EMT, lncRNA, TGF, SMAD and Cancers

Terrence P McGarty
White Paper No 161
June 2019

Long non coding RNA has the ability to interact in a variety of gene expression paths. We examine one recently proposed and use it as a basis to better grasp the TGF/SMAD functioning.

Notice

This document represents the personal opinion of the author and is not meant to be in any way the offering of medical advice or otherwise. It represents solely an analysis by the author of certain data which is generally available. The author furthermore makes no representations that the data available in the referenced papers is free from error. The Author also does not represent in any manner or fashion that the documents and information contained herein can be used other than for expressing the opinions of the Author. Any use made and actions resulting directly or otherwise from any of the documents, information, analyses, or data or otherwise is the sole responsibility of the user and The Author expressly takes no liability for any direct or indirect losses, harm, damage or otherwise resulting from the use or reliance upon any of the Author's opinions as herein expressed. There is no representation by The Author, express or otherwise, that the materials contained herein are investment advice, business advice, legal advice, medical advice or in any way should be relied upon by anyone for any purpose. The Author does not provide any financial, investment, medical, legal or similar advice in this document or in its publications on any related Internet sites.

Furthermore, this document contains references to and quotes from papers and documents under the premise of Fair Use in order to present ideas and understandings in context. The Author has attempted to make any and all references to such material separate from those of the author per se and has referenced the source expressly in all cases. These documents are for the dissemination of ideas and have no commercial intent. The Author would appreciate any communications relating to these documents and these should be sent to:

tmcgarty@telmarc.com.

Contents

1 INTRODUCTION

There is an ever-growing understanding of the role of long no-coding RNAs in the initiation and proliferation of many cancers. The nexus of these with TGF-β has been known also for a while. On top of this is the role of EMT, epithelial to mesenchymal transitions, is also critical.

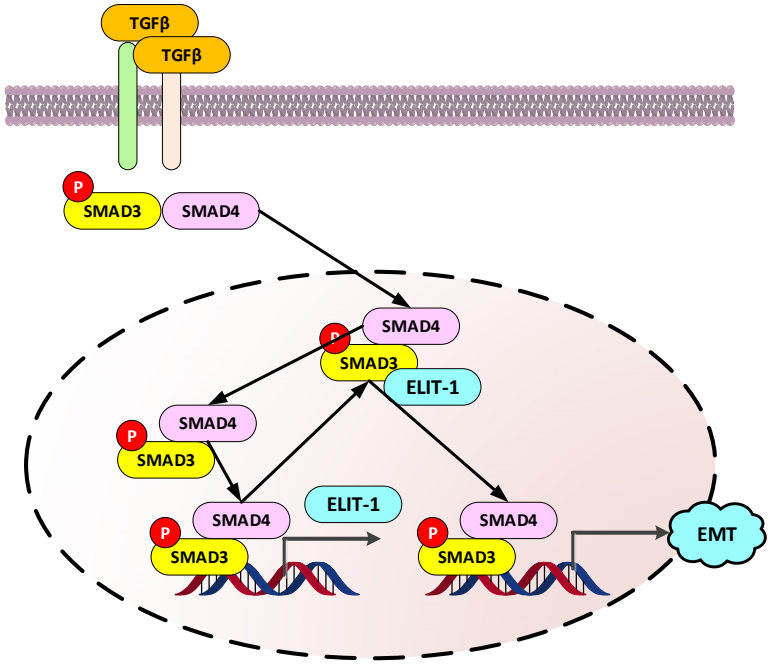
A recent paper by Sakai et al brings some of these elements together. They note:

TGFβ is involved in various biological processes, including development, differentiation, growth regulation, and epithelial–mesenchymal transition (EMT). In TGFβ/Smad signaling, receptor-activated Smad complexes activate or repress their target gene promoters. Smad cofactors are a group of Smad-binding proteins that promote recruitment of Smad complexes to these promoters. Long noncoding RNAs (lncRNA), which behave as Smad cofactors, have thus far not been identified.

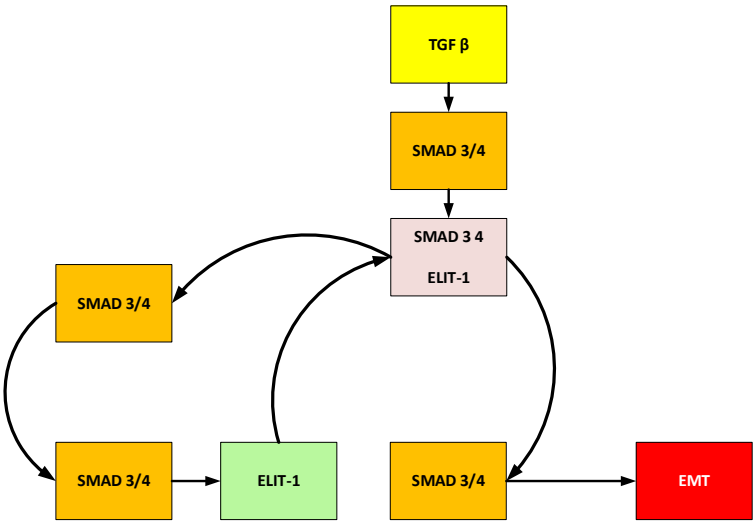
Here, we characterize a novel lncRNA ***EMT-associated lncRNA induced by TGFβ1*** ***(ELIT-1)****. ELIT-1 was induced by TGFβ stimulation via the TGFβ/Smad pathway in TGFβ-responsive cell lines. ELIT-1 depletion abrogated TGFβ-mediated EMT progression and expression of TGFβ target genes including Snail, a transcription factor critical for EMT.*

A positive correlation between high expression of ELIT-1 and poor prognosis in patients with lung adenocarcinoma and gastric cancer suggests that ELIT-1 may be useful as a prognostic and therapeutic target. RIP assays revealed that ***ELIT-1 bound to Smad3, but not Smad2****. In conjunction with Smad3, ELIT-1 enhanced Smad-responsive promoter activities by recruiting Smad3 to the promoters of its target genes including Snail, other TGFβ target genes, and ELIT-1 itself. Collectively, these data show that ELIT-1 is a novel trans-acting lncRNA that forms a positive feedback loop to enhance TGFβ/Smad3 signaling and promote EMT progression.*

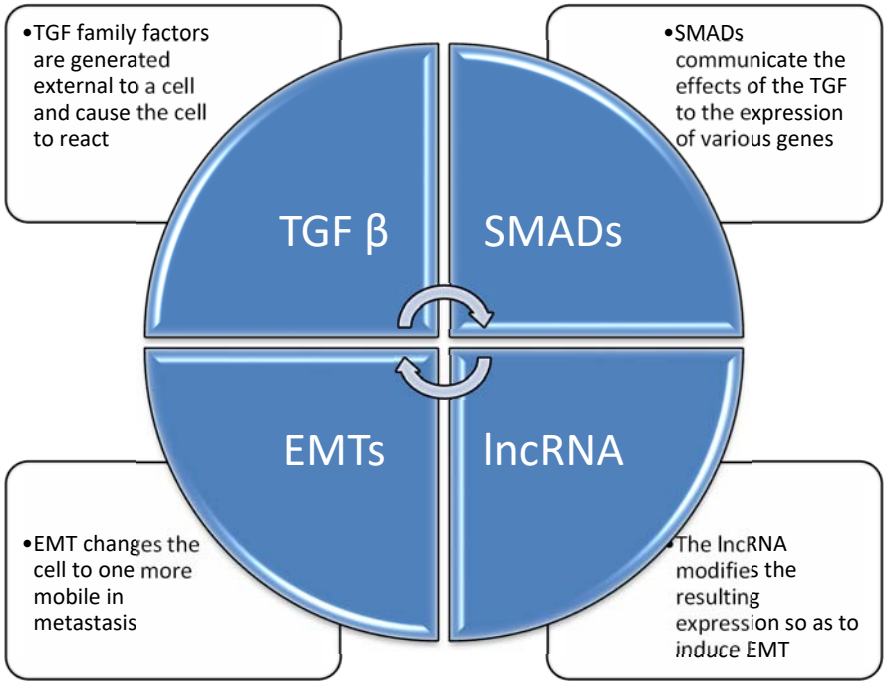
The above can be reflected in the following Figure modified from the above authors.



Now the feedback loop including the influence of the lncRNA can be depicted as below.



The elements we consider herein are shown below:



As Morikawa et al (2013) have noted:

The signaling pathways of TGF-β family members are key players in tumorigenesis and cancer progression. TGF-β can function both as a tumor-suppressing and a tumor-promoting factor during cancer progression. BMP signaling has been reported to play critical roles in oncogene-induced senescence, which is part of the tumorigenesis barrier and blocks cellular proliferation by inducing irreversible growth arrest. Interestingly, BMP signaling induces differentiation of certain cancer-initiating cells, such as glio-mainitiating cells, while TGF-β/activin signaling maintains their stem cell-like properties.

Since Smad proteins are central mediators of the signal transduction, studies on global and genome-wide binding sites of Smad proteins may reveal important insights into their complex biological functions. Identification of an appropriate antibody is the first and most important step for ChIP-chip and ChIP-seq analyses, because the quality of ChIP data depends crucially on the quality of the antibody used.

Since MH1 and MH2 domains are conserved among R-Smads, several specific antibodies for Smad proteins recognize their linker region. However, linker regions are targets of posttranslational modification and protein interactions, as discussed above. It is possible that such changes may attenuate the affinities of antibodies under specific conditions.

Understanding TGF/SMAD controls is a key element in the understanding of many malignancies. However, interference with these controls is also a critical factor. It is well known that there are many miRNAs which can interfere with normal homeostasis but also lncRNAs have the ability to do so as well. Understanding the processes associated with malignancies is often a very complex and challenging area. The impact of the various non-coding RNAs combined with the multiplicity of epigenetic factors in general cause a great deal of complexity

in normal gene product pathways. Moreover, these apparent irregularities may be key to explaining why some successful cancer therapeutics fail on sets of patients. What may be a logically and generally biologically correct methodology may get subjected to interference from a set of yet to be fully understood intermediaries.

In this Note we use the opening references as a sounding board for this complex set of interactions. The TGF/SMAD pathway is a critical one, but is just one of many. We have previously examined lncRNAs in prostate cancer, PCa, but they have impacts in a broad set of malignancies. The challenge is to have some measure of the existence of and impact of these epigenetic players.

2 lncRNA

Long non-coding RNAs are a collection of RNAs which are long in length but are not translated to any protein. They operate perforce of their bonding and inhibition/activation capabilities[1].

Liang et al note:

Recently, emerging evidence suggested that long noncoding RNAs (lncRNAs) played essential roles in human diseases, especially in tumors. LncRNAs are defined as a class of non-coding RNAs (ncRNAs) that are longer than 200 nucleotides. Although lncRNAs lack crossspecies conservation, increasing evidence suggests that lncRNAs play important roles in a variety of cellular process, and therefore may contribute to tumor initiation, metastasis, and chemoresistance. LncRNA HOTAIR, which is one of the most famous lncRNAs, played significant role in lung cancer, renal cancer, esophageal cancer ovarian cancer, and so on. LncRNA-MALAT1 promoted lung cancer metastasis and could serve as a treatment target.

Schmitt and Chang reflect on a paper by Yang et al:

The human genome generates more than 10,000 long non-coding RNA (lncRNA) molecules, yet the functions of only several dozens of these transcripts are known. In a study published by Yang et at. describe two lncRNAs that bind to, and govern the function of, the androgen receptor — a transcription factor that activates the expression of thousands of genes in response to the hormone androgen. The authors find that inhibition of these two lncRNAs can block the growth of prostate cancer cells that are resistant to hormone therapy owing to a mutation in the androgen receptor.

The number of identified lncRNAs has grown significantly since then. However, their function often remains obscure. Unlike miRNAs which seem better understood, the lncRNAs appear to have a multiplicity of yet to be determined functions.

Long non-coding RNA, lncRNA, are the long RNAs recently discovered, most of which whose function is yet unknown, which can actually control gene transcription. The lncRNAs range from 200 to well over 100,000 nucleotides. In Weinberg's latest edition of Cancer, he presents about one page only to lncRNAs, and such is an example of their newness and lack of understanding[2].

We know that there are over 20,000 genes expressible in the human genome but that these genes comprise about 2% of the total DNA. The question always has been; what does the rest of the DAN do, if anything? lncRNA may be one of many answers to this question.

2.1 LNCRNA OVERVIEW

[1] See McGarty, lncRNA and Prostate Cancer, 2013.

[2] Weinberg, 2013, p 26.

We begin with a brief summary of some of the details of lncRNA. From the recent book by the Kovalchuk's, the authors state that lncRNA have several functions:

1. Regulation of expression of neighboring genes
2. Blocking of splicing proteins-coding genes using antisense transcripts
3. Interaction with proteins making them more or less capable of fulfilling specific functions
4. Serving as precursors for smaller ncRNAs.

Kornienko et al present an excellent overview of these functions and we summarize here in their words some key elements of them:

Regulation of transcription is considered to be interplay of tissue and developmental-specific transcription factors (TFs) and chromatin modifying factors acting on enhancer and promoter sequences to facilitate the assembly of the transcription machinery at gene promoters. With a growing number of lncRNAs implicated in transcriptional gene regulation, this view may need refinement to include networks of tissue and developmental-stage specific lncRNAs that complement known regulators to tightly control gene expression and thereby organism complexity.

Transcriptional regulation by lncRNAs could work either in cis or in trans, and could negatively or positively control pc gene expression. lncRNAs work in cis when their effects are restricted to the chromosome from which they are transcribed, and work in trans when they affect genes on other chromosomes.

