Adipose-PAS interactions in the context of its localised bio-engineering potential (Review)

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Abstract. Adipocytes are a known source of stem cells. They are easy to harvest, and are a suitable candidate for autogenous grafts. Adipose derived stem cells (ADSCs) have multiple target tissues which they can differentiate into, including bone and cartilage. In adipose tissue, ADSCs are able to differentiate, as well as providing energy and a supply of cytokines/hormones to manage the hypoxic and lipid/hormone saturated adipose environment. The plasminogen activation system (PAS) controls the majority of proteolytic activities in both adipose and wound healing environments, allowing for rapid cellular migration and tissue remodelling. While the primary activation pathway for PAS occurs through the urokinase plasminogen activator (uPA), which is highly expressed by endothelial cells, its function is not limited to enabling revascularisation. Proteolytic activity is dependent on protease activation, localisation, recycling mechanisms and substrate availability. uPA and uPA activated plasminogen allows pluripotent cells to arrive to new local environments and fulfil the niche demands. However, overstimulation, the acquisition of a migratory phenotype and constant protein turnover can be unconducive to the formation of structured hard and soft tissues. To maintain a suitable healing pattern, the proteolytic activity stimulated by uPA is modulated by plasminogen activator inhibitor 1. Depending on the physiological settings, different parts of the remodelling mechanism are activated with varying results. Utilising the differences within each microenvironment to recreate a desired niche is a valid therapeutic bio-engineering approach. By controlling the rate of protein turnover combined with a receptive stem cell lineage, such as ADSC, a novel avenue on the therapeutic opportunities may be identified, which can overcome limitations, such as scarcity of stem cells, low angiogenic potential or poor host tissue adaptation.

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Abbreviations: ADSC, adipose derived stem cell; PAS, plasminogen activation system; uPA, urokinase plasminogen activator; EC, endothelial cell; MSC, mesenchymal stem cell; BMSC, bone marrow MSC; DFAT, dedifferentiated fat cells; PPARγ, peroxisome proliferator-activated receptor γ; CEBP, CCAAT/enhancer binding protein; TNF-α, tumour necrosis factor-α; Wnt, Wingless; MCP-1, monocyte chemoattractant protein 1; uPAR, uPA receptor; FPRL-1, human formyl peptide receptor like-1; PAI-1, plasminogen activator inhibitor 1; LRP, lipoprotein receptor-related protein; ECM, extracellular matrix; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; HIF, hypoxia inducible factor; SMC, smooth muscle cell; WISP1, Wnt-1 inducible signalling pathway protein 1; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; PRF, platelet rich fibrin

Key words: adipocytes, uPA, PAI-1, PAS

Contents

1. Introduction

Epimorphosis, the regeneration of a specific part of an organism, such as a limb, does not occur in humans, and is limited to regrowth of the tips of the digits (1). The underlying process, specifically dedifferentiation (2), provides an interesting prospect for generation of scaffolds (3), facilitation of wound healing (4) and guided tissue regeneration (5,6). Stem cell therapy is now a well established field; however, treatments based on stem cell therapies are limited, primarily due to the ethical concerns regarding the sourcing of appropriate stem cells (7). Pluripotent cells are difficult to harvest without prior planning, and require the application of differentiation factors, several of which have multiple, and occasionally unpredictable effects on the cells (8). Multipotent cells are difficult to harvest due to their scarcity within each individuals body and

the requirements for cell surface marker screening, prolonged incubation to develop colonies and narrow therapeutic range limit their use (9). These challenges can also result in poor tissue integration and survival (10). By exploring the adipocytic tissue niche through embryological, physiological and pathological processes, the aim of the present review is to summarize the potential of dedifferentiation of mature adipocytes for therapeutic use. The physiological processes governing adipocyte development and adipose tissue maintenance are summarized to provide an understanding of some of the well established baseline physiological processes that serve as checks for adipocytic lineage commitment. These checks have been shown to possess a degree of pliability, and this is subsequently explored. Fundamental signalling elements are discussed, to provide an in depth look at pathways involved in adipocyte regeneration. The similarities between regeneration and growth of adipocyte tissue are compared to the developmental potential of the adipose lineage. Furthermore, the effects of molecules responsible for maintaining a homeostatic balance of the adipose tissue through these developmental, regenerative and pathological processes on the adjacent vasculature, and the interactions with the immune system are examined. To create a possible framework for clinical utilization of these findings, as well as to stimulate further research in the field, the potential for modulation of these pathways by repurposing currently available techniques used in regenerative medicine are highlighted.

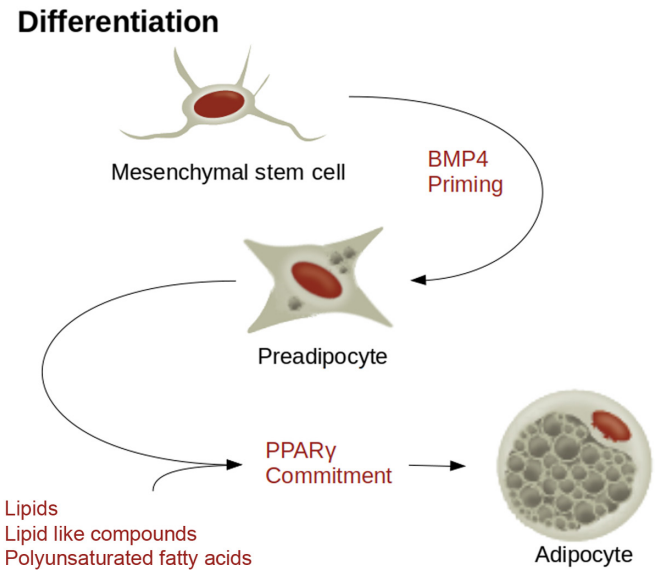

Figure 1. Stimuli mediating differentiation of MSCs to adipocytes. MSC, mesenchymal stem cell.

