

## Review

## Noncoding RNAs in cancer and cancer stem cells

Tianzhi Huang<sup>1,2,3,4</sup>, Angel Alvarez<sup>1,2,3,4</sup>, Bo Hu<sup>1,2,3,4</sup> and Shi-Yuan Cheng<sup>1,2,3,4</sup>**Abstract**

In recent years, it has become increasingly apparent that noncoding RNAs (ncRNA) are of crucial importance for human cancer. The functional relevance of ncRNAs is particularly evident for microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). miRNAs are endogenously expressed small RNA sequences that act as post-transcriptional regulators of gene expression and have been extensively studied for their roles in cancers, whereas lncRNAs are emerging as important players in the cancer paradigm in recent years. These noncoding genes are often aberrantly expressed in a variety of human cancers. However, the biological functions of most ncRNAs remain largely unknown. Recently, evidence has begun to accumulate describing how ncRNAs are dysregulated in cancer and cancer stem cells, a subset of cancer cells harboring self-renewal and differentiation capacities. These studies provide insight into the functional roles that ncRNAs play in tumor initiation, progression, and resistance to therapies, and they suggest ncRNAs as attractive therapeutic targets and potentially useful diagnostic tools.

**Key words** ncRNA, miRNA, lncRNA, cancer cell, cancer stem cell

It is estimated that up to 90% of the genome is actively transcribed into RNAs. However, only 1.5%–2.0% of the human genome is estimated to consist of protein-coding genes<sup>[1]</sup>. In recent years, it has become apparent that the non-protein-coding portion of the genome is not spurious transcriptional noise, as originally believed. Increasing evidence suggests that the proverbial “dark matter” of the genome may play a major biological role in health and disease, particularly in cancer<sup>[2]</sup>. The oncogenic and tumor suppressive functions of noncoding transcripts are particularly evident for the most widely studied class of noncoding RNAs (ncRNAs) called microRNAs (miRNAs)<sup>[3,4]</sup>, which are implicated in a variety of cancer processes<sup>[5-7]</sup>. However, miRNAs may just represent the tip of the iceberg of known and newly discovered ncRNA species. Other

ncRNAs include small interfering RNA (siRNA), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcribed ultraconserved regions (t-UCRs), large intergenic noncoding RNAs (lincRNAs), and other species<sup>[2]</sup>.

ncRNAs can be subdivided into two major classes based on their transcript size: small ncRNAs and long ncRNAs (lncRNAs), as shown in **Table 1**<sup>[8,9]</sup>. snoRNAs are intermediate in size, 60 to 300 bp. Small ncRNAs, typically less than 200 nucleotides in length, include the well-documented miRNAs, siRNAs, piRNAs, and the recently described transcription initiation RNAs (tiRNAs)<sup>[10,11]</sup>. Mammalian genomes also transcribe numerous lncRNAs, which are longer than 200 nucleotides and lack translational activity<sup>[12-15]</sup>. lncRNAs consist of a very heterogeneous group of RNA molecules that may be involved in a broad spectrum of cellular processes that utilize different modes of action. Accumulating studies have also described the potential functions of lncRNAs in cancer for their involvement in oncogene and tumor-suppressor pathways<sup>[16,17]</sup>.

Virtually all cancers consist of phenotypically and genetically heterogeneous cells. Among these heterogeneous cancer cells are a subpopulation of cells with stem cell-like properties, namely self-renewal and multipotency. These cancer stem cells (CSCs), or tumor-initiating cells, are capable of driving tumor growth and differentiating into multiple cell types to produce tumor heterogeneity<sup>[18]</sup>.

In this short review, we focus on the roles of miRNAs and lncRNAs in the context of cancer and CSCs, briefly describe recent studies of other ncRNAs in cancer, and discuss their potential therapeutic applications.

**Authors' Affiliations:** <sup>1</sup>The Ken & Ruth Davee Department of Neurology, <sup>2</sup>Northwestern Brain Tumor Institute, <sup>3</sup>The Robert H. Lurie Comprehensive Cancer Center, <sup>4</sup>Center of Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA.

**Corresponding Authors:**

Bo Hu, The Ken & Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA. Tel: +1-312-503-1031; Fax: +1-312-503-5603; Email: bo.hu@northwestern.edu.

Shi-Yuan Cheng, The Ken & Ruth Davee Department of Neurology, Northwestern Brain Tumor Institute, The Robert H. Lurie Comprehensive Cancer Center, Center of Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA. Tel: +1-312-503-3403; Fax: +1-312-503-5603; Email: shiyuan.cheng@northwestern.edu.

**doi:** 10.5732/cjc.013.10170

**Table 1. Types of ncRNAs**

Name	Size	Functions	Illustrative examples	Reference(s)
<b>Small ncRNA</b>				
miRNAs	19–24 bp	Translation inhibition, mRNA degradation	<i>miR-15/16, miR-21</i>	[61,66]
piRNAs	26–31 bp	Epigenetic modification	piRNAs targeting <i>RASGRF1</i> and <i>LINE1</i>	[144,145]
tiRNAs	17–18bp	RNAPII backtracking, nucleosome marking, and gene regulation	Associated with the <i>CAP1</i> gene	[11]
TSSa-RNAs	20–90 bp	Transcription maintenance	Associated with <i>RNF12</i> and <i>CCDC52</i> genes	[146]
PROMPTs	<200 bp	Transcription activation	Associated with <i>EXT1</i> and <i>RBM39</i> genes	[147]
snacRNA	89–135 bp	Potentially maintaining stemness and differentiation	<i>Snora61</i>	[120]
<b>Intermediate ncRNA</b>				
snoRNA	60–300 bp	Synthesis and processing of cytoplasmic ribosomal RNAs; post-transcriptional modification of rRNA and snRNA by 2'-O-methylation and pseudouridylation	<i>snoRNA U50</i>	[125,126]
<b>Long ncRNA</b>				
lincRNAs	>200 bp	Epigenetic modification	<i>HOTAIR, DD3</i>	[102,134]
T-UCRs	>200 bp	Regulation of miRNA and mRNA levels?	<i>uc.283+, uc.338, uc160+</i>	[148]
Other lincRNAs	>200 bp	Chromosome inactivation, telomere regulation, imprinting	<i>XIST, TSIX, TERRAs, p15AS, H19, HYMAI</i>	[149]

