have been implicated. For example, Sfrp1 has been suggested to be among the factors responsible for elevated intraocular pressure in glaucoma (Wang et al. 2008). Similarly, inhibition of the elevated Sfrp1 levels alleviates the inflammation and destruction of periodontal tissue that occurs in periodontitis (Li and Amar 2007). Furthermore, there are also reports linking other Sfrp family members to diseases. This is the case, for example, of Sfrp4 and osteomalacia, a condition in which the bones becomes soft and flexible often due to the lack of vitamin D. Unexpectedly, Sfrp4 plays an important role in homeostasis of phosphorus and inorganic phosphate (P, inhibiting vitamin D synthesis and thus intestinal P, absorption (Berndt and Kumar 2007). How this occurs is unclear, but it certainly offers an additional example of the pleiotropic activities of Sfrps. Furthermore, Sfrp3 seems upregulated in metastatic renal cell cancer, which suggests that inhibition of Sfrp signaling in this context would not be beneficial to patients (Hirata et al. 2010).

It is thus evident that basic studies of the interactions between the different Sfrp molecules and Wnt-related and unrelated proteins will be of key importance to understand the role of this family of soluble factors in a wide variety of diseases. These studies will also help to define whether, how and in which conditions Sfrps can be useful therapeutic targets or therapeutic agents themselves.
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