Long non-coding RNAs and TGF-β signaling in cancer

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Abstract
Cancer is driven by genetic mutations in oncogenes and tumor suppressor genes and by cellular events that develop a misregulated molecular microenvironment in the growing tumor tissue. The tumor microenvironment is guided by the excessive action of specific cytokines including transforming growth factor-β (TGF-β), which normally controls embryonic development and the homeostasis of young or adult tissues. As a consequence of the genetic alterations generating a given tumor, TGF-β can preserve its homeostatic function and attempt to limit neoplastic expansion, whereas, once the tumor has progressed to an aggressive stage, TGF-β can synergize with various oncogenic stimuli to facilitate tumor invasiveness and metastasis. TGF-β signaling mechanisms via Smad proteins, various ubiquitin ligases, and protein kinases are relatively well understood. Such mechanisms regulate the expression of genes encoding proteins or non-coding RNAs. Among non-coding RNAs, much has been understood regarding the regulation and function of microRNAs, whereas the role of long non-coding RNAs is still emerging. This article emphasizes TGF-β signaling mechanisms leading to the regulation of non-coding genes, the function of such non-coding RNAs as regulators of TGF-β signaling, and the contribution of these mechanisms in specific hallmarks of cancer.

KEYWORDS
non-coding RNA, signal transduction, Smad, transcription, transforming growth factor-β

1 | INTRODUCTION

Cancer represents a large group of diseases that affects many organs. Cancers are characterized in their onset by genetic mutations in key genes that subsequently unleash a cohort of cell biological processes; the various pathological processes ultimately lead to the growth of malignant tissue in the form of tumors and even further, but infrequently, to disseminating cells into metastases in nearby or distant organs.1,2 Secreted growth factors control the communication between cells and the organization of tissues. For these reasons, growth factor genes, upon mutagenic alteration, can act as initiators of the malignant process (oncogenes), but also as functional mediators of the malignant evolution through various stages.1,3 Since its discovery, transforming growth factor β (TGF-β) has been linked functionally with and continues to provide new lessons on mechanisms that govern cancer development.4-7

2 | TGF-β SIGNALING IN CANCER

TGF-β is the prototype member of a large family of polypeptide growth factors that has exhibited evolutionary conservation in...
all animals since the emergence of multicellularity. TGF-β signaling pathways participate in developmental morphogenetic programs and contribute to young or adult organismic homeostasis; the growth factors of this family regulate differentiation, proliferation, and motility. TGF-β (encompassing 3 isoforms, TGF-β1/2/3) is secreted from many cells via a well controlled mechanism that delivers a latent, inactive form of the growth factor, together with other proteins, to the extracellular matrix. Upon activation, TGF-β signals via its receptors, serine-threonine and weak tyrosine kinase enzymes, known as type II (TGFβRII) and type I (TGFβRI) receptors. When TGF-β binds to TGFβRI, recruitment of TGFβRI is followed by trans-phosphorylation of serine and threonine residues in TGFβRII by TGFβRI kinase, conformational activation of TGFβRI, which subsequently phosphorylates Smad2 and Smad3, members of the Smad family of signal transducers and latent transcription factors. The phosphorylated Smad2 and Smad3 interact with Smad4 to generate trimeric complexes that associate directly with DNA and many transcription factors that mediate the regulation of target gene expression.

A negative feedback mechanism is mediated by the inhibitory Smad7. TGF-β induces Smad7 expression, which inhibits signaling via direct interaction of Smad7 with TGFβRI. Smad complexes, and several ubiquitin ligases that ubiquitylate and degrade either the receptor, upon its internalization, or the active Smad complexes. The TGF-β receptor complex also recruits ubiquitin ligases that then, via ubiquitylation, activate protein kinases that lead to downstream engagement of the mitogen-activated protein (MAP) kinases. The same ubiquitylation-dependent mechanism also controls a cleavage and translocation of the cytoplasmic, protein kinase domain of TGFβRI, to the nucleus for further signaling in association with Smad and other transcriptional cofactors. The coordinated activity of Smads, phosphorylation inputs generated by TGFβ-mediated MAP kinase activation and nuclear TGFβRI intracellular domain mediate the diverse biological actions of TGF-β.

