Activatin' Human Adipose Progenitors in Obesity

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Obesity relates to the abnormal accumulation of body fat and is associated with a number of morbidities including diabetes, dyslipidemia, cardiovascular disease, and cancer. Great strides have been made in our understanding of the molecular basis for these associations, and a number of hypotheses have been put forward. One seemingly counterintuitive hypothesis is the "adipose tissue expandability hypothesis," which suggests that the relative accumulation of adipose mass per se may not be directly causative in obesity-related metabolic abnormalities; instead, adipose tissue function and plasticity are the key determinants. Although adipose tissue is arguably the most plastic organ in our bodies, it cannot expand indefinitely—particularly in the face of years of chronic fuel surplus. Furthermore, when such a limit is reached, this would inevitably set a limit on the body's capacity to safely store neutral triglycerides. Consequently, with continued nutritional overload, excess fat is deposited in ectopic sites, in tissues not specialized to store large amounts of triglycerides. The result is lipotoxicity and metabolic dysregulation of peripheral tissues (1). Similar metabolic consequences may also be predicted when one considers impaired adipose tissue function (dysregulated adipose metabolism and endocrinology) or indeed when there is a paucity of adipose tissue.

Recent evidence points to substantial adipose tissue remodeling, particularly in cases associated with metabolic dysregulation. Obese dysfunctional adipose tissue is characterized by adipocyte hypertrophy, increased angiogenesis, immune infiltration, increased extracellular matrix deposition/fibrosis, and chronic inflammation. In contrast, growth of healthy adipose tissue mass is determined by a complex coordination of adipocyte hypertrophy, adipocyte hyperplasia, adipocyte death, and angiogenesis. Furthermore, all these events appear to be orchestrated in a cell-type, context-dependent, and temporal manner. Indeed, in weight stable individuals, adipocyte turnover is thought to be balanced by the acquisition of new adipocytes from adult adipose tissue progenitors (mesenchymal stem cells and preadipocytes) via adipogenesis (2). There is also evidence that this process is titrated, presumably to appropriately match the requirements for fuel storage, such that not all progenitors become adipocytes simultaneously (3). What then are the physiological signals that regulate and control adipose

tissue expansion in response to nutritional surplus? Can these be distinguished from those pathological signals that limit further adipose expansion?

Although much has been elucidated with regards to the signals that regulate stem cell lineage determination, adipogenesis, and angiogenesis, we know surprisingly little of the signaling networks that coordinate all of these events in vivo in human adipose tissue. To do so will require knowledge of adipose cellularity under specific nutritional and pathological circumstances. Longitudinal animal studies suggest that increases in adipocyte size precede increases in adipocyte number. However, such longitudinal data (over decades) from humans are rare. Nonetheless, cross-sectional studies suggest that compared with nondiabetic obese subjects, obese diabetics have fewer but larger adipocytes (2,4). This is consistent with reports of an inverse correlation between differentiation capacity of primary human progenitors/preadipocytes and BMI (5–7).

In contrast, determining the number of adipose precursors (preadipocytes) in lean and obese adipose tissue has been challenging and often resulted in seemingly contradictory findings (6,7). Since different surface markers were used to define the progenitor population, a direct comparison cannot be made. One hypothesis that may reconcile these observations differentiates multipotent adipose stem cells (progenitors) from committed preadipocytes. It considers that although the multipotent stem cell (CD133+) number is greater, the immature adipocyte or preadipocyte cell number (aP2+/CD68−) is decreased in obese subjects. With the recent success in identifying and locating adipose progenitors in both murine and human tissues, it is hoped that these issues can now be addressed. Of note, bona fide multipotent adipose progenitors exhibit a distinct molecular signature, including the expression of stem cell markers (Lin−,CD29+,CD34+,Sca-1+,CD24+), developmental transcription factors, extracellular matrix genes, antiangiogenic factors, and signaling cascade components (3,8,9).

Many growth factors have been implicated in determining the size of progenitor pools (in embryonic and adult tissue). Some, such as Wnts, bone morphogenetic proteins (BMPs), and transforming growth factor (TGF)-β have also been implicated in regulating lineage determination and adipogenesis, primarily in rodent models. However, very few factors have been specifically demonstrated to promote proliferation of human adipose progenitors. In the accompanying original article, Zaragosi et al. (12) present their findings on activin A, a putative promoter of self-renewal and antiadipogenesis in human adipose progenitors.

