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Non-Coding RNAs and Cancer

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1. Introduction

The question of which regions of the human genome constitute its functional elements—those expressed as genes or serving as regulatory elements—has long been a central topic in biology. In the 1970s and 1980s, early cloning-based methods revealed the presence of more than 7000 genes in human genome [1], and large-scale analyses of expressed sequence tags (ESTs) in the 1990s suggested that the estimated number of human genes range from 35,000 to 100,000 [2]. The completion of the human genome project narrowed the focus considerably by highlighting the surprisingly small number of protein-coding genes, which is now conventionally cited as less than 25,000 [3]. While the number of protein-coding genes (20,000–25,000) has maintained broad consensus, recent studies of the human transcriptome have revealed an astounding number of non-coding RNAs (ncRNAs) [4-6]. In fact, the increased sensitivity of genome tiling arrays provides an even more detailed view, revealing that the extent of non-coding sequence transcription is at least four times greater than coding sequence, and that the abundance of non-coding transcripts had been previously overlooked. The RNA world hypothesis proposes that early life was based on RNAs, which subsequently devolved the storage of information to more stable DNA, and catalytic functions to more versatile proteins. Consequently, despite crucial roles in the ancient processes of translation and splicing, RNA is assumed to have been largely relegated to an intermediate between gene and protein, encapsulated in the central dogma 'DNA makes RNA makes protein' [7]. However, the finding that most of the genome in complex organisms is transcribed and the discovery of new classes of regulatory non-coding RNAs (ncRNAs) challenges this assumption and suggests that RNAs have continued to evolve and expand alongside proteins and DNA.

ncRNAs are considered as RNA transcripts that do not encode for a protein. In the past decade, a great diversity of ncRNAs has been observed. Depending on the type of ncRNA, transcription can occur by any of the three RNA polymerases (RNA Pol I, RNA Pol II, or RNA Pol III). General conventions divide ncRNAs into two main categories: small ncRNAs

less than 200 bp and long ncRNAs greater than 200 bps [8]. Within these two categories, there are also many individual classes of ncRNAs (**Table1**), although the degree of biological and experimental support for each class ranges substantially and should be evaluated individually. The relevance of ncRNAs in gene regulation has been rapidly unveiling during the last decade. However, the functional elements in the primary sequence of noncoding genes that determine their role as RNA molecules remain unknown. Protein-coding genes have a defined language with a set of grammatical rules: three nucleotides forms a codon that translates into a specific amino acid [9]. Aberrations in codons of a protein-coding gene can be interpreted in terms of the amino acids they encode. We can recognize a mutation in a codon and determine its contribution to a given disease. In contrast to the genetic code for protein synthesis, 'the ncRNA alphabet' – a specific set of RNA sequences or structural motifs important for ncRNA function – remains to be largely elucidated. However, it has become increasingly apparent that the ncRNAs are of crucial functional importance for normal development, physiology and disease [10]. The functional relevance of the ncRNAs is particularly evident for a class of small non-coding RNAs called microRNAs (miRNAs) [11-12]. In human diseases, particularly cancer, it has been shown that epigenetic and genetic defects in miRNAs and their processing machinery are a common hallmark of disease [13-16]. However, miRNAs are just the tip of the iceberg, and other ncRNAs such as small nucleolar RNAs (snoRNAs), PIWI-interacting RNAs (piRNAs), large intergenic non-coding RNAs (lincRNAs) and, overall, the heterogeneous group of long non-coding RNAs (lncRNAs), might also contribute to the development of many different human disorders. Here we discuss the most recent genetic studies on ncRNAs and their related proteins in the context of cancer and we will analyze the new regulatory elements of the noncoding language to interpret their contribution to the pathogenesis of cancer.

2. MicroRNAs

In 1993, Victor Ambros and colleagues discovered a gene, *lin-4*, that affected development in *Caenorhabditis elegans* and found that its product was a small nonprotein-coding RNA [31]. The number of known small RNAs in different organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster*, plants, and mammals—including humans—has since expanded substantially, mainly as a result of the cloning and sequencing of size-fractionated RNAs. MiRNAs are single stranded RNAs (ssRNAs) of 19–25 nucleotides in length that are generated from endogenous hairpin transcripts [32]. They play an important role in the negative regulation of gene expression by base-pairing to partially complementary sites on the target messenger RNAs (mRNAs), usually in the 3' untranslated region (UTR). Binding of a miRNA to the target mRNA typically leads to translational repression and exonucleolytic mRNA decay, although highly complementary targets can be cleaved endonucleolytically. A genomic analysis of miRNAs has revealed that more than 50% of mammalian miRNAs are located within the intronic regions of annotated protein-coding or non-protein-coding genes [33]. These miRNAs could therefore use their host gene transcripts as carriers, although it remains possible that some are actually transcribed separately from internal promoters. Other miRNAs, located in intergenic regions, apparently have their own transcriptional regulatory elements and thus constitute

Category	Name	Supporting data	Function	Role in cancer	Refs.
Housekeeping RNAs	Ribosomal RNAs (rRNA)	high	ribosome structure	no	17-18
	Transfer RNAs (tRNA)	high	protein translation	no	17-18
	Small nuclear RNAs (snRNA)	high	splicing	no	17-18
	Small nucleolar RNAs (snoRNA)	high	post-translational modification	yes	17-18
Short non coding RNAs (above 200nt in size)	MicroRNAs	high	translational repression	yes	20,21
	Tiny transcription initiation RNAs	high	may regulate gene expression	not known	18
	Repeat associated small interfering RNAs	high	gene regulation, transposon control and viral defence	not known	18
	Promoter-associated short RNAs	high	may regulate gene expression at chromatin level	not known	18, 22,24
	Termini-associated short RNAs	high	may regulate gene expression at chromatin level	not known	18, 22,24
	Antisense termini associated short	high	may regulate gene expression at chromatin level	not known	17, 22,24
	Piwi-interacting RNAs	high	regulate transposon activity and chromatin state	yes	23
	Transcription start site antisense RNAs	moderate	may regulate transcription	not known	17
	Retrotraspon-derived RNAs	high	may regulate transcription	not known	24
	3`UTR-derived RNAs	moderate	may regulate transcription	not known	17
	Splice-site RNAs	poor	not known	not known	18
Long non coding RNAs (over 200nt in size)	Long or large intergenic ncRNAs	high	epigenetic regulation, protein complex subcellular compartments or localization	yes	25,26
	Transcribed ultraconserved regions	high	not known	yes	27
	Pseudogenes	high	competitive endogenous RNAs	yes	25, 28
	Enhancer RNAs	high	not known	yes	29
	Long intronic ncRNAs	moderate	not known	not known	17,18
	Repeat associated ncRNAs	high	not known	not known	23
	Antisense RNAs	high	gene expression	not known	28
	Promoter associated long RNAs	moderate	may regulate gene expression at chromatin level	not known	22, 30
	Long stress-induced non-coding transcripts	moderate	epigenetic regulation, protein complex subcellular compartments or localization	yes	17, 18

Table 1. Non coding RNA in human genome.

