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Role of TIEG1 in Biological Processes and Disease States

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Abstract A novel TGFβ Inducible Early Gene-1 (TIEG1) was discovered in human osteoblast (OB) cells by our laboratory. Over the past decade, a handful of laboratories have revealed a multitude of organismic, cellular, and molecular functions of this gene. TIEG1 is now classified as a member of the 3 zinc finger family of Krüppel-like transcription factors (KLF10). Other closely related factors [TIEG2 (KLF11) and TIEG3/TIEG2b] have been reported and are briefly compared. As described in this review, TIEG1 is shown to play a role in regulating estrogen and TGFβ actions, the latter through the Smad signaling pathway. In both cases, TIEG1 acts as an inducer or repressor of gene transcription to enhance the TGFβ/Smad pathway, as well at other signaling pathways, to regulate cell proliferation, differentiation, and apoptosis. This review outlines TIEG1's molecular functions and roles in skeletal disease (osteopenia/osteoporosis), heart disease (hypertrophic cardiomyopathy), and cancer (breast and prostate). J. Cell. Biochem. 102: 539–548, 2007.

Key words: TIEG1; bone; heart; estrogen; TGFβ; cancer; apoptosis

DISCOVERY AND CHARACTERIZATION OF TIEG1 (KLF10)

The TGFβ Inducible Early Gene-1 (TIEG1) was initially identified in normal human fetal osteoblasts (hFOB) following TGFβ treatment using differential display PCR [Subramaniam et al., 1995]. A near-full length cDNA for TIEG1 was isolated from a human OB cDNA library. Subsequent studies revealed that TIEG1 mRNA increased within 30 min of TGFβ treatment of human osteoblasts and reached a maximum of approximately 10-fold above control levels at 120 min post-treatment. This regulation of TIEG1 mRNA was very transient, with a rapid return to normal (pretreated) levels within 3 h, and was independent of new protein synthesis. TIEG1 protein levels follow closely behind the mRNA levels. Sequence analysis indicated that TIEG1 mRNA encodes a 480 amino acid protein that contains a 3 zinc finger, Sp1-like DNA binding domain and several proline-rich Src homology-3 (SH3) binding domains at the C-terminal end [Subramaniam et al., 1995]. The regulation of TIEG1 expression in hFOB cells is growth factor/cytokine specific with high induction levels by TGFβ and bone morphogenetic protein-2 (BMP-2), moderate induction by epidermal growth factor (EGF), and no induction by other growth factors/cytokines (e.g., IGF-1, IGF-2, FGF, TNFα, IL-6, IL-1B) [Subramaniam et al., 1995; Hefferan et al., 2000].

TIEG1 GENE AND PROTEIN STRUCTURES, AND EVOLUTIONARY CONSERVATION

The TIEG1 and early growth response-alpha (EGRα) genes [Blok et al., 1995] were independently discovered and found to be very homologous [Fautsch et al., 1998b]. The EGRα cDNA was first cloned from a human prostate cDNA library on the basis of its differential expression in androgen-dependent and androgen-independent human prostate cell lines [Blok et al., 1995]. Our laboratory demonstrated that both the TIEG1 and EGRα proteins are the

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products of a single gene located on human chromosome 8q22.2 [Subramaniam et al., 1998; Fautsch et al., 1998b]. It was subsequently shown that TIEG1 and EGRα are transcribed from differentially regulated, alternative promoters but use common exons for almost all of their coding regions [Fautsch et al., 1998b]. Thus, TIEG1 and EGRα differ in sequence only by 12 amino acids at the amino termini. The functional consequences of this difference in structure are not yet known. Northern analysis of mRNA from various human tissues and several cell lines revealed that TIEG1 is the predominant transcript expressed and regulated by growth factors and cytokines.

The TIEG1 gene spans 8 kb and contains 5 exons. Computer analysis of the 5′-upstream region of TIEG1 shows no TATA box or initiator sequence but does show consensus sequence similarities to binding sites for several transcription factors including Sp1, JunB, and aromatic hydrocarbon/receptor-ligand complexes. Analysis of genomic regions containing 5′-flanking regions revealed that both the TIEG1 and EGRα promoters have significant activity in human fetal osteoblast cells. As depicted in Figure 1, the TIEG1 protein contains a 3 zinc finger motif with 3 unique repression domains (R1–R3) [Subramaniam et al., 1995; Fautsch et al., 1998a; Cook et al., 1999]. Thus, TIEG1 is classified as a member of the Krüppel-like family of transcription factors (KLF10), all of which bind to GC rich Sp1-like sequences to regulate gene transcription. The more global actions of TIEG1 result in the inhibition of cell proliferation and induction of apoptosis [Tachibana et al., 1997; Cook et al., 1998].

