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Bone Morphogenetic Proteins and Adipocyte Differentiation

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Bone morphogenetic proteins (BMPs) regulate many processes during embryogenesis and morphogenesis. However, the role of this family of proteins in adipogenesis appears to be underappreciated in the field of developmental biology. In the past fifteen years, our concept of adipose tissue has undergone a radical transformation: initially viewed as an inert mass for energy storage, adipose tissue is now seen as an endocrine organ with an important role in the regulation of whole-body energy homeostasis. Interest in adipocyte differentiation has increased markedly in the past few years with major emphasis on transcriptional controls during adipocyte formation. Clearly, much remains to be learned about adipogenesis. In this review, we summarize recent progress on the roles of BMPs in adipogenesis in mesenchymal stem cells and committed preadipocytes, and ultimately their potential involvement in the regulation of energy metabolism.

Introduction

Obesity is a public health problem worldwide, which results from an imbalance between energy intake and expenditure. Adipose tissue plays an important role in the regulation of energy balance, thereby contributing to the development of obesity. Therefore, improved knowledge of adipocyte biology is urgently needed to counter the growing epidemic of obesity. The past two decades have seen much progress toward defining the transcriptional events controlling adipocyte differentiation (recently reviewed in (Otto & Lane, 2005; Farmer, 2006; Rosen & MacDougald, 2006)). However, relatively little is known about how extracellular signals regulate these processes. One group of proteins of great interest in studies of the regulation of adipose development is the bone morphogenetic proteins (BMPs).

Bone Morphogenetic Proteins

Discovery, members, receptors, and signal transduction

More than 55 years ago, Pierre Lacroix hypothesized that bone may contain a substance, called osteogenin, which could initiate bone growth (Lacroix, 1945). This hypothesis was later validated by Marshall Urist's seminal work, in which the demineralized and lyophilized segments of rabbit bone implanted intramuscularly were shown to induce formation of new bone(Urist, 1965). This bone induction process unfolds in a cascade that recapitulates most, if not all, of the events occurring during development of the embryonic skeleton (Olsen *et al.*, 2000; Reddi, 1998). These experiments, performed in the 1960s, were followed by the cloning

of the first BMPs more than 20 years later, and the demonstration that these factors could reproduce the bone-forming activity of Urist's bone extracts (Reddi, 1998; Reddi, 1997). The amino acid sequences revealed homology to transforming growth factor (TGF)-β1, suggesting that BMPs and osteogenin are members of the TGF-β superfamily. In their landmark work, Wozney and colleagues cloned BMP-2, BMP-2B (a.k.a., BMP-4) and BMP-3 (also called osteogenin; Wozney *et al*., 1988). Subsequently, Ozkaynak and colleagues cloned Osteogenic protein-1 and -2 (OP-1 or BMP-7, and OP-2 or BMP-8; Ozkaynak *et al*., 1992). Comprising an ever-growing number of identified homologues, the BMPs represent almost one third of the TGF-β superfamily, with more than 30 members already described (Reddi, 1998; Reddi, 1997; Hogan, 1996; Luo *et al*., 2005). The members of this subfamily of secreted molecules are termed BMPs, osteogenic proteins (OPs), cartilage-derived morphogenetic proteins (CDMPs), or growth and differentiation factors (GDFs). They have been classified into several subgroups according to their structural similarities (Figure 1).

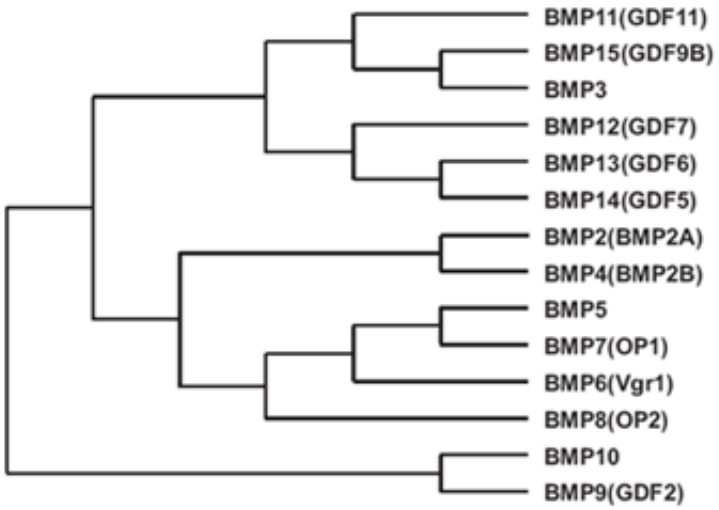

Figure 1. Phylogenetic classification of the 14 types of human BMPs.

