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Review Article

Identification and characterization of adipose surface epitopes

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Adipose tissue is a central regulator of metabolism and an important pharmacological target to treat the metabolic consequences of obesity, such as insulin resistance and dyslipidemia. Among the various cellular compartments, the adipocyte cell surface is especially appealing as a drug target as it contains various proteins that when activated or inhibited promote adipocyte health, change its endocrine function and eventually maintain or restore whole-body insulin sensitivity. In addition, cell surface proteins are readily accessible by various drug classes. However, targeting individual cell surface proteins in adipocytes has been difficult due to important functions of these proteins outside adipose tissue, raising various safety concerns. Thus, one of the biggest challenges is the lack of adipose selective surface proteins and/or targeting reagents. Here, we discuss several receptor families with an important function in adipogenesis and mature adipocytes to highlight the complexity at the cell surface and illustrate the problems with identifying adipose selective proteins. We then discuss that, while no unique adipocyte surface protein might exist, how splicing, posttranslational modifications as well as protein/protein interactions can create enormous diversity at the cell surface that vastly expands the space of potentially unique epitopes and how these selective epitopes can be identified and targeted.

Introduction

Adipose tissue is the primary organ storing and releasing energy at times of energy surplus or demand and an important endocrine organ [1]. Adipose tissue can be divided into white (WAT) and brown adipose tissue (BAT). Both depots are morphologically and functionally different. WAT is organized in discrete depots largely categorized as subcutaneous or visceral adipose tissue depots [2]. On the other hand, BAT dissipates energy as heat through the action of the inner-mitochondrial uncoupling protein 1 (UCP-1). In addition, the third type of adipocytes termed beige or brite adipocytes can appear in white adipose depots under β-adrenergic stimulation or chronic cold exposure [3–5]. These cells express similar genes as brown adipocytes (e.g. UCP-1, CIDEA, PAT2 and PGC1α). However, beige adipocytes are thought to derive from different progenitor cells [2].

Importantly, too little and too much of adipose tissue, especially white adipose tissue, can cause severe metabolic complications, where lipodystrophy, referring to the lack of adipose tissue, causes similar metabolic impairments as obesity [6]. While hereditary and acquired lipodystrophy are relatively rare, the rates of obesity are steadily increasing worldwide [7]. In 2016, more than 1.9 billion adults were overweight and more than 650 million obese, with obesity defined by a body mass index (BMI) above 30 [8]. However, a greater health risk than the excessive body weight *per se* are the diseases associated with obesity, such as type 2 diabetes, cardiovascular disease, and certain types of cancer [9]. Interestingly, genes and pathways associated with the regulation of body weight associate with the central nervous system rather than adipose tissue [10–12]. In contrast, the development of

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insulin resistance, eventually resulting in the metabolic syndrome, is largely driven by changes in adipose tissue, as illustrated by the elegant work of Drs. C. Ronald Kahn and Philipp Scherer and many others, which showed that maintaining adipose endocrine and lipid storing function prevents the progression form obesity towards insulin resistance and the metabolic syndrome [13–18].

Thus, pharmacological approaches to maintain adipose function could dissociate body weight gain from the development of the metabolic syndrome and activation of brown and beige adipocytes could be utilized to reduce body weight gain and resolve metabolic abnormalities [19]. A steadily increasing amount of literature has identified potential therapeutic targets in adipose tissues, in various cellular compartments. However, for most, pharmacological utilization is limited by essential functions of these proteins in tissues outside adipose, raising safety concerns due to undesired side effects. To overcome this bottle neck, a crucial step is to identify adipose tissue-specific epitopes, allowing tissue-selective drug delivery. Cell surface proteins integrate all extracellular inputs to co-ordinate a cellular response and are ideally located at the outside of the cell, allowing easy access by drugs. Thus, targeting the cell surface does not only provide a unique opportunity to deliver cargo to adipocytes, but is an attractive target for pharmacotherapy itself. To date, more than 1200 cell surface proteins have been described. However, albeit we and others have tried extensively, no proteins were identified that are exclusively expressed in either brown or white adipocytes [20].

In the first part of this review, we will highlight some important and well-described cell surface proteins and their role in adipocyte differentiation and mature adipocytes, to underscore the significance and pharmacological potential of the cell surface. We do not discuss the advantages or disadvantages of targeting white versus brown or beige adipocytes in detail, as there are several recent reviews highlighting the functional differences and pharmacological benefits of either of those adipocyte types [3,21–23]. In the second part, we will discuss techniques that can be utilized to identify novel adipose selective cell surface epitopes distinguishing between distinct adipocyte subtypes and different progenitor populations.

Important cell surface regulators of (pre-)adipocyte function

Adipose tissue hypertrophy, in response to excessive caloric intake, can exceed the maximal lipid storing capacity of individual adipocytes, leading to adipocyte cell death and the development of local and systemic inflammation and insulin resistance [13]. However, hyperplasia, the *de novo* generation of adipocytes from precursors to store excessive calories, is not associated with these pathological changes. Thus, to maintain healthy adipose tissue in the context of obesity, one appealing approach is to promote the differentiation of preadipocytes into mature adipocytes, distributing lipid storage into more adipocytes thereby preventing lipid-induced cell death. Initially, Rodeheffer et al. identified and Berry et al. characterized a subpopulation of early adipocyte progenitors defined as Lineage (CD45, CD31 and/or not Ter119)$^-$CD29$^+$CD34$^+$Sca-1 (Ly6A)$^+$CD24$^+$ in white adipose tissue of mice [24,25]. Since then, many studies found distinct adipocyte progenitor cells (APCs) with various cell surface proteins in white and BAT [26–32] (Table 1). Furthermore, preadipocytes with different functions were identified using cell surface proteins [28,34,35] and single-cell RNA sequencing (scRNAseq) [36–38,41–43]. For example, Ly6C$^-$CD9$^-$PDGFRβ$^+$ cells were shown to be highly adipogenic [35], whereas CD142$^+$ cells (Aregs) were shown to be anti-adipogenic APCs in human and mouse [37]. CD55 and CD34 were also identified as markers for APCs [36,37] and DPP4$^+$ cells were demonstrated to give rise to both ICAM1$^+$ and CD142$^+$ preadipocytes [38]. Moving forward, methods such as cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) approaches that integrate cell surface markers with transcriptomics will allow even more detailed insights into the heterogeneity of adipocyte progenitor populations [44]. Together, these data suggest that selective enhancement of individual (pre-)adipocyte subpopulations could provide an interesting approach to maintain adipose function.

The surfome is composed of a very complex mixture of transmembrane, membrane-anchored and extracellular proteins that mediate cell–matrix interactions, signal transduction and shuttling of metabolites and ions, among other functions. Receptors can be divided into three main categories (Figure 1). The largest category of receptors are G protein-coupled receptors (GPCRs) [45], followed by enzyme-linked receptors [46] and ion-channel linked receptors [47]. However, it would not be possible to discuss all types of cell surface proteins and their function in adipose tissue. Therefore, we highlight here the role of some important transmembrane receptor families to underscore the complexity of signal integration and transduction as well as the heterogeneity of inputs received by the cells (Figure 2).

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Table 1. Specific cell surface marker sets for different cell types in adipose tissue

Cell types	Markers	Fat depots	
White adipocytes	ASC-1	S/VAT, S/VAT*	[20]
	Prohibitin/annexin 2 complex	S/VAT	[33]
Brown adipocytes	P2RX5	BAT, CS/LC*	[20]
	PAT2	BAT, CS/LC*	[20]
Adipocyte progenitor cells	Lin(–):Ter119(–)CD29(+):CD34(+):Sca-1(+):CD24(+)	S/VAT	[24]
		iBAT	[26]
	PDGFRα(+):Lin(–):CD29(+):CD34(+):Sca-1(+):CD24(+)	SAT	[25]
	PDGFRα(+):CD34(+):Sca-1(+):CD24(–):PDGFRβ(–)	VAT	[27]
	Lin(–):CD34(+):CD38(+)	S/VAT, iBAT	[29]
	Zfp423(+):CD45(–):CD29(+)Sca-1(+):CD24(–)	SAT	[30]
	Zfp423(+):CD45(–):CD29(+)Sca-1(–):CD24(+/–)	VAT	
	PDGFRα(+):PDGFRβ(–)	SAT	[31]
	PDGFRα(+):PDGFRβ(–), PDGFRα(–):PDGFRβ(–)	VAT	
	Lin(–):Gp38(+):PDGFRα(+):CD9(low)	VAT	[34]
	Lin(–):CD34(+):CD44(+):PDGFRα(+):CD9(low)	VAT*	
	Lin(–):Ly6c(–):CD9(–):PDGFRβ(+)	VAT	[35]
	Lin(–):Ter119(–):Sca-1(+):CD55(high)	VAT	[36]
	Lin(–):Ter119(–):Sca-1(+):CD29(+):CD34(+):CD142(–)	S/VAT, SAT*	[37]
	Lin(–):CD26/DPP4(+):CD142(–)	S/VAT, SAT*	[38]
	Lin(–):CD34(+):CD44(+):ΔDecorin(+)	WAT	[39]
Preadipocytes	PDGFRα(+):Lin(–):CD29(+):CD34(+):Sca-1(+):CD24(–)	SAT	[25]
	PDGFRα(+):PDGFRβ(–), PDGFRα(+):PDGFRβ(+)	SAT	[31]
	PDGFRα(+):PDGFRβ(–), PDGFRα(–):PDGFRβ(+)	VAT	
	Lin(–):PDGFRβ (+):Zfp423(+)	S/VAT	[32]
	Lin(–):Ter119(–):Sca-1(+):CD55(low)	VAT	[36]
	Lin(–):CD54/ICAM1(+):CD142(–)	S/VAT, iBAT	[38]
Endothelial cells	Prohibitin	S/VAT, WAT*	[40]

SAT, subcutaneous white adipose tissue; VAT, visceral white adipose tissue; iBAT, interscapular brown adipose tissue; CS, carotid sheath; LC, longus colli; Lin, CD45 and CD31; *, Human samples; WAT, white adipose tissue.

G protein-coupled receptors

GPCR are the most abundant receptor super family in the human genome with more than 800 genes coding for GPCRs. GPCRs are divided into five groups namely rhodopsin, secretin, adhesion, glutamate and frizzled/taste2 based on their structure and sequence homology [45]. More than one-third of all drugs target GPCRs [48,49], highlighting their pivotal role in cellular function. Thus, it is not surprising that they also play an important role in adipose tissue regulating various, and sometimes even opposing, effects in fat.

GPCRs consist of an extracellular N-terminus and three extracellular loops followed by seven transmembrane α helices. Intracellularly there are three loops, a short amphipathic α helix and the C-terminus [50]. A diverse set of ligands, from ions to nucleotides and proteins, can bind to GPCRs. Upon ligand binding, receptor conformational changes occur and the activated receptor interacts and activates heterotrimeric G proteins. Activated G protein subunits (Gα and Gβγ) transduce then the signal [50]. However, G protein independent pathways also exist, multiplying signaling complexity [51,52]. Here, we discuss examples of GPRCs playing important roles in adipose tissue.