Similar to its actions in adult homeostasis, TGF-β signaling limits the development of hyperplastic, pre-malignant lesions in many organs. Once tumorogenesis has progressed, TGF-β cooperates with diverse oncogenic pathways and facilitates the development of aggressive, less differentiated, and invasive tumors. TGF-β also facilitates cancer metastasis. Homeostatic signaling fighting against hyperplastic growth is exemplified by the ability of TGF-β to induce the expression of cyclin-dependent kinase inhibitors, including CDKN1A (p21(CIP1)), CDKN1B (p27(KIP1)), CDKN2B (p15(INK4B)). These cell cycle inhibitors stall the epithelial, endothelial, lymphocytic, and erythropoietic cell cycle in the early G1 phase. In hepatocytes, prostate, and other epithelial cell types, TGF-β can also induce apoptosis via coordinated signaling actions: (i) Smad-mediated induction of pro-apoptotic genes (Bim, DAPK); (ii) activation of MAP kinases and cytochrome c release from mitochondria, leading to pro-caspase activation. Furthermore, the TGF-β receptors and the Smad genes can be mutated in various tumors.

Genetic alterations cause either complete loss of responses or preferential loss of the cytostatic and pro-apoptotic responses to TGF-β by malignant cells.

Once malignancy progresses, TGF-β secretion by cancer cells, cancer-associated fibroblasts, or in some cases even from immune cells, is abundantly observed. In carcinomas, EMT is potently induced by TGF-β and contributes to the invasive and pro-metastatic phases of tumor development. TGF-β inhibits the proliferation and differentiation of B and T lymphocytes, causing a local immune suppression that promotes expansive tumor growth and invasiveness. TGF-β can indirectly stimulate neo-angiogenesis that feeds the growing malignancy and facilitates invasiveness and metastatic dissemination. These multi-faceted effects of TGF-β have, in recent years, stimulated several clinical trials. As a combinatorial treatment, together with more classical chemo- or radio-therapy, TGF-β pathway inhibitors have shown ability to limit expansion of various tumors.

3 | LONG NON-CODING RNAs

The majority of the biological activities of TGF-β can be explained by regulation of expression of a large cohort of mRNAs and their encoded proteins. In recent years, attention has been given to the functional roles of non-protein-coding RNAs. Among the various non-coding RNAs, much focus has been given to microRNAs (miRNAs); TGF-β signaling regulates miRNA gene expression and miRNA maturation from precursor transcripts, whereas various miRNAs can regulate TGF-β signaling in the context of cancer. Here, we focus exclusively on long non-coding RNAs (lncRNAs), whose regulation by TGF-β signaling and functional participation in multiple responses to TGF-β form an emerging field.

lncRNAs are structurally identical to mRNAs. They are transcribed by RNA polymerase II into 250 nt or longer RNAs; they have 5' modified caps and poly-adenylated tails at their 3'-end, and localize in the nucleus, cytoplasm or both (Figure 1). In lncRNAs almost universally carry open reading frames, which are small, initiating with non-optimal start codons, embedded in the lncRNA sequence far away from the 5'-end and are often considered incapable of encoding polypeptides. The non-coding capacity of lncRNAs is bioinformatically attested and only rarely experimentally tested. Examples of lncRNAs encoding for functional polypeptides exist. The putative lncRNA LOC100507537 encodes for the 34 amino acid-long peptide “dwarf open reading frame,” which associates with and activates the sarcoplasmic reticulum calcium pump sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) in cardiomyocytes, thus regulating heart muscle contraction. In the context of cancer, the homeobox B cluster antisense RNA 3 (HOXB-AS3) encodes a 53 amino acid-long polypeptide; the polypeptide binds to arginine-rich sequences in the hnRNP A1 splicing factor regulating alternative splicing of the pyruvate kinase M. By inducing expression of pyruvate kinase isoform M2, the HOXB-AS3 polypeptide facilitates manifestation of the oncogenic Warburg effect in colorectal cancer.
IncRNAs are classified based on the position of their gene relative to protein-coding genes. Antisense IncRNAs are transcribed from the opposite DNA strand of a protein-coding gene and partially overlap with mRNAs. Intronic IncRNAs are completely embedded in the intron of a protein-coding gene. Divergent IncRNAs do not overlap with mRNAs but share promoter-enhancer sequences with a protein-coding gene and are transcribed in the opposite direction relative to the mRNA. Enhancer RNAs (eRNAs) or ncRNA-activating, are encoded by genes that overlap characterized enhancer sequences and regulate expression of the genes that are controlled by the enhancer. Finally, intergenic IncRNAs map as independent genes far away from protein-coding genes.