BMPs activate a signaling cascade that regulates cell proliferation and differentiation. BMPs are dimeric molecules and their conformation is critical for biological action (Reddi, 1998; Rosen *et al*., 1996; Wozney, 1998; Karsenty, 2000). Mature BMP monomers consist of about 120 amino acids, with seven canonical cysteine residues. There are three intrachain disulfides and one interchain disulfide bond. The cysteine knot is the critical central core of the BMP molecule. Upon dimerization, the two subunits are proteolytically cleaved at a consensus Arg-X-X-Arg site to yield carboxy-terminal mature dimers (Wozney *et al*., 1988; Israel *et al*., 1992; Kingsley, 1994). This cleavage, which is thought to occur before secretion, is mediated by subtilisin-like convertases (Cui *et al*., 1998; Constam & Robertson, 1999). It was recently shown that a downstream sequence adjacent to the cleavage site determines the cleavage efficiency, while the N-terminal region controls the stability of the processed mature protein (Constam & Robertson, 1999). Analysis of the crystal structure of two TGF-β family members, TGF-β2 and BMP-7, has revealed that the core of the monomer is a cystine knot involving six cysteine residues that are invariably spaced in the C-terminal region of all family members (Schlunegger & Grutter, 1992; Griffith *et al*., 1996). The mature BMPs fulfill their signaling function by binding to a heterodimeric complex of two transmembrane serine-threonine kinase receptors, termed type I and type II (Hogan, 1996; Kingsley, 1994; Massague & Weis-Garcia, 1996). In vertebrates, seven type I receptors and five type II receptors have been found so far. Among these different isoforms, three type I receptors (BMPR-Ia/ALK-3, BMPR-Ib/ALK-6, and ALK-2) and three type II receptors (BMPR-II, ActR-2a, and ActR-2b) mediate most of the effects of BMPs (Kishigami & Mishina, 2005). The kinase activity of the type II receptor is constitutive, while ligand binding is required for type I receptor kinase activation (Ruberte *et al*., 1995; Liu *et al*., 1995). Optimal ligand binding is achieved when both type I and type II receptors are present, although BMPs can bind to each of them weakly and subsequently recruit the second subunit. The specificity of signaling is primarily determined by type I receptors, whereas the specificity of ligand binding is established by combinations of both types of receptors. The activated receptor kinases subsequently phosphorylate transcriptional factors, called Smads. There are eight members in the Smad family (Massague & Chen, 2000). Phosphorylated Smads 1, 5 and 8 are functional mediators of BMP signaling in partnership with Smad 4, whereas Smad 6 and Smad 7 function as antagonists to inhibit TGF-β/BMP superfamily signaling (Massague & Chen, 2000). The phosphorylated Smad 1 enters as a heteromeric complex with Smad 4 into the nucleus, where they activate the expression of target genes in concert with other co-activators (Massague, 1998; Heldin *et al*., 1997). BMPs also activate the p38 MAPK pathway via the

MAPKKK cascade. This pathway leads to activation of the transcription factor ATF-2 and regulates expression of a variety of growth-related genes (reviewed in Canalis *et al*., 2003). Interestingly, activation of p38MAPK by BMP-7 during epithelial cell morphogenesis is negatively regulated by Smad1 (Hu *et al*., 2004), suggesting that cross-talk between these two signaling pathways may occur in certain cell types. (Figure 2).