They continue:

lncRNAs can inhibit general protein-coding (pc) gene expression in trans

(a) by preventing transcription factor (TF) activity (7SK lncRNA) or

(b) by inhibiting RNAPII binding to DNA (B2 lncRNA). Xist lncRNA is transcribed from the X inactivation center (XIC) and inactivates a whole chromosome in cis

(c) by recruiting epigenetic modifiers (EM). lncRNAs can regulate specific genes, acting in trans like HOTAIR

(d) or in cis like HOTTIP

(e) by directly recruiting epigenetic modifiers to certain genomic loci.

In both cases the lncRNA binds EMs via a specific sequence or structure and targets them to promoter regions via DNA/RNA interaction elements to affect expression of the respective pc gene. Transcription of a lncRNA through a pc gene promoter or a cis-regulatory element (RE) affects pc gene expression in cis independent of the lncRNA product (f) by mechanisms discussed in the text. Both DNA strands are shown as separate boxes to indicate lncRNA transcription over the pc gene promoter in the antisense orientation.

Thus, the lncRNAs have become an interesting target for examination especially as we learn more about why certain cancers return after targeted pathway control. lncRNAs are one of the many epigenetic elements which make understanding the process of cancer development and metastasis so complex.

2.2 FUNCTIONS OF LNCRNA

lncRNA are complex in their function and are being discovered at a rapid pace. We present herein some details that may assist in gaining a better understanding of how they operate and how they are classified. The lncRNAs have many functions. Although they do not encode into proteins, they have the ability to interfere and facilitate many other intra-nuclear processes. It must be remembered that this is still a work in progress, the understanding of lncRNAs.

We rely upon some of the recent summary literature which describes these newly observed entities in some detail. From Kornienko et al we have an overview of classification:

Transcriptional regulation by lncRNAs could work either in cis or in trans, and could negatively or positively control gene expression. lncRNAs work in cis when their effects are restricted to the chromosome from which they are transcribed, and work in trans when they affect genes on other chromosomes.

Thus, the classification of cis and trans is a critical distinction. In addition, they may activate or suppress, and do so directly or via agents. The authors then proceed to define cis and trans. They state as follows:

Regulation in trans: *Some significant examples of lncRNAs that act in trans are those that can influence the general transcriptional output of a cell by directly affecting RNAPII activity …Regulation in trans can also act locus-specifically. While the ability of lncRNAs to act locus-specifically to regulate a set of genes was first demonstrated for imprinted genes where lncRNA expression was shown to silence from one to ten flanking genes in cis*

Regulation in cis: *In contrast to trans-acting lncRNAs, which act via their RNA product, cis-acting lncRNAs have the possibility to act in two fundamentally different modes:*

(i) The first mode depends on a lncRNA product.

(ii) The second mode of cis regulation by lncRNAs involves the process of transcription itself, which is a priori cis-acting

The authors describe several mechanisms for its action. We report their comments as follows which are mechanisms by which lncRNA transcription silences gene expression**.**

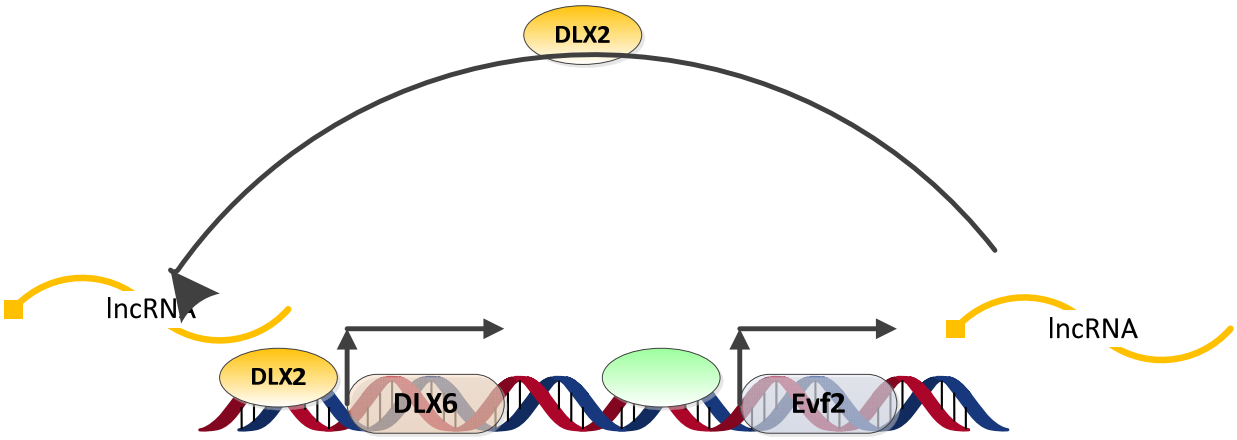
Mechanism	*Description*
Transcription-mediated silencing, also referred to as 'transcriptional interference' (TI)	This defined here as a case in which the act of transcription of one gene can repress in cis the functional transcription of another gene.
Mechanisms by which lncRNA transcription silences gene expression	Transcription-mediated silencing, also referred to as 'transcriptional interference' (TI), is defined here as a case in which the act of transcription of one gene can repress in cis the functional transcription of another gene.
Transcriptional interference acting by promoter nucleosome repositioning	DNA in the nucleus is organized into chromatin with the organizational scaffold consisting of nucleosomes, each with two copies of H3, H4, H2A and H2B histones. Nucleosomes can be densely packed, interfering with protein-DNA interactions, or relaxed, facilitating these interactions. The transcription process, which generates single-stranded DNA as RNAPII progresses along a gene locus, can directly affect nucleosome positioning.
Transcriptional interference acting by promoter histone modifications	Promoter associated nucleosomes carry post-translational histone tail modifications that reflect the activity state of the promoter and also influence accessibility of DNA binding factors involved in transcription.
Transcriptional interference acting by promoter DNA methylation	In mammalian genomes DNA methylation is generally associated with silent CpG island promoters, but the majority of CpG island promoters remain methylation free independent of their expression status.
Transcriptional interference in the absence of chromatin changes at the silenced promoter	In addition to RNAPII acting as a carrier of chromatin modifying enzymes, other TI models predict that RNAPII from one promoter traversing across another promoter can interfere with its activity without introducing chromatin changes.
lncRNA transcription creating a permissive chromatin environment	Enhancers are genetic elements that bind transcription factors facilitating transcription machinery assembly at nearby promoters.
lncRNA transcription and locus activation	Other examples indicate that lncRNA transcription activates gene expression by blocking access of repressor complexes to chromatin.

They continue as follows:

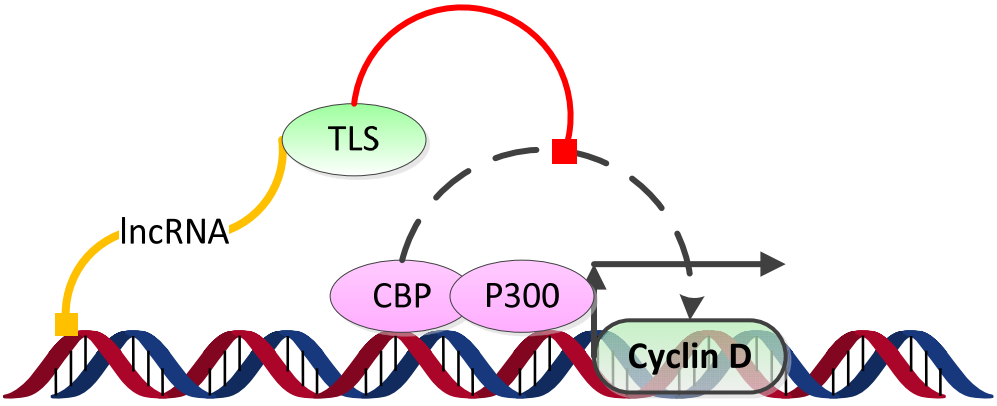
Modes of action include cotranscriptional regulation (e.g., through either the interaction of factors with the nascent lincRNA transcript or the act of transcribing through a regulatory region), regulation of gene expression in CIS or in TRANS through recruitment of proteins or molecular complexes to specific loci, scaffolding of nuclear or cytoplasmic complexes, titration of RNA-binding factors, and pairing with other RNAs to trigger posttranscriptional regulation.

The two latter mechanisms are illustrated in the cytoplasm (where they are more frequently reported) but could also occur in the nucleus. Additional mechanisms will presumably be proposed as additional functions of lincRNAs are discovered.

The following are two examples of how lncRNA may either activate or suppress gene transcription. Case 1 is an activation shown below.



Case 2 is a suppression mode of operation as shown below.



Now from Nie et. al. we have the following summary of lncRNAs. This is but a short list of what is currently known.

lncRNA Name	*Function/Characterization*
AIR	Imprinted, monoallelically expressed from the paternal allele, interacts with histone methyltransferase G9a
AK023948	Antisense transcribed from the intron of SIR-like adaptor gene (SLA), significantly downregulated in most of papillary thyroid carcinoma
ANRIL	Antisense transcript of INK4n/ARF/INK4a and p15/CDKN2B, required for the PRC2 recruitment to and silencing p15INK4b tumor suppressor gene
BACE1AS	Antisense transcript for beta-secretase-1, directly implicated in the increased abundance of Abeta 1-42 in Alzheimer's disease
CCND1/	Transcribed from 5' end of Cyclin D1 gene, induced by DNA damage and
CUDR	Upregulated in drug-resistant human squamous carcinoma, regulates drug sensitivity, cellular transformation and apoptosis
Cyclin D1	binding to TLS protein, leading to allosteric changes and repression of Cyclin D1 and anti-sense transcripts of tie-1 related to vascular malformation
DHFR	Transcribed from upstream of DHFR gene, regulates DHFR expression by forming triple helix with the promoter and disassociating pre-initiation complex
Evf-1	Activates transcriptional activity by directly influencing Dlx-2 activity
Evf-2	An alternatively spliced form of Evf-1 activates transcriptional activity by di-
GAS5	Growth arrest-specific transcripts, controls apoptosis and cell cycle, down-regulated in breast cancers
H19	Imprinted at the lgf2 locus; controls igf2 expression *in cis,* implicated in both tumor suppressors and oncogenes
HAR1	REST target gene, decreased in the neurons of Huntington's disease
HOTAIR	Intergenic transcript of HoxC locus, gene silencing in trans through interacting with PCR2 and LSD1 complex, involved in breast cancer metastasis
HOTAIRM1	Antisense intergenic RNA myeloid 1, transcribed antisense to the HOXA genes, plays a role in the myelopoiesis through modulation of gene expression in the HOXA cluster
KCNQ1OT1	Tissue-specific imprinted genes within the Kcnq1 domain, interacting with both PRC2 and G9a leading to gene silencing in a lineage-specific manner
KRAS1P	Transcript of KRAS pseudogene, overexpression of KRAS1P 3'-UTR, increases KRAS mRNA abundance and accelerates cell growth
LincRNA ROR	Expressed in the induced pluripotent stem cells (iPSCs), involved in the conversion of lineage-committed cells by interacting with reprogramming complexes
MALAT-1/NEAT2	Expressed in many cancers, regulates alternative splicing of pre-mRNA and promotes cell motility through transcriptional and post-transcriptional regulation of motility related gene expression
MEG3	Imprinted transcripts, highly expressed in human pituitary, stimulates p53-mediated transactivation and suppresses tumor growth in the absence of p53
Myc	Antisense transcript to myc gene, may be targeting the sense transcripts for immediate degradation
P15AS	Antisense transcript of p15, highly expressed in leukemia, epigenetically silences the tumor suppressor gene p15 directly influencing Dlx-2 activity
p21NAT	Antisense to cdkn1a/p21, requires Ago1 for epigenetic silencing of Cdkn1a/p21
PCGEM1	Prostate tissue-specific and prostate-associated, overexpressed in prostate cancers, regulates cell proliferation and apoptosis, promotes colony formation
PTENP1	Transcript of PTEN tumor suppressor pseudogene, PTENP1 3'-UTR exerts a tumor suppressive function by acting as a decoy for PTEN-targeting miRNAs
SRA-1	Alternative splicing of SRA-1, loss of coding frame, an increased expression is associated with tumor metastasis
TERRA	Telomeric UUAGG repeat-containing RNA, inhibits telomerase activity, also regulates Xist and HOTAIR
Tie-1AS	Expressed temporally and spatially in vivo with its native gene tie-1, binds tie-1 mRNA, regulating tie-1 transcripts; imbalance of sense

Tsix	Antisense transcript to Xist, prevents Xist stabilization and inhibits the interaction between Rep A and PRC2, silencing Xist expression
TUG1	Ubiquitously expressed in human and mouse cell types and tissues, involves eye development, upregulated by p53, repressed cell proliferation via bound to PRC2
UCA1	Urothelial carcinoma-associated transcript, upregulated in bladder carcinoma and embryo, influences cell growth and promotes invasion
VL30-1	A mouse noncoding retroelement RNA, binds and releases PSF from a proto-oncogene, thus activating Rab23 proto-oncogene transcription
Xist	Mosaic expression, spreads on Xi *in cis,* interacts with BRCA1, correlated with breast cancer, cervical, ovarian, and testis tumors
Zeb2NAT	Antisense to Zeb2, regulates splicing of the IRES-containing intron of Zeb 2, involved in EMT

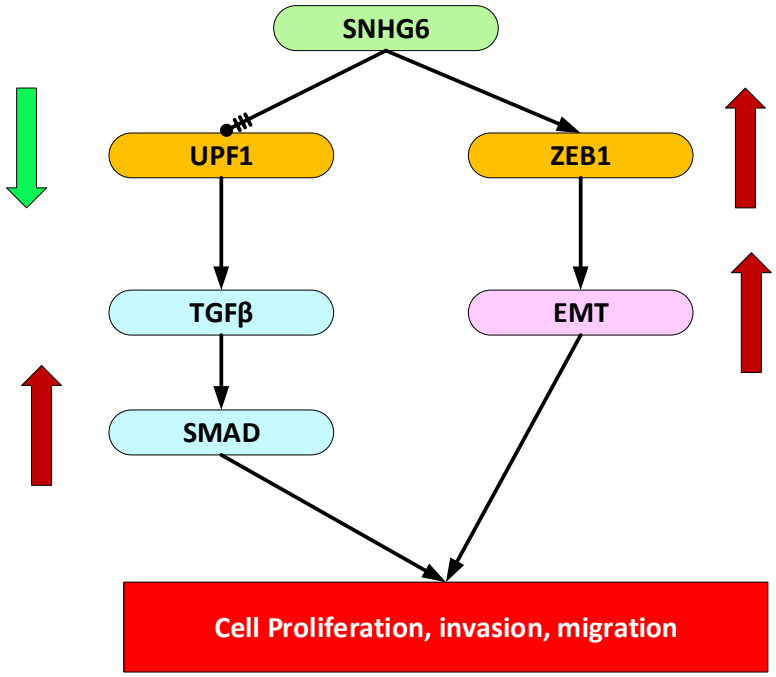
A similar result is from Ulitsky and Bartel which shows the number of identified lncRNAs determined by a number of investigators. The numbers go from just over 3,000 to almost 15,000. The functions of these lncRNAs is still less well understood than for example the miRNAs. They do however play a significant role in epigenetic control, especially in cancer dynamics.

