Chapter 18
TGF-β and Metabolic Homeostasis

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Abstract Metabolic homeostasis is achieved via a concerted and integrative action of various organ systems. The harmonious functionality of the collaborative organs maintains tight regulation of glucose levels. This intricate balance of glucose production and utilization maintains whole body glucose homeostasis and energy balance. In disease states, such as diabetes and obesity, dysfunction in one or more organ systems disturbs the metabolic homeostasis and propagates disease pathology. Understanding the avenues that dictate metabolic homeostasis and the pathways that disrupt this harmony are thus of great medical significance. We propose that the TGF-β signaling network plays an integral role in metabolic homeostasis by virtue of its actions on several organ systems that constitute the metabolic machinery. TGF-β levels are elevated in metabolic disease, which supports the utility of therapeutics aimed at targeting the TGF-β pathway to combat these diseases. Considering the complexity of the TGF-β signaling network, a rational approach is vital to designing anti-TGF-β modalities to combat metabolic diseases.

Keywords Adipocytes • Adipose tissue • Diabetes • Homeostasis • Insulin • Metabolism • Mitochondria • Obesity • Pancreas • Smad3 • TGF-β • β-cells

18.1 Metabolic Homeostasis

For living organisms to maintain a state of metabolic homeostasis there must be a steady state of nutrient and energy intake and its utilization. Macro- (carbohydrate, lipid and protein) as well as micro-nutrients (vitamins, minerals, and trace elements) are the components of the diet which provide the energy, molecular building blocks,

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co-enzymes, and co-factors needed to maintain properly functioning metabolic processes of nutrient digestion, absorption, utilization, and excretion. There are specific "metabolic tissues" that are primarily responsible for the breakdown and utilization of these nutrients. Because of their critical importance in supporting the life of an organism, the normal physiology of these tissues is well characterized, in particular the insulin responsive tissues (adipose tissue, liver, skeletal muscle) and the sole insulin producing tissue, the pancreas. However, we have gained insight into the importance of these tissues in metabolic homeostasis through their pathological contexts as well (Biddinger and Kahn 2006). In particular, the obese state, along with its metabolic comorbidities, has shed light on the integrated role of these organ systems, although this has also created the problem of determining which of these tissues is the primary site responsible for disease states such as type II diabetes (Yang and Trevillyan 2008; Olefsky and Glass 2010).

The adaptation of a stomach and adipose tissue has made it possible to eat in the meal format, where, as opposed to grazing, an organism consumes food in discrete settings until they reach a level of "fullness," stop eating while still fueling their cells to carry out their basic physiological processes due to adequate nutrient stores within the body.

After a meal, carbohydrates are broken down to glucose molecules and are either utilized by the intestine or liver (the first organs they encounter) or are sent to all other tissues where they can be utilized for the synthesis of ATP after conversion to pyruvate via glycolysis. In order for glucose to gain entry into insulin sensitive tissues, i.e., adipose tissue and skeletal muscle, glucose needs insulin, an endogenously secreted peptide hormone from the β-cells of the pancreas. Insulin binds its receptor on the cell surface of insulin responsive tissues. The receptor, being a receptor tyrosine kinase, autophosphorylates and begins a signal transduction cascade that involves insulin receptor substrates (IRS), phosphatidylinositol 3 phosphate (PI3K), and Akt. Activation of this pathway by phosphorylation releases premade vesicles containing glucose transporter 4 (GLUT4) to the cell surface thereby allowing for the entry of glucose into the cell (Chang et al. 2004). This glucose is used for the basic cellular needs, and what remains is converted to triacylglycerol through the process of de novo lipogenesis occurring primarily in the liver and adipose tissue.

Fatty acids consumed in a meal undergo a different metabolic process than carbohydrates, but, in part share the same fate being utilized for immediate energetic needs or stored in liver as glycogen or in adipose as triacylglycerol for later use. Fatty acids are typically found in a meal as triglycerides, which cannot enter the intestinal cells. In the small intestines, these triacylglycerols are bound by pancreatic lipases that break them down into component fatty acids and monoacylglycerols. However, these lipases are only able to work at the surface of these fats. Emulsification by bile salts created in the liver and released from the gall bladder creates more surface area for these lipases to work ultimately allowing for their entry in enterocytes of the small intestines to be reformed into triglycerides and packaged into chylomicrons before release into the lymphatic circulation. Unlike glucose, fatty acids do not need insulin to gain entry to adipocytes. Rather,

lipoprotein lipase on the cell surface releases fatty acids into the cell where they can be stored as triacylglycerol in a lipid droplet or oxidized.

