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REVIEW

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Mechanisms of TGF-β-mediated apoptosis

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Abstract Transforming growth factor-beta (TGF-β) is a multifunctional cytokine, whose numerous cell and tissue activities include cell-cycle control, the regulation of early development, differentiation, extracellular matrix formation, hematopoesis, angiogenesis, chemotaxis, immune functions, and the induction of apoptosis. TGF-β-mediated growth inhibition and apoptosis can be correlated with its function as a tumor suppressor. The apoptosis-inducing capacity has been investigated in many cell types. Data from cell-culture experiments and in vivo studies argue for a pivotal role of TGF-β-mediated apoptosis in the maintenance of B- and T-cell homeostasis. The importance of TGF-β in the control of liver cell apoptosis and cell death of prostate epithelial cells has been confirmed in many studies. Inactivation of TGF-β in animal models via a knockout approach or neutralizing antibodies suggests that TGF-β-mediated apoptosis plays an important part during tissue formation and remodeling and during the phase of ontogenetic neuron death. The molecular mechanisms involved in these processes seem to involve the activation of SMAD proteins. Many studies have described an interaction of TGF-β with other signalinging cascades as exemplified by the requirement of AP1 transcription factor for the induction of apoptosis in liver cells**.** The aim of this review is (1) to summarize and classify data in the TGF-β apoptosis literature with respect to the affected cell types, (2) to provide insights into the intracellular mechanisms involved in TGF-β-mediated apoptosis, and (3) to set TGF-β-mediated apoptosis in a physiological context.

Keywords TGF-β · Apoptosis · Regulation · Caspase · SMAD

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Introduction

Transforming growth factor-beta (TGF-β, also TGF-β1) was originally discovered by virtue of its capacity to induce anchorage-independent growth of normal rat kidney cells and fibroblast cell lines, i.e., to induce transformation (Moses et al. 1981; Roberts et al. 1981). It soon became apparent that the biological activity of TGF-β was not restricted to this effect on cell growth and that it was instead multifunctional. The numerous cell and tissue roles of TGF-β include cell-cycle control, the regulation of early development, differentiation, extracellular matrix formation, hematopoesis, angiogenesis, chemotaxis, and immune functions (Böttner et al. 2000; Dünker and Krieglstein 2000; Lawrence 1996; Mummery 2001; Saltis 1996). TGF-β represses growth of many epithelial cell types, whereas the growth of mesenchymal cells is stimulated (Gold 1999). TGF-β-mediated growth inhibition and apoptosis can be correlated with its function as a tumor suppressor (Gold 1999; Hata et al. 1998). A simplified view of the TGF-β signaling pathway is shown in Fig. 1. TGF-β binds to its membrane-bound receptors, TGF-β receptors 1 and 2 (TGFβRI, TGFβRII), which form a heterotetrameric complex. TGFβRI is phosphorylated by TGFβRII in the so-called GS-domain. Upon this activating phosphorylation, TGFβRI phosphorylates the receptor-activated SMAD proteins (SMAD2 and SMAD3), which form a heteromeric complex with the co-SMAD SMAD4 and enter the nucleus for transcriptional regulation thereby involving other components of the transcriptional machinery. An autoregulatory feedback loop is established by the induction of inhibitory SMAD proteins (SMAD6 or SMAD7) that prohibit the activation of receptor-activated SMADs (the currrent view of the TGF-β signal transduction pathway is best reviewed by Massague 2000). In many human tumors, the components of the TGF-β pathway are in some way defective, either because of an inactivating mutation within the TGF-β receptors (Larisch et al. 2000) or with regard to one of the downstream elements of the pathway, e.g., SMAD4/DPC4, which is important for intra-

cellular TGF-β mediated signal transduction (Taketo and Takaku 2000). Although the apoptosis-inducing capacity of TGF-β is well established in vivo and for a plethora of cell lines, we feel that there is major lack in summarizing the various data for interpretation and in classifying them into a physiological context. The aim of this review is (1) to summarize and classify data in the TGF-β apoptosis literature with respect to the affected cell types, (2) to provide insights into the intracellular mechanisms involved in TGF-β mediated apoptosis, and (3) to set TGF-β-mediated apoptosis in a physiological context.

TGF-β-induced apoptosis

TGF-β-induced apoptosis in cells of the immune system

TGF-β induces growth arrest and apoptosis in lymphocytes of human and mouse origin (Chaouchi et al. 1995; Kehrl et al. 1986; Lomo et al. 1995; Wahl et al. 1988). The mouse pre-B lymphoma cell line WEHI 231 responds to TGF-β in a dose-dependent manner (Brown et al. 1998). Upon TGF-β treatment, cell growth is inhibited, but with increasing doses of TGF-β, a substantial number of the cells die by apoptosis. A broad-spectrum caspase inhibitor (BD-fmk), but not specific caspase inhibitors, can completely block this apoptotic cell loss. Whereas cellular viability is maintained, the growth arrest persists, thereby distinguishing the growth inhibitory effects of TGF-β from an apoptotic TGF-β pathway. In the same cell line, TGF-β- and IgM-induced apoptosis can be inhibited by CD40 stimulation (Patil et al. 2000). Analysis of this phenomenon has revealed an upregulation of SMAD7 protein in response to CD40 stimulation. SMAD7 is an inhibitory SMAD that blocks TGF-β mediated SMAD signaling by preventing the activating phosphorylation of receptor-activated SMADs (Heldin et al. 1997; Massague 2000; Fig. 1). The activation of SMAD7 is dependent on NFκB, as pathway-specific inhibitors block the CD40-stimulated expression of SMAD7 (Patil et al. 2000). Further experiments demonstrating the importance of SMAD-dependent signal transduction in this process have been performed by inducing the ectopic overexpression of either SMAD7 or dominant negative mutants of SMAD2 and SMAD3. Patil et al. (2000) have shown that interference with the SMAD pathway inhibits TGF-β induced cell death in the pre-B lymphoma cell line WEHI 231, whereas IgM-induced apoptosis is not affected.

The induction of apoptosis has also been shown in two interleukin-2-dependent T-cell lines, OVA-7 and CTLL-2 (Weller et al. 1994). Cycloheximide prevents TGF-β-induced apoptosis in CTLL-2 cells, but not in OVA-7 cells, indicating that protein biosynthesis is required in CTLL-2 cells. The Bcl-2 and c-myc mRNA level remains unaltered during this process (see Fig. 2), differentiating it from the cell death induced by interleukin-2 deprivation in these cell lines, when Bcl-2 and c-myc mRNA levels are strongly reduced (Weller et al. 1994).

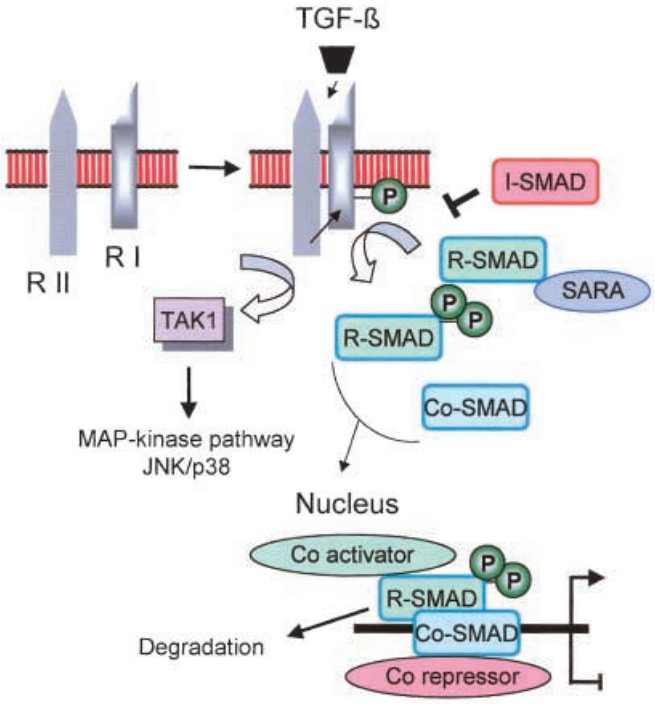

Fig. 1 Schematic representation of the TGF-β signal transduction pathway. Ligand binding to TGF-β receptors leads to the formation of heterotetrameric complexes of receptors I and II (*RI, RII*). TGF-βRII phosphorylates and activates TGF-βRI. Activated TGF-βRI phosphorylates receptor-activated SMADs (*R-SMADs*), which are released from a complex with *SARA* (SMAD anchor for receptor activation), a component important for targeting the R-SMADs to the receptor. The R-SMADs form a complex with a *Co-SMAD* and enter the nucleus to interact with other co-activators or co-repressors for efficient transactivation or repression. Downregulation of the activated pathway is achieved in two ways. Inhibitory SMADs (*I-SMAD*) are upregulated and prevent the activation of R-SMADs at the receptor level. After activation, R-SMADs are targeted for degradation via the ubiquitin-proteasome pathway. Another TGF-β signal, which is however not so well understood, is mediated via *TAK1* (TGF-β activated kinase 1). TAK1 may mediate TGF-β effects with respect to the activation of distinct MAP-kinase pathways (*JNK* Jun N-terminal kinase, *p38* stress activated protein kinase).

L1210 leukemic cells respond to TGF-β with a partial arrest of the cell cycle at the G1/S transition and with the induction of apoptotic cell death (Motyl et al. 1998). The apoptotic process is accompanied by two phases of generation of reactive oxygen species (ROS): a rapid (60 min) and a delayed (24 h and 48 h) phase after TGF-β administration. Bcl-2 protein level decreases upon TGF-β treatment, whereas the bax protein level increases, shifting the intracellular balance between death-promoting and death-inhibiting factors toward death induction (Fig. 2).