Activins are members of the TGF-β protein superfamily of secreted growth factors, which includes BMPs, Nodal, and TGF-β. They act in a paracrine/autocrine manner to regulate cell proliferation, differentiation, and apoptosis during embryonic development, tissue remodeling, inflammatory immune response, wound repair, and reproduction. Activins exist in a number of dimeric configurations,

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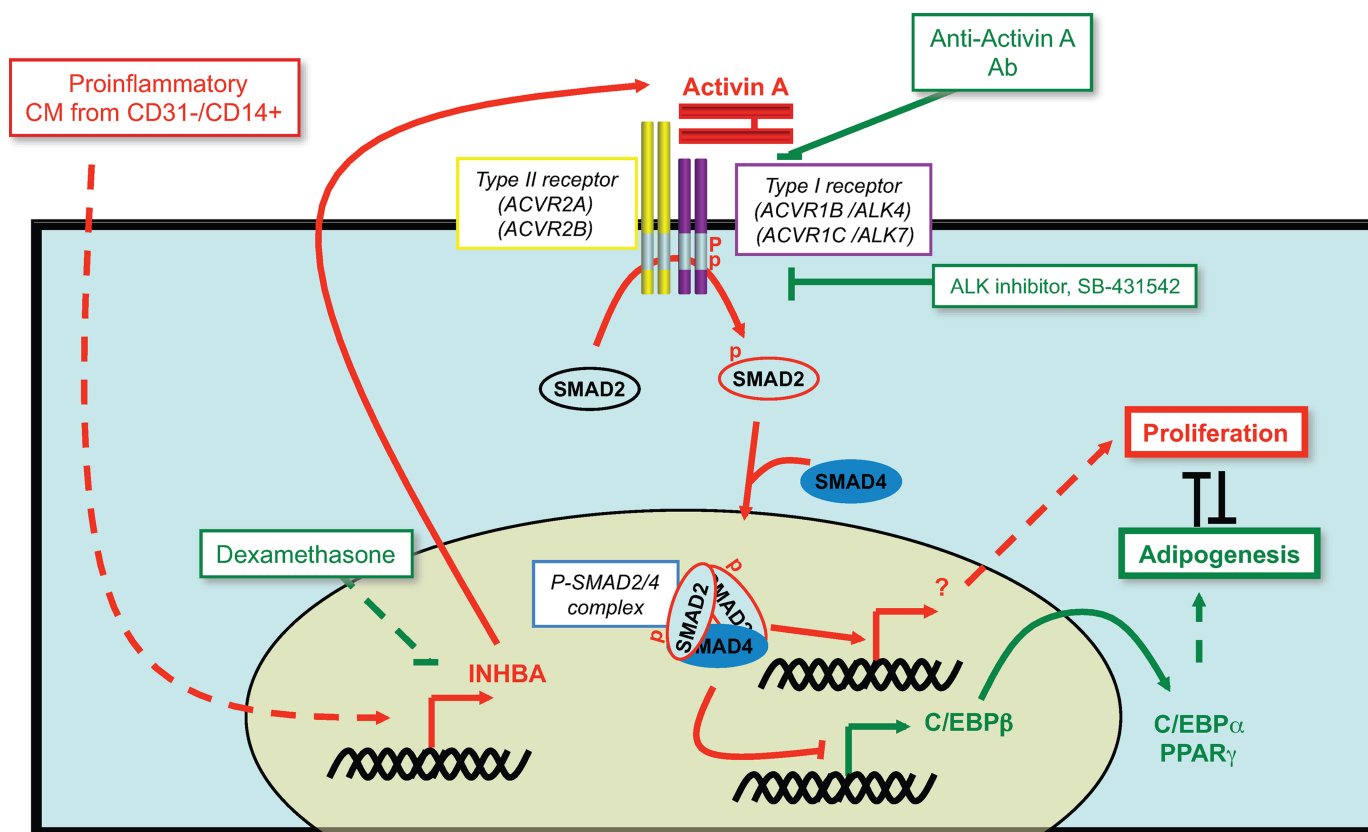Corresponding author: Jaswinder K. Sethi, jks30@cam.ac.uk.

