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Vaspin (serpinA12) in obesity, insulin resistance and inflammation[†]

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Abbreviations: BMI, body mass index; SNP, single nucleotide polymorphisms; KLK, kallikrein; ROS, reactive oxygen species; RCL, reactive center loop; HUVEC, human umbilical vein endothelial cells; VSMC, vascular smooth muscle cells; HAEC, human aortic endothelial cells

Abstract

While genome wide association studies as well as candidate gene studies have revealed a great deal of insight into the contribution of genetics to obesity development and susceptibility, advances in adipose tissue research have substantially changed the understanding of adipose tissue function. Its perception has changed from passive lipid storage tissue to active endocrine organ regulating and modulating whole body energy homeostasis and metabolism, inflammatory and immune responses by secreting a multitude of bioactive molecules, termed adipokines.

The expression of human vaspin (serpinA12) is positively correlated to BMI and insulin sensitivity and increases glucose tolerance *in vivo*, suggesting a compensatory role in response to diminished insulin signaling in obesity. Recently, considerable insight has been gained into vaspin structure, function and specific target tissue dependent effects and several lines of evidence suggest vaspin as a promising candidate for drug development for the treatment of obesity related insulin resistance and inflammation. These will be summarized in this review with a focus on molecular mechanisms and pathways.

Background

In parallel to ongoing research efforts on how to provide sufficient food for the predicted ten-plus billion people on this planet in the near future, the WHO's latest report on human obesity states 1.4 billion people as overweight, 30% of them obese [1]. There are now >60% more people overweight than undernourished (~850 million) and more deaths are linked to overweight than underweight [2]. To categorize obesity, the body mass index (BMI) is the most commonly used indicator of body mass and provides a basis to differentiate weight-ranges [3]. Obesity is generally defined by a BMI of $>30 \text{ kg/m}^2$ with overweight beginning at $>25 \text{ kg/m}^2$.

Overweight is caused by a chronic imbalance of energy intake and energy expenditure. Environmental, social and economical changes have massively changed dietary and physical activity patterns. Driven by globalized food markets, a transition to a westernized nutrition and an urbanized lifestyle, obesity is the fastest growing health problem worldwide [4]. While undernutrition issues remain unsolved in developing countries, access to cheap, energy-dense and nutrient-poor processed foods is in part changing the face of malnutrition putting especially the poor and the young in danger of going from undernourished to overweight [5]. Obesity seriously impairs the quality and length of life by raising morbidity and premature mortality [6-8]. With obesity, the rates of several common adverse medical conditions such as hypertension, type 2 diabetes, cardiovascular disease, stroke and cancer are significantly increased [9]. For most of these comorbidities the causal relationship to overweight and obesity has been established in weight loss studies demonstrating a diminished or eliminated degree of these conditions after body weight reduction [10, 11].

With over 50% of the European population being overweight or obese, direct costs of obesity have put a serious burden on healthcare systems and are accounting for an estimated 7% of total healthcare costs (~60 billion €) in the EU [12, 13]. Additionally, the indirect illness related economic costs due to loss of productivity are estimated to significantly exceed 100 billion € per year in the EU [13]. Furthermore, with childhood obesity becoming more and more prevalent, the peak of the obesity epidemic is obviously yet to come. Consequently, identifying the various factors and understanding their function in the regulation of both energy intake and expenditure is an utmost important step towards developing obesity treatments, whether they will be based on environmental/lifestyle or pharmaceutical intervention.