The Ramos B-cell-lymphoma cell line also undergoes apoptosis in response to TGF-β treatment. The apoptotic process in this cell line is accompanied by Bcl-X_L downregulation and the activation of caspase 3 (Saltzman et al. 1998). Downregulation of the anti-apoptotic Bcl-X_L protein may, in this case, play a key role in cell-death in-

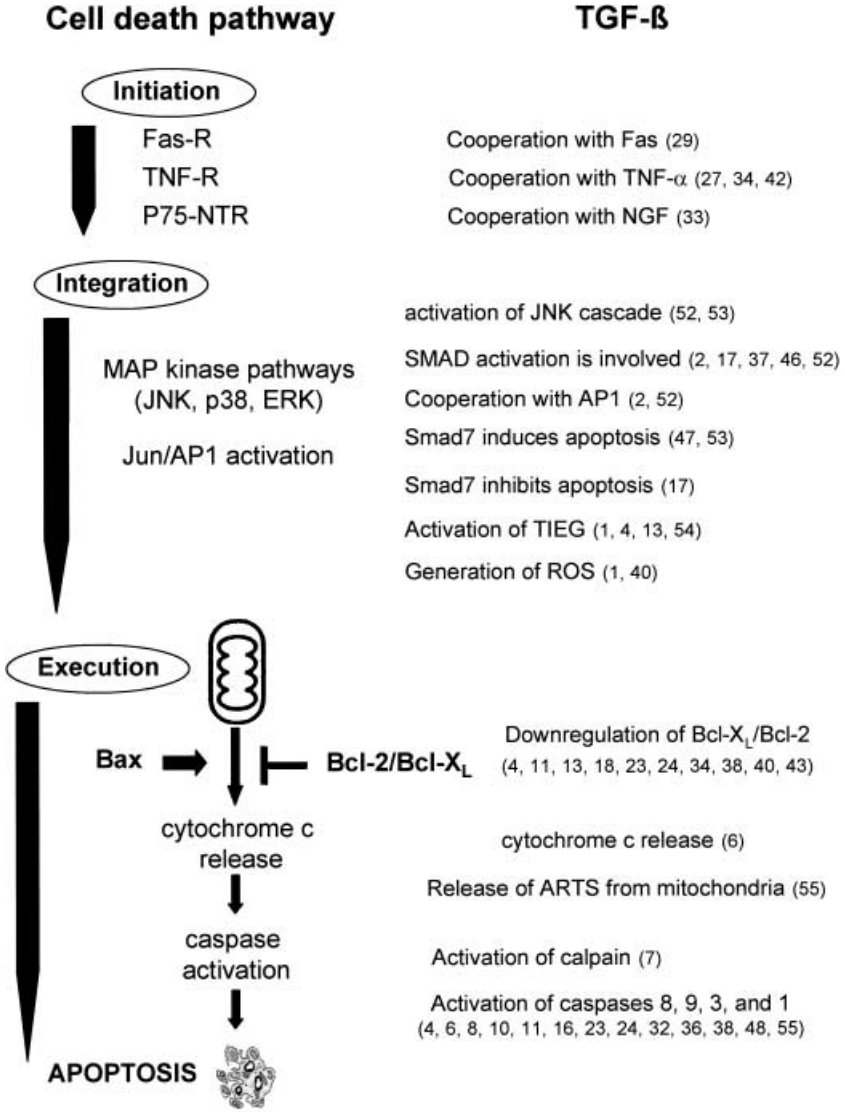

Fig. 2 Schematic representation of the apoptosis pathway. The apoptotic cascade can be divided into three phases (*Initiation, Integration, Execution*). In the initiation phase, apoptosis is triggered by stress signals or specific factors acting through a subset of receptors. During the integration phase, signals from several signaling pathways are balanced, and the decision is made regarding whether the execution of cell death should be initiated. During the execution phase, proteases of the caspase family are activated and degrade specific substrates leading to the self-destruction of the cell. Several key steps and mediators are known (*left*) for each phase. The events in which the TGF-β pathway is involved are delineated *right*. The *numbers* indicate the respective references in Table 1 (for a review of apoptosis mechanisms, see Jarpe et al. 1998)

duction, as the intracellular balance between pro- and anti-apoptotic molecules is disturbed.

Another mechanism of TGF-β-induced cell death has been proposed following the finding that TGF-β downregulates NFκB activity by the induction of the expression of IκBα, a specific inhibitor of NFκB (Arsura et al. 1996). IκBα binds to and inactivates NFκB, a transcription factor that is involved in the mediation of survival signals. NFκB inhibition results in the repression of c-myc expression in WEHI B cell lymphoma cells and increases cell death, which can be blocked by the ectopic expression of c-myc (Arsura et al. 1996).

Several Eppstein Barr virus (EBV)-negative B-cell lymphoma cell lines (BL-41, Ramos, and CAPA-2) respond to TGF-β by the induction of apoptosis. The expression of c-myc and Bcl-2 following TGF-β treatment has been investigated and found to be unchanged. However, another B-cell lymphoma cell line (CA46) and several preparations of normal human tonsilar B-cells do not undergo apoptotic cell death after TGF-β stimulation. These data suggest that there is a more complex regulatory network of as yet uncharacterized intra- and/or extracellular factors cooperating with TGF-β to induce cell death (Chaouchi et al. 1995).

One possibility is that immunosuppressants such as cyclosporine A can sensitize lymphocytes to Ca^{2+}-mediated cell death (Andjelic et al. 1997). Together with Ca^{2+}, cyclosporine induces the secretion of TGF-β in B-cells. The synergistic action of TGF-β and Ca^{2+} subsequently results in the apoptotic cell death of both T- and B-cells, thus leading to the speculation that TGF-β might play a role in the cyclosporine A immunosuppression pathway (Andjelic et al. 1997).

In another study, the upstream caspases involved in TGF-β-mediated cell death have been studied in more detail (Inman and Allday 2000). The TGF-β-induced cell death of the EBV-negative Burkitts lymphoma cell line BL41 involves the activation of caspases 2, 3, 7, 8, and 9. The authors demonstrate that the activation of the other caspases is dependent on caspase 8 activation. How-

ever, activation of caspase 8 in this cell system is independent of death-receptor activation by Fas or tumor necrosis factor α (TNF-α). Bcl-X_L and Bad protein levels were reduced after 24 h, whereas the protein level of bax remained relatively unchanged.

The importance of TGF-β for the maintenance of T-/and B-cell homeostasis is best documented by in vivo studies. TGF-β1 knockout mice die 2–3 weeks after birth and show massive inflammation (Kulkarni et al. 1993; Shull et al. 1992). Transgenic mice overexpressing a dominant negative mutant of the TGFβRII, specifically on T-cells, show a hyperplasia of CD8+ T-cells resulting in a massive expansion of lymph nodes and spleen (Lucas et al. 2000). However, TGF-β1 knockout mice have a smaller thymus and spleen than their wild-type littermates (Shull et al. 1992; Christ et al. 1994), indicative of a depletion of T-cells, perhaps through induced apoptosis. A recent publication from the group of Sharon Wahl (Chen et al. 2001) has expanded these findings by localizing TGF-β1 to mitochondria; this presumably mediates a protective effect on T-cell apoptosis. These results are contradictory to other findings, and further investigations are required into the possible ways in which TGF-β can mediate both pro- and anti-apoptotic stimuli.

The targeted deletion of TGFβRII in B-cells has revealed the various roles of TGF-β concerning the homeostasis and responsiveness of B-cell subpopulations (Cazac and Roes 2000). Cazac and Roes (2000) have found that the B-cell response to a weak antigen shows a unusual hyper-responsiveness of B-cells manifested by extremely high IgG3 antibody production. Hyperplasia of peripheral B-cells in the peritoneum and in Peyer's patches has been observed, revealing that the defect in B-cell homeostasis is the result of a defective cell-death program.

Taken together, there is thus strong evidence from cell-culture experiments and in vivo data that TGF-β is a key mediator of B- and T-cell homeostasis by regulating proliferation processes and apoptosis in these cells.

TGF-β-induced apoptosis in cells of the digestive system

As apoptotic cell death is a well-recognized phenomenon in the differentiation and maintenance of the liver, its underlying mechanism has been extensively studied in various cell types originating in the liver. Rat liver epithelial cells undergo growth arrest and apoptosis upon TGF-β treatment (Teramoto et al. 1998). Both p53 and bax are overexpressed during the apoptotic process. Several growth factors are unable to counteract this process, whereas interestingly, TGF-α enhances TGF-β mediated apoptosis. TGF-α alone also induces bax and p53 expression leading to apoptosis without TGF-β co-treatment. Thus TGF-α and TGF-β cooperate to induce apoptosis in rat liver epithelial cells possibly via the induction of p53 and bax. However, there are also reports of p53-independent pathways of TGF-β-induced apoptosis (Yamamoto et al. 1996).

TGF-β-induced cell death in Hep3B cells can be prevented by treatment with insulin (Chen and Chang 1997; Chuang et al. 1994) or by overexpression of IRS-1 (Tanaka and Wands 1996). This insulin-mediated survival signal acts via activation of PI 3-kinase and Akt. Constitutively active Akt can mimic the anti-apoptotic effect of insulin, and the anti-apoptotic effect of insulin can be inhibited by a dominant negative Akt. This kinase pathway seems to influence the activation of caspase 3 downstream of activated SMADs, because SMAD activation and translocation into the nucleus remains unaffected by the PI 3-kinase/Akt pathway (Chen et al. 1998).

