

Transforming Growth Factor- β

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Contributor: Vivian Weiwen Xue

Transforming growth factor- β (TGF- β) was originally identified as an anti-tumour cytokine. However, there is increasing evidence that it has important roles in the tumour microenvironment (TME) in facilitating cancer progression. TGF- β actively shapes the TME via modulating the host immunity. These actions are highly cell-type specific and complicated, involving both canonical and non-canonical pathways. In this review, we systemically update how TGF- β signalling acts as a checkpoint regulator for cancer immunomodulation. A better appreciation of the underlying pathogenic mechanisms at the molecular level can lead to the discovery of novel and more effective therapeutic strategies for cancer.

Keywords: TGF- β ; cancer immunity ; tumour microenvironment

1. Introduction

Transforming growth factor-beta (TGF- β) is a secretory cytokine that has pleiotropic roles in cancer progression through controlling cell proliferation, differentiation, apoptosis, and migration. The TGF- β family of cytokines consists of three different isoforms, TGF- β 1, TGF- β 2, and TGF- β 3, with each of them having a unique expression mode and executing distinct functions. For example, TGF- β 2 can deplete interleukin 6 (IL-6) function and induce apoptosis ^[1], TGF- β 3 affects the differentiation of mesenchymal stromal cells (MSCs) ^[2], while TGF- β 1 plays an important role in cancer progression and tumour microenvironment (TME) development ^{[3][4]}.

TGF- β executes its functions through canonical and non-canonical pathways (Figure 1). In the canonical TGF- β /Smad pathway, TGF- β ligands bind to a heterotetrameric TGF- β receptor complex, composed of dimers of type I (TGFR1) and type II (TGFR2) TGF- β receptors. The ligated TGF- β and receptor complexes then become phosphorylated and activated before recruiting and phosphorylating Smads, including Smad2 and Smad3. Smad2/3 phosphorylation induces the subsequent recruitment of Smad4 to the Smad2/3 complex which then translocates to the nucleus. Smad3 is the transcription factor (TF) and the component of the complex that directly binds to DNA, and the Smad complexes then cooperate with other cofactors to regulate multiple downstream mechanisms through directing transcriptional activation and suppression ^[5]. For example, TGF- β mediates the transcriptional regulation of effector genes including the cyclin-dependent kinase (CDK) inhibitors p15^{INK4} ^[6] and p21^{Cip1} ^[7] to modulate cell cycle arrest. Besides, the TGF- β /Smad axis also promotes the stemness and epithelial-mesenchymal transition (EMT) of cancer cells by restoring mesenchymal phenotypes and upregulating the expression of genes, such as *Snail* and *Vim* ^{[8][9]}. Meanwhile, the TGF- β /Smad pathway also has a negative feedback mechanism mediated through Smad7 competitive binding to TGFR1 and blocking the TGF- β /Smad pathway signalling ^[10]. For the non-canonical TGF- β pathway, the activated TGF- β crosstalks with other signalling pathways, such as Rho, phosphoinositide 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) signalling cascades, to promote EMT ^[11], cancer invasion ^[12], and angiogenesis ^[13]. In consequence, both the canonical and non-canonical TGF- β pathways play an important role in cancer progression ^[14].

Figure 1. Transforming growth factor- β (TGF- β) signalling pathways in tumorigenesis. The dual roles of TGF- β signalling pathways have been demonstrated in tumorigenesis. TGF- β is a tumour suppressor in TME development of early-stage cancer and a tumour promoter in malignancy processes of advanced-stage cancer. Schematic diagramme (above) showing TGF- β signalling and its role in cancer tumorigenesis and progression as well as tumour suppression. TGF- β binds to TGFR2 which then complexes with TGFR1 to activate downstream signalling. TGF- β can activate both Smad-dependent canonical and Smad-independent non-canonical signalling cascades. The TGF- β activated TGFR1 phosphorylates the Smad2/3 complex which then associates Smad4, before translocating to the nucleus to regulate the transcription of different targeted genes involved in tumour suppression during tumorigenesis (e.g., p15^{INK4b} and p21^{Cip1}), as well as tumour progression (e.g., *Snail* and *Vimentin*). The Smad-dependent TGF- β canonical signalling pathway can also be antagonised by Smad7 through inhibiting the binding of the Smad2/3 complex with Smad4. In the Smad-independent non-canonical signalling cascades, the activated TGFR1/2 receptors induce downstream signalling through the Ras/Raf/MAPKs (JNK/p38/ERK), PI3K/AKT, MAPK37(TAK1)/NF- κ B, and Rho family of small GTPase-dependent