Similar to mRNAs, the biological functions of IncRNAs permeate all essential cell biological processes, and their actions are often linked to cancer development. These functions range from the control of stemness and differentiation, including genomic imprinting and the mechanism of X chromosome inactivation, to immunity and programmed cell death. Mechanistically (Figure 1), IncRNAs regulate gene expression by acting as scaffolds, guides, or decoys or by base-pairing with DNA, through formation of triple helices. IncRNAs associate with nuclear proteins and affect nucleosome remodeling, including histone modifications catalyzed by protein methyltransferases, such as the polycomb repressor complex 2 (PRC2). IncRNAs can regulate mRNA splicing, stability, or translation. A widely established function of IncRNAs, especially when they are located in the cytoplasm, is the “sponge” or competing endogenous (ce) RNA function; this indicates their ability to base-pair with miRNAs (Figure 1), and thus shield the action of the miRNAs toward target mRNAs. Most of these molecular mechanisms of action have been demonstrated in the context of cancer cell biology.

4 | IncRNAs ACT AS EFFECTORS OF TGF-β SIGNALING

The list of IncRNAs, described as effectors of TGF-β signaling, is constantly growing (Figure 2). Table 1 summarizes TGF-β-regulated IncRNAs and their roles in different cancer types. Furthermore, IncRNAs acting as effectors of TGF-β signaling have been reported in a plethora of different cancer types. One of the first IncRNAs, demonstrated to be modulated by TGF-β, is the IncRNA-activated by TGF-β (IncRNA-ATB) in hepatocellular carcinoma (HCC). TGF-β upregulates IncRNA-ATB in order to favor EMT and establish a pro-metastatic program. IncRNA-ATB acts as a sponge for the epithelial-specific miR-200. miR-200 was previously established as a negative regulator of the EMT transcription factors ZEB1/2, and, accordingly, IncRNA-ATB acts by enhancing ZEB1/2 expression. Moreover, IncRNA-ATB stabilizes interleukin-11 mRNA, leading to increased cytokine signaling mediated by STAT3, which potentiates tumor colonization in secondary tissues to ensure efficient metastasis. Similar to HCC, IncRNA-ATB is induced by TGF-β in MCF7 breast cancer cells and was established as a marker of poor prognosis in breast cancer; IncRNA-ATB promotes EMT by sponging miR-200 and thus, upregulating Twist1 expression, the latter being another transcription factor of the EMT program. In intrahepatic cholangiocarcinoma, the TGF-β-induced long noncoding RNA (TLINC) boosts a pro-migratory phenotype and positively regulates interleukin-8, reinforcing a pro-inflammatory tumor microenvironment. In pancreatic ductal adenocarcinoma (PDAC), TGF-β induces the mir-100-let-7a-2-mir-125b-1 cluster host gene (MIR100HG), a IncRNA that gives rise to mir-100, let-7a-2, and mir-125b-1 miRNAs. mir-100 and mir-125b promote PDAC progression and EMT, by downregulating p53 and apoptotic pathways and upregulating the pro-survival phosphatidylinositol 3’-kinase/Akt signaling pathway. In colorectal cancer, the taurine


At the transcriptional level, regulating the cell cycle inhibitor Cdkn1a, both transcriptionally and post-transcriptionally. 34 At the transcriptional level, EPR directly binds to the Cdkn1a promoter and interacts with Smad3 during early TGF-β signaling, thereby activating Cdkn1a transcription. 34

Upon sustained TGF-β signaling Cdkn1a levels return to basal, a response that coincides with the delayed EPR downregulation. At the post-transcriptional level, EPR associates with the RNA-binding protein KHSRP (KH-type splicing regulatory protein) and prevents its binding to Cdkn1a mRNA, thereby increasing Cdkn1a stability. 34