FIG. 1. Activin A–mediated signal transduction promotes cell proliferation and inhibits adipogenesis. Activin receptor specificities and intracellular signal transducers appear complex and incompletely delineated, in part because activin A receptors can also transduce signals of other TGF-β superfamily members. They may also be tissue- and context-specific. Nonetheless, activin A has been reported to bind both type IIA (activin receptor type-2A; ActRIIA or ACVR2A) and type IIB (activin receptor type-2B; ActRIIB or ACVR2B) surface receptors. This receptor-ligand interaction leads to the recruitment, transphosphorylation, and activation of the type I activin receptor (ACVR1B or ALK-4). The receptor then interacts with and phosphorylates cytoplasmic SMAD2, which multimerizes with SMAD4 and translocates to the nucleus where these proteins can act as transcription factor complexes to induce a subset of genes implicated in stem cell renewal or differentiation. SMAD2 is required to promote activin A–induced cell proliferation and to suppress adipogeic expression of C/EBPβ.
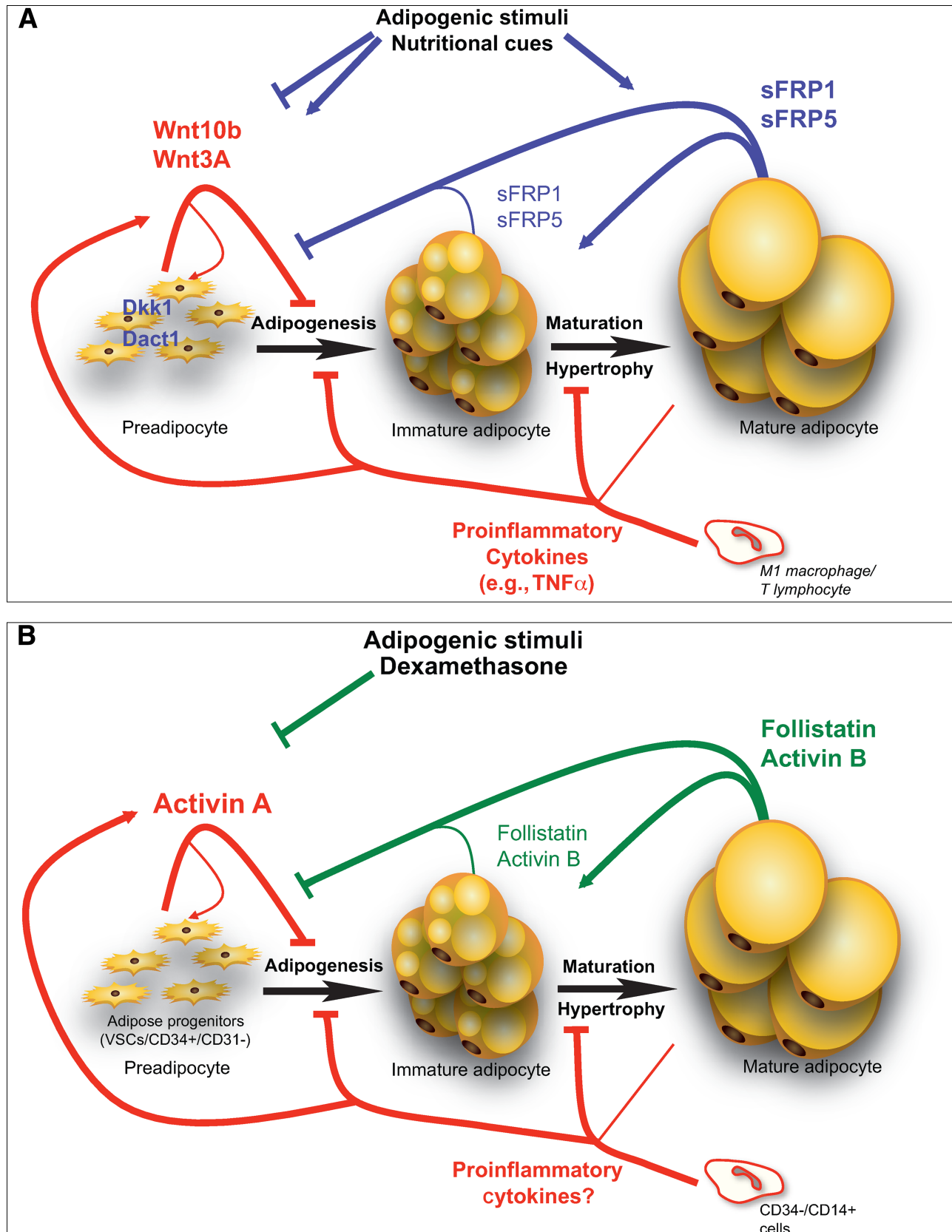
often associated with neutralizing binding proteins. Activin A is a homodimer of inhibin βA subunits encoded by the *INHBA* gene. Similarly, activins B, C, and E are homodimers of *INHBB*, *INHBC*, and *INHBE* gene products, respectively. Activin AB is heterodimer of inhibin βA and inhibin βB subunits. Interestingly, the inhibin βA subunit is widely expressed, whereas the inhibin βB subunit is highly expressed in adipocytes, and the βC and βE subunits are predominantly expressed in the liver. To date, only activin B and activin E homodimers have been implicated in glucose homeostasis (10,11).

Zaragosi et al. now report that *INHBA* mRNA is not only detected in human adipose tissue, but unlike *INHBB*, it is preferentially expressed in primary adipose progenitors (human multipotent adipose-derived stem [hMADS] cells and CD34+/CD31− stromal vascular fraction [SVF] cells) and localized to SVFs of omental, subcutaneous, and mesenteric white adipose depots. In vitro, activin A expression and secretion is required for optimal proliferation during cell propagation, but it is downregulated as cultured hMADS and primary human preadiocytes undergo adipogenic conversion (12). Although this observation seems consistent with the known mitogenic action of activin A (on embryonic and gonadal stem cells), it contrasts with a lack of effect on clonal expansion during murine preadipocyte differentiation (13,14).