signalling pathways. The TGF- β activated JNK/p38/ERK-MAPKs also crosstalk with Smad2/3/4 to modulate downstream signalling to influence cancer development. The Rho family of small GTPase-dependent signalling pathways (e.g., Rho/ROCK) are involved in epithelial–mesenchymal transition (EMT). The activated PI3K/AKT pathway induces mTOR to promote cell proliferation and EMT. The TGF- β signals also activate NF- κ B signalling to modulate inflammatory response.

Accumulating evidence has indicated that TGF- β has a dual function in cancer progression, with the different TGF- β signalling pathways switching between the two phenotypes of tumour suppression and tumour promotion [15]. More importantly, the role of TGF- β in the immunosuppressive TME and immune cells has attracted increasing attention. In this review, we will evaluate the complex role of TGF- β in cancer progression, focusing primarily on its function in the regulation of immune responses and TME development.

2. TGF- β in Cancer Initiation and Progression

2.1. Role of TGF- β as Tumour Suppressor

TGF- β plays multiple roles in the early stages of carcinogenesis, one of which is to modulate cell proliferation arrest. For example, TGF- β can induce cell cycle arrest via the cAMP response element-binding protein (CREB) to mediate histone acetylation and transcriptional activation of plasminogen activator inhibitor type-1 in a p53/Smad-dependent manner to maintain the anti-proliferative effects to suppress cancer tumorigenesis [16]. Besides, TGF- β can also activate the forkhead box O1 (FOXO1) transcription factor to induce the expression of the cyclin-dependent kinase inhibitor p21^{Cip1} at the transcriptional level to enforce a G0/G1 phase cell cycle arrest. This is mediated via the TGF- β /FOXO1/p21^{Cip1} axis to keep cells in a quiescent or senescent state [17]. TGF- β can also promote normal cell differentiation as an indirect tumour suppressive antiproliferative mechanism by promoting the activity of the stress-activated p38MAPK signalling pathway [18]. In agreement, haematopoietic stem and progenitor cells (HSPCs) expressing high levels of active TGF- β 1 protein and p38MAPK activity lose their haematopoietic stem cell (HSC) self-renewal and multi-lineage capacity and differentiate into progenitor cells [18].

Mechanistically, the role of TGF- β as a tumour suppressor during early tumorigenesis is mediated primarily through its function to cause cell cycle and proliferative arrest, to induce differentiation and apoptosis, and to block paracrine factor production [17]. The anti-proliferative tumour suppressive function of TGF- β is mediated primarily through the induction of CDK inhibitor expression and the suppression of c-Myc expression, at the transcriptional level, to arrest cell cycle progression at the G1 and S phases of the cell cycle. For example, in normal epithelial cells, TGF- β induces the expression of the cyclin-dependent kinase inhibitor (CKI) p15^{INK4b}, which competes with cyclin Ds for the formation of cyclin D complexes with CDK4/6, and of p21^{Cip1}, which inhibits the activity of the cyclin E/A-CDK2 complexes directly. Specifically, the Smad3/4 complexes associate and cooperate with FOXO transcription factors to transcriptionally activate the promoters of the *CDKN2B* gene, which encodes p15^{INK4b}, and of *CDKN1A*, which encodes p21^{Cip1}. The induced CKI p15^{INK4b} can also displace p27^{Kip1} from the cyclin D-CDK4/6 complexes, releasing the Cip/Kip CKI (e.g., p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}) to inhibit the cyclin E/A-CDK2 complexes to restrict cell cycle progression through late G1 and S phases of the cell cycle. Indeed, TGF- β has also been shown to stimulate the expression of CKIs, such as p15^{INK4b}, p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}, in a cell type- and context-dependent manner. For example, TGF- β induces the expression of p21^{Cip1} in T cells, p57^{Kip2} in haematopoietic stem/progenitor cells, and p15^{INK4b} and p21^{Cip1} in astrocytes, neural progenitor cells, and epithelial cells to cause cell cycle and proliferation arrest. The TGF- β -induced Smad3/4-containing protein complexes also repress the expression of transcription of c-Myc, a potent oncogene which plays a pivotal role in cell cycle entry and proliferation. Besides cell cycle progression and proliferation, TGF- β also limits proliferation indirectly through promoting differentiation. For instance, TGF- β signalling also represses the expression of Id proteins (inhibitor of differentiation/DNA binding), which inhibit some key differentiation pathways. In addition, TGF- β signalling can also trigger apoptosis, programmed cell death to restrict cell proliferation. Consistently, TGF- β can promote apoptosis through inducing the expression of modulators of programmed cell death, including the death-associated protein kinase (DAPK), the growth arrest and DNA damage 45 β factor (GADD45 β), the death receptor FAS, etc., and often in a cell type-dependent manner. Lastly, TGF- β can also restrict epithelial cell proliferation and tumorigenesis by inhibiting the production of mitotic growth factors, such as hepatocyte growth factor (HGF), by the fibroblasts and immune cells in the local TME. Together, these findings suggest that TGF- β targets different gene targets via discrete signalling pathways in distinct cell types and settings to function as a pleiotropic suppressor of cancer onset [17].