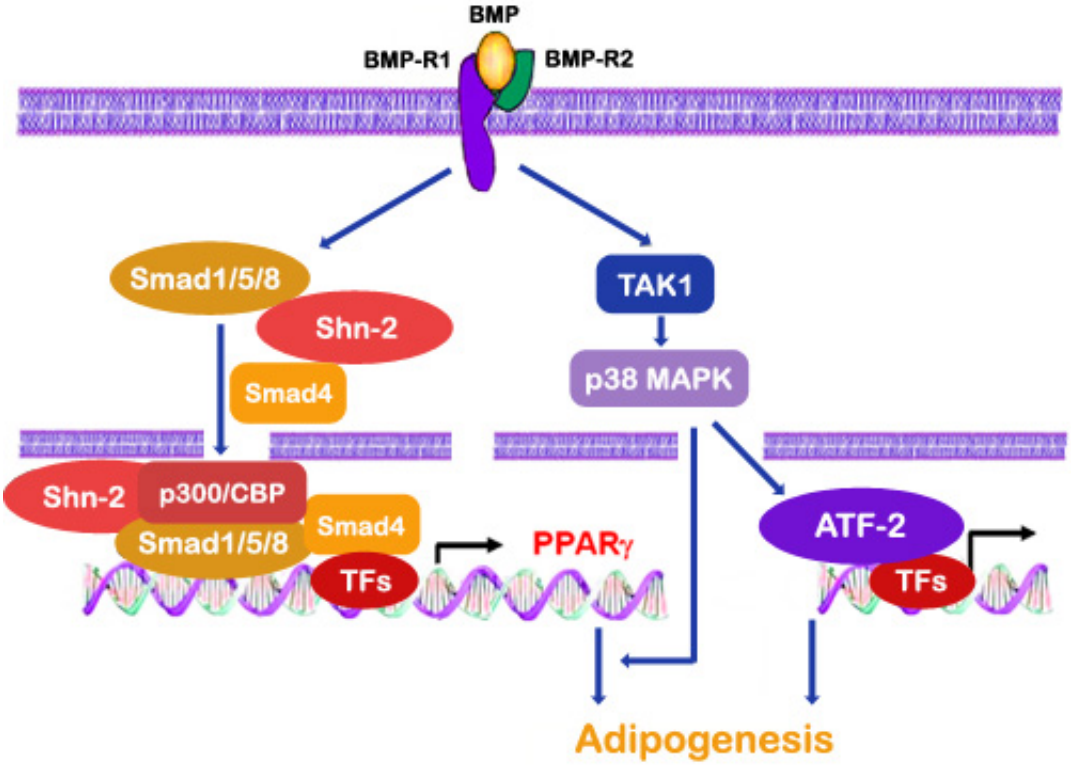

Figure 2. Molecular mechanisms utilized by BMPs to regulate adipogenesis.

Role of BMPs in development

Expression of BMPs is not restricted to developing skeleton but is also present in a wide range of tissues during development. Recent developmental and genetic studies have revealed their pleiotropic actions beyond bone morphogenesis. For example, BMP-2 expression can be detected in the mouse as early as 8.5 days postcoitum (dpc) in mesodermal cells of the amnion and chorion cells of the visceral endoderm, the allantois, and the lateral plate mesoderm underlying the head fold (Lyons *et al*., 1995; Zhang & Bradley, 1996; Schultheiss *et al*., 1997). From 8.5 to 9.5 dpc, BMP-2 is also expressed in the dorsal surface ectoderm underlying the neural tube (Dudley & Robertson, 1997). Around 9.5 and 10.5 dpc, its expression can be detected in the outer myocardial layer of the heart, in the apical ectodermal ridge, and in the zone of polarizing activity of the developing limb (Zhang & Bradley, 1996; Schultheiss *et al*., 1997; Dudley & Robertson, 1997; Lyons *et al*., 1990). Starting at 12.5 dpc, BMP-2 expression is also observed in the mesenchymal condensation that will give rise to the ribs and vertebrae, in the tooth buds, in the developing eye, and in the whisker follicles (Dudley & Robertson, 1997; Lyons *et al*., 1990; Lyons *et al*., 1989). Later during development, BMP-2 transcript can be observed in hypertrophic chondrocytes of long bones and in forming digits (Solloway *et al*., 1998). At the different stages of development, BMP-4 is expressed in the allantois, the amnion, the posterior part of the primitive streak, the neural tube area, the mesoderm around the developing gut, in the myocardium, the branchial arches, the developing eye, the otic vesicles, the neuroepithelium, and the dorsomedial telencephalon (Schultheiss *et al*., 1997; Dudley & Robertson, 1997). BMP-4 transcripts can also be detected in both the epithelium and mesenchyme tissues of the developing

limb bud and tooth bud (Jones *et al*., 1991; Furuta *et al*., 1997). BMP-6 has a broad pattern of expression also, as detected in the branchial pouch, the endodermal component of the visceral yolk sac, the roofplate of the neural tube, and the epithelium of the branchial pouch of the developing heart, as well as in the developing kidney and skin (Lyons *et al*., 1995; Lyons *et al*., 1989; Jones *et al*., 1991; Furuta *et al*., 1997). In the developing skeleton, BMP-6 is expressed preferentially in hypertrophic chondrocytes (Solloway *et al*., 1998; Iwasaki *et al*., 1997). Broad expression of BMP-7 is detected in the ectoderm of the periphery of the embryo, the surface ectoderm and the notochord, the neuroepithelium extending toward the prospective forebrain, and the developing gut. BMP-7 is also expressed in the atrial and ventricular chambers (Lyons *et al*., 1995; Schultheiss *et al*., 1997; Dudley & Robertson, 1997). During eye development, BMP-7 expression is detected in the surface ectoderm, the lens placode, and the optic vesicle (Lyons *et al*., 1995; Dudley & Robertson, 1997). BMP-7 is also expressed in the developing kidney, the Wolffian ducts, the ureteric bud epithelium, the pretubular aggregates and the podocytes of the glomeruli (Luo *et al*., 1995; Dudley *et al*., 1995). In the developing skeleton, BMP-7 is expressed in the developing limb, in the mesenchymal cells localized between the developing digits, and in the chondrogenic cells (Dudley & Robertson, 1997; Furuta *et al*., 1997; Luo *et al*., 1995; Dudley *et al*., 1995). Interestingly, several BMPs are not highly expressed in developing skeletal structures, but seem to be specifically expressed in particular tissues. For instance, BMP-15 is expressed in the ovaries, testis, and hypothalamus (McGrath *et al*., 1995; Dong *et al*., 1996), and BMP-10 expression appears to be restricted to the heart (Neuhaus *et al*., 1999). Finally, several BMPs are expressed more specifically in the developing nervous system, such as BMP-12 and BMP-11 (Lee, 1991; Lee *et al*., 1998; Nakashima *et al*., 1999). Thus, the expression of BMPs is often widespread and dynamic as development proceeds and is frequently localized in areas of epithelial–mesenchymal interaction, suggesting that BMPs could have a broad range of physiologic functions during embryonic and post-embryonic development.