Analyses of TGF-β induced cell death in the Hep3B hepatoma cell line have confirmed that apoptosis is accompanied by the activation of caspase-family proteases (Hung et al. 1998). The broad-spectrum caspase inhibitor ZVAD-FMK blocks TGF-β-induced cell death in a concentration-dependent manner. Application of the more specific caspase inhibitor DEVD-FMK has revealed that the activation of caspase 3 is necessary for TGF-β-induced cell death. Immunoblotting has confirmed the cleavage of the caspase-3-specific substrate poly(ADP-ribose)polymerase (PARP) after TGF-β treatment (Hung et al. 1998).

Interestingly, McGinnis et al. (1999) have shown that PARP and procaspase 3 are substrates of calpain in vitro and in vivo. This finding suggests that TGF-β-mediated apoptosis may not depend on the SMAD pathway, but may involve another mechanism directly activating calpain and subsequently the caspase cascade. In this context, Gressner and coworkers (1997) have been able to show that TGF-β-induced cell death in primary rat hepatocytes can be inhibited by the administration of calpain inhibitors.

Analysis of the apoptotic process in isolated primary hepatocytes of the rat has revealed that transcriptional activation is necessary for cell-death induction (Inayat-Hussain et al. 1997). The application of cycloheximide, which blocks de novo protein sythesis, can block TGF-β-mediated apoptosis induction. Caspase 3 is activated, whereas caspase 1 is not increased in this process.

Shima and coworkers (1999) have detected the activation of caspase 8, 9, and 3 in response to TGF-β administration to HuH-7 hepatoma cells. The apoptotic process is accompanied by a decreased level of Bcl-X_L protein and a low Bax/Bcl-X_L ratio. TGF-β treatment results in a reduced level of XIAP, an inhibitor of apoptosis previously shown to interact with the TAK1/TAB1 complex (TGF-β-activated kinase/TAK-binding protein), downmodulating the kinase activity of TAK1 (Shibuya et al. 1996). Interestingly, the potent growth factor, epidermal growth factor (EGF), completely abolishes the TGF-β-induced apoptosis of HuH-7 cells by an unknown mechanism, as XIAP and Bcl-X_L levels remain unaffected (Shima et al. 1999).

The investigation of human (HepG2), mouse (55.1c), and rat (FTO-2B) hepatoma cell lines has revealed differences in their response to TGF-β (Buenemann et al. 2001). The activation of the SMAD path-

way, monitored by measurement of luciferase activity from a SMAD-driven promoter element, is similar in all three cell lines, in which transient expression of TGF-β induced early gene (TIEG) is observed. Whereas HepG2 cells are completely resistant to TGF-β-induced apoptosis and growth arrest, 55.1c cells show only modest apoptosis. FTO-2B cells undergo G1 arrest and massive apoptosis in response to TGF-β. Since the SMAD pathway and TIEG expression, which are involved in TGF-β-mediated signaling and the induction of cell death, are similar in all three cell lines, the defect in the cell-death pathway in HepG2 and 55.1c must lie downstream of SMAD and TIEG (Buenemann et al. 2001).

The role of TIEG in apoptosis induction has been investigated in Hep 3B cells (Ribeiro et al. 1999). Like TGF-β, TIEG induces the generation of ROS and the loss of the mitochondrial membrane potential preceding the apoptotic death of TIEG-transfected cells.

In the rat hepatoma cell line FaO, TGF-β induces apoptosis via the activation of caspases 2, 3, 7, and 8 (Freathy et al. 2000). The activation of caspase 3 and 7 is blocked by a broad-spectrum caspase inhibitor; however, the assembly of the apoptosome complex is not disturbed. The process of cytochrome C release and apoptosome assembly triggered by TGF-β is not accompanied by the downregulation of Bcl-XL protein, an event often described for other cell systems. Freathy et al. (2000) speculate that another Bcl-2-family-independent mechanism, such as the generation of ROS, may be involved. However, they do not address this question further by conclusive experiments.

In primary hepatocytes, TNF-α and EGF antagonize the pro-apoptotic effect of TGF-β (Roberts et al. 2000). Pretreatment of hepatocytes with EGF or TNF-α suppresses TGF-β-induced cell death by 73% and 50%, respectively. Various survival signals are involved in this process. Whereas EGF relays signals via phosphoinositide 3-kinase (PI 3-kinase) and extracellular signal regulated kinase (ERK), the suppression of TGF-β-induced apoptosis by TNF-α is mediated by ERK and p38 MAP kinase. Again, this study provides strong evidence that TGF-β signaling interacts with other signaling pathways to regulate cellular survival.

In fetal hepatocytes, TGF-β induces growth arrest in the G1 phase of the cell cycle (Sanchez et al. 1995). Moreover, TGF-β induces apoptosis of fetal hepatocytes via a mechanism that requires new protein synthesis (Sanchez et al. 1997). Together with EGF, TGF-β promotes the differentiation of these cells (Sanchez et al. 1998). In the context of these studies, it has been observed that, regardless of the concentration of TGF-β, 50% of the cells always survive. These cells are less differentiated with respect to liver-specific transcription factor activity and are still able to undergo growth arrest in response to TGF-β, whereas they are totally resistant to TGF-β-mediated apoptosis (Sanchez et al. 1999). These data suggest a role for TGF-β during liver development and differentiation.

TGF-β-induced cell death in the reproductive system

Apoptosis occurs in specific uterine cells during the estrous cycle, blastocyst implantation, and decidualization. Active proliferation of endometrial stromal cells during the decidualization of early pregnancy are followed by programmed cell death in the antimesometrial region of the decidua. The control of the proliferation and pattern formation of cellular structures in the placenta after blastocyst implantation depends on the synthesis and secretion of endometrial growth factors (Moulton 1994). The mRNAs for TGF-β1 and TGF-β2 have been detected in decidual cells on day 6.5 of pregnancy. To investigate the role of TGF-β in the apoptosis of decidual cells, Moulton (1994) isolated endometrial stromal cells from progestin-pretreated ovariectomized rats. Application of exogenous TGFβ1 to the cultured cells induced cell death in a concentration-dependent manner. TGF-β2 showed the same effect. Speculating that a paracrine/autocrine mechanism of cell-death induction was responsible for the observed cell death in vivo, Moulton (1994) tested the hypothesis with high density cultures of endometrial cells, assuming that a high concentration of secreted TGF-β would increase cell death. With antibodies against TGF-β1 and -β2, only the active form of TGF-β2 could be detected within the cell culture supernatant, indicating that TGF-β2 was responsible for the TGF-β-mediated effects in vivo.

TGF-β has been shown to induce apoptosis in normal prostate cells and prostatic carcinoma cells in vitro and in vivo (Hsing et al. 1996; Kyprianou and Isaacs 1989; Landström et al. 1996). Castration of Dunning R3327 PAP rats leads to the upregulation of TGF-β1 and both TGF-β receptors, correlating with increased apoptosis (Landström et al. 1996). SMAD proteins are involved in this process as revealed by another study showing enhanced SMAD expression and activated SMAD2 and nuclear SMAD6 and SMAD7 in areas with a large number of apoptotic cells (Brodin et al. 1999).

One example of in vitro studies with prostate cancer cells is the TGF-β-induced apoptosis in PC-3 cells (Rajah et al. 1997). The apoptotic process here is accompanied by an increase in the expression of insulin-like growth-factor-binding protein 3 (IGFBP-3), another inhibitor of cell growth. IGFBP-3 is thought to act via the sequestration of free IGFs and, thereby, the hindrance of the activation of IGF receptors. Rajah et al. (1997) could induce apoptosis by IGFBP-3 treatment of the cells. Furthermore, they could inhibit TGF-β-mediated apoptosis with neutralizing antibodies against IGFBP-3. In this cell line, TGF-β-induced cell death may be mediated via IGFBP-3 (Rajah et al. 1997). In a study by Hsing and coworkers (1996), TGF-β-induced cell death has been analysed in nontumorigenic NRP-152 cells and tumorigenic NRP-154 prostatic epithelial cells. In this cell model, dexamethasone (Dex) enhances TGF-β-mediated apoptosis, whereas IGF-I counteracts apoptosis induction. This may support the data of Rajah and coworkers (1997) who have shown that TGF-β-mediated cell death

is induced via the upregulation of IGFBP-3, which sequesters IGF-I and hinders the survival promoting effect of this growth factor.

Apoptosis is an important process during the remodeling of the breast after postlactational involution. TGF-β mRNA is expressed at low levels in the mammary gland of pregnant mice but increases dramatically within the first 2 days following weaning and continues to be elevated throughout postlactational involution (Rosfjord and Dickson 1999). The high expression of TGF-β in transgenic mice results in the lack of secretory lobule development and an inability to lactate accompanied by increased apoptotic cell death within the mammary gland. These data indicate that TGF-β is important for tissue remodeling in vivo, possibly by the regulation of apoptotic cell death during distinct phases during the animal life cycle (Rosfjord and Dickson 1999).

Additional evidence from in vitro studies supports the role of TGF-β as a death-inducing factor in the mammary gland. TGF-β induces apoptosis in c-myc overexpressing mouse mammary epithelial cells (Amundadottir et al. 1996; Nass et al. 1996). Cell death is accompanied by a substantial downregulation of Bcl-2 and Bcl-X_L mRNA. TGF-β can also directly induce cell death in MCF-7 breast cancer cells (Chen et al. 1996).