2.2. Role of TGF- β as Tumour Progression Promoter

Conversely, TGF- β can also function as an oncogene and promote cancer cell progression by activating the PI3K/AKT/mTOR pathway [19]. Appropriately, TGF- β is associated with poor prognosis and cancer progression in osteosarcoma [19]. Alternatively, TGF- β 1 has also been shown to activate Golgi membrane protein 1 (GOLM1; also called

GP73) through lipid rafts to suppress Smad-dependent tumour-suppressive signals and, at the same time, induce the extracellular signal-regulated kinase (ERK) MAPK signalling pathway to promote tumorigenesis and progression in liver cancer [20]. In addition, TGF- β also regulates tissue infiltration and migration by enhancing bFGF signalling through the FGFR/FRS2/ERK axis in paediatric medulloblastoma [21]. The importance of MAPK in mediating the oncogenic function of TGF- β is exemplified by the fact that the Smad4 phosphatase Wip1 can restrain the TGF- β -induced cell growth arrest, migration, and invasion and enhances the tumorigenicity of cancer cells by repressing Smad4 activity via antagonising MAPK function. Mechanistically, Wip1 selectively dephosphorylates Smad4 at a specific MAPK phosphorylation site to inhibit its nuclear accumulation and stability [22].

Increasing evidence has indicated that TGF- β can aggravate EMT, a key process involved in metastasis during cancer progression. For example, the TGF- β /Smad pathways can promote EMT and contribute to lung cancer progression by driving the expression of the DNA binding protein family member ID1 and EMT-related transcriptional factor Snail. Besides that, TGF- β can also induce EMT via the PI3K/AKT axis in a tuberous sclerosis protein complex-dependent manner [23]. Furthermore, TGF- β also promotes EMT and enhances cancer cell invasion and migration via activating Rho and Rho-associated protein kinases (ROCKs), whereas the malignant phenotypes induced by TGF- β via the Rho/ROCK signalling pathway can be blocked by miR-335-5p and farnesyl pyrophosphate synthase inhibitors in non-small cell lung cancer (NSCLC) [24][25]. The activation of MAPKs and downstream genes is another crucial mechanism by which TGF- β mediates cellular responses. TGF- β induces Ras and recruits Raf to the plasma membrane. The TGF- β -activated Ras and Raf then transmit signals via MAP kinase kinases (MKKs) to MAPKs to promote EMT. Cho et al. have found that the activated TGF- β signals induce the degradation of downstream Raf kinase inhibitory protein and promote the activation of the MAPK signalling pathway, which in turn results in the transcriptional suppression of p53 to facilitate EMT [26]. Moreover, the TGF- β pathway can also crosstalk with NF- κ B to increase the expression of Twist to enhance EMT, and this cooperation between TGF- β and NF- κ B pathways is again mediated by MAP kinase kinase kinase 7 (MAP3K7), an upstream MAPK family member [27][28]. Recently, CCAAT enhancer-binding protein alpha (C/EBP α) has been identified as a “gatekeeper” gene in TGF- β -induced EMT. More specifically, the TGF- β -induced downregulation of C/EBP α allows epithelial cells to undergo malignant transformation of breast epithelial cells, while abundant C/EBP α expression can effectively prevent breast cancer tumourigenesis [29].