The protein sequence of human TIEG1 (hTIEG1) was shown to be closely related to three mouse sequences (mGIF, GC-binding protein and mTIEG1) [Fautsch et al., 1998a]. Due to the extremely high level of nucleotide sequence identity (more than 96%), these three sequences likely represent homologous genes. In fact, the predicted amino acid sequence of the murine TIEG1 protein is 85% identical to hTIEG1. Like hTIEG1, the mGIF cDNA was recently characterized as a negative-acting transcription factor that can be induced by another TGFβ family member, *G*lial Cell-*D*erived *N*eurotrophic *F*actor (GDNF), in murine neuroblastoma cells [Yajima et al., 1997]. The possibility that there are multiple TIEG-related genes in the mouse was suggested by the presence of multiple bands on genomic Southern blots [Yajima et al., 1997; Fautsch et al., 1998a]. The recent discovery and characterization of mTIEG3 [Wang et al., 2004], which is highly homologous to mTIEG2, opens the possibility that three mouse TIEG proteins (mTIEG1, mTIEG2, and mTIEG3) may have evolved. These genes have different expression patterns depending on the cell/tissue type but likely serve the same functions as their two human counterparts (hTIEG1 and hTIEG2), perhaps explaining discrepancies in the tissue distribution of the mouse and human homologs. Additional evidence that mTIEG1/mGIF is the mouse homolog of hTIEG1 was suggested by the conservation of the exon/intron structure of the mTIEG1 and hTIEG1 genes [Fautsch et al., 1998a]. Furthermore, although only one mTIEG1 transcript has been observed, other potential first exons for the mTIEG1 gene have been identified [Fautsch et al., 1998a] giving rise to the possibility that other transcripts may exist which code for proteins with alternative amino termini, similar to the case of human TIEG1 and EGRα [Fautsch et al., 1998a]. However, none of these alternative first exons directly corresponds to a mouse homolog of EGRα.

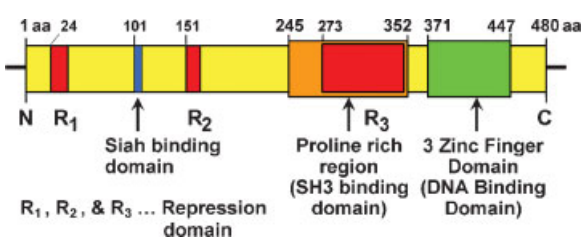

Fig. 1. TIEG1 protein structure.

OTHER KNOWN TIEG FAMILY MEMBERS: TIEG2 (KLF11) AND TIEG3 (TIEG2b)

As mentioned above, another closely related (e.g., sequence homologous) gene/protein to TIEG1 (KLF10) [Subramaniam et al., 1995] called TIEG2 (now classified as KLF11) was described by Cook et al. [1998]. TIEG2 is a ubiquitously expressed transcription factor which also contains a Krüppel-like 3 zinc finger motif at the C-terminal end of the protein. Similar to the TIEG1 gene, the TIEG2 gene encodes a nuclear protein which binds GC rich/

Sp1-like sequences to regulate gene expression and inhibit cell proliferation. At least in some cases, TIEG1 and TIEG2 can regulate the same genes [Johnsen et al., 2002c]. Like TIEG1, TIEG2 contains 3 repressor domains [Cook et al., 1999], but can activate gene transcription (monoamine oxidase B gene) when bound to Sp1 sites along with the Sp1 protein [Ou et al., 2004]. To date, one additional gene that is homologous to TIEG1, and more so to TIEG2, has been identified and classified as TIEG3 [Wang et al., 2004]. TIEG3 also belongs to the Krüppel-like family of transcription factors and contains a 3 zinc finger motif at the C-terminal end with 96% homology to TIEG2 and 86% homology to TIEG1. It also has 3 conserved repressor motifs and is induced by TGFβ to repress gene transcription. The uniqueness or redundancy of TIEG1, -2, and -3 actions, as well as other closely related Krüppel-like factors such as KLF13, remains unclear.

TIEG1 PROTEIN, LOCALIZATION, HALF-LIFE, AND UBIQUITINATION

Using a TIEG1 specific polyclonal antibody and immunoprecipitation methods in normal human fetal osteoblast cells (hFOB cells), TIEG1 was shown to encode a 72-kDa protein whose levels are transiently increased within 2 h of TGFβ treatment [Subramaniam et al., 1998]. Polarized confocal microscopic analysis of hFOB cells revealed that TIEG1 protein was localized in the nucleus in untreated cells. As expected, the levels of TIEG1 protein in the nucleus increase when the cells are treated with TGFβ for 2 h. Similar analyses of untreated human keratinocytes revealed that TIEG1 was localized in the cytoplasm but was translocated to the nucleus after H_2O_2 treatment [Subramaniam et al., 1998].