independent transcription units. Animal miRNAs are processed from longer primary transcripts (pri-miRNAs) that can contain multiple miRNAs [34,35]. Few pri-miRNA transcripts have been studied in detail, but in general miRNAs are regulated and transcribed similar to protein encoding genes by (Pol) II with the exception of the rapidly evolving RNA polymerase (Pol) III transcribed miRNA cluster [36]. MiRNA processing occurs in three essential steps (**Figure 1**). First, the nuclear endoribonuclease protein Drosha recognizes the miRNA hairpins in the primary transcript and cleaves each hairpin ~11 nt from its base [37-38]. It has been proposed that Drosha may recognize the pri-miRNA through the stem-loop structure and then cleave the stem at a fixed distance from the loop to liberate the pre-miRNA. How is the Drosha enzyme able to discriminate the pri-miRNA stem-loop structure from the other stem-loop cellular RNAs? Both cell culture experiments and in vitro Drosha cleavage assays have shown that proteins associated with Drosha confer specificity to this process. In fact, Drosha has been found to be part of a large, ~650-kDa protein complex known as the Microprocessor [39], where Drosha interacts with its cofactor DGCR8 (the DiGeorge syndrome critical region gene 8 protein) in the human and interacts with Pasha in *Drosophila melanogaster* [40]. The next step in miRNA biogenesis is recognition of the ~60 nt pre-miRNA by exportin-5 and export into the cytoplasm in a ran-guanine-GTP-dependent manner [41-43]. The Exp5/Ran-GTP complex has a high affinity for pre-miRNAs,
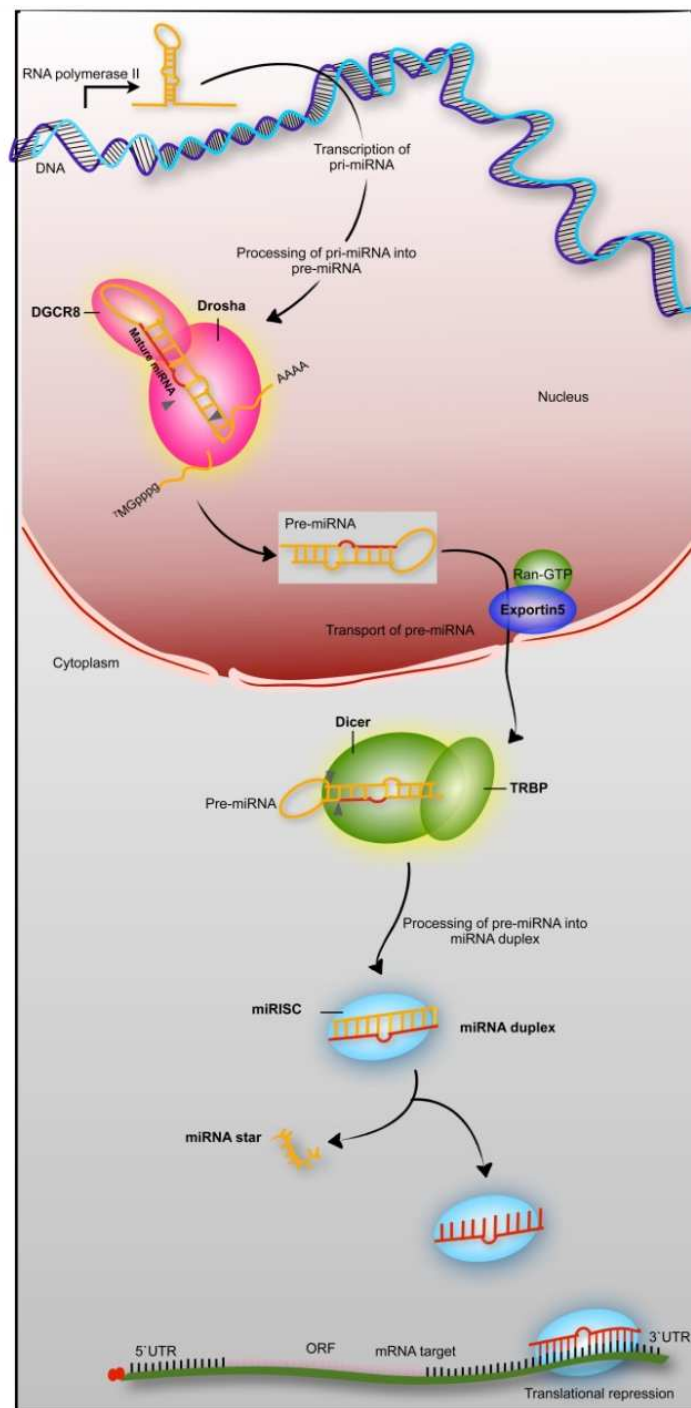
Figure 1. miRNA biogenesis and function. The primary miRNA (pri-miRNA) is transcribed by RNA pol II from its genomic location and cleaved by the microprocessor complex, which comprises Drosha and DGCR8. The resulting pre-miRNA is actively transported to the cytoplasm by exportin 5 (Expt.5), where the pre-miRNA undergoes further processing into the mature miRNA by Dicer and its co-factors, protein activator of interferon-induced protein kinase (PACT) and TAR RNA binding protein (TRBP). Normally, one strand of this duplex is degraded (miRNA star), whereas the other strand accumulates as a mature miRNA. From the miRNA-miRNA duplex, only the miRNA enters preferentially in the protein effector complex, formed by the RNA-induced silencing complex (RISC) and miRgonaute and binds with partial complementarity to the 3' untranslated region (UTR) of target messenger RNAs (mRNAs) to mediate translational repression.

protecting them from the moment they are generated in the nucleus until they are ready for the next cleavage step in the cytoplasm, where GTP is hydrolyzed to guanosine diphosphate (GDP); at that point, the Exp5/Ran-GDP complex releases its cargo. Third, the endoribonuclease protein Dicer cleaves the pre-miRNA into ~22 nt duplexes and, with the help of cofactors such as TAR RNA binding protein (TRBP) and protein activator of the interferon-induced protein kinase (PACT), preferentially incorporates one of the duplex strands Into the RNA induced-silencing complex (RISC) [44-50]. The final product is a miRNA-miRNA duplex that needs to be unwound to act as a single-stranded guide in the RISC to recognize its target mRNAs. It was originally proposed that an ATP-dependent helicase (known as unwindase) separates the two small RNA strands, after which the resulting single-stranded guide is loaded into Ago proteins. However, it was later shown that *Drosophila* Ago2 [51], as well as human Ago2 [52], directly receive double-stranded small RNA from the RISC-loading complex. Ago2 then cleaves the passenger strand, thereby liberating the single-stranded guide to form mature Ago2-RISC. In mammals, miRNAs guide the RISC to complementary target sites in mRNAs, where endonucleolytically active Ago proteins cleave the RNA [53] (**Figure 1**). Finally, RISC can cleave [54-55] degrade [56-57] or suppress translation [58-59] of target mRNAs depending on the complementarity between miRNA and mRNA. Imperfect base pairing between small RNAs and their target mRNAs leads to repression of translation and/or deadenylation (removal of the polyA tail of the target), followed by destabilization of the target [60], whereas perfect base pairing usually leads to mRNA degradation.