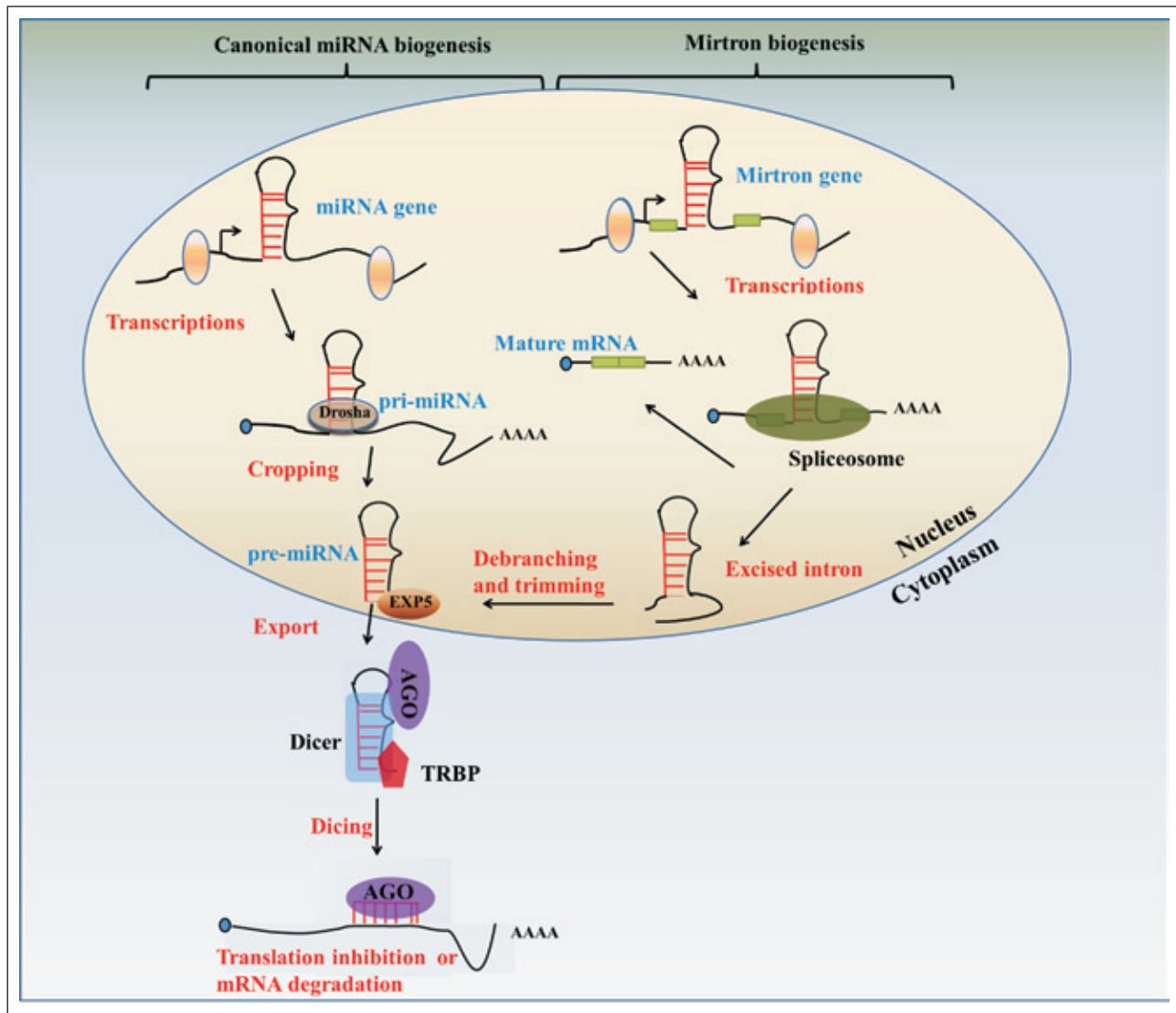
ncRNA, noncoding RNA; miRNAs, microRNAs; piRNAs, PIWI-interacting RNAs; tiRNAs, transcription initiation RNAs; TSSa-RNAs, TSS-associated RNAs; PROMPTs, promoter upstream transcripts; snacRNA, small non-polyadenylated (NPA) conserved RNA; snoRNA, small nucleolar RNAs; lincRNA, large intergenic noncoding RNAs; T-UCRs, transcribed ultraconserved regions; RASGRF1, RAS-protein-specific guanine nucleotide-releasing factor 1; LINE1, long interspersed element-1; CAP1, adenylatecyclase-associated protein 1; RNF12, ring finger protein 12; CCDC52, coiled-coil domain containing 52; EXT1, exostosin 1; RBM39, RNA-binding motif protein 39; HOTAIR, homeobox (HOX) transcript antisense RNA; XIST, X-inactivation specific transcript; TSIX, antisense transcript of XIST; TERRAs, telomeric repeat-containing RNAs; HYMAI, hydatidiform mole associated and imprinted.