TGF-β regulates lncRNA expression not only in carcinomas but also in tumors of diverse tissue origin. For example, LINC00115 is overexpressed in glioblastoma and is upregulated by TGF-β in glioma stem-like cells, in order to facilitate their self-renewal. 35 LINC00115 interferes with the binding of miR-200 to its target mRNAs ZEB1 and ZNF596 (zinc finger protein 596), leading to increased expression of these proteins and potentiation of downstream pro-tumorigenic signals that elicit tumor growth. 35 Table 1 presents additional lncRNAs which are not discussed here in the interest of space.

5 | lncRNAs ACT AS REGULATORS OF TGF-β SIGNALING

In addition to being effectors of TGF-β signaling, lncRNAs modulate several components of the pathway, thereby affecting the magnitude of its response, during tumor progression (Figure 2). Several lncRNAs can regulate TGF-β signaling in a wide range of cancers (Table 2). In HCC cells, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) alters the balance between phosphorylated (p-) and de-phosphorylated Smad2 and Smad3 levels. 36 MALAT1 interacts with p-Smad2 and p-Smad3, through the protein known as SET domain containing 2 (SETD2), which serves as a scaffold that facilitates complex formation between the phosphatase PPM1A (protein phosphatase, Mg2+/Mn2+ dependent 1A) and p-Smad2/3. Thus, MALAT1 promotes the termination of TGF-β signaling, by inducing the PPM1A-mediated de-phosphorylation of activated p-Smad2/3. 36 In colorectal cancer, the cancer susceptibility candidate 9 (CASC9) is an lncRNA that predicts poor survival for patients. 37 The pro-tumorigenic function of CASC9 is due to the increased stabilization of TGFβ2 levels, which lead to active TGF-β2 signaling and enhanced p-Smad3 levels. 37 The positive contribution of CASC9 to TGF-β signaling depends on its binding to the protein cleavage and polyadenylation specific factor 3 (CPSF3), an mRNA-processing factor, which is capable of directly interacting with TGFβ2 mRNA. 37 Liver fibrosis-associated lncRNA 1 (lnc-LFAR1) potentiates TGF-β signaling by enhancing TGFβ1, Smad2, and Smad4 mRNA levels in the intrahepatic cholangiocarcinoma cell line QBC939. 38 Moreover, lnc-LFAR1 exerts pro-EMT functions, by enhancing vimentin and downregulating E-cadherin protein levels and reinforces migration and invasion of QBC939 cells. 38 In colorectal cancer, the small nucleolar RNA host gene 6 (SNHG6) activates the TGF-β pathway by reducing UPF1 (UPF1 RNA helicase and ATPase), a regulator of Smad7, leading to reduced Smad7 expression and, therefore, increased p-Smad2/3 levels. 39 Thus, SNHG6 promotes cell proliferation, migration, and
| LncRNA          | Type of regulation | Function                                           | Mechanism of action                                      | Cancer type/ cell line                      | Ref. |
|-----------------|--------------------|----------------------------------------------------|----------------------------------------------------------|--------------------------------------------|------|
| TUG1            | Up                 | Induces EMT in vitro; metastasis in vivo           | Enhances Twist1                                          | Colorectal cancer                         | 31   |
| LINC00273       | Up                 | Promotes invasion and metastasis                   | Activates ZEB1 via sponging mir200a-3p                   | A549 adenocarcinoma cells                 | 51   |
| LINC00115       | Up                 | Promotes cell self-renewal                         | Upregulates ZEB1 and ZNF596, via sponging mir-200       | Glioma stem-like cells                    | 35   |
| EPR             | Up (early) Down (late) | Inhibits cell proliferation                      | Positively regulates Cdkn1a                             | NMuMG breast epithelial cells             | 34   |
| MIR155HG        | Up                 | Promotes EMT                                       | Regulates the mir-155-5p/ SOX10 axis                     | Laryngeal squamous cell carcinoma         | 52   |
| MACC1-AS1       | Up                 | Fatty acid oxidation-dependent stemness and chemoresistance | De-represses stemness and FAO genes, via sponging miR-145-5p | Gastric cancer                           | 53   |
| HCP5            | Up                 | Promotes EMT                                       | Upregulates Snail and Slug by sponging mir-203          | Lung adenocarcinoma                      | 54   |
| PTAF            | Up                 | Promotes EMT and invasion                          | Enhances SNAI2 by targeting mir-25                       | Ovarian cancer                           | 55   |
| MIR100HG        | Up                 | Hosts pro-tumorigenic miRNAs                       | miR-100 and miR-125b downregulate p53 and apoptotic pathways and activate the PI3K pathway | PDAC                                       | 30   |
| TLINC           | Up                 | Promotes cell migration and pro-inflammatory tumor microenvironment | Positively regulates pro-inflammatory cytokines | Intrahepatic cholangiocarcinoma          | 29   |
| MEG8            | Up                 | Induces EMT                                        | Upregulates SNAI1 and SNAI2, by epigenetically suppressing miR-34a and mir-203 | A549, LC-2/ad, Panc1 cells               | 56   |
| UCA1            | Up                 | Promotes cell proliferation                        | Upregulates HKK2                                         | HCC                                       | 57   |
| UCA1            | Up                 | Promotes EMT                                       | Enhances Slug by targeting miR-1 and mir-203a            | Glioma                                   | 58   |
| UCA1            | Up                 | Promotes EMT                                       | Unknown                                                 | Gastric cancer                           | 59   |
| NKILA           | Up                 | Inhibits cell migration and invasion               | Suppresses MMP14 by inhibiting the NF-κB pathway        | Esophageal squamous cell carcinoma        | 60   |
| NKILA           | Up                 | Inhibits EMT                                       | Blocks the NF-κB pathway                                 | MCF7 breast cancer cells                 | 61   |
| LINP1           | Down               | Inhibits EMT                                       | Enhances CDH1 and represses mesenchymal genes (mechanism unknown) | Lung cancer                              | 62   |
| incRNA-ATB      | Up                 | Promotes EMT                                       | Upregulates Twist1 by sponging mir-200                  | MCF7 breast cancer cells                 | 28   |
| Inc-MMP2-2      | Up                 | Regulates cell migration and invasion              | Promotes MMP2 expression                                 | A549 lung adenocarcinoma exosomes        | 63   |
| TBILA           | Up                 | Promotes tumor progression in vitro and in vivo    | Enhances RhoA and S100A7-JAB1 pathway activation         | Non-small cell lung cancer               | 64   |
| H19             | Down               | Increases tumorigenic potential in vivo            | Unknown                                                 | Tumor-initiating hepatocytes              | 65   |
| H19             | Up                 | Enhances cell invasion in vitro and metastasis in vivo | Upregulates Slug and inhibits CDH1 via mir-675         | Hep3B HCC cells                          | 66   |
| has2as          | Up                 | Promotes EMT and cancer stemness                   | Induces has2, by facilitating Smad2/3 binding to its promoter | NMuMG breast epithelial cells            | 67   |
| EPB41L4A-AS2    | Down               | Inhibits cell migration and invasion               | Inhibits TGFBR1 expression                               | Head and neck squamous cell carcinoma    | 68   |