Social and economical changes have created an obesogenic environment around the globe. But the susceptibility to obesity among individuals living in the same obesogenic environment

is very diverse, indicating genetic factors that are predisposing to obesity. Commonly, obesity is classified into two main subgroups, monogenic and polygenetic obesity where the latter represents the most common form of obesity. And while genome wide association studies as well as candidate gene studies have revealed a great deal of insight into the contribution of genetics to obesity development and susceptibility, advances in adipose tissue research have substantially changed the understanding of adipose tissue function. The perception of adipose tissue has changed from passive lipid storage tissue to active endocrine organ regulating and modulating whole body energy homeostasis and metabolism, inflammatory and immune responses. This crosstalk with all major organs is mainly achieved by secreting a multitude of bioactive molecules, termed adipokines (reviewed in [14]). As a consequence, condition, quantity and location of adipose tissue is reflected by the profile of adipokines it secretes and alterations in these secretion-patterns lead to disregulated metabolic (reviewed in [15, 16]), energetic (reviewed in [17]), immunologic (reviewed in [18, 19]), cardiovascular (reviewed in [20]) and cancerogenic (reviewed in [21]) processes and finally induce the various comorbidities of obesity. While healthy, adipose tissue secretion of adipokines is coordinated in an anti-inflammatory, insulin-sensitizing and cardioprotective pattern, but with increasing fat mass this secretion pattern flip-flops into a pro-inflammatory, insulin-resistant, atherogenic and along these lines fatal systemic environment.

Thus, adipokines comprise great potential as novel targets for pharmacological treatment of obesity and related metabolic diseases. Very few adipokines such as adiponectin and leptin, have been extensively studied so that a substantial body of knowledge with respect to function and mode of action is established (reviewed in [22, 23]). Therefore, the identification of novel adipokines and the molecular characterization of their structure, function, mode of action and molecular targets remain the major challenges in basic obesity research. Several lines of evidence suggest visceral adipose tissue derived serpin (vaspin) as a promising candidate for drug development and will be summarized with a focus on molecular mechanisms and pathways in this review.

Vaspin, a novel adipose tissue derived serpin family member

Serpins (serine protease inhibitors) represent the largest, most broadly distributed and most functionally diverse superfamily of peptidase inhibitors. Both intracellular and extracellular serpins exist, exhibiting important regulatory roles in sensitive and fine-tuned processes such as blood coagulation, inflammation and host defense, fibrinolysis and ischemia protection [24, 25]. Many serpins function as active serine protease inhibitors, but there are also non-

inhibitory serpins exerting effects as receptor ligands or chaperones. Over 1500 identified genes belong to the serpin family, and in humans 36 confirmed serpins have been categorized into nine clades (A to I) [24, 25]. Already eight members of the serpin family derived from six clades have been identified in the adipocyte secretome or proteome with the majority of them acting as extracellular protease inhibitors such as α 1-anti-trypsin (serpinA1), anti-chymotrypsin (serpinA3), plasminogen activator inhibitor (PAI1, serpinE1) or complement 1 inhibitor (serpinG1) [26-28]. With HSP47 (serpinJ6), a chaperone protein assisting in protein folding, also a non-inhibitory serpin has been identified [28].

Despite only moderate sequence homology, serpins exhibit well conserved structural core elements comprising a helical N-terminal domain, a C-terminal β -sheet domain, the functionally fundamental exposed reactive center loop (RCL) serving as the substrate for target proteases and the central five-stranded β -sheet (sheet A; Figure 1, left). Remarkably, the native serpin conformation (exposed RCL) is not the most stable and serpins are able to insert the RCL into the central β -sheet A and adopt a hyperstable conformation. The serpin inhibition mechanism is based on the stabilization of the protease-serpin complex as an acyl-enzyme intermediate. After protease attack and proteolytic cleavage within the RCL (Figure 1, middle), the RCL is rapidly inserted into β -sheet A resulting in the hyperstable serpin conformation and significant deformation of the protease (Figure 1, right). The distortion of the catalytic center prevents final deacylation due to the inability of the protease to stabilize the required tetrahedral transition state.

We have recently solved the crystal structure of human vaspin (PDB ID: 4IF8; <http://www.rcsb.org/pdb/>) [29]. As expected, vaspin features all typical structural core domains of native serpins with three β -sheets, nine α -helices and an exposed flexible RCL (extending from glycine 364 to proline 381 and not resolved in the crystal structure, Figure 2a). Conserved small side-chain amino acids within the hinge region together with a non-charged amino acid at position P14 within the RCL suggest vaspin to represent an inhibitory serpin targeting serine proteases presumably attacking between methionine 378 (P1) and glutamate 379 (P1') in the RCL sequence (Figure 2b) [29]. With respect to potential protease targets, the P1' glutamate is of special interest, as this represents a highly unusual residue at this position and very likely determines protease specificity.

