sFRPs: a declaration of (Wnt) independence

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Secreted Frizzled-related proteins (sFRPs) are signalling molecules well-known as antagonists of the Wnt pathway, but recent studies indicate that they may have additional functions unrelated to Wnt. A new study demonstrates that mammalian sFRP2 can act as an enhancer of collagen processing *in vitro* and *in vivo*, augmenting myocardial injury-driven fibrosis. These findings underscore the biological versatility of sFRP family members.

In the past decades, the Wnt signalling pathway has emerged as a key player in embryogenesis and postnatal development, affecting both normal and pathological processes[1]. The Wnt proteins constitute a large family of secreted glycoproteins that activate a number of cell-intrinsic signalling cascades by binding to the Frizzled seven-transmembrane receptors and to LRP5 or LRP6, which function as co-receptors[2]. Activation of the Wnt signal is antagonized by the secreted Frizzled-related proteins (sFRPs), the largest family of Wnt inhibitors, whose members share sequence similarity with the cysteine-rich domain (CRD) found in the extracellular region of Frizzled[3]. sFRPs bind the Wnt ligands through their CRD, thereby preventing their binding to Frizzled receptors[4] (Fig. 1a). In humans, this family consists of five members, sFRP1 to sFRP5, and orthologues of these genes have been found in all vertebrate species.

Recent studies, however, provide evidence that sFRPs also bind proteins distinct from Wnt, and exert other biological functions. For example, Sizzled belongs to the sFRP family, on the basis of its sequence homology with Frizzled receptors[5], but studies in fish and frog embryos demonstrated that it does not block Wnt signalling *in vivo*, but rather acts as a negative-feedback regulator of the bone morphogenetic protein (BMP) signalling pathway[6,7]. In vertebrates, a gradient of BMP activity is set by the BMP antagonist Chordin which, in turn, is inactivated through cleavage by evolutionarily conserved Tolloid (TLD) metalloproteinases. Sizzled was shown to inhibit Chordin cleavage by binding to TLD-like proteinases, leading to Chordin stabilization and a reduction of BMP signalling activity[6,7] (Fig. 1b). In a surprising twist, Kobayashi *et al.* demonstrate that mammalian sFRP2 functions as an enhancer of collagen processing and cardiac fibrosis by regulating the procollagen-C proteinase activity of Tolloid-like metalloproteinases[8].

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In mammals, there are four TLD-like proteinases: BMP1, mTLD, mTLL1 and mTLL2. Of these, only BMP1 and mTLL1 can cleave Chordin[9]. As Sizzled itself is absent in mammals, the question arises as to whether mammalian sFRPs can influence the activity of TLD family members. To start addressing this issue, Kobayashi *et al.* explored the ability of mammalian sFRP2 to inhibit cleavage of Chordin by TLD proteinases. In contrast to a previous report[6], they found no obvious sFRP2-dependent differences in the extent to which Chordin was cleaved by BMP1 or mTLL1. Instead, they showed that sFRP2 directly enhances the procollagen C-proteinase activity of TLD-like metalloproteinases (Fig. 1c). Cleavage of the C-propeptide from procollagen is a prerequisite for the release of mature triple-helical collagen molecules into the extracellular matrix (ECM). These new findings show that although the non-mammalian sFRP, Sizzled, inhibits both the Chordinase and procollagen C-proteinase activities of TLD-like proteinases, mammalian sFRP2 enhances the latter activity, but is ineffective in modulating Chordin cleavage.

Using a number of *in vitro* biochemical assays, the authors then demonstrated that the TLD-like proteinase BMP1 and sFRP2 physically interact through the CRD of sFRP2. sFRP2 binds to the non-protease domain of BMP1, presumably inducing conformational changes that facilitate collagen cleavage (Fig. 1c). Furthermore, sFRP2 binds to procollagen, thus facilitating the enzymatic reaction by bringing together the proteinase and its substrate (Fig. 1c). A similar mode of interaction was found for Sizzled, which binds to the non-protease domain of the TLD proteinases, resulting in inhibition of both Chordin and collagen cleavage. Whether other sFRPs can affect TLD proteinase-mediated activities remains to be seen.

