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STEAP4: its emerging role in metabolism and homeostasis of cellular iron and copper

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Abstract

Preserving energy homeostasis in the presence of stressors such as proinflammatory cytokines and nutrient overload is crucial to maintaining normal cellular function. Six-transmembrane epithelial antigen of the prostate 4 (STEAP4), a metalloreductase involved in iron and copper homeostasis, is thought to play a potentially important role in the cellular response to inflammatory stress. Genome-wide association studies have linked various mutations in *STEAP4* with the development of metabolic disorders such as obesity, metabolic syndrome, and type 2 diabetes. Several studies have shown that expression of *Steap4* is modulated by inflammatory cytokines, hormones, and other indicators of cellular stress, and that STEAP4 may protect cells from damage, helping to maintain normal metabolic function. STEAP4 appears to be particularly relevant in metabolically oriented cells, such as adipocytes, hepatocytes, and pancreatic islet cells. These cells struggle to maintain their function in iron or copper overloaded states, presumably due to increased oxidative stress, suggesting STEAP4's role in metal homeostasisis critical to the maintenance of cellular homeostasis in general, and in preventing the onset of metabolic disease. In this review, we explore genetic associations of *STEAP4* with metabolic disorders, and we examine STEAP4 tissue expression, subcellular localization, regulation, structure, and function as it relates to metabolic diseases. We then examine how STEAP4's role as a regulator of cellular iron and copper may relate to type 2 diabetes.

Keywords

T2D; STEAP4; islets; beta cells; cytokines; low-grade inflammation; iron homeostasis; iron overload; copper

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Introduction

Six-Transmembrane Epithelial Antigen of the Prostate 4 (STEAP4) is anintegral membrane protein that functions as a metalloreductase involved in the transport of copper and iron (Ohgami *et al.* 2006; Grunewald *et al.* 2012). The expression of *Steap4*, also known as TNFα- induced adipose-related proteins (TIARP) (Moldes *et al.* 2001) or six-transmembrane protein of prostate 2 (STAMP2) (Korkmaz *et al.* 2005), is modulated in response to inflammation, and metabolism of fatty acids and glucose. Several studies have identified genetic variants in *STEAP4* that are associated with numerous metabolic disorders. In line with the hypothesis that defects in STEAP4 are implicated in metabolic disorders, expression of *Steap4* is associated with protection against inflammatory-mediated cellular damage. How the metalloreductase actions of STEAP4 may or may not be associated with STEAP4's putative protective effects are discussed below.

STEAP4 Structure and Function

STEAP4 is a metalloreductase

Reduction of extracellular Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{1+} are prerequisites for the transport of each of these metals across the membrane, into the cell. Steap4 and the related Steap2 and Steap3 family members are integral membrane metalloreductases that move electrons from cytosolic NADPH to extracellular iron or copper. STEAP4 (Figure 1) is composed of two domains, an N-terminal oxidoreductase domain present on the cytoplasmic face and, as its name implies (STEAP4: Six Transmembrane Epithelial Antigen of the Prostrate 4), a C-terminal transmembrane domain composed of six membrane spanning α-helices that envelop a single heme binding site (Ohgami*et al.* 2005; Kleven *et al.* 2015). Early hypotheses based upon homology to other ferric- and oxidoreductases suggested the oxidoreductase domain would catalyze electron flow from NADPH to an unidentified flavin, that would then donate electrons to the transmembrane domain where they would move through the heme group to an extracellular metal binding site, reducing iron and copper (Ohgami *et al.* 2005, 2006).

Mutational, kinetic and crystallographic analysis now definitively show that the N-terminal domain does indeed bind and oxidize NADPH (Gauss *et al.* 2013; Kleven *et al.* 2015). While the N-terminal domain does reduce flavin, contrary to earlier predictions, it does not harbor a high affinity flavin binding site (Gauss *et al.* 2013). Instead, the major constituents of the high affinity FAD binding site are found on the cytosolic face of the transmembrane domain of STEAP3 (Kleven *et al.* 2015). Because the residues involved in FAD binding in the STEAP3 transmembrane domain are strictly conserved among all Steap family members (Steap1–4), it was concluded that Steap family members in general, including STEAP4, bind flavin primarily through the cytosolic face of the transmembrane domain. This work also strongly suggests that FAD is the preferred flavin for all Steap family members (Kleven *et al.* 2015), a conclusion that was recently verified for STEAP1 (Kim et al, 2016).

In initial studies, the specific activity of Steap4 for reduction of copper suggested copper might also be a physiologically relevant substrate (Ohgami *et al.* 2006). Importantly, Gauss et al subsequently determined the affinity of rat Steap4 for iron and copper, and found

similar K_m values for each of these substrates. Further, the affinity of rat Steap4 for both iron and copper is equal to or greater than those of other characterized mammalian ferric and cupric reductases (Gauss *et al.* 2013). Kleven et al also identified a conserved Fe^{3+} binding site on the extracellular face of STEAP3. Again, because these sequence motifs are present in STEAP4 and all other Steap family members, this strongly suggests the presence of a conserved metal binding site on the extracellular face (or endosome/organelle membrane) of STEAP4 (Kleven *et al.* 2015). Further, biochemical and structural studies also suggest that STEAP3 and STEAP4 each function as homodimers (Sendamarai *et al.* 2008; Gauss *et al.* 2013; Kleven *et al.* 2015).