18.1.1   Adipose Tissue

In obesity, chronic over nutrition leads to the need to store excess amounts of energy, manifest as increased storage of triacylglycerols in adipose tissue, with "spillover" resulting in ectopic fat deposition in liver, skeletal muscle, and pancreas (Cusi 2010; Unger and Scherer 2010). Adipocytes respond to this need to store excess energy in a depot-specific manner: subcutaneous adipocytes (under the skin) grow by hyperplasia (i.e., increasing the amount of adipocytes able to store triacylglycerols via proliferation of progenitor cell pools) (Ibrahim 2010). However, adipocytes of the visceral cavity (intra-abdominal adipose tissue) accommodate excess lipid by physically expanding the size of each cell (i.e., hypertrophy) (Ibrahim 2010). Hypertrophic adipocyte growth is considered a cellular stress in terms of both sheer and mechanical stress as the surface area to volume ratio changes, but also with regard to disruption of the normal physiological processes occurring within the cellular machinery (Bays et al. 2008; Cusi 2010; Ghorbani et al. 1997; Nishimura et al. 2007; Khan et al. 2009; Kim et al. 2007b). For example, hypertrophic adipocytes are susceptible to ER-stress (Guo et al. 2007), decreased secretion of insulin sensitizing agents such as adiponectin (Greenberg and Obin 2006), decreased insulin sensitivity (Biddinger and Kahn 2006), increased secretion of inflammatory mediators (Gutierrez et al. 2009; Halberg et al. 2009; Jager et al. 2007; Kang et al. 2008; Karalis et al. 2009; Kim et al. 2007a; Lacasa et al. 2007; Lumeng and Saltiel 2011; McGillicuddy et al. 2011; Odegaard et al. 2007; Olefsky and Glass 2010; Perfield et al. 2010; Shi et al. 2006; Shoelson et al. 2006), increased lipolysis and increased cell death (Cinti et al. 2005; Greenberg and Obin 2008; Greenberg and Obin 2006). The latter three processes initiate a vicious cycle that exacerbates the negative impact of adipocyte hypertrophy on metabolism as cell death triggers production of inflammatory mediators and releases free fatty acids into the local environment. Moreover, inflammatory mediators promote increased lipolysis and insulin resistance by blocking insulin receptor signaling through inhibitory phosphorylation of the insulin receptor, thereby blocking the anti-lipolytic actions of insulin. Increased release of free fatty acids via lipolysis promotes the secretion of inflammatory mediators through free-fatty acid activation of Toll-like receptor 4 (TLR4), resulting in transcription of inflammatory mediators (TNFα, IL-6, etc.), thereby propagating the signal which promotes insulin resistance and leads to the recruitment of many immune cell populations (Fain 2006; Weisberg et al. 2003; Zeyda and Stulnig 2007; Suganami et al. 2007; Shaul et al. 2010; Strissel et al. 2010; Strissel et al. 2007). All of the aforementioned processes have led to the hypothesis that insult to adipose tissue is the primary lesion leading to systemic hyperglycemia, hyperinsulinemia, insulin resistance, and diabetes mellitus type II.

18.1.2 Liver

As described above, it is clear that the liver plays a vital role in metabolism. The liver is the only organ in the body that can produce glucose and therefore plays an extremely important role in metabolic homeostasis during times of fasting or between meals. In times of fasting, the drop in blood glucose concentration triggers the liver to respond by utilizing glycogen stores to produce glucose for release into circulation, to maintain the blood glucose concentration within the tight range of 80–100 mg/dL. After a meal, the liver gets the first look at nutrients comprising what was consumed. Some carbohydrates are utilized to replace any glycogen stores that may have been depleted in between meals in a process regulated by the anabolic actions of insulin, before the rest are sent to the peripheral tissues for maintenance of blood glucose concentration, metabolic use or if a surplus exists, eventual conversion to triacylglycerol for storage in adipose tissue. Pathologic features of obesity that impact the ability of the liver to function normally are hepatic steatosis as well as inflammation. Hepatic steatosis can occur endogenously by increased synthesis of triacylglycerol from excess carbohydrates as well as from ectopic lipid deposition (i.e., lipid spillover from saturated adipose tissue) (Cusi 2010; Unger et al. 2010; Unger and Scherer 2010). This creates controversy when trying to ascribe the pathogenic blame of metabolic dysregulation. For example, while it is recognized that adipose tissue becomes temporarily saturated with lipid before it is able to expand its cellular pools, spilling lipids over into the liver, the liver can also produce endogenous fatty acids from de novo lipogenesis. This makes it difficult to determine the source and kinetics of ectopic fat found in liver. Be that as it may this lipid, regardless of the source, induces local inflammation within the liver and recruitment of Kupffer cells followed by the same inflammation induced insulin resistance in liver as in adipose tissue (Saberi et al. 2009). However, unlike adipose tissue, when the liver is insulin resistant, it can no longer respond to the signal from insulin after a meal to stop producing glucose and store glycogen. Therefore, insulin resistance in the liver is a primary cause of hyperglycemia, making some argue that the liver's contribution to metabolic dysregulation is more important than that of adipose tissue.