Subsequent immunohistochemical studies have demonstrated that TIEG1 protein is expressed in epithelial cells of the placenta, breast, pancreas, skeletal muscle, heart, glial cells, fibroblasts, pancreatic carcinoma cells, cerebral cortex cells, myeloid cells, smooth muscle cells, osteoblast cells, and other cells of the bone marrow [Yajima et al., 1997; Subramaniam et al., 1998; Kobori et al., 2002; Mitsumoto et al., 2003; Bender et al., 2004; Noti et al., 2004, 2005; Luo et al., 2005]. All cells of the kidney displayed negative staining for this protein. Interestingly, a breast cancer stage specific expression of TIEG1 protein is found in breast cancer biopsies [Subramaniam et al., 1998].

Using yeast-2-hybrid and co-immunoprecipitation assays, an E3 ubiquitin ligase, Seven in Absentia homologue-1 (SIAH1) protein, was found to interact with TIEG1 [Johnsen et al., 2002c]. TIEG1 and SIAH1 interact through a conserved SIAH-binding motif in TIEG1 [House et al., 2003]. Co-expression of SIAH1 results in proteasomal degradation of TIEG1 but not TIEG2. Importantly, co-expression of SIAH1 completely reverses the repression of Smad7 promoter activity by TIEG1. Furthermore, overexpression of a dominant negative SIAH1 stabilizes TIEG1 resulting in enhancement of TGFβ/Smad-dependent transcriptional activity [Johnsen et al., 2002c]. These findings suggest that the ability of TGFβ to modulate gene transcription may be regulated by proteasomal degradation of the downstream effector protein, TIEG1, through the SIAH pathway. In this manner, the rapid turnover of TIEG1 may serve to limit the duration and/or magnitude of TIEG1 and TGFβ responses.

ROLE OF TIEG1 IN THE TGFβ-SMAD PATHWAY

To ascertain if TIEG1 plays a role in the TGFβ pathway, human osteosarcoma MG-63 cells were stably transfected with varying amounts of a TIEG1 expression vector and were shown to overexpress varying levels of TIEG1 mRNA and protein. The overexpression of TIEG1 caused gene expression and cell proliferation changes which mimicked those of the MG-63 cells treated with TGFβ (e.g., increased alkaline phosphatase activity, decreased levels of osteocalcin mRNA and protein, and decreased cell proliferation) [Hefferan et al., 2000]. The degree of these changes correlated with the level of TIEG1 expression. These data support a primary role for TIEG1 as an important transcription factor in the TGFβ signaling pathway.

Since TIEG1 encodes a three zinc-finger Krüppel-like transcription factor, whose overexpression has been shown to mimic the effects of TGFβ in human osteosarcoma and pancreatic carcinoma cells [Tachibana et al., 1997; Cook et al., 1999; Cook and Urrutia, 2000], a potential role for TIEG1 in the TGFβ signal transduction pathway was indicated. Studies utilizing transient transfection of TIEG1 and a Smad binding element (SBE) reporter, which is known to be regulated by TGFβ, enhanced the

TGFβ induction of SBE reporter activity [Johnsen et al., 2002a]. Additionally, TIEG1 expression also enhanced the induction of the endogenous TGFβ regulated genes p21 and PAI-1 [Johnsen et al., 2002a]. The ability of TIEG1 to enhance TGFβ action was shown to be Smad dependent since TIEG1 had no effect on SBE transcription in the absence of Smad4 expression or when an inhibitory Smad protein, Smad7, was overexpressed [Johnsen et al., 2002a]. Furthermore, TIEG1 overexpression enhanced the TGFβ induced Smad2 phosphorylation. Lastly, TIEG1 bound to, and repressed, a specific element in the proximal promoter of the inhibitory Smad7 gene. Interestingly, similar to TIEG1, we observed that TIEG2 is also able to enhance TGFβ/Smad signaling and repress Smad7 gene expression [Johnsen et al., 2002c]. However, the effects of TIEG1 and TIEG2 are distinct, at least in part, because TIEG1, and not TIEG2, is targeted for degradation by SIAH1 [Johnsen et al., 2002c]. Thus, as outlined in Figure 2, TIEG1 increases the activity of the TGFβ/Smad signal transduction pathway by relieving negative feedback through repression of the inhibitory Smad7.