As with most "preadipocyte" genes, activin A can inhibit adipogenesis in vitro (12–14). However, its mechanism of

action seems to be context dependent. Previous reports have suggested that activin A targets CCAAT/enhancer-binding protein β (C/EBPβ) activity (13,15). In contrast, Zaragosi et al. suggest that activin A prevents adipogenic induction of both C/EBPβ isoforms (LAP and LIP). They further support this with evidence that C/EBPβ-LAP overexpressing hMADS cells are resistant to the antiadipogenic actions of activin A (12). Futher mechanistic insights are provided using both pharmacological inhibition of activin receptor kinase activity (ALK inhibitor SB-431542), as well as SMAD2 small interfering RNA. This is not only consistent with the limited knowledge of activin A–mediated signal transduction (Fig. 1), but also demonstrates that these signal tranducers are involved in activin A–induced proliferaton and antiadipogenesis in hMADS. What still remains unclear is the identity of the activin-induced SMAD2 target genes that inhibit C/EBPβ expression and/or promote proliferation. Moreover, given the roles of activin A in nonadipose lineage determination (16,17), it also remains to be formally demonstrated that activin A does not alter the multipotency of human adipose progenitors. If multipotency of progenitors is lost after activin A treatment, this could represent the alternative possibility that in obese-inflamed adipose tissue, activin A plays a role in reducing adipogenic potential of progenitors but also promotes tissue fibrosis.

Zaragosi et al. also found that in the white adipose tissue of obese nondiabetic individuals, *INHBA* expression was

FIG. 2. Local autocrine and paracrine signals regulate progenitor proliferation and titrate adipogenesis. Remarkable parallels are emerging between the Wnt/β-catenin signaling network (A) and the activin signaling network (B). These suggest that maturing adipocytes may produce proadipogenic signals that inhibit progenitor proliferation and promote preadipocyte recruitment.

increased compared with that in lean individuals, although it is not clear whether these subjects are exhibiting the early hallmarks of adipose tissue inflammation. Further investigation suggests that in progenitors, activin A was induced by signals secreted from adipose tissue–derived CD34−/CD14+ cells (i.e., macrophages, monocytes, and polymorphonuclear neutrophils). Given that activin A is known to be regulated by proinflammatory cytokines (18), this potentially provides additional support for the notion that a proinflammatory environment contributes to limiting further adipose expansion (19). An additional fascinating observation is that activin A expression in adipose progenitors is reduced by the synthetic glucocorticoid dexamethasone. This presents the intriguing possibility that anti-inflammatory steroids may promote adiposity, in part, via downregulation of INHBA.

As with any new discovery, the report by Zaragosi et al. raises many new questions. For example, if activin A is important in the physiological regulation of adipose tissue growth, is its production regulated by nutritional cues and during the development of normal adiposity? How does the presence of proadipogenic activin B (from adipocytes) affect activin A bioactivity in adipose progenitors? Does the activin network mediate the paracine cross talk between maturing adipocytes and preadipocytes as has been proposed for the Wnt network (Fig. 2 and ref. 20).

Given its ubiquitous expression and diverse biological functions, a detailed understanding of local/tissue-specific regulation of activin A activity is also required before we can consider it as a candidate for antiobesity therapeutics. In obesity, what proportions of adipose-derived activin A contribute to circulating levels? Are these levels influenced by sex or reproductive age, liver dysfunction or cancer? Does adipose expression and activity of activin A correlate inversely with adiposity, particularly in the absence of adipose inflammation? Is it differentially expressed in adipose tissue from obese healthy subjects relative to obese diabetic subjects? Finally, because the biological activity and bioavailability of activin A is influenced by the presence of functional antagonists and binding proteins, such as inhibins and follistatin (14), it will be pertinent to define the in vivo levels of activins, follistatin, and inhibins in the same subjects before making any inferences as to the potential consequences of differential expression in lean, obese, and obese-diabetic adipose tissue.

In summary, given that limitations in fat storage can lead to metabolic disorders, there is a clear need to decode the molecular mechanisms that regulate adipose tissue expandability and to discriminate between the normal physiological cues and those involved in the metabolic syndrome. Clearly, unlocking adipose-specific signals that prevent adipose progenitors from differentiating in response to nutritional surplus is attractive for biomarker discovery, targeted therapeutics, and tissue engineering alike. However, much remains to be addressed before activin A can be proposed as a candidate biomarker for obesity and/or a therapeutic target for the associated metabolic complications.

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