Vaspin, a beneficial serpin in obesity and diabetes

Vaspin (serpinA12) was first identified as a putative member of the serpin family specifically expressed in the visceral adipose tissue of the rat type-2 diabetes model Otsuka Long-Evans

Tokushima Fatty rat [30]. In this animal model expression levels of vaspin reached a maximum at the age when obesity and insulin plasma concentrations peaked and subsequently decreased with worsening of diabetes and concomitant body weight loss [31]. A study in Wistar rats confirmed significant expression of *vaspin* mRNA and protein in visceral and less pronounced in subcutaneous adipose tissue only after high fat diet induced obesity and development of insulin resistance [32]. In human adipose tissue, *vaspin* mRNA expression was found to be associated with obesity, insulin resistance and type 2 diabetes [33] and elevated vaspin serum concentrations are associated with obesity and impaired insulin sensitivity in humans [34, 35]. Already in childhood higher vaspin serum levels are associated with insulin resistance [36].

In addition to expression in white adipose tissue, we and others have demonstrated vaspin expression on the mRNA or protein level in several other tissues such as liver [36], stomach [37], pancreas [29, 36], skin [36, 38, 39] and hypothalamus [37]. In various studies, mean serum vaspin concentrations have been reported as ~1 ng/ml, ranging from 0.01 to 6.74 ng/ml [34, 35, 40, 41].

A number of interesting single nucleotide polymorphisms (SNP) within and around the *vaspin* gene have been identified influencing vaspin serum concentrations. We have identified several SNPs that map between the *vaspin* and *kallistatin* (*serpinA4*) gene and are significantly associated with vaspin serum concentrations. Of these, rs11160190 reached the lowest P-value in a meta-analysis ($P=3.8 \times 10^{-41}$) [42]. A minor allele of SNP rs77060950 genetically defines a distinct group in the Japanese population that exhibits significantly higher serum levels (>10 ng/ml) by regulating *vaspin* transcriptional activity [34]. While both of these SNPs do not alter the *vaspin* coding sequence and thus protein structure, activity or half-life, the rare *vaspin* functional variant rs61757459 results in a truncated protein due to an early stop codon and thus affects circulating vaspin levels [43]. The change from arginine to a premature stop codon (vaspin.R211X) revealed expression of a truncated protein in both pro- and eukaryotic cell lines. Bacterial expression resulted in non-refoldable inclusion body formation and after expression in human embryonal kidney cells the truncated vaspin could only be detected in cell lysates, but not in supernatants. Since the N-terminal signal peptide and regulating secretory elements are still present, the vaspin fragment seems to be unable to adopt an autonomous protein folding and subsequently undergoes lysosomal degradation [43]. The most intriguing finding in the initial publication on vaspin was that administration of vaspin to obese mice improved glucose tolerance and altered gene expression of candidate genes for insulin resistance, such as *leptin*, *resistin*, and *Tnf- α* , *glucose transporter-4* and

adiponectin [31]. Furthermore, administration of insulin significantly up-regulated *vaspin* mRNA in subcutaneous adipose tissue and to a lesser extent reduced expression in visceral adipose tissue. The authors suggested that vaspin expression may represent a compensatory mechanism to antagonize the action of unknown proteases up-regulated in obesity and with increasing insulin resistance, which blunt insulin action. However, proteases investigated in this study (trypsin, elastase, urokinase, factor Xa, collagenase, and dipeptidyl peptidase) were not inhibited by vaspin [31].