Interestingly, TIEG1 was still capable of increasing Smad pathway activity in the absence of Smad7 [Johnsen et al., 2002b]. While Smad7 is known to block both TGFβ and bone morphogenetic protein (BMP) signaling, TIEG1 specifically enhanced only the TGFβ pathway. Similarly, while both TIEG1 and the related Krüppel-like factor, FKLF2 (KLF13), repressed Smad7 transcription, only TIEG1 was capable of enhancing Smad signaling. In order to identify additional regulatory targets of TIEG1 important for this enhancement of the Smad pathway, microarray analyses were used to show that Smad2 is also a TIEG1 target gene [Johnsen et al., 2002b]. TIEG1 was subsequently shown to increase the transcription of Smad2, but not Smad3 or Smad4 [Johnsen et al., 2002b]. Furthermore, while the TGFβ/Smad pathway remains intact in Smad2 null cells, TIEG1 enhancement of Smad signaling is dramatically reduced [Johnsen et al., 2002b]. Therefore, as outlined in Figure 2, a new model was proposed whereby TIEG1 enhances Smad signaling by a dual mechanism involving both the repression of the inhibitory Smad7 gene as well as induction of Smad2 gene expression.

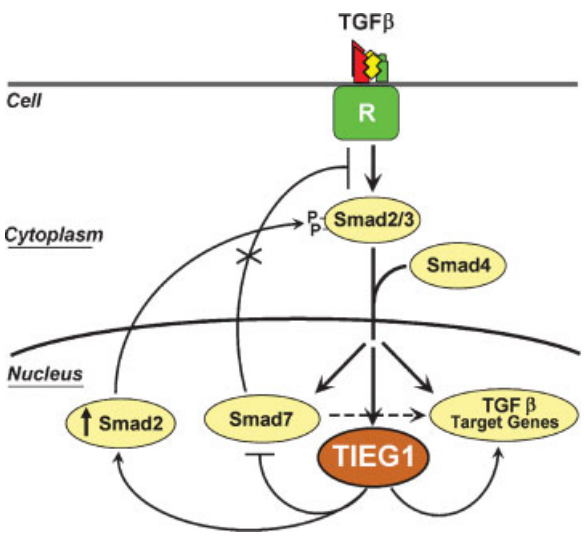

Fig. 2. Role of TIEG1 in the regulation of TGFβ-Smad signaling.

ROLE OF TIEG1 IN APOPTOSIS

Members of the TGFβ family of peptides are known to exert antiproliferative effects and induce apoptosis in many epithelial cell types. In the exocrine pancreas, these peptides not only regulate normal cell growth, but alterations in these pathways have been associated with neoplastic transformation. Therefore, the identification of molecules that regulate cell proliferation and apoptotic cell death in response to TGFβ is necessary for a better understanding of normal morphogenesis as well as carcinogenesis of the pancreas. Tachibana et al. [1997] characterized the expression and function of TIEG1 in exocrine pancreatic epithelial cells. The gene was expressed in both acinar and ductular epithelial cell populations from the exocrine pancreas. Overexpression of TIEG1 in the TGFβ-sensitive epithelial cell line PANC1 and hepatoma cell lines was sufficient to induce apoptosis [Tachibana et al., 1997; Ribeiro et al., 1999]. The TIEG1-induced apoptosis appears to be similar to the p53 induced mitochondrial apoptosis pathway which occurs in pancreatic epithelial cells [Ribeiro et al., 1999], as well as other epithelial cells [Chalaux et al., 1999], lymphoma cells [Sebestyen et al., 2005], leukemic cells [Jin et al., 2004], and retinal cells [Franke et al., 2006]. Together, these results support a role for TIEG1 in linking TGFβ-mediated signaling cascades to the regulation of cell growth, including antiproliferation and apoptosis.

ROLE OF TIEG1 IN THE TGFβ MEDIATED ANTIPROLIFERATIVE RESPONSE

Studies from our laboratory have demonstrated that TIEG1 expression (both mRNA and protein) is lost during the progression of breast cancer [Subramaniam et al., 1998; Reinholz et al., 2004]. A tetracycline inducible TIEG1 overexpressing breast cancer cell line and TIEG1 null mouse embryo fibroblasts (MEFs) were used to establish whether TIEG1 plays a central role in eliciting the antiproliferative effects of TGFβ [Johnsen et al., 2004]. Similar to TGFβ treatment, TIEG1 overexpression increases the expression of the cyclin dependent kinase inhibitor p21 and significantly decreases cellular proliferation. Interestingly, while cellular proliferation of wild-type MEFs is inhibited by TGFβ, the cell proliferation of TIEG1 null MEFs is stimulated by TGFβ. Furthermore, TIEG1 null MEF cells display a decrease in Smad dependent transcription with a concomitant prolonged increase in Smad7 expression compared to wild-type cells. These data strongly support that TIEG1 plays a central role in the anti-proliferative response via the TGFβ-Smad pathway and may explain how the reduced levels of TIEG1 expression correlate with the stages/development of cancer, as discussed below and elsewhere [Subramaniam et al., 1998; Reinholz et al., 2004].