Genetic disruptions of BMPs have resulted in various skeletal and extraskeletal abnormalities during development (Zhao, 2003; Table 1). Targeted disruption of BMP-2 in mice resulted in embryonic lethality (Zhang & Bradley, 1996). BMP-4 null animals exhibit no mesoderm induction, and gastrulation is impaired (Winnier *et al*., 1995). Homozygous BMP-6-deficient mice have no skeleton-patterning defect and only a mild delay of sternum ossification that could be traced to the formation of the mesenchymal condensations (Solloway *et al*., 1998). Targeted deletion of BMP-7 revealed critical roles for this molecule in kidney and eye development, in addition to patterning abnormalities of the ribs and a preaxial polydactyly of the hind limbs (Luo *et al*., 1995; Dudley *et al*., 1995). Interestingly, mutations in BMP-5 and BMP-14 were the first mutations to be identified in the BMP family, and the abnormalities they cause seemed to indicate that the wild-type proteins would function primarily if not exclusively in the developing skeleton. Kingsley *et al*. demonstrated that the gene mutated or deleted in the Short Ear mouse mutant was the BMP-5 gene (Kingsley *et al*., 1992). The brachypodism (bp) phenotype, characterized by a reduction in the length of several long bones and the replacement of two bones in most digits by a single skeletal element, is caused by frameshift mutations in the BMP-14 gene (Storm *et al*., 1994). BMP-14 has been shown to induce the formation of tendon and ligament structures in the classic subcutaneous implantation assay (Wolfman *et al*., 1997). Moreover, a mutation in the human BMP-14 gene has been implicated in two recessive chondrodysplasias: Hunter-Thompson chondrodysplasia and Grebe chondrodysplasia (Langer, Jr. *et al*., 1989; Thomas *et al*., 1996; Thomas *et al*., 1997). Mutations in the BMP-14 gene also cause autosomal dominant brachydactyly type C (Polinkovsky *et al*., 1997). Mice carrying null mutations in both BMP-14 and BMP-5 have skeleton-patterning defects that are not seen in any of the single mutants, but these abnormalities are extremely localized and do not disturb the differentiation of osteoblasts or chondrocytes (Storm & Kingsley, 1996). Mice carrying heterozygous mutations in both BMP-7 and BMP-4 have a higher frequency of rib cage and digit abnormalities than single heterozygotes (Katagiri *et al*., 1998), suggesting that BMP-7 and BMP-4 may act jointly in the formation of the affected mesenchymal condensations. BMP-5/-6 double mutant mice display only a slight exacerbation of the sternal defect present in the single mutants (Solloway *et al*., 1998). BMP-5/-7 double mutant mice die early during embryogenesis, prior to the onset of skeletogenesis. Genetic inactivation of other members of the BMP subfamily has revealed abnormalities in non-skeletal tissues (Zhao,

2003). Surprisingly, a recent gene deletion study has demonstrated that BMP-3 may act as an inhibitory regulator of bone density (Daluiski *et al*., 2001). Thus, these collective data have demonstrated the functional heterogeneity of BMPs in skeletogenesis and non-skeletal tissue development (Table 1).

Effect of BMPs on lineage determination in mesenchymal stem cells

Stem cells are defined as progenitor cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Mesenchymal stem cells (MSCs) are adherent bone marrow stromal cells or fibroblast-like cells that maintain their self-renewal stem-cell phenotype and give rise to differentiated progenitor cells belonging to the osteogenic, chondrogenic, adipogenic, myogenic and fibroblastic lineages (Prockop, 1997; Pittenger *et al*., 1999; Caplan & Bruder, 2001). Although primarily residing in within the bone marrow compartment (Caplan & Bruder, 2001; Friedenstein *et al*., 1966; Baksh *et al*., 2004), MSCs have been isolated from periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle and deciduous teeth (Barry & Murphy, 2004). MSCs represent a very small fraction (e.g., 0.001-0.01%) of the total population of nucleated cells in the marrow (Pittenger *et al*., 1999). Currently, the biologic properties of MSCs, specifically with respect to their existence in the adult organism and their postulated biological niche, are not well understood. Nonetheless, several signaling pathways have been implicated in regulating stem cell self-renewal and lineage commitment (Molofsky *et al*., 2004; Wang & Wynshaw-Boris, 2004; Kleber & Sommer, 2004; Reya & Clevers, 2005). BMPs play important roles both in regulating cell proliferation and differentiation during development and in stem cell biology (He, 2005; Varga & Wrana, 2005; Zhang & Li, 2005). For example, BMPs are known to induce osteoblast differentiation of MSCs and subsequent bone formation (Reddi, 1998; Luo *et al*., 2005; Zhao, 2003; He, 2005; Urist, 1997; Cheng *et al*., 2003) by regulating a distinct set of downstream targets (Peng *et al*., 2003; Peng *et al*., 2004; Luo *et al*., 2004; Si *et al*., 2006).