3. MicroRNAs and cancer

Cancer is a multistep process in which normal cells experience genetic changes that progress them through a series of pre-malignant states (initiation) into invasive cancer (progression) that can spread throughout the body (metastasis). The dysregulation of genes involved in cell proliferation, differentiation and/or apoptosis is associated with cancer initiation and progression. Genes linked with cancer development are characterized as oncogenes and tumor suppressors. Recently, the definition of oncogenes and tumor suppressors has been expanded from the classical protein coding genes to include miRNAs [61-62]. MiRNAs have been found to regulate more than 60% of mRNAs and have roles in fundamental processes, such as development [63], differentiation [64], cell proliferation [65], apoptosis [66], and stress responses [67]. Over the past few years, many miRNAs have been implicated in various human cancers. The first evidence that miRNAs are involved in cancer comes from the finding that miR-15 and miR-16 are downregulated or deleted in most patients with chronic lymphocytic leukemia [68]. This discovery has projected miRNAs to the center stage of molecular oncology and, in the past few years, a myriad of genome-wide miRNA expression profiling analyses have shown a general dysregulation of miRNA expression in all tumors (**Table 2**) [69]. Surprisingly, the use of miRNA profiles is newly becoming highly preferred to the traditional mRNA signature for a variety of reasons. First, the remarkable stability of miRNAs, due to their short length, has allowed scientists to perform analyses also in samples considered to be technically challenging, such as formalin fixed specimens. High sensitive and refined miRNA detection technique provide high reliability in the use of miRNAs as a diagnostic tools. Finally, miRNA fingerprints have demonstrated the ability to

identify the tissue of origin for cancer that have already spread in multiple metastatic sites, thereby reducing patient's psychological burden and overall procedure costs. To date, over 1000 miRNAs have been reported in humans (miRbase: 1527 at November 2011), and both loss and gain of miRNA functions contribute to cancer development through a range of different mechanisms that we will discuss in the following sections.

Cancer	Authors	Samples	Main findings	miRNA signature
Breast Cancer	Iorio et al. 2005	10 normal 76 tumors	First miRNA signature of breast cancer. 15 miRNAs predict the nature of the sample analyzed with 100% accuracy.	**miR-21, -155** were up-regulated while **miR-10b, -125b, -145** were down-regulated.
	Mattie et al. 2006	20 tumors	Unique sets of miRNAs are associated with ErbB2 and ER/PR status.	ErbB2 status (**let-7f, let-7g, miR-107, -10b, -126, -154, -195**) ER/PR status (**miR-142-5p, -200a, -205, -25**).
	Blenkiron et al. 2007	93 tumors	Striking differences in miRNA expression between breast tumor molecular subtypes (luminal A, luminal B, basal-like, HER2+ and normal breast-like).	-
	Sempere et al. 2007	> 100 pairs tumor-normal	In situ hybridization method to reveal the spatial distribution of miRNA expression in archived formalin-fixed breast tumors	20 miRNAs differentiate matched normal/tumor. **miR-21** up-regulated in all tumor specimens, **miR-145** and **miR-451** were the only miRs high expressed in normal specimens and low expressed in tumor specimens. **let-7a, miR-21,-141, -214** preferentially expressed in luminal cells, **miR-145** and **-205** in myoepithelial cells.
	Foekens et al. 2008	299 tumors	Identification of miRNAs associated with metastatic capability.	**miR-7, -128a, -210, -516-3p** associated with tumor aggressiveness in ER-positive patients with lymph node-negative disease. **miR-210** associated with early relapse in ER-negative patients with lymph node-negative disease and with poor outcome.
	Volinia et al 2012	80 IDC 8 DCIS 6 normal	Identification of miRNA dysregulation during the transition from ductal carcinoma in situ to invasive ductal carcinoma	**Let-7d, miR-210,** and **-221** down-regulated in DCIS and up-regulated in IDC. **miR-210, -21, -106b*, -197,** and **let-7i** associated with prognosis. Only miR-210involved in the invasive transition.
Lung Cancer	Yanaihara et al. 2006	104 pairs tumor-normal	miRNA expression profiles are diagnostic and prognostic markers of lung cancer.	43 miRNAs discriminate between normal ad tumors. **let-7a-2, mir-155, -17-3p, -145, -21** were associated with adenocarcinoma patients survival.
	Raponi et al. 2009	10 normal 61 squamous	miRNAs may have greater clinical utility in predicting the prognosis of patients with squamous cell lung carcinomas than mRNA-based signatures.	15 miRNAs that were differentially expressed including **miR-17/92** cluster, **miR-155**, let-7. **miR-146b** has the strongest prediction accuracy for stratifying prognostic groups.
	Landi et al. 2010	290 tumors 165AC-125SQ	miRNA expression profiles can distinguish adenocarcinoma (AC) from squamous carcinoma (SQ) and predict survival.	**let-7g** and **miR-26** were the most down-regulated in SQ versus AC. **miR-25, -34c-5p, -34a, -191** and **let-7e** strongly predicted SQ survival for the 107 male smokers with early-stage SQ tumors. **miR-21** is overexpresssed in AC and may be a marker of tumor progression in adenocarcinoma.
	Tan et al. 2011	34 pairs tumors-normal	miRNA expression profiles of squamous carcinoma vs normal tissues.	**miR-210, -182, -486-5p, -30a, -140-3p** discriminate between cancerous and normal lung tissues. **miR-31** was associated with poor survival in squamous cell carcinoma.
	Lu et al. 2012	527 tumors	Identification of miRNA signatures that predict prognosis of stage I NSCLC.	Two miRNA signatures that are highly predictive of recurrence/relapse free survival were identified. The first contained 34 miRNAs derived from 357 stage I NSCLC patients independent of cancer subtype, whereas the second containing 27 miRNAs was adenocarcinoma specific.
	Guan et al. 2012	14 different dataset	A meta-analysis reviews 14 microRNA expression profiling studies that compared the microRNAs expression profiles in lung cancer tissues with those in normal lung tissues.	184 miRNAs discriminate lung cancer tissues from normal tissues; 61 miRNAs were reported in at least two studies. Up-regulated miRNA: **miR-210** was reported in 9 studies and **miR-21** in 7 studies.Down-regulated miRNA: **miR-126** was reported in 10 studies and **miR-30a** in 8 studies.
Thyroid Cancer	Pallante et al. 2006	10 normals 30 tumors	Identification of the miRNA expression profile of papillary carcinomas.	A significant increase in **miR-221, -222** and **-181b** was detected in papillary carcinomas in comparison with normal thyroid tissue.
	Visone et. al 2007	10 normals 76 tumors	Identification of the miRNA expression profile of anaplastic carcinomas from normal thyroid tissues.	A significant decrease in **miR-30d, -125b, -26a, -30a-5p** was detected in anaplastic carcinomas compared to normal thyroid tissue.
	Nikiforova et al. 2008	60 normals 60 tumors	Identification of miRNA signature for the different thyroid tumors subtypes: Oncocytic, conventional follicular, papillary and medullary carcinomas.	**miR-187, -221, -222, -146b, -155, -224, -197** were the most differentially overexpressed in thyroid tumors vs. hyperplastic nodules and combination of them classified the different subtypes.
	Yip et al. 2011	32 tumors	Identification of a specific signature in aggressive (17) compared with nonaggressive papillary carcinomas (15).	Upregulation of **miR-146b, -221, -222, -155, -31** and downregulation of **miR-1, -34b, -130b, -138** downregulation. **miR-146** overexpression was associated with aggressive behavior in BRAF-positive tumors.
	Kitano et al 2011	47 tumors	miRNAs expression signature for samples representing difficult to diagnose histologic subtypes of thyroid neoplasm (21 benign, 26 malignant).	Out of 34 differentially expressed miRNA, **miR-126** and **miR-7** had high diagnostic accuracy and could be helpful in the classification of benign and malignant thyroid tumors.