Rhodopsin GPCRs

The biggest group of GPCRs are rhodopsin GPCRs [53]. We will discuss several receptor families to highlight the heterogeneity of these adipocyte cell surface receptors and their prominent role in adipose tissue.

Adenosine receptors

Adenosine and purinergic receptors fulfill various functions in the human body from the cardiovascular system to the central nervous system [54]. Within the adipose tissue, adenosine is released from adipocytes [55,56] and can bind to four different GPCRs (A1R, A2aR, A2bR and A3R). A1R and A3R are coupled to $G_{i/o}$

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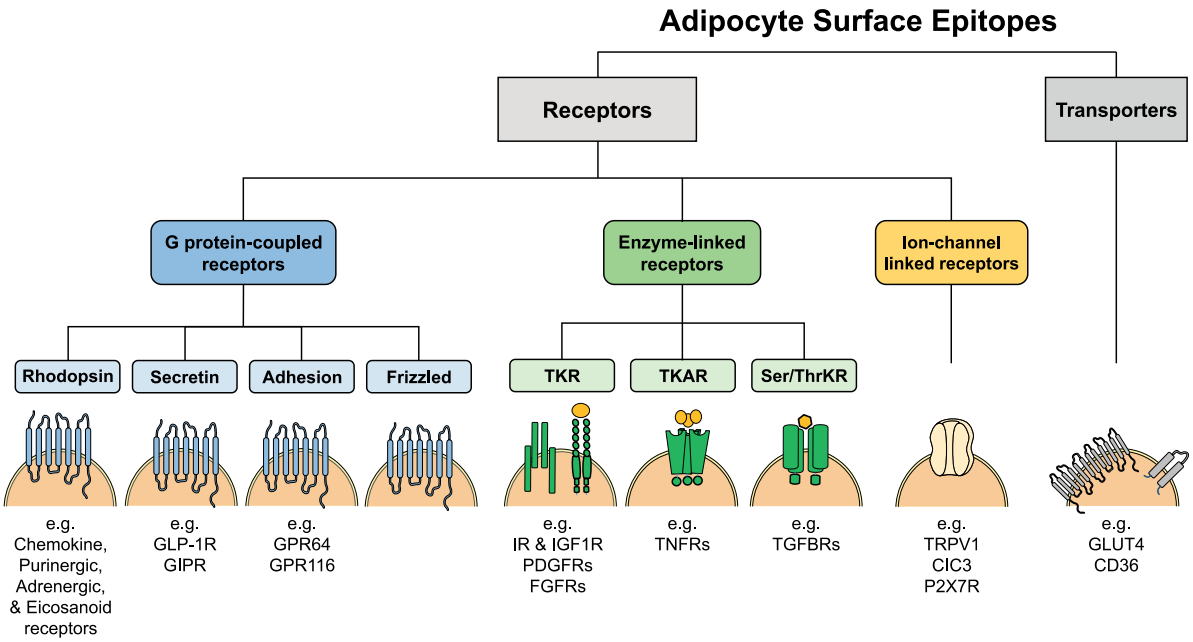

Figure 1. Receptor families expressed on adipocytes.

TKR, tyrosine kinase receptor; TKAR, tyrosine kinase-associated receptor; Ser/ThrKR, serine/threonine kinase receptor; GLP-1, Glucagen-like peptide 1; GIPR, Glucose-dependent insulinotropic polypeptide receptor; GPR, G protein-coupled receptor; IR, insulin receptor; IGF1R, insulin-like growth factor 1 receptor; PDGFRs, platelet-derived growth factor receptors; FGFRs, fibroblast growth factor receptors; TNFR, tumor necrosis factor receptor; TGFBR, transforming growth factor beta receptor; TRPV1, transient receptor potential vanilloid type 1 channel; CIC3, chloride channel 3; P2X7R, ionotropic purinergic receptor 7; GLUT4, glucose transporter 4.

proteins. Thus, their activation inhibits cyclic adenosine monophosphate (cAMP) production and decreases protein kinase A (PKA) activation while A2aR and A2bR are coupled to G_s proteins and their activation stimulates cAMP production and increases PKA activation. Additionally, some adenosine receptors can activate MAP kinases, PLC and Ca^{2+} signaling [57]. Earlier studies demonstrated that A1R is expressed in mature ob1771 and rat adipocytes while no expression was observed in undifferentiated ob1771 and rat preadipocytes. On the other hand, A2 receptors are expressed in preadipocytes and their expression decreases with differentiation [58,59]. A similar trend was seen with A2 receptors in 7F2 preosteoblasts, which can differentiate into adipocytes [60]. However, unlike murine white adipocytes, murine brown adipocytes show high A2aR expression, which was also reported for human adipocytes [61]. Interestingly, hamster brown adipocytes show similar levels of A1R and A2aR with no detectable expression of A2bR [61], indicating differences in adenosine receptor expression between different species.

With regards to adipogenesis, activation of the A2R with NECA (adenosine receptor agonist) in rat white preadipocytes increased differentiation in corticosterone treated ob1771 preadipocytes [58,59]. However, subsequent studies reported contradictory results, as activation of A2bR in human preadipocytes and murine stromal vascular fraction (SVF) inhibited adipogenesis. Moreover, knockdown of A2bR in mouse preadipocytes increased differentiation. This inhibition of differentiation by A2bR activation was associated with sustained krüppel like factor 4 (KLP4) expression as the ability of A2bR to inhibit differentiation is lost upon knockdown of KLP4 [62]. Furthermore, the transfection of 7F2 preosteoblasts with A1R promoted adipogenesis while transfection with A2bR decreased adipogenesis and increased osteogenesis [60]. The different effects of A2bR on differentiation in these studies could be explained by the different cell lines used and by the fact that NECA is a non-selective adenosine receptor agonist. Interestingly, no effect on brown preadipocyte differentiation was observed using brown preadipocytes from A2aR knockout mice [61]. A direct role of A3R in adipogenesis has not been reported so far. However, A3R knockout mice show less abdominal and total body fat [63].

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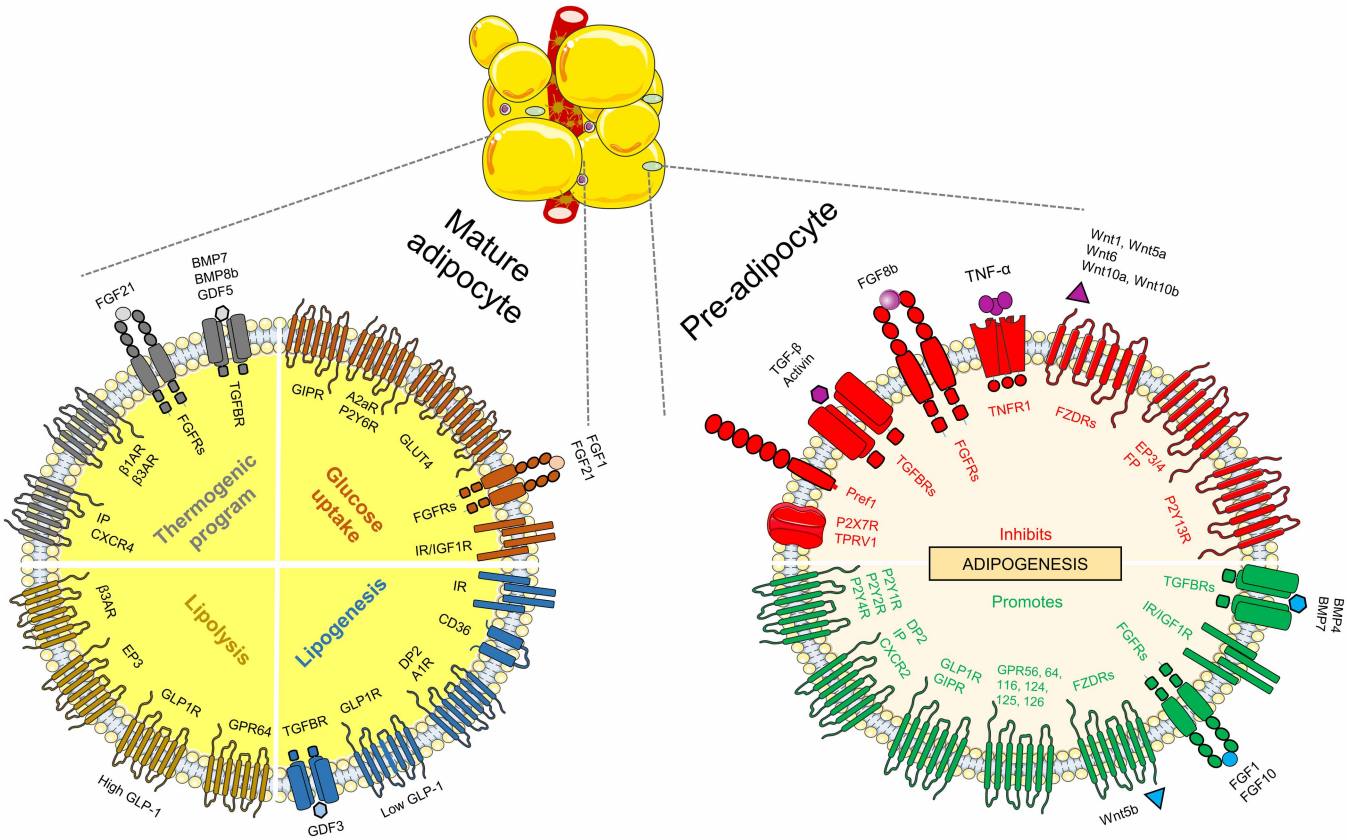

Figure 2. Receptors regulating pre- and mature adipocytes function.

Right side: receptors involved in preadipocyte differentiation. Left side: receptors promoting glucose uptake, thermogenesis, lipolysis and lipogenesis in mature adipocytes. IR, insulin receptor; IGF1R, insulin-like growth factor receptor; βAR, beta adrenergic receptor; AR, adenosine receptor; TGFBR, transforming growth factor beta receptor; P2YR, metabotropic purinergic receptor; P2XR, ionotropic purinergic receptor; FZDR, frizzled receptor; TNFR1, tumor necrosis factor alpha receptor 1; GLP1R, glucagon-like peptide-1 receptor; GIPR, glucose-dependent insulinotropic peptide receptor; CXCR2, CXC chemokine receptor 2; TPRV1, transient receptor potential vanilloid type-1; Pref1, preadipocyte factor 1; EP, prostaglandin E2 receptor; FP, prostaglandin F receptor; IP, prostaglandin I2 receptor; DP2, prostaglandin D2 receptor 2; GLUT4, glucose transporter type 4; BMP, bone morphogenetic protein; GDF, growth differentiation factor; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; GLP-1, Glucagon-like peptide-1.