Mechanistically, then, the current view is that the Steap4 oxidoreductase domain draws two electrons from cytoplasmic NADPH (oxidizing it to $NADP^+$), passing these to FAD at the cytosolic face of the transmembrane domain, reducing it to $FADH_2$. $FADH_2$, in turn, passes electrons, one at a time, through the transmembrane heme, to the cell surface metal binding site where Fe^{3+} is reduced to Fe^{2+}, or Cu^{2+} to Cu^{1+} (Kleven, et al. 2015). Fe^{2+} and Cu^{1+} are then ready for transport across the membrane by their respective transporters.

Steap4 and cellular uptake of iron and copper

In healthy individuals there is little extracellular free iron. Most free iron is bound by the 80 kDa protein, transferrin. Transferrin serves to solubilize Fe^{3+}, which would otherwise complex with OH^- and precipitate (rust).

To meet their iron needs, erythroid cells in particular are dependent upon the transferrin cycle. In this cycle, iron-loaded transferrin (Tf) binds to the cell surface transferrin receptor (TfR) (Lawrence *et al.* 1999). The Tf:TfR complex then enters the endosome via receptor mediated endocytosis. Within the low-pH endosome, iron is released from Tf and reduced from Fe^{+3} to Fe^{+2} by Steap3, permitting transport across the endosomal membrane by divalent metal iron transporter 1 (DMT1), which is selective for Fe^{2+}. The apo-Tf:TfR complex is then recycled to the cell surface, where, at neutral pH, the apo-Tf is released to participate in the cycle once again (Andrews *et al.* 2015). Other cells with reduced iron needs can, however, take advantage of non-transferrin bound iron. For non-transferrin bound iron, other metal transporters such as Zip8 or Zip14 might also play a role (Zhao *et al.* 2010; Wang *et al.* 2012a; Wang & Knutson 2013; Kleven *et al.* 2015).

Copper transporter 1 (CTR1) is the major copper transporter involved in cellular copper uptake in mammals (Kaplan & Lutsenko 2009; Wang *et al.* 2011; Kidane *et al.* 2012). Thus, while the specific identity of the iron and copper transporters is not known, overexpression of mouse Steap4 in HEK-293 cells stimulates cellular uptake of these metals. In fact, in this assay, Steap4 shows the highest copper and iron uptake values of any member of the Steap family (Ohgami*et al.* 2006). This strongly suggests that Steap4 plays a role in the cellular uptake of iron and copper, and that STEAP4 may be critical to both iron and copper homeostasis at the cellular level, and within the body in general.

Iron and the innate immune system

Iron withholding is an important strategy of the innate immune system. Transferrin, for example, serves to sequester Fe^{3+} from pathogenic invaders, for which iron is often the rate

limiting nutrient. Thus, several proteins involved in iron transport and homeostasis, including hepcidin, ferritin and transferrin are up or down regulated in individuals suffering from chronic infection.

The increased expression of STEAP4 in response to inflammatory cytokines (see below) suggests Steap4 is also linked to inflammation and the innate immune response. Increased expression of cell surface Steap4 might therefore be expected to increase iron and/or copper import into the cell. While this strategy might reduce the concentration of circulating Fe^{3+} (and Cu^{2+}) available to pathogens, it might also be expected to result in increased intracellular Fe^{2+}, which if mishandled could lead to increased oxidative stress. The upregulation of Steap4 and potentially iron import by proinflammatory cytokines suggests, at least at the systemic level, that STEAP4-mediated iron transport into the cell is beneficial, perhaps because it reduces the concentration of circulating iron.

STEAP4 Tissue and Cellular Expression

One approach to understanding STEAP4's role in metabolic dysfunction begins with determining the tissues and organs in which it is expressed. Reviews of tissue expression patterns showed that STEAP4 is found to varying levels in most organs with the exception of the central nervous system (Gomes *et al.* 2012; Grunewald *et al.* 2012). More specifically, analysis of metabolic tissues has revealed that STEAP4 is found in adipose tissue, hepatocytes, and pancreatic islets/beta-cells. These tissues are discussed in more detail below.

STEAP4 in Adipocytes

Relative to other cell types, the effects of STEAP4 expression and misexpression are most studied in adipocytes. STEAP4 expression is higher in mature adipocytes rather than young undifferentiated preadipocytes (Moldes*et al.* 2001; Chen *et al.* 2009; Moreno-Navarrete *et al.* 2011; Narvaez *et al.* 2013; Sikkeland & Saatcioglu 2013). Emerging evidence has demonstrated a role for STEAP4 in the cellular response to nutritional and inflammatory signals (Wellen *et al.* 2007). When overexpressed, STEAP4 has been shown to reduce inflammation and better regulate glucose metabolism in a mouse model of streptozotocin-induced diabetes (Chuang *et al.* 2015). In another study, STEAP4 overexpression shifted macrophage polarization to enhance protection of adipose tissue, leading to reduced insulin resistance in diabetic $ApoE^{-/-}/LDLR^{-/-}$ mice (Han *et al.* 2013).

