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COMMENTS ON THE CROSS-TALK OF TGFβ AND EGF IN CANCER

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INTRODUCTION

Transforming growth factor-β (TGFβ) acts as a potent tumor suppressor and tumor promoter in a context-dependent manner [1]. Tumor suppressive functions include inhibition of cell proliferation, induction of apoptosis and regulation of autophagy. As tumors develop they switch their response to TGFβ and utilize this factor as a potent promoter of cell motility, invasion, metastasis and tumor stem cell maintenance. It has been widely proved that misregulation of TGFβ, i.e. TGFβ over-expression, TGFβ receptor or Smad2/4 loss or mutation, can result in tumor development [2]. Epidermal Growth Factor (EGF) is another potent regulator of cell functions, with predominantly pro-mitogenic role in tumorigenesis [3]. EGF promotes also cell survival, angiogenesis and differentiation. Deregulation of human epidermal growth factor receptor (ErbB/HER) pathways by over-expression or constitutive activation can promote tumorigenesis, including angiogenesis and metastasis and is associated with poor prognosis in many human malignancies [4].

TGFβ and EGF initiate signaling events via acting on different receptors. However, recent reports indicate that many of the components in their intracellular signaling pathways may be targeted by both growth factors. Understanding the molecular mechanisms of how TGFβ and EGF signaling interact at different stages of cancer is important for development of novel therapeutics. In this report, we focus on TGFβ and EGF signaling, with emphasis on the cross-talk between their regulatory pathways.

TGFβ SIGNALING AND CANCER

TGFβ signaling is initiated by the binding of TGFβ ligands to type II TGFβ receptor (TβR-II). TβR-II forms a dimer and recruits type I TGFβ receptor (TβR-I). Upon formation of a heterotetrameric complex of 2 TβR-II and 2 TβR-I, TβR-I becomes activated, and phosphorylates a number of substrates. TGF-β elicits its biological effects by coordinated activation of the well studied canonical Smad pathway and several non-Smad signaling pathways (Fig. 1) [5]. In Smad-dependent pathway, TβR-I mediated C-terminal phosphorylation of the receptor-regulated Smads, Smad2 at Ser465/476 and Smad3 at Ser433/435. Following phosphorylation, Smads 2 and 3 form heteroligomeric complexes with the co-Smad, Smad4, enter the nucleus, and in cooperation with cofactors and other sequence specific transcription factors both positively and negatively regulate gene expression. TGFβ also activate several Smad-independent pathways, including TAK1, RAS, PI3K, PLC, PP2A, SHC, Rho, Rac and protein synthesis via eEF1A1. TGFβ plays dual role in cancer development. It acts as a tumor suppressor in normal epithelial cells and in early stage of tumor progression. In advanced cancers the growth inhibitory function of TGFβ is selectively lost, and TGFβ induces many activities that lead to growth, invasion and metastasis of cancer cells [1, 2].

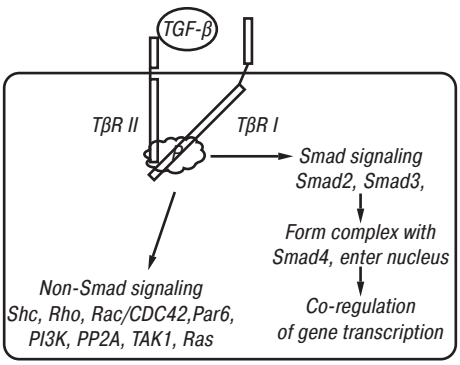

Fig. 1. Schematic presentation TGFβ signaling pathway. TGFβ binds the type II TGFβ receptor (TβRII) and this complex is able to recruit and transactivate the type I TGFβ receptor (TβRI). The ligand-bound TβRII/TβRI receptor complex subsequently activates downstream Smad-dependent and Smad-independent signaling. In the Smad-dependent signaling cascade, activated Smad2 and Smad3 are able to bind Smad4 as homo- and heterodimers, then translocate to the nucleus where they act as co-activators or co-repressors of transcription. The Smad-independent pathways regulated by TGFβ are known to include Shc, RHO, RAC/CDC42, RAS, TAK1, PI3K, PAR6, PP2A and protein synthesis (eEF1A1).