2. Adipocytes

Origins. Adipocytes are present throughout the body within adipose tissue (11). They primarily originate from the somitic mesoderm and posterior lateral plate mesoderm, and they share their origins with cardiac, vascular, muscle and connective tissues embryonically (12). Adipocytes are mesenchymal in nature, and they are present at a variety of stromal and visceral positions, in response to hormonal, physical (13,14) as well as sex-specific stimuli (15), making them adaptable to a wide range of environments. Adipocyte tissue is tasked with supporting endothelial glands (16,17) and epithelial membranes (18), as well as modulating the immune response (19), and they are composed of highly versatile cells. An important factor supporting the functional variety of adipocytes, which promotes their use in multiple clinical applications, is their origin from mesenchymal stem cell (MSC) progenitors, similar to that of osteoblasts, chondrocytes and myocytes (20).

MSCs have been shown to serve an adjuvant role with beneficial effects as a clinical adjunct in non-healing ulcers (21), tendon scarring (22) and osseous titanium implants (23), as well as in a variety of osseous, titanium, polymer and cartilaginous scaffolds (24,25). Injected aspirates rich in MSCs from bone marrow and adipose harvests have been shown to increase the speed of repair and longevity of torn tendons, non-union fractures, and osteoarthritis (26,27). MSCs obtained from a variety of sources, such as bone marrow and amniotic fluid, share a similar proteome (28). Cell cultures from the bone marrow, adipose tissue and umbilical cords also show similar growth patterns and cellular architecture, differing by the ease of harvest and size of the initial inoculum, which affects the subsequent growth rate and viability of cultures and grafts (29).

Adipose derived stem cells (ADSCs) are easy to obtain due to the abundance of adipose tissue, as well as the fact that the isolation time is reported to be as short as 30 min (30). The allogenic nature of ADSCs makes them more suitable for clinical use compared with the considerably harder to harvest umbilical cord MSCs (31), whereas bone marrow MSC (BMSCs) require a 24 h incubation to isolate suitable cultures on plastic (32).

Mature adipocytes can also be utilised clinically, for instance to form dedifferentiated fat (DFAT) cells, using a ceiling culture method, which provides a physical dedifferentiation stimulus on the cultured adipocytes. This physical stimulus activates the Wnt pathway, resulting in MSC-like protein expression and pluripotency (33). Activation of the Wnt pathway causes downstream peroxisome proliferator-activated receptor γ (PPARγ) inhibition (34). In addition to the ceiling culture method, there are experimental pharmacological approaches such as Chir98014 and Chir99021 that have been developed to achieve the same results (35).

ADSCs have been shown to be more versatile in adapting to surgical use than BMSCs (36). There is evidence to show that the versatility of ADSCs may be due to an intrinsic dedifferentiation potential, which is also partly involved in the wound healing response of adipocytes (37). Deeper insights into differentiation and dedifferentiation may shed light on the adipose mechanisms that control the roles of adipocytes in tissue repair.

Differentiation. PPARγ is considered the master regulator of proadipogenic differentiation since all stimuli of adipogenesis converge on it (38). Activation of PPARγ is both necessary and sufficient to induce adipocyte differentiation from MSCs (39). PPARγ has also been shown to be responsible for vascularisation, cardiac and placental development, and monocyte function (40,41). There are several molecules that can stimulate PPARγ mediated adipogenic differentiation (39,42). These include

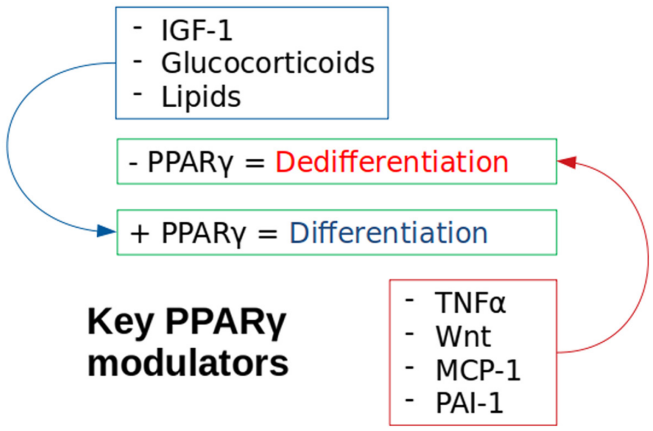

Figure 2. Significant molecules involved in regulation of PPARγ. PPARγ, peroxisome proliferator-activated receptor γ.

a variety of lipids and lipid-like compounds, including naturally occurring polyunsaturated fatty acids (Fig. 1). Co-stimulators such as CCAAT/enhancer binding protein β (CEBPβ) can significantly increase the speed of this process.

MSC sensitivity to PPARγ is reliant on a cascade of signalling factors. Bone morphogenic protein 4 signalling determines the adipose lineage, whereas CEBPβ stimulation by insulin like growth factor-1 or glucocorticoids stimulates the preadipocytes from a growth arrested state to re-entry back into the cell cycle, at which point PPARγ commits them to differentiate into terminal adipocytes (43). It is hypothesized that a potent factor in the induction of commitment to an adipocyte lineage is the environment (44). Plating MSCs at a high density, used to mimic heavy loading pressure in a bone environment or epiphyseal plates, was shown to preferentially induce differentiation into osteoblasts, as opposed to low density plating, which resulted in adipocytes (45). Similarly, the coculture of MSCs with mature adipocytes provides a positive feedback mechanism as terminal adipocytes are a source of PPARγ, stimulating further MSCs to differentiate into adipocytes (46). The molecules responsible for adipocyte differentiation can also serve as targets for their dedifferentiation (Fig. 2).