In contrast, reducing or eliminating STEAP4 negatively impacts adipose tissue. STEAP4 expression has also been implicated in translocation of glucose transporter 4 (GLUT4) to the cell surface, correlating reduced or abolished STEAP4 with increased insulin resistance and the pathophysiology of type 2 diabetes (T2D) (Cheng *et al.* 2011; Qin *et al.* 2011). At the whole animal level, Steap4 KO mice are prone to developing obesity, insulin resistance, glucose intolerance (Wellen *et al.* 2007), and hyperglycemia, hallmarks of metabolic syndrome and T2D. Thus, STEAP4 appears to play a protective role against metabolic and proinflammatory stress in adipocytes and adipose tissue.

STEAP4 Expression in Hepatocytes

In hepatocytes, the lack of STEAP4 has been suggested to correlate with dysfunctional responses to fat and nutrient influx, and the onset of fatty liver disease (Wellen *et al.* 2007). On the other hand, expression of STEAP4 in hepatocytes has been suggested to cause a suppression of lipogenesis and gluconeogenesis (Wang *et al.* 2012b). Overexpression of STEAP4 has been shown to ameliorate steatosis and insulin resistance caused by high fat diet (Kim *et al.* 2015). Hepatic STEAP4 overexpression also decreases hepatitis B virus X-protein signaling and subsequent metabolic dysfunction (Kim *et al.* 2012a). In mice with STEAP4 deficiency, liver size is elevated, hepatic insulin receptor signaling is impaired, and rates of fatty liver disease are increased (Wellen *et al.* 2007). As with adipose tissue, STEAP4 thus appears to play a protective role against metabolic and inflammatory stresses.

STEAP4 in pancreatic islets

Steap4 is expressed in pancreatic beta-cell lines (Berner *et al.* 2015) and primary mouse islets (Sharma *et al.* 2015) and is upregulated by exposure to cytokines or by free fatty acids (Sharma *et al.* 2015). In human islets, *STEAP4* expression is reduced in obesity and hyperglycemia (HbA1c), but elevated in donors with high white blood cell count (Gordon *et al.* 2017). Islet *STEAP4* expression also appears to be slightly higher in women compared to men (Gordon *et al.* 2017). To date, little is known about the function or localization of STEAP4 in islets and warrants further study.

Cellular localization of STEAP4

STEAP4 seems to localize specifically to the Golgi apparatus network and the plasma membrane of cells (Korkmaz *et al.* 2005; Yoo *et al.* 2014). One study suggests that STEAP4 co-localizes with caveolin-1, which is known to play a role in insulin signaling in adipose tissues (Chambaut-Guerin & Pairault 2005). Studies of osteoclasts also suggest that STEAP4 can be found in endosomes important in osteoclast development and function (Zhou *et al.* 2013). Further, with regard to the subcellular distribution of STEAP4, rat STEAP4 shows ferric and cupric reductase activity at acidic pH suggesting it also functions within intracellular organelles, particularly endosomes and granules, to reduce these metals (Gauss *et al.* 2013). As STEAP4 is examined for localization in more cell types, it would be intriguing if the STEAP4 subcellular distribution differed with cell types, i.e., was found in endosomes and/or the plasma membrane of some cell types, but in the Golgi, ER, nuclear membranes or other organelles in other cell types. While purely speculative as this time, this would suggest STEAP4 has tissue-specific actions that may differ by cell type.

Regulatory Influences on STEAP4 Expression

With potential roles in metabolism, inflammation, cell growth, and cancer, STEAP4 expression is regulated by a number of different factors. Known regulatory factors are described below and summarized in Figure 2.

Cytokines

Studies have suggested STEAP4 plays a fundamental role in cellular homeostasis during inflammatory stress, and a key regulator of STEAP4 expression is cytokine exposure.

Several reports show that *Steap4* mRNA increases in a dose-dependent manner with TNFα exposure, suggesting TNFα accelerates STEAP4 synthesis (Moldes *et al.* 2001; Chen *et al.* 2009; Tanaka *et al.* 2012a, b). Similar to the effect of TNFα, IL-6 exposure also results in an increase in *Steap4* mRNA (Fasshauer *et al.* 2004) and STEAP4 protein levels in human adipocytes (Chen *et al.* 2010). Moreover, simultaneous exposure to multiple cytokines including IL-1β, TNFα, and IL-6 stimulates *STEAP4* expression in a synergistic manner in adipocytes (Kralisch *et al.* 2009). *Steap4* expression is also increased in hepatocytes when cells are exposed to IL-17 or TNFα, and together, these cytokines produce a synergistic effect (Sparna *et al.* 2010; Wu *et al.* 2015). The only cytokine/chemokine found to inhibit *STEAP4* expression thus far is leptin (Chen *et al.* 2010).