## Biogenesis and Function of miRNA and lncRNA

Sequence analysis of miRNA positions in the human genome reveals the majority of miRNAs are located in intergenic regions<sup>[19,20]</sup>, although a few located within exonic or intronic regions have been described<sup>[21]</sup>. The canonical pathway for miRNA biogenesis begins with transcription of primary miRNA (pri-miRNA) by RNA polymerase II (**Figure 1**). The pri-miRNA is processed by the RNase Drosha in the nucleus into ~70 nucleotide precursor miRNA (pre-miRNA) products, which locally fold into stable secondary hairpin loops with ~2 nucleotide 3'-overhangs<sup>[22]</sup>. The pre-miRNAs are then exported to the cytoplasm by the Ran-GTP-dependent transporter Exportin 5<sup>[23]</sup> for further cleavage by the RNase Dicer, which is assisted by its partner TAR RNA-binding protein (TRBP) or protein activator of the interferon-induced protein kinase, also known as protein kinase interferon-inducible double-stranded RNA-dependent activator (PRKRA), to recognize and bind pre-miRNAs<sup>[24]</sup>. The resulting imperfect miRNA:miRNA\* duplex is then unwound and one strand is selected as the mature miRNA, based on sequence thermodynamic properties<sup>[25]</sup>, whereas the complementary passenger strand is usually

subjected to degradation. Then, TRBP recruits Argonaute 2 to form the RNA-induced silencing complex (RISC)<sup>[26,27]</sup>, where the mature miRNA recognizes its mRNA target by pairing the miRNA seed region (positions 2–8) to complementary sequences located primarily in the target 3'-untranslated regions (3'-UTRs) and sometimes in the coding regions<sup>[28]</sup>. In addition, a special type of miRNA known as mirtrons is regulated through a non-canonical pathway (**Figure 1**). Mirtrons are produced from spliced introns that mimic the structural features of precursor miRNAs and enter the miRNA-processing pathway independent of Drosha-mediated cleavage<sup>[29,30]</sup>. In mammalian cells, miRNAs regulate the expression of target genes by inducing mRNA degradation or translational repression, depending on the sequence complementarity between the small RNA and the target mRNA<sup>[31]</sup>. miRNAs control a wide range of pathologic and physiologic processes, including development, differentiation, cellular proliferation, programmed cell death, oncogenesis, and metastasis<sup>[32]</sup>. Therefore, dysregulation of miRNA expression can lead to pathogenesis, including many types of cancer<sup>[33]</sup>.

Similar to miRNA primary transcripts, lncRNAs are frequently transcribed by RNA polymerase II and polyadenylated. In the genome, lncRNAs are located in sense or antisense orientations relative to the protein-coding genes, either within the introns or intergenic regions.



**Figure 1.** Canonical and non-canonical miRNA biogenesis pathways. In the canonical pathway (left), long primary miRNAs (pri-miRNAs) are transcribed by RNA polymerase II and are then capped and polyadenylated, forming RNA with a hairpin secondary structure. Cropping is the first step in the maturation mediated by the RNase III enzyme Drossha and produces a ~65 nucleotide hairpin RNA with a 2–3 nucleotide overhang termed precursor—miRNA (pre-miRNA). pre-miRNA is recognized and exported into cytoplasm by the Exportin-5 (EXP5)–Ran-GTP complex for further processing by Dicer with its partner TAR RNA-binding protein (TRBP) and Arogonaute proteins 1–4 (AGO). Dicer processing generates the miRNA:miRNA\* duplex. One strand of the miRNA duplex is selected to form the RNA-induced silencing complex (RISC), which mediates translation inhibition or mRNA degradation. In the non-canonical pathway (right), some miRNAs called mirtrons are embedded in short introns and bypass the Drossha procession. After the splicing and production of the mature mRNA, the excised intron is debranched and trimmed to generate the pre-miRNA that can be EXP5 and exported by EXP5–Ran-GTP complex, and then subsequently enter the canonical pathway for miRNAs biogenesis.

lncRNAs are involved in diverse cellular functions<sup>[34,35]</sup>. For example, lncRNAs can serve as the precursors of small ncRNAs to produce miRNA and endo-siRNA, or serves as a “miRNA sponge” to inhibit miRNA activity<sup>[36,37]</sup>. lncRNAs can also act as scaffolds during the formation of cellular substructures or protein complexes<sup>[38]</sup>. The best-described function of lncRNAs is the regulation of gene expression. The interaction of lncRNAs with proteins can influence protein activity and localization. For example, nuclear factor of activated T cells (NFAT) is a cellular transcription factor; the lncRNA noncoding

repressor of NFAT (NRON) interacts with NFAT and regulates its nuclear-cytoplasmic trafficking, resulting in repression of NFAT target gene expression<sup>[39]</sup>. Furthermore, lncRNAs regulate gene transcription by recruiting transcription factors to bind the promoters of their target genes and activate or repress gene expression<sup>[40]</sup>. A very recent study revealed that numerous lncRNAs were induced by Toll-like receptors, which recognized microbial products and induced antimicrobial defense signaling and adaptive immune response<sup>[40-42]</sup>. The lncRNA lincRNA-Cox2 was found to play a key role in activation

and repression of distinct classes of immune genes. Transcriptional repression of target genes was dependent on interactions of lincRNA-Cox2 with heterogeneous nuclear ribonucleoprotein (hnRNPs) A/B and A2/B1<sup>[44]</sup>. This finding indicates that lincRNAs can serve as repressors and activators of genes through their physical interactions with hnRNPs. On the other hand, lincRNAs can also occupy the binding sites of general transcription factors and block transcription<sup>[41]</sup>. Intriguingly, a number of studies demonstrate that lincRNAs are also key components of epigenetic networks, where they can interact with chromatin remodeling complexes and induce local or global changes in chromatin packaging<sup>[43,44]</sup>. For example, lincRNA *Xist*, 17 kilobases in length, was transcribed from the X chromosome. *Xist* recruits the polycomb repressive complex 2 (PRC2) to switch off gene expression from one X chromosome in each female cell<sup>[45]</sup>. However, exactly how *Xist* establishes binding pattern during the initiation of X chromosome inactivation remains unknown. Recently, Engreitz *et al.*<sup>[46]</sup> used a high-throughput “chromosome conformation capture” technology to identify DNA sequences in close proximity to each other within the nucleus, and found that the early binding sites for *Xist* correspond to loci spatially nearby the *Xist* transcription site, rather than those that are close along the linear sequence. They demonstrated that *Xist* recruited PRC2 to spread across and silence active genes using a targeting mechanism based on three-dimensional chromosome conformation, which was exploited to extrude *Xist* onto its early binding site targets where it then helped to modify and reorganize the X chromosome architecture<sup>[46]</sup>. Overall, lincRNAs are also emerging as important regulatory molecules in gene expression at a transcriptional, post-transcriptional, and epigenetic level.

