Association of Secreted Frizzled-Related Protein 4 with Obesity and Type II Diabetes

Mukesh Kumar Shrewastwa¹, Viyatprajna Acharya², Arun Kumar Mahat³

¹Department of Biochemistry, Nepalgunj Medical College, Kohalpur, Nepal
²Department of Biochemistry, KIMS & PBMH, Bhubaneswar
³Department of Dental Surgery, Nepalgunj Medical College, Kohalpur, Nepal

ABSTRACT

Secreted frizzled-related protein 4 (SFRP4) is a secreted protein family member similar to the sequence of frizzled receptors of wingless-related integration site (Wnt) signaling pathways which regulate various functions from fetal growth to adulthood. SFRPs are recognized as antagonists of Wnt signaling and are thought to be affiliated with Wnts. Further research revealed their interaction with frizzled receptors and functional differences were transferred to these proteins, the power of Wnt signaling without flexibility. Also, SFRP4 is linked to many diseases including obesity, type 2 diabetes (T2D), and cancer. In addition, SFRP4 acts as a biomarker for the diagnosis of T2D and its expression is observed before the clinical diagnosis of T2D.

This review is mainly focused on the role of SFRP4 in obesity and its role in β-cell failure that leads to T2D. SFRP4 acts on adipose tissue that causes increased production of adipokines which creates oxidative stress in the pancreas with low levels of antioxidant enzymes in pancreatic β-cells resulting in failure of insulin exocytosis. Inflammation caused by obesity is an important factor in the pathogenesis of insulin resistance and metabolic syndrome. Pro-inflammatory cytokines may induce insulin resistance in adipose tissue, bone tissue and liver by blocking the transmission of insulin signals. SFRP4 secretion is caused by interleukin 1-β (IL1-β). This review also highlights the molecular mechanisms by which SFRP4 leads to T2D. Understanding the cellular pathway and identifying SFRP4 may help to eliminate or reduce the chances of developing T2D.

INTRODUCTION

Diabetes mellitus (DM) is one of the major causes of morbidity and mortality throughout the world and the prevalence is uninterruptedly increasing day by day. According to the International Diabetes Federation (IDF) incidence of diabetes, especially type 2 (T2DM), will be rising from 366 million in 2011 to 552 million by 2030, which predicts one out of ten adults being affected by the disease.¹ Europe spends at least 131 billion dollars per year on healthcare due to diabetes,² which says that not only an important health problem, it is also a socio-economic problem and a major health problem for the public of South East Asians, especially India, Nepal etc. Therefore, the need for prevention as well as the discovery of new risk prediction factors for diabetes has become a major challenge for contemporary
laboratory medicine. Compared to the year 2000, in the year 2030, the prevalence of DM will become double in urban dwellers of developing countries. Complications associated with DM range from nagging problems of polyphagia, polydipsia and polyuria to increased rates of hospitalization, cataract and blindness, renal failure and non-traumatic amputations. The kidneys, autonomic nervous system, eyes, heart and blood vessels may be damaged due to chronic hyperglycemia. Type 1 and type 2 are two basic etiopathogenic categories of diabetes. Absolute deficiency of insulin secretion in the body is seen in type 1 diabetes whereas the body is mainly resistant to insulin action or an inadequate compensatory insulin secretory response occurs in type 2 diabetes. It happens due to the aberrant expression of several genes and their products. T2DM is a heterogeneous disorder characterized by insulin resistance and pancreatic β-cell dysfunction. Patients with impaired glucose tolerance (IGT) and pancreatic β-cell dysfunction. Patients with impaired glucose tolerance (IGT) and with newly diagnosed T2DM are found to have defective β-cell function. T2DM naturally occurs due to progressive β cell dysfunction, but the detailed molecular mechanism has not been well understood.