ROLE OF TIEG1 IN DNA REPLICATION AND CANCER

Studies using immunohistochemistry showed that cells in normal breast epithelium display high expression of TIEG1 protein, those in the in situ carcinoma display less than one-half of the levels, and those in the invasive carcinoma show a complete absence of the TIEG1 protein [Subramaniam et al., 1998]. TIEG1 is located on the chromosome 8q22.2 locus, the same locus as genes involved in osteopetrosis and acute myeloid leukemia. This locus is also very close to the c-*myc* gene locus as well as a locus of high polymorphism in cancer biopsies. The correlation between the levels of TIEG1 protein and the stage of breast cancer, its prime location on human chromosome 8q22.2, and past studies revealing its role in pancreatic carcinoma, suggests that TIEG1 may play a major role as a tumor suppressor in a variety of cancers.

Since TIEG1 plays an important role in cell proliferation and apoptosis, and its levels inversely correlate with breast cancer stages, the mRNA levels of TIEG1 and its target genes, Smad7, Smad2, and BARD1, were examined using real-time RT-PCR in 14 normal human breast, as well as five non-invasive, 57 invasive (including 29 with outcome data), and five metastatic human breast tumor tissues [Reinholz et al., 2004] (Fig. 3). TIEG1 and Smad7 mRNA levels were lower in all non-invasive tumors compared to normal breast tissues but Smad7 mRNA levels increased in more advanced stages of cancer. TIEG1, BARD1, and Smad2 mRNA levels were lower in invasive cancers compared to normal breast tissues. In addition, TIEG1, Smad2, and BARD1, provided discriminatory ability to distinguish between normal and tumor samples, N− and N+ tumors, and N−/good (no recurrence for at least 5 years) and N−/bad (recurrence within 3 years) outcome patients. Interestingly, the TIEG1 mRNA levels accurately discriminated between normal breast tissue and primary tumors with a sensitivity and specificity of >90%. TIEG1, in combination with Smad2, distinguished between N+ and N− primary tumors with a sensitivity and specificity of ~80%. TIEG1 in combination with BARD1 discriminated between N-/bad outcome from N−/good outcome tumors with a sensitivity and specificity of >80%. As outlined in Figure 3, our results support the hypothesis that the differential gene expression of TIEG1 and its induced target genes, Smad2, and BARD1 (both of which are tumor suppressor genes), as well as a repressed gene, Smad7, play a significant role in the proliferation of breast cancer cells [Reinholz et al., 2004].

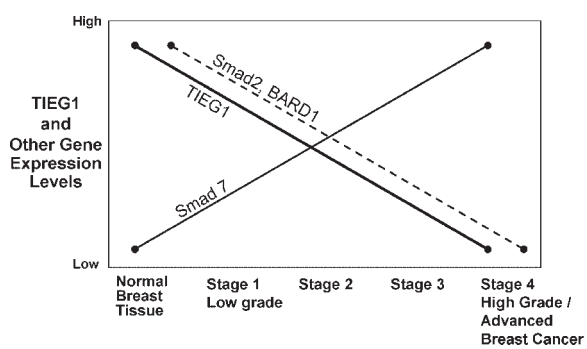

Fig. 3. Correlations between TIEG1 and its target gene expression levels in advancing breast cancer stages.

TIEG1 also appears to play a role in other cancers. In addition to the relationships between TIEG1 (and its target genes, BARD1 and Smad 2, 7) in breast cancer as described above [Reinholz et al., 2004], TIEG1 appears to play a role in human prostate cancer (as EGRα) [Eid et al., 1998], pancreatic cancers [Cook and Urrutia, 2000; Antonello et al., 2002], and human colorectal cancer cells [Chen et al., 2003], with the levels displaying an inverse correlation with the development and progression of the cancer and TIEG1 playing an important role in the regulation of cell proliferation. These past studies, involving TIEG1 overexpression in osteoblast, osteosarcoma, and pancreatic carcinoma cells, indicate that TIEG1 expression inhibits DNA synthesis, similar to a tumor suppressor-like gene, and plays a role in apoptosis [Tachibana et al., 1997; Hefferan et al., 2000].