18.1.3 β-Cells

β-cells comprise about 65–80 % of the islets of Langerhans within the pancreas, where they make and store insulin. As mentioned, the liver is a strong determinant of basal blood glucose concentration. However, when blood glucose levels increase rapidly, as they do after a meal, for example, the pancreas senses this and β-cells release two waves of insulin. The first wave releases the premade insulin rapidly, within minutes, while, the second wave occurs about 30 min post-prandially.

The β-cells also release C-peptide and Amylin, and the pancreas in general secretes many endocrine and exocrine products. Here, we will focus on the role of β-cells in insulin/glucose homeostasis. β-cell destruction by auto-antibodies leads to diabetes mellitus type I, while "β-cell fatigue" is the final step in the progression from obesity, tissue-specific insulin resistance, hyperglycemia, whole-body insulin resistance, and ultimately diabetes mellitus type II (Biddinger and Kahn 2006). The former typically occurs in adolescents and young adults, while the latter is a much more chronic process which takes years to develop. However, in the current obesigenic environment, increasing numbers of adolescents are being diagnosed as type II diabetics.

18.1.4 Skeletal Muscle

Muscle is able to aid in gluconeogenesis via transfer of amino acid substrates to the liver for conversion into carbon skeletons for glucose production; however, the large size of skeletal muscle and its responsiveness to insulin are what create the major role for this tissue in glucose homeostasis as the predominant site of glucose utilization (Thiebaud et al. 1982). As with liver and adipose tissue, inflammation and free fatty acids interfere with the function of insulin receptor signaling in skeletal muscle, leading to decreased glucose uptake and hyperglycemia (Kennedy et al. 2009; Nieto-Vazquez et al. 2008; Senn 2006; Weigert et al. 2004). It has been dogma for some time that skeletal muscle insulin resistance is the most critical component to developing whole-body insulin resistance and diabetes mellitus type II (DeFronzo and Tripathy 2009); however as mentioned above, the contributions of multiple tissue types mean that it is not difficult to see how intimately intertwined adipose tissue, muscle, β-cells, and liver are in maintaining metabolic regulation. Euglycemic/hyperinsulinemic clamp studies have made it possible to tease apart the relative contribution of liver and skeletal muscle to hyperglycemia/insulin resistance phenotype in human subjects as well as experimental settings that utilize animal models (Borai et al. 2011). However, the timing of both adipose tissue's deleterious contributions as well as failure of β-cells to compensate by increasing production of insulin remains to be elucidated.

Bariatric surgeries, in which parts of the intestinal tract are removed or bypassed, have shed light onto the importance of the intestinal-brain axis and related hormones in metabolic homeostasis, but as this is beyond the scope of this chapter, we point you to the work of Andreelli et al. for a more detailed review (Andreelli et al. 2009).

In summary, metabolic homeostasis is the ability to maintain body weight and physiological processes via mechanisms which balance energy intake, expenditure, and storage. These processes involve many tissues and organ systems, and dysregulation of one part can affect the whole organism leading to metabolic complications, weight gain, and/or disease.

18.2   Role of TGF-β Signaling in Metabolic Tissues

The ability to properly specify, differentiate, and/or proliferate in the early developmental stages of metabolic tissues is of critical importance to metabolic homeostasis. TGF-β and their family members: BMPs, activins, and GDFs have all been well characterized in their roles in determination of cellular fate, in particular, in early development and stem cell biology (James et al. 2005; Chng et al. 2011; Wu et al. 2008; Watabe and Miyazono 2009; Takenaga et al. 2007; Willems and Leyns 2008; Kitisin et al. 2007; Varga and Wrana 2005; Mishra et al. 2005). Here, we review the importance of the TGF-β signaling pathway in metabolic homeostasis based on its important actions on two organs—the adipose tissue and the pancreatic β-cell.