Reference	***Data for Transcript Reconstruction***	***Genomic Features and Filters***	***Coding-Potential Filters***	***Number of lincRNAs***
Khalil et al., 2009	Chromatin marks, tiling arrays	Collection of approximate exonic regions, chromatin domain > 5kb	CSF	3,289 loci
Jia et al., 2010	cDNAs	Overlap with mRNAs allowed		5,446 transcripts
Orom et al., 2010	cDNAs	Restricted to loci >1 kb away from known protein-coding genes, > 200 nt mature length	Manual curation based on length, conservation and other characteristics of the ORFs	3,019 transcripts from 2,286 loci
Cabili et al., 2011	RNA-seq	Multi-exon only, > 200 nt mature length	PhyloCSF, Pfam	8,195 transcripts (4,662 in the stringent set)
Derrien et al., 2012	cDNAs	Overlap with mRNAs allowed (intergenic transcripts reported separately), > 200 nt mature length	Manual curation based on length, conservation and other characteristics of the ORFs	14,880 transcripts from 9,277 loci, including 9,518 intergenic transcripts

Sigova et al., 2013	RNA-seq, cDNAs, chromatin marks,	Antisense overlap with mRNA introns allowed, > 100 nt mature length	CPC	3,548 loci from embryonic stem cells, and 3,986 loci from endodermal cells

2.3 LINEAR LNCRNA

From Wang et al the following depicts the impact of a specific lncRNA, SNHG6, on colon cancer proliferation. It activates the TGF/SMAD pathway by binding to UPF1 and inhibiting while simultaneously upregulating ZEB1. The result is proliferation and EMT.



As Liz and Esteller have noted:

The lncRNA category comprises a very heterogeneous subclass that was first described by genome-wide sequencing of cDNA libraries of the mouse genome. It is becoming clear that a large number of lncRNAs are determining regulators of normal tissue physiology and the processes of diseases such as cancer and neurological disorders, despite so often being branded as transcriptional noise. As a result, much effort is currently being made to characterize those genetic, epigenetic and transcriptional changes that occur during disease…

In spite of their importance, the mechanisms that govern the control of lncRNAs are largely unknown but there is some evidence that demonstrate that they are regulated by a similar mechanism to that controlling protein-coding genes and microRNAs. However, there are some discrepancies about the transcriptional regulation of lncRNAs by epigenetic mechanisms, especially DNA methylation.

Thus, the lncRNA can be pervasive and their effects significant yet current understanding seems somewhat lagging.

2.4 LNCRNA AND CANCERS

Huarte has noted that lncRNAs play a significant role in cancers. The author notes:

Multiple lncRNAs are subjected to transcriptional regulation by factors that control fundamental aspects of cellular homeostasis. Much of the evidence for this comes from genome-wide studies that reveal that transcription factors, such as p53, MYC, or the estrogen receptor, or signaling cascades such as Notch, specifically regulate the expression of a substantial number of lncRNAs.

For example, after DNA damage or oncogenic stress, the transcription factor p53, a preserver of cellular homeostasis, initiates a tumor suppressor program that involves the induction of many genes, including dozens of lncRNAs.

Some of these are direct transcriptional targets of p53. Among them, the mouse tumor protein p53 pathway corepressor 1 lincRNA-p21 (officially known as Trp53cor1) promotes apoptosis by contributing to p53-dependent transcriptional repression through its interaction with the protein heterogeneous nuclear ribonucleoprotein K (Hnrnpk). In addition, the human lncRNAs (PANDAR) and LINC-PINT38 act as regulators of p53-dependent apoptosis and cell cycle arrest, depending on the cellular context, by mediating transcriptional and epigenetic repression of gene expression, respectively.

In contrast, the lncRNA induced by p53 lncRNA activator of enhancer domains (LED), contributes to p53 transcriptional regulation by interacting with p53 transcriptional enhancers.

Consistent with their role in the p53 response, several human p53-regulated lncRNAs are downregulated in colorectal cancer, or, similarly to LED, epigenetically silenced in acute lymphocytic leukemia, among other tumor types, suggesting a role for them as tumor suppressors. In addition, other lncRNAs are involved in the p53 network without necessarily being transcriptional targets of p53, such as the maternally expressed gene 3 (MEG3) lncRNA, which is downregulated in multiple cancers, is involved in p53 regulation and has a concomitant effect on cell survival and proliferation.

In contrast to lncRNAs that are involved in the p53 tumor-suppressor pathway, the expression of numerous lncRNAs is regulated by the proto-oncogene MYC. Some of them, such as MYCLo-1 and MYCLo-2 (officially known as ELFN1 Antisense RNA 1, ELFN1-AS1), upregulated in colorectal cancer, are involved in MYC-dependent gene repression of some cell-cycle regulator genes. MYC resides in the 8q24 genomic region, which is the most frequently amplified region in human cancers. This 2-Mb region also contains several cancer-associated SNPs within enhancers that form tissue-specific long-range chromatin loops with the MYC gene. Several lncRNAs are expressed from this transcriptionally active region.

Among these, prostate cancer–associated transcript 1 (PCAT1) and prostate cancer–associated noncoding RNA 1, PRNCR1, contain SNPs that confer predispositions to prostate and both breast and prostate cancers, respectively, and the rs6983267 SNP, which resides in the colon cancer–associated transcript 2 CCAT2 gene and predisposes a host to prostate and colorectal cancer, shows allele-specific effects on the expression levels of another lncRNA contained in the region, colon cancer associated transcript 1, CCAT1, also known as CARLo-5.

Several of the lncRNAs in the amplified region regulate the transcription of MYC. For instance, CCAT1 has a role in MYC transcriptional regulation by promoting long-range chromatin looping. In contrast, the oncogenic lncRNA PCAT1, which promotes the proliferation of prostate cancer cells and is also co-amplified with MYC, induces MYC expression by a post-transcriptional mechanism and impairs double-stranded DNA break repair by inhibiting expression of the breast cancer 2 gene, BRCA2.

Also contained inside the 8q24 genomic region is PRNCR1. This has been linked to prostate cancer, and together with PCGEM1, it induces cell proliferation. They both interact with the androgen receptor protein, promoting androgen-receptor–mediated gene activation programs18. These results, nonetheless, have been questioned in another study92.

Finally, a recent study performed in mice using chromosome engineering demonstrated that amplification of the lncRNA plasmacytoma variant translocation 1 Pvt1, which is contained in the same genomic region and adjacent to Myc, correlates with c-Myc gene copy number gain, and that such a gain in Pvt1 increases Myc protein levels, indicating a probable cis-regulatory mechanism of Myc regulation by Pvt1. Although these few studies represent only a small sample, they illustrate the large diversity of strategies by which lncRNAs may regulate tumor suppressors or oncogenes to influence cancer phenotypes.

Prensner and Chinnaiyan have discussed many lncRNAs and their related cancers. The Table below is based on their work:

lncRNA	**Function**	**Cancer type**	**Cancer phenotype**	**Molecular interactors**
HULC	Biomarker	Hepatocellular	Not known	Unknown
PCA3	Biomarker	Prostate	Not known	Unknown
ANRIL/p15AS	Oncogenic	Prostate, leukemia	Suppression of senescence via INK4A	Binds PRC1 and PRC2
HOTAIR	Oncogenic	Breast, hepatocellular	Promotes metastasis	Binds PRC2 and LSD1
MALAT1/NEAT2	Oncogenic	Lung, prostate, breast, colon	Unclear	Contributory to nuclear paraspeckle function
PCAT-1	Oncogenic	Prostate	Promotes cell proliferation; inhibits BRCA2	Unknown

lncRNA	Function	Cancer type	Cancer phenotype	Molecular interactors
PCGEM1	Oncogenic	Prostate	Inhibits apoptosis; promotes cell proliferation	Unknown
TUC338	Oncogenic	Hepatocellular	Promotes cell proliferation and colony formation	Unknown
uc.73a	Oncogenic	Leukemia	Inhibits apoptosis; promotes cell proliferation	Unknown
H19	Oncogenic; tumor suppressive	Breast, hepatocellular	Promotes cell growth and proliferation; activated by cMYC; downregulated by prolonged cell proliferation	Unknown
GAS5	Tumor suppressive	Breast	Induces apoptosis and growth arrest; prevents GR-induced gene expression	Binds GR
linc-p21	Tumor suppressive	Mouse models of lung, sarcoma, lymphoma	Mediates p53 signaling; induces apoptosis	Binds hnRNP-k
MEG3	Tumor suppressive	Meningioma, hepatocellular, leukemia, pituitary	Mediates p53 signaling; inhibits cell proliferation	Unknown
PTENP1	Tumor suppressive	Prostate, colon	Binds PTEN-suppressing	Unknown

Liz and Esteller have noted:

Unlike microRNAs, the length of lncRNAs allows them to fold into intricate structures, by which they may perform their function as RNA sequences by themselves through secondary and tertiary structural determinants.

The lncRNAs are about 200-400 nucleotides in length. Unlike miRNA, which are on the order of 20 to 22, the lncRNA is long enough to have significant conformations bonding parts.

In general, lncRNA transcripts exhibit low-level but tissue-specific expression and, with some exceptions, their nucleotide sequence is poorly conserved. In the last few years, several studies have shown the importance of lncRNAs to normal physiology as well as to the control of gene expression, wherein they modulate key cellular processes such as cell proliferation, senescence, migration and apoptosis.

The issue is; are lncRNA epigenetic or a separate function. We have argued for their epigenetic classification. The tissue specific expression raises questions as regards to local cell environment as well.

The intrinsic ability of lncRNAs to interact with DNA, RNA and proteins by acting as guides, tethers, decoys and scaffolds offers the most compelling explanation of their ability to regulate gene expression, including epigenetic transcriptional control by their association with chromatin remodeling complexes, splicing, translation and protein stability.

Moreover, an increasing body of experimental evidence suggests connections between microRNAs and lncRNA. A new function for lncRNAs has become apparent from their ability to regulate microRNA activity by acting as either competitive endogenous RNAs for microRNAs or as microRNA sponges.

Considering that lncRNAs control key cellular processes, knowledge of the mechanisms by which lncRNA expression is regulated is the first step towards understanding the basic principles by which they exert their functions. Furthermore, many studies have shown that lncRNA expression is altered in a variety of human cancer types and that their expression pattern may be associated with metastasis and disease prognosis.

The complexity of the relationship between miRNA and lncRNA is compelling but it adds dramatically to the complexity of understanding the workings of the general pathways. Also, the inter-individual variability needs explanation for it most likely is a significant driver in the inter-patient responses to various therapeutics.

The expression of specific lncRNAs with oncogenic features is closely linked to the ability to promote matrix invasion of cancer cells and tumor growth. In spite of their importance, the mechanisms that govern the control of lncRNAs are largely unknown but there is some evidence that demonstrate that they are regulated by a similar mechanism to that controlling protein-coding genes and microRNAs.

However, there are some discrepancies about the transcriptional regulation of lncRNAs by epigenetic mechanisms, especially DNA methylation. In this context, genome studies involving 5Cm, H3K4me3, H3K9me3, H3K27me3 and H3K36me3 epigenetic marks associated with transcription start sites of lncRNAs common to various human cell types showed that the pattern of DNA methylation and H3K9me3 differs considerably between mRNAs and lncRNAs and that it does not seem to play a role in lncRNA expression.

Despite this disparity, it has been clearly demonstrated that, in addition to microRNAs, another singular class of lncRNAs transcribed from ultra-conserved regions of the genome is also subject to transcriptional silencing driven by epigenetic mechanisms such as DNA methylation and histone modifications. This highlights the importance of epigenetic disruption of lncRNA in human tumorigenesis.

As with proteins, over the past decade many studies have shown that some lncRNAs are under the regulation of microRNAs to reduce their stability. Given that the proper activity of these lncRNAs is essential to key cellular processes, changes in their expression can alter diverse

molecular pathways altering physiological processes. In this context, the role of HOTTIP has been found to be the most significant oncogenic lncRNA in human hepatocellular carcinoma (HCC).

