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Overlapping activities of TGF-β and Hedgehog signaling in cancer: Therapeutic targets for cancer treatment☆

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ABSTRACT

Recent advances in the field of cancer therapeutics come from the development of drugs that specifically recognize validated oncogenic or pro-metastatic targets. The latter may be mutated proteins with altered function, such as kinases that become constitutively active, or critical components of growth factor signaling pathways, whose deregulation leads to aberrant malignant cell proliferation and dissemination to metastatic sites. We herein focus on the description of the overlapping activities of two important developmental pathways often exacerbated in cancer, namely Transforming Growth Factor-β (TGF-β) and Hedgehog (HH) signaling, with a special emphasis on the unifying oncogenic role played by GLI1/2 transcription factors. The latter are the main effectors of the canonical HH pathway, yet are direct target genes of TGF-β/SMAD signal transduction. While tumor-suppressor in healthy and pre-malignant tissues, TGF-β is often expressed at high levels in tumors and contributes to tumor growth, escape from immune surveillance, invasion and metastasis. HH signaling regulates cell proliferation, differentiation and apoptosis, and aberrant HH signaling is found in a variety of cancers. We discuss the current knowledge on HH and TGF-β implication in cancer including cancer stem cell biology, as well as the current state, both successes and failures, of targeted therapeutics aimed at blocking either of these pathways in the pre-clinical and clinical settings.

Contents

1. Introduction

Cancer represents one of the leading causes of mortality in developed countries. Despite the encouraging development of cancer therapies over the past decades, the number of cancer-related deaths keeps rising. Deregulated cellular processes in tumors, including proliferation, invasion, angiogenesis, epithelial–mesenchymal transition (EMT) and tumor immune evasion, all occur as a consequence of aberrant activation of, or abnormal response to, signaling pathways such as those driving Transforming Growth Factor (TGF-β) and Hedgehog (HH) responses.

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TGF-β signaling is involved in the maintenance of normal tissue homeostasis and exerts tumor-suppressive roles in healthy and pre-malignant tissues (reviewed in Javelaud et al., 2008; Massague, 2008). However, malignant cells may take advantage of TGF-β signaling which promotes tumor growth, invasion and metastasis in advanced tumor stages, as it is the case in various malignancies including breast, lung, colon, prostate cancers and melanoma. The HH signaling cascade is critical as morphogen during embryonic development, and in the adult for tissue homeostasis and repair. Consistent with its role as a regulator of cell proliferation, differentiation and apoptosis, aberrant HH signaling is involved in a variety of cancer types, including basal cell carcinoma (BCC) of the skin, medulloblastoma, rhabdomyosarcoma, glioma, lung and pancreas cancers (reviewed in Varjosalo & Taipale, 2008; Javelaud et al., 2012). In this review, we focused our attention on the overlapping activities of these two pathways in cancer and cancer stem cell biology, with a particular interest for GLI transcription factors, effectors of the canonical HH cascade and direct target genes of the TGF-β/SMAD pathway. We then describe the pharmacological and therapeutic strategies targeting either pathways, to provide an update on the specificity and toxicity of the inhibitors assessed in preclinical and clinical studies. Preliminary and promising results obtained in ongoing trials are exposed for the most relevant molecules, as they represent major advances for the treatment of specific tumors, exemplified by the recent FDA approval of an HH inhibitor for the treatment of BCC (Dlugosz et al., 2012).

2. TGF-β signaling in cancer

2.1. TGF-β superfamily members and functions

Mammalian Transforming Growth Factor-β (TGF-β) family members include more than 35 structurally related secreted proteins, including TGF-βs stricto sensu, activins and inhibins, Bone Morphogenetic Proteins (BMPs), Growth Differentiation Factors (GDFs) such as myostatin (GDF8), Glial-Derived Neurotrophic Factors (GDNFs), Nodal, Lefty and the Müllerian Inhibitory Substance/Anti-Müllerian Hormone (MIS/AMH) (reviewed in Zi et al., 2012). Members of the TGF-β family play fundamental roles during embryonic development and for maintenance of tissue homeostasis as they regulate diverse cellular processes, such as proliferation, differentiation, migration and extracellular matrix synthesis (reviewed in Verrecchia & Mauviel, 2007; Massague, 2008). TGF-β was first described in the early 80s in the cell culture media of sarcoma virus transformed mouse fibroblasts as an activity that induced the anchorage-independent growth of non-malignant cells. It was demonstrated in subsequent studies that TGF-β can act as a potent tumor suppressor and inhibitor of cell proliferation (reviewed in Siegel & Massague, 2003). Deregulation of TGF-β signaling is implicated in several diseases, including cancer, impaired wound healing, developmental defects, auto-inflammatory diseases and neurodegenerative disorders (reviewed in Massague, 2008).