Dedifferentiation. Mature adipocytes have been shown to exhibit a plastic phenotype in a variety of conditions (33,37). Their dedifferentiation has been shown to function as a physiological process in the mammary glands (47) and hair cycling (48). It also readily occurs in association with pathological situations, notably inflammatory diseases (49), dermal fibrosis (50), cancer (51) and wound healing (52,53).

Inhibition of the PPARγ molecule is a method of forced dedifferentiation of mature adipocytes and has been achieved using tumour necrosis factor-α (TNF-α) (54) and wingless 3a (Wnt3a), a Wnt pathway activator (35). Notably, a decrease in PPARγ production in insulin resistant adipocytes has also been reported following an increase in insulin stimulated release of monocyte chemoattractant protein-1 (MCP-1) (55). TNF-α is present in the adipocyte environment, where its systemic levels are increased under inflammatory conditions such as obesity or insulin resistance (56), and locally under the same states following release of MCP-1 (57,58).

MCP-1 stimulation of integrin mediated cell adhesion and migration has been shown to be abrogated by a naturally occurring truncated soluble urokinase plasminogen activator receptor (uPAR) lysis product termed $D2D3_{88-274}$, which inhibits the human formyl peptide receptor like-1 (FPRL-1) G-protein coupled receptor (59,60). MCP-1 is also known to signal through a chemokine CC motif receptor 2 G-protein coupled receptor that is present in adipocytes (61). This pattern of activation of multiple G-protein activation coupled receptors is reminiscent of that observed with alarmins, which are damage associated molecular patterns (62). FPRL-1 activation stimulates αvβ3 integrin production (63), whereas $D2D3_{88-274}$ formation occurs after urokinase plasminogen activator (uPA)-plasminogen activator inhibitor-1 (PAI-1)-uPAR binding within the plasminogen activation system (PAS), and is necessary for fibroblast-to-myofibroblast differentiation (64). There are also reports of $D2D3_{88-274}$ being chemotactic itself, by activating FPRL1 with LXA4R, thus highlighting the importance of cytokine receptors and PAS in orchestrating inflammatory responses (65).

The αvβ3 vitronectin specific integrin has been found to allow MSC to activate Wnt signalling and maintain pluripotency (66), whereas Wnt signalling in turn activates PAI-1 production and inhibits PPARγ production (67). Since MCP-1-mediated stimulation of monocyte chemotaxis is reliant on FPRL-1 (59), and this in turn activates Wnt signalling through integrin production and activation, it is hypothesized that this may be the system by which MCP-1 exerts an inhibitory effect on PPARγ and subsequent adipose dedifferentiation (Fig. 3).

The activation of the Wnt signalling pathway has an inhibitory effect on PPARγ production and therefore inhibited adipocytic differentiation (68). 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, an AMP-activated protein kinase activator, enhances lipoprotein receptor-related protein (LRP)6 as well as β-catenin expression, the latter of which activates the Wnt/β-catenin pathway causing inhibition of expression of PPARγ, CEPBα, as well as their downstream transcription targets, fatty acid binding protein 4 and lipoprotein lipase (69). Wnt expression was also found to directly stimulate PAI-1 expression (67). Integrin secretion was found to be mediated by the Wnt/β-catenin signalling pathway via LRP (70).

TNF-α stimulated adipocyte dedifferentiation was found to be mediated by PAI-1, since PAI-1 deficiency caused an upregulation of PPARγ in TNF-α stimulated cells, resulting in abrogation of the dedifferentiation caused by TNF-α (71). The mechanism by which PAI-1 is upregulated is hypothesized to be mediated through TNF receptor stimulated production of reactive oxygen species, which ultimately propagates nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) PAI-1 gene activation (72).

Due to the multiple sources indicating PAI-1 and Wnt involvement in dedifferentiation of adipocytes, or maintenance of MSC pluripotency, their interaction is discussed further below.

3. PAS

Systemic functions. PAI-1 is a member of the PAS. The primary protease of this system is plasmin, which is responsible for catalysing the lysis of fibrin, glycoproteins and other