(Continues)
invasion in vitro and colorectal tumor growth in vivo.\textsuperscript{29} In HCC, the nuclear enriched abundant transcript 1 (NEAT1) acts as a ceRNA for miR-139-5p, thereby protecting TGFBI mRNA from miR-139-5p-induced degradation.\textsuperscript{40} Thus, NEAT1 is an activator of TGF-\(\beta\) signaling and promotes HCC growth.\textsuperscript{40} In ovarian carcinoma, the LINK-A is frequently overexpressed and positively correlated to TGF-\(\beta\) pathway, such as KLF11 represses Smad7; the transcription factor KLF11 represses Smad7 and enhances TGF-\(\beta\) signaling, which promotes liver fibrosis.\textsuperscript{47} In keloid fibroblasts, the TGF-\(\beta\) pathway, as described above, potentiates or diminishes the responses of the pathway itself. We categorize these IncRNAs into 2 subclasses: first, IncRNAs that are transcriptionally upregulated by TGF-\(\beta\), which then enhance TGF-\(\beta\) signaling output, forming positive feedback loops; second, TGF-\(\beta\)-induced IncRNAs, with inhibitory roles on TGF-\(\beta\) responses, thereby belonging to negative feedback loops. Examples of IncRNAs that form positive feedback loops with TGF-\(\beta\) are the IncRNAs PCAT7, ELI1, HOTAIR, lincRNA-p21, MALAT1 and lincRNA-ATB (Table 3). In prostate cancer, PCAT7 (prostate cancer-associated transcript-7) is upregulated by TGF-\(\beta\) via the transcriptional complex of Smad3 with Sp1 and then positively regulates TGF-\(\beta\) signaling by sponging miR-324-5p, leading to enhanced TGFBR1 expression, as TGFBR1 is downregulated by miR-324-5p.\textsuperscript{45} In endothelial progenitor cells, the TGF-\(\beta\)-induced MALAT1 described earlier, is required for the induction of endothelial-to-mesenchymal transition, a process similar to the EMT that has been implicated in the dissemination of tumor cells to metastatic sites. Mechanistically, MALAT1 binds to the tumor suppressor miR-145 and sequesters it away from its target mRNAs TGFBR2 and Smad3, resulting in increased TGF-\(\beta\) activation.\textsuperscript{46} In hepatocytes, lincRNA-p21 is involved in a positive feedback loop, whereby TGF-\(\beta\) induces its expression, in order to strengthen the magnitude of the pathway, by sponging miR-30, leading to increased KLF11 levels, as miR-30 downregulates KLF11; the transcription factor KLF11 represses Smad7, and thus enhances TGF-\(\beta\) signaling, which promotes liver fibrosis.\textsuperscript{47} In keloid fibroblasts, the TGF-\(\beta\)-induced