Adenosine was shown to inhibit lipolysis in rat adipocytes [64]. A1R was later demonstrated to be required to inhibit lipolysis, as the administration of an adenosine analog to wild type mice reduced free fatty acid (FFA) and glycerol levels, which was blunted in A1R knockout mice. Furthermore, increased lipolysis was observed upon depletion of adenosine, using adenosine deaminase, in mouse adipocytes but not in adipocytes from A1R knockout mice [65]. Additionally, antagonizing the A1R receptor promoted lipolysis in rat adipocytes [66] further confirming the need for a functional A1R to inhibit lipolysis. On the other hand, mice overexpressing A1R exhibit reduced FFA. Moreover, these mice showed improved insulin sensitivity upon high-fat diet (HFD) feeding in comparison with controls [67]. Another well-characterized adenosine receptor is the A2b receptor. Chronic activation of A2bR for 4 weeks upon HFD feeding improved glucose tolerance and insulin sensitivity. This improvement in insulin sensitivity was accompanied by reduced adipose tissue inflammation, which is attributed to increased M2 macrophage activation [68,69]. In line with this, the deletion of the A2bR in HFD fed mice further impairs glucose tolerance and insulin sensitivity [69]. Interestingly, activation of A2aR improved glucose homeostasis and reduced adipose tissue inflammation in mice [70]. Moreover, agonizing A2aR protected mice from diet-induced obesity (DIO) and induced beiging of WAT [61].

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Purinergic receptors

Purinergic receptors are divided into two types. The ionotropic ligand-gated cation channels (P2XR), which are mainly activated by adenosine tri-phosphate (ATP) and the metabotropic GPCRs (P2YR) which are activated by endogenous nucleotides. Seven P2XRs have been identified (P2X1–7) and eight P2YRs (P2YR1, 2, 3, 4, 6, 11, 12, 13 and 14). The P2YR1, 2, 4 and 6 are coupled to G_q proteins and activate PLC-β, increasing cytosolic Ca^{2+} levels. P2Y11R is coupled to G_s proteins and can, therefore, stimulate cAMP production while P2YR12, 13 and 14 are bound to G_i proteins and hence inhibit cAMP production [71,72]. All purinergic receptors are expressed in human adipose tissue-derived mesenchymal stem cells (MSCs) and mature adipocytes derived from these MSCs except P2X1R and P2X2R [73]. Another report examined human subcutaneous adipocytes and showed that they express all purinergic receptors except P2X1R, P2X2R, P2X3R and P2Y14R [74], while we could not detect the significant expression of P2X5R in white adipocytes [20].

ATP was shown to potentiate the differentiation of bone marrow-derived human MSCs into adipocytes. This effect is dependent on P2YRs as inhibition of P2YRs with pertussis toxin negated ATP effects on differentiation. Furthermore, P2Y1R and P2Y4R activation increased adipogenesis [75]. Another study showed that uridine tri-phosphate (UTP), which activates P2Y2R and P2Y4R, as well as uridine diphosphate (UDP), activating P2Y6R, promote adipogenesis and suppresses osteogenesis in rat bone marrow-derived MSCs. This effect was mediated by P2Y2R and not P2Y4R or P2Y6R as silencing P2Y4R (via siRNA) and antagonizing P2Y6R did not affect differentiation. Moreover, this pro-adipogenic effect of P2Y2R was mediated through ERK1/2 signaling [76]. Conversely, P2Y13R inhibits adipogenesis and MSCs from P2Y13R knockout mice showed increased adipogenesis [77].

Unlike adenosine receptors, purinergic receptors are not thoroughly characterized in mature adipocytes. Activation of P2Y6R increased glucose uptake via elevated glucose transporter (GLUT) 4 translocation in primary and 3T3-L1 adipocytes [78]. Moreover, purinergic receptors are implicated in the inflammatory response of adipocytes [79]. For a more detailed outlook on adenosine and purinergic receptors' role in the adipose tissue, please see [80].

Eicosanoid receptors

Prostaglandin receptors are the best-described members of this receptor class in adipose tissue [81]. There are nine types of prostaglandin (PG) receptors: The PGD receptors (DP1–2), the PGE receptors (EP1–4), the PGF receptor (FP), the PGI receptor (IP) and the TXA receptor (TP) [82]. Several types of prostaglandin receptors are expressed in preadipocytes [83] and were shown to regulate adipogenesis. However, depending on the PG receptor activated, adipogenesis can be either promoted or inhibited. For example, activation of EP4 and EP3 receptors inhibit adipogenesis in both 3T3-L1 preadipocytes and mouse embryonic fibroblasts (MEFs) [84–86]. Similarly, activation of the FP receptor suppresses differentiation in 3T3-L1 preadipocytes [87], primary rat inguinal adipocyte precursor cells [88], human orbital preadipocytes [89] and mouse MSCs [90]. On the other hand, IP and DP receptor actions promote adipogenesis, which was demonstrated in various cell models, including human and mouse adipose precursor cells [91]. This pro-adipogenic effect is mediated via an increase in C/EBPβ and C/EBPδ [92,93]. Moreover, activation of DP2 enhances differentiation and lipid accumulation in 3T3-L1 and MEFs, where increased lipid accumulation is due to a suppression of lipolysis [94].

Prostaglandin receptors also regulate mature adipocyte function. The deletion of the EP3 receptor in mice impaired insulin sensitivity and promoted adipose tissue inflammation. Moreover, EP3 knockout mice were obese with high levels of serum triglycerides and insulin. Interestingly, EP3 receptor is down-regulated in genetically and diet-induced obese mice, [86]. In line with this, antagonizing EP3 receptors in a human adipocyte cell line (SGBS) decreased pro-inflammatory gene expression, while activation of the EP3 receptor (via PGE2) promoted inflammatory gene expression [95]. Conversely, agonizing EP4 receptors in *db/db* mice improved insulin sensitivity and glucose tolerance concomitant with a decreased inflammatory profile in adipose tissue. This phenotype was mainly due to a switch from pro-inflammatory M1 to anti-inflammatory M2 macrophages in adipose tissue [96]. Activation of DP2 receptors in 3T3-L1 adipocytes inhibited lipolysis through inhibition of the cAMP/PKA pathway and promoted triglyceride accumulation [94]. This is in line with *in vivo* data, as mice overexpressing PGD2 (a ligand for the DP2 receptor) exhibited increased body weight gain under HFD in comparison with controls. Moreover, these mice showed decreased serum triglycerides and improved insulin sensitivity [97]. Lastly, activation of the IP receptor, in human multipotent adipose-derived stem cells, promoted the transition from white to beige adipocytes [98]. All in all, these results demonstrate that prostaglandin receptors can modulate adipose tissue function in various ways.

Adrenergic receptors

β-adrenergic receptors are among the best-described receptors in adipose tissue, regulating cold- and diet-induced thermogenesis in BAT [4]. There are three β-adrenergic receptors (β1AR, β2AR and β3AR) in mice and humans [99,100]. All three types are expressed in white and brown adipocytes with β3AR showing the highest expression of all β-adrenergic receptors in adipose of mice [101]. Activation of β-adrenergic receptors occurs via the release of catecholamines from the sympathetic nerve terminals. This leads to the induction of lipolysis and BAT thermogenesis [102]. The contribution of each receptor was dissected using knockout and overexpression studies.

β1AR plays a crucial role in both cold- and diet-induced thermogenesis. This was demonstrated using β1AR knockout mice. These mice were hypothermic when cold challenged and gained significantly more weight under HFD, in comparison with controls, indicating a deficit in cold- and diet-induced thermogenesis. Moreover, β1AR knockout mice developed insulin resistance [103]. Furthermore, overexpression of the β1AR, under the control of the aP2 promoter, partially protected mice from DIO [104]. Deletion of the β2AR did not impair cold- or diet-induced thermogenesis, but glucose homeostasis [105]. Activation of β3AR in brown adipocytes promoted lipolysis and increased oxygen consumption [106], and even when mice were housed at thermoneutrality, reduced fat mass and improved glucose tolerance upon HFD feeding [107]. Counterintuitively, β3AR knockout mice are cold tolerant with only a modest increase in adiposity [108], which is exacerbated under HFD [109]. This could be explained by increased β1AR and UCP-1 expression in BAT in comparison with control mice. Moreover, UCP1 expression can be induced by activation of β3AR or β1AR (but not β2AR) in human brown adipocytes derived from multipotent adipose-derived stem cells. Thus, β1AR can substitute for the action of β3AR in β3AR knockout mice [110].

Beige adipocytes are therapeutically interesting to reduce body fat and β3AR agonist treatment-induced beiging of certain WAT depots [111]. Moreover, β3AR knockout mice showed an inability to recruit beige adipocytes in WAT [112,113]. However, this was shown to be dependent on the genetic background, as β3AR knockout mice on a FVB/N background normally developed beige adipocytes upon cold exposure, while β3AR knockout mice on a C57BL/6 and 129Sv background did not [114]. Additional data showed that β1ARs are required for cold-induced beiging [115]. All in all, β-adrenergic receptors have a prominent role in adipose tissue and are interesting therapeutical targets for combating obesity. However, the great limitation for the use in humans is the important role of adrenergic receptors in the human heart raising strong safety concerns regarding side effects upon β-adrenergic receptor activation in humans [116]. Nevertheless, adipose restricted β3AR activation would be a promising therapeutic approach to reduce body weight and restore glucose and lipid homeostasis.

In addition to β-adrenergic receptors, two α-adrenergic receptors have been identified. α2-adrenergic receptor (α2AR) exhibits anti-lipolytic effects and inhibits cAMP production, thus, antagonizing the effects of β-adrenergic receptors [117–119]. An increase in α2AR and the ratio between α2AR/βAR was found in adipocytes from obese humans [120–126]. Moreover, in animal models, the α2AR/βAR ratio is correlated with obesity and an increase in α2AR is associated with adipose hypertrophy [120,121,123–128]. Overexpression of α2AR in the adipose tissue of mice lacking β3AR, which resembles the situation in humans where there is low β3AR and high α2AR expression, showed that these mice are more susceptible to HFD induced weight gain. Surprisingly, these mice exhibited normal glucose and insulin levels and the increase in fat mass was due to adipose tissue hyperplasia rather than hypertrophy [129]. Conversely, the α1-adrenergic receptor regulates lactate production and glycogenolysis and is not linked to lipolysis [118].

Chemokine receptors

Chemotactic cytokines or chemokines compose a family of secreted proteins that were classically thought to direct the migration of leucocytes. However, it is now clear that chemokines regulate the physiology of most cell types. Chemokine actions are mediated via binding to chemokine receptors, which are divided into typical chemokine receptors (GPCRs) and atypical chemokine receptors. To date, there are 18 typical chemokine GPCRs described [130] and here we will discuss selected examples that regulate adipocytes/adipose tissue function.

CXCR2 plays a role in neutrophil recruitment, as well as in adipocytes. The knockdown of CXCR2 inhibits adipogenesis in immortalized preadipocytes [131]. In line with this, CXCR2 knockout mice show smaller and fewer adipocytes in different fat depots, possibly due to a reduction in adipogenesis. Interestingly, this

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phenotype is only seen in female, and not male, mice [132]. Moreover, CXCR2 knockout mice are protected from HFD induced insulin resistance [133].

