Restricted Adipogenesis in Hypertrophic Obesity
The Role of WISP2, WNT, and BMP4

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The current global diabetes epidemic is driven by obesity. However, many obese individuals do not develop insulin resistance or the metabolic complications. Inappropriate expansion of the subcutaneous adipose cells leads to hypertrophic obesity characterized by a dysregulated adipose tissue with insulin resistance and inflammation. Here, we discuss the limited expandability of the subcutaneous adipose tissue, which, when exceeded, promotes ectopic fat accumulation and metabolic complications.

Hypertrophic obesity is associated with an inability to recruit and differentiate new subcutaneous adipose cells. This is not due to lack of precursor cells but to an inability to induce their commitment and differentiation through inactivation of canonical WNT signaling and allowing bone morphogenetic protein 4 (BMP4) to initiate commitment of precursor cells. The WNT-inducible secreted protein 2 (WISP2) links WNT and BMP4 signaling. It is highly expressed in early adipogenic precursor cells and inhibits adipogenesis through dual mechanisms; cytosolic WISP2 forms a complex with ZNF423, a transcriptional activator of peroxisome proliferator–activated receptor γ (PPARγ), and this complex is dissociated by BMP4 allowing ZNF423 to enter the nucleus and initiate PPARγ activation. However, WISP2 is also a secreted protein that, through unclear mechanisms, directly inhibits PPARγ activation. WISP2 expression in human subcutaneous adipose tissue is associated with hypertrophic obesity, markers of ectopic fat accumulation, and degree of insulin resistance.

Thus, WISP2 is a novel secreted adipokine at the crossroad between WNT and BMP4 signaling that can play a critical role for the development of hypertrophic obesity and associated metabolic complications. Identifying the receptor and signaling pathways for WISP2 can open up new avenues for treatment.

HYPERTROPHIC OBESITY IS ASSOCIATED WITH A DYSREGULATED AND DYSFUNCTIONAL ADIPOSE TISSUE

The current global epidemic of obesity is a huge challenge to society and imposes increasing costs on the health care system because obesity is associated with several negative consequences to our health, including type 2 diabetes (T2D), cardiovascular disease, and cancer (1). However, it is not only the degree of obesity that is important but also the distribution of fat; an abdominal distribution augments the metabolic complications at a given BMI (2). This finding has raised much interest in the potential role of regional differences in adipose tissue metabolism and, in particular, the role of intra-abdominal and visceral fat in causing the metabolic complications (3,4). However, increased amounts of (intra) abdominal/visceral fat is also associated with other ectopic fat accumulations and may, thus, be a marker rather than causally related to the metabolic complications of obesity.

The limited expandability of the subcutaneous adipose tissue leads to inappropriate adipose cell expansion (hypertrophic obesity) with local inflammation and a dysregulated and insulin-resistant adipose tissue (4,5). The inability to store excess fat in the subcutaneous adipose tissue is a likely key mechanism for promoting ectopic fat accumulation in tissues and areas where fat can be stored, including the intra-abdominal and visceral areas, in the liver, epi/pericardial area, around vessels, in the myocardium, and in the skeletal muscles (6). Many studies have implicated ectopic fat accumulation and the associated lipotoxicity as the major determinant of the metabolic complications of obesity driving systemic insulin resistance, inflammation, hepatic glucose production, and dyslipidemia (7,8).

Support for the concept that factors other than degree of obesity play a key role for the negative metabolic consequences come from the many studies showing that BMI is not a good determinant of these complications at an individual level and that the complications are, in fact, also seen in many nonobese individuals while many obese individuals are metabolically healthy (9,10).

We here review basic pathways regulating adipogenesis, focusing on the limited expandability of the subcutaneous adipose tissue and the development of hypertrophic obesity with a dysregulated adipose tissue and ectopic fat accumulation. Understanding mechanisms that limit subcutaneous adipogenesis in humans should provide novel targets for the treatment of obesity-related metabolic complications.

DEFINING OBESITY

The most commonly used definition of obesity is BMI and, in Caucasians, BMI $\geq$30 kg/m^2 is the accepted cutoff point. However, there are clear ethnic differences and, for instance, in the Japanese and other Asian populations, obesity-related risks are already seen at BMI $\geq$25 kg/m^2. In fact, a pronounced epidemic of T2D is seen in the Asian populations—in spite of their lower BMI. Furthermore, studies have convincingly shown that Asian populations are characterized by an early accumulation of ectopic fat in the liver and intra-abdominal depots suggesting an associated, and likely genetic, impairment in subcutaneous

adipogenesis (11–13). Furthermore, in Caucasians, around 30% of obese individuals do not show insulin resistance or the associated metabolic complications characterizing the metabolic syndrome and, likewise, at least a similar proportion of nonobese individuals exhibit metabolic complications as if they were obese (14–16).