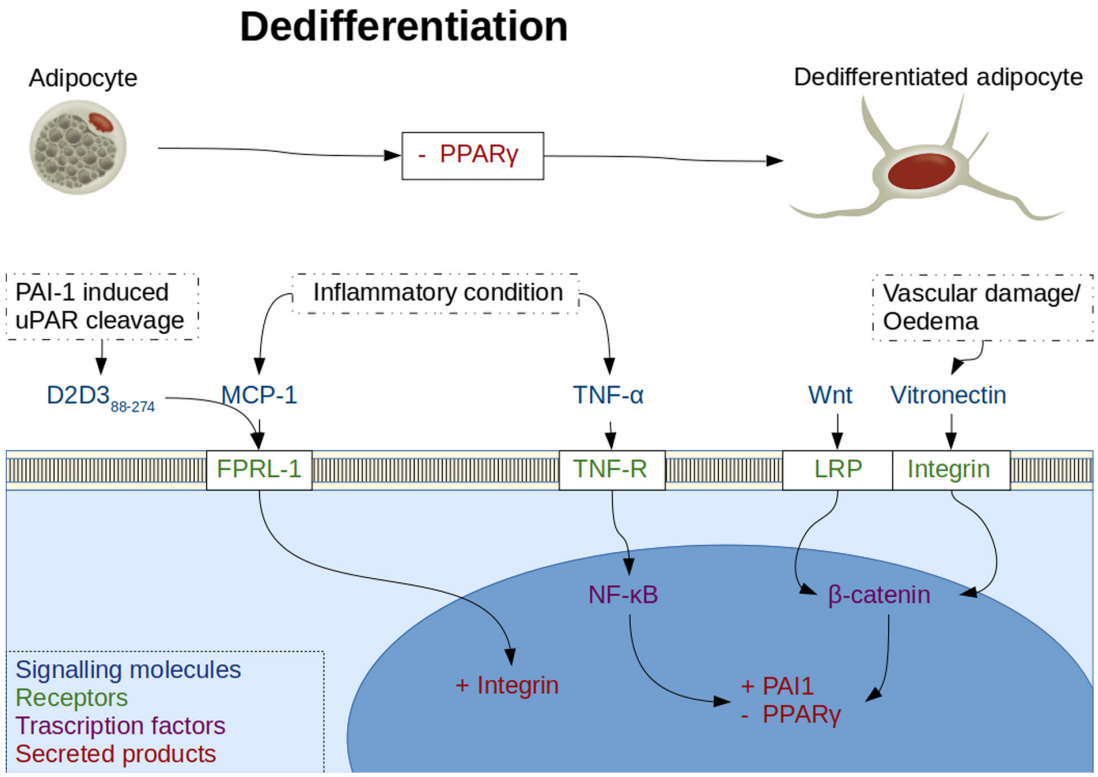

Figure 3. Primary pathways identified in the literature, which are either physiologically or pathologically involved in mediating adipocyte dedifferentiation.

components of the extracellular matrix (ECM), but requires activation from a precursor state (73).

The cleavage of the plasmin precursor, plasminogen, is mediated by uPA and the tissue-type plasminogen activator (tPA), activity of both of which is regulated by the PA-I family of proteins, of which PAI-1 is the most rapidly acting and abundant (74).

Under hypoxic conditions, the adipose tissue actively utilises the PAS, both in physiological (75) and pathological (76) conditions, and this can be exploited to improve our understanding of the complexity of this signalling framework. Adipocytes secrete increased quantities of uPA under hypoxic conditions in order to initiate the degradation of the extracellular environment, in preparation for macrophages, neutrophils and endothelial cells (ECs) necessary for the resolution of the hypoxic state (77). In adipose tissue, a distance of 120 μm from the capillary is the limit of oxygen diffusion, while a single adipose cell can reach sizes of up to 150 μm (78).

uPA activates plasminogen, and can itself initiate the extracellular remodelling process (79). Once plasminogen is present, the ECM degradation begins to cascade. uPA is truncated allowing it to form dimers that have a lower affinity to cell surface uPAR, causing increased plasminogen and matrix metalloproteinase (MMP) activation due to lower PAI-1 affinity (80).

MMPs are secreted either as membrane anchored or free proteins, and in both instances as inactive zymogens, requiring lysis for activation (81,82). Plasmin, along with membrane bound and soluble uPA can activate MMP extracellularly to allow for more specific targeting of the local extracellular glycosaminoglycans and peptidoglycans, which is necessary for correctly guiding the invasion of ECs, host immune cells and stem cells (83). The hypoxic stimulus is known to potentiate the dedifferentiation of chondrocytes (84), and formation of DFAT cells in ceiling culture (85).

In the context of adipose organs, continuous remodelling is important physiologically to maintain stability during adaptation to storage capacities of dietary nutrients, and this puts pressure on the vasculature and connective tissues (86). These pressures are correlated between the adipocytes and ECs, which promote both adipocyte-mediated stimulation of EC angiogenesis and EC stimulation of preadipocyte formation *in vitro* (87). Hypoxic conditions arise spontaneously as the adipose organ grows to meet the physiological demands (88). PAS elements serve a key role in mediating multiple aspects of these interactions, which are most clearly observed in remodelling events following injury. Adipose harvested MSC migration requires uPAR activation (89), whereas PAI-1 as well as αvβ3 are strongly expressed by preadipocytes, resulting in a loss of a migratory phenotype and maturation within the cell cluster (90).

Hypoxia primed neutrophils start adhering and migrating towards the affected ECs (91), whereas uPA activates proteolysis in association with intracellular remodelling via vitronectin-integrin binding (92). This allows new blood vessels to extend towards the hypoxic locale. There is an increased expression of vascular endothelial growth factor (VEGF) under hypoxic conditions, either due to colder local temperatures (93), trauma or oncogenesis (94). VEGF is also known as a vascular permeability factor, as beyond angiogenesis, VEGF often causes a vascular leak, which can lead to oedema (95). The increased perfusion towards a hypoxic locale by VEGF allows for influx of neutrophils and serum contents, high molecular weight proteins (96), such as vitronectin (97) and fibronectin (98) (Fig. 4). Subsequently upon exposure to