Nevertheless, there is also evidence that TGF- β can also suppress tumour progression by generating a lethal EMT. Specifically, David et al. have found that TGF- β induces the expression of SRY-box transcription factor 4 (SOX4) to promote pro-apoptotic events via Smad4-mediated repression of kruppel-like factor 5 (KLF5). This in turn induces the lethal effects and phenotype transformation of EMT in TGF- β -sensitive pancreatic ductal adenocarcinoma (PDA) cells [30]. In agreement, the pro-apoptotic regulator WT1 is another gene that has been reported to be induced during TGF- β /Smad4-derived lethal EMT [31].

2.3. Role of TGF- β in Normal and Cancerous Cells

Overall, these findings suggest that TGF- β plays distinct and sometimes contrasting roles during cancer initiation and development. In general, in non-cancerous cells, TGF- β normally functions as a tumour suppressor to limit cell proliferation and promote apoptosis in order to exert growth inhibition during early stages of carcinogenesis. During later stages of cancer development, TGF- β often switches to a cancer promoting role, which is essential for tumour progression and metastasis. In concordance with this conjecture, one transcription factor that plays a key role during this TGF- β functional switch is distal-less homeobox 2 (Dlx2) [32]. In normal mammary epithelial cells, Dlx2 neutralises the TGF β -induced cell cycle arrest and apoptosis by multiple mechanisms. Essentially, Dlx2 functions as a transcriptional repressor of TGFBR2 gene expression, restricting the downstream canonical Smad-dependent TGF- β signalling and expression of the cell cycle inhibitors, such as p21^{Cip1}, and enhancing the expression of the potent mitogenic transcription factor c-Myc. Conversely, Dlx2 can also directly drive the expression of the epidermal growth factor (EGF) family member betacellulin to promote cell survival by stimulating EGF receptor (EGFR) signalling. Moreover, Dlx2 also further supports tumour growth and metastasis. These results establish Dlx2 as a critical switch in shifting TGF- β from its tumour-suppressive functions in early tumorigenesis to its tumour-promoting functions during cancer progression [32].

2.4. Regulation and Role of TGF- β in the Tumour Microenvironment

The TME provides the structures and materials for tumour maintenance and progression, and comprises the cellular and extracellular materials surrounding the tumour mass. Essentially, TME serves as a platform for cancer cells to reprogramme infiltrating stromal cells, thereby promoting tumorigenesis, as well as cancer invasion, metastasis, and resistance to therapy. TGF- β is secreted in a latent form as a large latent complex (LLC) which is primarily embedded in extracellular matrix (ECM) and is partially anchored on the cell surface [33][34]. It will remain as an inactivated complex until it is processed further to release the active TGF- β . Within the TME, a myriad of cell types can produce TGF- β , and the

generation and processing of this important cytokine is mediated through diverse and complex mechanisms. For example, TGF- β produced by epithelial cells is induced via matrix metalloprotease (MMP) stimulation and EGFR activation [35]. In addition, TGF- β secretion in macrophages is regulated by the Notch signalling pathways [36]. Moreover, signal transducer and activator of transcription 6 (STAT6) and Furin can also cooperate to induce the production of TGF- β by T lymphocytes [37]. Furthermore, inactive forms of TGF- β can be stimulated and released by integrin-mediated activation, protease cleavage, and latency-associated peptide (LAP) dissociation upon X-ray irradiation [38][39][40]. It is also worth noting that a recent study has suggested a novel integrin-activated form of TGF- β that does not require release from the latent TGF- β complex [41]. Together, these observations illustrate the complexity and diversity of the mechanisms by which TGF- β are generated and activated. Moreover, diverse cell types are involved in modulating the effects of TGF- β on the development and the reprogramming of the TME, as well as during cancer progression. For example, migratory dendritic cells (DCs) can activate TGF- β via an integrin-dependent manner and promote naïve CD8 $^{+}$ T cell differentiation into epithelial resident memory T cells (eTRM) [42]. Notably, the interactions between non-immune cells and immune cells are also essential for TGF- β activation and function. It has been found that keratinocytes help to preserve epithelial-resident DCs and eTRM by inducing integrin expression and thereby TGF- β activation [43]. Besides that, tumour-initiating cells also release IL-33 to induce macrophage differentiation, which ultimately promotes TGF- β secretion and signalling to cancer stem cells (CSCs) to create a CSC niche for the maintenance of a stem cell pool within the TME [44]. In concordance, Takasaka et al. have also observed that integrin $\alpha\beta 8$ is highly expressed on the cancer cell surface and that the integrin $\alpha\beta 8$ -expressing tumour cells can evade host immunity by regulating TGF- β activation in immune cells [45]. On the contrary, integrin $\beta 1$ -mediated TGF- β activation may also drive tumor suppression. In a human melanoma xenograft model, cell surface integrin $\beta 1$ -activation can increase TGF- β activity, which culminates in stromal activation and angiogenesis but also an accumulation of intra-tumoral CD8 $^{+}$ T cell infiltration within the TME. The latter recruitment of CD8 $^{+}$ T lymphocytes can result in an attenuation of tumour growth and long-term survival, suggesting a role of TGF- β in immune surveillance against tumours [46].