The current understanding of the limited expandability of the subcutaneous adipose tissue states that accumulation of excess fat in the subcutaneous adipose tissue, and appropriately protecting the stored lipid droplets with the PAT (perilipin, adipophilin, and tail-interacting protein of 47 kDa) family of proteins (17), is the most beneficial way of storage from a metabolic point of view. This is also supported by the large prospective Dallas Heart Study in which markers of ectopic fat accumulation, but not subcutaneous fat, predicted risk of developing T2D in obese individuals (4).

The concept of lipid storage "overflow" as a consequence of the limited expansion of the subcutaneous adipose tissue has received much experimental support in both human and animal studies. Lipoatrophy is characterized in humans by pronounced insulin resistance, ectopic fat accumulation, and development of T2D (18); in animal models, this can be overcome by transplanting adipose tissue (19). Furthermore, the animal model generated by Scherer and colleagues (20), with overexpression of adiponectin in the subcutaneous adipose tissue, induced massively obese mice with perfectly normal insulin sensitivity and metabolism.

Importantly, the adipose tissue in these mice was characterized by many small adipose cells—hyperplastic obesity. These and many other studies support the concept that the ability to recruit new adipose cells in the subcutaneous adipose tissue during lipid accretion prevents inappropriate adipose cell expansion (hypertrophic obesity) with infiltration of inflammatory cells, insulin resistance, and a dysfunctional adipose tissue with reduced insulin receptor substrate 1, GLUT4, lipoprotein lipase activation, adiponectin, and other markers of reduced PPARγ activation (5,14). The recruitment and activation of the inflammatory cells in the adipose tissue may be a consequence of both necrotic enlarged adipose cells and tissue factors promoting a proinflammatory phenotype (21,22).

HYPERTROPHIC OBESITY IS ASSOCIATED WITH RISK FOR T2D

Hypertrophic obesity is associated with inflammation and increased cytokine production and release, which can inhibit PPARγ activation, the key mediator of the fully differentiated and insulin-sensitive adipose cell phenotype (23). Overall, the adipose tissue becomes dysfunctional both in terms of taking up lipids from the bloodstream via lipoprotein lipase activation, secreting adiponectin and other apparently protective adipokines and, instead, increasing the secretion of insulin-antagonistic and proinflammatory molecules also associated with increased lipolysis and free fatty acid release.

Promoting adipose cell recruitment in the subcutaneous adipose tissue rather than merely inflating the cells would be protective of the obesity-associated metabolic complications. In fact, this is a fundamental mechanism of action of the thiazolidinediones (24), also leading to reduced ectopic fat while the subcutaneous depot becomes expanded and less insulin resistant (24,25), but their unwanted side effects have limited the usefulness of this class of drugs. To further support this concept, we and others have shown

that subcutaneous fat cell size in humans is negatively correlated with insulin sensitivity measured with the euglycemic clamp irrespective of degree of obesity measured as BMI (26). Furthermore, subcutaneous adipose cell size has been shown to be an independent predictor of future T2D in prospective studies (27).

Our recent work (28) has also identified a link between a genetic predisposition for T2D, defined as being a healthy first-degree relative (FDR) to subjects with T2D. Nonobese FDR were less insulin-sensitive than individuals with the same BMI and amount of body fat but lacking a known family history. In contrast, no difference was seen when we examined individuals with or without a family history of overweight/obesity. Importantly, FDRs were found to have considerably larger subcutaneous fat cells than either the control group or a similar group of individuals with a family history of overweight/obesity (28). Although insulin resistance in FDRs is well established from other studies, our work adds the novel information that they, also when nonobese, have a dysfunctional adipose tissue characterized by inappropriate hypertrophy of the adipose cells. We have made independent verification of this finding in other cohorts and also found that the gene expression pattern in these nonobese FDRs reflects that seen in obese individuals with increased inflammation and other perturbations discussed above (A.H. and U.S., unpublished observations).

The finding that FDRs have an inappropriate (for amount of body fat and BMI) expansion of the adipose cells suggests a reduced ability to recruit new adipose cells and to rather store the excess lipids in existing fat cells through expansion. This concept would make these individuals particularly susceptible to the current lifestyle promoting an inflamed and dysregulated subcutaneous adipose tissue with increased propensity for ectopic fat accumulation, insulin resistance, and risk for T2D. Further support for this concept comes from studies showing that individuals with T2D have, for a given amount of body fat, relatively less subcutaneous adipose tissue and more fat in ectopic sites (29). Similarly, individuals with T2D have more liver fat than nondiabetic subjects at the same BMI (30).

Taken together, familial risk for T2D is associated with markers of an impaired adipogenesis in the subcutaneous adipose tissue, making them more vulnerable to the negative consequences of today's lifestyle. Although this impairment in adipogenesis is likely to be genetic, we have yet to identify specific risk genotypes involved.