Nutrients

Exposure to nutrients also plays a fundamental role in the regulation of STEAP4. Adipocyte exposure to high serum and fatty acids markedly increases *Steap4* expression, whereas glucose and insulin treatment show no effect on expression (Waki & Tontonoz 2007; Wellen *et al.* 2007). Supporting these findings, islet expression of *STEAP4* also increased in response to 48-hour exposure to free fatty acids but not to high glucose (Sharma *et al.* 2015). Moreover, *Steap4* expression was increased following meals in normal lean mice, but this effect was lost in obese mice (Wellen *et al.* 2007).

It is not clear, however, whether STEAP4 is elevated or depressed in conditions of obesity resulting from chronic nutrient excess. Initial studies found that *STEAP4* levels in adipocytes and white adipose tissue were increased in obesity (Arner *et al.* 2008; Catalan *et al.* 2012). Other studies, however, demonstrated that STEAP4 protein and/or gene expression is downregulated in obese patients (Zhang *et al.* 2008; Moreno-Navarrete *et al.* 2011; Kim *et al.* 2015; Ozmen *et al.* 2016; Xu *et al.* 2016). Additionally, STEAP4 expression in both fat and muscle was reduced among the most insulin resistant individuals independent of BMI (Elbein *et al.* 2011). Further, in human pancreatic islets, reduced *STEAP4* gene expression correlates with increased BMI (Gordon *et al.* 2017) among non-diabetic donors, but not among donors with T2D. Donors with T2Dshowed reduced *STEAP4* gene expression with increased HbA1c, a key indicator of chronic hyperglycemia (Gordon *et al.* 2017). On balance, these findings suggest that reduced STEAP4 expression is associated with obesity.

Hormones

In addition to cytokine and nutrient exposure, STEAP4 expression is regulated by hormones such as growth hormone (GH) and testosterone. Maneschi et al. established that an increase in testosterone increases STEAP4 expression in visceral adipose tissue (Maneschi *et al.* 2012). Other research has shown that STEAP4 expression is markedly increased in prostate cancer tissues in which testosterone production is high (Korkmaz *et al.* 2005). Interestingly, testosterone is important in normal glucose metabolism, insulin signaling, and the prevention of metabolic syndrome, and it has been suggested that STEAP4 acts synergistically with testosterone to achieve this effect (Maneschi *et al.* 2012; Vignozzi *et al.* 2012). Not surprisingly, an increase in GH, which impacts glucose homeostasis among other effects, has also been shown to increase the expression of STEAP4 in a dose dependent manner (Fasshauer *et al.* 2003). Although the exact connection between GH and insulin

resistance is unclear, an excess of GH hinders the action of insulin in tissues, which may act as a stressor to promote increased STEAP4 expression to counter the effect of testosterone.

Transcription Factors

At the transcriptional level, *STEAP4* expression is driven by several transcription factors and signaling cascades. Recent studies have shown that two factors involved in adipocyte differentiation, CCAAT/enhancer-binding protein α (C/EBPα) and liver-X-receptor-alpha activate the STEAP4 promoter, whereas PPARγ does not (Wellen*et al.* 2007). Other studies have demonstrated that STEAP4 expression is regulated by C/EBPα and STAT3 in the liver (Ramadoss *et al.* 2010). These transcription factors are activated in response to inflammatory and nutritional signals, suggesting that STEAP4 might play a protective role at the cellular or systemic level (Ramadoss *et al.* 2010). Similarly, in mesangial cells where high glucose induces Steap4 expression, the increased expression was found to be dependent upon S100B, JNK, PI3K, JAK2 and STAT3 (Chuang *et al.* 2015).

Regulatory and Protective Influences of STEAP4

Although STEAP4 functions as a metalloreductase at the molecular level, overexpression of STEAP4 has been shown to modulate the expression of several genes. For example, in the mesangial cells discussed immediately above, STEAP4 overexpression attenuates high glucose induced expression of collagen IV, fibronectin and COX2, as well as the expression of TGF-β, ERK1/2, Akt, Smad2/3 and STAT3 (Chuang *et al.* 2015). The observation that high glucose induces STAT3 dependent expression of STEAP4 (above), and that overexpression of STEAP4 in turn attenuates expression of STAT3, as well as other important signaling molecules like TGF-β, ERK1/2, Akt and Smad2/3, suggests STEAP4 may participate in a feedback loop that modulates the effects of high glucose on mesangial cells.

Steap4 is also reported to show protective effects at the systemic level. STEAP4 overexpression reduced rates of atherosclerosis and plaque formation in diabetic mice (Wang *et al.* 2014), while its deficiency promoted atherosclerosis (Freyhaus *et al.* 2012). Similarly, STEAP4 overexpression reduced migration of neutrophil-like HL60 (Tanaka *et al.* 2012a) and reduced IL-6 and IL-8 cytokine expression (Tanaka *et al.* 2012b), whereas siRNA knockdown of STEAP4 increased cytokine signaling in patients with rheumatoid arthritis (Tanaka *et al.* 2012b), again consistent with a role for Steap4 in a negative feedback loop. These protective effects have also been observed in adipocytes and hepatocytes as discussed above.