Perry and coworkers (1995) have shown that TGF-β is induced by tamoxifen treatment in MCF-7 and MBA-MD-231 breast cancer cell lines. Tamoxifen-induced cell death can be prevented by TGF-β neutralizing antibodies.

TGF-β also induces cell death in NIH-OVCAR-3 ovarian carcinoma cells. As in other TGF-β-treated cells, the Bcl-2 protein level is decreased (Lafon et al. 1996). Here, the apoptotic process can be inhibited by the administration of N-acetylcysteine, an antioxidative agent, thus revealing an important role for ROS in this cell line.

TGF-β-induced cell death in tissue formation and remodeling

TGF-β induces capillary morphogenesis in vitro and angiogenesis in vivo. Using a dominant negative TGF-βRII, Choi and Ballermann (1995) have shown that exogenous and endogenous TGF-β induces growth arrest and capillary formation accompanied by massive cell death during this morphogenetic process. This TGF-β effect is mediated via receptor activation, because cells transfected with the mutant receptor do not respond to TGF-β. The application of TGF-β neutralizing antibodies has substantiated this finding, indicating that TGF-β-mediated apoptosis may be of great relevance during embryogenetic tissue modeling (Choi and Ballermann 1995).

In human aortic smooth muscle cells, TGF-β treatment results in massive apoptotic cell death (Hishikawa et al. 1999). Since a TGF-β-responsive element exists in the connective tissue growth factor (CTGF) gene, Hishikawa et al. (1999) investigated the role of this factor in TGF-β-mediated apoptosis. They found that CTGF was upregulated by TGF-β. Inhibition of CTGF by antisense oligonucleotides inhibited TGF-β-induced cell death and reduced caspase 3 activation by TGF-β. These data suggest an important role of CTGF in TGF-β-mediated cell death in human aortic smooth muscle cells possibly via autocrine mechanisms (Hishikawa et al. 1999).

Apoptosis is believed to be important for proper wound healing. During the maturation of the wound, excess cell numbers are reduced by programmed cell death (Crowe et al. 2000). During wound healing, apoptotic cells are mainly localized in the granulation tissue beneath the advancing epithelial edge. TGF-β1/Scid double-knockout mice reveal a delay in each of the major phases of wound healing, including a delayed and reduced onset of apoptosis in cells localized in the granulation tissue (Crowe et al. 2000). Thus, the TGF-βs have been implicated in wound healing, possibly via their apoptosis-inducing capacity. The data also suggest a compensatory role for TGF-β2 and 3, as these are upregulated in TGF-β1/Scid knockout mice and may be responsible for succesful healing of the wound.

TGF-β3 knockout mice exhibit, among other distortions, a cleft palate (Proetzel et al. 1995). Detailed analysis of this phenotype has revealed that medial edge epithelial cells undergo apoptosis during the process of cleft fusion (Martinez-Alvarez et al. 2000). In palates of TGF-β3 knockout mice, apoptotic cell death is markedly reduced. This, together with other observations in these knockout animals, provides good evidence that the apoptosis-inducing capacity of TGF-β3 is necessary, at least in part, for successful palatal fusion.

TGF-β-induced cell death in the nervous system

The regulation of the balance of neuron survival and death is a permanent feature in nervous system development, maintenance, degeneration, and repair. The first evidence for a role of TGF-β in the regulation of apoptosis in the nervous system came from the following in vitro study. Experiments by de Luca and coworkers (1996) showed that cultured immature cerebellar granule neurons died apoptotically within several days in vitro unless they were cultured with high concentrations of potassium, which led to depolarization. All three TGF-β isoforms induced apoptosis when these neurons were maintained under low potassium levels. This effect could not be blocked by the application of CNTF or LIF, cytokines that enhance neuronal survival, or IGF-I, which normally prevents apoptosis after potassium withdrawal. Interestingly, such neurons cultured for several days in high potassium acquired resistance to TGF-β-mediated cell death. These findings imply that TGF-β limits the expansion of postmitotic neuronal precursor populations, on the one hand, by promoting cell death and, on the other hand, by supporting the survival of those neurons that have succesfully reached their target area and gained supportive synaptic connectivity (de Luca et al. 1996).

The role of TGF-β in ontogenetic neuron death in vivo was first demonstrated in a study in which chick embryos were used as a model system (Krieglstein et al. 2000). By application of TGF-β neutralizing antibodies in ovo, the ontogenetic death of ciliary, dorsal root, and spinal motor neurons was largely prevented. Moreover, in limb-bud ablation experiments, the classical protocol used to show the importance of target-derived survival factors (Hollyday and Hamburger 1976; Oppenheim 1991; Pettmann and Henderson 1998) during the phase of ontogenetic neuron death, the immunoneutralization of TGF-β rescued both motoneurons and dorsal root ganglion neurons that would normally die (Krieglstein et al. 2000).

In another study, it could be shown that TGF-β is essentially required to regulate programmed cell death in the central retina (Dünker et al. 2001). Application of TGF-β neutralizing antibodies resulted in a substantial decrease of TUNEL-positive cells in the central retina. This decrease was comparable with the effect of nerve growth factor (NGF) immunodepletion in chick embryonal retina (Dünker et al. 2001; Frade et al. 1996).

Furthermore, in primary rat oligodendrocyte progenitor cultures, TGF-β induces both growth arrest and apoptosis (Yu et al. 2000). TGF-β-induced cell death can be prevented when the cells are pretreated for 15 h with FGF-2, whereas TNF-α-induced cell death is not affected. Yu et al. (2000) have unfortunately not investigated the apoptosis-inducing cascade, their experiments being directed mainly at the mechanisms involved in the G1 arrest.

TGF-β inhibits cell proliferation of T24 glioma and 476–16 trigeminal neurinoma cells. Inhibition of cell growth is accompanied by apoptotic cell death (Marushige and Marushige 1994). The mode of cell death between these two cell lines seems to be different. Cells of the 476–16 line undergo apoptosis primarily after growth arrest in G1. Apoptosis can be stimulated by serum withdrawal and inhibited by mitogenic stimuli, such as insulin and platelet-derived growth factor. T24 cells undergo only a moderate growth arrest after TGF-β treatment and die by apoptosis when cells reach confluency in the culture dish. The kinase inhibitor staurosporine can accelerate TGF-β-induced apoptosis in both 476–16 and T24 cells, whereas phorbol ester treatment inhibits apoptotic cell death in 476–16 cells but stimulates that of T24 cells. These results clearly indicate that there are at least two different modes of TGF-β-induced apoptosis within cultured cells (Marushige and Marushige 1994).

In a study by de Luca et al. (1996), the authors investigated the role of TGF-β expression in the progression of malignant glioma by using an immunocompetent murine model. They generated cell lines stably overexpressing TGF-β or repressing endogenous expression of TGF-β. These cells were injected either subcutaneously or intracranially. TGF-β inhibited the induction of anti-tumor cytotoxicity when the tumor cells were applied subcutaneously but not when they were injected intracranially. Overproduction of TGF-β reduced the tumorigenicity of tumors induced by subcutaneous or intracranial injection in line with an increase of apoptosis within the tumor area. The opposite effect was observed with the antisense cell lines, which underproduced TGF-β. This resulted in a higher growth rate of the tumor and reduced apoptotic cell death within the tumor area. Furthermore, de Luca et al. (1996) could show that Fas/APO-1 was expressed in vitro and in vivo in the parental SMA 560 cell line. In vitro experiments showed that these cell line was sensitive to Fas- and TGF-β-induced cell death. Application of both factors synergistically potentiated apoptotic cell death (Ashley et al. 1998).

Synergistic effects

The cooperativity of TGF-β with other growth factors is one possible explanation for its multifunctional properties. Within the last few years, data have accumulated suggesting that TGF-β modulates the action of other growth factors, i.e., the neurotrophic function of GDNF. TGF-β synergizes with GDNF to promote the survival of peripheral and central nervous system dopaminergic neurons (Krieglstein et al. 1998; Unsicker and Krieglstein 2000). On the other hand, TGF-β and TNF-α act synergistically to induce cell death in Schwann cells (Skoff et al. 1998). Neither TNF-α nor TGF-β alone are capable of inducing cell death in these cells, despite these growth factors individually inducing cell death in a variety of different cell types.

TGF-β enhances growth inhibitory effects of Dex and 1α,25-dihydroxyvitamin D_3 (VD3) in monocytoid leukemia U937 cells (Kanatani et al. 1999). The expression of differentiation specific markers, such as CD11b and CD14 antigens, is enhanced by VD3. In cooperation with Dex, these genes are not induced, but the number of apoptotic cells (Apo2.7-positive cells) is increased. Both factors induce, together with TGF-β, an increased expression of $p21^{waf1}$ leading to a strong hypophosphorylation of the retinoblastoma-susceptibility gene product pRb. When cells are treated with TGF-β and Dex in combination, the anti-apoptotic Bcl-X_L protein is downregulated, whereas co-treatment with VD3 blocks the downregulation of Bcl-X_L. These data suggest that TGF-β is an indispensable cofactor either for differentiation processes or for the elimination of cells, depending on the cooperating growth factor (Kanatani et al. 1999).

In thecal/interstitial cells, TGF-α and TGF-β synergistically induce apoptosis, whereas each growth factor by itself has no effect on cell viability. In T/I cells treated with both growth factors, Bcl-2 mRNA levels decrease significantly, and the expression of ICE/caspase-1 is enhanced three-fold. Cells treated only with TGF-α or TGF-β show unaltered Bcl-2 and caspase-1 mRNA levels. Induction and repression processes are involved in the synergistic action of TGF-α and TGF-β. This perhaps could explain why only both growth factors together can induce cell death in these cells, one component being responsible for induction, the other for repression (Foghi et al. 1998).