Pathogenesis of T2DM goes parallel to adipose tissue dysfunction. Excessive body fat behaves as an endocrine organ where many adverse biochemical mechanisms occur. Adipocytes produce cytokines such as interleukin 6 (IL-6), tumor necrosis factor a (TNF-a), resistin, retinol-binding protein 4 (RBP-4), dipeptidyl peptidase 4 (DPP-4) and adipocyte fatty acid-binding protein (A-FABP) that activate inflammatory pathways which result in reduce the activity of the insulin-dependent receptors and affect gene expression and insulin production in human pancreatic islets which leads to insulin resistance and impaired glucose metabolism. Apparently healthy, no diabetic and overweight/obese subjects are at potential risk, by the fact that elevated levels of various chemokines may predict the occurrence of disease even several years before its diagnosis.

The β-cell function is controlled by IL-1β, a prototype pro-inflammatory cytokine, which also triggers and amplifies inflammatory responses. Expression of Secreted frizzled-related protein 4 (SFRP4) causes increased expression of inflammatory markers in pancreatic islets of donors. SFRP4 was recognized by Mahai et al. as one of the groups of T2DM-related genes improved for IL-1-associated genes, and human islets incubated with IL-1β revealed a 1.8-fold elevation of SFRP4 mRNA and a 3-fold increased secretion of protein into the medium.

Another study observed that increased expression of inflammatory markers in the pancreatic islets of donors correlates with overexpression of SFRP4. These results proved SFRP4 as an inflammatory mediator and were induced by IL-1β. Liu et al. showed that serum IL-1β levels were statistically significantly higher in T2DM and IGT subjects as a comparison to normal glucose tolerance (P<0.01) but not statistically significant between T2DM and IGT (P>0.05). Hence, the involvement of SFRP4 in the inhibition of insulin release due to IL-1β production was statistically observed (P <0.001) by Liu et al. It may be associated to chronic, low-grade inflammation in islets and defective insulin secretion.

Secreted frizzled-related protein 4 (SFRP4)

SFRP4 is a family of secreted proteins displaying sequence similarity to the extracellular domain of frizzled receptors. It is also recognized by its synonyms FRP-4, frpHE (FRP Human Endometrium), FRPHE; FRZB-2. Extracellular signaling molecules have remarkable roles in affecting cell migration, proliferation, differentiation, and morphogenesis of tissues during normal development. They are also involved in several neoplasms derived from aberrant growth regulatory pathways. These are the large family of palmitoylated secreted Wingless integrated (Wnt) glycoproteins, (mammalian homologues of the Drosophila wingless gene), with Frizzled (FZ) class proteins functioning as their receptors or as a Wnt receptor complex apparatus. Several members of Wnt and Frizzled families have a complicated set of interconnections, and further mechanisms occur to fine-tune them during particular periods of development or in certain tissues. SFRPs are expressed in a variety of embryonic and old tissues that encompass Wnt-binding domains and are soluble regulators of Wnt signaling pathways.

The secreted frizzled-related proteins (SERPs)

Wnt antagonists can be divided into two classes, the sFRP and the Dickkopf class on a work-based basis. The family of SFRP, Cerberus and WIF-1 (Wnt inhibitory factor-1) are included in the SFRP class. The SFRP family has five members in humans, sFRP1 to sFRP5. In line with sequential evolution of Wnt signaling pathways, the SFRP genetic family consists of two closely related subgroups. SFRP1, SFRP2, and SFRP5 form one small subgroup (subfamily
Structure and subcellular location of SFRP4 & its relationship to frizzled receptors