REDUCED ADIPOGENESIS IN HYPERTROPHIC OBESITY

It is well established that subcutaneous adipose cell size can differ markedly between individuals with the same BMI and amount of body fat (31), supporting the concept that adipogenesis is under differential regulation. Furthermore, it was recently shown that around 10% of our subcutaneous adipose cells undergo annual replacement (32) and that individuals with inappropriately enlarged cells have a reduced turnover rate (31). The data also suggest that adipose cell number in the subcutaneous depot is unlikely to change after puberty (32) although direct overfeeding studies to prove this concept were not performed. However, a recent study suggests that women may increase the number of fat cells in the thigh and gluteal subcutaneous regions following weight gain, which would exert a protective effect in weight increase (33). The caveat to that study is that the techniques used cannot

differentiate between new cell recruitment and lipid accumulation in preformed preadipocytes. Thus, at present, the best available data indicate that the subcutaneous adipose cell number may become fixed in early life and that lipid accretion after that is primarily a question of recruiting existing uncommitted stem cells in the adipose tissue into the adipose lineage, differentiating committed preadipocytes as well as filling up preexisting adipose cells.

In a recent large and extensive study, we analyzed adipogenesis in stromal-vascular cells from the abdominal subcutaneous depot (34). We found marked differences between the donors in the number of cells that underwent adipogenesis under identical conditions. In fact, in some individuals we saw only 5–10% of cells undergoing differentiation while, in others ~80% of the cells became adipogenic (34). Interestingly, poor differentiation was seen in individuals with large subcutaneous adipose cells (hypertrophic obesity), whereas small adipose cells were associated with good adipogenesis (Fig. 1) suggesting a causal relationship. As discussed below, this difference was not due to a lack of precursor cells in hypertrophic obesity but rather due to inappropriate signaling of pathways that promote precursor cell differentiation and/or enhanced inhibitory signals promoting dedifferentiation, as has been suggested to be the case for β-cells in diabetes (35).

ADIPOGENESIS: TIGHTLY REGULATED BY CELL COMMITMENT AND DIFFERENTIATION

Figure 2 shows a schematic figure of the overarching regulation of adipogenesis from recruiting mesenchymal stem cells and other uncommitted precursor cells into

| Cell size, μm | 106 | 53 |
| BMI, kg/m² | 26.4 | 19.3 |
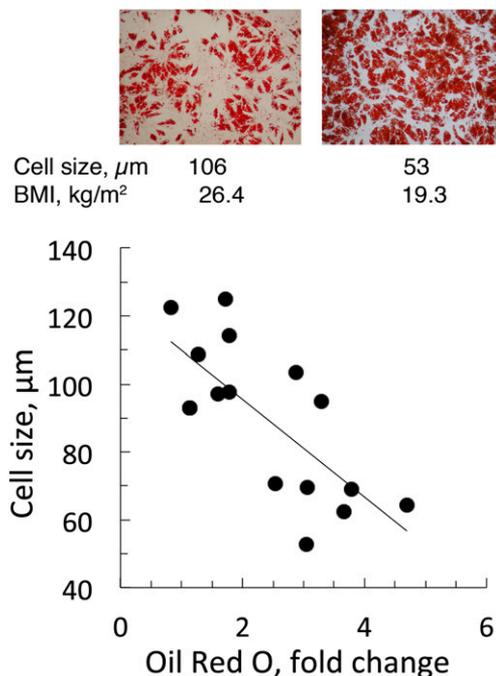
FIG. 1. Differentiation of human preadipocytes from the abdominal subcutaneous adipose tissue is related to the adipose cell size of the donors. Purified stromal-vascular cells were differentiated for 21 days in vitro and then stained with Oil Red O to show the lipid droplets (*top*). The negative correlation between the amount of lipids accumulated during differentiation and the adipose cell size of the donors is shown (*bottom*). Data is from ref. 34.

preadipocytes (commitment) and the subsequent differentiation of these cells into mature and functional adipose cells. A key signaling pathway for maintaining precursor cells uncommitted and undifferentiated is the wingless-type mouse mammary tumor virus integration site family (WNT) signaling pathway (23,36).

The transcriptional program regulating subsequent differentiation is well characterized, and PPARγ and CCAAT/enhancer-binding protein-α have been identified as the key regulators of terminal differentiation (37). However, there is an array of other regulatory factors of importance for this process.

WNT SIGNALING, ADIPOGENESIS, AND INSULIN ACTION

Secreted ligand proteins of the WNT-signaling family regulate several developmental pathways and can influence cell fate through autocrine and paracrine mechanisms. Their conservation throughout the animal kingdom emphasizes their importance for cell proliferation, cell fate, differentiation, and organism development (38). Furthermore, activating mutations in the WNT signaling pathway, which promote proliferation, are common in many different forms of cancer.