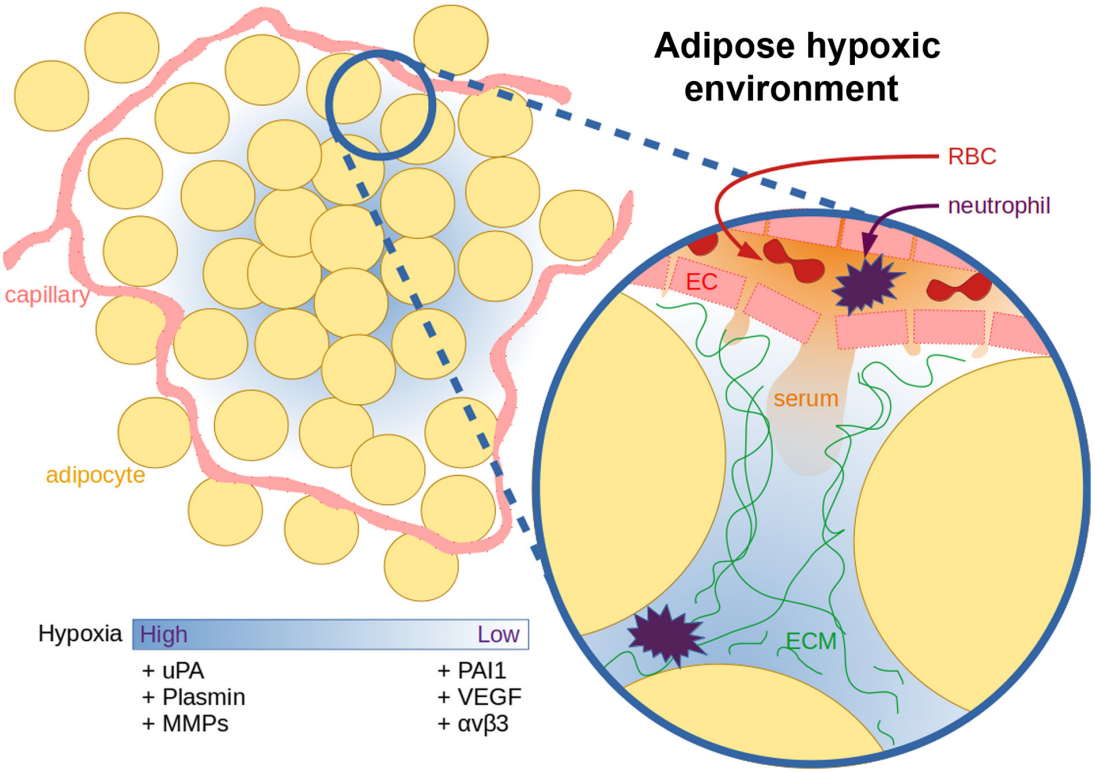

Figure 4. The adipocyte tissue niche and hypoxia resulting from growth, hyperplasia or damage to the vasculature. The resulting response is magnified showing vascular ingress towards the hypoxic location, vascular perfusion, neutrophil infiltration and ECM degradation. The primary molecules regulated by the hypoxic environment are highlighted. ECM, extracellular matrix.

serum components, such as vitronectin and fibronectin, integrin signalling along with uPA/uPAR activation leads to Wnt activation and PAI-1 secretion (99).

Self-regulation and signal propagation. PAI-1 is found bound to vitronectin, in a latent state which prevents its autolysis (100,101). The affinity of PAI-1 is lower to vitronectin than it is to uPA. This can result in the release of PAI-1 from vitronectin upon secretion of uPA, making vitronectin available to bind to integrins, which co-localise to uPAR on the lipid raft and mediate uPA stimulated extracellular proteolysis and motility (102).

The uPA/uPAR/integrin complex allows for directional endocytosis and proteolysis along the path of ECM degradation. The presence of PAI-1 at the uPA/uPAR/integrin complex can cause LRP-mediated intracellular recycling. This severs the extracellular connection of uPA/uPAR/integrin and vitronectin to the degrading ECM, and thus reduces the mobility caused by uPA/uPAR/integrin association (103).

Vitronectin cellular attachment is mediated by integrin αvβ3, which can also act in concert with αvβ5 to bind fibronectin (104). Vitronectin itself is secreted by the liver and the central nervous system (105), and is primarily found in the serum, although it is present in trace amounts in the ECM (106), particularly in the lamina elastica of vessels (107). Fibronectin is more commonly found in areas of high cellular growth or turnover (108), such as in a wound (109). Collagen, the third major ECM component, which binds to the cell surface via integrins, is abundant throughout the ECM, its function is altered as a result of changes in the function with its conformation and composition (110).

Atherosclerotic vessels are a common complication of hypoxia inducing conditions, such as obesity and diabetes (111). Studies have shown that cold induced catabolism and hypoxia inducible factor (HIF) expression by adipocytes inhibits the formation of atherosclerotic plaques through lipid digestion (112). Hypoxia inducible factor expression is known to stimulate PAI-1 expression (113). However, increased PAI-1 expression results in a hypofibrinolytic state, which leads to fibrosis and thrombosis of the fatty plaques (114). The inhibition of low-density lipoproteins by statins can reduce the adipocytic commitment of fibroblast progenitors into adipose cells (115) resulting in reduced atherosclerotic plaque formation (116). HIF expression can also stimulate PAI-1 expression resulting in angiogenesis (117). Increased vitronectin presence at sites of vessel injury, such as atherosclerotic plaques, are expected to be involved in the mechanism underlying of increased platelet adhesion, and coupled with the increased PAI-1 secretion, it may serve as an explanation for the rapid thrombosis at these loci (118), much beyond the rate of PAI-1 induced angiogenesis.

Integrin binding to collagen and fibronectin can increase the secretion of uPA, uPAR and PAI-1; however, αvβ3 binding to vitronectin was found to downregulate uPA and uPAR antigen levels and upregulate PAI-1 (99) (Fig. 5). The attachment of the uPA/uPAR complex to vitronectin and other ECM proteins is performed via binding of the complex to integrins, which is activated by the ECM proteins (119).