TGF-β induces cell death in transformed fibroblasts and synergizes with TNF-α in this process (Schulz and Bauer 2000). Pretreatment of cells with TNF-α followed by application of TGF-β causes a faster induction of apoptosis and vice versa. The underlying mechanism seems to be the reduction in the level of endogenous survival factors.

The results from an interesting study conducted on MCF-7 cells (Tobin et al. 2001) lend support for the putative role of the TGF-β pathway in TNF-α-induced apoptosis. A dominant negative TGF-β receptor was induced to be overexpressed in order to disturb TGF-β signaling in the MCF-7 cells. These MCF-7 cells were relatively resistant to TNF-α-induced cell death, a resistance possibly mediated by the increased Bcl-2 expression in these cells. Levels of bax and Bcl-X_L remained unchanged (Tobin et al. 2001). These data suggest that TGF-β sensitizes cells for other pro-apoptotic stimuli by the downregulation of anti-apoptotic Bcl-2.

Another study has demonstrated a synergism between EGF and TGF-β in the induction of apoptosis in porcine thyroid follicles (Bechtner et al. 1999). However, this does not seem to be "real" synergism as EGF induces the expression of TGF-β in this cell system, and therfore, the increased TGF-β level could account for the increase in apoptotic cells. Nevertheless, the induction of cell death by TGF-β is dose-dependent. The application of IGF could not rescue the cells from TGF-β-induced cell death (Bechtner et al. 1999).

Cell death induced by TGF-β effectors

TGF-β sends signals to the nucleus via SMAD-family member proteins (Massague 2000). The overexpression of DPC4 (also called SMAD4) results in the induction of apoptosis in transiently transfected MDCK cells (Atfi et al. 1997). Coexpression of SMAD3 and SMAD4 results in an enhancement of apoptosis. SMAD4 and SMAD3 together could enhance TGF-β-mediated transactivation. Atfi et al. (1997) have shown that supertransactivation of TGF-β-responsive genes by SMAD3/SMAD4 coexpression can be blocked by the dominant negative c-jun (TAM67). Similar effects have been obtained with the dominant negative MEKK1, a kinase involved in the activation of the JNK-MAP kinase pathway. These findings suggest that TGF-β signaling processes are dependent on other signal pathways for an optimal outcome and that ectopic expression of SMAD proteins can induce apoptosis (Atfi et al. 1997).

SMAD2 and SMAD3 are highly homologous members of the receptor-activated SMADs. Yanagisawa and coworkers (1998) found that, upon overexpression in stably transfected cell lines, cell death was strongly induced by SMAD3, but to a much lesser extend by SMAD2, in the presence of TGF-β. Interestingly, human lung epithelial cells downregulated SMAD3 expression after TGF-β administration, whereas SMAD2 expression remained unaffected. These data demonstrated, for the first time, the functional differences between these two TGF-β effector molecules in vivo (Yanagisawa et al. 1998).

The importance of the SMAD pathway for TGF-β-mediated apoptosis has been substantiated by a study of Yamamura and coworkers (2000). They investigated cell-death induction in M1 and Hep3B cells and found that the overexpression of dominant negative SMAD3 inhibited TGF-β-induced apoptosis (Yamamura et al. 2000). The same effect could be achieved by the overexpression of the inhibitory SMAD7. More interestingly, an interaction between SMAD proteins and the AP1 transcription factor was found to be involved. The inhibition of AP1 by the overexpression of a dominant negative FosB also caused substantial inhibition of TGF-β-mediated apoptosis. Moreover, the overexpression of JunD-FosB potentiated TGF-β-mediated cell death, corroborating the importance of other signaling pathways for TGF-β-mediated apoptosis.

Although SMAD7 has been described as an inhibitory SMAD protein that prevents the phosphorylation of receptor-activated SMADs, thereby inhibiting their nuclear translocation and the TGF-β-induced response (ten Dijke et al. 2000), SMAD7 has been shown to possess the capacity to induce apoptosis in prostatic carcinoma cells (Landström et al. 2000). Ectopic expression of SMAD7 induces apoptosis in PC-3U cells. The expression of SMAD7 antisense mRNA abolishes TGF-β-induced cell death in these cells. The same effect can be observed in TGF-β-treated DU 145 and HaCaT cells when SMAD7 antisense mRNA is expressed. Taken together, these results provide evidence for a new effector role of SMAD7 during TGF-β-induced apoptosis (Landström et al. 2000). Although this seems to be contradictory to the results obtained by Patil and coworkers (2000) who have described an anti-apoptotive effect in B-cells, the view of SMAD7 as an apoptosis-inducing protein is substantiated by the findings of Lallemand et al. (2001). SMAD7 seems to induce the JNK-signaling cascade specifically, whereas p38 and p42/p44 MAPK are not affected. The activation of the JNK pathway however is not involved in the inhibitory function of SMAD7 that prevents the activation of SMAD2/3 at the receptor level, whereas it is necessary for SMAD7-induced apoptosis (Lallemand et al. 2001).

The adapter protein Daxx seems to be another essential component of the TGF-β cell-death pathway (Perlman et al. 2001). Daxx, which is normally associated with the Fas receptor and mediates the activation of JNK and Fas-induced apoptosis, has been found to bind directly to the cytoplasmic domain of TGFβRII. Treatment of AML12 mouse hepatocytes with antisense oligonucleotides for Daxx results in substantial inhibition of TGF-β-mediated apoptosis. The induction of apoptosis and the activation of JNK by TGF-β application can be abolished by the overexpression of the dominant negative c-terminal part of the Daxx protein, indicating that JNK activation via Daxx is an important upstream event during TGF-β-mediated apoptosis (Perlman et al. 2001).

Table 1 Listing of TGF-β-mediated cell-death induction with investigated cell type and mechanistic events that have been found during this process (*No.* numbers in Fig. 2)

No.	Cell type	TGF-β iso-form	Genes/proteins upregulated (↑)/ downregulated (↓)/ unchanged (–)/ other modifications	Activated caspase	Other factors	Co-operation	Reference
1	Hep3B	-β1	bcl-xl (–), bax (–), TIEG ↑	ND	TIEG induces apoptosis and ROS upregulation	–	Ribeiro et al. 1999
2	Hep3B	-β1	ND	ND	SMAD and AP1 important for apoptosis induction	–	Yamamura et al. 2000
3	RLE	-β1	p53 ↑, bax ↑, AP1 activity ↑	ND	Dex inhibits apoptosis	TGF-α	Teramoto et al. 1998
4	Hepatoma cells	–	TIEG ↑, bcl-X_L ↓	3	Dex, insulin, phenobarbital inhibit apoptosis	–	Buenemann et al. 2001
5	Primary rat hepatocytes	-β1	ND	ND	TNF-a and EGF suppress apoptosis through ERK/PKB activation	–	Roberts et al. 2000
6	Hepatoma cells	-β1	Cytochrome c release; formation of apoptosome complex	2, 3, 7, 8	–	–	Freathy et al. 2000
7	Primary hepatocytes	-β1	ND	ND	Calpain inhibitor blocks apoptosis	–	Gressner et al. 1997
8	Hepatoma cells	-β1	ND	3	z-VAD-fmk blocks apoptosis	–	Hung et al. 1998
9	Fetal hepatocytes	-β1	–	ND	Cell death is dependent on differentiation status of fetal hepatocytes	–	Sanchez et al. 1999
10	Rat hepatocytes	-β1	–	3	Cycloheximide blocks TGF-β induced apoptosis	–	Inayat-Hussain et al. 1997
11	Human hepatoma cells	-β1	bcl-X_L ↓, bax (–), XIAP ↓	3, 8, 9	Sequential activation of caspase 8, 9, and 3; bcl-X_L downregulation precedes caspase activation; EGF inhibits apoptosis	–	Shima et al. 1999
12	Gastric cancer cells	-β1	p53 (–)	ND	ND	–	Yamamoto et al. 1996
13	Mv1Lu	-β1	TIEG ↑, bcl-2 ↓	ND	TIEG induces apoptosis	–	Chalaux et al. 1999
14	Normal B-cells, B-cell lymphoma	-β1	c-myc (–), bcl-2 (–)	ND	–	–	Chaouchi et al. 1995
15	B-cell lymphoma phosphorylation	-β1	Decreased Rb	ND	bcl-2 overexpression does not protect from apoptosis	–	Wang et al. 1996
16	WEHI 231	-β1	cdk/cyclin A activity ↓	Yes, not specified	BD-fmk inhibits apoptosis	–	Brown et al. 1998
17	WEHI 231	-β1	Apoptosis is SMAD dependent, SMAD7 inhibits apoptosis	ND	CD40 inhibits apoptosis and induces NFκB dependent SMAD7 activation	–	Patil et al. 2000
18	U937	-β1	$p21^{waf1}$ ↑, $p27^{kip1}$ ↑, bcl-X_L ↓, phosphorylated Rb ↓	ND	Combination with Dex is proapoptotic; combination with VD3 anti-apoptotic	Dex and VD3	Kanatani et al. 1999
19	OVA-7 T-cell line	-β2	bcl-2 (–), c-myc (–)	ND	–	–	Weller et al. 1994
20	In vivo, T-cells	–	ND	ND	Dominant negative TGFβRII expression causes hyperplasia of $CD8^+$ T-cells	–	Lucas et al. 2000
21	In vivo, T-/B-cells	-β1	ND	ND	Massive inflammation of TGF-β1 knockout mice; disturbed cell death	–	Shull et al. 1992; Kulkarni et al. 1993
22	In vivo, B-cells	–	ND	ND	Hyperplasia of peripheral B-cells	–	Cazac and Roes 2000
23	Burkitt lymphoma cells	-β1	bad ↓, bcl-X_L ↓, bcl-2 (–), bax (–)	2, 3, 7, 8, 9	fas and TNF-receptor independent; caspase8 dependent	–	Inman and Allday 2000