Another well-studied member of the chemokine GPCRs in adipose tissue is CXCR4. CXCR4 is expressed on adipocytes [134] and targeted deletion of CXCR4 in fat depots of mice using the aP2-Cre promoter (AdCXCR4KO mice) resulted in elevated body weight gain upon HFD feeding in comparison with controls, due to increased fat mass. Moreover, AdCXCR4KO mice are cold intolerant with reduced BAT activity upon HFD feeding, due to decreased mitochondrial biogenesis and expression of oxidative phosphorylation genes in BAT of mice fed a HFD and housed at room temperature (25°C) and upon acute cold exposure. However, AdCXCR4KO mice are not glucose intolerant or insulin-resistant despite increased adiposity and cold intolerance. This phenotype is only observed when CXCR4 is deleted in adipocytes and not in myeloid leucocytes (macrophages) [135]. Moreover, the administration of a CXCR4 antagonist in mice led to decreased M1 macrophage recruitment to WAT of obese mice, resulting in reduced inflammation and improved insulin sensitivity in WAT (and other tissues) and improved systemic glucose tolerance [136]. In line with this, antibody-mediated blocking of CXCL12 (a ligand for CXCR4) led to improved adipose tissue and whole-body insulin sensitivity [137].

Thus, these two examples demonstrate the intricate role of chemokine receptors in adipose tissue and that more studies are needed to further elucidate their potential as targets for combating obesity and its co-morbidities.

Secretin GPCRs

Secretin GPCRs include various receptors that are pharmacological targets for the treatment of cardiovascular disease, psychiatric disorders and diabetes [138]. Some of these receptors play an important role in maintaining adipose tissue function. Two good examples are glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptors.

GLP-1 receptor

GLP-1 is produced from enteroendocrine L cells in the small intestine. It is produced after nutrient ingestion and has important effects on different organs [139] including adipose tissue. GLP-1 is well known for its antidiabetic effect in healthy and diabetic individuals [140]. The actions of GLP-1 are mediated via the GLP-1 receptor which is expressed in several tissues like the pancreas, central nervous system and adipose tissue. Isolated plasma membranes from rat adipose tissue [141], 3T3-L1 adipocytes [142,143] as well as human adipose tissue [144] have been shown to express GLP-1 receptor. Interestingly, the adipose tissue GLP-1 receptor isoform seems to differ from the pancreatic isoform [145,146]. Early studies demonstrated that GLP-1 can have either lipolytic [145] or lipogenic effects [147] in isolated rat adipocytes and rat adipose tissue explants, respectively. Later it was demonstrated that low doses of GLP-1 have a lipogenic effect, while high doses have a lipolytic effect in isolated human adipocytes [146]. Another prominent role of GLP-1 is to enhance insulin-stimulated glucose uptake, which was demonstrated in 3T3-L1 [143] and isolated rat adipocytes [148]. In isolated rat adipocytes, GLP-1 stimulated glucose uptake on its own and had an additive effect together with insulin [149]. Moreover, GLP-1 treatment increased GLUT1 and GLUT4 protein levels in 3T3-L1 adipocytes [150] and increased the phosphorylation of the insulin receptor substrate 1 (IRS-1) and other signaling molecules downstream of the insulin receptor (IR) [151]. This increased insulin sensitivity is seen in *ob/ob* mice due to the alleviation of endoplasmic reticulum stress [152]. Furthermore, GLP-1 receptor activation promotes preadipocyte proliferation and differentiation by inhibiting apoptosis [153].

In vivo, administration of GLP-1 receptor agonist (exenatide) increased lipolysis in Wistar rats [154]. Additionally, the adenoviral administration of GLP-1 reduced adipose tissue inflammation in *ob/ob* mice [155]. In humans, GLP-1 receptor expression was increased in visceral fat of obese individuals with insulin resistance [144]. Moreover, exenatide administration ameliorated adipose tissue insulin resistance [156] and decreased inflammation in visceral fat [157]. However, the exact physiological role of the GLP-1 receptor in adipocytes remains to be elucidated.

GIP receptor

Another prominent member of the secretin receptor family is GIP. The GIP receptor is expressed on rat and 3T3-L1 adipocytes [158]. GIP, like GLP-1, is an incretin that stimulates insulin secretion from pancreatic β-cells upon meal ingestion. GIP increased glucose uptake and fatty acid synthesis in isolated rat adipocytes [159]. Moreover, GIP stimulated lipoprotein lipase activity in explants of rat adipose tissue [160].

Mechanistically, this is based on the activation of AKT and inhibition of AMP-dependent protein kinase (AMPK) and LKB1 phosphorylation in the presence of insulin, as seen in human adipocytes [161]. Like insulin, GIP promotes AKT phosphorylation leading to GLUT4 translocation in 3T3-L1 adipocytes. Additionally, knockdown of GIP receptor in 3T3-L1 preadipocytes inhibited adipogenesis [162]. Interestingly, global GIP receptor knockout mice are protected from DIO and insulin resistance [163]. Surprisingly, the deletion of the GIP receptor in adipose tissue via the aP2-Cre promoter ($GIPR^{adipo-/-}$) did not result in differences in body fat upon HFD feeding. However, $GIPR^{adipo-/-}$ mice showed improved insulin sensitivity and a reduction in lean mass, as well as reduced interleukin (IL)-6 expression in adipose tissue and decreased hepatosteatosis upon HFD feeding [164].

Adhesion GPCRs

The human genome encodes more than 30 adhesion GPCRs. Adhesion GPCRs are characterized by long N-termini containing adhesion domains (e.g. epidermal growth factor-like repeats) capable of mediating cell–cell and cell–matrix interactions [165]. Adhesion GPCRs play different roles in adipocytes/adipose tissue physiology. Most adhesion GPCRs are expressed in human and mouse adipose tissues [166]. Knockdown of GPR56, GPR64, GPR116, GPR124, GPR125 and GPR126 decreased adipogenesis as seen by decreased lipid accumulation. Additionally, GPR64 activation decreased adiponectin secretion and glucose uptake and increased lipolysis in 3T3-L1 adipocytes [166]. Knockdown of GPR116 also inhibited the differentiation of 3T3-L1 preadipocytes and adipose tissue-specific deletion of GRP116 resulted in decreased epididymal adipocyte size. Furthermore, plasma adiponectin levels were decreased and resistin levels increased, suggesting impaired adipocyte function. Additionally, these mice were glucose intolerant upon chow diet and HFD feeding and insulin-resistant upon HFD feeding [167].

Frizzled GPCRs

Frizzled receptors

Frizzled receptors are crucial for cell proliferation and differentiation as well as regulation of cell polarity [168]. The 10 mammalian frizzled (FZD) receptors are seven transmembrane receptors, with best-known function in inhibiting adipogenesis. FZD receptors mainly act as receptors for the 19 Wnt proteins. The initiation of the signaling cascade begins when Wnts bind to two receptors. The first interaction is with the cysteine-rich domain of the FZD receptor and the second one is with the low-density lipoprotein receptor-related protein (LRP) 5 or 6 [169]. This results in the stabilization of β-catenin and its translocation to the nucleus where it regulates gene expression. In addition, FZD receptors also initiate non-canonical signaling independent of β-catenin [169]. Of note, not all Wnt actions are through FZD/LRP receptors [170].

In MSCs, Wnt signaling inhibits adipogenesis and stimulates osteoblastogenesis. Wnt1 also inhibited adipogenesis of 3T3-L1 preadipocytes. This was mediated by inhibition of PPARγ and C/EBPα. Similarly, 3T3-F442A preadipocytes overexpressing Wnt1, injected subcutaneously into athymic mice, failed to develop into adipose tissue [171]. In line with this, activation of the FZD1 receptor stabilized β-catenin, promoted osteoblastogenesis and inhibited adipogenesis. Activation of FZD2 receptors also inhibited adipogenesis but did not affect osteoblastogenesis, which appeared dependent on β-catenin in the case of FZD1 receptor and β-catenin independent in case of FZD2 receptor [172].

Enzyme-linked receptors

Enzyme-linked receptors are receptors with intrinsic intracellular kinase activity. These can be tyrosine kinase receptors (e.g. IR), serine/threonine kinase receptors (e.g. TGF-β receptors) or receptors which do not have intrinsic intracellular activity. However, they can associate with intracellular molecules possessing kinase activity (e.g. TNF-α receptor) (see below). In all of these categories, there are receptors that play a crucial role in adipose tissue and few selected examples of each are described below.

Tyrosine kinase receptors

IR and IGF-1R

IR and insulin-like growth factor (IGF-1) receptor 1 (IGF-1R) signaling are among the best-studied signaling cascades in preadipocytes and adipocytes. To this end, it is of important to highlight their role in adipose tissue here. However, a detailed review of their role in adipose tissue would exceed the scope of this review. IR

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and IGF-1R belong to the tyrosine kinase receptor superfamily. However, unlike other members of the family, they exist as a covalent disulfide-linked dimer prior to ligand binding. Upon ligand binding, the tyrosine kinase domain phosphorylates tyrosine residues on the intracellular part of the receptor [173]. These phosphorylated residues act as a binding region for a multitude of adaptor and signaling proteins that regulate the pleiotropic effects of insulin/IGF-1 action. Importantly, the IR exists as two splice variants (IR-A and IR-B) and both can form heterodimers with the IGF-1R, creating six different combinations, which have been shown to differentially regulate metabolic or mitogenic effects of insulin/IGF signaling [174–176]. Moreover, we previously showed that the surface proteoglycan Glypican-4 interacts with the IR in preadipocytes and thereby regulates IR binding affinity to insulin [177], providing further complexity in the regulation of insulin action in these cells.

With respect to adipogenesis, both the IR and IGF-1R are expressed in pre and mature adipocytes [178,179]. It was already shown in the 1980s that IGF-1 is required for the differentiation of 3T3-L1 preadipocytes into mature adipocytes. This could also be achieved by using supraphysiological amounts of insulin [180], which remains part of the standard differentiation cocktail for adipocytes. Antibody-mediated blockage of the IGF-1R in human MSCs decreased proliferation and lipid accumulation [181]. However, there is also a role of the IR in adipogenesis as pluripotent stem cells from IR knockout mice differentiated poorly in comparison with control cells, as assessed by lipid accumulation and gene expression [182]. Thus, insulin/IGF signaling plays an important role in adipogenesis and the complex regulation of this signaling network through multiple receptor heteromers and modulatory surface proteins suggests adipose selective combinations could be explored to selectively modulate adipose function.