The authors present an interesting Table relating lncRNA, miRNA and diseases.

lncRNA	***miRNA***	***Disease***
H19	Let-7	Pancreatic carcinoma
PTCSC3	miR-574	Thyroid cancer
CASC2	miR-21	Glioma
HOST2	Let-7b	Ovarian cancer
LincRNA-RoR	miR-145 miR-205	Endometrial cancer stem cells Breast cancer
HULC	miR-372	Hepatocellular carcinoma
loc285194	miR-211	Osteosarcoma tumors
ncNRFR	Let-7	Colonic malignant transformation
T-UCR Uc.283+A	miR-195	Colorectal cancer and neuroblastoma

2.5 CIRCULAR RNA

It is worth commenting on other non-coding RNAs, especially circular RNA. Adhikary et al note:

The importance of circular RNAs (circRNAs) in pathological processes like cancer is evident. Among the circRNAs, recent studies have brought circPVT1 under focus as the most potent oncogenic non-coding RNA. Recent studies on various aspects of circPVT1, including its biogenesis, molecular alteration and its probable role in oncogenesis, have been conducted for research and clinical interest.

In this review, a first attempt has been made to summarise the available data on circPVT1 from PubMed and other relevant databases with special emphasis on its role in development, progression and prognosis of various malignant conditions. CircPVT1 is derived from the same genetic locus encoding for long non-coding RNA lncPVT1; however, existing literature suggested circPVT1 and lncPVT1 are transcripted independently by different promoters.

The interaction between circRNA and microRNA has been highlighted in majority of the few malignancies in which circPVT1 was studied. Besides its importance in diagnostic and prognostic procedures, circPVT1 seemed to have huge therapeutic potential as evident from differential drug response of cancer cell line as well as primary tumors depending on expression level of the candidate. circPVT1 in cancer therapeutics might be promising as a biomarker to make the existing treatment protocol more effective and also as potential target for designing novel therapeutic intervention.

It would be interesting to see how these non-coding RNAs ultimately interact with various cancers.

2.6 ELIT-1 AND OTHER LNCRNAS

It appears that ELIT-1 is a new lncRNA with the interesting characteristics of interfering with the TGF/SMAD pathways. This type of behavior is not uncommon. Schmitt and Chang had described a specific lncRNA recently:

The human genome generates more than 10,000 long non-coding RNA (lncRNA) molecules, yet the functions of only several dozens of these transcripts are known. In a study published on Nature's website today, Yang et at.1 describe two lncRNAs that bind to, and govern the function of, the androgen receptor — a transcription factor that activates the expression of thousands of genes in response to the hormone androgen. The authors find that inhibition of these two lncRNAs can block the growth of prostate-cancer cells that are resistant to hormone therapy owing to a mutation in the androgen receptor…

A large proportion of lncRNAs associate with regulators of chromatin (DNA-protein complexes), and several are known to 'tag' specific chromosomal regions with particular chromatin marks that modulate the availability of the associated genes for expression4. Several prostate-cancer-specific lncRNAs have been identified, and some are associated with distinct subtypes of the disease5.

In 2012, the US Food and Drug Administration approved the use of the lncRNA PCA3 for the detection of prostate cancer6. But, despite the discovery of multiple cancer-related lncRNAs, functions for most of these remain unknown. Yang et at. report that PRNCR1 and PCGEM1, two lncRNAs seen at high levels in many aggressive prostate cancers, enhance androgen-receptor-associated transcriptional programs to promote this cancer's growth.

Schmitt and Chang continue:

The authors' ChIRP analysis (a recently developed method of RNA localization7) reveals that these lncRNAs localize to distal androgen- response elements on chromatin, co-localizing with the androgen receptor. In a surprisingly intricate series of events, PRNCR1 interacts with the acetylated carboxy terminus of the androgen receptor and recruits the enzyme DOT1L to methylate the amino terminus of this receptor; this step is necessary for the subsequent association of the androgen receptor with PCGEM1. PCGEM1 then interacts with the protein Pygo2, which can bind to H3K4me3 — a methyl mark on the chromatin- associated histone H3 protein that is prevalent at gene-promoter sequences.

From Wellner et al:

Invasion and metastasis of carcinomas is promoted by the activation of the embryonic 'epithelial to mesenchymal transition' (EMT) program, which triggers cellular mobility and subsequent dissemination of tumour cells. We recently showed that the EMT-activator ZEB1 (zinc finger E-box binding homeobox 1) is a crucial promoter of metastasis and demonstrated that ZEB1

inhibits expression of the microRNA-200 (miR-200) family, whose members are strong inducers of epithelial differentiation. Here, we report that ZEB1 not only promotes tumour cell dissemination, but is also necessary for the tumour-initiating capacity of pancreatic and colorectal cancer cells.

We show that ZEB1 represses expression of stemness-inhibiting miR-203 and that candidate targets of miR-200 family members are also stem cell factors, such as Sox2 and Klf4. Moreover, miR-200c, miR-203 and miR-183 cooperate to suppress expression of stem cell factors in cancer cells and mouse embryonic stem (ES) cells, as demonstrated for the polycomb repressor Bmi1. We propose that ZEB1 links EMT-activation and stemness-maintenance by suppressing stemness-inhibiting microRNAs (miRNAs) and thereby is a promoter of mobile, migrating cancer stem cells. Thus, targeting the ZEB1–miR-200 feedback loop might form the basis of a promising treatment for fatal tumours, such as pancreatic cancer.

3 TGFB

Transforming Growth Factor β is one of a multiplicity of powerful growth factors. These GF have powerful impacts on cells and their over expression is often found in malignant environments. Fundamentally the GF is produced by a source cell and then is attached to a target cell via a receptor and from that a cascade of response ensue.

As Morikawa et al (2016) have noted there are 33 knowns human TGF polypeptides. There are 3 TGF-β isoforms, BMP or bone morphogenetic proteins, 10 of them, growth and differentiation factors, GDPs, some 10 of them, some inhibins (5), Mullerian inhibiting substance, and Lefty A and B, Nodal, and myostatin. These ligands bind to a set of receptors and the result in a set of various useful and at time deleterious cellular actions. TGF-β are also known to regulate lncRNAs as we have seen herein. Also, TGF-β is known to be an active driver of EMT.

TGF is a family and its most well understood member is TGF-β1. To best understand its functioning, we must first understand its synthesis and activation and then as it migrates in the extracellular matrix, ECM, its impact on other cells. We first consider activation.

3.1 TGF ACTIVATION

The activation elements are best described by Kubiczkova et al:

Mature dimeric form of TGF-β, composed of two monomers stabilized by hydrophobic interactions and disulphide bridge, initiates intracellular signaling. The three TGF-βs are synthesized as pro-proteins (pro-TGF- βs) with large amino-terminal pro-domains (called latency associated proteins – LAPs), which are required for proper folding and dimerization of carboxy-terminal growth-factor domain (mature peptide).

This complex is called 'small latent complex' (SLC). After folding and dimerization, TGF-β dimer is cleaved from its propeptides in trans-Golgi apparatus by furin type enzymes; however, it remains associated with its pro-peptide through noncovalent interactions, creating 'large latent complex '(LLC). Most cultured cell types release latent TGF-β into extracellular matrix as LLC which in addition includes a 120–240 kDa glycoprotein called latent TGF-β binding protein (LTBP) [24]. LTBP is composed primarily of two kinds of cysteine-rich domains: EGF-like repeats (most of which are calcium-binding) and eight-cysteine domains.

LTBP participates in the regulation of latent TGF-β bioavailability by addressing it to the extracellular matrix (ECM). Nonactive TGF-β stays in ECM; its further activation is a critical step in the regulation of its activity. A number of papers have reported TGF-β activation by retinoic acid and fibroblast growth factor-2 (FGF-2) in endothelial cells, or by endotoxin and bleomycin in macrophages.

Further, a variety of molecules is involved in TGF-β activation. Proteases including plasmin, matrix metaloproteases MMP-2 and MMP-9, are TGF-β activators in vitro. Other molecules involved in the mechanism of activation are thrombospondin-1, integrins, such as αVβ6 or αVβ8,

or reactive oxygen species (ROS). Moreover, latent TGF-β present in conditional medium is activated by acid treatment (pH 4.5) in vitro. In vivo, a similar pH is generated by osteoclasts during bone resorption. Since the bone matrix deposited by osteoblasts is rich in latent TGF-β, the acidic environment created by osteoclasts in vitro might result in latent TGF-β activation.

The TGF comes out of the producing cell as a dimer and then works its way through the ECM. The authors continue:

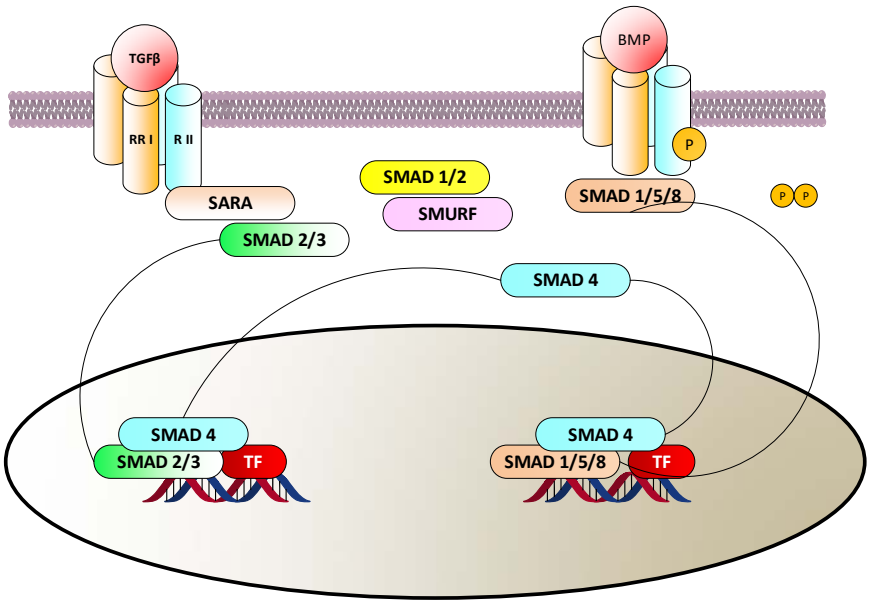
TGF-βs are synthesized as inactive precursors that contain pre-region (Signal peptide) and pro-region (N terminal peptide - LAP). Processing of inactive form starts with proteolytic cleavage that removes signal peptide from pre-pro-TGF-βs form. After dimerization, TGF-βs are cleaved by proteases (eg. Furin) into C-terminal mature peptides and N-terminal LAP (Latency Associated Peptide). TGF-βs with LAP form small latent complexes (SLP) that are transported to extracellular matrix where can further covalently bind to latent TGF-β binding protein (LTBP) to form a large latent complexes (LLC). LTBP is able to connect inactive TGF-β forms to ECM proteins.

This interaction is further supported by covalent transglutaminase-induced (TGase) crosslinks. Activation of TGF-β starts with release of LCC from ECM by proteases. Then, the mature protein is cleaved from LTBP, which is provided in vitro by acidic condition, pH or plasmin or in vivo by thrombospondin (TSP). Once the active TGF-β family member is released from the ECM, it is capable of signaling.

When the TGF gets to a cell with an appropriate and activated receptor set then it can initiate the SMAD pathways for internal cellular actions. We consider these next.

3.2 TGF PATHWAYS

Once the TGF has been produced it finds its way to a target cell with an appropriate receptor, composed of a complex of Type I and II dimers. From Cantley et al we present a slightly modified TGF/SMAD interaction. We demonstrate two of the TGF actions as shown below:



Now TGFβ is described in NCBI as follows[3]:

This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins.

Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression.

The SMAD transcription factor then is activated and its response leads to many of the resulting malignant changes. We shall review SMAD in the following section. NCBI continues:

The encoded preproprotein is proteolytically processed to generate a latency-associated peptide (LAP) and a mature peptide, and is found in either a latent form composed of a mature peptide homodimer, a LAP homodimer, and a latent TGF-beta binding protein, or in an active form consisting solely of the mature peptide homodimer. The mature peptide may also form heterodimers with other TGFB family members.

This encoded protein regulates cell proliferation, differentiation and growth, and can modulate expression and activation of other growth factors including interferon gamma and tumor necrosis factor alpha. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease.

As Derynck et al have noted:

Epithelial and hematopoietic cells have a high turnover and their progenitor cells divide continuously, making them prime targets for genetic and epigenetic changes that lead to cell transformation and tumorigenesis. The consequent changes in cell behavior and responsiveness

[3] https://www.ncbi.nlm.nih.gov/gene/7040

result not only from genetic alterations such as activation of oncogenes or inactivation of tumor suppressor genes, but also from altered production of, or responsiveness to, stimulatory or inhibitory growth and differentiation factors.

Among these, transforming growth factor β (TGF-β) and it signaling effectors act as key determinants of carcinoma cell behavior. The autocrine and paracrine effects of TGF-β on tumor cells and the tumor micro-environment exert both positive and negative influences on cancer development. Accordingly, the TGF-β signaling pathway has been considered as both a tumor suppressor pathway and a promoter of tumor progression and invasion. Here we evaluate the role of TGF-β in tumor development and attempt to reconcile the positive and negative effects of TGF-β in carcinogenesis.

Connolly et al have noted:

Many advanced tumors produce excessive amounts of Transforming Growth Factor-β (TGF-β) which, in normal epithelial cells, is a potent growth inhibitor. However, in onco-genically activated cells, the homeostatic action of TGF-β is often diverted along alternative pathways. Hence, TGF-β signaling elicits protective or tumor suppressive effects during the early growth-sensitive stages of tumorigenesis. However, later in tumor development when carcinoma cells become refractory to TGF-β-mediated growth inhibition, the tumor cell responds by stimulating pathways with tumor progressing effects.