## Cancer and CSCs

A tumor mass contains heterogeneous subsets of cells with diverse states of differentiation. CSCs are a small subpopulation identified in many types of human cancers<sup>[47]</sup>. CSCs can undergo a theoretically unlimited number of mitotic cycles, and through asymmetric cell division, form progeny that are either stem-like or more differentiated cell types, depending on intrinsic or microenvironmental factors<sup>[48]</sup>. CSCs are capable of initiating tumor formation, increasing tumor cell proliferation and expansion, and becoming differentiated tumor cells<sup>[14,48]</sup>. CSCs can be isolated based on their growth properties or by sorting using cell surface antigens, metabolic markers such as CD44, CD24 and CD133, and activity of aldehyde dehydrogenase 1 (ALDH1)<sup>[49]</sup>. Through the common sorting approach, CSCs have been isolated from hematologic malignancies<sup>[50]</sup>, breast tumors<sup>[51]</sup>, brain tumors<sup>[52]</sup>, colon cancer<sup>[53]</sup>, and other solid tumors<sup>[54]</sup>.

Current cancer therapeutics for most malignant tumors can reduce tumor size or inhibit further progression, but have a limited curative effect. CSCs, which are intrinsically resistant to conventional chemotherapy and radiation treatment, are hypothesized to lead to tumor recurrence. Thus current treatments are unlikely to result in long-term remission unless the CSCs are also targeted<sup>[47]</sup>. Treatment resistance results from multiple factors. Resistance to chemotherapy is partially attributed to the overexpression of transmembrane efflux

pump proteins, which are regulated by reactive oxygen species within cells<sup>[55]</sup>. However, Zielske *et al.*<sup>[56]</sup> revealed a controversial result from two patients on treatment for therapy-resistant CSCs. The CD44<sup>+</sup>CD24<sup>-low</sup> lineage and ALDH<sup>+</sup> breast CSCs from one patient were rapidly depleted 2 weeks after treatment with radiation, resulting in a significant decrease in tumor sphere frequency and tumorigenic capacity. In contrast, CSCs from the other patient showed enrichment after radiation and resistance to therapy, suggesting that CSC variance may exist in individual patients<sup>[56]</sup>. Therefore, therapeutics that target different CSC subtypes is likely required.

The mechanisms that regulate CSC growth, in conjunction with factors that facilitate resistance to radiation and chemotherapy, are of particular clinical importance given the role CSCs have in tumorigenesis and recurrence<sup>[18,47,48,54,57-60]</sup>. Stem cells undergo symmetric or asymmetric cell division, producing identical stem cell progeny or cells that are more differentiated. Moreover, the cycling of stem cells varies considerably, facilitating so-called kinetic resistance, whereby slow cycling or quiescent stem cells are unaffected by DNA damaging agents or radiation compare to the way more rapidly dividing cells are. CSCs also show great drug resistance through other mechanisms, including drug effluxing. Resistance to radiation can occur through the repair of damaged DNA, redistribution of cycling cells, re-oxygenation of hypoxic tumor regions, and repopulation of the tissue, commonly considered the “four Rs.” The role of ncRNAs in regulating these mechanisms of resistance, in addition to intrinsic resistance that CSCs may have, is an exciting area of research.

## miRNAs in Cancer and CSCs

Since the first characterized miRNAs, *miR-15* and *miR-16*, were found to be deleted in patients with chronic lymphocytic leukemia (CLL) in 2002<sup>[61]</sup>, a large number of miRNAs have been found to be dysregulated in virtually all cancers<sup>[62,63]</sup>. miRNAs regulate multiple cancer processes, such as transformation, tumor cell proliferation, epithelial-mesenchymal transition (EMT), invasion, and metastasis, mainly by inhibiting the expression of critical genes in pathways that regulate cell processes including cell cycle, apoptosis, and migration<sup>[62,63]</sup>.

miRNAs can function as either oncogenes or tumor suppressors. *miR-21* is a well characterized example of an oncogenic miRNA. *miR-21* is overexpressed in most types of malignancies, including breast cancer, glioblastoma, colorectal cancer, lung cancer, pancreatic cancer, and leukemia<sup>[64-66]</sup>. In glioblastoma, *miR-21* was revealed to target several important components of the epidermal growth factor receptor (EGFR) and phosphatase and tensin homolog (PTEN) signaling pathway in glioma cell lines. Inhibition of *miR-21* by specific antisense oligonucleotides in U251MG cells decreased the expression of EGFR and activated AKT, CYCLIN D, and BCL2<sup>[67,68]</sup>. Down-regulation of PTEN, followed by AKT activation, was also reported as a result of *miR-26a* overexpression in both NIH-3T3 fibroblast and LN-18 human glioblastoma cells<sup>[69]</sup>. In contrast, *miR-34* family members (*miR-34s*) are known as tumor suppressive miRNAs. The tumor suppressor *TP53* transcriptionally induces the



*miR-34* family in response to DNA damage<sup>[70]</sup>. *miR-34a* is encoded by a sequence on chromosome 1, whereas both *miR-34b* and *miR-34c* are processed from one primary transcript from chromosome 11<sup>[71,72]</sup>. *miR-34a* deletion was associated with metastasis and recurrence of prostate cancer<sup>[73]</sup>. Restoration of *miR-34a* expression in pancreatic cancer cells substantially repressed cell proliferation and invasion, and sensitized cells to chemotherapy and radiation<sup>[74]</sup>. *miR-34* could also be repressed by ZEB1, a transcriptional repressor of E-cadherin that is involved in promoting metastasis by remodeling cytoskeletal actin, which is required for tumor cell invasion<sup>[74-76]</sup>.