STEAP4 in Metabolic Disorders

Genome-wide Associations between STEAP4 and Metabolic Disorders

A number of genome-wide association studies have identified genetic variants of *STEAP4* associated with obesity and obesity-related disorders. For example, metabolic syndrome is known to impair glucose tolerance, insulin sensitivity, and other conditions linked with obesity. It was found that individuals with metabolic syndrome showed associations with several Single Nucleotide Polymorphisms (SNPs) within or near the *STEAP4* gene (Nanfang

et al. 2010; Guo *et al.* 2011a; Chen *et al.* 2014), particularly in the Uyghur people of western China who are genetically a mix of east Asian and western European genetic lineages (Yao *et al.* 2004). *STEAP4* variants were also associated with metabolic syndrome among the Han Chinese (Qi *et al.* 2015). Among western Europeans, an epidemiological study examined constitutive parameters of metabolic syndrome in French Caucasians and found that *STEAP4* variants were associated with higher triglyceride levels, fasting glucose, and fat intake, however, no relationship between *STEAP4* and the prevalence of metabolic syndrome was found (Miot *et al.* 2010).

Genetic variations in *STEAP4* have also been found to impact obesity and insulin secretion. A pair of studies associated a common variant in the *STEAP4* gene (rs1981529 Gly75Asp, 224A/G) with obesity in the Uyghur population (Guo *et al.* 2011b; Han *et al.* 2012). However, no relationship was found between *STEAP4* and severe obesity in a Norwegian cohort (Wangensteen *et al.* 2011). Finally, an islet-targeted genome-wide association study of Hispanic Americans implicated a *STEAP4* polymorphism with measures of acute insulin response to glucose (Sharma *et al.* 2015), suggesting a role for STEAP4 in insulin-secreting beta-cells. In each case, it is not known whether loss-of-function or gain-of-function mutations in *STEAP4* are related to disease status in these studies. Collectively, however, these associations suggest a potentially important role for STEAP4 in obesity and related metabolic disorders, and further mechanistic work is clearly needed.

STEAP4, Iron and T2D

Several excellent reviews detail cellular iron regulation and the relationship between iron and diabetes (Swaminathan *et al.* 2007; Simcox & McClain 2013; Backe *et al.* 2016). Further, iron regulation is governed by multiple processes involving heme vs. non-heme dietary iron intake, iron absorption, inflammation, and cellular iron regulation that are more complex than we can address within the scope of this review. For this reason, our discussion of iron will be largely limited to STEAP4. Critical to this discussion, however, is the understanding that while iron is essential for a variety of reasons (DNA synthesis, electron transport chain and ATP production, etc), excess iron results in the production of hydroxyl radicals (via Fenton Chemistry) and reactive oxygen species (ROS) in general. In turn, these ROS react indiscriminately with lipids, proteins and DNA, leading to oxidative damage and cellular stress. In short, iron is a double-edged sword that cuts both ways.

Not surprisingly then, long-standing evidence links T2D with numerous conditions of iron overload including hereditary hemochromatosis (Dymock *et al.* 1972; McClain *et al.* 2006) and blood transfusions associated with blood disorders such as beta-thalassemia major (Merkel *et al.* 1988; Dmochowski *et al.* 1993; Cario *et al.* 2003). Markers for elevated iron levels are also strongly associated with increased risk of T2D in the general population (Ford & Cogswell 1999; Fernandez-Real *et al.* 2002a) and in gestational diabetes (Lao & Tam 1997; Rawal *et al.* 2016). There is evidence that simply reducing iron levels by phlebotomy can reduce insulin resistance, improve insulin secretion, and lower blood glucose in patients with T2D (Facchini 1998; Fernandez-Real *et al.* 2002b).

Studies have shown that iron overload can cause insulin resistance in adipocytes (Dongiovanni *et al.* 2013; Wlazlo *et al.* 2013; Gao *et al.* 2015), impair hepatocytes (Ramm &

Ruddell 2005; Fargion *et al.* 2011), and cause beta-cell dysfunction (Cooksey *et al.* 2004). In contrast, when DMT1 was knocked out in beta-cells, the result was iron depletion and subsequent reductions in ROS, mitochondrial activity, and insulin secretion (Hansen *et al.* 2012). Consistent with this, under conditions of increased inflammatory stress, DMT1 deficiency was found to protect beta-cells from dysfunction and apoptosis (Hansen *et al.* 2012). These studies suggest that excessive iron uptake and the resulting oxidative stress inhibit normal beta-cell function, whereas inhibition of iron uptake is protective under conditions of inflammatory stress.