At late stages of malignancy, tumor progression is driven by TGF-β overload. The tumor microenvironment is a target of TGF-β action that stimulates tumor progression via pro-tumorigenic effects on vascular, immune, and fibroblastic cells. Bone is one of the richest sources of TGF-β in the body and a common site for dissemination of breast cancer metastases. Osteoclastic degradation of bone matrix, which accompanies establishment and growth of metastases, triggers further release of bone-derived TGF-β. This leads to a vicious positive feedback of tumor progression, driven by ever increasing levels of TGF-β released from both the tumor and bone matrix.

It is for this reason, that pharmaceutical companies have developed therapeutic agents that block TGF-β signaling. Nonetheless, the choice of drug design and dosing strategy can affect the efficacy of TGF-β therapeutics. This review will describe pre-clinical and clinical data of four major classes of TGF-β inhibitor, namely i) ligand traps, ii) antisense oligonucleotides, iii) receptor kinase inhibitors and iv) peptide aptamers. Long term dosing strategies with TGF-β inhibitors may be ill-advised, since this class of drug has potentially highly pleiotropic activity, and development of drug resistance might potentiate tumor progression.

Current paradigms for the use of TGF-β inhibitors in oncology have therefore moved towards the use of combinatorial therapies and short-term dosing, with considerable promise for the clinic.

3.3 DRIVERS OF EMT

Fuxe et al have noted:

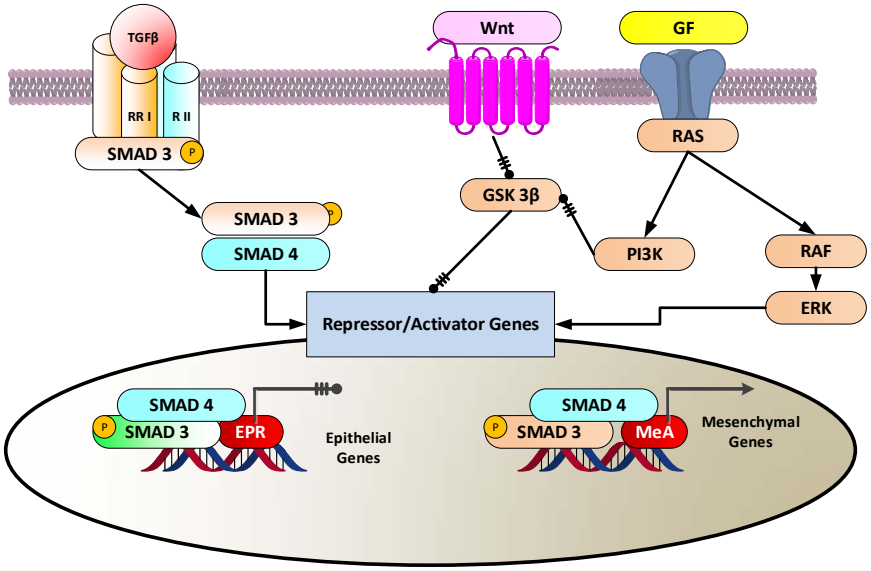
Transforming growth factor-beta *(TGF-β) is a major inducer of EMT during development and is overexpressed in many types of human cancer suggesting a role for TGF-β as an inducer of EMT in tumors. Paradoxically, TGF-β also has antiproliferative tumor suppressive effects and inactivating mutations or epigenetic silencing of various components of the TGF-β signaling pathway predisposes to cancer and inflammation indicating that the capacity of TGF-β to induce EMT is contextual.*

Concordantly, not all cultured epithelial cells undergo EMT in response to TGF-β. In contrast, few cultured cell lines, including Namru mammary gland epithelial cells (NMuMG) are highly sensitive to TGF-β induced EMT.

Accordingly, NMuMG cells are frequently used as a model system to study mechanisms of TGF-β induced EMT. TGF-β cooperates with Wnt, Hedgehog, Notch and Ras signaling pathways to induce complete EMT. Interestingly, these are pathways involved in the induction and maintenance of stem cell niches.

Thus, co-activation of stem cell pathways may shift the cellular response to TGF-β towards EMT. An explanation for this may lie in the subtle design of the TGF-β signaling pathway.

TGF-β binding to its receptors leads to activation of Smads, intracellular transcription factors and transducers of TGF-β signaling. Smad complexes are translocated into the nucleus where they regulate transcription of TGF-β target genes.



The Epithelial Repressor genes are Snail, Zeb and Twist whereas the Mesenchymal Activator genes are AP-1, TCF, Foxc2, SP1, and β catenin. The above is a broadly-based description of how TGF and SMAD manage to suppress epithelial stasis and activate mesenchymal. The authors detail this in the following Table.

Cofactor	Smad partner	Target genes	Role in EMT
Snail1	Smad3/4	Cxadr, Cdh1, Occln, Cldn3	repressor complex
Zeb1	Smad2/3/4	Cdh1	repressor complex
Zeb2	Smad1/2/3/5	Cdh1, Cldn4, Tjp3, Gjb2	repressor complex
Lef1/TCF	Smad3/4	Xtwn	Activator complex
β-catenin	Smad2/3	PAi-1, a-SMA	Activator complex
AP-1	Smad3	vimentin, eT-1, c-jun	Activator complex
SP1	Smad3	PAi-1, endoglin, a2 (i) collagen	Activator complex
HMGA2	Smad2/4	Snail	Activator complex

From Fuxe et al: Transcriptional crosstalk between TGF-β, Wnt and Ras signaling pathways in EMT. TGF-β binding to its receptor results in phosphorylation and nuclear translocation of Smad transcription factors, which achieve target gene specificity through interaction with transcriptional cofactors. EMT promoting transcription factors including epithelial repressors (EpR), such as Snail, Zeb and Twist, and mesenchymal activators (MeA), such as β-catenin (β-cat), AP-1, Foxc2, TCF and Sp1 interact with Smads, which results in the formation of EMT promoting Smad complexes (EPSC). These complexes drive EMT by repressing epithelial genes, such as E-cadherin, or activating mesenchymal genes, such as vimentin. Signals from Wnt and/or Ras pathways promote activation of Snail, Zeb, β-catenin and other EMT promoting transcription factors that can partner and form EPSC with Smads. Thus, the formation of EPSC represents a point of convergence between TGF-β, Wnt and Ras pathways. GSK-3β is a nodal protein, which negatively regulates stability of Snail and β-catenin. Activation of Wnt and Ras/PI3K/AKT pathways leads to inhibition of GSK-3β and thereby stabilization of Snail and β-catenin. TGF-β and Ras/Raf/ER K pathways also regulate EMT promoting transcription factors

As Ungefroren notes:

The transforming growth factor- (TGF-β) family of secreted growth factors controls many aspects of cell and tissue physiology in multicellular eukaryotes. Dysregulation of its pathway contributes to a broad variety of pathologies, including fibrosis and cancer. TGF-β acts as a powerful tumor suppressor in epithelial cells but during later stages of tumor development cancer cells eventually respond to this cytokine with epithelial-mesenchymal transition (EMT), invasion, metastasis, and immunosuppression. This collection of articles covers some important aspects of TGF-β signaling in cancer.

Two articles focus on the role of TGF-β in tumor immunity and pro- and anti-inflammatory signaling, with one analyzing its impact on T-cell biology and different T-cell subsets, while the other deals with modulation of anti-inflammatory signaling by TGFβ- receptors through proinflammatory signaling by immune receptors and the role of mechanotransduction in TGF-β -dependent immunosuppression. Another set of four chapters highlights the fact that context-dependent responsiveness to TGF-β is largely controlled by inputs from negative regulators and

cooperation with proinflammatory and proapoptotic pathways. This theme is extended to the regulation of Smad signaling by differential phosphorylation, eventually converting canonical Smad signaling to a mitogenic, fibrogenic and carcinogenic outcome. Last, it is discussed how another posttranslational modification, SUMOylation, can modify protein function and impact TGF-β -induced EMT, invasion and metastasis.

Connelly et al have noted:

Many advanced tumors produce excessive amounts of Transforming Growth Factor-β (TGF-β) which, in normal epithelial cells, is a potent growth inhibitor. However, in onco- genically activated cells, the homeostatic action of TGF-β is often diverted along alternative pathways. Hence, TGF-β signaling elicits protective or tumor suppressive effects during the early growth-sensitive stages of tumorigenesis. However, later in tumor development when carcinoma cells become refractory to TGF-B-mediated growth inhibition, the tumor cell responds by stimulating pathways with tumor progressing effects.

At late stages of malignancy, tumor progression is driven by TGF-β overload. The tumor microenvironment is a target of TGF-β action that stimulates tumor progression via pro-tumorigenic effects on vascular, immune, and fibroblastic cells. Bone is one of the richest sources of TGF-β in the body and a common site for dissemination of breast cancer metastases. Osteoclastic degradation of bone matrix, which accompanies establishment and growth of metastases, triggers further release of bone-derived TGF-β.

This leads to a vicious positive feedback of tumor progression, driven by ever increasing levels of TGF-β released from both the tumor and bone matrix. It is for this reason, that pharmaceutical companies have developed therapeutic agents that block TGF-β signaling. Nonetheless, the choice of drug design and dosing strategy can affect the efficacy of TGF-β therapeutics.

This review will describe pre-clinical and clinical data of four major classes of TGF-β inhibitor, namely i) ligand traps, ii) antisense oligonucleotides, iii) receptor kinase inhibitors and iv) peptide aptamers. Long term dosing strategies with TGF-β inhibitors may be ill-advised, since this class of drug has potentially highly pleiotropic activity, and development of drug resistance might potentiate tumor progression. Current paradigms for the use of TGF-β inhibitors in oncology have therefore moved towards the use of combinatorial therapies and short-term dosing, with considerable promise for the clinic.

4 SMAD

SMADs are a significant family of gene products that facilitate a variety of transcriptions. They are driven by the TGF family of ligands and have been known to have a place in cancer proliferation.

4.1 SMAD FAMILY

SMADs are a set of signal transducers that assist the TGF bindings to effect cellular action via expression of a variety of genes. As Hill notes:

The transforming growth factor-β (TGF-β) family of ligands elicit their biological effects by initiating new programs of gene expression. The best understood signal transducers for these ligands are the SMADs, which essentially act as transcription factors that are activated in the cytoplasm and then accumulate in the nucleus in response to ligand induction where they bind to enhancer/promoter sequences in the regulatory regions of target genes to either activate or repress transcription. …

The SMAD complexes have weak affinity for DNA and limited specificity and, thus, they cooperate with other site-specific transcription factors that act either to actively recruit the SMAD complexes or to stabilize their DNA binding. In some situations, these cooperating transcription factors function to integrate the signals from TGF-β family ligands with environmental cues or with information about cell lineage. Activated SMAD complexes regulate transcription via remodeling of the chromatin template. Consistent with this, they recruit a variety of coactivators and corepressors to the chromatin, which either directly or indirectly modify histones and/or modulate chromatin structure.

SMADs thus act in conjunction with other transcription factors. Moustakas et al have noted:

Smad proteins transduce signals from transforming growth factor-β (TGF-β) superfamily ligands that regulate cell proliferation, differentiation and death through activation of receptor serine/threonine kinases. Phosphorylation of ***receptor-activated*** *Smads (R-Smads) leads to formation of complexes with the common mediator Smad (Co-Smad), which are imported to the nucleus. Nuclear Smad oligomers bind to DNA and associate with transcription factors to regulate expression of target genes. Alternatively, nuclear R-Smads associate with ubiquitin ligases and promote degradation of transcriptional repressors, thus facilitating target gene regulation by TGF-β. Smads themselves can also become ubiquitinated and are degraded by proteasomes.*

Finally, the ***inhibitory Smads (I-Smads)*** *block phosphorylation of R-Smads by the receptors and promote ubiquitination and degradation of receptor complexes, thus inhibiting signalling.*

Namely there are multiple functions with various SMADs, some activate, some repress, some assist. Yet in all cases they have a close relationship with TGF.

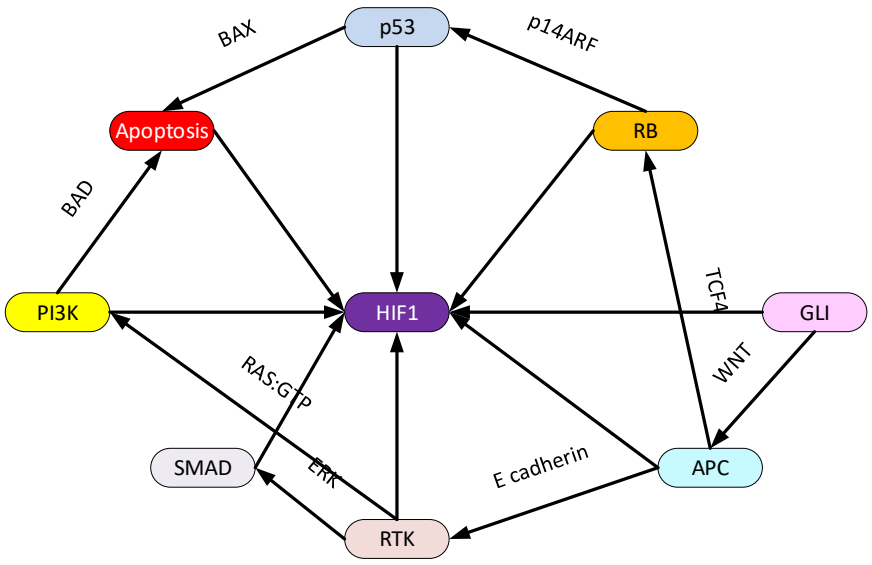
As Lamouille and Derynck have noted:

TGF-β family proteins signal through Smads, which combine with DNA sequence-specific transcription factors to activate or repress transcription. The Smad pathway, which, in the case of TGF-β, is mediated by Smad2 and Smad3 in combination with Smad4, is considered to be the major TGF-β family signaling pathway and accounts for the many changes in gene expression observed in response to TGF-β family proteins. TGF-β–induced non- Smad pathways have been identified and lead to the activation of Erk and JNK MAPK or RhoA, but how these pathways are activated in response to TGF-β is not well understood. In TGF-β– induced EMT, Smad signaling represents an essential pathway that confers changes in gene expression through cooperation with transcription factors such as Snail, Slug, and/or Id. Non-Smad signaling in response to TGF-β (e.g., activation of RhoA) also contributes to EMT and is important for the associated cytoskeletal and phenotypic changes.