Vaspin inhibits kallikrein 7

Based on the crystal structure data demonstrating that our expression system resulted in a most likely active protein and suggesting vaspin to be an inhibitory serpin, we aimed to identify target proteases and thereby unravel the presumed mechanistic basis of the intriguing beneficial metabolic effects of this serpin. Identification of a first target protease would further enable evaluation of the biological inhibitory activity in an assay based manner and thus the development of vaspin variants with modulated activity, specificity profiles or screen for activity modulating cofactors and molecules.

We started investigating potential target proteases of the kallikrein (KLK) family, as KLK5 has been reported to co-localize with vaspin in skin and a study on KLK7 substrate specificity was characterized using bovine insulin B-chain [44, 45]. Initial protease activity screens of the closely related KLKs 4, 5 and 7 using increasing molar excess of vaspin revealed specific inhibition of KLK7 and we could demonstrate stable complex formation and locate the cleavage site between the predicted methionine 378 and glutamate 379 [29].

With an assay system to assess the activity of vaspin we were able to generate first non inhibitory variants (vaspinT365R and vaspinA369P) as molecular tools to investigate protease inhibition dependence of observed vaspin effects in *in vitro* and *in vivo* experiments. As the inhibition mechanism of serpins is critically dependent on RCL length and flexibility [46], the introduction of a positively charged arginine (vaspinT365R) or a turn-inducing proline (vaspinA369P) in the hinge region of the RCL sufficiently slows loop insertion after proteolytic attack and prevents stable complex formation and inhibition (Figure 2b). These vaspin mutants serve as substrate molecules for KLK7 [29].

Vaspin, KLK7 and insulin action

After the identification of KLK7 as a target protease we furthermore investigated degradation of native human insulin by KLK7 and identified multiple cleavage sites within the A and

B chain congruent with or close to the cleavage sites of the insulin degrading enzyme. We found vaspin and KLK7 co-expressed in murine islets indicating a potential relevance of the vaspin-KLK7 interaction beginning with insulin secretion. Indeed, vaspin treatment of isolated islets resulted in increased insulin levels in islet supernatants under normal and increased glucose concentrations without affecting insulin secretion, as C-peptide levels were not changed [29].

We confirmed previously described improved glucose tolerance after acute vaspin administration in different mouse strains, C57BL/6NTac and diabetic *db/db* mice, and could demonstrate the dependence of this *in vivo* effect on the vaspin serpin activity using the mutant vaspinA369P. Application of the inactive mutant failed to improve glucose tolerance. Furthermore, after a glucose challenge we detected significantly increased insulin serum levels in mice after vaspin treatment, all together suggesting KLK7 inhibition by vaspin as one underlying physiological mechanism for its compensatory action on obesity-induced insulin resistance [29].

This mechanism of action would imply an acute and increased demand of vaspin protein before or parallel to postprandial increase in blood glucose levels and subsequent insulin release and, due to the suicide mechanism of serpin action, should result in reduced vaspin protein levels shortly after. Several studies support this general concept, as it has been shown that vaspin expression follows a circadian rhythm with expression highs preceding postprandial insulin concentration peaks [47] and acute insulin administration decreases vaspin serum concentrations in humans [48]. And while long-term insulin therapy seemed to slightly increase serum vaspin levels in diabetic OLEFT rats [31], patients on insulin treatment display lower serum vaspin levels [49]. Also, an acute glucose challenge significantly decreased vaspin serum levels in hyperinsulinemic, but not normoinsulinemic obese adolescents [36] and increases vaspin expression and secretion from omental fat pads in humans [50]. As aforementioned, insulin administration increased vaspin mRNA expression in white adipose tissue in mice [31]. These data support the hypothesis of compensation for depleted vaspin serum levels after acute vaspin demand and consumption due to the suicide mechanism of protease inhibition before or parallel to insulin action. Until now, there is no data on possible diurnal or meal related changes in KLK7 expression or serum levels.