Besides targeting cancer cells, TGF- β also exerts its effect on stromal cells of the TME through controlling their differentiation, angiogenesis, and metabolic reprogramming during tumourigenesis and cancer progression. For example, amphiregulin expressed by macrophages can induce TGF- β activation to mediate pericyte differentiation into myofibroblasts to direct tissue restoration during inflammation [47]. In addition, TGF- β -licensed mesenchymal stromal cells (TGF- β MSCs) can contribute to immunosuppression by altering the phenotypes of macrophages and by promoting regulatory T cell (Treg) expansion [48]. Previous studies have also showed a strong association between stromal TGF- β levels and poor prognosis in hepatocellular carcinoma (HCC), as well as colorectal cancer (CRC), suggesting an important role for stromal TGF- β in promoting an immunosuppressive TME [49][50]. Moreover, TGF- β is able to stimulate myofibroblasts and other stromal cells to boost the synthesis of collagen crosslinked enzymes, especially lysyl oxidases (LOs) and matrix metalloproteinases (MMPs), to improve collagen crosslinking during the early stages of carcinogenesis [51]. Collagen crosslinking mediated by the lysyl oxidase family of enzymes (LOX, LOXL1-4) contributes to the pathogenesis of idiopathic pulmonary fibrosis (IPF), a progressive scarring lung disease which predisposes patients to lung cancer, mostly NSCLC. A previous study has also shown that the novel lysyl hydroxylase 3 receptor recruits MMP9 to the surface of fibroblasts and activated TGF- β and actin alpha 2 (α -SMA) functions during fibroblast differentiation [52]. At the same time, TGF- β can also downregulate the synthesis and expression of MMP family members, such as MMP2, MMP7, and MMP8, to facilitate the regulation of the ECM [53]. In addition, increased matrix protein synthesis and reduced matrix proteinase activity due to increased TGF- β activity can also contribute to the tumour ECM remodelling and result in desmoplasia, which is commonly found in many types of tumours, particularly pancreatic and renal cell carcinomas, as well as sarcomas [54]. In agreement, bone marrow-derived mesenchymal stem cells (BM-MSCs) have been shown to inhibit the TGF- β /SMAD pathway to repress hypoxia-inducible factor 1 subunit alpha (HIF-1 α) and VEGF protein expression in the ECM to restrict liver fibrosis and early HCC tumourigenesis [55]. Similarly, pharmaceutical inhibition of the TGF- β signalling can promote the epithelial differentiation of human adipose-derived mesenchymal stem cells (ADSCs) during EMT through the downregulation of mesenchymal genes (e.g., Slug, zinc finger E-box binding homeobox 1 (ZEB1), integrin $\alpha 5$ (ITGA5), and vimentin (VIM)) and upregulation of epithelial genes (e.g., E-cadherin, epithelial cell adhesion molecule (EpCAM), zonula occludens-1 (ZO-1), occludin, deltaN p63 (δ Np63), transcription factor 4 (TCF4), and Twist family bHLH transcription factor (TWIST)) to reverse EMT [56]. Moreover, cancer-associated fibroblasts (CAFs) are an abundant and active population of stromal cells and play a major role in conditioning the TME. TGF- $\beta 1$ can drive the conversion of normal fibroblasts to CAFs in the TME. For example, CAFs have a key role in the tumourigenesis of oral squamous cell carcinoma (OSCC), the most common cancer of the oral cavity. In this context, TGF- β can induce the conversion of normal oral fibroblasts (NOFs) into CAFs, which are fibronectin type III domain-containing 1 (FNDC1), serpin peptidase inhibitor type 1 (SERPINE1), stanniocalcin 2 (STC2), and type I collagen, to promote the proliferation and invasion of OSCC cells, resulting in a more aggressive tumour phenotypes [57]. The transformation of fibroblasts, which is promoted by the TGF- β /Smad pathway, is also involved in the process of pulmonary fibrosis and early lung