Role in differentiation. PAI-1 is secreted by ADSCs and osteoblasts derived from ADSCs (120). Elevated levels of

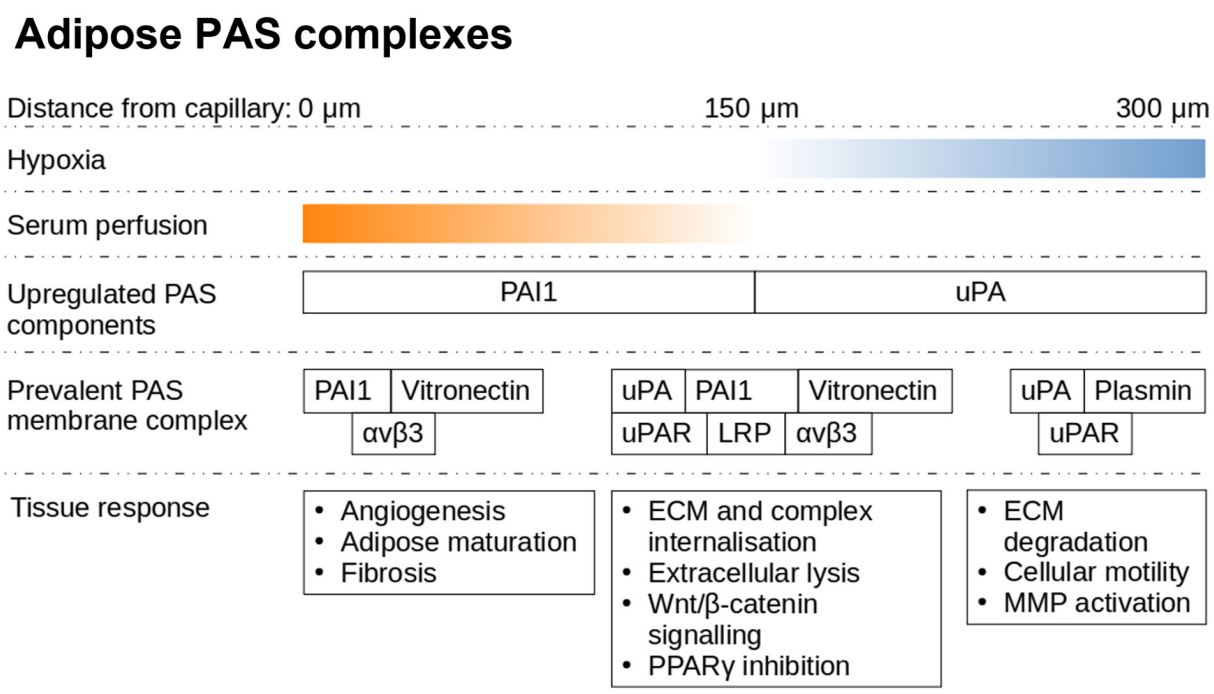

Figure 5. PAS activity is dependent on the microenvironment and available complex elements. PAS, plasminogen activation system.

PAI-1 secretion from adipose cells have also been observed during inflammatory states, such as at post-surgical abdominal sites (121), in murine models of obesity in both gonadal and subcutaneous adipocytes (122), and in aged (24-month-old murine) ADSCs (123).

The integrin binding site on vitronectin is shared by PAI-1, suggesting an interaction between signalling pathways (124). It has been shown that the extracellular signal-regulated kinase pathway is stimulated by αvβ5 integrin during chondrocyte dedifferentiation (125). Integrin αvβ1 has been identified in the proteome of dedifferentiated chondrocytes (126), whereas αvβ3 has been reported to mediate dedifferentiation of smooth muscle cells under hypoglycaemic conditions (127).

The uPA/PAI-1 complex on the uPAR causes clathrin/LRP1-mediated recycling (128,129); however, uPAR also undergoes constant rapid macropinocytic recycling, independent of the uPA/PAI-1 complex (130). A similar mechanism that induces cellular migration has been reported when β1-integrin colocalizes to LRP1 (131). Integrin/LRP signalling is mediated by growth factor receptor-bound-2 (132) as well as integrin linked kinase (133), both of which were found to stimulate Wnt signalling (132,133). LRP1 upregulation occurs under multiple pathological conditions, such as idiopathic pulmonary arterial hypertension, hypoxia-exposed mice and monocrotaline-treated rats. The homeostatic function of maintaining smooth muscle cell (SMC) proliferation seems to be controlled by LRP1-mediated promotion of SMC dedifferentiation (134). LRP5/6 activation is known to stimulate Wnt signalling, which in turn stimulates renal epithelial dedifferentiation (135). Interestingly LRP1 signalling was found to be crucial in the production of PPARγ and adipocyte differentiation, whereas silencing LRP1-mediated dietary lipid internalisation abrogated this (136). Therefore, PAI-1-stimulated LRP1 recycling could prevent lipid-mediated PPARγ production.

Wnt-1 inducible signalling pathway protein 1 (WISP1) is a downstream mediator of Wnt signalling (137) that has been found to be closely associated with integrins. αvβ5 activation is now known as a mediator of WISP1 in acute respiratory distress syndrome lung injury (138). A separate study has confirmed these findings across integrins αvβ5 and αvβ3 (139). Upregulated WISP1 was found to stimulate αvβ1 expression in transfected BMSCs (140). αvβ3 also allowed MSCs to activate the Wnt/β-catenin pathway (66).

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway is necessary to stimulate adipocyte differentiation from 3T3-L1 preadipocytes in the absence of other inputs, as AKT1 can stimulate PPARγ production (141). uPA and uPAR downregulation inhibits the PI3K/AKT pathway (142). The downregulation of AKT by uPA/uPAR RNA inhibition results in upregulation of PAI-1 (143). In fact, PAI-1 is a strong regulatory mechanism of adipocyte differentiation, as microRNA (miRNA)-mediated inhibition of PAI-1 secretion in ADSCs was sufficient to stimulate differentiation into adipocytes (144). miRNAs are mRNA binding and modulating sequences, and have been strongly associated with both stimulating adipocytic differentiation of 3T3-L1 cells (144,145) and inhibiting it (146). The pre-miRNA requires endonuclease activation. Once active, they can stimulate gene expression. miR-130 (147) and miR-27b (146) were found to stop adipocyte differentiation by inhibiting PPARγ production via targeting coding and untranslated mRNA regions. Stimulating the differentiation by miRNA has been found via other pathways, namely miR-21 inhibition of TGFBR2 secretion (148), miR-17-92 reduction of tumour-suppressor Rb2/p130 (145), and possibly miR-143 mediated reduction of ERK5 production (149). Embryonic stem cell differentiation into adipocytes is also inhibited by other uPA inhibitors, such as amiloride (150).