a

Cancer	Authors	Samples	Main findings	miRNA signature
Colon Cancer	Schetter et al. 2008	84 pairs tumor-normal	miRNA expression profiles and clinical correlation for colon cancer.	37 miRNAs differentially expressed in tumors: high **miR-21** was in adenomas and tumors with more advanced TNM staging. High **miR-21** expression was also associated with poor survival and therapeutic outcome.
	Schepeler et al. 2008	10 normals 49 tumors	miRNA expression profiles of colon cancer with different microsatellite status and prognosis.	**miR-145** showing the lowest expression in cancer relative to normal tissue. **miR-142-3p, -212, -151, -144** were associated with tumor microsatellite status. High expression of **miR-320** or **-498** associated with good prognosis.
	Arndt et al. 2009	8 cell lines 4 normals 45 tumors	miRNA expression profiles for colon cancer.	37 miRNAs discriminate between colorectal cancer and normal tissues. 22 miRNAs were differentially expressed between normal and early stage cancer including increases in **miR-21** and **-224** and decreases in **miR-133a** and **-145**. A differential expression in **miR-31, -7, -99b, -378*, -133a, -125a** discriminates between early and late stage.
Prostate Cancer	Ambs et al. 2008	16 normals 60 tumors	First miRNA expression profiles for prostate cancer.	Up-regulated miRs: **miR-32, -182, -31, -26a, -200c, -196a, -106b/25** cluster; down-regulated miRNAs included **miR-520h, -494, -490, -1/133a** cluster.
	Tong et al. 2009	40 pairs normal-tumor	miRNA expression profiles of paired microdissected malignant and non-involved areas from stage T2a/b, early relapse and non-relapse cancer patients.	**miR-23b, -100, -145, -221, -222** were significantly downregulated in malignant tissues. Patients with post-surgery elevation of prostate-specific antigen (chemical relapse) displayed a distinct profile of 16 miRNAs, as compared with those with non-relapse disease.
	Schaefer et al. 2010	76 pairs normal-tumor	miRNA expression profiles for prostate cancer and clinical correlation.	**miR-16, -31, -125b, -145, -149, -181b, -184, -205, -221, -222** were downregulated and **miR-96, -182, -182, -183, -375** were upregulated. Expression of 5 miRNAs correlated with Gleason score or pathological tumor stage.
Liver Cancer	Murakami et al. 2006	25 pairs normal-tumor	miRNA expression profiles in hepatocellular carcinoma and non-tumorous tissues.	**miR-18**, precursor **miR-18, -224, -199a*, -195, -199a, -200a, -125a** differentially expressed between cancer and normal tissues; **miR-92, -20, -18** and precursor **miR-18** were significantly higher in poorly differentiated tumors. In contrast, **miR-99a** exhibited a positive correlation between expression levels and degree of tumor differentiation.
	Budhu et al. 2008	241 pairs normal-tumor	miRNA expression profiles in liver cancer predict metastasis.	A unique 20-miRNA metastasis signature was identified that could predict primary neoplastic tissues with venous metastases from metastasis-free solitary tumors. **mir-219-1, -207,** and **-338** were most highly up-regulated, whereas **mir-34a, -30c-1, -148a** were most highly down-regulated in metastasis cases.
	Wang et al. 2008	4 normals 46 tumors	miRNA expression profiles in hepatocellular carcinoma and non-tumorous tissues.	**miR-224** overexpression identified in all tumors and **miR-200c, -200, -21, -224, -10b, -222** specific deregulation in benign or malignant tumors. **miR-96** was overexpressed in HBV tumors, and **miR-126*** was down-regulated in alcohol-related hepatocellular carcinoma. Down-regulations of **miR-107** and **miR-375** were specifically associated with HNF1alpha and beta-catenin gene mutations, respectively.
	Ji et al. 2009	241 pairs normal-tumor	miRNA expression profiles in hepatocellular carcinoma and non-tumorous tissues and significant correlation to survival.	Reduced **miR-26** in tumors and the expression of **miR-26a** and **miR-26b** in nontumor liver tissue was higher in women than in men. Low **miR-26** is associated to a short overall survival but a better response to interferon therapy.
	Toffanin et al. 2011	89 tumors	miRNA expression profiles in hepatocellular carcinoma and non-tumorous tissues: identification of tumor subtypes and new oncomiRs.	3 main clusters of hepatocellular carcinoma: beta-catenin gene mutated tumors (36%), interferon-response–related genes (33%), and tumors with abnormal activation of IGF and mTOR-(PI)3K pathways (31%). A subset of tumors in last subclass (9%) overexpressed a family of miRNAs from chr19q13.42: **miR-517a** and **miR-520c** (from ch19q13.42) increased proliferation, migration, and invasion of HCC cells in vitro.