Role of BMPs in Development of Adipose Tissue

Adipose tissue and obesity

For decades, adipose tissue was regarded as an inert mass for energy storage. However, this concept was radically revised in the past fifteen years. It is now known that adipose tissue not only serves as a fat depot, but also acts as a secretory/endocrine organ and plays a central role in the regulation of energy balance and thermoregulation. When energy input exceeds energy expenditure, the extra energy is stored in fat, leading to obesity. Modern sedentary life style and high-caloric diet have contributed to a high prevalence of obesity worldwide. According to the World Health Organization, more than 1 billion adults are overweight (body mass index (BMI) >25), and over 300 million rank as truly obese (BMI>30; Lev *et al*., 1993; Friedman, 2000). In developed countries, obesity has become a major heath issue with serious social, psychological, and physiological consequences. Thus, improved understanding on all aspects of adipose biology is required to counter the growing epidemic of obesity.

The adipose lineage arises from a multipotent stem cell population of mesodermal origin. These precursor cells reside in the vascular stroma fraction of adipose tissue and become committed to the adipocyte lineage under appropriate developmental cues (Otto & Lane, 2005). At the cellular and molecular levels, the program of white adipocyte differentiation can be delineated into at least four stages that include (a) preconfluent proliferation; (b) confluence/growth arrest; (c) hormonal induction/clonal expansion; and (d) permanent growth arrest/terminal differentiation (Gregoire *et al*., 1998; Cowherd *et al*., 1999; Rosen, 2005). The latter part of this process is under complex transcriptional control involving CCAAT/enhancer-binding protein (C/EBP) δ, β, and α, peroxisome proliferator-activated receptor (PPAR) γ and other transcription factors that are induced in a specific sequence. This leads to the synthesis of proteins characteristic of a fully differentiated phenotype, such as fatty acid synthase and glucose transporter 4. This process is tightly controlled by positive and negative stimuli, such as hormones, nutrients and extracellular environment (Rangwala & Lazar, 2000;

Gregoire, 2001; Koutnikova & Auwerx, 2001; Camp *et al*., 2002; Farmer, 2006; Rosen & MacDougald, 2006).

There are two functionally different types of adipose tissue in mammals: white adipose tissue (WAT), which is the primary site of storage of triglycerides and release of fatty acids, and brown adipose tissue (BAT), which is specialized in thermogenic energy expenditure through the expression of uncoupling protein-1 (UCP-1; Figure 3). The developmental patterns of both tissues are quite distinct. BAT develops during fetal life and possesses all the features of mature tissue at birth when the requirements for non-shivering thermogenesis are needed. In contrast, development of WAT in rodents takes place primarily after birth and its mass increases during post-natal life (Nedergaard *et al*., 1986; Moulin *et al*., 2001b). Morphologically, BAT can be distinguished from WAT by multi-locular lipid inclusions and numerous well-developed mitochondria. Indeed, brown fat cells have among the highest mitochondrial density of any cell type in mammals (Lindberg *et al*., 1967). With regard to the developmental origins of brown vs. white fat, the current hypothesis favors that white and brown preadipocytes are already determined toward differentiation into one or the other adipose cell type (Klaus, 2004; Moulin *et al*., 2001a; Moulin *et al*., 2001b). However, adipose tissues are organs of great heterogeneity and plasticity (Cinti, 2005). Both environmental and nutritional stimuli can cause anatomical interconversions of BAT and WAT. For example, BAT is transformed into WAT during development and, conversely, WAT can be turned into BAT during cold adaptation or after pharmacological treatment, such as with β3 adrenoceptor agonist (Cousin *et al*., 1992; Himms-Hagen *et al*., 2000). Nevertheless, one of the remaining questions in adipocyte biology is how and when the differentiation of BAT vs. WAT is regulated and specified.