Table 1 (continued)

No.	Cell type	TGF-β isoform	Genes/proteins upregulated (↑)/ downregulated (↓)/ unchanged (–)/ other modifications	Activated caspase	Other factors	Co-operation	Reference
24	Ramos	-β1	bcl-X_L ↓, bik ↑	3	–	–	Saltzman et al. 1998
25	WEHI	-β1	IκBα ↑, c-myc ↓	ND	Ectopic expression of c-myc inhibits apoptosis		Arsura et al. 1996
26	B- and T-cells	-β1	ND	ND	Ca^{2+} and cyclosporin induce TGF-β expression	Ca^{2+} cyclosporin	Andjelic et al. 1997
27	Schwann cells	-β1	ND	ND	ND	TNF-α	Skoff et al. 1998
28	Primary oligodendrocytes	-β1	$p21^{waf1}$ ↑, $p27^{kip1}$ ↑	ND	–	–	Yu et al. 2000
29	SMA 560 glioma cells	-β1	ND	ND	–	Fas	Ashley et al. 1998
30	Glioma and trigeminal neurinoma cells	-β1	ND	ND	Inhibition of apoptosis by insulin and platelet-derived growth factor	–	Marushige and Marushige 1994
31	Cerebellar granule neurons	-β1, -β2, -β3	ND	ND	Apoptosis under low potassium concentration; no reversion of apoptosis by CNTF, LIF, and IGF-I	–	de Luca et al. 1996
32	In vivo; neutralizing antibodies; chick embryo	–	ND	3	Cell death of DRG, CG, and motoneurons is reduced after TGF-β neutralization	–	Krieglstein et al. 2000
33	In vivo; neutralizing antibodies; chick embryo	–	ND	ND	Developmental cell death in the retina is reduced after TGF-β neutralization	NGF	Dünker et al. 2001
34	Transformed fibroblasts	-β1	Levels of anti-apoptotic proteins are reduced		Synergism with TNF-α	TNF-α	Schulz and Bauer 2000
35	Capillary endothelial cells	-β1	ND	ND	Dominant negative TGF-βRII blocks apoptosis	–	Choi and Ballermann 1995
36	Aortic smooth muscle cells	-β1	CTGF ↑	3	TGF-β effect is mediated by CTGF	–	Hishikawa et al. 1999
37	Lung epithelial cells	–	SMAD3 ↓	ND	SMAD3 induces apoptosis	–	Yanagisawa et al. 1998
38	Thecal interstitial cells	–	bcl-2 mRNA ↓, caspase1 expression ↑	1	–	TGF-α	Foghi et al. 1998
39	Thyroid follicle	-β1	ND	ND	EGF stimulates TGF-β1 expression	EGF	Bechtner et al. 1999
40	NIH-OVCAR-3 ovarian carcinoma cells	-β1	fos ↑, jun ↑, bcl-2 ↓,		ROS induction; N-acetylcysteine and ectopic bcl-2 expression inhibits apoptosis		Lafon et al. 1996
41	MCF-7, MBA-MD-231 breast cancer cells	–	ND	ND	–	–	Perry et al. 1995
42	MCF-7	-β1	bax (–), bcl-2 ↑, bcl-X_L (–) in DNRII expressing cells	ND	Dominant negative TGF-βRII reduces TNF-α mediated apoptosis	TNF-α	Tobin et al. 2001
43	Mammary epithelial cells	-β1	bcl-X_L ↓	ND	EGF counteracts TGF-β		Nass et al. 1996
44	Mammary gland	–	ND	ND	Remodeling of gland tissue after weaning involves apoptosis modulated by TGF-β; IGF-I and TGF-α inhibits remodeling and apoptosis	–	Rosfjord and Dickson 1999
45	Endometrial stromal cells	-β1, -β2	ND	ND	–	–	Moulton 1994

Table 1 (continued)

No.	Cell type	TGF-β isoform	Genes/proteins upregulated (↑)/ downregulated (↓)/ unchanged (–)/ other modifications	Activated caspase	Other factors	Co-operation	Reference
46	In vivo, prostate Dunning R3327 rat model	–	SMAD3, 4 ↑, activated SMAD2 ↑, SMAD6 ↑, SMAD7 ↑, nuclear localized SMAD7	ND	Castration paradigm	–	Brodin et al. 1999
47	Prostatic carcinoma cells	-β1	SMAD7 ↑	ND	SMAD7 induces apoptosis, antisense SMAD7 inhibits apoptosis	–	Landström et al. 2000
48	PC-3	-β1	IGFBP-3 ↑	Yes, not specified	IGFBP-3 induces apoptosis	–	Rajah et al. 1997
49	NRP-152/ NRP-154 Prostate epithelial cells	-β1, -β2, -β3	ND	ND	Dex enhances apoptosis; IGF-I inhibits apoptosis	Dex	Hsing et al. 1996
50	In vivo; TGF-β1-Scid double knockout	-β1	ND	ND	TGF-β2, and 3 compensate β1 loss; apoptosis during wound healing is disturbed	–	Crowe et al. 2000
51	In vivo; TGF-β3 knockout	-β3	ND	ND	Medial edge epithelial cell apoptosis is disturbed and responsible for palate fusion defect	–	Martinez-Alvarez et al. 2000
52	MDCK cells	–	JNK activation	ND	SMAD4 expression induces apoptosis; JNK activation; dominant negative jun can inhibit apoptosis	–	Atfi et al. 1997
53	Mv1Lu, COS-7, HepG2	–	JNK activation	ND	SMAD7 induces apoptosis; SMAD7-induced inhibition of receptor signaling is independent of JNK activation	–	Mazars et al. 2001
54	Pancreatic epithelial cells	–	–	–	TIEG induces apoptosis	–	Tachibana et al. 1997
55	NRP-1, COS-1, MCF-7 cells	-β1	–	3	ARTS sensitizes cells for TGF-β mediated apoptosis: upon TGF-β stimulation, ARTS translocates from mitochondria to the nucleus	–	Larisch et al. 2000
56	COS-1, AML12 cells	-β1	JNK activation	ND	Daxx adapter protein is reqired for TGF-β induced apoptosis and JNK activation	–	Perlman et al. 2001

Another downstream effector of TGF-β function is the SP1-like zinc-finger protein TIEG, which was first identified in human osteoblasts (Subramaniam et al. 1995). Ectopic overexpression of TIEG has been shown to induce apoptosis in epithelial cells, hepatocytes, and pancreatic epithelial cells (Chalaux et al. 1999; Ribeiro et al. 1999; Tachibana et al. 1997). TIEG action seems to involve the activation of ROS, as the blocking of ROS generation could block TIEG-mediated apoptosis (Ribeiro et al. 1999).

Recently, the group of Anita Roberts has described the finding of the new protein ARTS (apoptosis-related protein in the TGF-β signaling pathway), a new TGF-β downstream effector important for TGF-β-mediated apoptosis (Larisch et al. 2000). ARTS is a 32-kDa septin-like protein that has been located in mitochondria. ARTS sensitizes cells to TGF-β-stimulated apoptosis and affects only weakly fas or TNF-α-mediated apoptosis. Upon TGF-β stimulation, ARTS loses its mitochondrial localization and migrates to the nucleus. The inhibition of ARTS protein expression by antisense technology inhibits TGF-β-stimulated apoptosis substantially, showing the important role of ARTS during this process (Larisch et al. 2000).

Conclusions

Taken all the available data regarding TGF-β and TGF-β-mediated apoptosis together, it is clear that TGF-β itself is a death-inducing agent in vitro and in vivo (Table 1). However, it is also clear that the death-inducing capacity of TGF-β is context-dependent, i.e., it is restricted to certain cell types, to a certain state of differen-

tiation, and most notably, to the presence or absence of other growth factors. This may explain the contradictory results of some studies, e.g., when considering the role of SMAD7. Remaining unresolved issues include the way in which caspase activation is regulated by TGF-β or whether the downregulation of the anti-apoptotic proteins Bcl-2 and Bcl-X_L is a critical step during TGF-β-mediated apoptosis. Furthermore, a great deal of work remains concerning the regulatory molecules and their importance for TGF-β-induced apoptosis, in order to generate a complete and conclusive model that also explains the many interactions with other signaling pathways.