Most mammalian genomes contain 19 WNT genes with distinct signaling pathways and downstream effects. WNT signaling can be transduced by different receptor-ligand coupling processes, which interact with numerous other important signaling pathways.

The canonical WNT/β-catenin pathway is well characterized; binding of WNT ligands to Frizzled and its coreceptor, low density-lipoprotein receptor-related protein 5/6, results in a cascade of events leading to increased β-catenin levels (36,38). In the absence of WNT ligands, a multiprotein complex promotes β-catenin degradation through the proteasomal pathway. Stabilization of β-catenin enables it to interact with the nuclear transcription factors TCF/LEF and regulate transcription of many WNT-inducible genes (38).

Terminating the WNT signal is a prerequisite to allow induction of adipogenic differentiation. In contrast, maintaining this signaling pathway is important for the induction of myo- and osteogenesis (Fig. 2). Thus, these pathways need to be carefully regulated, and many fundamental questions about the molecular mechanisms remain unclear.

The importance of WNT activation for adipogenesis has not only been supported by in vitro data discussed below but also by in vivo data showing that adipocyte-specific overexpression of WNT10b in mouse models of diet-induced or genetic obesity leads to a reduced expansion of the adipose tissue (39).

WNT activation can be terminated by several secreted antagonists, including Dickkopf-1 (DKK1), WNT inhibitory factor-1, and secreted Frizzled-related proteins (sFRPs), although the exact mechanisms for the regulation and induction of these molecules are unclear. We have shown that PPARγ activation by thiazolidinediones increases DKK1 in differentiated 3T3-L1 adipocytes (40). However, this is obviously a late event and cannot account for the necessary early termination of WNT signaling needed to induce adipogenesis from precursor cells.

When we examined the temporal gene induction of PPARγ and key secreted WNT inhibitors during human subcutaneous adipose precursor cell differentiation, we found a rapid but transient increase in DKK1 that preceded PPARγ activation, whereas sFRP1 was increased more
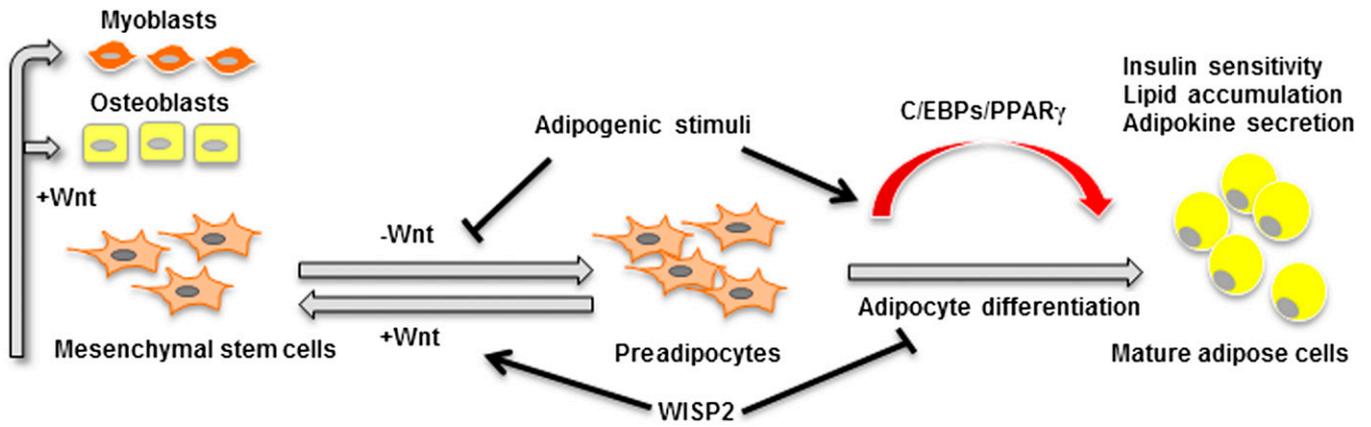
FIG. 2. Schematic figure of the overarching regulation of adipogenesis from mesenchymal stem cells and other uncommitted precursor cells. WNT/WISP2 silencing is a prerequisite for adipogenic commitment and subsequent differentiation of preadipocytes into mature adipose cells by activation of transcription factors of the C/EBP family and the key regulator of adipogenesis, PPARγ.

slowly (Fig. 3). This finding is in agreement with a previous study (41) and suggests that DKK1 may play a fundamental role in both terminating early WNT activation as well as in maintaining the WNT pathway inhibited because DKK1 is highly expressed in fully differentiated adipose cells (34). It should be added that there are several other known secreted antagonists of canonical WNT, but their role in the regulation of human adipogenesis is unclear. sFRP5 has attracted much recent attention as an anti-inflammatory and insulin-sensitizing molecule in mouse models (42), but its role in humans is unclear (43).