Notably, the mechanism by which sFRP2 enhances procollagen processing, through its binding to both procollagen substrates and the non-protease domains of the TLD proteinases (Fig. 1c), is similar to that seen in type 1 procollagen C-proteinase enhancer proteins (PCOLCEs)[10]. Both sFRPs and PCOLCEs contain an NTR module identified in several other proteins, including netrins, which is presumed to be involved in their interactions with matrix metalloproteinases.

Kobayashi *et al.* then compared procollagen processing in cultures of wild-type and *Sfrp2*-null fibroblasts. Consistent with their biochemical findings, the authors revealed marked reductions both in procollagen processing and in the deposition of collagen at the ECM in *Sfrp2*-null fibroblasts, further indicating that sFRP2 enhances the ability of TLD proteinases to process procollagen.

It is well-known that excessive collagen fibre formation results in fibrosis, abnormal tissue growth that may occur during the repair of damaged tissue, and has been linked to human diseases. An example of the damage caused by fibrosis is the effect of myocardial infarction — commonly known as a heart attack — which occurs when the blood supply to part of the heart is interrupted (Fig. 1c). The fibrosis that ensues in the heart leads to decreased elasticity and impaired cardiac contractile function (Fig. 1c). Kobayashi *et al.* next explored the clinical relevance of their findings and showed a significant upregulation of sFRP2 and BMP1 expression in the infarcted heart after coronary artery ligation in mice. Importantly, they observed markedly reduced fibrosis in the infarcted hearts of *Sfrp2*-null mice, indicating that sFRP2 enhances procollagen processing by BMP1 *in vivo*. The authors further investigated the functional consequences of decreased fibrosis in the hearts of *Sfrp2*-null mice and showed that 14 days after ligation, cardiac function (assessed by monitoring the ejection fraction) was improved in *Sfrp2*-null mice, compared with controls. The authors propose that sFRP2 inhibitors could constitute an effective therapeutic approach to control fibrosis and improve cardiac function in cases of myocardial infarction, which accounts for about 13% of deaths worldwide, and is the leading cause of death in developed countries.

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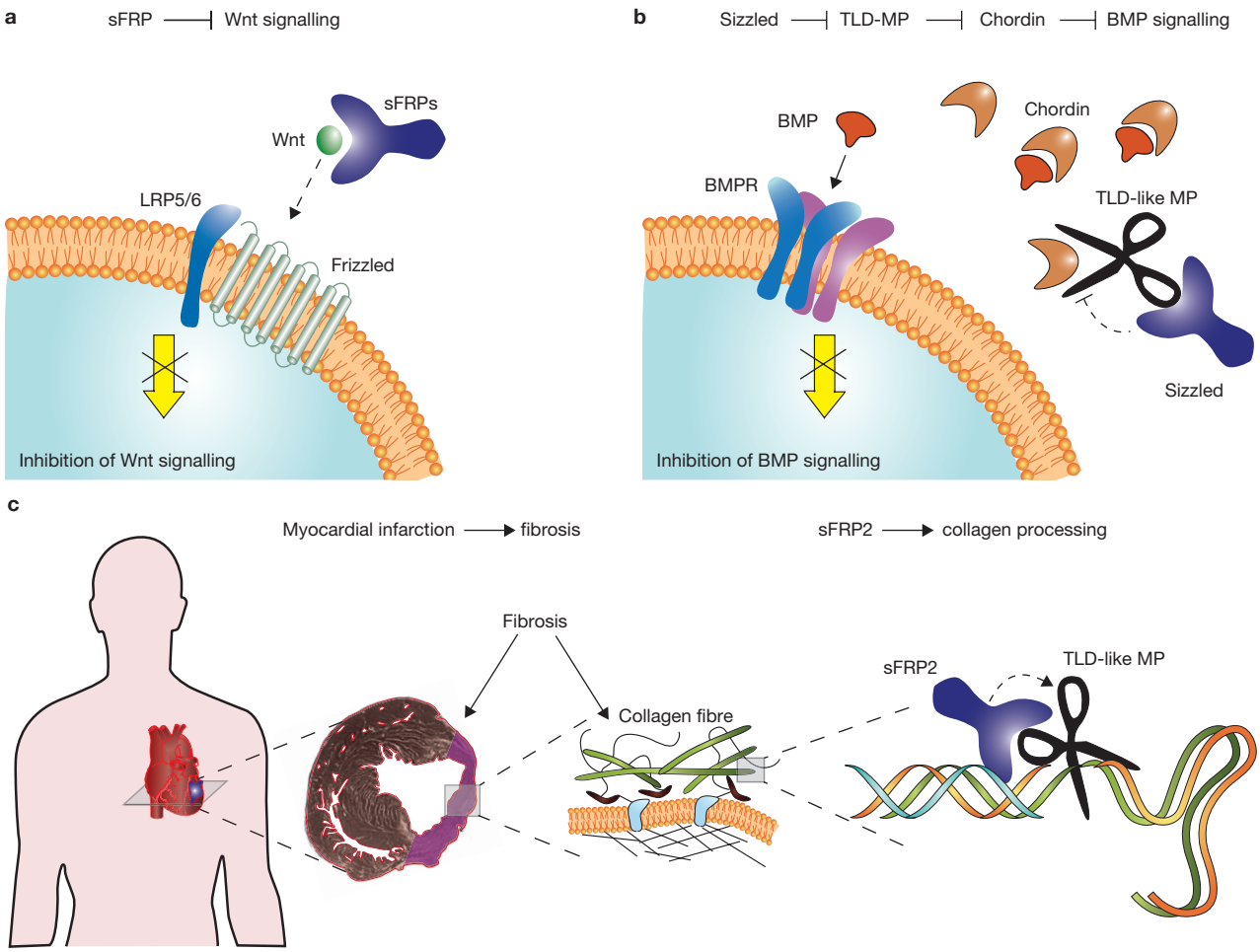