Importantly, the beneficial metabolic effects of vaspin might still be mediated by vaspin regulating additional or different proteolytic cascades that impact glucose uptake and insulin levels. Also, receptors for stable serpin-enzyme complexes are known, that mediate the clearing of these complexes from circulation and trigger signaling pathways increasing serpin

expression into cells [51, 52]. A receptor for the vaspin-protease complexes, KLK7 or yet unidentified proteases, is currently not known.

Vaspin in obesity related inflammatory skin diseases

A recent study investigated expression of vaspin in human skin in the context of obesity associated psoriasis and demonstrated pronounced expression in the epidermal layers of non leisional skin [38]. In leisional skin, vaspin expression was significantly decreased and the authors hypothesized a potential role of vaspin in the regulation of proteolytic cascades in skin desquamation and inflammatory cytokine activation. KLK7 was identified as *stratum corneum* chymotryptic enzyme [53], and its function in skin has been shown to include the desquamation process [53, 54] and also activation of the pro-inflammatory cytokine interleukin-1 β [55, 56]. Overexpression of *Klk7* in a transgenic mouse model resulted in increased epidermal thickness, hyperkeratosis and dermal inflammation, skin changes similar to inflammatory skin diseases in humans [57-59] and in humans KLK7 expression and activity in psoriatic lesions is increased in comparison to healthy or non leisional psoriatic skin [60], suggesting a potential role of KLK7 in the initiation or progress of psoriasis. We have recently demonstrated that also the pro-inflammatory adipokine chemerin is proteolytically activated by KLK7 and that both proteins are co-expressed in human skin with increased co-localization in psoriatic lesions [39]. We could also confirm vaspin expression in the stratum corneum and furthermore found a distinct co-localization of vaspin and KLK7 in healthy skin, which was considerably decreased in psoriatic lesions, which might indicate a potential role for the vaspin-KLK7 system in inflammatory and obesity-associated skin diseases such as psoriasis.

Vaspin and regulation of food intake

Interestingly, we found that central vaspin administration leads to reduced food intake and has sustained blood glucose-lowering effects in mice [37]. This has since been confirmed in rats, where acute injection of vaspin in the hypothalamus was shown to decrease orexigenic neuropeptide Y while increasing anorexigenic proopio-melanocortin expression [61]. Data in the patent literature indicate that the anorexic effect of vaspin in mice is also dependent on the serpin functionality, as a non-inhibitory vaspin mutant did not alter food intake compared to the native protein [62]. As the only known target protease KLK7 is also expressed at relatively high levels in the central nervous system and in cerebrospinal fluid [63, 64],

prolonged insulin stability could also in part explain the reduced food intake after vaspin administration, whether given centrally or intraperitoneally.

An initial study on vaspin and human eating behavior also demonstrated an association of vaspin serum concentrations and eating behavior factors assessed by the German version of the Three-Factor Eating Questionnaire [65]. Nonetheless, conclusions with regards to the underlying causality remain elusive and genetic variations of the *vaspin* gene influencing vaspin serum concentrations were not associated with human eating behavior [65].