18.2.1   Role of TGF-β Signaling in Adipose Tissue Biology

Obesity is principally characterized by fat accumulation in the adipose tissue depots (Gesta et al. 2007; Park et al. 2008). Mammalian adipose tissue has been traditionally classified as white adipose tissue (WAT) and brown adipose tissue (BAT). WAT functions predominantly as an energy storage depot characterized by large unilocular lipid droplets and serves as a prominent endocrine organ producing hormones that regulate feeding and satiety (Rosen and Spiegelman 2006). In contrast, BAT is involved in energy dissipation and is characterized by multilocular cells that harbor densely packed mitochondria and expression of uncoupling protein-1 (UCP1) (Cannon and Nedergaard 2004, 2011). Copious amount of brown fat exists in rodents, hibernating animals, and human infants—and until recently brown fat was considered to be nonexistent in adult humans. Recent findings that metabolically active brown fat exists in humans (Nedergaard et al. 2007; Cypess et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009), and that levels of brown fat activity might vary with age and adiposity, have re-stimulated interest concerning the therapeutic potential of augmenting brown fat to combat metabolic diseases such as diabetes and obesity (Nedergaard and Cannon 2010; Enerback 2010). Interestingly, lineage tracing data supports the notion that brown fat shares its developmental origin with muscle, and not white fat as it was long presumed (Atit et al. 2006; Timmons et al. 2007). Further, the transcription factor PRDM16 determines the fate of Myf5⁺-precursor cells towards the brown fat lineage (Seale et al. 2009; Seale et al. 2008).

18.2.2   Brown Adipocyte Induction in Traditional WAT Depots

Brown adipocytes are found interspersed within the WAT depots, in response to chemical or hormonal stimulation, cold exposure, or defined genetic manipulation

(Langin 2009; Lefterova and Lazar 2009; Frontini and Cinti 2010). Expression of the brown adipocyte marker UCP1 is increased in the multiple adipose tissue depots upon exposure to cold (Loncar 1991) or upon treatment with a β3-adrenoceptor agonist, CL 316,243 (Cousin et al. 1992). In agreement, β3-adrenoceptor knockout mice have reduced numbers of brown adipocytes in white fat upon cold exposure (Jimenez et al. 2003), whereas transgenic mice overexpressing the β1-adrenergic receptor in adipose tissue exhibit abundant appearance of brown fat cells in WAT and are resistant to obesity (Soloveva et al. 1997). Further, chronic treatment with CL 316,243, (Bloom et al. 1992) promotes thermogenesis and the appearance of multilocular brown adipocytes in WAT while protecting from high-fat diet induced obesity (Himms-Hagen et al. 1994). Also, infusion of CL 316,243 reduced abdominal fat, increased resting metabolic rate and induced abundant multilocular brown adipocytes expressing UCP1 in WAT of mice (Ghorbani et al. 1997) and Zucker fa/fa rats (Ghorbani and Himms-Hagen 1997). We have recently reviewed the various mechanisms that lead to browning of white fat (Yadav and Rane 2012).