PHENOTYPIC AND DISEASE IMPLICATIONS OF TIEG1 NULL MICE

Skeletal Disease

The skeletal phenotype of TIEG1 null mice. To elucidate the functions of TIEG1 in skeletal development and maintenance and potential involvement in skeletal diseases, we generated TIEG1 knockout mice [Subramaniam et al., 2005; Bensamoun et al., 2006a]. Three-point bending tests on mixed background, knockout animals indicated that the femurs of female TIEG1$^{-/-}$ mice are significantly weaker than those of wild-type animals [Bensamoun et al., 2006a]. pQCT analysis of tibias of TIEG1$^{-/-}$ mice revealed significant decreases in bone content, density, and size compared to wild-type mice. Micro-CT analysis of the femoral head and vertebrae revealed increases in femoral head trabecular separation and decreases in cortical bone thickness and vertebral bone volume in TIEG1$^{-/-}$ mice relative to wild-type controls. pQCT analysis of the TIEG1$^{-/-}$ tibias revealed marked decreases in multiple bone parameters in both the diaphyseal and metaphyseal regions. In addition, electron microscopy indicated a significant decrease in osteocyte number in the femurs of TIEG1$^{-/-}$ mice, suggesting that defects in osteoblast differentiation might exist. As summarized in Figure 4, these data demonstrate that the bones of TIEG1$^{-/-}$ mice display an osteopenic phenotype with significantly weaker bones and reduced amounts of cortical and trabecular bone suggesting an important role for TIEG1 in skeletal development and/or homeostasis.

Subsequently, we generated a congenic mouse strain by breeding the TIEG1$^{-/-}$ mice against a C57BL6 background for over 10 generations. pQCT and DXA studies on congenic TIEG1$^{-/-}$ mice at 2 months of age demonstrated that only the female, but not male, TIEG1$^{-/-}$ bones exhibit a marked decrease in nearly all of the parameters examined to date relative to wild-type littermates (Subramaniam, et al. in preparation). These latest findings confirm the results observed in mixed breed animals and

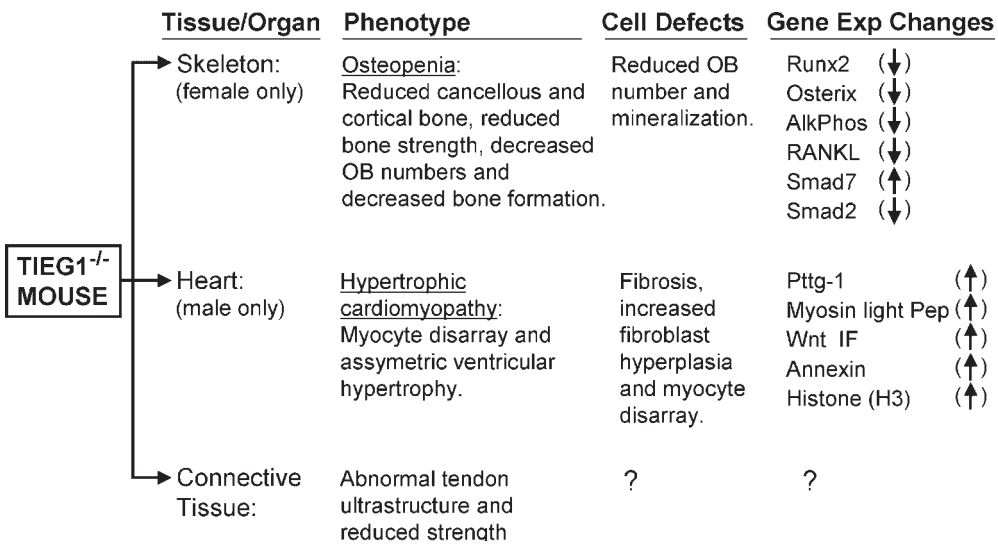

Fig. 4. TIEG1$^{-/-}$ mouse phenotype (diseases).

demonstrate that only female TIEG1$^{-/-}$ mice are osteopenic with no changes observed in male animals. Additional studies indicate that differentiation and function of the bone forming osteoblasts are markedly reduced as discussed below.

TIEG1 regulation of osteoblast differentiation. To better assess the effects of TIEG1 ablation on OBs, calvarial OBs were isolated from neonatal TIEG1$^{-/-}$ and wild-type mice and cultured in vitro. The TIEG1$^{-/-}$ cells displayed reduced expression of important OB differentiation markers including osterix and osteocalcin as well as a reduced capacity to mineralize bone [Subramaniam et al., 2005]. Further, the TIEG1$^{-/-}$ OBs were also shown to have reduced expression of Runx2 (Hawse et al. in preparation; Fig. 4). Additionally, we have demonstrated that TIEG1$^{-/-}$ mice display an osteopenic bone phenotype suggesting an important role for TIEG1 in osteoblast differentiation and bone formation [Bensamoun et al., 2006a].

