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JB Commentary

Implication of TGF-β as a survival factor during tumour development

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Transforming growth factor (TGF)-β is a pleiotropic secretory protein which inhibits and potentiates tumour progression during early and late stage of tumourigenicity, respectively. However, it still remains veiled how TGF-β signalling reveals its two faces. Hoshino *et al.* (Autocrine TGF-β protects breast cancer cells from apoptosis through reduction of BH3-only protein, Bim, *J. Biochem.* 2011;149:55–65) demonstrated a new aspect of TGF-β as a survival factor in highly metastatic breast cancer cells from which TGF-β1 and TGF-β3 are abundantly expressed. They found that TGF-β suppressed the expression of BH3-only protein Bim which promotes programmed death signalling via release of cytochrome c from mitochondria. Further interestingly, forkhead box C1 (Foxc1) whose expression is suppressed upon TGF-β stimulation is involved in the expression of Bim. Based on their results, autocrine TGF-β signalling in certain breast cancers promotes cell survival via inhibition of apoptotic signalling. Thus, the inhibitors for activin receptor-like kinase (ALK)5 kinase might exert a curative influence on certain types of metastatic breast cancers.

Keywords: Bim/Foxc1/metastatic breast cancer.

Abbreviations: Akt, v-akt murine thymoma viral oncogene homolog; Arts, apoptosis-related protein in the TGF-β signaling pathway; Bcl-XL, B-cell leukemia/lymphoma x; Bim, bcl-2 homology domain 3-only protein; DAPK, death-associated protein kinase; Daxx, death-domain associated protein; DEC1, deleted in esophageal cancer 1; Foxc1, forkhead box C1; Id3, inhibitor of differentiation 3; iTreg, inducible regulatory T cell; JNK, c-Jun N-terminal kinase; MKP2, MAP kinase phosphatase 2; mTOR, mechanistic target of rapamycin; Par6, partitioning defective 6 homolog; PDGF-B, platelet derived growth factor B; PI3K, phosphoinositide 3-kinase; S6K, ribosomal protein S6 kinase; SHIP, SH2 containing inositol phosphatase; Smad, Sma- and Mad-related protein; TAK1, TGF-β activated kinase 1; TGF-β, transforming growth factor-β; Th, T helper; TIEG1, TGF-β-inducible early growth response protein 1; TRAF6, TNF receptor-associated factor 6; TSC22, TGF-β-stimulated clone 22; XIAP, X-linked inhibitor of apoptosis.

Transforming growth factor-β (TGF-β) belongs to a family of cytokine that regulates cell proliferation and differentiation of many different cell types. TGF-β is found to possess critical roles during embryogenesis and in maintaining tissue homeostasis during adult life. Besides, TGF-β is negatively and positively implicated in inflammatory responses via inactivation of Th1 and Th2 cells, and activation of Th17 and iTreg cells. Therefore, deregulated TGF-β signalling has been involved in cancer, fibrosis and auto-immune diseases (*1–5*).

After TGF-β binds to specific cell surface receptors TGF-β type I (activin receptor-like kinase; ALK5) and TGF-β type II receptors (TβRII), canonical Smad pathways are mainly activated to transduce its signal to nucleus, where the target genes for TGF-β/Smad signal are transcriptionally regulated together with other transcription factors, co-activators and/or co-repressors. On the other hand, non-Smad pathways via ras/Erk, TRAF6/TAK1/JNK or p38, PI3K/Akt/mTOR/S6K and Par6 upon TGF-β stimulation are also evident recently (*6–11*). During tumour progression, TGF-β has dual roles in tumourigenicity. TGF-β-mediated G1 arrest in the cell cycle is implicated in the anti-oncogenic functions of TGF-β in normal and premalignant tissues, whereas TGF-β secreted from tumours or stroma cells around tumours potentiates invasion and metastasis of various tumour cells (*8*, *12–16*).