Alterations in TGFβ signaling have been associated with solid tumor initiation, progression and metastasis [1, 2, 5]. $TGF\beta_1$ overexpression has been reported in breast, colon, esophageal, gastric, lung, pancreatic and prostate cancer. High levels of $TGF\beta_1$ have also been detected in the serum of patients with colon and liver cancer. However, downstream signaling component mutations, loss of expression and attenuation have been detected in many solid carcinomas. In particular, it has been shown that the central mediators of TGFβ signaling $TGF\beta R_1$, $TGF\beta R_2$, Smad2 and Smad4 are frequently lost, mutated or attenuated in human carcinomas. Intragenic mutation, down-regulation and loss of $TGF\beta R_2$ expression have been observed in bladder, breast, colon, esophageal, lung, ovarian, pancreatic and prostate cancers. Loss of expression, down-regulation and mutation have also

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Abbreviations: EMT – epithelial-mesenchymal transition; EGF – epidermal growth factor; TGFβ – transforming growth factor-β.

been demonstrated in association with TGFβR1 in biliary, bladder, breast, gastric, liver, ovarian, pancreatic and prostate cancers. Smad4 mutation, deletion and loss of expression has been reported in biliary, bladder, breast, cervical, colon esophageal, intestine, liver, lung, ovarian and pancreatic cancers. Smad2 deregulation seems to be less frequent than Smad4, however mutation and deletion has been observed in cervical, colon, liver and lung cancer. Interestingly, with the exception of gastric, extravillus trophoblast and colon cancer, Smad3 is often maintained in human carcinomas suggesting that it may have a role different from Smad2 in carcinoma cells that favors tumor progression.

EGF SIGNALING AND CANCER

The epidermal growth factor (EGF) receptor family consists of four related receptors: EGFR (EGFR or ErbB1), ErbB2 (Neu/HER2), ErbB3 (HER3) and ErbB4 (HER4). These receptors are differently activated by ligands including EGF, TGFα, amphiregulin (SDGF), βcellulin (BTC), epiregulin (EREG), heparin-binding EGF-like growth factor (HB-EGF) and the neuregulins (NRG1, NRG2, NRG3 and NRG4) [3, 6]. Binding of ligands to the extracellular domains of ErbB receptors initiates their homodimerization or heterodimerization with other ErbB receptors, and phosphorylation of tyrosine residues within their cytoplasmic domains. Autophosphorylation of receptors leads to a number of protein-protein interactions, that in turn activate downstream growth and survival signals such as the mitogen-activated protein kinase (MAPK) and phosphoinositol 3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/AKT) pathways (Fig. 2) [6].

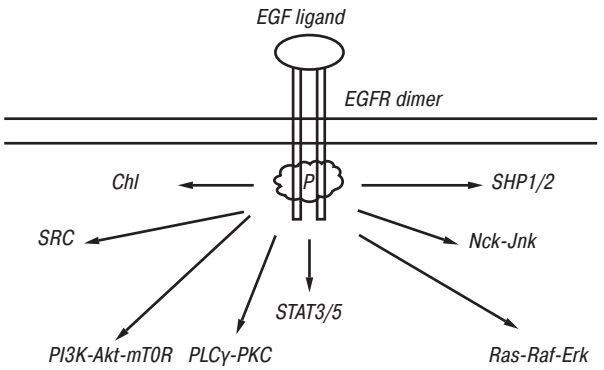

Fig. 2. Schematic presentation of EGFR signaling pathway. EGFR signaling pathway is initiated by binding of ligands to the extracellular domain of ErbB receptors, which results in receptor dimerization, tyrosine kinase activation and transphosphorylation (P). The activated ErbB receptors are able to interact with different signaling molecules that transmit the signal in the cell, including Chl, Src, PI3K, PLC, STAT, Ras, Nck-Jnk and SHP1/2.