Compelling evidence suggests critical roles that miRNAs play in regulating stem cells and in CSC biology. The regulation of stem cells by miRNA has been partially revealed through their regulation of pluripotency and differentiation. For example, the balance between stemness and differentiation is maintained by the reciprocal regulation *miR-145* and the embryonic stem cell genes *NANOG*, *OCT4*, *SOX2* and *KLF4*<sup>[77-81]</sup>. In fact, the use of feedback mechanisms that drives stemness while inhibiting differentiation, or *vice versa*, is a common occurrence in stem cell signaling. In embryonic stem cells, signaling from transcription factors OCT4, NANOG, SOX2, and MYC are regulated with LIN28 to maintain embryonic stem cells in their pluripotent state<sup>[82-84]</sup>. Alternatively, *let-7* is activated upon differentiation and inhibits LIN28 (by binding to the gene's 3'-UTR) and MYC, creating a bi-stable switch between stemness and differentiation<sup>[85,86]</sup>. These interlinked pathways have been exploited to create induced-pluripotent stem cells by different combinations of transcription factors or miRNAs<sup>[81-83]</sup>.

Interlinked ncRNA and protein signaling pathways are aberrantly perturbed and result in transformation and oncogenic activity. For example, *let-7* represses cell proliferation and is often down-regulated in many tumors<sup>[87,88]</sup>. The role of *let-7* and LIN28 in inhibiting or enhancing oncogenic signaling via RAS, NF- $\kappa$ B, and high mobility group AT-hook 2 (HMGA2) make these pathways attractive therapeutic targets<sup>[88-90]</sup>. Studies that demonstrate the important feedback inhibition of enhanced LIN28 activity or repressed *let-7* in regulating stem cell activity<sup>[86,91]</sup> highlight how these miRNAs are important to oncogenesis. LIN28 inhibits the tumor suppressor activity in breast cancer and regulates the ALDH1, a marker for breast and ovarian CSCs<sup>[92]</sup>. Recently, a comparative analysis of miRNA expression profiles between embryonic stem cells and breast CSCs showed an overlap between the two groups, and 37 miRNAs were found to be differentially expressed in CD44<sup>+</sup>CD24<sup>-low</sup> breast CSCs. In breast CSCs, three clusters of *miR-200* family miRNAs, *miR-200c-141*, *miR-200b-200a-429*, and *miR-183-96-182*, were significantly down-regulated<sup>[76]</sup>. Recently, *miR-22* was found to suppress the expression of *miR-200* family members in breast CSCs via direct targeting of chromatin remodeling enzymes such as ten-eleven translocation (TET) family members. Inhibition of TETs leads to hypermethylation of the *miR-200* promoter and induction of EMT and stemness in breast CSCs<sup>[93]</sup>. In CD133<sup>+</sup> colon CSCs, a distinct miRNA signature of 11 overexpressed and 8 underexpressed miRNAs was shown to be involved in self-renewal and differentiation<sup>[94,95]</sup>. In glioma CSCs, *miR-128* could specifically block the self-renewal capacity of CSCs through down-regulation of BMI1, whereas *miR-125* promoted cell

proliferation by regulating CDK6 and CDC25<sup>[96,97]</sup>. In EpCAM-positive hepatocellular CSC, *miR-181* expression was shown to be involved in the regulation of differentiation by targeting transcription factors CDX2 and GATA6<sup>[98,99]</sup>. In pancreatic carcinoma, CSCs showed a signature of 210 miRNAs involved in self-renewal and differentiation, including *miR-99a*, *miR-100*, *miR-125b*, *miR-192*, and *miR-429*<sup>[100]</sup>. *miR-34* is down-regulated in pancreatic CSCs, and restoring *miR-34* in these CSCs repressed self-renewal by blocking the expression of the BCL2 and NOTCH signaling pathways<sup>[101]</sup>. Taken together, these findings demonstrate that miRNAs play a critical role in regulating CSC self-renewal, differentiation, and tumorigenesis.

## lncRNAs in Cancer Cells

In addition to well-characterized miRNAs, lncRNAs have also emerged as important regulators in oncogenic and tumor suppressor pathways (Table 2)<sup>[102]</sup>. Accumulating evidence provides mechanistic insight demonstrating how lncRNAs regulate important cellular signaling pathways in cancer cells at transcriptional, post-transcriptional, and epigenetic levels<sup>[103]</sup>. Among the better characterized oncogenic lncRNAs is *lincRNA-HOTAIR* (*HOX* antisense intergenic RNA), which is located in the mammalian homeobox C (*HOXC*) locus on chromosome 12q13.13<sup>[104]</sup>. *HOTAIR* was highly up-regulated in primary and metastatic breast tumors. Depletion of *HOTAIR* resulted in an altered pattern of H3K27 methylation and decreased invasiveness, whereas restoration of *HOTAIR* had the opposite effect<sup>[105]</sup>. Furthermore, the expression level of *HOTAIR* in primary breast tumors was a powerful predictor of patient outcomes and correlated with metastasis and low survival rates<sup>[106]</sup>. Expression of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), located at chromosome 11q13.1, associates with metastasis and poor prognosis in patients with non-small cell lung cancer (NSCLC)<sup>[106]</sup>. Studies in cervical and lung cancers implicate *MALAT1* in regulating the invasive potential of cancer cells<sup>[107,108]</sup>. Most recently, Yang *et al.*<sup>[109]</sup> reported that two lncRNAs, *PRNCR1* and *PCGEM1*, were highly expressed in a large number of aggressive prostate cancers and drive androgen receptor (AR)-associated transcriptional programs to promote prostate cancer growth. In this study, *PRNCR1* was found to directly interact with the acetylated carboxyl terminus of AR and recruit the enzyme DOT1L, resulting in methylation of the amino terminus of AR and subsequent interaction with *PCGEM1*. *PCGEM1* then induced PYGO2, an important component of the Wnt signaling transcriptional complex, to activate the expression of AR-targeted genes by binding to H3K4me3 chromatin marks in the genes' promoter sequences<sup>[109,110]</sup>.