b

Cancer	Authors	Samples	Main findings	miRNA signature
Ovarian Cancer	Iorio et al. 2007	5 cell lines 15 normals 69 tumors	miRNA expression profiles of ovarian cancer vs normal and tumor subtype-specific miRNA signature.	29 miRNAs differentially expressed between normal and tumor with a classification rate of 89%. 4 up-modulated: miR-200a, -200b, -200c, -141; 25 down-modulated: miR-199a, -140, -145, -125b-1 among the most significant.
	Yang et al. 2008	10 normal cells 10 tumors	miRNA expression profiles of ovarian cancer vs normal ephitelial cells.	Up-regulation of miR-214, -199a*, -200a and down-regulation of miR-100; alterations of the first three miRNAs is associated with late-stage and high-grade ovarian tumors. miR-214 induces cell survival and cisplatin resistance by targeting the PTEN/Akt pathway.
	Wu et al. 2009	10 pairs normal-tumor	miRNA expression profiles of endometrioid ovarian cancer vs normal tissues.	17 up-regulated (miR-205, -449, -429) and 6 down-regulated (miR-204, -99b, -193b) miRNAs in endometrioid adenocarcinoma samples.
	Marchini et al. 2011	144 tumors	miRNA expression profiles of stage I ephitelial ovarian cancer assess the existence of a miRNA signature associated with overall and progression-free survival.	34 miRNAs were associated with survival. Between them miR-200c, -199a-3p, -199a-5p were highly associated with overall and progression-free survival.
	Devor et al. 2011	4 normal 23 tumors	miRNA signature in ovarian cancer vs normal and in serous (9) and endometrioid (14) subtypes	7 miRNAs was down-regulated and 13 miRNAs up-regulated in both adenocarcinomas. miR-133b the most repressed miRNA in both adenocarcinomas; miR-205 the most expressed miRNA. miR-135b, -200a, -200b, -200c, -141, -429 significantly overexpressed in both types of endometrial cancers.
Gastric Cancer	Luo et al. 2009	2 cell lines 3 normals 24 tumors	miRNA expression profiles of gastric cancer vs normal.	19 miRNAs down-regulated and 7 miRNAs up-regulated. miR-433 and miR-9 were remarkably down-regulated in the carcinoma samples.
	Ueda et al. 2010	160 pairs normal-tumor	miRNA expression profiles of gastric cancer vs normal and miRNA signature in histological subtypes	22 miRNAs upregulated and 13 downregulated in gastric cancer. Diffuse-type and intestinal-type subtypes were discriminated by miRNA expression. miR-125b, -199a, -100 were the most important microRNAs involved progression signature. Low let-7g and miR-433 and high expression of miR-214 were associated with unfavourable outcome in overall survival independent of clinical covariates, including depth of invasion, lymph-node metastasis, and stage.
	Brenner et al. 2011	45 tumors	miRNA expression profiles of primary tumor of patients with recurrent and non-recurrent gastric cancer.	miR-451, -199a-3p, -195 differentially expressed in gastric tumors from patients with good prognosis vs bad prognosis. High expression of each miR was associated with poorer prognosis for both recurrence and survival. miR-451 showed a positive predictive value for non-recurrence of 100%.
	Kim et al. 2011	34 normals 90 tumors	miRNA signature distinguishes gastric cancer from normal stomach epithelium from healthy volunteers, and a chemo-reresistance miRNA signature that is correlated with time to progression after cisplatin/fluorouracil therapy	30 miRNAs inversely correlated with time to progression of disease after chemotherapy whereas 28 miRNAs positively correlated. Among the upregulated miRNAs associated with chemosensitivity: let-7g, miR-342, -16, -181, -1, -34 known to regulate apoptosis.
Esophageal Cancer	Guo et al. 2008	31 pairs normal-tumor	miRNA expression profiles of esophageal cancer vs normal tissues.	46 miRNAs differently expressed between the cancerous and adjacent normal tissues. A minimal set of 7 distinguishes malignant from normal esophageal tissues: miR-25, -424, -151 showed up-regulation and miR-100, -99a, -29c, -140* showed down-regulation. High miR-103/107 correlated with poor survival.
	Yang et al. 2009	32 pairs normal-tumor	miRNA expression profiles of esophageal cancer progression from Barret`s low to high grade dysplasia to adenocarcinoma.	111 miRNAs differentiated the adenocarcinoma tissues with 100% accuracy. 11 miRNAs may be important in the progression from low-grade to high-grade dysplasia. let-7b/a/c/f, miR-345, -494, -193a were modulated in the progression from high-grade dysplasia to adenocarcinoma, and all of them were down-regulated in esophageal adenocarcinoma.
	Mathe et al. 2009	170 tumors	Identification of miRNAs involved in major histologic types of esophageal carcinoma and significant associations with prognosis.	In adenocarcinoma patients: high expression of miR-21, -223, -192, -194 and low miR-203 levels. In squamous carcinoma: high expression of miR-21 and low miR-375 levels. High miR-21 in normal tissue of squoamous carcinoma and low levels of miR-375 in cancerous tissue of adenocarcinoma patients with Barrett's were strongly associated with worse prognosis.
	Fassan et al. 2011	14 normals 23 dysplasias	miRNA expression profiles in Barrett`s subtypes (7 low grade dysplasia, 5 high grade dysplasia, 11 Barrett`s adenocarcinomas)	Up-regulation of miR-215, -560, -615-3p, -192, -326, -147 and down-regulation of miR-100, -23a, -605, -99a, -205, let-7c, -203.
	Feber et al. 2011	45 tumors	miRNA expression profiles provide prognostic utility in staging esophagus cancer patients and elucidate steps in the metastatic pathway and allow for development of targeted therapy.	Up-regulation of miR-143, -199a_3p, -199a_5p, -100, -99a predicted a worse survival. miR-99b, -199a_3p and _5p also associated with the presence of lymph node metastasis.

c

Table 2. miRNA profiling in cancer.

4. Oncogenic microRNAs

Although studies linking miRNA dysfunctions to human diseases are in their infancy, a great deal of data already exists, establishing an important role for miRNAs in the pathogenesis of cancer. Many miRNAs have been shown to function as oncogenes in the

majority of cancers profiled to date (**Table 3**). *MiR-21* displays a strong evolutionary conservation across a wide range of vertebrate species in mammalian, avian and fish clades [70]. It has been demonstrated that a primary transcript containing *miR-21* (i.e., *pri-miR-21*) is independently transcribed from a conserved promoter that is located within the intron of the overlapping protein-coding gene *TMEM49* [71]. Several studies suggest that this miRNA is oncogenic [72-74] and that it may act as an antiapoptotic factor. For example, Chan et al. have found that miR-21 is commonly and markedly up-regulated in human glioblastoma and that inhibiting miR-21 expression leads to caspase activation and associated apoptotic cell death [72]. Moreover, Zhu and collaborators provided the first evidence that *miR-21* regulates invasion and metastasis, at least in part, by targeting metastasis-related tumor suppressor genes such as TPM1, programmed cell death 4 (PDCD4) and maspin [73]. Furthermore, examination of human breast tumor specimens revealed an inverse correlation of *miR-21* with PDCD4 and maspin [74]. The final proof of miR-21 oncogenic activity came from the Slack laboratory where the first conditional knock-in of miR-21 overexpressing mice was generated. The mice developed a severe pre-B-cell lymphoma but when miR-21 was reduced to endogenous levels, the mouse tumors completely disappeared, defining the concept of "oncomiR addition" [75].

Another important oncogenic miRNA is represented by *miR-155*. Several groups have shown that *miR-155* is highly expressed in pediatric Burkitt's lymphoma [76], Hodgkin's disease [77], primary mediastinal non-Hodgkin's lymphoma [77], chronic lymphocytic leukemia (CLL) [78], acute myelogenous leukemia (AML) [79], lung cancer [80], pancreatic cancer [81], and breast cancer [80]. Dr. Croce laboratory reported that *miR-155* transgenic mice develop acute lymphoblastic leukemia/high-grade lymphoma and that most of these leukemias start at approximately nine months, irrespective of the mouse strain, preceded by a polyclonal pre-B-cell proliferation [82].

Another example of "oncomiR" is represented by *miR-221&222* cluster that is highly upregulated in a variety of solid tumors, including thyroid cancer [83], hepatocarcinoma [84], estrogen receptor negative breast tumor [85], and melanoma [86]. Elevated *miR-221&222* expression has been causally linked to proliferation [85-87], apoptosis [88-89], and migration [89] of several cancer cell lines. We recently reported that the hepatocyte growth factor receptor (MET) oncogene, through c-Jun transcriptional activation, upregulates *miR-221&222* expression, which, in turn, by targeting *PTEN* and *TIMP3*, confers resistance to tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) and enhances tumorigenicity of lung and liver cancer cells [89]. The results suggest that therapeutic intervention involving the use of miRNAs should not only sensitize tumor cells to drug-inducing apoptosis but also inhibit their survival, proliferation, and invasion [89].