We recently performed a detailed study to characterize WNT activation and its termination during adipogenic differentiation of human subcutaneous adipose precursor cells from different donors (34). Low ability of these cells to undergo differentiation was associated with elevated β-catenin levels and no clear induction of DKK1. In contrast, cells that underwent good differentiation exhibited clear evidence of terminated WNT activation with both low β-catenin levels and high cellular induction of DKK1 protein (Fig. 4). Importantly, when we added exogenous DKK1 to cells with a low differentiation capability, they underwent a markedly improved differentiation together with induction of endogenous DKK1 and reduced β-catenin levels (Fig. 4).
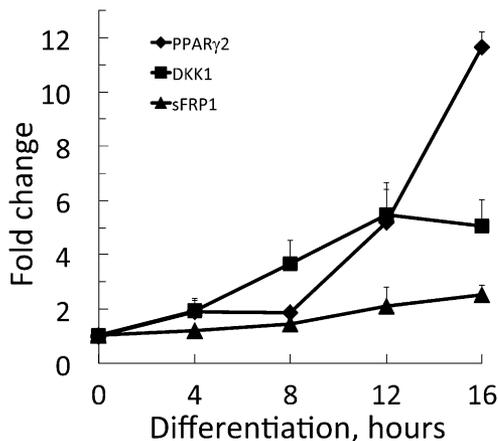
Taken together, these findings show that a poor ability of adipose precursor cells to differentiate is associated with markers of a maintained WNT activation and lack of induction of the secreted WNT inhibitor DKK1. Furthermore, inhibiting WNT with DKK1 increases adipogenic differentiation, showing that the low ability to differentiate is not due to lack of precursor cells but rather to an inability to terminate WNT activation. A low degree of differentiation characterizes adipose precursor cells from individuals with large adipose cells suggesting that hypertrophic obesity is indeed a consequence of the impaired ability of precursor cells to differentiate.

Interestingly, several large studies have associated genetic variation of the WNT-associated transcription factor TCF7L2 with T2D (44) and genetic variants of TCF7L2 also appear to have a negative impact on changes in BMI and visceral and nonvisceral adipose tissue during lifestyle intervention (45). TCF7L2 is a nuclear transcription factor that binds β-catenin and increases expression of WNT target genes. Interestingly, it is also a target of PPARγ activation through the induction of the transcriptional

FIG. 3. Temporal increase of PPARγ2, DKK1, and secreted sFRP1 mRNA levels following addition of differentiation cocktail to abdominal preadipocytes. Note that DKK1 is more rapidly induced than PPARγ2 and sFRP1. Data are means of 3 experiments.
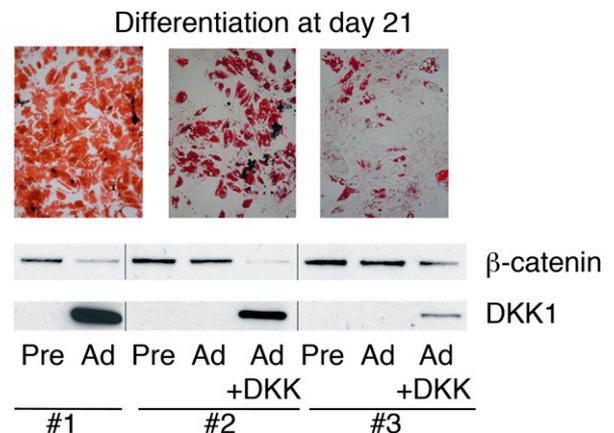

FIG. 4. Differentiation of human preadipocytes from different donors with high to low (top, left to right) accumulation of lipid droplets. High differentiation is associated with inhibited WNT activation seen as reduced β-catenin levels and induction of DKK1 protein. Intermediate or low differentiation has low inhibition of β-catenin and induction of DKK1. Adding exogenous DKK1 (+DKK) induced DKK1 protein in the cells, reduced β-catenin, and improved differentiation (not shown). For additional data, see ref. 34. Ad, adipocytes; Pre Ad, preadipocytes.

coregulator TLE3, which antagonizes β-catenin binding and helps terminate WNT activation (46).

Taken together, WNT activation plays a profound role in regulating adipogenesis, and it remains activated in adipogenic precursor cells in hypertrophic obesity.

BMP4 INDUCES COMMITMENT OF ADIPOSE PRECURSOR CELLS

WNT inhibition is necessary but not sufficient to induce commitment of mesenchymal stem cells and other adipogenic precursor cells. Additional signals have to be turned on or repressed in order to start the differentiation process. The BMPs have been shown to play an important role for the induction of both white adipogenesis (BMP2 and 4) and brown adipogenesis (BMP7) (34,47,48). We here focus on white adipogenesis and the effect of BMP4, although BMP2 can have similar effects. However, BMP2 is not increased in human preadipocytes undergoing differentiation in contrast to BMP4.