Anti-inflammatory effects of vaspin

On the cellular level, published data are mainly focused to vaspin effects on vascular endothelial cells and smooth muscle cells and demonstrates anti-inflammatory action and anti-atherogenic effects in the context of obesity associated inflammation and cardio-vascular disease. Multiple lines of evidence suggest that vaspin protects endothelial cells from inflammation and apoptosis (Figure 3). A first study investigating the anti-inflammatory potential of vaspin on human umbilical vein endothelial cells (HUVEC) found no beneficial effects on TNF- α -induced inflammation and activation of downstream inflammatory pathways such as NF- κ B, JNK and p38-MAPK (p38) [66]. But subsequent work on vascular smooth muscle cells (VSMC) could demonstrate vaspin inhibition of Tnf- α -induced expression of adhesion molecules in VSMC and subsequently decreased lymphocyte adhesion by a reducing reactive oxygen species (ROS) generation and activation of NF- κ B and protein kinase C [67]. Furthermore, vaspin attenuates high glucose-induced VSMC proliferation and chemokinesis also by blocking ROS production as well as inhibition of Mapk, Pi3k/Akt, insulin receptor signaling and Nf-kb signaling pathways [68]. In a similar manner, vaspin protects human aortic endothelial cells (HAEC) against free fatty acid (FFA)-induced apoptosis by blunting the phosphatidylinositol 3-kinase (PI3K)/AKT pathway [69]. Methylglyoxal is a harmful glucose metabolite due to the inherent formation of advanced glycation endproducts and represents an additional factor promoting diabetes induced vascular inflammation and apoptosis [70, 71]. In HUVEC, vaspin was shown to inhibit methylglyoxal induced NADPH oxidase (NOX) activation and subsequent ROS production, thus inhibiting apoptosis inducing caspase-3 cleavage and activation [72]. In addition to the inflammatory response, migration of smooth muscle cells plays an important role in the progress of atherosclerosis and vaspin has been shown to inhibit platelet-derived growth factor (PDGF)-BB induced VSMCs migration through inhibition of p38/Hsp27 signals also due to decreased ROS production [73]. Furthermore, nitric oxide (NO) and NO synthase

(NOS) represent key regulators of vascular structure and tone. In HAEC, vaspin has been found to increase both NOS activity and NO levels by diminishing the cellular concentration of the NOS inhibitor asymmetric dimethylarginine (ADMA) [74]. These effects were based on activation of the transcription factor STAT3 and subsequent upregulation of gene expression of the ADMA metabolizing enzyme dimethylarginine dimethylaminohydrolase (DDAH).

Importantly, none of the mentioned studies above have provided a mechanism of how the extracellular vaspin signal is transduced into the cell. A recent study has reported, that anti-apoptotic effects of vaspin in HAEC appear at least in part to be mediated as a ligand of a membrane localized complex of 78 kDa glucose-regulated protein (GRP78) and voltage-dependent anion channel (VDAC) [75]. Also in H4-II-E-C3 rat hepatocytes, GRP38 seems to be involved in vaspin induced intracellular signaling, e.g. activation of Akt and Ampk [76].

In adipose tissue, vaspin was reported to exert anti-inflammatory effects due to inhibition of pro-inflammatory adipocytokine expression (e.g. *resistin* and *Tnf- α*) in murine white adipose tissue [31], and a recent study reported vice versa *Tnf- α* mediated reduction of vaspin expression in 3T3-L1 adipocytes during differentiation [77]. Nevertheless, distinct direct effects and underlying molecular pathways of vaspin action on adipocytes, e.g. adipogenesis, adipocyte differentiation and adipose tissue function remain unknown.

Vaspin and first links to osteoporosis

Two studies have provided first evidence of anti-osteoporosis potential of vaspin. A fine tuned equilibrium between bone resorption by osteoclasts and bone formation by osteoblasts is fundamental for skeleton strength and integrity with osteoporosis developing when bone resorption exceeds bone formation. Vaspin seems to protect osteoblasts from apoptosis by upregulating apoptosis inhibitor Bcl-2 and decreasing expression of apoptosis activator Bax via activation of the ERK pathway [78]. On the other hand, osteoclast formation was found to be diminished by vaspin via blocking of receptor activator of nuclear factor- κ B ligand (RANKL) induced activation of nuclear factor of activated T cells c1, matrix metalloproteinase-9 and cathepsin K in two types of osteoclast precursors [79]. Again, whether these intriguing effects of the adipokine vaspin on bone metabolism are dependent on inhibition of a protease, KLK7 or yet unidentified targets, or rely on interaction with a membrane bound receptor, as a free ligand or in complex with its target protease, remains to be investigated.