Our results now show that TGF-β can increase protein synthesis in EMT through the mTOR pathway, leading to the regulation of S6K1 and 4E-BP1 activities. The activation by TGF-β of a pathway that leads directly to increased protein synthesis stands in contrast with the changes in gene transcription through the Smad pathway.

Thus, in addition to changes in gene expression, TGF-β signaling through mTOR leads to the enhanced translation of proteins that contribute to the behavior of cells that undergo EMT. Accordingly, studies using rapamycin have implicated mTOR in the regulation of collagen synthesis. A characterization of the relative changes in protein levels that are independent of changes in gene expression and can be blocked by rapamycin will provide insight into the contribution of mTOR signaling to the cell's response to TGF-β.

Therelationships of SMAD and other key genes is summarized in the following Figure.



Now there are a set of differing SMADs as noted by Moustakas et al who state:

Functionally, Smads fall into three subfamilies:

(i) receptor-activated Smads (R-Smads: Smad1, Smad2, Smad3, Smad5, Smad8), which become phosphorylated by the type I receptors;

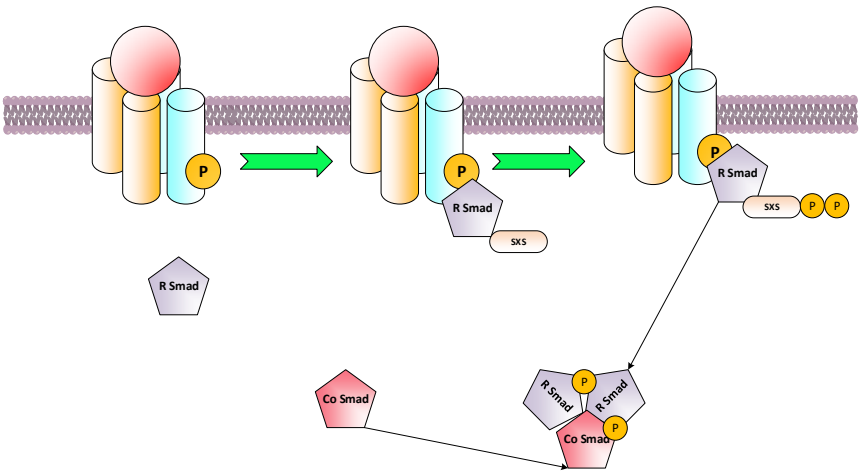
(ii) common mediator Smads (Co-Smads: Smad4), which oligomerise with activated R-Smads; and

(iii) inhibitory Smads (I-Smads: Smad6 and Smad7), which are induced by TGF-b family members. The latter exert a negative feedback effect by competing with RSmads for receptor interaction and by marking the receptors for degradation.

We shall see that TGF and its elements react with all but SMAD4 which is internal to the cell and operates in conjunction with the other SMADs.

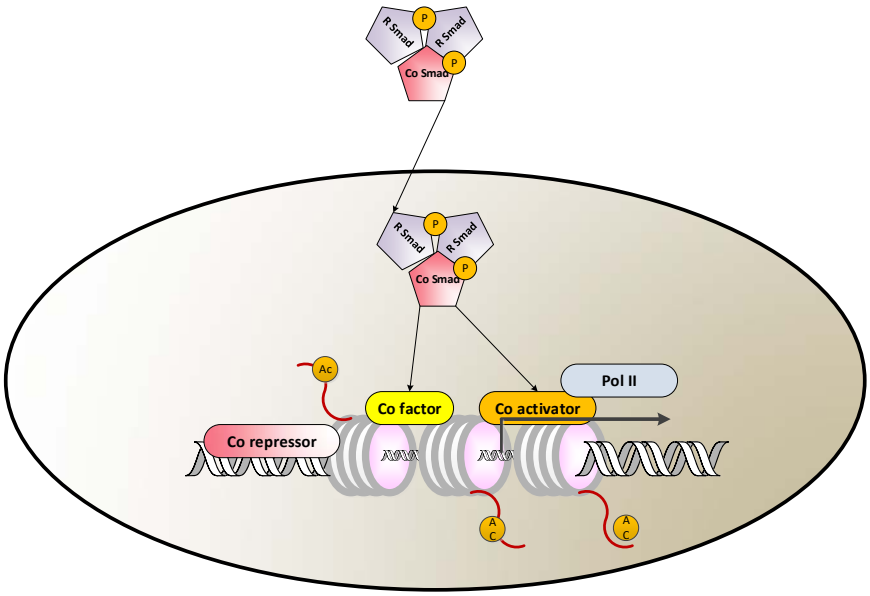
4.2 SMAD FUNCTIONING

We now detail some of the specific SMAD functioning. We see R Smads, Co Smads and I Smads. The Figure below shows the action in the cell cytoplasm. There is a ligand binding a phosphorylation and then a binding with a Co Smad.



Feng and Derynck

This amalgam then enters the nucleus and acts upon the DNS which is still wrapped in histones and via co-factors and polymerase can result in the expressing of the targeted gene.

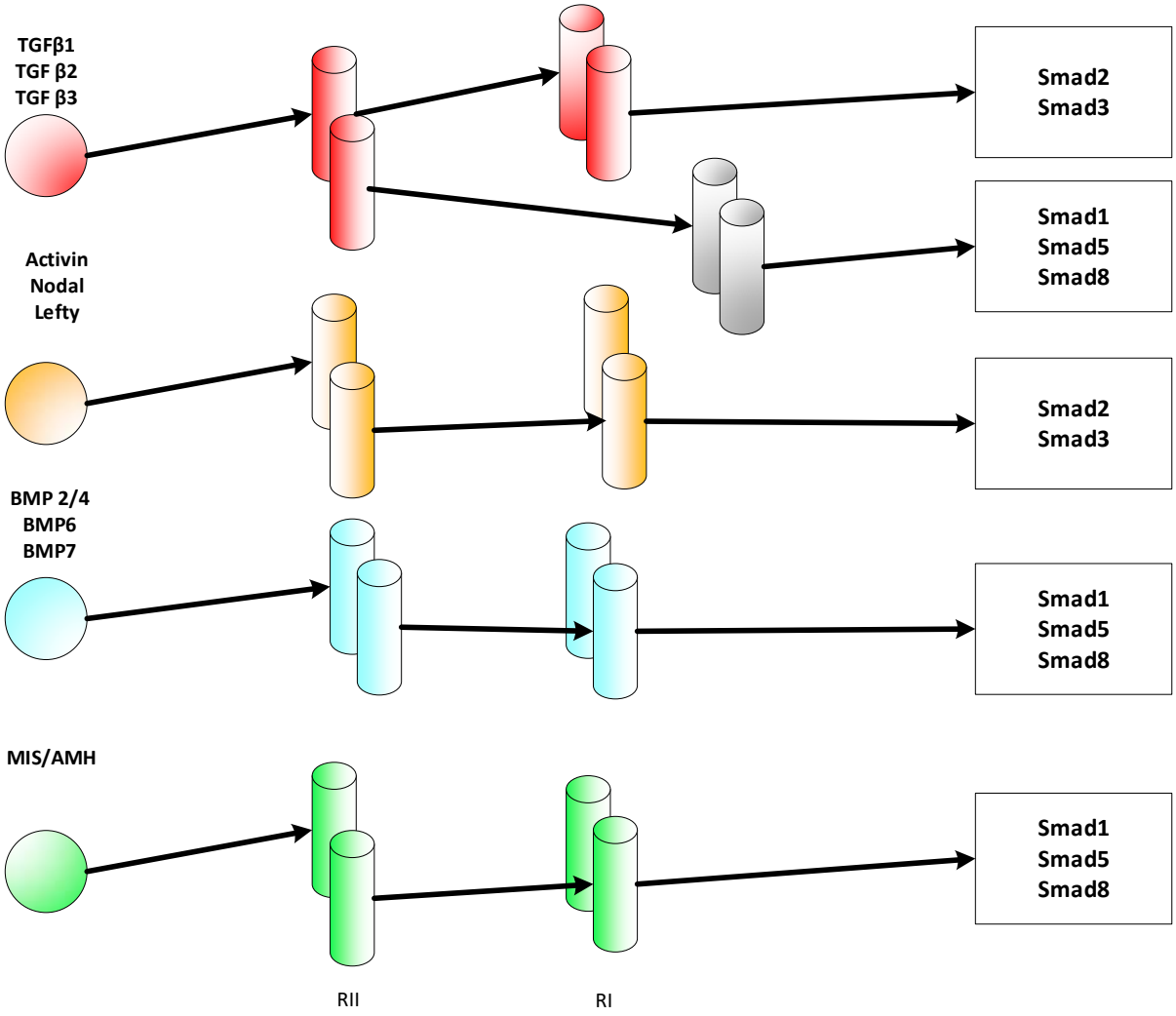


4.3 SUPERFAMILY

Finally, we summarize the SMAD family as a means to best understand its interactions. We use the Figures as modified from Fang and Derynck. First the families of the various SMADs. The authors note:

The TGF-β family comprises many structurally related differentiation factors that act through a heteromeric receptor complex at the cell surface and an intracellular signal transducing Smad complex. The receptor complex consists of two type II and two type I transmembrane serine/threonine kinases. Upon phosphorylation by the receptors, Smad complexes translocate into the nucleus, where they cooperate with sequence-specific transcription factors to regulate gene expression. The vertebrate genome encodes many ligands, fewer type II and type I receptors, and only a few Smads. In contrast to the perceived simplicity of the signal transduction mechanism with few Smads, the cellular responses to TGF-β ligands are complex and context dependent. This raises the question of how the specificity of the ligand-induced signaling is achieved. We review the molecular basis for the specificity and versatility of signaling by the many ligands through this conceptually simple signal transduction mechanism.

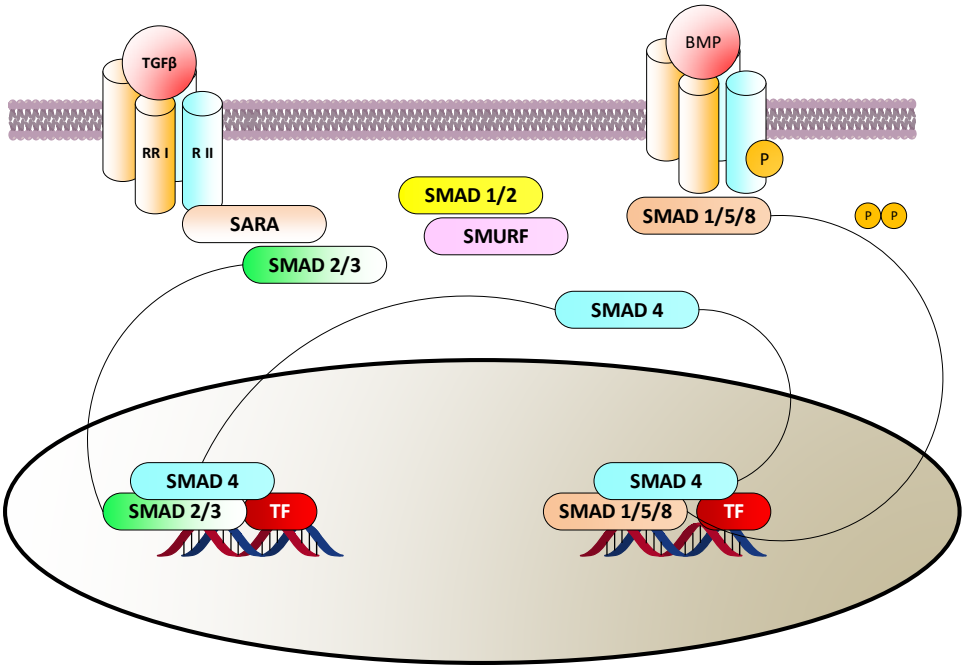
Namely the TGF receptors are a type I and type II each in a dimer configuration and the TFG ligand attaches to the receptor complex. We have shown this above. But now the specific TGF as a ligand activates a different SMAD pathway activity as we shown in the Figure.



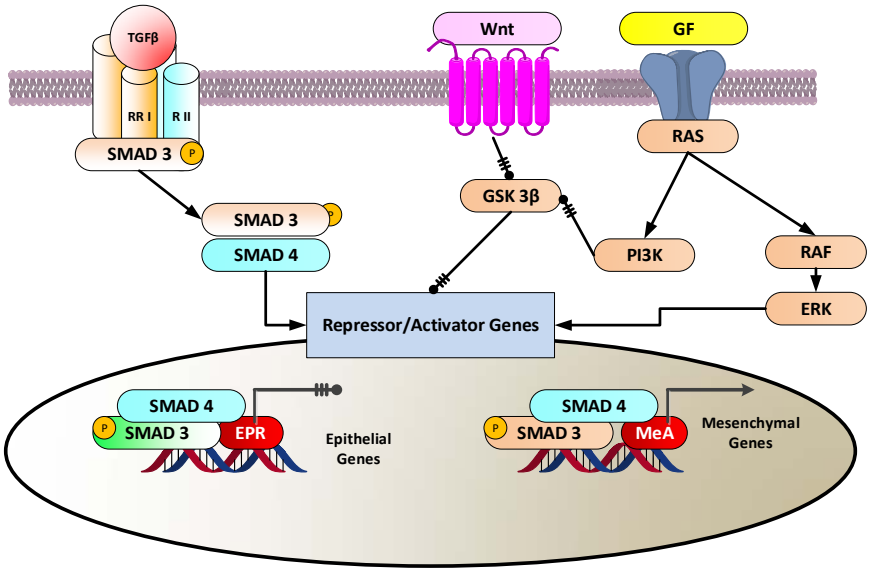
We can summarize these in some detail in the following Table. Note that there are interactions with RII and RI complexes as shown in the Figure. There are multiple TGF ligands and in turn multiple SMAD reactions.