The change in uPAR colocalization to integrins from α3β1 to αvβ5 blocks the uPA signalling and activation of

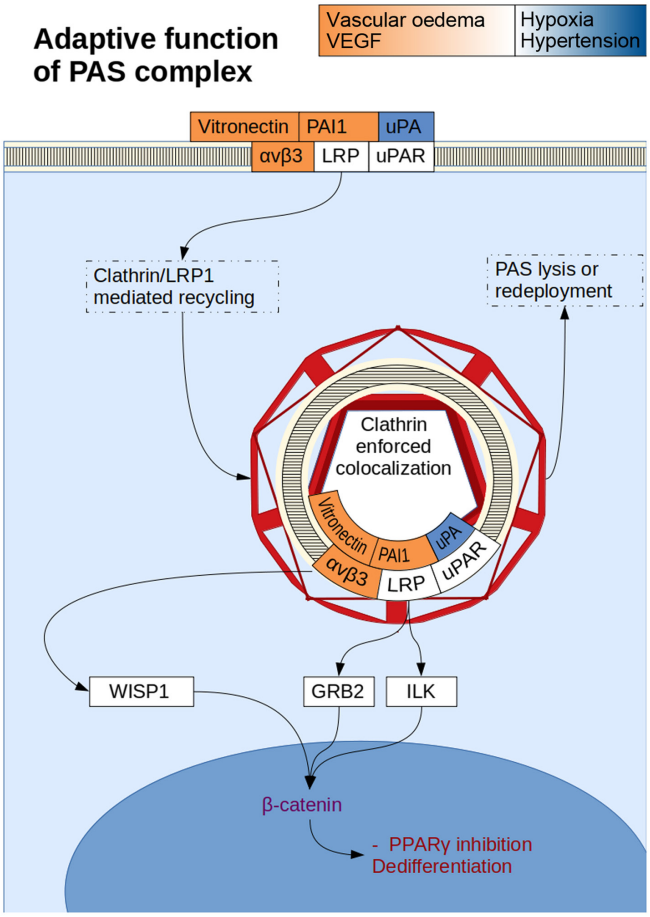

Figure 6. PAI-1/LRP mediated clathrin recycling of the uPA/uPAR complex and the activation of the vitronectin/αvβ3 complex can be simultaneous due to the different binding sites between PAI-1/uPA and PAI-1/LRP, and uPA/uPAR itself also has a separate integrin binding domain. The resultant vacuole is internalised with the plasma membrane, and is isolated from the ECM, allowing only for non-ECM dependent signalling. PAI, plasminogen activator inhibitor 1; LRP, lipoprotein receptor-related protein; uPA, urokinase plasminogen activator; uPAR, uPA receptor.

ERK or AKT (151), which functionally represents a shift from a laminin rich environment to a vitronectin rich environment. Interestingly, Wnt signalling can be inhibited by α3β1 complexes (152), while activation of αvβ5 complexes stimulates Wnt signalling (153). uPAR complex internalisation by the cell can result in Wnt mediated β-catenin gene transcription due to nuclear colocalization, suggesting that uPAR in complex with β-catenin can be a potent activator of stemness (154). PI3K/AKT signalling appears more independent of uPAR internalisation, and tends towards maturation. αvβ3/vitronectin complexes have been shown to activate a PI3K/AKT induced stemness profile, whereas while α3β1/Laminin inhibits this (155) (Fig. 6). PAI-1/LRP mediated clathrin recycling of the uPA/uPAR complex and the vitronectin/αvβ3 complex can be simultaneous due to different binding sites between PAI-1/uPA and PAI-1/LRP, while uPA/uPAR itself also has a separate integrin binding domain. The resultant vacuole is internalised with the plasma membrane, and is isolated from the ECM, allowing only for non-ECM dependent signalling.

Clinical applications. Wound closure is initiated by thrombin, which activates fibrinogen to create a fibrin clot (156). The activation of uPA, uPAR and PAI-1 has been suggested as the mechanism underlying MSC invasiveness into a fibrin clot of a wound (157). Fibronectin is an extracellular glycoprotein, which creates a provisional matrix for cellular adhesion to a wound environment via integrin binding (158). This in turn, has been shown to stimulate uPA, uPAR and PAI-1 secretion (99). Adipocytes bind to fibronectin via integrins (159). Preadipocyte differentiation into adipocytes is marked by a loss of the attachment to ECM components resulting in a rounded shape, increased lipid content and reduced Wnt/βcatenin signalling (160).

Fully differentiated adipocytes were reported to lower PAI-1 concentration, and the subsequent addition of PAI-1 to an osteoblast/fully differentiated adipocyte coculture did not cause spontaneous transition of adipocytes to osteoblasts (161). Nonetheless, PAI-1 has been found to push differentiated MSC lineages, of not only adipocytes but also fibroblasts, back into the partially dedifferentiated growth arrest state (162), as well as being necessary for dedifferentiating osteosarcoma (163) into more malignant stem like cells, suggesting the prevalence of this molecule in maintaining pluripotency. It is also worth noting that the dedifferentiating stimulus wrought by PAI-1 may partially explain why it is involved in several types of tumours associated with a poor prognosis (104).