Besides, TGF-β can control tumour development through influence on apoptotic pathways. In particular, TGF-β-induced apoptotic programmes have been much elucidated in spite that TGF-β-induced apoptosis can be observed in context-dependent or cell-type specific manner (*8*, *13*). Indeed, apoptotic pathways can be transmitted in either Smad-dependent or Smad–independent fashion (Fig. 1). For example, death-associating protein kinase (DAPK) and SH2-domain containing inositol-5-phosphatase (SHIP) are respectively induced in Hep3B and MPC-11 cells through TGF-β-mediated Smad-dependent pathway to activate programmed cell death signal in these cells (*17*, *18*). On the other hand, Daxx, Arts and XIAP/TAK1/p38 or JNK pathways have been implicated in Smad-independent apoptosis upon TGF-β stimulation (*19*). Interestingly, either TRAF6-mediated or Smad7-mediated TAK1 activation is required for TGF-β-induced apoptosis in the prostate cancer although its detail mechanism must be

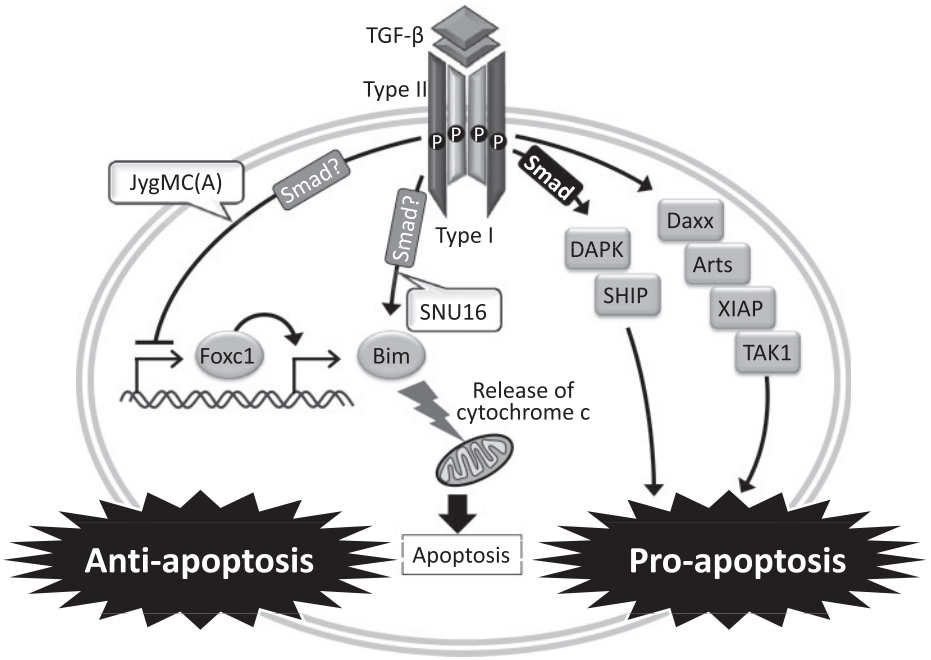

Fig. 1 TGF-β positively and negatively regulates apoptotic reaction in context- or cell type-dependent manner. Hoshino *et al.* (*26*) demonstrated that TGF-β inhibits apoptosis via inhibition of Bim expression in JygMC(A) cells, whereas Ohgushi *et al.* (*23*) found that TGF-β accelerates apoptotic reaction via the induction of Bim in SNU16 cells. It remains veiled whether Bim expression in JygMC(A) and SNU16 cells is regulated via TGF-β/Smad pathway. Besides, TGF-β activates DAPK, SHIP, Daxx, Arts, XIAP and TAK1 to potentiate apoptosis.

elucidated further (*20*–*22*). Id3, TIEG1 (TGF-β-inducible early growth response protein 1) and TGF-β-stimulated clone 22 (TSC-22) have been reported to contribute to TGF-β-mediated apoptosis although it remains unclear to show if Smad pathway is needed or not (*19*). MAP kinase phosphatase (MKP)2 also potentiates programmed cell death signal via TGF-β/Smad pathway (*8*). In human gastric epithelial cell line SNU16, TGF-β induces Bim, a proapoptotic Bcl-2 homology domain 3 (BH3)-only protein, to potentiate apoptosis via its binding to an anti-apoptotic protein Bcl-X_L (*23*). TGF-β signal ultimately results in activation of caspases by activation and inhibition of pro- and anti-apoptotic members of the Bcl-2 family, respectively (*8*, *19*).