Figure 1 The biological versatility of sFRPs. (**a**) sFRPs inhibit Wnt signalling by binding to the Wnt ligand, thereby preventing its interaction with the Frizzled receptor and its co-receptors LRP5/6. (**b**) Sizzled blocks BMP signalling by binding to the non-protease domain of the TLD-like metalloproteinase (MP, illustrated as scissors), which subsequently degrades Chordin. By binding to Sizzled, Chordin is stabilized and able to bind to the BMP ligand, which leads to inhibition of BMP signalling. (**c**) Myocardial infarction is characterized by increased fibrosis (shown in purple within the ventricular wall), which markedly reduces cardiac function. The dense, collagenous tissue that characterizes fibrosis results from the release of mature triple-helical collagen molecules into the extracellular matrix. Kobayashi *et al.* now show that sFRP2 enhances procollagen processing (cleavage) by the TLD proteinases, *in vitro* and *in vivo*. sFRP2 binds both the procollagen and the TLD proteinases, thus facilitating the enzymatic reaction by bringing together the proteinase and its substrate.

Although several studies have pinpointed the exact biochemical and/or functional specificity of sFRP–Wnt interactions, it is clear that sFRPs can bind a diverse array of signalling molecules besides the Wnts in a rather promiscuous fashion[11]. First, sFRPs and Frizzled proteins, through their CRDs, are able to form homo- and heteromeric complexes. For example, sFRP1 functions as an axon guidance cue by interacting with Frizzled-2 in a Wnt-independent manner[12]. In addition, sFRPs can bind to fibronectin–integrin complexes, or to RANKL, the activating receptor of NFκB[11]. Whether the effects mediated by sFRP2 to regulate procollagen processing[8] or the modulation of fibronectin–integrin complexes by other sFRPs[13] are totally Wnt-independent is still unclear, but it is an attractive hypothesis, given that these proteins co-exist in the extracellular matrix.

One question that remains, however, is why there is a need for five distinct sFRPs in humans. Many biological signalling networks, such as Wnt, evolved from simpler signalling cascades through gene duplications. Conceivably, the modularity of sophisticated signalling networks results in their members adopting both redundant and distinct functions. Along these lines, sFRPs are likely to share some biological functions such as Wnt signalling inhibition (Fig. 1a), whereas they adopt additional specific ones, such as axon guidance regulation (sFRP1), Bmp signalling modulation (Sizzled, Fig. 1b) or fibrosis enhancement (sFRP2, Fig. 1c). Thus, by virtue of extensive protein–protein interactions, sFRP family members facilitate distinct signalling outcomes both in Wnt-dependent and Wnt-independent manners. Other biological functions of sFRPs and their possible clinical implications remain to be revealed.

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