The EGF family members play important roles in normal physiological processes including ontogeny, morphogenesis, migration, differentiation and proliferation. Deregulation of EGF family members and related signaling molecules can contribute to tumorigenesis, invasion and metastasis. In particular, ErbB2 and EGFR have been implicated in development of many types of human cancer [3, 4, 6]. Genetic changes that have been detected in human tumors include gene amplification leading to receptor overexpression, activating kinase domain mutations mainly in EGFR, but also in ErbB2, in-frame deletions in the extracellular domain of EGFR (EGFR vIII), and coexpression of ErbB ligands and receptors in tumors. Each alteration promotes constitutive activation of the receptors.

CROSS-TALK OF TGFβ AND EGF IN CANCER

Signaling pathways do not act in isolation, but interplay with each other and form complex signaling networks. Recent studies have shown that ErbB signaling, which activates both the MAPK (including Erk1/2, JNK1/2/3, and p38/MAPKs) and phosphatidylinositol-3 kinase (PI3K)/Akt pathways, communicates intimately with TGFβ/Smad in controlling mammary epithelial cell biology and breast cancer development [7]. Other common Smad-dependent regulatory mechanisms include phosphorylation of Smad2 and Smad3 by PKC and PKG. MAPKs and Akt bind to and/or phosphorylate Smads to control their intracellular distribution and transcriptional activity [2, 7]. MAPKs and Akt also phosphorylate and regulate a variety of Smad binding partners in the nucleus, indirectly affecting the Smads. We focus below on the cross-talk involving TGFβ and Smads from one side, and MAPK and Akt from the other side (Fig. 3).

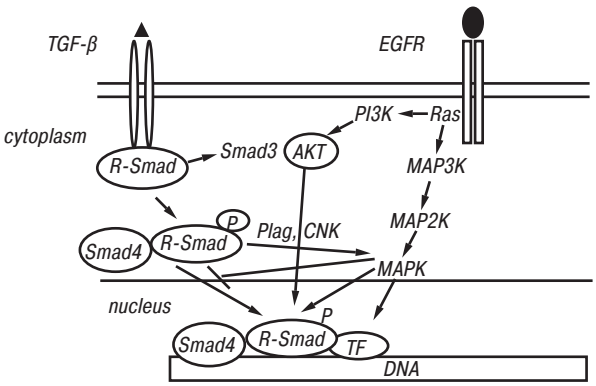

Fig. 3. Cross-talk between TGFβ and EGF signaling pathway. The MAPK and PI3K/Akt are linkers (common targets) of TGFβ and EGF pathways. Their interplay is modulated primarily by Smad functions. MAPKs and Akt bind and/or phosphorylate R-Smads to control their intracellular distribution and transcriptional activity. MAPKs and Akt also phosphorylate and regulate a variety of Smad binding partners in the nucleus, indirectly affecting the Smads. TGFβ stimulates Erk1/2 activation by regulating Plag1 and CNK.

TGFβ/SMADS AND THE ERBB/MAPK PATHWAY

A consensus is that HER2/Ras can antagonize TGFβ-induced apoptosis and cell cycle arrest, while allowing for the pro-migratory and pro-invasive functions of TGFβ. Therefore, both positive and negative regulations exist between the two pathways. The synergy between the TGFβ and HER2/Ras/MAPK pathways often leads to the secretion of TGFβ, which in turn promote epithelial-to-mesenchymal transition (EMT) and cell invasion, whereas JNK kinases seem to negatively regulate the autocrine expression of TGFβ1 [7,

8]. MEK/Erk has been reported to positively regulate SMAD3 gene transcription in epithelial and smooth muscle cells [7].