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References

Amundadottir LT, Nass SJ, Berchem GJ, Johnson MD, Dickson RB (1996) Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis. Oncogene 13:757–765

Andjelic S, Khanna A, Suthanthiran M, Nikolic-Zugic J (1997) Intracellular Ca^{2+} elevation and cyclosporin A synergistically induce TGF-beta 1-mediated apoptosis in lymphocytes. J Immunol 158:2527–2534

Arsura M, Wu M, Sonenshein GE (1996) TGF beta 1 inhibits NF-kappa B/Rel activity inducing apoptosis of B cells: transcriptional activation of I kappa B alpha. Immunity 5:31–40

Ashley DM, Kong FM, Bigner DD, Hale LP (1998) Endogenous expression of transforming growth factor beta1 inhibits growth and tumorigenicity and enhances Fas-mediated apoptosis in a murine high-grade glioma model. Cancer Res 58:302–309

Atfi A, Djelloul S, Chastre E, Davis R, Gespach C (1997) Evidence for a role of Rho-like GTPases and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in transforming growth factor beta-mediated signaling. J Biol Chem 272:1429–1432

Bechtner G, Froschl H, Sachse A, Schopohl D, Gartner R (1999) Induction of apoptosis in porcine thyroid follicles by transforming growth factor beta1 and epidermal growth factor. Biochimie 81:315–320

Bottner M, Krieglstein K, Unsicker K (2000) The transforming growth factor-betas: structure, signaling, and roles in nervous system development and functions. J Neurochem 75:2227–2240

Brodin G, Dijke P ten, Funa K, Heldin CH, Landström M (1999) Increased Smad expression and activation are associated with apoptosis in normal and malignant prostate after castration. Cancer Res 59:2731–2738

Brown TL, Patil S, Basnett RK, Howe PH (1998) Caspase inhibitor BD-fmk distinguishes transforming growth factor beta-induced apoptosis from growth inhibition. Cell Growth Differ 9:869–875

Buenemann CL, Willy C, Buchmann A, Schmiechen A, Schwarz M (2001) Transforming growth factor-beta1-induced Smad signaling, cell-cycle arrest and apoptosis in hepatoma cells. Carcinogenesis 22:447–452

Cazac BB, Roes J (2000) TGF-beta receptor controls B cell responsiveness and induction of IgA in vivo. Immunity 13: 443–451

Chalaux E, Lopez-Rovira T, Rosa JL, Pons G, Boxer LM, Bartrons R, Ventura F (1999) A zinc-finger transcription factor induced by TGF-beta promotes apoptotic cell death in epithelial Mv1Lu cells. FEBS Lett 457:478–482

Chaouchi N, Arvanitakis L, Auffredou MT, Blanchard DA, Vazquez A, Sharma S (1995) Characterization of transforming growth factor-beta 1 induced apoptosis in normal human B cells and lymphoma B cell lines. Oncogene 11:1615–1622

Chen H, Tritton TR, Kenny N, Absher M, Chiu JF (1996) Tamoxifen induces TGF-beta 1 activity and apoptosis of human MCF-7 breast cancer cells in vitro. J Cell Biochem 61:9–17

Chen RH, Chang TY (1997) Involvement of caspase family proteases in transforming growth factor-beta-induced apoptosis. Cell Growth Differ 8:821–827

Chen RH, Su YH, Chuang RL, Chang TY (1998) Suppression of transforming growth factor-beta-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway. Oncogene 17:1959–1968

Chen WJ, Jin W, Tian H, Sicurello P, Frank M, Orenstein JM, Wahl SM (2001) Requirement for transforming growth factor β1 in controlling T cell apoptosis. J Exp Med 194:439–453

Choi ME, Ballermann BJ (1995) Inhibition of capillary morphogenesis and associated apoptosis by dominant negative mutant transforming growth factor-beta receptors. J Biol Chem 270: 21144–21150

Christ M, McCartney-Francis NL, Kulkarni JM, Ward JM, Mizel DE, Mackall CL, Gress RE, Hines KL, Tian H, Karlson S, Wahl SM (1994) Immune dysregulation in TGF-β1 deficient mice. J Immunol 153:1936–1946

Chuang LY, Hung WC, Chang CC, Tsai JH (1994) Characterization of apoptosis induced by transforming growth factor beta 1 in human hepatoma cells. Anticancer Res 14:147–152

Crowe MJ, Doetschman T, Greenhalgh DG (2000) Delayed wound healing in immunodeficient TGF-beta 1 knockout mice. J Invest Dermatol 115:3–11

Dijke P ten, Miyazono K, Heldin CH (2000) Signaling inputs converge on nuclear effectors in TGF-beta signaling. Trends Biochem Sci 25:64–70

Dünker N, Krieglstein K (2000) Targeted mutations of transforming growth factor-beta genes reveal important roles in mouse development and adult homeostasis. Eur J Biochem 267: 6982–6988

Dünker N, Schuster N, Krieglstein K (2001) Transforming growth factor beta modulates programmed cell death in the retina of the developing chick embryo. Development 128:1933–1942

Foghi A, Teerds KJ, Donk H van der, Moore NC, Dorrington J (1998) Induction of apoptosis in thecal/interstitial cells: action of transforming growth factor (TGF) alpha plus TGF beta on bcl-2 and interleukin-1 beta-converting enzyme. J Endocrinol 157:489–494

Frade JM, Rodriguez-Tebar A, Barde YA (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383:166–168

Freathy C, Brown DG, Roberts RA, Cain K (2000) Transforming growth factor-beta(1) induces apoptosis in rat FaO hepatoma cells via cytochrome c release and oligomerization of Apaf-1 to form a approximately 700-kd apoptosome caspase-processing complex. Hepatology 32:750–760

Gold LI (1999) The role for transforming growth factor-beta (TGF-beta) in human cancer. Crit Rev Oncog 10:303–360

Gressner AM, Lahme B, Roth S (1997) Attenuation of TGF-beta-induced apoptosis in primary cultures of hepatocytes by calpain inhibitors. Biochem Biophys Res Commun 231:457–462

Hata A, Shi Y, Massague J (1998) TGF-beta signaling and cancer: structural and functional consequences of mutations in Smads. Mol Med Today 4:257–262

Heldin CH, Miyazono K, Dijke P ten (1997) TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 390:465–471

Hishikawa K, Nakaki T, Fujii T (1999) Transforming growth factor-beta(1) induces apoptosis via connective tissue growth factor in human aortic smooth muscle cells. Eur J Pharmacol 385:287–290

Hollyday M, Hamburger V (1976) Reduction of the naturally occurring motor neuron loss by enlargement of the periphery. J Comp Neurol 170:311–320

Hsing AY, Kadomatsu K, Bonham MJ, Danielpour D (1996) Regulation of apoptosis induced by transforming growth factor-beta1 in nontumorigenic rat prostatic epithelial cell lines. Cancer Res 56:5146–5149

Hung WC, Chang HC, Chuang LY (1998) Transforming growth factor beta 1 potently activates CPP32-like proteases in human hepatoma cells. Cell Signal 10:511–515

Inayat-Hussain SH, Couet C, Cohen GM, Cain K (1997) Processing/activation of CPP32-like proteases is involved in transforming growth factor beta1-induced apoptosis in rat hepatocytes. Hepatology 25:1516–1526

Inman GJ, Allday MJ (2000) Apoptosis induced by TGF-beta 1 in Burkitt's lymphoma cells is caspase 8 dependent but is death receptor independent. J Immunol 165:2500–2510

Jarpe MB, Widmann C, Knall C, Schlesinger TK, Gibson S, Yujiri T, Fanger GR, Gelfand EW, Johnson GL (1998) Anti-apoptotic versus pro-apoptotic signal transduction: checkpoints and stop signs along the road to death. Oncogene 17:1475–1482

Kanatani Y, Kasukabe T, Okabe-Kado J, Yamamoto-Yamaguchi Y, Nagata N, Motoyoshi K, Honma Y (1999) Role of CD14 expression in the differentiation-apoptosis switch in human monocytic leukemia cells treated with 1alpha,25-dihydroxyvitamin D3 or dexamethasone in the presence of transforming growth factor beta1. Cell Growth Differ 10:705–712

Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS (1986) Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. J Immunol 137:3855–3860

Krieglstein K, Farkas L, Unsicker K (1998) TGF-beta regulates the survival of ciliary ganglionic neurons synergistically with ciliary neurotrophic factor and neurotrophins. J Neurobiol 37:563–572

Krieglstein K, Richter S, Farkas L, Schuster N, Dunker N, Oppenheim RW, Unsicker K (2000) Reduction of endogenous transforming growth factors beta prevents ontogenetic neuron death. Nat Neurosci 3:1085–1090

Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S (1993) Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 90:770–774

Kyprianou N, Isaacs JT (1989) Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Mol Endocrinol 3:1515–1522

Lafon C, Mathieu C, Guerrin M, Pierre O, Vidal S, Valette A (1996) Transforming growth factor beta 1-induced apoptosis in human ovarian carcinoma cells: protection by the antioxidant N-acetylcysteine and bcl-2. Cell Growth Differ 7:1095–1104

Lallemand F, Mazars A, Prunier C, Bertrand F, Kornprost M, Gallea S, Roman-Roman S, Cherqui G, Atfi A (2001) Smad7 inhibits the survival nuclear factor kappaB and potentiates apoptosis in epithelial cells. Oncogene 20:879–884

Landström M, Eklov S, Colosetti P, Nilsson S, Damber JE, Bergh A, Funa K (1996) Estrogen induces apoptosis in a rat prostatic adenocarcinoma: association with an increased expression of TGF-beta 1 and its type-I and type-II receptors. Int J Cancer 67:573–579

Landström M, Heldin NE, Bu S, Hermansson A, Itoh S, Dijke P ten, Heldin CH (2000) Smad7 mediates apoptosis induced by transforming growth factor beta in prostatic carcinoma cells. Curr Biol 10:535–538

Larisch S, Yi Y, Lotan R, Kerner H, Eimerl S, Tony PW, Gottfried Y, Birkey RS, Caestecker MP de, Danielpour D, Book-Melamed N, Timberg R, Duckett CS, Lechleider RJ, Steller H, Orly J, Kim SJ, Roberts AB (2000) A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. Nat Cell Biol 2:915–921

Lawrence DA (1996) Transforming growth factor-beta: a general review. Eur Cytokine Netw 7:363–374

Lomo J, Blomhoff HK, Beiske K, Stokke T, Smeland EB (1995) TGF-beta 1 and cyclic AMP promote apoptosis in resting human B lymphocytes. J Immunol 154:1634–1643