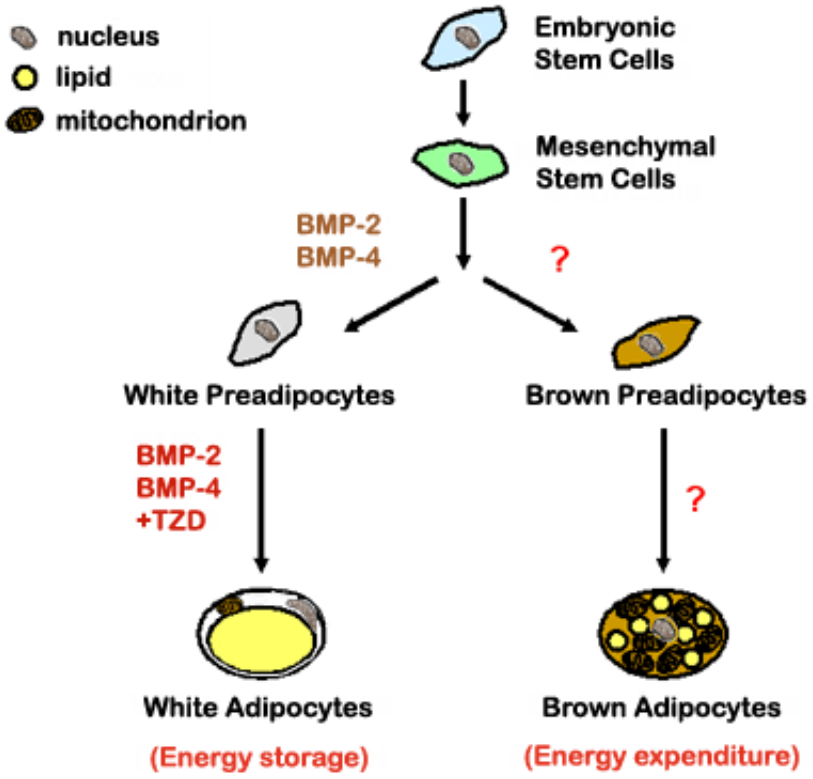

Figure 3. Role of BMPs in adipose development

Understanding the role of BMPs in adipose development from knockout animal models

In the past two decades, although genetic ablation approaches have revealed the important function of BMPs in different aspects of embryogenesis and organogenesis (Zhao, 2003), the specific effects of BMPs on adipose development can not be evaluated in these models, partly because of embryonic lethality in many of the KO animals or because severe defects in other tissues/organs obscure the adipose phenotype. Interestingly, however, several of the KO models with null mutations ablating either ligands, receptors, or downstream components of the BMP signaling system showed defects in mesoderm formation. Since mesoderm is the primordium of adipose tissue, these results provide indirect evidence for a role for BMPs in the development of adipose tissue. For example, mice homozygous for BMPR-Ia died at embryonic day 8 due to a lack of mesoderm formation (Mishina *et al*., 1995). Similar phenotypes were found in mice without functional BMPR-II (Beppu *et al*., 2000). Of the different BMP receptor isoforms, BMPR-Ia is particularly interesting to adipocyte biology since it has been shown to specialize in adipocyte differentiation *in vitro* (Chen *et al*., 1998). Other BMP KO mouse models with mesodermal defects include mutants of BMP-4 (Winnier *et al*.,

1995), Alk-2 (Gu *et al*., 1999; Mishina *et al*., 1999), Smad1 (Lechleider *et al*., 2001; Tremblay *et al*., 2001) and Smad5 (Chang *et al*., 1999; Yang *et al*., 1999; Chang & Matzuk, 2001). Although a good *in vivo* model to test the direct function of BMPs in development of adipose tissue is currently unavailable, several *in vitro* studies suggest important roles for BMPs in adipocyte differentiation, including effects of the dosage and type of BMP, the type of precursor cells and the presence of other regulators. Recently, Schnurri-2 (Shn-2) KO mice provided the first in vivo evidence that BMP signaling is critical for adipose development (Jin *et al*., 2006). BMP-2 induces nuclear translocation of Shn-2, where it functions as a scaffold to bring together other transcription factors to regulate adipogenesis. Shn-2 KO mice display reduced WAT, a tendency toward reduction of brown fat mass, and improved glucose tolerance and insulin sensitivity.

Induction of adipogenesis by BMPs in MSCs

As mentioned above, several studies have demonstrated important roles for BMPs in the control of commitment of stem cells to various lineages (Varga & Wrana, 2005; Zhang & Li, 2005). BMPs have been shown to have different roles in adipogenesis depending on the cell stage and the dosages of BMPs. In bone marrow stromal cells, the predominant effect of BMPs, in particular BMP-2, is to promote osteogenic differentiation and inhibit adipogenesis (Gimble *et al*., 1995; Chen *et al*., 2001; Pereira *et al*., 2002; Sciaudone *et al*., 2003; Song *et al*., 2003); however, low concentrations of BMPs modestly stimulate adipocyte differentiation (Chen *et al*., 2001). The effects of BMPs in the pluripotent mesenchymal cell line C3H10T1/2 are more complex and tightly controlled by the dosages and types of BMPs used in the system as well as by the presence of other extracellular and intracellular factors. The C3H10T1/2 cell line was established from 14- to 17-day-old C3H mouse embryos (Reznikoff *et al*., 1973). These cells display fibroblastic morphology and are functionally similar to mesenchymal stem cells, having the ability to differentiate into multiple lineages, including myoblast, adipocyte, chondrocyte, and osteoblast (Taylor & Jones, 1979; Wang *et al*., 1993; Ahrens *et al*., 1993). In these cells, low concentrations of BMP-2 and BMP-7 induce adipogenic differentiation whereas high concentrations promote differentiation toward chondrocyte and osteoblast (Wang *et al*., 1993; Asahina *et al*., 1996). Stable expression of cDNAs encoding different BMPs induces C3H10T1/2 cells to differentiate into osteogenic, chondrogenic and adipogenic lineages (Ahrens *et al*., 1993; Bachner *et al*., 1998). These BMPs appear to have differential effects on adipogenesis in this system, with BMP-4 having the greatest effect on induction of lipid accumulation and expression of markers for mature adipocytes (Bachner *et al*., 1998). Implantation of C3H10T1/2 cells treated with BMP-4 into nude mice results in the formation of a fat pad morphologically resembling WAT (Tang *et al*., 2004). Furthermore, *in vitro* studies demonstrate that BMP-4 is required for stable commitment of pluripotent C3H10T1/2 cells to the adipocyte lineage (Bowers *et al*., 2006) and this process involves downregulation of the expression of matrix metalloproteinase (MMP)-3 and MMP-13 (Otto *et al*., 2006). More recently, Taha *et al*. showed that BMP-4 could induce adipocyte differentiation in mouse embryonic stem cells (Taha *et al*., 2006).