18.2.3   TGF-β Signaling Modulates Brown Adipocyte Induction in WAT

The TGF-β superfamily, inclusive of TGF-β, activins, and BMPs, regulates the developmental programs of many, if not all, diverse cell types (Attisano and Wrana 2002; Feng and Derynck 2005; Massague et al. 2000). TGF-β transmits its signals via dual serine/threonine kinase receptors and transcription factors called Smads, with Smad3 being the principal facilitator of TGF-β isoform derived signals (Feng and Derynck 2005). We recently illustrated an important role of the TGF-β/Smad3 signaling pathway in regulating glucose and energy homeostasis (Yadav et al. 2011). *Smad3*^{−/−} mice exhibit enhanced insulin sensitivity and increased glucose uptake in WAT. When challenged with high-fat diet, *Smad3*^{−/−} mice gained less weight, exhibited enhanced glucose tolerance and insulin sensitivity and were protected from hepatic steatosis. Further, *Smad3*^{−/−} mice displayed significantly reduced fat mass, smaller adipocyte size and reduced expression of WAT-specific genes. *Smad3*^{−/−} mice also exhibited reduced levels of inflammatory cytokines and less inflammatory macrophage infiltration into WAT with a switch in macrophage spectrum from inflammatory M1 to protective M2 macrophages (Lumeng et al. 2007). Interestingly, *Smad3*^{−/−} epididymal WAT possessed a dark-red color and was comprised of smaller multi-locular adipocytes interspersed within larger uni-locular white adipocyte depots (Yadav et al. 2011), a morphology that resembled brown adipocytes (Cousin et al. 1992). Further, we observed that many cells in the *Smad3*^{−/−} WAT stained positive for UCP1 and expressed increased mRNA levels of BAT markers (Seale et al. 2009), i.e. PGC-1α, UCP1, and PRDM16. Physiologically, *Smad3*^{−/−} mice were able to maintain significantly higher body temperature, when exposed to cold for an extended time and displayed elevated rates of fatty acid oxidation.
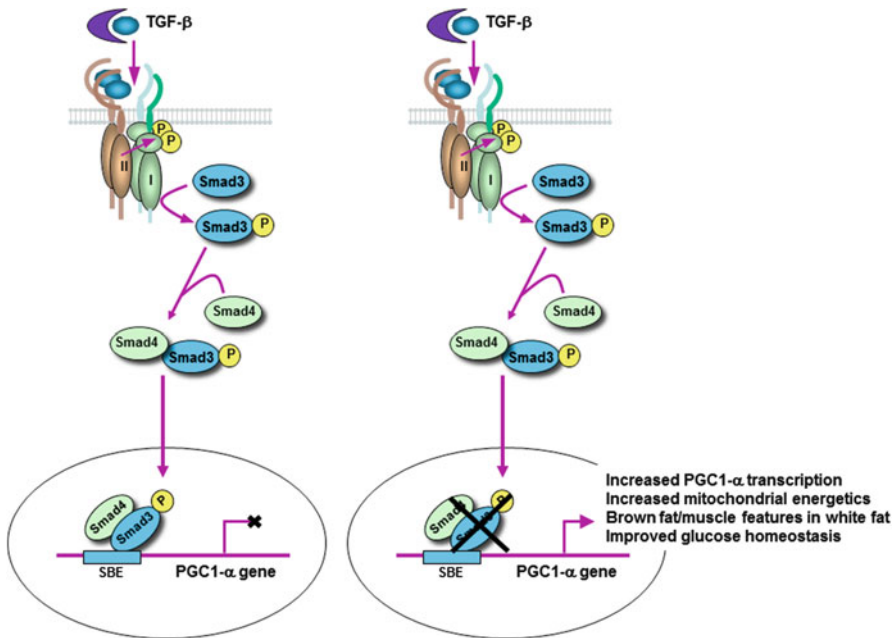
Fig. 18.1 Smad3 regulates PGC1-α promoter in the *white* adipose tissue. Binding of the TGF-β ligand to TβRII results in activation of the TβRI kinase and subsequent phosphorylation of Smad3. Phospho-Smad3 translocates to the nucleus along with Smad4. Smad3 binds to Smad-binding elements (SBE) on the PGC1-α promoter and represses PGC1-α gene transcription (*left panel*). Loss of Smad3 binding to the promoter enhances PGC1-α transcription and is associated with improved functionality of the *white* adipocytes

The molecular signature that we obtained in *Smad3⁻/⁻* white fat and in WAT from mice treated with the TGF-β neutralization antibody (1D11) provided interesting insight into the development of brown adipocytes within the WAT. Thus, WAT from *Smad3⁻/⁻* mice and mice treated with 1D11 antibodies expressed a preponderance of genes that represent brown fat, mitochondrial and skeletal muscle (Yadav et al. 2011). It is possible that TGF-β acts on a pool of cells that represents a common progenitor for white, brown, and muscle cells. Smad3 co-occupies the genome with cell-type-specific master transcription factors, including Myod1 in myotubes, PU.1 in pro-B cells and Oct4 in ESCs (Mullen et al. 2011). It is probable that in WAT, Smad3 interacts with factor/s that control the induction of cells with BAT/muscle features. Sequence analysis revealed the presence of Smad-binding elements on the promoter of PGC-1α, a transcriptional co-activator that regulates genes involved in energy metabolism and is a direct link between external physiological circuits and the regulation of mitochondrial biogenesis (Lin et al. 2005). Chromatin immunoprecipitation assays provided evidence of Smad3 binding to the PGC-1α promoter (Yadav et al. 2011), and we observed that TGF-β represses the PGC-1α promoter in a Smad3-dependent manner (Fig. 18.1). *Smad3⁻/⁻* WAT exhibited increased mitochondrial DNA copy number and functionality. Specifically, the *Smad3⁻/⁻* mitochondria

displayed increased rates of basal respiration, which was consistent with the increase in the basal rate of oxygen consumption in intact *Smad3^{-/-}* primary adipocytes. *Smad3^{-/-}* mice also showed increased metabolic rate and exhibited lower respiratory exchange ratio in agreement with the observed increase in mitochondrial function and lipid oxidation.