The miR-106b-25 polycistron is composed of the highly conserved miR-106b, miR-93, and miR-25 that accumulate in different types of cancer, including gastric, prostate, and pancreatic neuroendocrine tumors, as well as neuroblastoma and multiple myeloma. Petrocca and collaborators [90] demonstrated that E2F1 regulates miR-106b, miR-93, and miR-25, inducing their accumulation in gastric tumors. Conversely, miR-106b and miR-93 control E2F1 expression, establishing a negative feedback loop that may be important in preventing E2F1 self-activation and apoptosis. On the other hand, miR-106b, miR-93, and

miRNA	Target	Tumor
miR-21	PTEN	cholangiocarcinoma
	TPM1	breast cancer
	PDCD4	breast cancer
	SPRY1	
	RECK, TIMP3	glioblastoma
	p63, JMY, TOPORS, TP53BP2, DAXX, HNRPK, TGFβRII	glioblastoma
	MARKS	prostate cancer
	ANP32A, SMARCA4	prostate cancer
miR-155	SOCS1	breast cancer
	CEBPB, PU.1 ,CUTL1, PICALM	AML
	BACH1, ZIC3	
	ETS1, MEIS1	human cord blood CD34+
	C-MAF	lymphocytes
	HGAL	diffuse large B-cell lymphoma
	JMJD1A	nasopharyngeal carcinoma
	WEE1	breast cancer
	TP53INP1	pancreatic cancer
	SMAD1, SMAD5, HIVEP2, CEBPB, RUNX2, MYO10	
	FOXO3A	breast cancer
	hMSH2, hMSH6, hMLH1	colon cancer
	SMAD5	diffuse large B-cell lymphoma
miR-221/222	p27(KIP1)	glioblastoma, prostate and thyroid carcinoma
	p57 (KIP2)	normal fibroblast
	PTEN, TIMP3	non small cell lung cancer and hepatocellular carcinoma
	FOXO3A	breast cancer
	KIT	Endothelial cells
	ESR1	breast cancer
	PUMA	glioblastoma
	TRSP1	breast cancer
	PTPμ	glioblastoma
	DICER	breast cancer
	APAF1	non small cell lung cancer
miR-106a~363 miR-106b~25	BIM, p21	gastric cancer
	E2F1	prostate cancer
	PTEN	prostate cancer
miR-17-92	TSP-1, CTGF	colon
	E2F2, E2F3	prostate/Burkitt lymphoma/testis carcinoma/
	BIM PTEN	c-Myc induced lymphoma
	HIF1α	lung cancer
	PTPRO	cervix tumor cell line
	p63	myeloid cells
	BIM, PTEN, PRKAA1, PPP2R5e,	T-cell acute lymphoblastic leukaemia
	JAK1	endothelial cells
	HBP1	breast cancer
	p21(WAF1)	Ras induced senescent-fibroblasts
	TGFβII, SMAD4	glioblastoma
	MnSOD, GPX2, TRXR2	prostate
miR-10a/10b	HOXB1, HOXB3	pancreatic cancer
	HOXD10	breast cancer
	KLF4	esophageal cancer
	TIAM1	breast cancer
	NF1	Ewing's sarcoma

Table 3. *-oncomiRs*

miR-25 overexpression causes a decreased response of gastric cancer cells to TGFβ by downregulating p21 and Bim, the two most downstream effectors of TGFβ-dependent cell cycle arrest and apoptosis, respectively.

Another example of a miRNA locus with oncogenic properties is represented by the *miR-17-92* cluster, which consists of six miRNAs: miR-17-5p, -18, -19a, -19b, -20a, and -92-1. The miR-17-92 cluster is located in a region frequently amplified in several types of lymphoma and solid tumors [91-92]. It has been shown that mice deficient for miR-17-92 die shortly after birth with lung hypoplasia and a ventricular septal defect. This cluster is also essential for B cell development; its absence, in fact, leads to increased levels of the proapoptotic protein Bim and inhibits B cell development at the pro-B-to-pre-B transition [93]. All together these studies indicate that many miRNAs have oncogenic activity. Importantly, their knockdown through the use of antisense oligonucleotides, inhibits the development of cancer-associated phenotypes, laying the groundwork for the creation of miRNA-based therapies [94-96].

5. Tumor suppressor microRNAs

The first evidence that miRNAs are involved in cancer comes from the finding that miR-15 and miR-16 are downregulated or deleted in most patients with chronic lymphocytic leukemia (CLL) (**Table 4**) [68]. They are transcribed as a cluster (*miR-15a–miR-16-1*) that resides in the 13q14 chromosomal region. Deletions or point mutations in region 13q14 occur at high frequency in CLL, lymphoma, and several solid tumors [97]. Their expression is inversely correlated to *BCL2* expression in CLL [98]. The tumor suppressor function of *miR-15a/16-1* has also been addressed in vivo. In immunocompromised nude mice, ectopic expression of *miR-15a/16-1* was found to cause dramatic suppression of tumorigenicity of MEG-01 leukemic cells that exhibited a loss of endogenous expression of *miR-15a/16-1*. Furthermore, Klein et al. [99] generated transgenic mice with a deletion of the *miR-15a–miR-16-1* cluster, causing development of indolent B-cell-autonomous, clonal lymphoproliferative disorders, recapitulating the spectrum of CLL-associated phenotypes observed in humans. Recently, Bonci et al. reported that the *miR-15a–miR-16-1* cluster targets not only *BCL2* but also *CCND1* (encoding cyclin D1) and *WNT3A* mRNA, which promote several prostate tumorigenic features, including survival, proliferation, and invasion [100]. Together, these data suggest that *miR-15a/16-1* genes are natural antisense interactors of *BCL2* and probably other oncogenes and that they can be used to suppress tumor growth in therapeutic application for a variety of tumors [100].

In mammalians, the miR-34 family comprises three processed miRNAs that are encoded by two different genes: miR-34a is encoded by its own transcript, whereas miR-34b and miR-34c share a common primary transcript. The miR-34 family has been shown to form part of the p53 tumor-suppressor network: their expression is directly induced by p53 in response to DNA damage or oncogenic stress [101-102]. He et al. identified different miR-34 targets such as cyclin E2 (CCNE2), CDK4, and MET. Silencing these selected miR-34 targets through the use of small interfering RNAs (siRNAs) led to a substantial cell cycle arrest in G1.

Moreover, ectopic miR-34 delivery caused a decrease in levels of phosphorylated retinoblastoma gene product (Rb), consistent with lowered activity of both CDK4 and CCNE2 complexes [102]. BCL2 and MYCN were also identified as miR-34a targets and likely mediators of the tumor suppressor phenotypic effect in neuroblastoma [103]. It has been also reported that p53 activation suppressed the EMT-inducing transcription factor SNAIL via induction of the miR-34a/b/c genes. In fact, suppression of miR-34a/b/c by anti-miRs caused up-regulation of SNAIL and cells displayed EMT markers, enhanced migration and invasion [104].

MicroRNA-122 (miR-122) is a liver-specific microRNA and is frequently downregulated in liver cancer [105]. Xu et al. reported that restoration of miR-122 in hepatocellular carcinoma cells could render cells sensitive to chemotherapeutic agents adriamycin or vincristine through downregulating antiapoptotic gene Bcl-w and cell cycle related gene cyclin B1 [106]. Another group found that over-expression of miR-122 inhibits hepatocellular carcinoma cell growth and promotes the cell apoptosis by affecting Wnt/β-catenin signalling pathway [107]. Coulouarn et al. showed that miR-122 is specifically repressed in a subset of primary hepatocellular tumors that are characterized by poor prognosis [108]. They further reported that loss of miR-122 resulted in an increase of cell migration and invasion and that restoration of miR-122 reverses this phenotype [108]. The final understanding of the tumor suppressor role for mir-122 role in liver cancer came from a recent study where miR-122 knockout mice were studied. When miR-122 KO mice aged, hepatic inflammation ensued, preceding the progressive onset of fibrosis and, eventually, tumors resembling human liver cancer. These pathologic manifestations were associated with hyperactivity of oncogenic pathways and hepatic infiltration of inflammatory cells that produce pro-tumorigenic cytokines, including IL-6 and TNF [109].