### TABLE 1 (Continued)

| LnRNA          | Type of regulation | Function                  | Mechanism of action                                                                 | Cancer type/ cell line                  | Ref. |
|----------------|--------------------|---------------------------|-------------------------------------------------------------------------------------|----------------------------------------|------|
| Inc-Spyr1      | Down               | Suppresses EMT            | Alternative splicing of FGFRs, via binding to U2AF65                                 | NMuMG breast epithelial cells          | 33   |
| MEG3           | Up                 | Induces EMT               | Represses CDH1 and miR-200 by facilitating recruitment of JARID2 and EZH2 on their promoters | A549, LC-2/ad cells                    | 69   |
| LINC01186      | Down               | Inhibits EMT              | Suppresses mesenchymal markers and induces CDH1, (mechanism unknown)                | A549 lung adenocarcinoma cells         | 70   |
| IncRNA-LET     | Down               | Represses cancer cell stemness | Decreases NF90 stability leading to miR-145 upregulation                          | Urinary bladder cancer                 | 71   |
| linc00673      | Up                 | Induces EMT               | Upregulates ZEB1, by sponging miR-150-5p                                             | Non-small cell lung cancer             | 72   |
| LINC01133      | Down               | Inhibits EMT and metastasis | Blocks SRSF6 function                                                              | Colorectal cancer                      | 73   |
| IncRNA-HIT     | Up                 | Enhances EMT, migration, invasion | Represses CDH1                                                                        | NMuMG breast epithelial cells          | 32   |
| MALAT1         | Up                 | Induces EMT               | Represses CDH1 via binding to SUZ12                                                  | Bladder cancer                         | 74   |
| IncRNA-Smad7   | Up                 | Inhibits apoptosis         | Unknown                                                                              | NMuMG, JygMC(A) breast cancer cells    | 75   |