We also found that elevated TGF-β levels associate with poor metabolic profile in human subjects showing a significant positive association between TGF-β and human adiposity (Yadav et al. 2011). In addition, positive correlations of circulating TGF-β were observed with fat mass, fasting insulin levels, and HOMA insulin resistance index. Importantly, administration of a TGF-β pan neutralization antibody (1D11) suppressed body weight gain, size of fat depots and fat mass, adipocyte cell size and levels of triglyceride, resistin, and leptin in mouse models of obesity and diabetes (Yadav et al. 2011). Further, treatment with 1D11 improved glucose and insulin tolerance, suppressed hyperglycemia and hyperinsulinemia, ameliorated hepatic steatosis, and increased protein levels of BAT/mitochondrial markers in the WAT (Yadav et al. 2011).

The TGF-β superfamily member, BMP7, is implicated in brown adipogenesis (Tseng et al. 2008). BMP proteins promote differentiation to either white adipocytes or brown adipocytes (Schulz et al. 2011). BMP7 triggers commitment of mesenchymal progenitor cells to a brown adipocyte lineage, and implantation of these cells into nude mice results in development of adipose tissue containing mostly brown adipocytes (Tseng et al. 2008). Subpopulation of adipogenic progenitors residing in murine brown fat, white fat, and skeletal muscle were isolated, and it was shown that muscle and white fat derived Sca-1(+) cells were able to differentiate into brown-like adipocytes upon stimulation with BMP7 (Schulz et al. 2011). Also, BMP7 knockout embryos show a marked reduction of brown fat, whereas adenoviral-mediated expression of BMP7 in mice results in a significant increase in brown fat mass and leads to an increase in energy expenditure and a reduction in weight gain (Tseng et al. 2008). However, the role of BMP7 in promoting browning of white fat remains unclear. A more recent study identified Zfp423, a BMP-Smad signaling effector, as a transcriptional regulator of both brown and white preadipocyte differentiation (Gupta et al. 2010), although whether Zfp423 plays a role in promoting brown fat features in white fat is unknown. TGF-β regulates adipocyte differentiation via a Smad3-C/EBP interaction (Choy and Derynck 2003; Choy et al. 2000). Further, Schnurri-2 interacts with Smads and C/EBP during BMP2-regulated adipogenesis (Jin et al. 2006). These studies suggest an important role for TGF-β superfamily members in WAT and BAT biology—one that is analogous to the role played by myostatin during muscle development (McPherron and Lee 2002). Further, the recently described role for TGF-β in glucose-induced cell hypertrophy (Wu and Derynck 2009) may have ramifications in conditions associated with hyperglycemia, glucose intolerance, and insulin resistance.

Circulating TGF-β levels are elevated in cardiovascular disease and hypertension (Gordon and Blobe 2008) and TGF-β and BMI are closely associated in human adipose tissue during morbid obesity (Alessi et al. 2000). Predisposition to certain cancers, atherosclerosis, myocardial infarction, hypertension, and stroke is correlated

with the presence of TGF-β polymorphisms (Grainger et al. 1999). Further, polymorphisms such as the T29C polymorphism, which results in a Leu-Pro substitution at codon 10, are correlated with elevated circulating TGF-β levels. Interestingly, the T29C polymorphism is associated with increased BMI, elevated fasting insulin and glucose levels, and higher HOMA insulin resistance indices (Rosmond et al. 2003). Moreover, the *SMAD3* gene was recently identified in a type 2 diabetes genome-wide association study (Perry et al. 2009) which further supports the notion of TGF-β/Smad3 pathway as a potential target in diabetes and obesity. TGF-β antagonist approaches are being clinically evaluated to treat diseases, such as cancer, fibrosis, scarring, diabetic nephropathy, where elevated TGF-β levels are implicated. The occurrence of elevated TGF-β levels in obese individuals combined with the beneficial effect of the anti-TGF-β neutralization antibody in mouse models of obesity and diabetes offer treatment alternatives for these diseases.

18.2.4   Role of TGF-β Signaling in Pancreatic Islet β-Cell Function

The pancreatic islet β-cell, due to its unique function of insulin synthesis and glucose-stimulated insulin secretion (GSIS), is a prime target of affliction in diabetes (Weir and Bonner-Weir 2004). In addition, a majority of patients with diabetes mellitus type II develop β-cell dysfunction in concert with insulin resistance in target organs of insulin action: liver, muscle, and adipose tissue (Herman and Kahn 2006). Improved mechanistic understanding of normal β-cell function and insulin action is needed to enable early diagnosis of β-cell dysfunction and insulin resistance and to facilitate development of new rational therapeutics for diabetes.