It has been confirmed that the linker region in Smad proteins is important for integrating ErbB/MAPK signals with the TGFβ pathway. Human cancer cells overexpressing oncogenic Ras are often resistant to TGFβ-induced cytostasis. MAPK/Erk-mediates Smad2/3 linker phosphorylation and Smad nuclear exclusion, which is considered as the reason for the attenuation of TGFβ induced cytostasis by MAPK [9]. MAPKs (especially Erk1/2) also phosphorylate the linker of Smad1/5, which almost always blocks Smad1/5 nuclear translocation. Phosphorylation in Smad1 linker region by Erk creates a docking site for the Smad1/5-specific E3 ubiquitin ligase, Smurf1. Smurf1 binding not only causes ubiquitination and degradation of the Smads but also occludes their interaction with the nuclear pore complex, thereby preventing Smad nuclear translocation [9]. In addition to R-Smads, MAPKs also phosphorylate and regulate the Co-Smad, Smad4, and the inhibitory Smad7. For instance, MAPK/Erk decreases Smad4 protein stability [9]. JNK and p38 seem to preferentially phosphorylate tumor-derived mutant Smad4 and promote its proteasomal degradation *http://www.nature.com/ cr/journal/v19/ n1/full/cr2008302a.html* - bib58. Erk, JNK, and p38 have all been implicated in the transcriptional regulation of Smad7, therefore indirectly regulating TGFβ signaling. On the flip side, TGFβ also regulates MAPK/Erk signaling. Addition of TGFβ stimulates Erk1/2 activation in most of the cells. Our lab identified Plag1 and CNK contribution to TGFβ induced Erk1/2 activation [8].

MAPKs phosphorylate a number of nuclear transcription factors, many of which can physically interact with Smads and regulate TGFβ responses. The best-characterized ones in this category are the AP-1 proteins, including members of the Jun, Fos, Maf, and ATF sub-families [9]. Functional interaction between Smad and the Jun/Fos family proteins has been widely studied, and their relationship can be synergistic or antagonistic depending on their target genes and other binding partners. Thus, TGFβ and EGF may coordinate their actions by the cross-talk between Smad-dependent signaling and Erk1/2 activation.

TGFβ AND THE ERBB/PI3K/AKT PATHWAY

PI3K pathway promotes cell survival, growth, and motility through Akt-mediated phosphorylation of a number of proteins. Oncogenic mutations and protein overproduction of PI3K and Akt are commonly found in human cancers. The activity of PI3K is counteracted by the tumor suppressor protein PTEN. Loss-of-function mutations of *PTEN* also occur at a high frequency in human cancers [7].

The PI3K/Akt activity is known to alleviate TGFβ-induced apoptosis and/or cell cycle arrest in multiple types of cells. Interestingly, Smad3, but not Smad2, seems to be the primary target of inhibition by PI3K/Akt, consistent with the indispensable function of Smad3 in mediating the pro-apoptotic effects of TGFβ. However, exactly how PI3K/Akt modulates Smad3 activation remains unanswered.

On the other hand, the PI3K/Akt pathway is also subjected to TGFβ regulation. Akt activity increases in response to TGFβ treatment, which seems to be required for a variety of TGFβ-induced activities, such as cell migration of HER2-expressing breast cancer cells, EMT of normal mammary epithelial cells, cell survival of mouse hippocampal neurons and mesenchymal cells, as well as growth stimulation of certain fibroblasts [7, 9]. It is to be noted that Akt activation by TGFβ is cell type-dependent and very likely indirect, often requiring either MAPKs or autocrine actions of secreted molecules.

Alteration of PTEN function represents another route for TGFβ to influence Akt activity. TGFβ has been shown to transcriptionally downregulate PTEN in Smad4 null pancreatic cancer cells, which, again, seems to rely on the function of the Ras/MAPK pathway [7, 8, 9]. In the same cells, TGFβ elicits EMT by dislodging β-catenin from the adherence junctions, a process that involves TGFβ-dependent PTEN dissociation from β-catenin and Akt activation. On the other hand, TGFβ/Smad can reduce Akt activity in hematopoietic cells by inducing the expression of SHIP (SH2 domain-containing 5' inositol phosphatase), a lipid phosphatase that removes the 5 position phosphate from PIP_3.