cancer tumorigenesis. Consistently, it has been shown that TGF- β 1 can induce lung fibroblast transition to myofibroblasts and the expression of α -SMA via the Rho/ROCK and TGF- β /Smad pathways in lung fibroblast–myofibroblast transformation [58]. Furthermore, CAFs also interact with immune cells in the TME, including macrophages, mast cells, natural killer (NK) cells, DCs, myeloid-derived suppressor cells (MDSCs), tumour-associated neutrophils (TANs), and T lymphocytes, to promote tumour progression and immune evasion [59][60]. For example, CAFs and tumour-associated macrophages (TAMs) have been shown to cooperate in driving neuroblastoma progression and invasion [59]. Similarly, CAFs from the HCC TME can enhance the generation of regulatory DCs, to facilitate tumour progression and immune evasion through the production of high levels of TGF- β , impairment of T cell proliferation, and expansion of the Treg population [59]. In addition, the activation of TGF- β signalling in CAFs has been linked to immunosuppression mediated through the induction of a unique set of ECM genes. Moreover, the immunosuppression triggered by TGF- β signalling in CAFs is caused by immune checkpoint blockade and has been shown to be able to lead to anticancer immunotherapy failure in melanoma and bladder cancer [61]. Together, these findings provide solid evidence that TGF- β plays an unparalleled role in mediating tumour growth, metastasis, and chemoresistance and immune evasion through the TME and is therefore a promising target for anticancer intervention.

Besides deregulation of TGF- β expression and activity itself, changes in the expression of components of the TGF- β downstream signalling pathways can also modulate tumorigenesis, cancer progression, and chemoresistance. DC-STAMP domain-containing 1-antisense 1 (DCST1-AS1) and annexin A1 are two genes functioning downstream of TGF- β that play key roles in EMT and cancer chemotherapy resistance. DCST1-AS1 regulates the expression of EMT-related genes including MMP2, MMP9, and E-cadherin. DCST1-AS1 has been found to bind to annexin A1 directly in triple-negative breast cancer (TNBC) cells to modulate the transcription of its downstream regulatory genes involved in EMT and cancer drug resistance [62]. In addition, the TGF- β /MAPK pathway also participates in the transcriptional activation of ETS1 to modulate TGF- β -mediated chemoresistance in liver cancer [63]. Conversely, Ma et al. have found that DNA methyltransferase inhibitor treatment can restore the decreased expression of TGFBR2 caused by hypermethylation on the gene promoter, and this treatment can successfully promote cell cycle arrest and inhibit cancer proliferation in esophageal squamous cell carcinoma (ESCC) [64]. Moreover, the epigenetic silencing of the TGF- β gene, *TGFB1*, also contributes to trastuzumab resistance in Her2+ breast cancer [65]. Another point worth mentioning is the function of non-coding RNAs in TGF- β pathway regulation and their effects on tumorigenesis. The microRNA miR-495 is one that inhibits the expression of homeobox C6 and the function of TGF- β 1. Its overexpression in CSCs contributes to EMT reversion, apoptosis, and the decreased proliferation and migration of CSCs [66]. The miRNA-200 family is another important group of microRNAs that promote cyst formation and ovarian cancer spread through targeting TGF- β expression [67]. Moreover, some recent studies show that circular RNAs, such as circCACTIN and circCCDC66, also affect cancer progression by regulating the TGF- β pathway [68][69], and circular RNA cSERP1 can increase the chemosensitivity of cancer cells by inducing Smad7 and blocking the TGF- β pathway [70].

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