BMP4 is a member of the transforming growth factor-β superfamily, and members of this family were originally identified by their ability to induce ectopic bone formation, but they are now known to have different pleiotrophic developmental actions. The effect of BMP4 on adipogenic commitment was first shown with the multipotent stem cell line established from mouse embryos, C3H10T1/2, which differentiated to adipocytes in the presence of BMP4 (49). Similarly, we have found that incubating human subcutaneous adipose precursor cells with BMP4 induced commitment of precursor cells and in addition, also promoted subsequent adipogenesis (34). This was also seen in adipose precursor cells from individuals with hypertrophic obesity supporting that mesenchymal stem cells and other early precursor cells are present in the adipose tissue and can be recruited following appropriate proadipogenic signals.

Not only is BMP4 able to promote both commitment and subsequent differentiation when added to the culture medium but, interestingly, BMP4 is also induced in human adipose precursor cells during differentiation (34). Furthermore, when we added the BMP4 inhibitor noggin to the cells, the ability to undergo differentiation was markedly reduced supporting that endogenous BMP4 also promotes preadipocyte differentiation and activation of PPARγ. Interestingly, when a clone of committed adipose precursor cells was isolated from the C3H10T1/2 cells, which readily underwent adipogenic differentiation, these cells were characterized by having a high expression of endogenous BMP4 (49).

Taken together, both WNT and BMP4 signaling play important roles in adipogenesis by regulating commitment as well as subsequent PPARγ activation and differentiation. The finding that human committed preadipocytes also markedly increase and are apparently dependent on endogenous BMP4 during differentiation is intriguing. This suggests that BMP4, as a secreted molecule, could be part of an auto/paracrine system whereby differentiated adipose cells can crosstalk with precursor cells to promote their adipogenic commitment and differentiation under periods of lipid accretion. This process would obviously be impaired in hypertrophic obesity, and this possibility is under study in our laboratory.

REGULATION OF RESPONSIVENESS TO BMP4 BY BMP INHIBITORS

BMP4 signaling is a complicated process which is not only dependent on the induction and secretion of BMP4 but also on the ability to respond because cells also express and secrete many inhibitors that can antagonize BMP4 in different ways.

The amount of BMP4 available for signaling is tightly regulated by the complex BMP receptor signaling pathways and the number of structurally distinct BMP antagonists that alter the ability to bind to their receptors. Very little is currently known about the regulation of the endogenous BMP antagonists and their effects in adipogenesis.

Activin A is a secreted homodimer of inhibin βA subunits and is expressed in human multipotent adipose-derived stem cells and in preadipocytes. Activin A has a positive effect on proliferation of adipocyte precursor cells while it inhibits differentiation, and its expression declines after induction of differentiation (50).

Noggin is a secreted inhibitor that binds to the BMP receptors to block BMP action. However, the biological functions of noggin in adult tissue homeostasis are mostly undetermined, but it inhibits BMP signaling and, consistent with the proadipogenic effect of BMP4, we found differentiation of stromal precursor cells from human adipose tissue to be reduced in the presence of noggin (34). Thus, noggin seems to play an important role as an endogenous inhibitor of BMP4 signaling and action. Additional BMP inhibitors are chordin and chordin-like 1, but their role in adipogenesis is unclear. Knockdown of the BMP and activin membrane-bound inhibitor increased PPARγ mRNA levels in human preadipocytes (51), and the BMP inhibitor Gremlin1 is more highly expressed in omental than subcutaneous adipose tissue and is rapidly downregulated during initiation of preadipocyte differentiation (52).

Taken together, BMP4 plays an important role in adipogenesis by promoting both commitment and differentiation, but the molecular mechanisms for the regulation of BMP4 cellular responsiveness and the specific role of the many cellular inhibitors are unclear. Current data suggest that BMP4 may be an integral part of a positive feedback signal leading to the recruitment and differentiation of new adipose cells in the subcutaneous adipose tissue under periods of weight increase. Differential expression and regulation by the BMP inhibitors would then be strong candidates for the apparent failure of endogenous BMP4 to recruit new cells in hypertrophic obesity.

## WISP2: AT THE CROSSROAD BETWEEN WNT AND BMP4

BMP4 promotes commitment and adipogenic differentiation of precursor cells, whereas canonical WNT activation is inhibitory to both these processes. To make progress in understanding adipogenesis, we need to understand the apparent crosstalk between these pathways.