Several lncRNAs are involved in the regulation of p53 tumor suppressor signaling. Maternally expressed gene 3 (*MEG3*), an imprinted lncRNA on chromosome 14q32.3 in humans, was shown to activate p53 and facilitate p53 signaling, including enhancing p53 interactions with the promoters of its target genes<sup>[111]</sup>. Frequent hypermethylation within the *MEG3* promoter is widely observed in human cancers, including pituitary tumors<sup>[10]</sup>, meningioma<sup>[112]</sup>, and leukemia<sup>[113]</sup>. Overexpression of *MEG3* suppresses cell proliferation in meningioma and hepatocellular carcinoma cell lines<sup>[112,114]</sup>,

**Table 2. Examples of lncRNAs in cancer**

lncRNA	Cancer types	Functions	Molecular interactors	Reference(s)
<b>Oncogene</b>				
<i>ANRIL/p15AS</i>	Prostate, leukemia	Suppression of senescence via INK4A	PRC1, PRC2	[139,150]
<i>HOTAIR</i>	Breast, hepatocellular	Promotes metastasis	PRC2, LSD1	[104,105]
<i>MALAT1/NEAT2</i>	Lung, prostate, breast, colon	Promotes metastasis	Unknown	[106–109]
<i>PCGEM1</i>	Prostate	Inhibits apoptosis; promotes cell proliferation	Unknown	[151]
<i>TUC338</i>	Hepatocellular	Promotes cell proliferation	Unknown	[152]
<i>H19</i>	Breast, hepatocellular	Promotes cell proliferation	Unknown	[139–141]
<b>Tumor suppressor</b>				
<i>GAS5</i>	Breast	Induces apoptosis and growth arrest	GR	[153,154]
<i>linc-p21</i>	Mouse model, lung cancer cells, sarcoma, lymphoma	Induces apoptosis	hnRNP-K	[115]
<i>MEG3</i>	Meningioma, hepatocellular	Inhibits cell proliferation	Unknown	[10,111–114]
<i>PTENP1</i>	Prostate, colon	Binds PTEN-suppressing miRNAs	Unknown	[155]

ANRIL, antisense noncoding RNA in the INK4 locus; HOTAIR, HOX antisense intergenic RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; NEAT2, nuclear enriched abundant transcript 2; PCGEM1, prostate-specific transcript 1; TUC338, transcribed ultra-conserved region 338; GAS5, growth arrest-specific 5; MEG3, maternally expressed 3; PTENP1, phosphatase and tensin homolog pseudogene 1; PRC, polycomb complex; LSD1, lysine-specific demethylase 1; GR, glucocorticoid receptor.

suggesting that MEG3 functions as a putative tumor suppressor. In addition, some lncRNAs are under the regulation of p53. For example, *linc-p21*, a murine lncRNA located near the p21 gene, has also emerged as an important gene in p53 pathways. In murine lung cancer, sarcoma, and lymphoma, *linc-p21* expression was induced by p53 stimulation and repressed p53 target genes via interaction with hnRNP-K, a protein that binds the promoters of p53 downstream genes<sup>[115]</sup>. However, it is still unknown whether the human homolog of *linc-p21* has a similar mechanism, although *lincRNA-p21* seems to be conserved and was also up-regulated in human fibroblasts after induction of DNA damage<sup>[115]</sup>.

## Other ncRNA Species in Cancer Cells and CSCs

In addition to miRNAs and lncRNAs, other ncRNA species such as piRNAs and snoRNAs are gaining a greater appreciation for their role in carcinogenesis<sup>[33]</sup>. piRNAs are small ncRNAs of 24–30 nucleotides that are found in the germ line of flies and vertebrates<sup>[116]</sup>. piRNAs are generated independent of Dicer processing and interact with the PIWI subfamily of the argonaute protein family that are implicated in maintaining germ line genome stability<sup>[116]</sup>. Recently, *piRNA-651* was found to be up-regulated in several cancer cell lines including gastric, lung, mesothelial, breast, liver, and cervical cancer cell lines<sup>[117]</sup>. The inhibition of *piRNA-651* by antisense oligonucleotides led to the repression of proliferation in gastric cancer cells and cell cycle arrest at the G<sub>2</sub>/M phase<sup>[117]</sup>. In contrast, *piRNA-823* was significantly decreased in gastric cancer tissues when compared with non-cancerous tissues. Restoration of *piR-823* in gastric cancer cells inhibited cell proliferation and tumor growth in

a xenograft nude mouse model<sup>[118]</sup>. Both piRNAs and PIWI proteins can play an important role in tumor growth. It was recently shown that ectopic expression of germ line PIWI genes can drive brain tumor development in *Drosophila*<sup>[119]</sup>. This evidence indicates that the piRNA pathway is not limited to germ line cells, but also plays roles in tumorigenesis, although the specific functions of piRNAs are largely unknown.