4. Perspectives

Whilst detection of increased secretion of PAS components within the tissue can be indicative of oncogenesis (74,143) and diabetic dysregulation in obese patients (61), localised activation is both physiological and necessary for maintenance and healthy tissue development. Currently, the fast-developing fields of aesthetic and regenerative medicine, as well as dentistry, are keenly focused on the bio-engineering potential of natural products. Transplants of adipocytes, platelet rich fibrin or platelet rich plasma are used for acceleration of healing, aiming to stimulate fibroblasts or osteoblasts, and multiple approaches are often combined to increase their effectiveness (164). It is hypothesized that adipocytes primed with PAS system components may be used to improve outcomes of bone regeneration, soft connective tissue regeneration and wound healing.

Instances where establishing a sufficient blood supply is of high concern, such as an osseoinductive transplant, a collagenous cellular carrier implant, or for procedures such as bone distraction, extensive surgical flaps, or any other surgical intervention where scarring and grafting is an issue, may benefit from the possible therapeutic applications of receptive stem cells. When the protraction of healing is necessary to allow for complete angiogenesis, and adequate deposition of ECM to support the unformed tissue, uPA can ensure that the microenvironment is maintained in a state of turnover (129). Degradation of the ECM increases permeability to both cells and signalling molecules. However, local inflammatory mediators tend to stimulate ECM degradation and uPA release. However, at present, the clinical use for uPA alone is limited to acellular pathologies, such as large thrombi (165) or thinning of tuberculous pleural thickening (166).

The ability for adipocytes to secrete uPA and PAI-1 to modulate and maintain their pluripotent microenvironment

has been explored, and this may be conducive in wound healing or bio-engineering stents (77). It is hypothesized that balancing the vascularisation and remodelling properties of uPA, with the dedifferentiating properties of PAI-1 production and recycling in association with integrins may be achieved by cultivating an adipocyte harvest in the right ECM protein environment (89).

The MSC like fraction can be boosted by synthetically overloading harvested adipocytes with PAI-1 or any of the other Wnt activating PPARγ inhibitors and re-introducing this population into a wound or surgical microenvironment in order to become more susceptible to local differentiation factors and conduit healing or growth (157).

Alternatively, whole tissue adequately prepared to stimulate activation of the PAS, such as in a wound healing or hypoxic environment, may create a DFAT rich graft which would be significantly more conducive to local cellular populations or ECM architectures in mesenchymal lineage use cases (21).

For directed mobility via extracellular degradation, there needs to be present a steady stream of uPA to counteract any baseline PAI-1 secretion, to overcome the background PAI-1 recycling of the uPA/uPAR/integrin complex, which undergoes intracellular recycling along with LRP and downstream targets. Such interventions are difficult to perform *in vivo* due to the delicate nature of surgical sites. Thus, it is more prudent to identify self-regulating stents, which can stabilise harvested adipocytes in a required state (3).

PAI-1 is found to be stable for 145 h when bound to vitronectin, and for 2 h when expressed in isolation *in vitro* (167). This suggests that PAI-1 can cause a dormant inhibition of cellular motility during a latent phase of the cell cycle when there are no uPA stimulating environmental queues. However, when uPA is released, the motility, sensing, and endocytic potential inhibited by the latent PAI-1 secretion could explain a physiological receptiveness to changes observed during pathological processes tending to homeostasis (150).

Studies have shown that platelet rich plasma with ADSCs can significantly improve tissue incorporation of synthetic scaffolds and their neovascularisation (168). Additionally, ADSCs with platelet rich fibrin (PRF) have exhibited a certain degree of improvement in restoring salivary function following gland irradiation, which neither ADSC nor PRF alone achieved (164). This effect could be due to the presence of fibrin in the platelet rich plasma, which in turn activates uPA and PAI-1 secretion through integrin activation of Wnt. Fibrin has been shown to enhance Wnt signalling (169,170), whereas PRF-stimulated BMSC healing of alveolar bone defects was found to be mediated by expression of Wnt3a (171), further suggesting the promising clinical applications of this approach. The high vitronectin content of plasma, which has a selective PAI-1 up-regulatory mechanism, could account for the multipotent stimuli that modifies the local cell population for faster healing outcomes.

5. Conclusions

Adipocytes can be seen as a versatile cell lineage which harbour mesenchymal stem potential in the form of MSCs, ADSCs and the induced DFAT cells. The formation of the latter has been found to rely on inhibition of PPARγ. Wnt signalling has been shown to be stimulated by TNF-α and integrins, notably αvβ3 via fibrin. The proteolytic cascade activated by PAS during inflammation is mediated by multiple Wnt activators, acting on Wnt to stimulate uPA release for ECM degradation, PAI-1 release for endocytosis, uPAR for localisation to LRP, and integrins to provide motility and directional specificity.

Since Wnt activation also inhibits PPARγ expression, there is a high chance that adipose cells exposed to a fibrin or vitronectin rich wound environment would undergo dedifferentiation. PAI-1 expression has also been linked to dedifferentiation, which could be explained by its ability to stimulate endocytotic clathrin basket mediated recycling of uPA/uPAR/integrin complexes. After wound resolution, in order to reach homeostasis and inhibit uPA mediated extracellular ECM degradation, PAI-1 needs to be released following its internalisation, suggesting that activation of Wnt signalling is necessary in wound healing in order to produce PAI-1, and consequently inhibit PPARγ. Coupled with Wnt mediated stimulation of PAS expression to allow for remodelling of the ECM and cellular motility, there is a strong suggestion that Wnt is central to the success and high versatility of adipose tissue and more importantly DFAT cells in pilot studies. An initial dedifferentiating priming of adipose to DFAT cells from a lipoaspirate harvest in a PRF could be followed by cytokine, mineral or ECM exposure to initiate target cell differentiation prior to clinical use, taking the DFAT cells one step closer to use in a clinically applicable environment.

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MWS, AE and conceived the topic of the review MN wrote the manuscript. MN and AE reviewed and revised the manuscript. All authors have read and approved the final manuscript. Data sharing is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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