6 | IncRNAs Form Feedback Loops With TGF-\(\beta\) Signaling

Some of the IncRNAs whose expression is regulated by the TGF-\(\beta\) pathway, as described above, potentiate or diminish the responses of the pathway itself. We categorize these IncRNAs into 2 subclasses: first, IncRNAs that are transcriptionally upregulated by TGF-\(\beta\), which then enhance TGF-\(\beta\) signaling output, forming positive feedback loops; second, TGF-\(\beta\)-induced IncRNAs, with inhibitory roles on TGF-\(\beta\) responses, thereby belonging to negative feedback loops. Examples of IncRNAs that form positive feedback loops with TGF-\(\beta\) are the IncRNAs PCAT7, ELI1, HOTAIR, lincRNA-p21, MALAT1 and lincRNA-ATB (Table 3). In prostate cancer, PCAT7 (prostate cancer-associated transcript-7) is upregulated by TGF-\(\beta\) via the transcriptional complex of Smad3 with Sp1 and then positively regulates TGF-\(\beta\) signaling by sponging miR-324-5p, leading to enhanced TGFBR1 expression, as TGFBR1 is downregulated by miR-324-5p.\textsuperscript{45} In endothelial progenitor cells, the TGF-\(\beta\)-induced MALAT1 described earlier, is required for the induction of endothelial-to-mesenchymal transition, a process similar to the EMT that has been implicated in the dissemination of tumor cells to metastatic sites. Mechanistically, MALAT1 binds to the tumor suppressor miR-145 and sequesters it away from its target mRNAs TGFBR2 and Smad3, resulting in increased TGF-\(\beta\) activation.\textsuperscript{46} In hepatocytes, lincRNA-p21 is involved in a positive feedback loop, whereby TGF-\(\beta\) induces its expression, in order to strengthen the magnitude of the pathway, by sponging miR-30, leading to increased KLF11 levels, as miR-30 downregulates KLF11; the transcription factor KLF11 represses Smad7, and thus enhances TGF-\(\beta\) signaling, which promotes liver fibrosis.\textsuperscript{47} In keloid fibroblasts, the TGF-\(\beta\)-induced
IncRNA-ATB described earlier, facilitates TGF-β-dependent responses, by acting as a ceRNA for miR-200c, leading to ZNF217 upregulation and increased secretion of TGFβ2.48 Conversely, TGFβ2-AS1 and Inc-TSI participate in negative feedback loops with TGF-β signaling (Table 3). TGF-β induces the expression of TGFβ2 antisense RNA 1 (TGFβ2-AS1) in human immortalized...
keratinocytes and lung adenocarcinoma cells. TGFβ2-AS1, in turn inhibits Smad-mediated transcriptional responses, via interaction with the TGFβ superfamily. During renal fibrosis, the kidney-specific TGFβinteracting long noncoding RNA (lnc-TSI) is upregulated by TGFβ and forms a negative loop, by binding to the MH2 domain of Smad3, thereby blocking the association of Smad3 with TGFβ receptors and inhibiting receptor signaling. This mechanism seems to not involve the function of Smad7, and results in lower TGFβ-induced renal fibrogenesis. Although some of these examples do not stem from studies of cancer biology, they are useful as they illustrate the importance of feedback control of the TGFβ signaling pathway, a mechanism whereby previously well established proteins are now demonstrated to cooperate with IncRNAs in order to elicit their full action.

7 CONCLUDING REMARKS

The large spectrum of biological actions engaging the TGFβ signaling pathway during cancer development has necessitated the elucidation of many target genes of this pathway, and their functions. Whereas the first 35 of TGFβ signaling research focused on protein-coding genes, the past 5 have demonstrated the important function of IncRNAs. Most of the studied IncRNAs act either as regulators of chromatin modifications and transcriptional control or as sponges that limit the abundance of miRNAs. We anticipate the elucidation of completely new mechanisms of action of IncRNAs downstream of TGFβ in cancer. Equally interesting is the large number of IncRNAs that regulate specific steps of TGFβ signaling. Whereas TGFβ ligand expression is a frequent target for regulation by IncRNAs, examples of very intricate mechanisms, such as regulation of Smad phosphorylation or Smad translocation to the nucleus, have been described to engage IncRNAs. In cancer, differentially expressed oncogenic IncRNAs that modulate TGFβ signaling could serve as biomarkers to stratify patients that may benefit from anti-TGFβ-based therapies. Placing such IncRNAs together with protein-based mechanisms into the biology of specific tumors is a challenging task. Completion of this task promises a more coherent understanding of the mistakes made as cancer cells aim to survive and spread their biological potential in multiple organs of the afflicted patients.

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