Ligand	*R II*	*R I*	*SMAD Activation*
TGF-β1 TGF-β2 TGF-β3	TβRII	TβRI	Smad2 Smad3
TGF-β1 TGF-β2 TGF-β3	TβRII	ALK1	Smad1 Smad5 Smad8
Activin Nodal Lefty	ActRII ActRIIB	ACTRIB ALK7	Smad 2 Smad 3
BMP 2/4 BMP 6 BMP 7	BMPRII BMPRIIB	BMPRIA BMPRIB	Smad1 Smad5 Smad8
MIS/AMH	MISRII	ALK2	Smad1 Smad5 Smad8

The specific actions can be combined as we show below. Here we show both TGFβ1 and BMP each on their own receptors and thus activation separate SMADs and in turn the SMADs entering the nucleus and assisting as transcription factor adjuncts in gene expression.

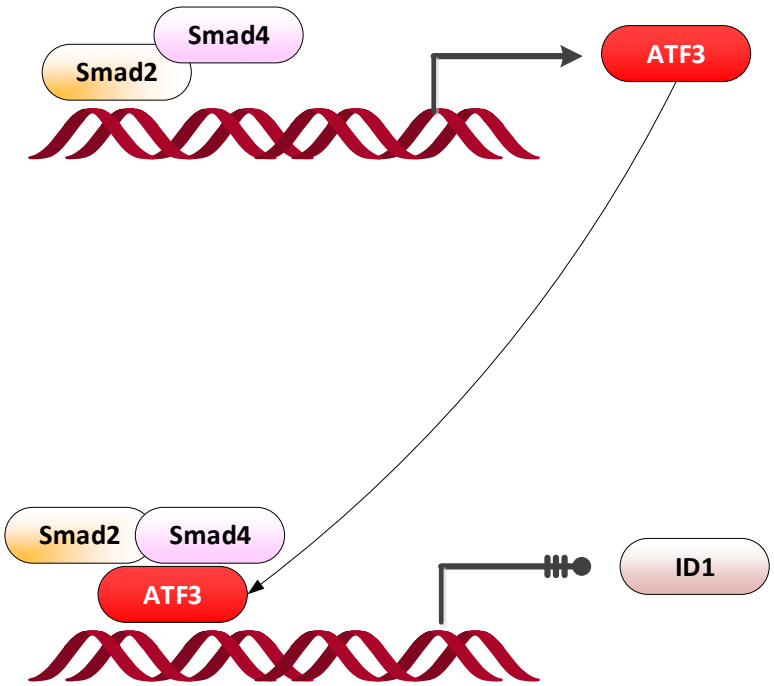


From Fuge et al we also have a detailed description of other ligand and receptors and their actions on other pathway elements and in turn on Smads.:

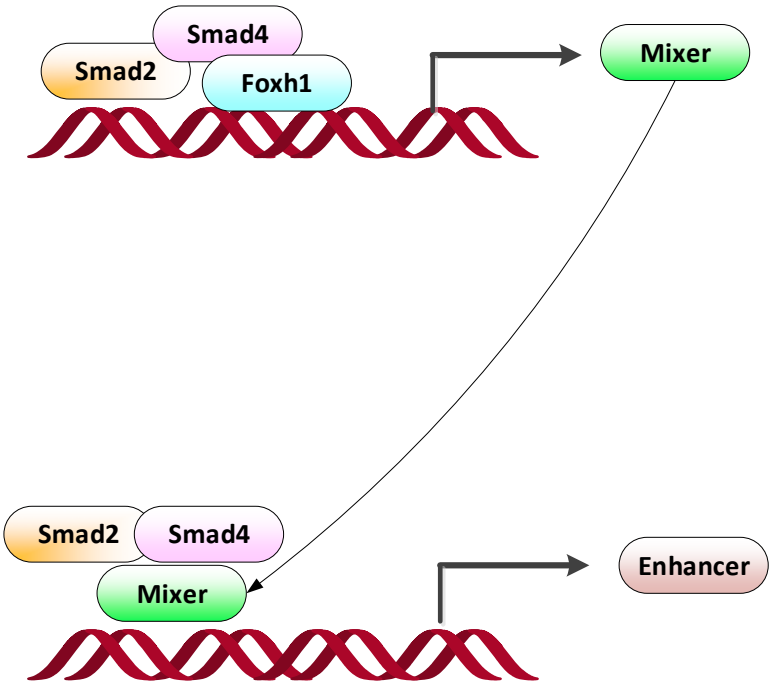


4.4 ACTIONS

SMADs can effect a multiplicity of actions. Below from Hill we show a self-enabling (negative) control:



The next is the self-enabling (positive) control.


Smad4
Smad2
Foxh1
Mixer
Smad2
Smad4
Mixer
Enhancer

5 EMT REDUX

We have examined the epithelial mesenchymal transition, EMT, extensively elsewhere. Our intent here is to highlight some of the nexus with TGF and SMADs[4].

Singh and Settleman have noted:

Tumors are cellularly and molecularly heterogeneous, with subsets of undifferentiated cancer cells exhibiting stem cell-like features (CSCs). Epithelial to mesenchymal transitions (EMT) are trans-differentiation programs that are required for tissue morphogenesis during embryonic development. The EMT process can be regulated by a diverse array of cytokines and growth factors, such as transforming growth factor (TGF)-p, whose activities are dysregulated during malignant tumor progression.

Thus, EMT induction in cancer cells results in the acquisition of invasive and metastatic properties. Recent reports indicate that the emergence of CSCs occurs in part as a result of EMT, for example, through cues from tumor stromal components. Recent evidence now indicates that EMT of tumor cells not only causes increased metastasis, but also contributes to drug resistance. In this review, we will provide potential mechanistic explanations for the association between EMT induction and the emergence of CSCs. We will also highlight recent studies implicating the function of TGF-β-regulated noncoding RNAs in driving EMT and promoting CSC self-renewal. Finally, we will discuss how EMT and CSCs may contribute to drug resistance, as well as therapeutic strategies to overcome this clinically.

From Craene and Berx we have the following:

During epithelial to mesenchymal transition (EMT) epithelial cells are converted to migratory and invasive cells. This process has been considered to be a fundamental event in morphogenesis as it is intimately involved in the generation of tissues and organs during embryogenesis of both vertebrates and invertebrates. A similar process is recapitulated during wound healing, a classical example of a process in adulthood in which EMT is important. It has now been more than 10 years since EMT was first demonstrated to be closely related to cancer progression. …

Endogenous expression of EMT-TFs has been found in various tissues and is not necessarily coupled to the dedifferentiation of tumour cells. Therefore, their actual role is broader than the name EMT-TF suggests. A thorough understanding of their molecular function can increase our knowledge of their specific contribution in the context of cancer. Indeed, EMT-TFs have been involved not only in migration and invasion but also in the suppression of senescence and apoptosis, attenuation of cell-cycle progression and resistance to radiotherapy and chemotherapy.

Importantly, these nuclear drivers of EMT seem to be involved in both differentiation and dedifferentiation, and this implies that their function is compatible with both growth suppression

[4] See McGarty, EMT and Cancer

and stimulation, depending on the context… In addition to transcriptional induction of EMT-TF and activation of the miR-200 family, TGFp also controls EMT through the regulation of a crucial translational checkpoint at the elongation stage of EMT effector transcripts… So far, TGFp-mediated EMT induction clearly exemplifies how the action of one growth factor results in a regulatory network connecting transcriptional, noncoding and translational levels of EMT control. Further understanding of how the various methods of EMT induction and their associated sophisticated control mechanisms are interconnected and linked to different signal transduction pathways should help us to better understand tumour progression.

As Lamouille and Derynck have noted:

Epithelial to mesenchymal transition (EMT) occurs during development and cancer progression to metastasis and results in enhanced cell motility and invasion. Transforming growth factor-β (TGF-β) induces EMT through Smads, leading to transcriptional regulation, and through non-Smad pathways.

We observe that TGF-β induces increased cell size and protein content during EMT. This translational regulation results from activation by TGF-β of mammalian target of rapamycin (mTOR) through phosphatidylinositol 3-kinase and Akt, leading to the phosphorylation of S6 kinase 1 and eukaryotic initiation factor 4E–binding protein 1, which are direct regulators of translation initiation. Rapamycin, a specific inhibitor of mTOR complex 1, inhibits the TGF-β–induced translation pathway and increase in cell size without affecting the EMT phenotype. Additionally, rapamycin decreases the migratory and invasive behavior of cells that accompany TGF-β–induced EMT.

The TGF-β–induced translation pathway through mTOR complements the transcription pathway through Smads. Activation of mTOR by TGF-β, which leads to increased cell size and invasion, adds to the role of TGF-β–induced EMT in cancer progression and may represent a therapeutic opportunity for rapamycin analogues in cancer. …

Our observations on the activation of mTOR signaling and the increase of cell size in response to TGF-β during EMT may have considerable clinical relevance. Indeed, increased autocrine TGF-β expression and responsiveness leading to TGF-β–induced EMT are considered to be important initiators of the invasive behavior of cancers during cancer progression.

Our findings that TGF-β–induced EMT is accompanied by increased cell size and that the increased cell size and the EMT-associated increase in invasive behavior are mediated by increased mTOR signaling in response to TGF-β suggest that rapamycin analogues can be used to antagonize some key features of EMT that contribute to cancer progression.

We find the last observation of interest. Relating histological, cytological and genetic relationships is a slowly evolving field. All too often the pathologist reports on phenotypical characteristics and the underlying genotypical structure is ignored or poorly understood. Observations of this type are essential for establishing a nexus between the two.

Finally, Yang and Weinberg in an excellent paper on EMT have noted:

In cancer cells, the TGF-b signaling pathway induces multiple EMT-inducing transcription factors, including Slug, SIP1, and Goosecoid, via activation of Smads.

The Wnt pathway and loss of E-cadherin from adherens junctions activate b-catenin, which in turn induces several EMT-inducing transcription factors as well, such as Slug, Twist1, and Goosecoid. Multiple tyrosine kinase receptor (TKR) pathways, including FGFR, EGFR, PDGFR, and HGFR, can induce the expression of Snail and Slug through the Ras- MAPK pathway.

The Wnt and tyrosine kinase receptor pathways also modulate Snail nuclear transport and degradation through GSK3b.

The Notch pathway is induced by and required for TGF-β-induced EMT. Activated ROS, through Rac1b, is capable of promoting an EMT in tumor cells, though the inducing signal is unknown.

Among all the EMT-inducing transcription factors, Snail, Slug, SIP1, and E47 directly suppress E-cadherin transcription, while Twist1, Goosecoid, and FOXC2 seem to function directly. FOXC2 is induced in tumor cells expressing Twist1, Snail, and Goosecoid and mediates mesenchymal differentiation. Although almost all the aforementioned pathways are associated with cell migration and invasion, the specific inducers of migration and invasion during EMT are not well understood. Solid lines indicate direct transcriptional or posttranscriptional regulations. Dashed lines indicate indirect regulation.

One can see that the TGF-β pathway is intertwined with a significant mass of others.

6 OBSERVATIONS

The following are some observations based upon the examination of the paper discussed herein.

6.1 FEEDBACK AND NON-CODING RNAS

The example we commenced with in this Note was an interesting one since it did not block or simply activate but it created a positive feedback path that amplified the overall result. It is well known that many genes can act in this way but the interaction of the lncRNA seems somewhat unique. One suspects that there may be many others. It does beg the question of what drives the expression of the lncRNA.

6.2 EPIGENETIC FACTORS

The gene is often wrapped tightly in a histone ring which may have acetylation and methylation on it. However, the opening of the histone rings and wraps need to be accomplished for both the lncRNA as well as the gene to be expressed. The question then is; what are the epigenetic factors expressed here and further what are the epigenetic factors relating to the lncRNA? Namely, is the lncRNA a mis-expressed gene product resulting from some methylation blockage or a commonly occurring genetic product?

6.3 THERAPEUTIC TARGETING

If there is a causative relationship between the presence of the lncRNA and a cancer, is there some therapeutic target available. On one extreme it may be akin to a kinase inhibitor but then again, we are looking at something in the nucleus and not the cytoplasm. Likewise does the presence of this lncRNA express itself as a surface marker recognizable by the immune system approach?

6.4 IMMUNE SYSTEM RESPONSE

Multiple immune response mechanisms have been employed across a wide set of malignancies. However as is often the case not every patient responds equally. The recent work seems to indicate a significant role for TGF in this lack of response.

Mariathasan et al have reported:

Therapeutic antibodies that block the programmed death-1 (PD-1)– programmed death-ligand 1 (PD-L1) pathway can induce robust and durable responses in patients with various cancers, including metastatic urothelial cancer.

However, these responses only occur in a subset of patients. Elucidating the determinants of response and resistance is key to improving outcomes and developing new treatment strategies.