On the contrary, the mechanism(s) for TGF-β-mediated cell survival has not been elucidated fully although certain cancer cells are known to be able to proliferate in the presence of TGF-β. The glioblastoma cell line U373MG which is highly responsive to TGF-β can grow via TGF-β-mediated PDGF-B secretion because PDGF-B acts as a growth factor for glioblastoma (*24*). Azuma *et al.* found that introduction of Smad7, which is an endogenous inhibitor of TGF-β family signalling, perturbs migration and invasion of highly metastatic breast cancer cell line JygMC(A). However, they did not elucidate how Smad7 blocks proliferation, invasion and metastasis of JygMC(A) cells either *in vitro* or *in vivo* experiment (*25*). To that end, Hoshino *et al.* tried to address the molecular mechanism(s) by which Smad7 interferes with tumour progression of highly metastatic breast cancer cell lines. They showed that an ALK5 kinase inhibitor SB431542 promotes apoptosis of breast cancer cell lines in addition that exogenous TGF-β protects their programmed cell death. Indeed, the high level of TGF-β1 and TGF-β3 mRNAs could be detected in these cell lines. Therefore, they proposed that autocrine TGF-β signal helps these cell lines to proliferate. To further understand how TGF-β acts as a survival signal in these cell lines, they performed the DNA micro array analysis to focus on Bim whose expression is inhibited and enhanced in JygMC(A) cells by exogenous TGF-β and SB431542, respectively. Consistent with the expression of Bim by TGF-β signal, the reduced expression of Bim rescued SB431542-induced cell death in JygMC(A) cells. However, the suppression of Bim expression was not directly regulated upon TGF-β signal pathway. Therefore, they further analysed how Bim expression is regulated in JygMC(A) cells upon TGF-β stimulation. The transcription factor forkhead box C1 (Foxc1) was then found to be up-regulated and down-regulated by SB431542 and exogenous TGF-β, respectively. Indeed, the knockdown of Foxc1 in JygMC(A) cells perturbed SB431542-induced apoptosis. In their current model for the survival of certain breast cancer cells, autocrine TGF-β suppresses the expression of Foxc1, followed by the reduction of Bim expression to protect programmed cell death in certain breast cancers (*26*). Recently, their idea was supported by Du *et al.* (27) who proposed that Foxc1 inhibits metastasis in breast cancer cell line MDA-MB231. However, contradictory evidences have also been reported that Foxc1 promotes tumour progression and becomes a potential prognostic biomarker for poor survival in a basal-like breast cancer (BLBC) (*28*, c*29*). Furthermore, the introduction of Foxc1 in Hela cells lacking Foxc1 alleles restored TGF-β-mediated growth inhibition in addition that Foxc1 was induced in several tumour cell lines by TGF-β (*30*) in the

opposite way to Hoshino *et al*. (*26*). In addition, TGF-β induces Bim to potentiate apoptosis via its binding to an anti-apoptotic protein Bcl-X_L in a human gastric epithelial cell line SNU16 (*23*).These contradictory evidences might be explained by context-dependent or cell type-dependent effects of TGF-β.

An anti-apoptotic factor differentially expressed in chondrocytes 1 (DEC1) whose expression is induced in JygMC(A) cells by TGF-β promotes cell survival and metastasis of breast cancer cells as well (*31*). Thus, increase of DEC1 expression and decrease of Foxc1expression by TGF-β might be critical for JygMC(A) cells to survive and metastasize. Thus, SB431542 or other related small compounds to block TGF-β signalling might be useful tools for anti-cancer chemotherapy for certain breast cancers.

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Conflict of interest
None declared.

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