TARGETING TGFβ AND EGF PATHWAYS IN CANCER TREATMENT

The genetic and preclinical studies support targeting TGFβ signaling as therapeutic strategy for combating cancer. To date there have been investigated three approaches to inhibit the TGFβ signaling. They are: (1) inhibition at the translational level using antisense oligonucleotides, (2) inhibition of the ligand-receptor interaction using monoclonal antibodies, and (3) inhibition of the receptor-mediated signaling cascade using inhibitors of TGFβ receptor kinases [1, 2]. For each of these approaches, several drugs have been developed and are either in pre-clinical or in early stages of clinical trials. Some of these have already been shown to be efficacious in limiting tumor invasion and metastasis in vivo. Among these drugs are antisence oligos used for treatment of gliomas, and TβR-I kinase inhibitors used for treatment breast cancer. One of the challenges of anti-TGFβ therapy will be in targeting the tumor promoting arm of TGFβ signaling while maintaining the tumor suppressive arm.

EGFR and ErbB receptors have been especially explored as targets for cancer treatments, because overexpression and mutations of these receptors are frequently observed in human malignancies. A variety of small molecule kinase inhibitors targeting EGFR (e.g. erlotinib: Tarceva™) and monoclonal antibodies targeting EGFR (e.g. cetuximab: Erbitux) and HER2 (e.g. trastuzumab: Herceptin™) have been

developed and some of them are used for treatment of lung and breast cancer [3]. Anti-EGFR therapy has shown significant efficacy for some patients. However, no therapeutic response was seen in high number of other cancer patients. In addition, patients initially responsive to anti-EGFR therapy develop resistance over time of treatment. Potential mechanisms of resistance to EGFR-targeted therapy may be dependent on EGFR gene amplification and mutations, and on activation of alternative signaling pathways which bypass the EGFR pathway.

Using targeted agents to inhibit multiple signaling pathways has emerged as a new paradigm for anticancer treatment. This approach is based on preclinical and clinical data showing potent anti-tumor activity of single drugs inhibiting multiple molecular targets or combination therapies involving multiple drugs with selective or narrow target specificity [10]. In a study comparing the multi-targeting tyrosine kinase inhibitor SU11248—whose targets include VEGFR and PDGFR—with agents targeting only PDGFR (imatinib) or VEGFR (SU10944) in mouse xenograft models, the most robust anti-tumor and anti-angiogenic effects were observed with SU11248. Moreover, the efficacy of imatinib combined with SU10944 was generally similar to that of SU11248 monotherapy, suggesting that the anti-tumor and anti-angiogenic effects of SU11248 included additional effects related to VEGFR and PDGFR inhibition [10]. This suggests that optimal therapeutic approaches to cancer may involve targeting multiple molecules in different signaling pathways. Therefore, combination of drugs targeting EGF and TGFβ or development of drugs targeting common components of both pathways may confer better therapeutic effects than single treatments.

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REFERENCES

1. **ten Dijke P, Hill CS.** New insights into TGF-β-Smad signalling. Trends Biochem Sci 2004; **29**: 265–73.

2. **Massagué J.** TGFβ in cancer. Cell 2008; **134**: 215–30.

3. **Scaltriti M, Baselga J.** The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006; **12**: 5268–72.

4. **Lurje G, Lenz HJ.** EGFR signaling and drug discovery. Oncology 2009; **77**: 400–10.

5. **Derynck R, Zhang YE.** Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003; **425**: 577–84.

6. **Salomon DS, Brandt R, Ciardiello F, *et al.*** Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995; **19**: 183–232.

7. **Kretzschmar M, Doody J, Timokhina I, *et al.*** A mechanism of repression of TGFbeta/Smad signaling by oncogenic Ras. Genes Dev 1999; **13**: 804–16.

8. **Bhaskaran N, Souchelnytskyi S.** Systemic analysis of TGFbeta proteomics revealed involvement of Plag1/CNK1/RASSF1A/Src network in TGFbeta1-dependent activation of Erk1/2 and cell proliferation. Proteomics 2008; **8**: 4507–20.

9. **Guo X, Wang XF.** Signaling cross-talk between TGF-β/BMP and other pathways. Cell Research 2009; **19**: 71–88.

10. **Faivre S, Djelloul S, Raymond E.** New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. Semin Oncol 2006; **33**: 407–20.