The central role of insulin action in adipose tissue and the fact that most other signaling cascades in one way or another impact on insulin action, requires a brief overview over its impact on adipose tissue. More detailed information can be found elsewhere [183]. IR and IGF-1R both play a crucial role in adipose tissue. Their function has been studied in great detail using conditional ablation in adipose tissues using different Cre-expressing mouse lines. Using adiponectin-Cre mice, the IGF-1R knockout slightly reduces BAT mass, but does not impact on its function as assessed by its ability to maintain body temperature under cold exposure. Meanwhile, the size of WAT is reduced by 25% with concurrent reduction in leptin and adiponectin levels. The effect of IR deletion in adipose tissue is more pronounced. In adipose-specific IR knockout mice, WAT mass is greatly reduced (by 90%). These mice are insulin resistant and exhibit compensatory β-cell hyperplasia throughout life. Interestingly, BAT of IR knockout mice is increased (by 50%) with the appearance of large unilocular adipocytes. Moreover, BAT function is impaired. The deletion of both the IR and IGF-1R resulted in a more severe phenotype with an almost complete absence of WAT and an 85% reduction in BAT mass. These double knockout mice were also highly cold intolerant [184]. The deletion of the IGF-1R and IR using the aP2-Cre promoter resulted in different phenotypes than with the adiponectin-Cre promoter. aP2-Cre-mediated IGF-1R knockout mice showed an increase in WAT mass with an increase in overall growth related to a modest increase in IGF-1 levels [185]. Deletion of the IR or both the IR and IGF-1R using the aP2-Cre promoter resulted in a modest decrease in WAT with an improved glucose tolerance under HFD [186,187]. These differences are thought to results from incomplete deletion using the aP2 promoter, further highlighting the requirement of fine balanced insulin/IGF-1 action in adipose tissue.

The difference in the phenotype observed between the adiponectin-Cre IR knockout and IGF-1R knockout could be due to differences in expression of these receptors during adipogenesis. The IGF-1R is higher expressed in preadipocytes than the IR [188,189], while at this stage adiponectin expression is low and no gene deletion is expected [190,191]. However, IR expression increases with differentiation and is more expressed in mature adipocytes than the IGF-1R [192] and at this time adiponectin expression is high [193] ensuring high recombination efficacy. Interestingly, IR and IGF-1R regulate identical gene expression in murine brown adipocytes [188]. Thus, the differences seen *in vivo* could be a result of different ligand concentration and availability as well as different extent and timing of receptor expression.

PDGF receptors

Platelet-derived growth factor receptors (PDGFR) α and β are class III tyrosine kinase receptors. Upon ligand binding, dimerization of the receptor occurs followed by autophosphorylation of the receptor on tyrosine residues, initiating downstream signaling [194]. PDGFRα was suggested as a marker for adipocyte progenitors [195] and both PDGFRα and β are expressed in 3T3-L1 preadipocytes, while their expression diminishes upon differentiation [196]. The role of PDGFRs in adipogenesis is controversial. PDGF-AA promoted adipogenesis

while PDGF-BB inhibited adipogenesis in 3T3-L1 cells [197]. Early studies suggested that PDGF enhances differentiation of 3T3-L1 preadipocytes [198] and acts anti-apoptotic [199]. Others showed that PDGF inhibits differentiation of human adipose stromal cells [200], human preadipocytes and murine 3T3-L1 preadipocytes [201]. Inhibition of adipogenesis was accompanied with an increase in the inhibitor κB kinase β (IKKβ) in human subcutaneous preadipocytes [202]. Moreover, blocking PDGFRα and β promoted adipogenesis via suppression of phosphatidylinositol-3-kinase (PI3K) in human MSCs [203]. Thus, increasing evidence suggests an inhibitory role of PDGFR signaling in adipogenesis. In addition, PDGFRα and β differentially impact on preadipocyte fate as PDGFRα$^+$ cells give rise to both beige and white adipocytes in murine abdominal WAT under β3 adrenergic stimulation and HFD feeding [27]. This was further corroborated by another study showing that adipocyte precursors with dominant PDGFRα expression and signaling differentiated to beige adipocytes while adipocyte precursors with dominant PDGFRβ expression and signaling differentiated into white adipocytes in mice [31]. However, PDGFRβ$^+$ mural preadipocytes can differentiate to beige adipocytes under prolonged cold exposure in mice, indicating that PDGFR expression does not mark distinct developmental lineages [32]. These data demonstrate the intricate role of PDGFRs in adipogenesis and cell fate determination and highlight the complex interplay between receptor/ligand combinations in the regulation of cellular outcome.

FGF receptors

Fibroblast growth factor (FGF) receptors (FGFRs) are single-pass transmembrane receptors with an intracellular tyrosine kinase domain. There are seven FGFRs made from four different genes (FGFR1–4) by alternative splicing. Activation of FGFRs results in activation of PI3K-AKT, RAS-RAF-MAPK, STAT and PLCγ pathways [204].

In murine and human WAT, FGFR1 is highest expressed, followed by FGFR2, while expression of FGFR3 and 4 were not detected [205–207]. FGFR1 and 2 expression levels gradually declined during differentiation in 3T3-L1 adipocytes [208]. FGFR1 was shown to regulate adipogenesis in murine and human preadipocytes as well as MSCs [209–211]. *Pdgfrα*-CreER^{T2};*Fgfr1*$^{flox/flox}$ mice lacking *Fgfr1* in APCs showed adipocyte hypertrophy in response to HFD feeding to compensate deficits in adipogenesis [212].

FGFR1 also regulates glucose and lipid metabolism in mature adipocytes [213,214]. The FGF1-induced glucose-lowering effect was blunted in adipose FGFR1 knockout (aP2-Cre;*Fgfr1*$^{flox/flox}$) mice [215]. Furthermore, antibody-mediated activation of FGFR1c increased glucose uptake in murine WAT and BAT [214]. FGFR1, but not FGFR2, deficiency in white adipocytes increased adipocyte lipase activity in response to fasting, resulting in hepatosteatosis [216]. Furthermore, lack of the FGFR1 in white adipocytes increased lipogenesis and lipid droplet size due to accelerated phospholipid biosynthesis [217]. These findings suggest that FGFR1 signaling regulates glucose and lipid metabolism in mature white adipocytes.

Paracrine FGFs, such as FGF1, 6 and 9, that have heparin-binding domains can directly activate FGFRs. In contrast, endocrine FGFs (FGF19 subfamily) including, FGF19, 21 and 23 lacking this domain require the co-receptor Klotho to activate FGFRs [205,206,218,219]. βKlotho, a type I membrane protein is up-regulated during adipocyte differentiation [208]. In contrast, obesity decreased the expression of βKlotho as well as FGFR1 in WAT [220,221]. The association of βKlotho with FGFR1 is necessary for FGF21-induced glucose uptake in WAT and BAT as well as cultured adipocytes [205,219,222–224]. FGFR1/βKlotho signaling also mediates the FGF21 effects on BAT thermogenesis [213,223,225], albeit its action includes non-adipocyte cells within BAT [226,227]. In contrast, FGFR3 regulates UCP1 dependent thermogenesis in brown adipocytes [228].

Tyrosine kinase-associated receptors

These receptors possess no intrinsic intracellular kinase activity, but signal through the recruitment of kinases upon ligand binding.

TNF-α receptors

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine [229] and high TNF-α levels in adipose tissue are associated with type 2 diabetes and insulin resistance [230,231]. In adipose tissue, the effects of TNF-α are mediated via two distinct receptors: TNF-α receptor (TNFR) 1 and 2. Both receptors belong to the TNFR superfamily and are expressed in many cell types including pre- and mature adipocytes [232]. Upon ligand binding to TNFR1 or TNFR2, homo-trimerization of these receptors occurs [233]. TNFR1 and 2 do not possess an intracellular catalytic domain and therefore depend on intracellular adaptor proteins to further transduce the signal. Activation of TNFRs can induce apoptosis or promote cell survival and a pro-inflammatory response.

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Both receptors are proteolytically cleaved to produce soluble forms [232]. These soluble receptor forms sequester ligands from binding to cell surface receptors inhibiting signal transduction [234].

TNF-α inhibits adipocyte differentiation [235–237], during the first 24–36 h after induction (commitment phase) as the addition of TNF-α after this time period did not impair differentiation [238]. The inhibitory action of TNF-α is mediated via TNFR1 as the deletion of TNFR1 in preadipocytes blocks TNF-α effects [237]. Mechanistically, TNF-α blocked C/EBPα and PPARγ expression and promoted expression of anti-adipogenic genes. Moreover, TNF-α induced the de-differentiation of mature adipocytes *in vitro* [239,240].

TNF-α also inhibited insulin action in murine adipocytes by inhibiting tyrosine phosphorylation of the IR and IRS-1 [241], which could mediate the observed de-differentiation/delipidation described above. Moreover, TNF-α down-regulates several proteins involved in insulin action (e.g. GLUT4) [242–244], which appears to be mediated by TNFR1, as deletion of TNFR1 blunted the effects of TNF-α treatment [245]. Moreover, *ob/ob* mice lacking TNFR1 showed improved insulin sensitivity [246] and global TNF-α knockout mice had improved insulin sensitivity in comparison with controls [247]. A more detailed review on the role of TNF-α in the adipose tissue can be found in [232].

Serine/threonine kinase receptors

Transforming growth factor beta (TGF-β) receptors (TGFBRs) are transmembrane serine/threonine kinase glycoproteins with well-established roles in adipocyte differentiation and function. There are two receptor types (I and II) with five type I and seven type II receptors. Binding of a TGFBR ligand results in an interaction of receptor type I and II. In the canonical signaling pathway, the signal is then propagated through the phosphorylation of Smad proteins. However, other non-canonical signaling pathways have been reported including β-catenin/tcf4, p38 MAPK, ERK and JNK [248]. Preadipocytes express both TGFBRs and expression of these receptors decreases during differentiation [249].

Activation of the TGF-β superfamily receptors has different effects on adipogenesis, depending on the ligand/receptors activated. Bone morphogenetic protein (BMP) 4 induced mouse embryonic stem cells to differentiate into adipocytes [250]. Moreover, the treatment of C3H10T1/2 pluripotent stem cells with BMP4 triggered commitment to the adipocyte lineage. Furthermore, treatment of C3H10T1/2 with BMP4 in culture followed by transplantation of those cells in the subcutaneous adipose tissue of athymic mice resulted in the formation of WAT indistinguishable from normal adipose tissue [251]. Interestingly, BMP4 treatment suppressed UCP-1 expression while increasing lipid accumulation in brown preadipocytes [223]. However, the role of BMP4 on the differentiation of brown and beige adipocytes is controversial [252]

BMP7, which is another member of the TGF-β superfamily, also promotes adipogenesis [253,254]. In brown preadipocytes, the addition of BMP7, in the absence of an induction cocktail, induced differentiation and induction of UCP-1. This pro-adipogenic role of BMP7 includes suppression of adipogenic inhibitors like Pref-1 and Wnt10a, while increasing expression of pro-adipogenic genes like PPARγ, C/EBPα and aP2. BMP7 also drove brown adipogenesis in mesenchymal progenitor cells [255].

Other members of the TGF-β superfamily inhibit adipogenesis. TGF-β1 inhibits adipogenesis in both 3T3-L1 [256] and 3T3-F442A cells [249]. TGF-β1 also reduced lipid accumulation in primary cultures of pig subcutaneous adipose tissue [257]. Interestingly, inhibition of TGFBR1 promoted beiging in undifferentiated cells of the epididymal murine SVF. Similarly, subcutaneous transplantation of SVF cells from adipose tissue-specific TGFBR1 knockout mice into nude mice showed that knockout of the TGFBR1increases beiging in HFD fed mice after β-adrenergic stimulation [258]. Moreover, there are additional receptors of this family that showed mixed effects on adipogenesis and are reviewed in detail elsewhere [248].