miRNA	Target	Tumor
	BCL2	CLL
	COX-2	colon cancer
	CHEK1	follicolar lymphoma
	CEBPβ, CDC25a, CCNE1	fibroblast
	VEGF, VEGFR2, FGFR1	fibroblast
miR-15/16	FGF2, FGFR1	cancer associated fibroblast
	CCNE1	
	FGFR1, PI3KCa, MDM4, VEGFa	multiple myeloma
	WIP1	
	BMI-1	ovarian cancer
	CCND1, CCND2, CCNE1	lung cancer
	SIRT1	colon cancer
	BCL2, NOTCH, HMGA2	
	MYC	fibroblast
miR-34	AXL	lung cancer
	MET	ovarian cancer
	NANOG, SOX2, MYCN	embryonic fibroblast
	SNAIL	colon cancer

Table 4. Tumor suppressor *miRS*

6. MetastamiRs

Metastasis is the result of cancer cells detaching from a primary tumor, consequently adapting to distant tissues and organs, and forming a secondary tumor [110] and this ability of cancer cells to metastasize is a hallmark of malignant tumors [111-112]. To successfully metastasize, a tumor cell must complete a complex set of processes, including invasion, survival and arrest in the circulatory system, and colonization of foreign organs. Despite great advancements in knowledge of metastasis biology, the molecular mechanisms are still not completely understood. Several miRNAs have been shown to initiate invasion and metastasis by targeting multiple proteins that are major players in these cellular events, thus they have been denominated as metastamiRs (**Table 5**). It seems that these metastasis-associated miRNAs do not influence primary tumor either in development or initiation steps of tumorigenesis, but they regulate key steps in the metastatic program and processes, such as epithelial-mesenchymal transition (EMT), apoptosis, and angiogenesis. Ma et. al reported that miR-10b is highly expressed in metastatic breast cancer cells and positively regulates cell migration and invasion. Overexpression of miR-10b in otherwise non-metastatic breast tumors initiates robust invasion and metastasis [113]. The team led by Joan Massague found that miR-335, miR-126, and miR-206 are metastasis-suppressors in breast cancer [114]. MiR-126 and miR-206 restoration reduced overall tumor growth and proliferation, whereas miR-335 inhibits metastatic cell invasion through targeting of the progenitor cell transcription factor *SOX4* and extracellular matrix component tenascin C [114]. Others miRNAs with prominent roles in breast cancer metastasis have been reported. It has been reported that miR-31 inhibited multiple steps of metastasis including invasion, anoikis, and colonization leading to almost complete reduction of lung metastasis [115]. Clinically, miR-31 levels were lower in breast cancer patients with metastasis. In addition, miR-9, which is up-regulated in breast cancer cells, directly targets CDH1, the E-cadherin-encoding messenger RNA, leading to increased cell motility and invasiveness [116].

Another important aspect of the metastatic dissemination is represented by the epithelial-to-mesenchymal transition (EMT) that allow neoplastic cells to abandon their primary site and survive in the new tissue. During EMT, an epithelial neoplastic cell looses cell adhesion by repressing E-cadherin expression and thereby the cell increases its motility. Numerous studies have shown that different microRNAs are modulated during EMT and one of the best-studied example is represented by the miR-200 family. These miRs are commonly lost in aggressive tumors such as lung, prostate, and pancreatic cancer. It has been shown that miR-200 family members directly target ZEB1 and ZEB2, transcription repressors of E-cadherin [117]. In fact, in the highly aggressive mouse lung cancer model where KRAS is constitutively activated and p53 function is perturbed, miR-200 ectopic expression prevented metastasis by repressing ZEB1 and ZEB2 and preventing E-cadherin down-regulation [117]. However, overexpression of the miR-200 family is associated with an increased risk of metastasis in breast cancer and this overexpression promotes metastatic colonization in mouse models, phenotypes that cannot be explained by E-cadherin expression alone [118]. By using proteomic profiling of the targets of mesenchymal-to-ephitelial (MET)-inducing miR-200, the authors discovered that miR-200 globally targets secreted proteins in breast cancer cells. Between the 38 modulated target genes, Sec23a,

which is involved in transporting protein cargo from the endoplasmic reticulum to the Golgi, shows a superior association with human metastatic breast cancer as compared to the currently recognized miR-200 targets ZEB1 and the EMT marker E-cadherin. EMT is first acquired in the onset of transmigration and then reversed in the new metastatic site. Korpal et al. have shown that the miR-200 status predicts predisposition of the cancer to successful metastasis [119].

| miRNA	Target	Tumor
miR-10a/10b	HOXB1, HOXB3	pancreatic cancer
	HOXD10	breast cancer
	KLF4	esophageal cancer
	TIAM1	breast cancer
	NF1	Ewing's sarcoma
miR-9	PRDM1/BLIMP-1	lymphomas
	CDH1	breast cancer
	CAMTA	glioblastoma
miR-31	ITGA5, RDX, RHOA FZD3, M-RIP, MMP16	breast cancer
	SATB2	cancer associated fibroblast
miR-200 family	ZEB1, ZEB2	breast cancer
	ERRFI-1	bladder cancer
	ZEB1, CTNNB1	nasopharyngeal carcinoma
	BMI-1	pancreatic cancer
	PLCγ1	breast cancer
	FAP1	
	SUZ12	breast cancer
	FLT1/VEGFR1	lung cancer
	JAG1, MALM2, MALM3	
	FN1, LEPR, NTRK2, ARHGAP19	breast and endometrial cancer
	p38α	ovarian cancer

Table 5. *metastamiRS*

7. Other non-coding RNAs: Biology and implications in cancer

7.1. snoRNAs: From post-transcriptional modification to cancer

Small nucleolar RNAs (snoRNAs) have, for many years, been considered one of the best-characterized classes of non-coding RNAs (ncRNAs) [120-123] but despite the common assumption that snoRNAs only have cellular housekeeping functions, in the past few years, independent reports have converged in implicating snoRNAs in the control of cell fate and oncogenesis [124-130]. SnoRNAs are small RNAs of 60-300nt in lenght that specifically accumulate in the nucleolar compartment of the cell where are in charge of the 2'-O-ribose methylation and pseudouridylation of specific ribosomal RNA nucleotides, essential