To date, a single report links STEAP4 with changes in iron regulation associated with metabolic disorders. STEAP4 and lipocalin-2 (aka NGAL, which sequesters bacterial siderophores) were both negatively correlated with serum iron levels and positively correlated with markers of inflammation in obese individuals (Catalan *et al.* 2012). These findings suggest that increased STEAP4 expression from low-grade inflammation in obesity could drive down circulating free iron levels and presumably drive up cellular iron accumulation. Considering the detrimental effect of iron overload and STEAP4's role in iron transport, it could be argued that knocking out STEAP4 might be beneficial under conditions of stress by reducing iron accumulation and reducing ROS production as occurs for DMT1. However, the opposite appears to be true; STEAP4 deficiency leads to dysfunction in several tissues including liver hepatocytes and adipocytes and exacerbates inflammatory environments. How changes in STEAP4 expression or activity would impact cellular function in these metabolic tissues, particularly in conditions of iron overload, acute vs. chronic inflammation, or nutrient-associated stress, is yet another open question warranting future study.

STEAP4, Copper and Type-2 Diabetes

Copper is also important for many biological functions. Copper is necessary for the activity of superoxide dismutase, an important antioxidant enzyme that scavenges the free radical superoxide (McCord & Fridovich 1969). Beta-cells are thought to be especially susceptible to oxidative stress because of very low anti-oxidant enzyme activity (Karunakaran & Park 2013). Thus, copper import by Steap4 may facilitate increased activity of cytosolic superoxide dismutase, protecting the cell against oxidative stress.

Copper is also a cofactor for cytochrome c oxidase, and is thus critical for mitochondrial electron transport and ATP synthesis (Kim *et al.* 2012b, Xu et al, 2013). Cytochrome c oxidase activity is thus reduced in copper deficient cells, which could lead to mitochondrial dysfunction. Supporting this idea, increasing dietary copper in Cohen diabetic rats restored insulin secretion and improved glycemia by increasing respiratory-chain enzyme cytochrome c oxidase (Weksler-Zangen *et al.* 2013).

However, a classic study of biological metals in humans links elevated levels of circulating copper with T2D (Walter *et al.* 1991), and a recent systematic review also concludes increased copper is associated with both type 1 and type 2 diabetes (Qiu *et al.* 2016). In addition, when diabetic (db/db) mice with elevated copper levels were given a copper chelator, the mice showed reduced copper and ROS levels and improved glucose tolerance

(Tanaka *et al.* 2009). Thus, the evidence appears to favor a link between increased circulating copper and diabetes, but the mechanism for this link is not well understood.

To date, there has been little study of STEAP4 with respect to copper homeostasis. Evidence of a role for STEAP4 in copper transport comes fromi) the demonstration of Steap4 reduction of Cu^{2+} to Cu^{+} with a Cu^{+}-sensitive chelating dye (Ohgami *et al.* 2006), ii) measurements of cellular copper uptake utilizing transiently transfected HEK-293T cells with STEAP4 expression plasmids (Ohgami, et al. 2006), and iii) the subsequent determination of a high affinity (physiologically relevant, low K_m) for copper (Gauss, et al. 2013). In this regard, we point out that increased intracellular copper levels and reduced circulating levels are not mutually exclusive, as STEAP4 mediated transport of circulating copper into the cell would explain both observations. This might also contribute to a functional electron transport chain, or the reduction in ROS in cells expressing STEAP4. Future studies will hopefully precisely pinpoint the links between STEAP4, copper homeostasisand metabolic disorders.

Interacting Partners for STEAP4

While the ferric reductase activity of Steap4 can explain a number of observations related to over, under, or mis-expression of *STEAP4*, it is difficult to rationalize the reported protective effects of STEAP4 with potentially increased iron import and production of ROS. This suggests Steap4 might possess metal-independent actions, and that understanding these actions will be important in reconciling these apparently contradictory observations. In this light, the literature suggests that STEAP proteins in general, and Steap4 in particular, may be involved in protein-protein interactions that modulate STEAP activity, or the activities of the interacting partners. For example, STEAP3 has been reported to interact with NIX and MYT1 kinase, localizing these proteins at the plasma membrane, and suggesting a role in apoptosis or cell cycle control. STEAP3 is also reported to interact with translationally controlled tumor protein (Amzallag *et al.* 2004), and there is strong genetic evidence for an interaction between STEAP3 and transferrin receptor (Jabara *et al.* 2016). Importantly, Steap3 can also form a heterodimer with STEAP4(Kleven 2015). Increased STEAP4 expression could then conceivably modulate the activity of STEAP3 and the activities or subcellular locations of proteins interacting with STEAP3 (NIX, MYT1), thus potentially impacting metal homeostasis, apoptosis and cell cycle control.

Similarly, Steap4 has also been reported to interact with BNIP3L (Passer *et al.* 2003), focal adhesion kinase-1(Tamura & Chiba 2009) and S100B (Chuang *et al.* 2015), again suggesting possible links to apoptosis, differentiation and cell cycle progression. In addition, STEAP4 is a reported target of the rhomboid protease RHBDL4/RHBDD1 (Wan *et al.* 2012). Finally, large scale interactome studies and their related data bases (BioGrid, IntAct, STRING, etc.) provide an expanded list of potential interactions that might help explain the protective effects. Importantly, most of these potential interactions have not been characterized with respect to metalloreductase activity, metal import and the protective effects of STEAP4, and much additional work is clearly needed. However, one possible explanation for the protective activity of Steap4 is that it localizes one or more of these interacting partners to

the membrane, thus modulating the activity of the respective signaling pathway, completing a regulatory feedback loop.