In adipose tissue, activin receptor-like kinase 7 (ALK7), is a TGFBR1that is activated by growth differentiation factor 3 (GDF3) [259,260]. Mice lacking ALK7 receptor have reduced fat mass upon HFD feeding reminiscent of *Gdf3* knockout mice [259]. Conversely, activation of the ALK7 receptor increased adiposity by suppression of lipolysis [261]. These data demonstrate the crucial function of TGFBR superfamily in adipose tissue.

Ion-channel linked receptors

Ion-channel linked receptors are transmembrane proteins that undergo conformational changes upon activation, allowing selective ions to pass through the channel and across the membrane [262]. This group of receptors plays a role in various tissues including adipose. Activation of transient receptor potential vanilloid type 1

channel inhibits adipogenesis [263]. Similarly, blockage of the chloride channel 3 on human subcutaneous pre-adipocytes by tamoxifen inhibits the proliferation of these cells [264].

K^+ channels regulate the proliferation of human preadipocytes [265]. Furthermore, activation of the ionotropic purinergic cation channel P2X7R decreased adipogenesis and increased osteogenesis in rat MSCs [266]. Our group also demonstrated that P2RX5 is highly expressed in BAT in comparison with WAT and other tissues and therefore could be used as a cell surface marker for brown adipocytes. Yet it's function remains unknown [20]. Many other ions channels exist in adipose tissue and could be considered as pharmacological targets, which are discussed in [267].

Transporters

Apart from the groups/categories mentioned above, there are transporters that are pivotal for adipose tissue and whole body normal physiology but do not fit in the above-mentioned classification. Two good examples of these receptors are carbohydrate and fatty acid transporters which have been shown to play a crucial role in the adipose tissue.

GLUT4

Insulin action is the most important regulator of glucose uptake in adipose tissue, by mediating the translocation of the GLUT4 to the plasma membrane [268]. GLUT4 is a part of 14 member protein family that mainly share structural similarities [269].

GLUT4 was first identified in rat adipocytes by screening for proteins that translocate from the intracellular fraction to the plasma membrane upon insulin stimulation [270]. The gene for GLUT4 (*Slc2a4*) was later cloned from rat adipose tissue [271,272]. The pivotal role of GLUT4 in adipose tissue was demonstrated by overexpression and knockout studies [273–275]. The knockout of GLUT4 in adipose tissue (using aP2-Cre mice) resulted in glucose intolerance and insulin resistance. This insulin resistance was also observed in skeletal muscle and liver where GLUT4 expression is intact [273]. Moreover, overexpression of GLUT4 in adipose tissue enhanced glucose tolerance while increasing adiposity. Interestingly, this increase in adiposity was due to adipocyte hyperplasia with no change in adipocyte size, further strengthening the conclusion that a shift from hypertrophy towards hyperplasia has beneficial metabolic effects even in the context of obesity [274]. Additionally, adipose tissue overexpression of GLUT4 in mice lacking muscle GLUT4 restored insulin sensitivity and improved glucose tolerance. However, these mice showed an increased fat mass, increased serum FFA and leptin levels, but decreased adiponectin levels [275]. These results underscore the important role of adipose GLUT4 and the IR (see above) in regulating systemic glucose homeostasis, but also highlight that the adipocyte surface is continuously remodeled depending on the metabolic state of the organism.

CD36

Like GLUTs, fatty acid transporters also play an important role in adipocyte function. In fact only a small proportion of triacylglycerides stored in adipocytes derive from glucose through *de novo*-lipogenesis, whereas the vast majority is esterified from circulating lipids. One prominent fatty acid transporter is CD36 [276–278]. CD36 is a scavenger receptor, which exhibits a hairpin-like topology with two transmembrane domains and both termini facing intracellularly. CD36 is widely expressed in various cell types including adipocytes [279] and is not the only fatty acid transporter expressed in adipocytes. Other important fatty acid transporters are reviewed elsewhere [280]. Of note, CD36 is expressed on other cell types and in these cells, it plays different roles [281]. CD36 is complexed with prohibitin and annexin 2 at the plasma membrane of endothelial cells as well as adipocytes, mediating transendothelial fatty acid transport to adipocytes [33].

CD36 is induced upon adipocyte differentiation [278], but can be also detected in human preadipocytes [282]. Uptake of labeled oleate was reduced in adipocytes isolated from CD36 knockout mice [283]. Moreover, injection of CD36 knockout mice with fatty acid analogs (BMIPP and IPPA) showed impaired uptake in adipose tissue [284]. CD36 knockout mice were protected from DIO exhibiting reduced fat mass [285]. Under HFD feeding, CD36 knockout mice showed improved glucose tolerance and insulin sensitivity compared with controls. Interestingly, primary adipocytes isolated from CD36 knockout mice showed a reduced pro-inflammatory response to lipopolysaccharides. In accordance with this, adipose tissue from these mice showed reduced inflammation with less macrophage infiltration [286]. Moreover, gonadal adipocytes from CD36 knockout mice showed increased lipolysis and elevated insulin sensitivity [287]. However, this is in contradiction to another report showing that knockdown of CD36 in 3T3-L1 adipocytes impairs isoproterenol

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(β-adrenergic receptor agonist) stimulated lipolysis, which was also observed using adipose tissue cultures from CD36 knockout mice *ex vivo* [288].

In humans, CD36 deficiency is accompanied with mild fasting hyperglycemia, hyperlipidemia and insulin resistance [289]. Furthermore, common variants in the CD36 gene are associated with the metabolic syndrome [290]. Expression of CD36 in adipocytes is positively correlated with systemic and adipose tissue insulin sensitivity in obese non-diabetic individuals [282]. However, other studies found an up-regulation of CD36 in insulin-resistant individuals [291–294]. Thus, additional studies are needed to understand the regulation and role of CD36 in human adipose tissue and its contribution to the metabolic syndrome.

All receptors and transporters described above have important roles in regulating adipose tissue function and could provide interesting pharmacological targets. However, we chose these receptors/transporters to illustrate that while they possess interesting functions in adipose tissues, they also play important functions in cell types outside adipose. Thus, targeting them will have potentially severe side effects. Therefore, novel strategies are urgently needed to achieve adipocyte selective drug targeting to generate effective and safe pharmacotherapy for the metabolic syndrome.

Chasing adipose selective drug delivery

A multitude of studies have identified marker genes distinguishing adipocyte subtypes, such as white versus beige versus brown adipocytes [295–297]. Identification of such markers is important for the characterization of dissected tissues or cells in culture to estimate the relative contribution of one cell type versus the others or changes in the abundance of certain cell types in disease states. However, most of the identified markers are intracellular proteins, which limit their use for cell sorting or other applications aiming to work with living cells. To this end, cell surface proteins have the clear advantage that they are readily accessible by various molecules (small molecules, antibodies, peptides, aptamers, etc.) to stain, interfere with their function or hijack their cell type-specific expression to facilitate tissue-selective drug delivery. Unfortunately, to date, no unique cell surface protein for adipose tissue has been identified. We previously identified three surface markers for white, beige and brown adipocytes, respectively, which remain the most adipocyte selective surface proteins [20]. However, ASC-1 is also expressed in the central nervous system and P2RX5 in skeletal muscle, albeit at lower levels than in adipose. Low mRNA expression of PAT2 can be found outside beige/brown fat, but how that translates to protein levels remains unknown. Furthermore, we recently showed that the surface location of PAT2 is highly dependent on extracellular amino acid concentrations, and could strongly fluctuate *in vivo* [298].

To this end, we came to the conclusion that, given the large number of distinct cell types in the mammalian organism, and the key cellular functions mediated by most surface proteins, the identification of a cell surface protein that is uniquely expressed in adipocytes will most likely be unsuccessful. However, at the same time, we are convinced that cell type-specific cell surface epitopes exist that can be utilized to facilitate target drug delivery. In contrast with cell surface proteins, surface epitopes could present protein/protein interactions, posttranslational modifications or even lipid modifications, thereby greatly extending the potential repertoire of possible targets (Figure 3). However, the great challenge is that we do not know the nature of these epitopes nor do we have knowledge of the potential targeting reagents. In the following part, we will discuss ways to extend the spectrum of surface epitopes and ways to identify and target these.

Extending the complexity of surface epitopes

Alternative splicing

Alternative splicing is one of the most important mechanisms to expand the diversity of proteins. In the last decade, advances in next-generation sequencing technologies combined with computational analyses revealed that up to 95% of multi-exon transcripts undergo alternative splicing [299,300]. This, however, could be a strong underestimation as Vaquero-Garcia et al. suggest that the classical binary definition of splicing variants (e.g. include or skip an exon) is too simplified [301]. They developed novel algorithms to define previously annotated classical alternative splicing as well as unknown more complex transcript variants, which account for over 30% of tissue-dependent transcript variants [301]. In addition, splicing is highly tissue and cell type dependent [299,300]. Thus, this additional level of complexity amplifies the chance to detect cell type-specific protein isoforms.

FGFR2b is one of three FGFR2s and preferentially expressed on preadipocytes compared with mature adipocytes [302,303]. Its signaling is important for the commitment of preadipocytes towards beige/brown adipocytes [302,303]. On the other hand, FGFR2c is expressed on mature white adipocytes and represses

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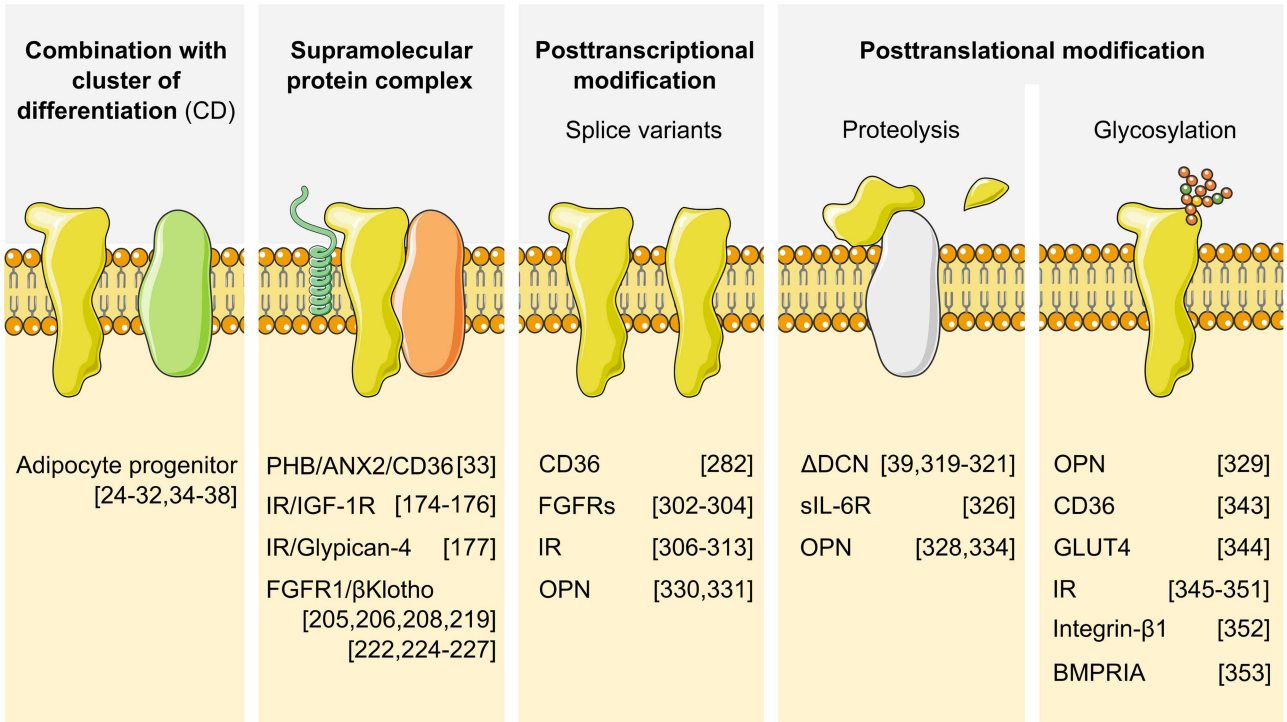

Figure 3. Extending cell surface epitope complexity beyond protein expression.