Effect of BMPs on differentiation of committed preadipocytes

BMPs can also stimulate differentiation in committed preadipocytes. For example, BMP-2 promotes adipogenesis of the committed white preadipocyte lines 3T3-F442A and 3T3-L1 (Ji *et al*., 2000; Rebbapragada *et al*., 2003). Although these effects are very moderate, they can be enhanced when PPARγ ligand is present in the medium (Sottile & Seuwen, 2000). The synergistic effect of BMP-2 and PPARγ ligand may be explained, at least in part, by the ability of BMP-2 to upregulate PPARγ expression (Hata *et al*., 2003). However, when given with retinoic acid, BMP-2 inhibits adipogenic differentiation and enhances osteogenic differentiation in 3T3-F442A cells (Skillington *et al*., 2002). Thus, the current literature suggests an important role for BMPs in white adipocyte differentiation, especially in multipotent mesenchymal cells and to a lesser extent, in committed preadipocytes. However, knowledge of whether or not BMPs have an effect on the differentiation and function of brown fat is entirely lacking (Figure 3).

Effect of BMPs on lipid metabolism

Compared to the substantial amount of data concerning the roles of BMPs in all aspects of embryonic development and organogenesis, very little attention has been given to the potential role of BMPs in whole-body energy homeostasis. Using a proteomics approach, Witthuhn and Bernlohr demonstrated a significant increase of GDF-3/Vgr-2, which is a member of the BMP family, in the serum of mice lacking fatty acid binding protein-4 (FABP-4; Witthuhn & Bernlohr, 2001). The increase in serum GDF-3/Vgr-2 protein correlates with a marked increase in adipose GDF-3/Vgr-2 mRNA. FABP-4 is the major fatty acid binding protein in adipocytes and plays an important role in the regulation of lipid metabolism and energy balance. More recently, expression of BMP-3 was found to be elevated in mice that are more susceptible to high fat diet-induced obesity (DIO) compared to those resistant to DIO (Koza *et al*., 2006). Together, these data suggest a link between adipocyte lipid metabolism and the expression of the BMP family of differentiation regulators.

Molecular mechanism by which BMPs regulate adipogenesis

As described above, there are at least two major signaling pathways by which BMPs elicit most of their biological functions. One pathway is the canonical Smad-dependent pathway, and a second pathway involves p38 MAPK activation. Both signaling machineries are present in both MSCs and preadipocytes. Hata *et al* reported that both Smad1 and p38 MAPK regulate the expression and activity of PPARγ during BMP-2-induced adipogenesis in C3H10T1/2 cells (Hata *et al*., 2003). Recently, Jin *et al* report that the BMP signaling intermediate Shn-2 is required for adipogenesis *in vivo* and *in vitro* (Jin *et al*., 2006). Shn-2 is a zinc finger-containing protein that enters the nucleus upon BMP-2 stimulation and, in cooperation with Smad1/4 and C/EBPα, induces PPARγ expression. In addition, ectopic expression of PPARγ can compensate for the loss of Shn-2 *in vitro*. A proposed model summarizing current knowledge on the molecular regulation of adipogenesis by BMPs is shown in Figure 2.

Conclusions and Perspectives

In addition to the pleiotropic effects of BMPs on different aspects of embryonic development and organogenesis, BMPs also play important roles in adipocyte differentiation. Although progress in understanding the molecular mechanisms by which BMPs regulate adipogenesis has been made, there are several questions remaining to be answered to fully understand the role of BMPs in these processes. Of particular interest, do BMPs also play a role in the regulation of brown adipogenesis? Do different BMPs have differential effects on brown vs. white fat differentiation? How is the specificity of BMP-receptor signaling achieved? What are the contributions of the different signaling pathways in the complex transcriptional cascade leading to adipogenesis?