Recent next-generation sequencing studies have highlighted differentially expressed ncRNA between stem cells and differentiated cells. For example, small non-polyadenylated (NPA)-conserved RNA (snacRNA) differs significantly between embryonic stem cells and differentiated cells<sup>[120]</sup>. It is unknown whether snacRNAs have functional activity in cancer cells or CSCs.

snoRNAs are a group of intermediate-sized ncRNAs (60–300 bp) characterized in eukaryotes. snoRNAs are enriched in the nucleolus, where they provide the cellular locale for the synthesis and processing of cytoplasmic ribosomal RNAs (rRNAs)<sup>[121]</sup>. In vertebrates, most snoRNAs are transcribed by RNA polymerase II from introns of protein-coding genes<sup>[122]</sup>. In addition, they can also be processed from introns of longer ncRNA precursors<sup>[123]</sup>. snoRNAs interact with specific proteins to form snoRNPs, which are responsible for post-transcriptional modification of rRNA and snRNA by 2'-O-methylation and pseudouridylation<sup>[121]</sup>. Recently, it was reported that human snoRNAs also have an important role in tumorigenesis. Various snoRNAs were shown to be differentially expressed in NSCLC when compared to the corresponding matched tissue<sup>[124]</sup>. A germ line homozygous 2-bp (TT) deletion of the snoRNA *U50* was found in prostate tumors and loss of *U50* is associated with tumorigenesis. Furthermore, frequent deletion of snoRNA *U50* was also observed in breast cancer<sup>[125,126]</sup>. Given that translation is often

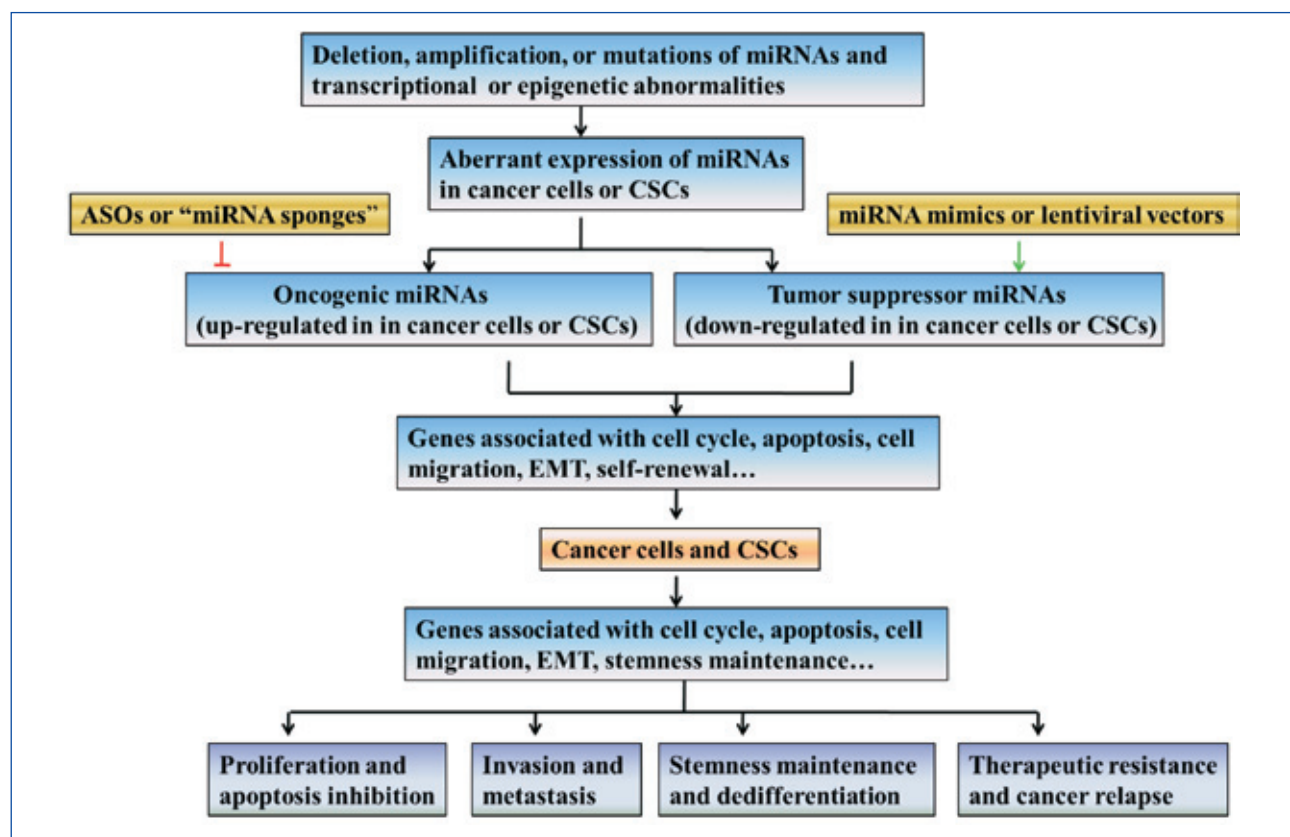
perturbed in cancer cells, it is possible that snoRNAs contribute to tumorigenesis by regulating protein translation<sup>[127]</sup>. Taken together, emerging evidence suggests that dysfunctions of other ncRNA species, such as piRNAs and snoRNAs, are also involved in the development and progression of cancer. However, the functional roles of these ncRNAs in the biology and tumorigenesis of cancer and CSCs are still in question. Therefore, further understanding the molecular mechanisms of how these aberrant ncRNAs contribute to the development and progression of cancer is warranted.

## Noncoding RNAs as Potential Therapeutics for Cancer

miRNAs have now been recognized as promising therapeutic targets for anticancer treatments<sup>[128]</sup>. This is because modulating the level of a single miRNA can eventually affect its multiple target genes, simultaneously altering oncogenic and tumor suppressor pathways. Oncogenic miRNAs (**Figure 2**), which are usually overexpressed in tumors when compared with healthy tissues, can be altered by several means. Based on the knowledge that miRNAs

control their targets through base pair complementarity, antisense oligonucleotides (ASOs) have been developed to inhibit miRNA function. In order to increase ASOs' stability and efficacy, different chemical modifications, such as locked nucleic acids (LNAs), anti-miRNA oligonucleotides (AMOs), and antagomirs, are incorporated in the skeleton of ASOs<sup>[129-131]</sup>. For example, a specific antagomir was used to knockdown the oncogene *miR-21* in breast cancer MCF-7 cells, resulting in insignificant inhibition of MCF-7 tumor growth *in vitro* and in tumor xenografts through inhibiting cell proliferation and inducing apoptosis<sup>[66]</sup>. Alternatively, "miRNA sponges" have been developed to inhibit miRNA activity in cancer cells and mouse models by incorporating the complementary binding sites of target miRNA into RNA transcripts expressed from strong promoters<sup>[132]</sup>. For instance, inhibition of *miR-22* by the specific sponge in LM2 cells, a highly metastatic breast cancer cell line derived from the MDA-MB-231 cell line, resulted in a reduction of breast cancer metastasis to the lung using xenograft models<sup>[93]</sup>.