Here we examined tumours from a large cohort of patients with metastatic urothelial cancer who were treated with an anti-PD-L1 agent (atezolizumab) and identified major determinants of clinical outcome. Response to treatment was associated with CD8+ T-effector cell phenotype and, to an even greater extent, high neoantigen or tumour mutation burden. Lack of response was associated with a signature of transforming growth factor β (TGFβ) signalling in fibroblasts.

This occurred particularly in patients with tumours, which showed exclusion of CD8+ T cells from the tumour parenchyma that were instead found in the fibroblast- and collagen-rich peritumoural stroma; a common phenotype among patients with metastatic urothelial cancer. Using a mouse model that recapitulates this immune-excluded phenotype, we found that therapeutic co-administration of TGFβ-blocking and anti-PD-L1 antibodies reduced TGFβ signalling in stromal cells, facilitated T-cell penetration into the centre of tumours, and provoked vigorous anti-tumour immunity and tumour regression.

Integration of these three independent biological features provides the best basis for understanding patient outcome in this setting and suggests that TGFβ shapes the tumour microenvironment to restrain anti-tumour immunity by restricting T-cell infiltration.

6.5 THERAPEUTIC IDENTIFICATION VIA EXCEPTIONS

Xie et al have recently summarized therapeutic efforts as follows:

MicroRNAs and long noncoding RNAs have long been investigated due to their roles as diagnostic and prognostic biomarkers of cancers and regulators of tumorigenesis, and the potential regulatory roles of these molecules in anticancer therapies are attracting increasing interest as more in-depth studies are performed. The major clinical therapies for cancer include chemotherapy, immunotherapy, and targeted molecular therapy.

MicroRNAs and long noncoding RNAs function through various mechanisms in these approaches, and the mechanisms involve direct targeting of immune checkpoints, cooperation with exosomes in the tumor microenvironment, and alteration of drug resistance through regulation of different signaling pathways. Herein we review the regulatory functions and significance of microRNAs and long noncoding RNAs in three anticancer therapies, especially in targeted molecular therapy, and their mechanisms…

miRNAs and lncRNAs, subcategories of ncRNAs, have primarily been investigated as biomarkers for predicting the initiation and development of cancer, but they have recently been discovered to be involved in the curative process of three clinically adopted therapies. These molecules enhance or suppress cancer cell responses to chemotherapy drugs and targeted drugs indirectly by modulating relevant pathways, and they also affect immune checkpoint blockage therapy directly by altering the expression of PD-1/PD-L1. Overexpressing miRNAs and lncRNAs by mimics and silencing these molecules by small interfering RNAs (siRNAs) verify their therapeutic capacity in suppressing aggressive cell phenotypes and alleviating drug resistance.

Furthermore, rapid advances in elucidating the roles of miRNAs and lncRNAs in anticancer therapies have revealed several opportunities and challenges to address in the future. One opportunity is cooperation with extracellular vesicles, especially exosomes. As mentioned above, exosome-mediated miR-503 reduced chemoresistance after it was transferred from endothelial cells to tumor cells.

Studies have demonstrated the communication shuttle function of exosomes between cells and that exosome-associated ncRNAs fulfill important jobs in regulating gene expression in cancer. However, more work on the therapeutic value of exosome associated ncRNAs in cancer is needed. Second, miRNA-miRNA and miRNA-lncRNA networks reveal the complexity of ncRNA mediated mechanisms in anticancer therapies, providing a better understanding of the ncRNA-mediated drug response and creative research approaches.

One outstanding problem is whether ectopic miRNAs and lncRNAs actually function in vivo, and more research utilizing convenient in vivo model systems are needed. Future studies will likely focus on ncRNA-based drug development and integrated clinical trials, which may lead to a cure for cancer. Additionally, the investigation of circular RNAs, another ncRNA research hotspot, is needed to improve our understanding of the ncRNA therapeutic network.

6.6 LNCRNA BLOOD BORNE MARKERS

For many cancers we now have a multiplicity of blood borne markers. Does the presence of the lncRNA produce any such marker? If so, then how specific can it be?

6.7 QUASI HORMONE ACTION

The production of a lncRNA may be perceived to act as a hormone by means of its modifying other genes and their expression. As Pardini and Calin have recently noted:

A special mention should be made for lncRNAs, since their biological roles and mechanisms of action are not yet completely understood, especially in the context of carcinogenesis. Assigning molecular, cellular, and physiological functions to lncRNAs is among the greatest challenges of the next decade, and there is now increased attention on their biological functions in hormonal signaling systems. lncRNAs are defined as non-protein coding RNA transcripts larger than 200 nucleotides, but this definition is quite vague since a universal scheme does not exist.

The working definition for lncRNAs includes all RNA molecules longer than 200 nucleotides, having little coding potential, transcribed by PolII, capped, spliced, and polyadenylated. The expression of lncRNAs is dependent on the cellular, tissue, and metabolic context. As a consequence, there are specific lncRNAs associated with specific cellular processes that may be inferred by their differential pattern of expression in tissues but also in different developmental time points or under specific stimuli.

It is a common belief that lncRNAs are mostly involved in transcriptional regulation and, therefore, reside principally in the nucleus. However, several lncRNAs act, or are even

exclusively localized, within the cytoplasm by working as post-transcriptional regulators in interaction with miRNAs, mRNAs, or proteins.

Interestingly, the EV cargo may be enriched in lncRNAs, as observed in plasma exosomes of patients with castration-resistant prostate cancer and in renal cancers. The scenario is even more complicated due to a large number of lncRNAs that have been implicated in competing endogenous RNA (ceRNA) mechanisms. This is possible since lncRNAs can function as sponges, able to bind and reduce the targeted effects of miRNAs on mRNAs.

One suspects that this concept may be extended also along a therapeutic target.

7 REFERENCE

1. Adhikary et al, Circular PVT1: an oncogenic non-coding RNA with emerging clinical importance, Jrl Clin Path 2019.
2. Baker, M. Long noncoding RNAs: the search for function, Nature Methods, VOL.8 NO.5, May 2011, p| 379.
3. Cabili, M.N., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., Regev, A., and Rinn, J.L. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 25,1915-1927.
4. Cantley et al, Signal Transduction, CSHL, 2013
5. Connolly et al, Complexities of TGF-β Targeted Cancer Therapy, Int Jrl Bio Sci, 2012; 8(7):964-978.
6. Crane and Berx, Regulatory networks defining EMT during cancer initiation and progression, Nature Reviews Cancer 13,97-110 (2013)
7. DeCraene and Berx, Regulatory networks defining EMT during cancer initiation and progression, Nature Reviews Cancer volume 13, pages 97–110 (2013)
8. Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D.G., et al. (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22, 1775-1789.
9. Derynck et al, TGF-β signaling in tumor suppression and cancer progression, Nature Genetics 29, 117–129 (2001)
10. Feng and Derynck, Specificity and Versatility in TGF-β Signaling Through Smads, Annu. Rev. Cell Dev. Biol. 2005. 21: 659-93
11. Fuxe et al, Transcriptional crosstalk between TGF-β and stem cell pathways in tumor cell invasion, Cell Cycle 9:12, 2363-2374; June 15, 2010
12. Griffith, D., Targeting aggressive prostate cancer, Eureka, http://www.eurekalert.org/pub_releases/2013-08/uoc--tap081213.php , 14 August 2013.
13. Gutman, M., J, Rinn, Modular regulatory principles of large non-coding RNAs, Nature, V 462, 14 Feb 2012.
14. Hill, Transcriptional Control by the SMADs, Cold Spring Harb Perspect Biol, 2016
15. Huarte, The emerging role of lncRNA in cancer, Nature Medicine 21 2015
16. Jia, H., Osak, M., Bogu, G.K., Stanton, L.W., Johnson, R., and Lipovich, L. (2010). Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA 16, 1478-1487.
17. Khalil, A.M., Guttman, M., Huarte, M., Garber, M., Raj, A., Rivea Morales, D., Thomas, K., Presser, A., Bernstein, B.E., van Oudenaarden, A., et al. (2009). Many human large

intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc. Natl. Acad. Sci. USA 106, 11667-11672.

18. Kornienko, A., et al, Gene regulation by the act of long non-coding RNA transcription, BMC Biology 2013, 11:59.
19. Kovalchuk, I., O., Kovalchuk, Epigenetics, FT Press (Upper Saddle River, NJ) 2012.
20. Kubiczkova et al, TGF-β – an excellent servant but a bad master, Journal of Translational Medicine 2012, 10:183
21. Lamouille and Derynck, Cell size and invasion in TGF-β–induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway, The Journal of Cell Biology, Vol. 178, No. 3, July 30, 2007 437–451
22. Lee, Epigenetic Regulation by Long Noncoding RNAs, Science Vol 338 14 December 2012
23. Li et al, Long noncoding RNAs in prostate cancer: mechanisms and applications, Molecular & Cellular Oncology 1:3, e963469; October 1, 2014
24. Liang et al, A novel long non-coding RNA-PRLB acts as a tumor promoter through regulating miR- 4766-5p/SIRT1 axis in breast cancer, Cell Death and Disease, 2018
25. Liz and Esteller, lncRNAs and microRNAs with a role in cancer development, Biochimica et Biophysica Acta (2015)
26. Mariathasan et al, TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells, Nature, Jan 2018
27. McGarty, EMT and Cancers, February, 2019, https://www.researchgate.net/publication/330222973_EMT_and_Cancers
28. McGarty, Growth Factors and Cancer, December 2018, https://www.researchgate.net/publication/329702571_Growth_Factors_Pathways_and_Cancers
29. McGarty, lncRNA and Prostate Cancer, July 2013, http://www.telmarc.com/Documents/White%20Papers/100%20lncrna.pdf
30. McGarty, T. P., Prostate Cancer Genomics, DRAFT, 2013, http://www.telmarc.com/Documents/Books/Prostate%20Cancer%20Systems%20Approach%2003.pdf
31. Morikawa et al, Genome-wide mechanisms of Smad binding, Oncogene (2013) 32, 1609–1615
32. Morikawa et al, TGF-b and the TGF-b Family: Context-Dependent Roles in Cell and Tissue Physiology, Cold Spring Harb Perspect Biol 2016;8:a021873
33. Moustakas et al, Smad regulation in TGF-b signal transduction, Journal of Cell Science 114, 4359-4369 (2001)
34. Orom, U.A., Derrien, T., Beringer, M., Gumireddy, K., Gardini, A., Bussotti, G., Lai, F., Zytnicki, M., Notredame, C., Huang, Q., et al. (2010). Long noncoding RNAs with enhancer-like function in human cells. Cell 143, 46-58.

35. Pardini and Calin, MicroRNAs and Long Non-Coding RNAs and Their Hormone-Like Activities in Cancer, Cancers 2019, 11, 378

36. Prensner and Chinnaiyan, The Emergence of linRNA in Cancer Biology, October 2011 Cancer Discovery | 391

37. Rinn, J., H. Chang, Genome Regulation by Long Noncoding RNAs, Annu. Rev. Biochem. 2012. 81:145–66.

38. Sakai et al, Long Noncoding RNA ELIT-1 Acts as a Smad3 Cofactor to Facilitate TGFβ/Smad Signaling and Promote Epithelial–Mesenchymal Transition, Cancer Research, Mol Cell Bio, June 2019

39. Schmitt and Chang, Long RNAs wire up cancer growth, Nature 2013

40. Shi X, Sun M, Liu H, Yao Y, Song Y. (2013) Long non-coding RNAs: A new frontier in the study of human diseases. Cancer Letters, August 2013.

41. Sigova, A.A., Mullen, A.C., Molinie, B., Gupta, S., Orlando, D.A., Guenther, M.G., Almada, A.E., Lin, C., Sharp, P.A., Giallourakis, C.C., and Young, R.A. (2013). Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells. Proc. Natl. Acad. Sci. USA 110, 2876-2881.

42. Singh and Settleman, EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer, Oncogene (2010) 29, 4741–4751

43. Sun M, Kraus WL. (2013) Minireview: Long Non-Coding RNAs: New "Links" Between Gene Expression and Cellular Outcomes in Endocrinology. Mol Endocrinol, August 2013.

44. Teng, G., F Papavasiliou, Long Noncoding RNAs: Implications for Antigen Receptor Diversification, Advances in Immunology, Volume 104, 2009.

45. Ungefroren, TGF-β Signaling in Cancer: Control by Negative Regulators and Crosstalk with Proinflammatory and Fibrogenic Pathways, Cancers 2019, 11, 384

46. Vogelstein and Kinzler, Cancer genes and the pathways they control, Nature Medicine Volume 10 Number 8 August 2004

47. Wang et al, LncRNA SNHG6 promotes proliferation, invasion and migration in colorectal cancer cells by activating TGF-β/Smad signaling pathway via targeting UPF1 and inducing EMT via regulation of ZEB1, Int Jrl Med Sci 2019; 16(1): 51-59.

48. Washeti, S., et al, Computational analysis of noncoding RNAs, WIREs RNA 2012. doi: 10.1002/wrna.1134.

49. Weinberg, R., The Biology of Cancer, Garland (New York), 2013.

50. Wellner et al, The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs, Nature Cell Biology volume 11, pages 1487–1495 (2009)

51. Wilusz et al, Long noncoding RNAs: functional surprises from the RNA world, Genes & Development 23:1494–1504 2009

52. Wu, L. et al, long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer, Am J Transl Res 2012; 4(2):127-150.

53. Xie et al, Potential Regulatory Roles of MicroRNAs and Long Noncoding RNAs in Anticancer Therapies, Mol Therapy Nucleic Acids Vol 13 Dec 2018

54. Yang et al, lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs, Nature, 29 Aug 2013

55. Yang and Weinberg, Epithelial-Mesenchymal Transition: At the Crossroads of Development and Tumor Metastasis, Developmental Cell 14, June 2008