In line with the pure amino-terminal sequence of FRP, Finch et al. used the degenerate oligonucleotides to separate the corresponding cDNA clones which then encoded a polypeptide consisting of 313 amino acids. It had a cysteine-rich domain (CRD) of about 110-120 amino acids (called a frizzled motif containing 10 cysteine residues), and was about 30-40% similar to a binding domain of Wnt ligand of Frizzled, the serpentine receptors regulating Wnt signalling. SFRPs are modular proteins that fold into two independent domains. The amino terminus has a signal-release peptide followed by CRD. The Lack of any clear transmembrane segments and the presence of a putative peptide signal propose that they are secreted. The structure of SFRP4 is shown in Fig. 1. An open reading framework of the FrzB-2 (SFRP4) gene, as well as conserved cysteine residues and the putative signal sequence was described in a study conducted by James I et al. in articular cartilage, which encodes the polypeptide of 346 amino acids in 2000. They also show the peptide sequence used to elevate the anti-FrzB-2 polyclonal antibody. The C-terminal has a netrin-like domain (NTR) and contains various potential threonine/serine phosphorylation sites (9 in humans) and a region with high serine and threonine content (10 of the last 26 residues). Therefore, this molecule is a potential target for protein phosphorylation via serine/threonine kinases. The NTR domain has six cysteine residues that make three disulphide bridges. Incorporating 10.99 kb in the short arm of 7 chromosomes and recorded from antisense strand in centromere to telomere orientation, the SFRP4 gene is encoded by six exons – 7p14-p13 (Fig. 2). SFRP4 is expressed in a variety of tissues usually including the endometrial stroma (high expression in the growth phase of the menstrual cycle), pancreas, stomach, colon, lung, bone tissue, testicles, ovary, kidneys, heart, brain, breast cancer, cervix, eye, bone, prostate, and liver.  

Mechanism of action of sFRP4

Various Wnt-binding sites have been presented on the surface of SFRP molecules, and/or on the basis of diverse post-translational and conformational modifications of the SFRP, SFRP-Wnt pairs could link with differential affinities. Both CTD and NTD domains are mandatory for complete Wnt blocking. The specificity of the Wnt ligand binding variation of multiple CRDs is given by the sequence variation between the conserved cysteine residues. But, Uren A et al. described in their study that the CRD is not always needed for Wg (Wnt) binding to SFRP-1. Adjacent non-CRD sequence or additional Wnt binding sites may be prevailing elsewhere that may result in interaction with Wnt protein. Though the C terminus does not participate in the binding of Wnt ligands, it may take part in a role in the SFRPs tertiary structure stabilization, in conferring the affinity to bind to Wnts, in turnover, or in helping to solubilize the protein. The secreted antagonist of the Wnt signaling pathway

The functions and activities of cells are coordinated through the processing of biological
information and interaction between cells with the help of different signaling molecules which regulate gene expression leading to the proliferation and differentiation of cells. Wingless-related integration site (Wnt) signaling is an evolutionarily conserved pathway that controls various functions during the development of the embryo such as cellular growth, proliferation, and differentiation.33, 34 There are two Wnt pathways: β-catenin dependent canonical pathway and the β-catenin independent noncanonical pathways.35

The effector molecules of the Wnt pathway consist of the Wnt ligands and frizzled receptors (FZDs).34 Wnt ligands are secreted cysteine-rich glycoprotein containing 340–400 amino acids with signal peptides at their N-terminal.37 These ligands trigger Wnt-mediated signaling by binding to FZD receptors. Wnt ligands and receptors have important roles in the development, differentiation, determination of polarity of tissue, control of the movement of cells, and patterning of the central nervous system.36

Various Wnt antagonists modulate Wnt signaling. These antagonists are classified into two main groups based on their molecular mechanism (Figure 3). The secreted frizzled-related proteins (SFRPs) and cerebrosis/Wnt inhibitory factors (WIF) bind directly to Wnt proteins as well as FZD receptors and block both canonical and non-canonical pathways.38 SFRPs are secreted glycoprotein, which have a characteristic cysteine-rich domain (CRD) that can interact with FZDs and Wnts. Signal sequence and CRD constitute the N-terminus while the C-terminal consists of a hydrophilic heparin-binding domain. The SFRPs act as soluble modulators and it is believed to change Wnt signals by competing with FZDs to bind Wnts.39 Their competitive potential is connected with the presence of 10 cysteine residues at conserved positions having homology to CRD domains on the extracellular part of FZD.40 41 Five members of SFRPs had been recognized for having antagonistic effects on Wnt signaling cascades in mammals and named SFRP1-5.38