Development of the endocrine and exocrine pancreas is controlled by factors that include members of the TGF-β superfamily (Kim and Hebrok 2001; Kim and MacDonald 2002). In addition, TGF-β signaling has been implicated in pancreatic diseases (Rane et al. 2006). BMP signaling plays an instructive role during early pancreatic development (Kim and Hebrok 2001; Kim and MacDonald 2002; Rane et al. 2006) and regulates mature β-cell function (Gannon 2007; Goulley et al. 2007), whereas activin signaling regulates islet morphogenesis and β-cell mass (Yamaoka et al. 1998; Zhang et al. 2004). TGF-β isoforms are expressed in the epithelium and mesenchyme of embryonic pancreas and in adult pancreas (Yamanaka et al. 1993). Islet cells demonstrate diffuse cytoplasmic immunostaining for TGF-β isoforms with most of the positive islet cells co-expressing insulin. TGF-β receptors (TβRI and TβRII) are present in the pancreatic epithelium and mesenchyme during early stages of development and postnatally in pancreatic islets and ducts. Further, Smad proteins are expressed in the pancreas which elucidates that components needed for activation of the canonical TGF-β signaling exist within the pancreas.

Disruption of TGF-β signaling at the receptor level using mice expressing the dominant-negative TGF-β type II receptor (DNTβRII) resulted in increased
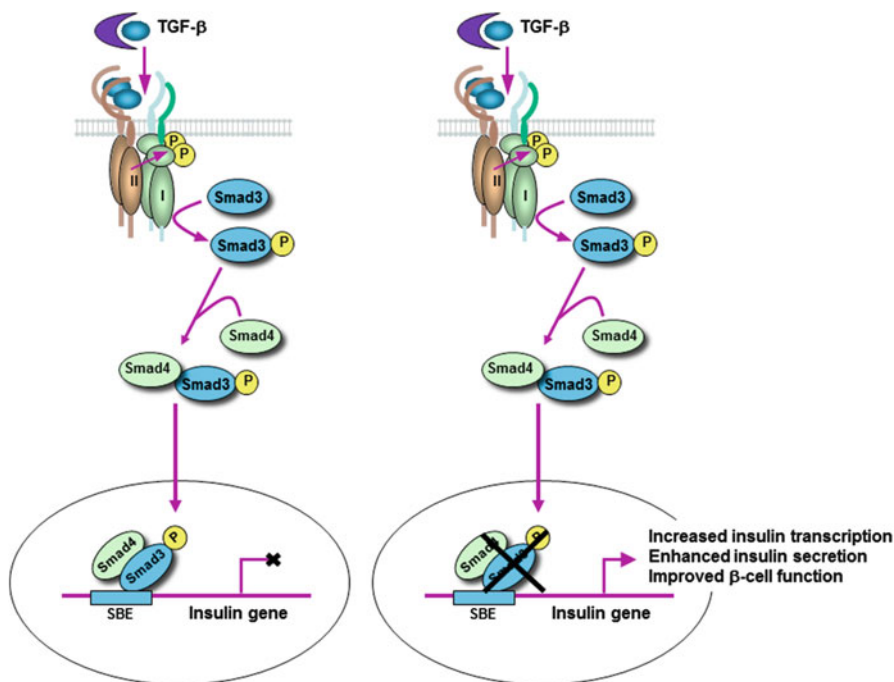
Fig. 18.2 Smad3 regulates the insulin promoter in pancreatic islet β-cells. Binding of the TGF-β ligand to TβRII results in activation of the TβRI kinase and subsequent phosphorylation of Smad3. Phospho-Smad3 translocates to the nucleus along with Smad4. Smad3 binds to Smad-binding elements (SBE) on the Insulin promoter and represses Insulin gene transcription (*left panel*). Loss of Smad3 binding to the promoter enhances Insulin transcription and is associated with improved functionality of the pancreatic islet β-cells

proliferation of pancreatic acinar cells and severely perturbed acinar differentiation (Bottinger et al. 1997). Additionally, DNTβRII mice exhibit increased endocrine precursors and proliferating endocrine cells, with an abnormal accumulation of endocrine cells around the developing ducts of mid-late stage embryonic pancreas (Tulachan et al. 2007). Transgenic mice expressing TGF-β in β-cells exhibit abnormal small islet cell clusters without formation of normal adult islets, although the overall islet cell mass is not significantly diminished (Lee et al. 1995).