Diversity in cell surface epitopes is created through combination of protein expression and protein/protein interactions. Additional diversity in cell surface epitopes is achieved through posttranscriptional and posttranslational modifications. Splicing can be tissue and/or cell type specific. Proteolysis can generate tissue-specific fragments from ubiquitously expressed proteins. Glycosylation is one representative for posttranslational modifications further increasing surfome diversity.

thermogenic gene expression [304]. In line with this, adipocyte-specific FGFR2c knockout mice showed reduced hypertrophy in visceral WAT and reduced plasma FFA levels [304]. These findings indicate that splice variants of the same gene can facilitate very different or even opposing functions. Intriguingly, neutralization of FGFR2c with monoclonal antibodies did not reduce plasma FFA levels or induced thermogenic gene expression in WAT, although it suppressed body weight gain caused by social isolation in KKA^{y} diabetic mice [305]. This suggests that systemic inhibition of FGFR2c has different effects than adipose selective inactivation, further strengthening our conclusion that tissue-selective targeting is essential for next-generation therapeutics.

Another example for a metabolically relevant alternatively spliced receptor is the IR [306,307]. As already mentioned above, the IR is found as two splice variants IR-A and IR-B. IR-B has 12 additional amino acids at the C-terminus of the alpha chain, which results from alternative splicing of exon 11 [306,308]. IR-A is predominantly expressed in fetal, tumor tissues and preadipocytes, whereas IR-B is preferentially expressed in postnatal tissues such as liver, muscle, fat and kidney. IR-A has been associated with the mitogenic function of insulin, whereas IR-B correlates with the metabolic aspect of insulin action [309,310]. Importantly, the abundance of IR-B in adipose is changed in obesity, type 2 diabetes and weight loss [311,312]. Moreover, the splicing of IR-B appears to be regulated by insulin but not glucose levels [311,313]. Nevertheless, currently, no animal models are available to verify potentially distinct functions of these splice variants *in vivo*.

Proteolytic cleavage

In addition to posttranscriptional modifications, posttranslational modifications, such as proteolysis and glycosylation that is described below, provide an additional layer of diversification. Proteolytic cleavage of cell surface proteins can result in shedding via sheddases to release the ectodomain of single-pass transmembrane proteins, such as Pref-1 [314]. Furthermore, processing by intracellular proteases can release intracellular domains as

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described for Notch [315]. However, it is important to keep in mind that albeit most attention is being paid to the liberated protein fragments, the residual transmembrane peptides provide potentially unique surface epitopes that could be targeted.

Decorin is a secreted proteoglycan mediating cell–matrix interaction [316,317]. Proteolytic cleavage generates different isoforms [318]. For example, a non-glycosylated isoform (termed ΔDCN) lacking the N-terminal methionine, suggests that it is generated by proteolysis rather than alternative splicing [39]. ΔDCN accumulates exclusively on the cell surface of human and murine perivascular PDGFRα^{-}PDGFRβ^{+} APCs within WAT and is absent on MSCs in other tissues. Based on these characteristics a ΔDCN targeting peptide was generated and used to specifically deliver cargo into subsets of APCs [319–321]. Moreover, ΔDCN was shown to act as a resistin receptor to facilitate proliferation and migration of 3T3-L1 preadipocytes [39].

Development of obesity is associated with alterations in the expression of proteases, including matrix metalloproteases (MMPs) [322–324] and A disintegrin and metalloproteinases (ADAMs) [325–327] in WAT, locally changing the bioactivity of transmembrane proteins and cytokines [326,328]. IL-6 *trans*-signaling, where IL-6-bound soluble IL-6 receptor (IL-6R) binds to gp130 on the cell surface, but not classical transmembrane IL-6R signaling, contributes to diet-induced macrophage infiltration into WAT rather than liver [326]. Furthermore, osteopontin is heavily posttranslationally modified, including proteolysis [328–330]. Its levels increase in WAT upon HFD feeding and β3 adrenergic stimulation through secretion by macrophages [331–333]. This results in the recruitment of macrophages [332], T cells [334] and APCs expressing CD44 that acts as an osteopontin receptor [333]. Intriguingly, not only full-length osteopontin but also MMP-cleaved derivatives potentiate T cell viability [334]. Moreover, MMP-cleaved osteopontin blunts insulin sensitivity of adipocytes compared with full-length osteopontin [328].

Protein glycosylation

With increasingly advanced proteomics and metabolomics technologies, the number of identified posttranslational modifications is rapidly expanding [335–339]. Protein glycosylation is one of the best-studied posttranslational modification, generating large proteomic diversity. This diversity depends on glycan composition and complexity of branching, which is cell type-specific [340,341]. Glycosylation regulates the biological activity of proteins, such as cell–cell and cell–extracellular matrix interactions, ligand binding affinity, protein trafficking as well as subcellular localization and protein stability [177,342–344]. The IR, for example, is highly glycosylated in the ectodomains of the α and β subunit [345–351], which is essential for proper processing of the receptor [347,349,350]. Furthermore, site-specific mutation of N-glycosylation sites within the IR revealed that glycation of the α and β subunit regulate IR trafficking and tyrosine kinase activity [345,348].

Interestingly, N-glycosylation of the α subunit of the IR differs between adipocytes and the brain [346], highlighting that glycan structures are differently produced by numerous glycan-related enzymes in a tissue-dependent pattern [340]. Moreover, glycosyltransferases are differentially regulated in the adipose tissue upon obesity and directly impact on adipocyte differentiation and function through modulation of different cell surface proteins such as beta 1 integrin and BMP receptors [352,353]. Beta-galactoside α-2,6-sialytransferase-1 (ST6GAL1) was identified as the most down-regulated glycosyltransferase during adipocyte differentiation in visceral adipose tissue of DIO mice [352]. ST6GAL1 mediates sialylation of beta 1 integrin resulting in inhibition of adipogenesis. Expression of β1,4-galactosyltransferase 5 (B4GalT5) in subcutaneous adipose tissue is positively correlated with type 2 diabetes and obesity in human and mice [353], but is predominantly expressed in the SVF rather than mature adipocytes. B4GalIT5 inhibits adipocyte differentiation by modifying glycan structures of the type 1 BMP receptor [353].

Taken together, the combination of splice variants, tissue-specific posttranslational modifications and protein/protein interactions create a very large theoretical diversity at the cell surface that should provide adipocyte-specific cell surface epitopes. However, as the nature of these tissue-selective epitopes are very hard to predict, a diverse set of methods, outlined below need to be combined to not only identify these epitopes, but also to generate targeting vehicles for tissue-selective drug delivery.

How to identify adipose selective surface epitopes

Database mining

Comparative gene expression analysis has been a long-used tool to identify markers for tissues, cell types or disease states (Figure 4A). However, classically this approach is limited in its ability to predict tissue selectivity

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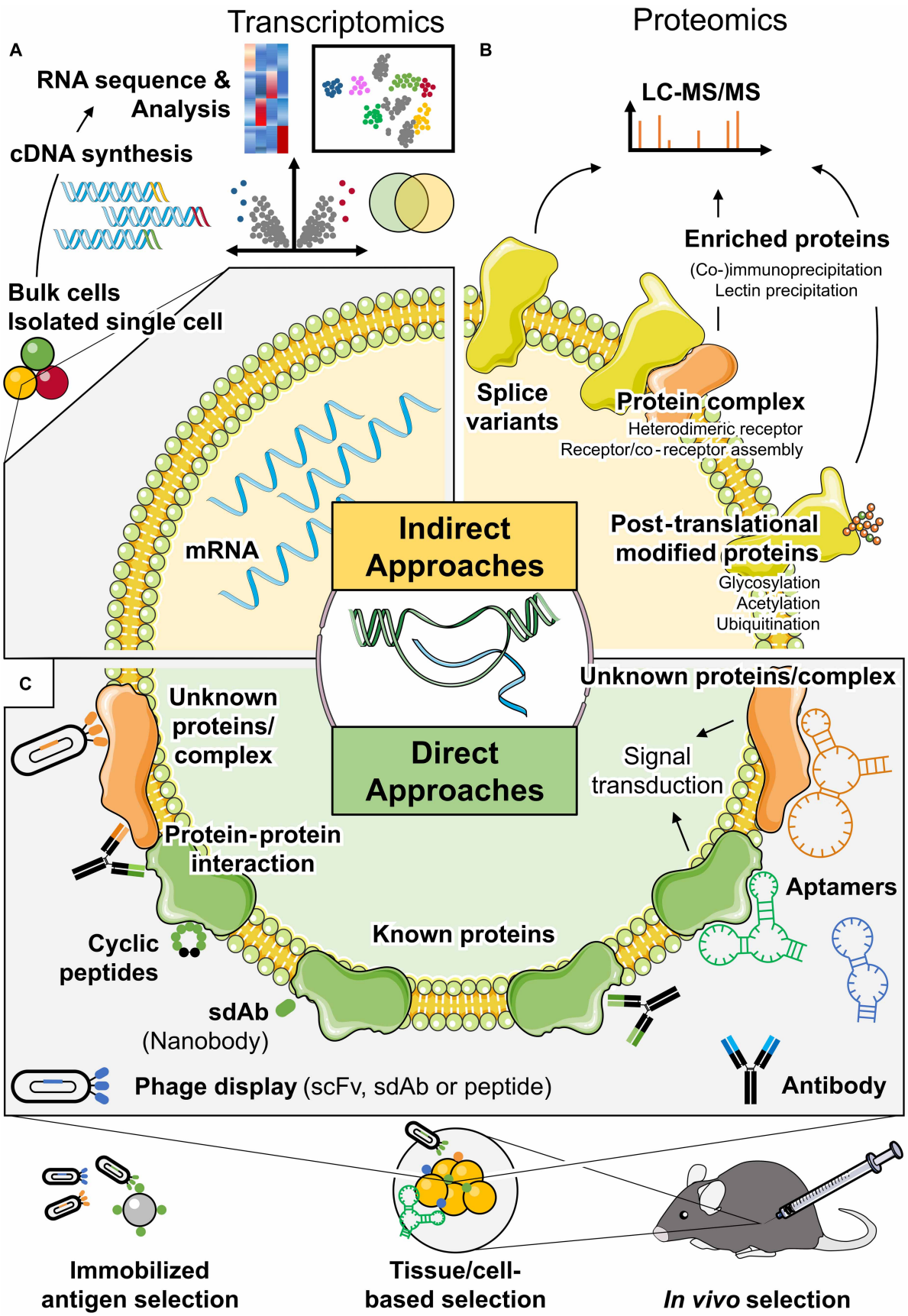

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Figure 4. Approaches for identification and characterization of cell surface epitopes.