Luca A de, Weller M, Fontana A (1996) TGF-beta-induced apoptosis of cerebellar granule neurons is prevented by depolarization. J Neurosci 16:4174–4185

Lucas PJ, Kim SJ, Melby SJ, Gress RE (2000) Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor. J Exp Med 191:1187–1196

Martinez-Alvarez C, Tudela C, Perez-Miguelsanz J, O'Kane S, Puerta J, Ferguson MW (2000) Medial edge epithelial cell fate during palatal fusion. Dev Biol 220:343–357

Marushige K, Marushige Y (1994) Induction of apoptosis by transforming growth factor beta 1 in glioma and trigeminal neurinoma cells. Anticancer Res 14:2419–2424

Massague J (2000) How cells read TGF-beta signals. Nat Rev Mol Cell Biol 1:169–178

Mazars A, Lallemand F, Prunier C, Marais J, Ferrand N, Pessah M, Cherqui G, Atfi A (2001) Evidence for a role of the jnk cascade in smad7-mediated apoptosis. J Biol Chem 276: 36797–36803

McGinnis KM, Gnegy ME, Park YH, Mukerjee N, Wang KK (1999) Procaspase-3 and poly(ADP)ribose polymerase (PARP) are calpain substrates. Biochem Biophys Res Commun 263:94–99

Moses HL, Branum EL, Proper JA, Robinson RA (1981) Transforming growth factor production by chemically transformed cells. Cancer Res 41:2842–2848

Motyl T, Grzelkowska K, Zimowska W, Skierski J, Wareski P, Ploszaj T, Trzeciak L (1998) Expression of bcl-2 and bax in TGF-beta 1-induced apoptosis of L1210 leukemic cells. Eur J Cell Biol 75:367–374

Moulton BC (1994) Transforming growth factor-beta stimulates endometrial stromal apoptosis in vitro. Endocrinology 134: 1055–1060

Mummery CL (2001) Transforming growth factor beta and mouse development. Microsc Res Tech 52:374–386

Nass SJ, Li M, Amundadottir LT, Furth PA, Dickson RB (1996) Role for Bcl-xL in the regulation of apoptosis by EGF and TGF beta 1 in c-myc overexpressing mammary epithelial cells. Biochem Biophys Res Commun 227:248–256

Oppenheim RW (1991) Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501

Patil S, Wildey GM, Brown TL, Choy L, Derynck R, Howe PH (2000) Smad7 is induced by CD40 and protects WEHI 231 B-lymphocytes from TGFbeta-induced growth inhibition and apoptosis. J Biol Chem 275:38363–38370

Perlman R, Schiemann WP, Brooks, MW, Lodish HF, Weinberg RA (2001) TGF-β-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation. Nat Cell Biol 3:708–714

Perry RR, Kang Y, Greaves BR (1995) Relationship between tamoxifen-induced transforming growth factor beta 1 expression, cytostasis and apoptosis in human breast cancer cells. Br J Cancer 72:1441–1446

Pettmann B, Henderson CE (1998) Neuronal cell death. Neuron 20:633–647

Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MW, Doetschman T (1995) Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet 11:409–414

Rajah R, Valentinis B, Cohen P (1997) Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism. J Biol Chem 272:12181–12188

Ribeiro A, Bronk SF, Roberts PJ, Urrutia R, Gores GJ (1999) The transforming growth factor beta(1)-inducible transcription factor TIEG1 mediates apoptosis through oxidative stress. Hepatology 30:1490–1497

Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB (1981) New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. Proc Natl Acad Sci USA 78:5339–5343

Roberts RA, James NH, Cosulich SC (2000) The role of protein kinase B and mitogen-activated protein kinase in epidermal growth factor and tumor necrosis factor alpha-mediated rat hepatocyte survival and apoptosis. Hepatology 31:420–427

Rosfjord EC, Dickson RB (1999) Growth factors, apoptosis, and survival of mammary epithelial cells. J Mammary Gland Biol Neoplasia 4:229–237

Saltis J (1996) TGF-beta: receptors and cell cycle arrest. Mol Cell Endocrinol 116:227–232

Saltzman A, Munro R, Searfoss G, Franks C, Jaye M, Ivashchenko Y (1998) Transforming growth factor-beta-mediated apoptosis in the Ramos B-lymphoma cell line is accompanied by caspase activation and Bcl-XL downregulation. Exp Cell Res 242:244–254

Sanchez A, Alvarez AM, Benito M, Fabregat I (1995) Transforming growth factor beta modulates growth and differentiation of fetal hepatocytes in primary culture. J Cell Physiol 165:398–405

Sanchez A, Alvarez AM, Benito M, Fabregat I (1997) Cycloheximide prevents apoptosis, reactive oxygen species production, and glutathione depletion induced by transforming growth factor beta in fetal rat hepatocytes in primary culture. Hepatology 26:935–943

Sanchez A, Pagan R, Alvarez AM, Roncero C, Vilaro S, Benito M, Fabregat I (1998) Transforming growth factor-beta (TGF-beta) and EGF promote cord-like structures that indicate terminal differentiation of fetal hepatocytes in primary culture. Exp Cell Res 242:27–37

Sanchez A, Alvarez AM, Lopez Pedrosa JM, Roncero C, Benito M, Fabregat I (1999) Apoptotic response to TGF-beta in fetal hepatocytes depends upon their state of differentiation. Exp Cell Res 252:281–291

Schulz A, Bauer G (2000) Synergistic action between tumor necrosis factor-alpha and transforming growth factor type-beta: consequences for natural antitumor mechanisms. Anticancer Res 20:3443–3448

Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, Ueno N, Irie K, Nishida E, Matsumoto K (1996) TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. Science 272:1179–1182

Shima Y, Nakao K, Nakashima T, Kawakami A, Nakata K, Hamasaki K, Kato Y, Eguchi K, Ishii N (1999) Activation of caspase-8 in transforming growth factor-beta-induced apoptosis of human hepatoma cells. Hepatology 30:1215–1222

Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D (1992) Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 359:693–699

Skoff AM, Lisak RP, Bealmear B, Benjamins JA (1998) TNF-alpha and TGF-beta act synergistically to kill Schwann cells. J Neurosci Res 53:747–756

Subramaniam M, Harris SA, Oursler MJ, Rasmussen K, Riggs BL, Spelsberg TC (1995) Identification of a novel TGF-beta-regulated gene encoding a putative zinc finger protein in human osteoblasts. Nucleic Acids Res 23:4907–4912

Tachibana I, Imoto M, Adjei PN, Gores GJ, Subramaniam M, Spelsberg TC, Urrutia R (1997) Overexpression of the TGFbeta-regulated zinc finger encoding gene, TIEG, induces apoptosis in pancreatic epithelial cells. J Clin Invest 99:2365–2374

Taketo MM, Takaku K (2000) Gastro-intestinal tumorigenesis in Smad4 mutant mice. Cytokine Growth Factor Rev 11:147–157

Tanaka S, Wands JR (1996) Insulin receptor substrate 1 overexpression in human hepatocellular carcinoma cells prevents transforming growth factor beta1-induced apoptosis. Cancer Res 56:3391–3394

Teramoto T, Kiss A, Thorgeirsson SS (1998) Induction of p53 and Bax during TGF-beta 1 initiated apoptosis in rat liver epithelial cells. Biochem Biophys Res Commun 251:56–60

Tobin SW, Brown MK, Douville K, Payne DC, Eastman A, Arrick BA (2001) Inhibition of transforming growth factor beta signaling in MCF-7 cells results in resistance to tumor necrosis factor alpha: a role for Bcl-2. Cell Growth Differ 12:109–117

Unsicker K, Krieglstein K (2000) Co-activation of TGF-ss and cytokine signaling pathways are required for neurotrophic functions. Cytokine Growth Factor Rev 11:97–102

Wahl SM, Hunt DA, Wong HL, Dougherty S, McCartney-Francis N, Wahl LM, Ellingsworth L, Schmidt JA, Hall G, Roberts AB (1988) Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. J Immunol 140:3026–3032

Wang H, Grand RJ, Milner AE, Armitage RJ, Gordon J, Gregory CD (1996) Repression of apoptosis in human B-lymphoma cells by CD40-ligand and Bcl-2: relationship to the cell-cycle and role of the retinoblastoma protein. Oncogene 13:373-379

Weller M, Constam DB, Malipiero U, Fontana A (1994) Transforming growth factor-beta 2 induces apoptosis of murine T cell clones without down-regulating bcl-2 mRNA expression. Eur J Immunol 24:1293–1300

Yamamoto M, Maehara Y, Sakaguchi Y, Kusumoto T, Ichiyoshi Y, Sugimachi K (1996) Transforming growth factor-beta 1 induces apoptosis in gastric cancer cells through a p53-independent pathway. Cancer 77:1628–1633

Yamamura Y, Hua X, Bergelson S, Lodish HF (2000) Critical role of SMADS and AP-1 complex in transforming growth factor-beta-dependent apoptosis. J Biol Chem 275:36295–36302

Yanagisawa K, Osada H, Masuda A, Kondo M, Saito T, Yatabe Y, Takagi K, Takahashi T, Takahashi T (1998) Induction of apoptosis by SMAD3 and down-regulation of SMAD3 expression in response to TGF-beta in human normal lung epithelial cells. Oncogene 17:1743–1747

Yu C, Takeda M, Soliven B (2000) Regulation of cell cycle proteins by TNF-alpha and TGF-beta in cells of oligodendroglial lineage. J Neuroimmunol 108:2–10