TIEG1 regulation of osteoclast differentiation: role of OPG/RANKL. When OBs from TIEG1$^{-/-}$ and wild-type mice were cultured with marrow and spleen cells from wild-type mice, significantly fewer osteoclasts developed in the TIEG1$^{-/-}$ OB co-cultures compared to the osteoclasts that developed in the wild-type OB co-cultures. The examination of gene expression in the TIEG1$^{-/-}$ OBs revealed that decreased RANKL and increased OPG levels, relative to wild-type OBs, are likely responsible for the reduced ability of these osteoblasts to support osteoclast differentiation [Subramaniam et al., 2005]. Thus, it appears from these studies that TIEG1 expression in OBs is critical for both osteoblast-mediated mineralization as well as OB support of osteoclast differentiation. Figure 4 summarizes the effects of loss of TIEG1 expression on the skeleton and Figure 5 depicts the actions of TIEG1 in osteoblasts and osteoclasts.

Heart Disease

Our laboratory recently reported a novel finding in the TIEG1$^{-/-}$ mouse implicating an important role for TIEG1 in cardiac hypertrophy [Rajamannan et al., 2007]. Male mice 4–16 months of age were characterized using echocardiography, transcript profiling by gene microarray, and immunohistochemistry. As depicted in Figure 4, the male, but not female, TIEG1$^{-/-}$ mice develop features of cardiac hypertrophy including asymmetric septal hypertrophy, increased ventricular size at 16 months of age, increased heart weight to body weight ratio, increased fibrosis and increased wall thickness relative to wild-type animals. Female mice did not develop hypertrophy or fibrosis. Masson Trichrome staining demonstrated evidence of myocyte disarray and myofibroblast fibrosis. Interestingly, cardiomyocyte specific Smad4 knockout mice also develop cardiac hypertrophy [Wang et al., 2005]. Although no significant differences in Smad2 or Smad7 were detected in TIEG1$^{-/-}$ hearts, the interdependence of the Smad4-pathway on TIEG1 (or vice versa) in the heart is not clear and will need to be clarified. Additional insights into the molecular mechanism by which TIEG1 regulates heart development were elucidated by microarray analysis of the left ventricles which

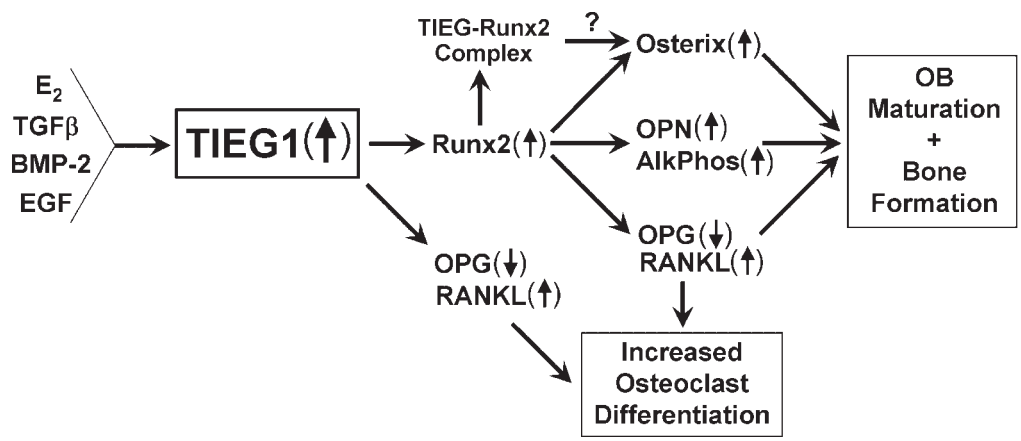

Fig. 5. Role in TIEG1 in OB and OC differentiation.

demonstrated that TIEG1$^{-/-}$ heart tissues display a 13.81-fold increase in pituitary tumor-transforming gene-1 (*Pttg1*; Fig. 4). Increases in Pttg1 and histone H3 protein levels were confirmed in the TIEG1$^{-/-}$ mouse hearts by RT-PCR. These data implicate TIEG1 and possibly its target gene, *Pttg1*, in the development of cardiac hypertrophy in the male TIEG1$^{-/-}$ mouse.

Connective Tissue Disease

Studies were also performed to examine the healing potential of flexor tendons in TIEG1$^{-/-}$ mice, and to further examine what role the TIEG1 pathway plays in flexor tendon repair [Tsubone et al., 2006]. Wild-type and TIEG1$^{-/-}$ mice showed healing of lacerated tendons, but the chronologic expression pattern of TGFβ was found to be different. TIEG1$^{-/-}$ tendons had delayed expression of TGFβ when compared with wild-type tendons. The collagen mRNA expression pattern was similar between both groups, but the overall expression level was different, with TIEG1$^{-/-}$ tendons having a lower expression of collagen type I mRNA. The mouse model described in this study provides a novel means for further understanding of the tendon healing process through isolated deletion of specific factors.