Another interesting issue that remains to be clarified is the osteogenic vs. adipogenic effects of BMPs. It has long been hypothesized that the osteoblast and marrow adipocyte lineages may have a close but reciprocal relationship (Nuttall & Gimble, 2004). Changes in the balance between osteogenesis and adipogenesis have been postulated as contributing factors to physiologic and pathologic conditions, such as aging and osteoporosis (Meunier *et al*., 1971; Burkhardt *et al*., 1987; Rozman *et al*., 1990; Kajkenova *et al*., 1997; Verma *et al*., 2002; Justesen *et al*., 2001; Moerman *et al*., 2004). A recent study indicated that aging activates adipogenic and suppresses osteogenic programs in marrow stem cells (Moerman *et al*., 2004). Clinically, a decrease in bone volume in age-related osteoporosis is usually accompanied by an increase in marrow adipose tissue (Meunier *et al*., 1971; Burkhardt *et al*., 1987; Rozman *et al*., 1990; Kajkenova *et al*., 1997; Verma *et al*., 2002; Justesen *et al*., 2001). Conversely, patients with progressive osseous hyperplasia have heterotopic bone formation within adipose tissue (Kaplan & Shore, 2000).

BMP subfamily	BMP* designation	Generic name	Phenotype of genetic disruption [References]	Functions in development
BMP2/4	BMP2	BMP2A	embryonic lethality. delayed primitive streak, small allantois, lack of amnion, heart defects, decreased number of primordial germ cells (PGCs). *[Zhang & Bradley, Dev. 122:2977 (1996)]*	bone & cartilage morphogenesis/heart
	BMP4	BMP2B	embryonic lethality. lack of allantois and PGCs, posterior trunction, head defects, lack of optic vesicle. *[Winnier et al. Genes Dev 9:2105 (1995)]*	bone morphogenesis, fat differentiation
BMP3	BMP3	osteogenin	increased bone density. *[Daluiski et al. Net Genet 27:84 (2001)]*	negative regulator of bone density
BMP7	BMP5	BMP5	short ear phenotype, defects in skeleton, lung and kidney. *[Kingsley et al. Cell 71:399 (1992); King et al. Dev Biol. 166:112 (1994)]*	bone morphogenesis
	BMP6	Vgr-1	mild delay of sternum ossification. *[Solloway et al. Dev Genet. 22:321 (1998)]*	bone morphogenesis, hypertrophy of cartilage/skin
	BMP7	OP-1	skeletal defects, kidney agenesis, eye defects. *[Dudley et al. Genes Dev. 9:2795 (1995); Luo et al. Genes Dev. 9:2808 (1995)]*	bone morphogenesis, eye and kidney development
	BMP8	OP-2	Defects in spermatogenesis and epididymis. *[Zhao et al. Dev 125:1103(1998)]*	bone formation
	BMP9	GDF-2	not known	cholinergic neuron differentiation, hepatocyte growth, hematopoiesis, bone formation
	BMP10	BMP10	not known	expression restricted to heart
	BMP11	GDF-11	Defects in A-P partterning of axial skeleton. *[McPherron et al, Nat Genet 22:260(1999)]*	A-P patterning of axial skeleton
GDF-5,6,7	BMP12	GDF-7 or CDMP-3	commissural interneurons of spinal cord. Hydrocephalic abnormalities growth defects in seminal vesicle. *[Lee et al, Genes Dev 12:3394(1998); Settle et al, Dev Bio 234:138(2001)]*	ligament and tendon development
	BMP13	GDF6 or CDMP2	defects in joints, cartilage, and ligament formation. *[Settle et al. Dev Bio 254(1):116-130(2003)].*	ectopic induction of tendon and ligament, cartilage development
	BMP14	GDF-5 or CDMP-1	Brachypodism in mice (limbs shortened with reduced number of bones). Dominant negative mutation in humans causes severe limb shortening and dysmorphogenesis. *[Storm et al. Nature 368:639(1994); Storm and Kingsley, Dev 122:3969(1996);Thomas et al. Nat Gen 17:58-64(1997)]*	joint formation, chondrogenesis
	BMP-15	GDF-9B	increased ovulation rate & infertility. Sterile due to defects in oogenesis. *[Dong et al, Nature 383:531 (1996)]*	ovulation and female fertility

* Note: BMP-1 is excluded because it functions as a protease rather than a bone-forming factor.

Table 1. Members of BMP Family and Their Functions in Development

One potential link between BMP-induced osteogenic and adipogenic differentiation of MSCs is the transcription factor PPARγ. Our earlier studies showed that PPARγ is readily up-regulated by osteogenic BMPs in pre-osteoblast progenitor cells (Peng *et al*., 2003). PPARγ insufficiency enhances osteogenesis (Akune *et al*., 2004), whereas the PPARγ agonist rosiglitazone causes bone loss in mice (Ali *et al*., 2005; Soroceanu *et al*., 2004). Nevertheless, it is not clear why osteogenic BMPs would upregulate PPARγ expression. Interestingly, Runx2 null mice exhibit both impaired bone formation and reduced adipogenesis (Kobayashi *et al*., 2000), suggesting that osteogenic and adipogenic differentiation are closely related. Therefore, it remains to be determined what exact roles, if any, PPARγ may play in BMP-induced osteogenesis and adipogenesis of MSCs. Ultimately, knowledge of the regulatory mechanisms behind BMP-regulated lineage divergence during MSC differentiation should aid us in understanding the causes of osteoporosis, aging, and other skeletal diseases, and may lead to the development of novel therapeutic strategies for human disorders, such as osteoporosis and obesity.

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