Tumor suppressor miRNAs are usually repressed in cancer cells. miRNA mimics or lentiviral vectors can be used to restore their expression levels. *miR-34* is a well-known tumor suppressor and is



**Figure 2.** miRNA in cancer and cancer stem cells (CSCs). Aberrant expression of miRNAs, either oncogenic or tumor suppressive, may be due to deletion, amplification, or mutations of miRNA genes, and dysregulation of transcriptional and epigenetic factors that regulate the miRNA genes. Dysregulation of genes linked to cell cycle, apoptosis, cell migration, epithelial-mesenchymal transition (EMT), and self-renewal results in carcinogenesis, invasion, metastasis, and maintenance of stemness. It is proposed that miRNA inhibition can knockdown the effects of oncogenic miRNAs, and miRNA mimics or lentiviral vectors expressing target miRNAs can restore the capabilities of tumor suppressor miRNAs. Therefore, miRNAs have great therapeutic potential against cancer progression, therapy resistance, and relapse. ASOs, antisense oligonucleotides.

down-regulated in many types of cancers. In gastric cancer cells, a *miR-34* mimic could arrest the cell cycle in the G<sub>1</sub> phase and induce apoptosis by attenuating its downstream target genes, including *BCL2*, *Notch*, and *HMG2*<sup>[133]</sup>. However, the delivery of the miRNA mimic into mice can only last a few days, limiting long-term efficacy. To overcome this dilemma, lentiviral vectors can be used to express sequences targeting miRNAs in cancer cells. Then these modified cancer cells with stable expression of miRNAs can be utilized for *in vivo* xenograft models. For example, expression of *miR-34a* by a lentiviral vector in pancreatic CSCs inhibited tumor sphere formation and tumor growth *in vivo*<sup>[101]</sup>. Therefore, miRNA mimic and lentiviral-expressed miRNAs have great potential in restoring tumor suppressor miRNAs in cancer and CSCs.

Similar to their smaller miRNA counterparts, lncRNAs represent an important untapped resource in terms of developing diagnostics and therapies. Many lncRNAs are expressed in a tissue- and cancer-type restricted manner. These lncRNAs can be exploited for the development of novel biomarkers. The highly expressed prostate lncRNA *DD3* has been shown to be a prognostic marker for prostate cancer<sup>[134]</sup>. *HOTAIR* expression was highly increased in primary and metastatic breast tumors, and expression levels of *HOTAIR* were directly correlated with poor outcomes for patients with breast cancer<sup>[135]</sup>. Similarly, the high expression levels of *HOTAIR* were also found in hepatocellular carcinomas<sup>[136]</sup>. Therefore, *HOTAIR* can serve as a biomarker to predict tumor recurrence in primary breast tumor and hepatocellular carcinomas.

In comparison to well-utilized miRNA, lncRNAs are just beginning to be incorporated into therapeutic strategies against cancer. Although our knowledge of the molecular mechanisms of lncRNA function is limited, some unique characteristics of lncRNAs make these ncRNAs potential candidates for therapeutic intervention. Many lncRNAs appear to adopt some characteristic secondary structures for protein binding, thereby providing possible avenues for cancer intervention<sup>[137]</sup>. For example, inhibition of the interactions between *HOTAIR* and the PRC2 or LSD1 complexes restricted the metastatic potential of breast cancer cells<sup>[138]</sup>. In addition, the certain lncRNAs are expressed in specific types of cancers, which limits the off-target effects and risk to normal tissues that are associated with transgene-based therapies. For example, *H19* is highly and

specifically expressed in many types of human cancers<sup>[139-141]</sup>. As such, a construct with a diphtheria toxin gene, driven by H19 specific promoter sequences, was developed and tested in treating bladder cancer. This plasmid was administered into tumor-bearing mice through intratumoral injection either as naked DNA or as a polyplex vector consisting of the cationic polymer polyethylenimine (PEI), which increases DNA uptake efficiency. Upon uptake in the tumor, high levels of diphtheria toxin were expressed, leading to a reduction in tumor growth<sup>[142,143]</sup>. Collectively, these studies indicate that lncRNAs provide novel strategies in cancer diagnostics and therapies.

## Conclusions

Dysregulation of ncRNAs is involved in regulation of cellular signaling in cancer and CSCs. A better understanding of the molecular mechanisms and pathways regulating ncRNAs and how they control tumor phenotype and malignancy offers promise for developing more effective cancer therapies. Emerging evidence demonstrates that lncRNAs are involved in self-renewal of embryonic and pluripotent stem cells, but it is still unknown whether ncRNAs drive the transformation process or what role they play in maintaining stemness and rendering therapeutic resistance of CSCs. Therefore, studies of ncRNA biology will ultimately yield further insights into the molecular mechanisms of tumorigenesis and lead to the development of new therapeutic strategies against cancer.

## Acknowledgments

This work was supported in part by grants from the US NIH grant CA130966, CA158911 to S. Y. Cheng, the Zell Scholar Award from the Zell Family Foundation and funds from Northwestern Brain Tumor Institute and Department of Neurology at Northwestern University Feinberg School of Medicine to S. Y. Cheng, and the Brain Cancer Research Award from the James S. McDonnell Foundation to B. Hu.

Received: 2013-09-26; revised: 2013-10-08;  
accepted: 2013-10-09.

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