Additional studies investigated the age-dependent changes in the architecture and mechanical properties of tendons isolated from TIEG1$^{-/-}$ mice [Bensamoun et al., 2006b]. The results revealed that the fascicles of the TIEG1$^{-/-}$ mice at 3 months of age exhibit decreased fast and static stresses compared with those of wild-type mice. Electron microscopy revealed an increase in fibril size in TIEG$^{-/-}$ mouse tendons relative to wild-type controls. These data indicate an important role for TIEG1 in tendon microarchitecture and strength in adult mice (Fig. 4).

OVERVIEW AND CONCLUSIONS

TIEG1 was originally discovered in our laboratory as an immediate response gene following TGFβ treatment of human osteoblasts. We further characterized this gene as a member of the Krüppel-like family of transcription factors (KLF10). We have shown that TIEG1 plays a major role in mediating the effects of TGFβ through regulation of the Smad signaling pathway. As shown in Figures 4 and 6, TIEG1 is known to induce and repress the expression of multiple genes in several cell types and functions as an inhibitor of cell proliferation and an inducer of apoptosis. TIEG1 expression is inversely correlated with the severity and stage of breast cancer, implying an important role for TIEG1 in the progression of this disease. It is likely that TIEG1 functions

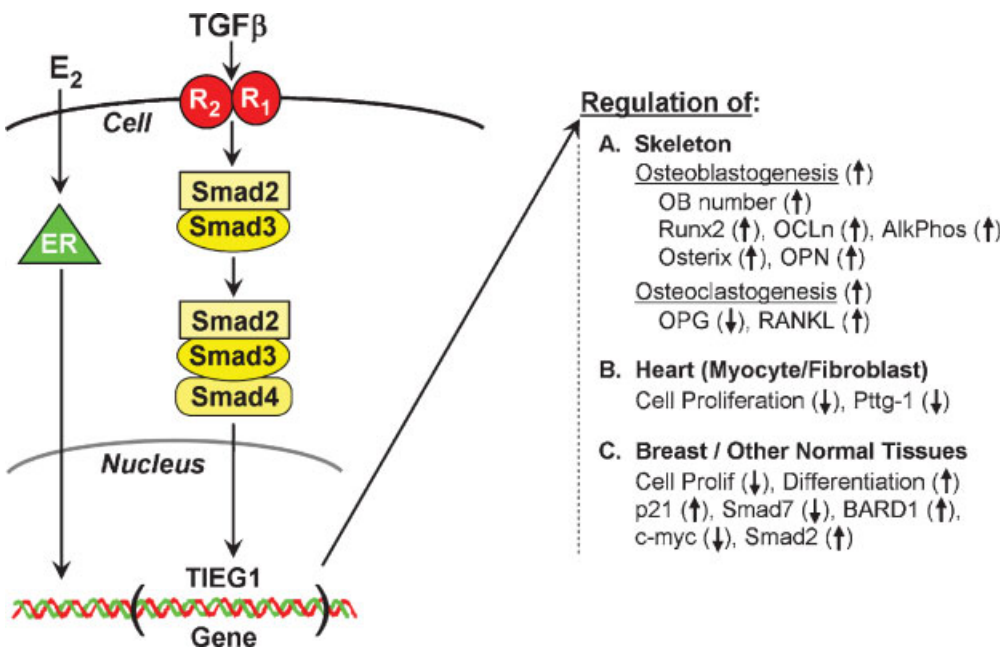

Fig. 6. E_2/TGFβ regulation of cellular proliferation and differentiation via TIEG1.

as a tumor suppressor and may play an important role in other types of cancer. Further experiments including long-term studies in which the TIEG1$^{-/-}$ mice are crossed with various mouse models of tumor formation will be necessary to definitively establish the role of TIEG1 in cancer. To date, deletion of TIEG1 in mice is known to result in multiple phenotypes including defects in the skeleton, heart, and connective tissues. TIEG1$^{-/-}$ mice display a severe osteopenic phenotype only in female animals, suggesting a potential role for TIEG1 in mediating estrogen signaling in bone. Conversely, male, and not female, TIEG1$^{-/-}$ mice develop cardiac hypertrophy nearly identical to the human disease, hypertrophic cardiomyopathy. TIEG1$^{-/-}$ mice have also been shown to have defects in the mechanical properties and healing potential of tendons. The known phenotypes of TIEG$^{-/-}$ mice are summarized in Figure 4. Taken together, these data implicate an important role for TIEG1 in multiple biological processes and disease states. However, the redundancy of TIEG1, TIEG2, and TIEG3 and the specific roles of these proteins in a variety of physiological processes remains largely unknown. Ongoing studies by other investigators are likely to identify novel functions of the TIEG family of proteins and uncover consequences of abnormalities in the expression, structure, and/or function of this important class of transcription factors in other diseases.

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