![FIGURE 3 Modulation of wingless-related integration site (Wnt) signaling by secreted frizzled-related proteins (SFRPs), Wnt inhibitory factors 1 (WIF1), and Dickkopfs (DKKs) family of Wnt modulators. (a) In the absence of antagonists, Wnts bind to the frizzled receptor and phosphorylated LRP5/LRP6 that get associated with axin and trigger the destruction complex to fall apart stabilizing cellular β-catenin. This stabilized β-catenin migrates to the nucleus and binds to TCF7/L2, a major transcription factor implicated in T2D and recruits coactivators of transcription including CBP, BRG1, BCL9, and Pygo to trigger target gene transcription. (b) In the presence of Wnt antagonists that either bind to Wnts (WIF1 or SFRPs) or frizzled receptors (DKK), the axin is released and gets associated with the destruction complex and phosphorylates cytoplasmic β-catenin at Ser45 by CKIα, ser33, 37, and threonine 41 by GSK3β. The binding site for E3Ubiquitin ligase β-Trep is created by phosphorylation at Ser33 and 37. This phosphorylated β-catenin is ubiquitinated and proteolytically cleaved by proteosomes. Ultimately, transcription of genes is blocked due to the association of groucho to TCF7L2.

Each SFRP has different binding specificity for different Wnt ligands. Various investigations have come across which have described different functions of SFRP proteins in Wnts diffusion,42 potentiating of Wnt signaling,43 and Wnt independence.44 Modulation of SFRPs in various pathological conditions including cancer and diabetes has been found. SFRP molecules enhances Wnt diffusion, hence, SFRP can be a positive modulator of Wnt signaling.42 Wnt signaling can also be activated by the diffusion of Wnt8 and Wnt43 and facilitated by SFRP molecules. Similarly, Wnt signaling was found to be disrupted in the mouse model by inactivating SFRP1 and SFRP2 affecting...
developmental changes. Diffusion of Wnts was disrupted by reducing SFRPs which impaired the peripheral area of mouse optic cups depending specifically on Wnt signaling. Nevertheless, such activating roles may be implicated to be tissue-specific responses because many studies advocate the antagonistic role of SFRPs and therefore, inhibition of these proteins could be utilized in treating various disease conditions like Wnt-dependent tumors and type 2 diabetes (T2D).

Many components of Wnt pathway are related to the metabolism of lipids and glucose, thus they might play a crucial role in the development of metabolic disorders. The relationship between Wnt signaling disturbances and type 2 diabetes is connected with adipogenesis and insulin signaling. Wnt can maintain pre-adipocytes in an undifferentiated state by inhibiting pro-adipogenic factors. Studies conducted on animals manifested a 50% reduction of adipose mass in over-expression of Wnt-10b, thus Wnt signaling pathway regulates adipogenesis negatively. Surprisingly, in a physiological state mature adipocytes produce Wnt ligands, like secreted frizzled-related protein5 (SFRP5), that are anti-inflammatory with insulin-sensitizing properties. In obesity and type 2 diabetes, low-grade inflammation in adipose tissue because of increased macrophage infiltration alters a proper Wnt action. In lean subjects, there is less number of macrophages in adipose tissue. Macrophages produce a negligible number of Wnt antagonists, such as Wnt5a and their activity is neutralized by SFRPS from adipocytes. On the contrary, in obesity, a large number of macrophages produce increased levels of Wnt5a and large mature adipocytes produce less SFRP5. Inflammation with Wnt signaling impairment induces insulin resistance in adipocytes via c-Jun N-terminal kinase (JNK) impeding with the substrate of insulin (IRS-1). In vitro study by Abiola et al. manifested that activation of Wnt signaling enhances insulin sensitivity in skeletal muscles because of inhibition of accumulation of intramyocellular lipids.