We recently described an important role of the TGF-β/Smad3 pathway in regulation of insulin gene transcription and β-cell function (Lin et al. 2009). We identified insulin as a TGF-β target gene and showed that Smad3 occupies the insulin gene promoter and represses insulin gene transcription (Fig. 18.2). In contrast, Smad3 siRNAs relieve insulin transcriptional repression and enhance insulin levels. Transduction of adenoviral Smad3 into primary human and nonhuman primate islets suppresses insulin content, whereas dominant-negative Smad3 adenoviral transduction enhances insulin levels. Consistent with this, Smad3-deficient mice exhibit moderate hyperinsulinemia and mild hypoglycemia. Moreover, Smad3

deficiency results in improved glucose tolerance and enhanced GSIS in vivo. In ex vivo perfusion assays, Smad3-deficient islets exhibited improved glucose-stimulated insulin release. Interestingly, Smad3-deficient islets harbor an activated insulin-receptor signaling pathway and TGF-β signaling regulates expression of genes involved in β-cell function. These studies emphasize TGF-β/Smad3 signaling as an important regulator of insulin gene transcription and β-cell function and suggest that components of the TGF-β signaling pathway may be dysregulated in diabetes.

We find that inhibition of Smad3 signaling leads to elevated GSIS ex vivo and in vivo. It is plausible that the effects of TGF-β/Smad3 on insulin gene transcription may be independent of its role in GSIS. Levels of expression of genes involved in insulin biosynthesis, pro-insulin processing, glucose sensing, glucose metabolism, incretin signaling, insulin exocytosis, and GSIS are significantly elevated in *Smad3*^{−/−} islets and, conversely, significantly repressed in cells harboring a constitutively active TβRI/Smad3 pathway. Interestingly, we observe an activated insulin receptor signaling pathway in *Smad3*^{−/−} islets, which is consistent with the enhanced β-cell function in *Smad3*^{−/−} mice.

TGF-β appears to exert a bimodal effect on GSIS depending on the dose, time of exposure, and concentration of coexisting glucose (Sekine et al. 1994; Sjoholm and Hellerstrom 1991). Therefore, at low glucose concentration TGF-β stimulates insulin release, whereas at a high glucose concentration the same dose of TGF-β significantly inhibits GSIS (Sjoholm and Hellerstrom 1991; Sekine et al. 1994). Interestingly, dual and opposing roles of TGF-β in cancer are believed to suppress primary tumors and paradoxically promote cancer metastases (Roberts and Wakefield 2003). Recent reports of the role of BMP4/BMPR1A and Smad7 in β-cell function further underscore the important role of TGF-β superfamily signaling in this cell type (Smart et al. 2006; Bertolino et al. 2008; Goulley et al. 2007). BMP4-BMPR1A signaling plays a key role in insulin secretion by positively regulating genes involved in glucose sensing, glucose-metabolism-coupled secretion, incretin signaling, proinsulin processing, and insulin exocytosis (Goulley et al. 2007). Our findings of TGF-β/Smad3 mediated regulation of genes associated with β-cell function are consistent with an important role of the TGF-β superfamily signaling in pancreas development, particularly, β-cell function.

The findings that amplified TGF-β/Smad3 signals repress insulin transcription and reduce insulin level and insulin secretion, whereas ablation of Smad3 leads to improved β-cell function suggesting that abnormal TGF-β signaling may promote pathogenesis of dysfunctional β-cells. We propose that TGF-β/Smad3 pathways may (Sekine et al. 1994) regulate β-cell function in settings of increased insulin demand, including insulin resistance, obesity, and during β-cell injury. Further, our results that reduced TGF-β/Smad3 signaling markedly enhance insulin content and insulin secretion suggest that pharmacological inactivation of TGF-β/Smad3 signaling might be useful for promoting β-cell differentiation and ameliorating β-cell failure during diabetes.

18.3 Perspectives and Conclusion

Together, our findings support the notion that the TGF-β signaling network plays important roles in the functionality of the pancreatic β-cell and that of the adipose tissue (Figs. 18.1 and 18.2). Further, we propose that this pathway serves an integral role in metabolic homeostasis. We believe that this role is not limited to the TGF-β isoforms and that there is evidence supporting an integrated role for various TGF-β superfamily isoforms in the process. In addition, we believe that other signaling pathways that intersect into, or, are off-shoots of, the TGF-β signaling network play a collaborative role to maintain metabolic homeostasis. As such, it is important to define the precise role of TGF-β signaling in regulating the actions of various metabolic organs—both via the canonical Smad-transcription factor loop and/or via non-canonical signaling pathways. TGF-β levels are elevated in metabolic disease, which supports the utility of therapeutics aimed at targeting the TGF-β pathway for these diseases. However, the complexity of the TGF-β signaling network warrants a cautious and rational approach to designing anti- TGF-β modalities to combat metabolic diseases.

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