We recently identified a secreted and WNT-inducible protein, WISP2, as a novel adipokine providing new insight into the crosstalk between WNT and BMP4 signaling (53). WISP2, a member of the CCN family, has previously been found to crosstalk with transforming growth factor-β and to prevent epithelial mesenchymal transformation in cancer cells (54). It is a secreted protein, highly expressed in mesenchymal stem cells and preadipocytes (53). However, it is also present in the cytosol in undifferentiated cells as well as in the nucleus, although mechanisms regulating the subcellular distribution are not known. WISP2 expression is considerably higher in the subcutaneous adipose tissue than in visceral fat, and the expression is positively correlated to adipose cell size of the donor, i.e., it is upregulated in the subcutaneous adipose tissue in hypertrophic obesity.

Furthermore, the expression is significantly higher in the subcutaneous, but not the visceral, adipose tissue in individuals with the metabolic syndrome than in equally obese subjects without these markers of metabolic risk (53). We also found a positive correlation between WISP2 expression in the subcutaneous adipose tissue and ectopic fat accumulation, measured as amount of intra-abdominal fat, in different cohorts. This finding is consistent with the lipid overflow hypothesis discussed above, since WISP2 is a powerful inhibitor of adipogenesis. In support of this, we found the inability of human subcutaneous precursor cells from individuals with hypertrophic obesity to undergo differentiation to be associated with an inability to suppress WISP2 (53). The inability to suppress WNT signaling in precursor cells from individuals with hypertrophic obesity would be consistent with our finding of an increased activation of WISP2, since it is a WNT-inducible molecule.

We found WISP2 to have dual functions in regulating both adipocyte commitment/recruitment and differentiation. Cytosolic WISP2 regulates BMP4-dependent adipogenic commitment by forming a complex with the important PPARγ transcriptional activator ZNF423/Zfp423 (55), thus inhibiting its nuclear localization (53). BMP4 dissociates this complex and allows ZNF423/Zfp423 to enter the nucleus and induce PPARγ, the first step of commitment of precursor cells. However, it does not necessarily lead to full adipogenic differentiation because secreted WISP2 also inhibits PPARγ activation through an unknown cellular receptor (53). The direct inhibitory effect of secreted WISP2 on PPARγ activation is similar to the effect of secreted WNT ligands and, in fact, these pathways may converge because we found WISP2 to also activate and phosphorylate low density-lipoprotein receptor-related protein 5/6, the coreceptor for Frizzled (38,53). Thus, WISP2 is likely to be an important mediator of canonical WNT activation in adipogenic precursor cells, keeping the cells undifferentiated and preventing PPARγ activation. Secreted BMP4 by differentiated adipose cells would then be able to crosstalk with ambient precursor cells and induce commitment and differentiation under periods of lipid accumulation. However, this is obviously not well functioning in hypertrophic obesity, possibly because of secreted inhibitors to BMP4 and/or increased WNT signaling antagonizing the effect of BMP4. These questions are currently under study in our laboratory. The identity of the WISP2 receptor is currently unknown, but it would appear an interesting target for drug development,
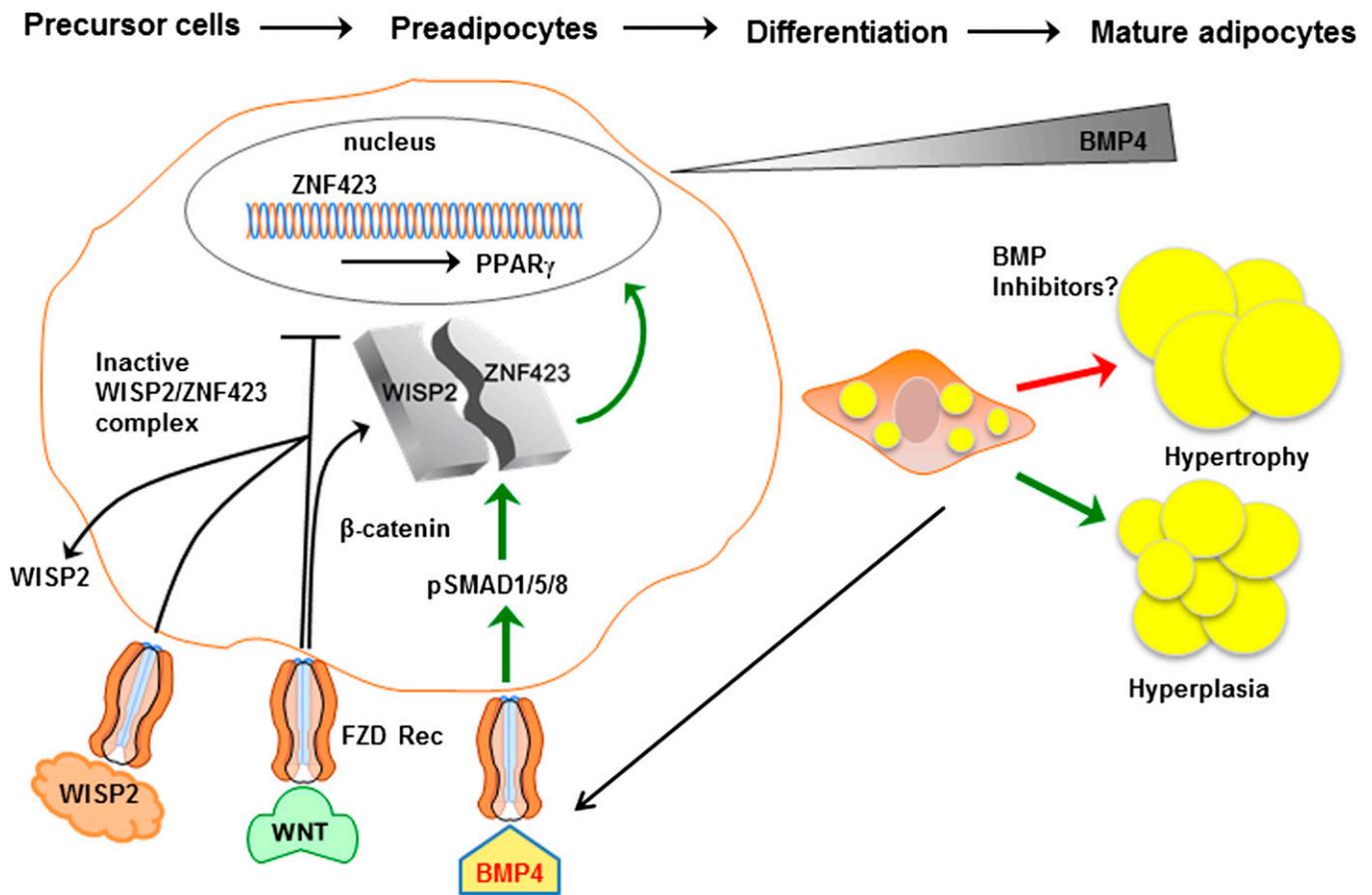
FIG. 5. Regulation of precursor cell commitment by BMP4 (*left*) and subsequent differentiation of the preadipocytes (*right*). Precursor cells are kept undifferentiated by WNT activation and the intracellular and secreted mediator WISP2. Cytosolic WISP2 binds the PPARγ transcriptional activator ZNF423/Zfp423 and retains it in the cytosol. BMP4 dissociates this complex whereby ZNF423/Zfp423 enters the nucleus and activates PPARγ. However, full activation of PPARγ also requires inhibition of extracellular WNT/WISP2, probably involving the rapid induction of the secreted WNT antagonist DKK1 (see Fig. 3). Committed preadipocytes undergo differentiation following PPARγ/c/EBP activation leading to increased BMP4 induction which, as a secreted protein, can activate commitment of other precursor cells through a paracrine action and also promote differentiation. The inability of this putative feedback regulation to prevent hypertrophic obesity may be due to increased expression of BMP inhibitors antagonizing the effect of the secreted BMP4. See the text for details. Adapted from Hammarstedt et al. (53). pSMAD1/5/8, phosphorylation of SMAD family members 1,5, and 8.