Gain and loss of vaspin function in animal models

Recently, first data on adipose tissue-specific (under control of the adipocyte *fatty acid binding protein* aP2-promoter) *vaspin* gain and loss of function in animal models have been published. In line with the hypothesis of vaspin representing a compensatory molecule in metabolically challenged or inflamed conditions, *vaspin* transgenic and knock-out mice exhibit inconspicuous phenotype under normal, e.g. chow fed conditions [76]. While activity and food intake were unaltered, vaspin transgenic mice under a high-fat diet gained less body weight with reduced liver and adipose tissue weight. As observed for acute vaspin administration, transgenic *vaspin* overexpression resulted in improved glucose and insulin tolerance without influencing adiponectin expression levels, a known insulin sensitizing adipokine. In the adipose tissue, macrophage infiltration and crown-like-structure occurrence was reduced and adipocyte hypertrophy was ameliorated. In agreement with these findings, vaspin knock-out mice featured the adverse, opposite phenotype under high fat diet [76].

Interestingly, the most pronounced effects under diet induced obesity were observed in the liver of vaspin transgenic mice. Liver lipid droplet number and size as well as total triglyceride content were significantly reduced in combination with a downregulation of gluconeogenic and lipogenic gene expression. Serum pro-inflammatory Il-6 levels were significantly downregulated and adipose tissue microarray analysis revealed an immune response gene expression pattern comparable to normal chow fed control mice [76]. Together, the findings in transgenic and knock-out mice corroborate the data from *in vitro* and *in vivo* studies on the modulation of glucose tolerance and on anti-inflammatory effects previously reported.

Summary

In conclusion, the reviewed data demonstrates that vaspin is a truly intriguing serpin with promising beneficial effects in obesity and related insulin resistance and inflammatory diseases of the skin and cardio-vascular system. The identification of further proteases and substrates thereof, which are inhibited and regulated by vaspin together with a detailed and better understanding of vaspin action may be the basis for future pharmacologic treatment strategies. Along these lines, the regulation of vaspin activity by small molecules, glucosaminoglycans or other cofactors, as described for many other inhibitory serpins (reviewed in [80]), has not been investigated yet and might lead to new strategies for pharmaceutical intervention and modulation of activity.

The unexpected finding of significant effects of adipose tissue specific vaspin overexpression on the liver of transgenic mice indicates that the understanding of vaspin target tissues and mechanisms of target tissue specific effects, whether as a ligand of membrane bound receptors or as a serine protease inhibitor, is far from complete. More research is needed to understand and ultimately utilize and translate this intriguing potential in obesity, diabetes or inflammation treatment.

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Figure 1. Serpin structure and inhibition mechanism. The exposed reactive center loop of the native serpin (left) is attacked by the target protease forming the initial Michaelis complex (middle) (PDB ID: 1OPH, [81]). After proteolytic cleavage, rapid insertion of the RCL into β -sheet A results in the stable acyl-enzyme complex and significant deformation of the protease catalytic center prevents deacylation (right) (PDB ID: 2D26, [82]).

Figure 2. Crystal structure of human vaspin. (a) The highly flexible RCL (including the unresolved residues 365-377 in the X-ray structure) are illustrated in red (PDB ID: 4IF8, [29]). (b) Detailed view of important residues within the RCL and the hinge region. The scissile bond residues P1 (M378) and P1' (G379) for KLK7 are presented in blue, mutations within the hinge region resulting in substrate vaspin variants for KLK7 (T365R and A369P) in green.

Figure 3. Anti-inflammatory and anti-apoptotic effects of vaspin. Molecular pathways that are affected by vaspin and underlie its beneficial effects in endothelial and vascular smooth muscle cells (pathways impaired are marked with a red symbol, induced effects with a green symbol). Vaspin inhibits TNF- α , PDGF, methylglyoxal and high glucose induced ROS formation and subsequently inhibits cell apoptosis (reduced caspase3 activity), monocyte adhesion (reduced ICAM1 expression), cytoskeletal reorganization and migration (reduced expression of activation of p38 and HSP27). Vaspin increases intracellular NO via STAT3 induced expression of DDAH and also by increasing AKT signaling. FFA induced apoptosis is inhibited also by increased IRS1/AKT signaling and reduced caspase3 activity. IR: insulin receptor; TNFR: TNF receptor; PDGFR: PDGF receptor.