STEAP4 in Cancer

In addition to its role in cellular or systemic homeostasis in the presence of inflammatory stress, STEAP4 is also associated with tumorigenesis. STEAP4 overexpression has been suggested to increase ROS, which may contribute to increased mutational rates and further prostate cancer progression (Jin *et al.* 2015). At the same time, elevated levels of STEAP4 in prostate cancer cells might also help meet the increased need for iron in rapidly multiply cells, signifying its role in cell growth and maintenance (Korkmaz *et al.* 2005). Thus, while STEAP4 may have a beneficial role in protection from inflammatory stress in a host of chronic metabolic and inflammatory diseases (Grunewald *et al.* 2012; Gomes *et al.* 2012; Wu *et al.* 2015; Jin *et al.* 2015; Tamura & Chiba 2009; Lindstad *et al.* 2010, 2016), its mis-expression may also promote cancer cell proliferation and cancer progression, adding to the complexity in understanding the role of STEAP4 in health and disease.

Conclusion

STEAP4 has emerged as a key player in inflammatory responses in metabolic tissues and in cellular iron and copper homeostasis. It is well established that iron and copper, among other trace metals, are essential to maintaining many cellular processes. Numerous studies have pointed to iron overload (Swaminathan *et al.* 2007; Simcox & McClain 2013; Backe *et al.* 2016) and copper overload (Walter *et al.* 1991; Tanaka *et al.* 2009; Qiu *et al.* 2016) as contributing factors to insulin resistance and beta-cell dysfunction. With such obvious links, it is somewhat surprising that STEAP4's function as a metalloreductase has not been examined in the context of metabolic diseases. Several other iron-regulating genes have been recognized as important to both inflammation and metabolic disorders including lipocalin-2 (Wang *et al.* 2007), hepcidin and ferritin (Andrews *et al.* 2015). It has yet to be determined whether STEAP4's protective effects against metabolic and inflammatory damageare mediated by iron regulation, copper regulation, immunomodulation, by some combination of these, or by some completely novel mechanism. Given the clear links between STEAP4 and numerous metabolic disorders, defining these putative functions of STEAP4 is a goal worthy of future discovery and discussion.

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Plain Language Summary

Problems with the cellular regulation of iron and copper have long been associated with diseases of metabolism, including type 2 diabetes. In this review, we examine a protein involved with iron and copper handling in cells called STEAP4 and its connections to inflammation and diseases of obesity.