allowing adipogenic cell recruitment and differentiation and thereby storing excess lipids appropriately in the subcutaneous adipose tissue rather than in ectopic sites.

In summary, hypertrophic obesity is due to an impaired ability to recruit and differentiate available adipose precursor cells in the subcutaneous adipose tissue. If this is a ubiquitous problem also involving other adipose depots in hypertrophic obesity is currently unknown, but animal data suggest that BMP4 also promotes adipogenesis in visceral precursor cells (56). Furthermore, a recent study in humans found that the visceral fat depot, the greater omentum, increased its size in obesity mainly through an increased cell number (57). Thus, the subcutaneous adipose tissue may be particular in its limited ability in certain individuals to undergo adipogenesis during weight increase.

Inability to promote subcutaneous adipogenesis under periods of affluence would favor lipid overflow and ectopic fat accumulation with negative metabolic consequences (3,4,6). Our recent findings have shown that the WNT-inducible molecule WISP2 plays a key role by both preventing the effect of BMP4 to induce precursor cell commitment as well as by exerting a direct extracellular inhibitory signal on PPARγ activation and adipose cell differentiation after its secretion. Thus, WISP2 is situated at the crossroad between WNT and BMP4 signaling and can play a critical role for the development of hypertrophic obesity. Our current work is aimed at identifying the extracellular receptor and intracellular signaling as well as the regulation of the subcellular distribution of this molecule.

Figure 5 illustrates schematically our current understanding of the crosstalk between BMP4, canonical WNT signaling and WISP2 in the regulation of adipogenic commitment and differentiation. Secretion of BMP4 by differentiating adipose cells would promote both autocrine and paracrine commitment/differentiation of available precursor cells leading to new cell recruitment. However, this expected positive feedback regulation is apparently inhibited by counterregulatory signals in hypertrophic obesity.

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