(**A**,**B**) Indirect approaches to identify the cell surface epitopes. (**A**) Transcriptomics can provide information to predict potential unique epitopes and splice variants. (**B**) Proteomics identifies protein complexes and posttranslational modifications as well as splice variants. (**C**) Approaches capable of not only identifying but also targeting adipocyte selective epitopes. Antibodies, peptides and aptamers can all recognize epitopes of unknown proteins and protein complexes in addition to known proteins. These high affinity molecules are obtained by enrichment from large libraries screened with *in vitro* and *in vivo* methods. scFv, single-chain variable fragments; sdAb, single-domain antibody.

as a given data set is often only compared with one or a hand full of other cells, tissues or states. Nevertheless, comparisons of differentially expressed genes between adipose tissue depots, have revealed many interesting candidate genes to study adipose function [354]. To identify genes with adipose selective expression we also utilized multiple microarray datasets available from databases, such as the SymAtlas database (http://www.biogps.org) [20]. To identify genes with adipose selective expression encoding cell surface proteins we first selected all genes with high correlation to white and brown adipocyte-specific reference genes, such as *Adiponectin* and *Ucp1* and then ranked those according to their adipose selectivity. Finally, transmembrane protein-encoding genes were selected and individually verified on mRNA and protein level from a large multi-tissue panel. However, this strategy did not consider splice variants or any other posttranscriptional modifications. Today, with rapidly increasing numbers of RNAseq data from numerous species, organs and even single cells, such as in the human cell atlas, and increasingly sophisticated mathematical models and computational tools, a more detailed analysis for potential selective surface proteins might be possible in the near future. Nevertheless, this simple approach was critically important to advance our understanding that tissue selectivity is most likely, at least in adipose tissue, not encoded by individual genes, but the result of a more complex interactions of protein modifications and interactions.

Proteomics

While comparative gene expression analysis has a very high sensitivity, it is only an indirect method to identify cell surface proteins, not taking into account regulatory steps post transcription. Thus, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been used to successfully identify proteins in small amounts of crude protein lysate from culture cells and tissues (Figure 4B). Moreover, proteomics enables us to provide unbiased comprehensive information by matching the molecular mass of peptide fragments composing proteins in biological samples against databases [355]. Thus, mass spectrometry is an alternative approach to screen for adipose selective surface proteins, especially when combined with cell surface biotinylation or other approaches to enrich for cell membrane proteins [356,357], allowing the identification of modified proteins and profiling of posttranscriptional and posttranslational modifications, such as splicing, methylation, ubiquitination, acetylation, phosphorylation and glycosylation [335–339]. However, thus far these approaches have not been successfully used to identify tissue-selective surface epitopes, largely as they also require comparative analysis and reference data for the full surfome of all tissues and cell types is not available.

This should, however, by no means devalue the enormous progress that had been made in understanding cellular and organismal function using these methods. However, omics technologies always rely on a comparison between datasets and while comparisons to closely related cell types or organs will significantly increase the probability of identifying cell type-specific proteins or protein modifications, this remains the search for the needle in the haystack. Furthermore, identifying a tissue-specific epitope would be only the first step, as subsequently targeting vehicles need to be developed that then allow for tissue-selective drug delivery.

Selecting adipose tissue targeting reagents

The biggest challenge for identifying adipocyte selective targeting reagents is that neither the selective epitope nor the characteristics of the targeting reagent are known. To this end, selection methods are required where neither the reagent nor the target need to be known in advance. Thus, the only applicable methods are those, where pools of molecules are incubated with adipocytes or exposed to adipose tissue *in vivo* and selectively bound molecules can be extracted and subsequently characterized (Figure 4C).

Antibody-drug conjugates are the most frequently used active drug delivery moieties [358] and phage displays allow to screen large libraries of antibodies or peptides [359]. The technology is based on the finding that

bacteriophages can display exogenous random peptides fused with their coating proteins on the surface [360]. Building upon these bacteriophages have been modified to display single-chain variable fragments (scFv) linking the light- and heavy-chain variable region (V_L and V_H, respectively) capable of binding to antigens [361]. However, antibodies and scFvs remain challenging to use due to low stability and low solubility. In this context, single-domain antibodies provide an attractive alternative (sdAb, also referred to as nanobody). They mimic heavy chain only antibodies naturally found in camelidae species. Due to their small size (~15 kDa), sdAb could be used to target otherwise hidden epitopes [362–364].

Antibody phage libraries encoding various scFv are generated from nonimmune (naïve), immune and synthetic libraries. Naive libraries are constructed from antibody genes in lymphocytes of healthy subjects while immune libraries are built from that of immunized donors, infected or cancer patients. Naive libraries are unbiased despite low antigen specificity. Antibody repertoires in immune libraries have high antigen specificity even though the library size is small. The use of synthetic libraries allows displaying artificial unnatural scFvs by the replacement of genes encoding complementary determining regions in the V_H with random oligonucleotides. These libraries are constructed from human as well as rodent genomes so that antibody phage displays can provide human and mouse antibodies. Similarly, random synthetic peptide libraries possess large structural diversity complementing these antibody approaches [39,40,319,320,365].

The real advantage of these libraries, however, is that they can be used for biopanning (reviewed in [366]), which is a method to screen phage libraries against immobilized antigen on plates or beads, cell-based screenings, tissue-based screenings and/or *in vivo* screenings. Using scFv phage libraries Edwards and colleagues identified a set of antibodies binding to the cell surface of human adipocytes [367]. Interestingly, however, none of the tested antibodies was selective to adipose tissue and cross-reacted with at least one additional tissue. In contrast with this *in vitro* biopanning, the use of *in vivo* biopanning has major advantages as it allows selecting for specific binding of a biomolecules to a target tissue, while in parallel negatively selecting against all other tissues and cell types in the body. Indeed, using this *in vivo* approach novel peptides targeting murine BAT [365], WAT [368], and the adipose tissue endothelium [40], APCs [39,319,320] as well as adipocytes [33] were developed. Furthermore, targeting the adipose vasculature allowed the delivery of functional peptides and liposome into the adipose intercellular space [40,369–373], suggesting that the surfome of the endothelium could also be a promising target in adipose tissue. Importantly, *in vivo* biopanning is not limited to phages but can be also performed with other 'barcoded'/retrievable molecules such as aptamers [366].

Aptamers are randomly synthesized short (typically 40–100 nucleotides) single-stranded deoxy- or ribonucleic acids (ssDNA/ssRNA) that can fold into very heterogeneous three-dimensional structures and bind a wide variety of targets. Compared with antibodies, aptamers are much smaller, typically between 6 and 30 kDa, have greater stability and are cheaper to produce. In addition, aptamers can be easily conjugated to small molecules, siRNAs and used to decorate liposomes to function as drug delivery reagents. [374–376].

Similar to biopanning aptamers are selected through the systematic evolution of ligands by exponential enrichment (SELEX), which has been developed by Tuerk and Gold [377]. The SELEX protocol enriches potential aptamers through positive and negative selection, followed by PCR based amplification, single strand aptamer preparation and repeated selection. To date *in vivo* SELEX has not been utilized to identify aptamers binding to metabolically relevant tissues, but *in vitro* SELEX has been used to select white adipocyte selective DNA aptamers [378], albeit adipose selectivity was not shown for these sequences. Thus, both *in vivo* biopanning using phage libraries or *in vivo* SELEX provide interesting strategies to identify adipocyte cell surface epitopes that are truly selective for adipose tissue and target the full epitope space available.

Limitation and perspective

The human body contains many different adipose depots, some, such as brown and dermal adipose tissue [2,3,379], with specialized functions beyond energy storage that are not explicitly discussed here. These depots can have unique cell surface topology, gene expression, cytokine profiles and association with the metabolic syndrome [380,381]. Thus, much more research is needed to understand the heterogeneity between, but also within each adipose depot and its role in obesity and its associated co-morbidities to develop highly potent tissue-selective drugs combating the metabolic syndrome. The adipose surfome also contains many more interesting proteins and epitope classes than the few receptors/transporters discussed above. Examples for these are proteoglycans such as Glypican-4 [177] and Syndecan-1 [382], but also angiotensin-converting enzyme 2 (ACE2), which is currently extensively studied, as it serves as the docking receptor for SARS-CoV, NL63 and

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SARS-CoV-2 viruses [383–385]. ACE2 is a single-pass transmembrane glycoprotein [386,387] that is expressed on the surface of various cell types [388] and is higher expressed in adipose tissue than in the lungs [389]. Furthermore, increased adipose tissue mass (obesity) is linked to increased severity and worse outcome of COVID-19 [390–396]. However, the exact role of adipose ACE2 in physiology and disease remains to be largely determined.

Nevertheless, it is important to stress that while we highlight the high probability for adipose selective epitopes and discuss potential approaches to target these, proof that truly adipose selective epitopes exist remains to be experimentally verified.

Concluding remarks

Adipose tissue plays a central role in maintaining whole-body glucose and lipid homeostasis and the adipocyte cell surface is the central hub integrating various environmental and endocrine inputs to orchestrate its function. Moreover, the cell surface of adipocytes is the key target for future therapeutic attempts aiming to selectively deliver drugs to adipose tissue. To this end, a more detailed characterization of the composition of the adipocyte cell surface between different depots and healthy versus diseased states is pivotal to achieve this task. It is becoming increasingly clear, however, that posttranscriptional, posttranslational modification as well as protein/protein interactions need to be considered when aiming to identify unique cell surface epitopes.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

All authors wrote the manuscript, edited the text and approved the final version of the manuscript.

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Abbreviations

APCs, adipocyte progenitor cells; ATP, adenosine tri-phosphate; BAT, brown adipose tissue; cAMP, cyclic adenosine monophosphate; DIO, diet-induced obesity; FFA, free fatty acid; GLUT, glucose transporter; GPCRs, G protein-coupled receptors; HFD, high-fat diet; KLP4, krüppel like factor 4; LRP, lipoprotein receptor-related protein; MEFs, mouse embryonic fibroblasts; MMPs, matrix metalloproteases; MSCs, mesenchymal stem cells; PKA, protein kinase A; SVF, stromal vascular fraction; UCP-1, uncoupling protein 1; UTP, uridine triphosphate.

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