TGF-β, stricto sensu, composed of three different isoforms in mammals: TGF-β1, TGF-β2 and TGF-β3, are encoded by distinct genes and share ~70% peptide sequence identity. TGF-β1 is the most ubiquitous and abundant subtype, with TGF-β2 and TGF-β3 having a more restricted tissue distribution. All isoforms are secreted as inactive precursors called latent TGF-βs (L-TGF-βs) which are then activated by conformational change induced by an acidic microenvironment or proteolytic cleavage by enzymes such as plasmin or matrix metalloproteinases (MMPs) (reviewed in Hyytiainen et al., 2004). Once active, TGF-βs bind their receptors and initiate intracellular signaling.

TGF-β isoforms display overlapping activities in vitro but distinct functions in vivo, as illustrated by the phenotype of isoform-specific null mice: *Tgf-β1* null mice die within three weeks of birth due to generalized inflammation, while *Tgf-β2* null mice die in the perinatal period due to heart and pulmonary insufficiency and *Tgf-β3* null mice die shortly after birth and display a cleft palate (reviewed in Bottinger & Kopp, 1998).

2.2. Canonical TGF-β signal transduction: the SMAD pathway

There are three types of TGF-β receptors: type I (also known as activin receptor-like kinases, ALKs), type II, and type III receptors. Type I and II receptors are transmembrane glycoproteins, each comprising a glycosylated extracellular domain, a short transmembrane domain, and an intracellular serine/threonine kinase domain. Mammalian cells express seven different type I and five different type II receptors that allow for discrete and precise control of ligand response specificity (reviewed in de Caestecker, 2004). Type II receptors, the ligand-binding receptors, are constitutively autophosphorylated on various serine residues, while type I receptors, the signal-transducing receptors, are distinguished by the presence of a conserved glycine- and serine-rich region (GS-region) located upstream of the kinase domain. Initiation of signal transduction requires ligand-dependent formation of a heterotetrameric complex between type I and II receptors. Type III receptors, betaglycan and endoglin (CD105), aid this process by presenting TGF-β ligands to type II receptors. Transphosphorylation of the type I receptor GS domain by the type II receptor kinase results in initiation of downstream signaling (reviewed in Xu et al., 2012) (Fig. 1).

Mammalian SMADs may be divided into three groups according to their function: receptor-associated SMADs (R-SMADs), common-mediator SMADs (Co-SMADs) and inhibitory SMADs (I-SMADs). SMAD1/2/3/5/8 belong to the group of receptor-activated SMADs, SMAD4 to the co-SMADs. SMAD6 and SMAD7 form the I-SMADs group (reviewed in Massague et al., 2005) (Fig. 1).

TGF-β binding to its receptors results in the direct phosphorylation of R-SMADs by the type I receptor in their C-terminal domain: the type I receptors for TGF-βs, Activin, Nodal and Myostatin (ALKs 4/5/7) phosphorylate SMADs 2 and 3, whereas BMPs and AMH type I receptors (ALKs 1/2/3/6) phosphorylate SMAD1/5/8. Recruitment of SMAD2/3 to the receptor requires SARA (SMAD Anchor for Receptor Activation), a FYVE domain-containing protein that facilitates SMAD2/3 localization near the cell membrane, and enhances their interaction with type I receptors. Other proteins that may facilitate R-SMAD/receptor interactions include the FYVE domain-containing protein HGS, the -β spectrin ELF, the clathrin adaptor Disabled-2, and Axin (reviewed in Massague et al., 2005) (Fig. 1).

R-SMADs form heterocomplexes with the co-SMAD SMAD4 that accumulate in the cell nucleus where they transactivate target genes, binding either directly to DNA or in association with other transcription factors, in conjunction with transcriptional coactivators or corepressors (reviewed in Javelaud & Mauviel, 2004; Xu et al., 2012).

I-SMADs interfere with TGF-β signaling by various mechanisms. They may for example suppress R-SMADs phosphorylation and activation by competitively interacting with TGF-β receptors. SMAD6 specifically inhibits BMP signaling by disrupting the SMAD1/Co-SMAD interaction and forming an inactive SMAD1/6 complex, while SMAD7 inhibits signals triggered by TGF-βs, Activins and BMPs. SMAD7 not only inhibits R-SMAD phosphorylation, but also recruits E3 ubiquitin ligases SMURF1 and SMURF2 to the receptor complex, leading to TGF-β receptor degradation. SMAD7 may also recruit the protein phosphatase PP1/GADD34 to the receptor complexes that dephosphorylate TβRI. All these mechanisms lead to TGF-β signaling blockade (reviewed in Javelaud & Mauviel, 2004). I-SMADs may also function as transcriptional repressors in the nucleus, by recruiting histone deacetylases (HDACs) to the target genes (reviewed in Xu et al., 2012). As I-SMAD expression may be induced by TGF-β, they participate in a negative feedback loop attenuating TGF-β signaling (reviewed in Itoh & ten Dijke, 2007) (Fig. 1).

2.3. Non-canonical pathways and interference with other transcription factors

TGF-β receptors, via a complex network of adaptor proteins whose expression may be cell-type specific, potentially activate other signaling pathways that complement, synergize or antagonize SMAD signaling,