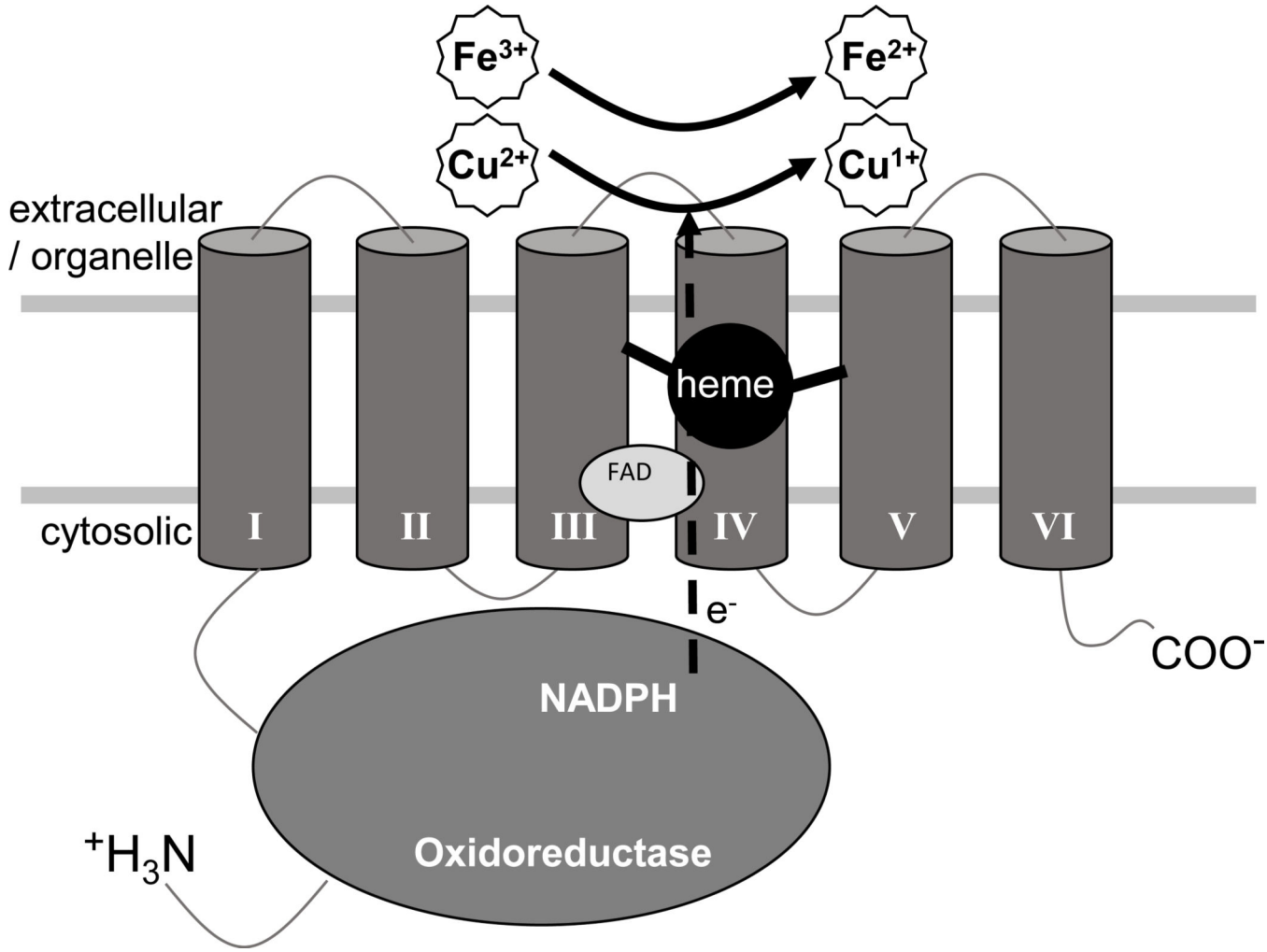

Figure 1.

Structure and function of STEAP4. Steap4 is composed of two domains, an N-terminal oxidoreductase domain, and a C-terminal transmembrane domain composed of six alpha-helices that coordinate a single heme. The N-terminal oxidoreductase domain draws two electrons from cytoplasmic NADPH and passes these to FAD at the cytosolic face of the transmembrane domain, reducing it to $FADH_2$. $FADH_2$, in turn, passes electrons, one at a time, through the heme in the transmembrane domain, to the cell surface metal binding site where Fe^{3+} is reduced to Fe^{2+}, or Cu^{2+} to Cu^{1+}, a prerequisite for transport into the cell or across organelle membranes.

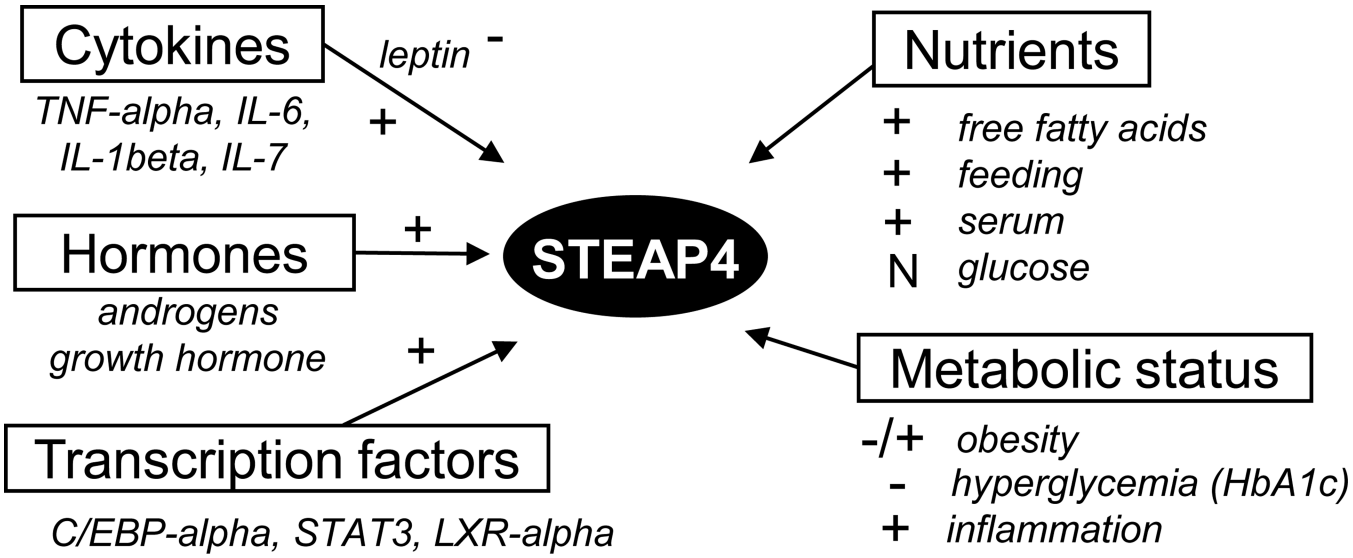

Figure 2.

Factors known to regulate STEAP4. "+" indicates a factor that upregulates STEAP4 expression; "−"indicates a factor that downregulates STEAP4 expression. "−/+" indicates both increased STEAP4 or decreased STEAP4 have been observed in obesity. N = no significant effect detected in adipose tissue (Wellen *et al.* 2007) or pancreatic islets (Sharma *et al.* 2015); note that high glucose increases STEAP4 expression in mesangial cells (Chuang *et al.* 2015).