Findings of Secreted frizzled-related protein 4 (SFRP4) in other studies

Secreted frizzled-related protein 4 (SFRP4) contains a cysteine-rich domain homologous to the putative Wnt-binding site of frizzled proteins —G protein-coupled receptor proteins related to Wnt signaling pathway. It has been found to be expressed in several cells and tissues, including adipocytes, myocardium, endometrium, kidney and ovary, however, its association with β cell dysfunction in diabetes is closely associated to expression in pancreatic islets. Many studies demonstrated that SFRP4 is a Wnt antagonist and inhibits Wnt signaling pathway, for example in human cancer cells and adipocytes. Number of studies showed different properties of SFRP4 in both, physiological and pathological states. It was found to have an inhibitory role in angiogenesis, tumor suppression, promotion of differentiation of the epidermis, development of polycystic kidney disease and preeclampsia. However, some authors have also suggested that activation of Wnt signaling in pancreatic cells plays a major role in pathogenic factors in T2DM. Enhanced Wnt signaling might play an adaptive role, for example, in promoting β-cell proliferation in early diabetes. On the other side, chronic pathway activation stimulates cell apoptosis. It also decreases the release of insulin. Therefore, increased expression of SFRP4 in β cells of diabetic subjects can be because of impaired Wnt signaling. Despite this interesting hypothesis, the role of SFRP4 in obesity and T2DM has not been understood enough till now. Expression of SFRP4 mRNA and its release from visceral adipose tissue is increased in obese and related with reduced insulin release. Taneera et al. observed a significant inverse correlation of SFRP4 expression in human pancreatic islets with insulin secretion and a positive relationship with glycated hemoglobin (HbA1c) levels. Previous independent results manifested that in human pancreatic islets, recombinant SFRP4 decreases in vitro insulin secretion by 30% and cell exocytosis by 50%, as well as that SFRP4 overexpression is associated with the occurrence of pro-inflammatory factors was also found. Recently Mahdi et al. noticed SFRP4 expression links inflammation and defective insulin secretion, therefore it demonstrates the purpose of SFRP4 investigation as a biomarker of pancreatic islet dysfunction in T2DM. In the same study, over-expression of SFRP4 in islets of diabetic subjects and its association with markers of inflammation, particularly IL-1β stimulating its synthesis, were reported. Decreased insulin release was explained by reduced expression of Ca2+ channels in the islets' cells and inhibition of insulin exocytosis (Fig. 4). Serum SFRP4 concentration was significantly correlated with fasting glucose, decreased insulin sensitivity index and lower disposition index in non-diabetic subjects and it was not influenced by BMI, sex and age of the patient. Interestingly, the authors concluded that SFRP4 is raised in the serum several years before clinical diagnosis of diabetes and its presence
increases the risk of developing diabetes by three-fold. Therefore, SFRP4 might be useful in predicting the risk early mainly in individuals who are apparently healthy which are also supported by studies done by Anand K et al. 60, Baldane S et al. 61 and Brix J et al.62

Figure 4. Potential role of SFRP4 in insulin resistance, according to Mahdi et al.11

CONCLUSIONS

Although in recent years, the link between low-grade inflammation mediated by adipose tissue and metabolic disorders has been well established; the discoveries of new potential biomarkers and pathways are still an open question for researchers. Presented cytokines and secreted frizzled-related protein 4 must meet general requirements for biomarkers prior to their application in routine clinical practice.63 A standardized assay (preferably adapted for automatic analyzers) with proven analytical performance, instead of manual ELISA tests and clinical performance and effectiveness evaluation is a must in order to confirm their diagnostic/therapeutic goals and prognostic value in assessing the risk of T2DM. Cost-effectiveness analysis is an important aspect that compares the changes in costs and health effects of introducing a new test. Despite the presented promising results on the contribution of secreted frizzled-related protein 4 in the pathogenesis of T2DM, more detailed large population-based studies are required to evaluate their clinical and diagnostic utility. They may build up an opportunity for the development of new therapeutic targets.

REFERENCES

glucose-stimulated insulin secretion in individuals with different glucose tolerance. Endocrine Journal 2015, 62(8), 733-740. [PubMed] [DOI]


15. sFRP4 Symbol Report | HUGO Gene Nomenclature Committee. [FULL TEXT]


42. Mii Y, Taira M. Secreted frizzled-related proteins enhance the diffusion of Wnt ligands and expand their signalling range. Development.2009; 136:4083-4088. [PubMed] [DOI] [FULL TEXT]


49. Laudes M. Role of Wnt signalling in the determination of human mesenchymal stem cells into preadipocytes. J Mol Endocrinol 2011; 46(2):R65–72. [PubMed] [DOI] [FULL TEXT]