modification for the efficient and accurate production of the ribosome [120-122]. The snoRNAs carry out their function in the form of small nucleolar ribonucleoproteins (snoRNPs), each of which consists of a box C/D or box H/ACA guide RNA, and four associated C/D or H/ACA snoRNP proteins (**Figure 2**). In both cases, snoRNAs hybridize specifically to the complementary sequence in the rRNAs, and the associated protein complexes then carry out the appropriate modification on the nucleotide that is identified by the snoRNAs. Biogenesis of vertebrate snoRNPs is remarkable and highly variable: in fact snoRNA gene organization ranges from independently transcribed genes, endowed with their own promoter elements, to intronic coding units lacking an independent promoter. In both yeast and animals, processing of intron-encoded snoRNAs is largely splicing-dependent; in contrast, the production of plant snoRNAs from introns seems to rely on a splicing-independent process [131]. Moreover, in both contexts (intergenic or intronic), genes can be either single or part of clusters. In the latter case, the generation of individual snoRNAs involves the enzymatic processing of polycistronic precursor RNAs. Such a processing, at least in yeast, appears to involve the same combination of endo- and exoribonucleases required for the maturation of monocistronic pre-snoRNAs [132-134]. The first indication that snoRNAs might have important roles in human disease was provided by the genetic studies on Prader–Willi syndrome (PWS), an inherited human disorder characterized by a complex phenotype, including mental retardation, decreased muscle tone and failure to thrive at birth, short stature, hypogonadism, sleep apnea, behavioral problems and hyperphagia (an insatiable appetite) that can lead to severe obesity [135]. The disease is caused by the genomic loss of the imprinted chromosomic 15q11-q13 locus which is normally only active on the paternal allele. The only characterized and conserved genes within this 121-kb-long genomic interval are the numerous HBII-85 snoRNA gene copies, thus suggesting that loss of expression of these repeated small C/D RNA genes might play a role in conferring some (or even all) phenotypes of the human disease and PWS-like phenotypes in mice (neonatal lethality, growth retardation and hypotonia). In fact, it has been shown that a site-specific deletion of the entire murine MBII-85 gene cluster led to post-natal growth retardation with low postnatal lethality (<15%) only seen in some genetic backgrounds, but no obesity [136]. Although all the imprinted C/D RNAs that have been tested accumulate within the nucleolus, none of them appear to act as RNA guides to modify rRNAs or spliceosomal U-snRNAs; they are called 'orphan C/D RNAs'. So far, the MBII-52 gene clusters have attracted much attention, given that the neuronal-specific MBII-52 small RNA is predicted to interfere (A-to-I RNA editing and/or alternative RNA splicing) with the post-transcriptional regulation of the pre-mRNA that encodes the $5-HT_{2C}$ (5-hydroxytryptamine 2C) receptor, playing a key role in regulating serotonergic signal transduction [137-138]. These observations raised the possibility that snoRNAs could have functions completely independent from their traditional activities and carry out other regulatory roles. The first insights into the potential roles of snoRNAs in cancer began with a study that identified C/D box snoRNA U50 and its host gene U50HG at the breakpoint in the t(3;6) (q21;q15) translocation in a diffuse large B cell lymphoma [139]. Moreover, snoRNAU50 gene has been found to undergo to a frequent copy number loss and a transcriptional downregulation in breast and prostate cancer samples [139,140]. In addition, a 2-bp deletion in U50 sequence also occurred both somatically and in germline, leading to increased incidence of homozygosity for the deletion in cancer cells [140].
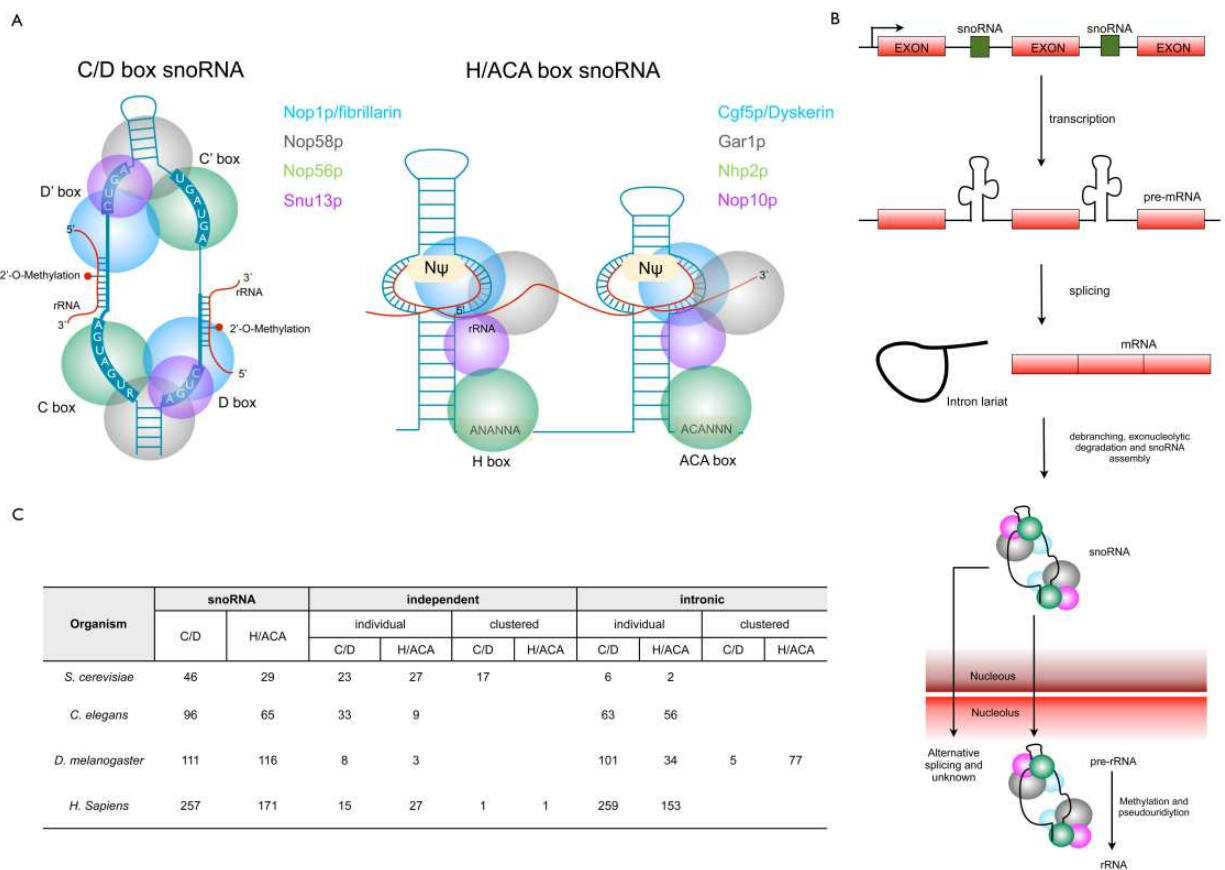
Figure 2. snoRNAs. A. Boxed sequences C and D (named from conserved, nuclease-resistant sequences that were originally identified in snoRNA U3) are hallmarks of the C/D box snoRNAs; boxed sequences H (Hinge region) and ACA are hallmarks of the H/ACA box snoRNAs. These conserved boxed sequences are important for the associations with protein components that are required to form the functional small nucleolar ribonucleoprotein (snoRNP) complexes and for accumulation in the nucleolus. C/D box snoRNAs associate with several proteins, including fibrillarin, which is the methyl transferase that is involved in the 2'-O-methylation of particular ribonucleotides, and H/ACA box snoRNAs associate with proteins such as the pseudouridine synthase dyskerin. Antisense sequences within the C/D box and H/ACA box snoRNAs guide the snoRNP complex to the appropriate nucleotide within the target RNA (most often ribosomal RNA). In a minority of cases both C/D-associated and C'D'-associated antisense sequences within the same C/D box snoRNA can act as guides for 2'-O-methylation of the target RNA. The eukaryotic H/ACA box snoRNAs contain two hairpin domains with complementary regions flanking the uridine to be converted in the target rRNA, at a position 14–16 nucleotides upstream of the conserved H and/or ACA box. Most mammalian snoRNAs are encoded within the introns of genes producing 5' terminal oligopyrimidine (5'TOP) RNAs. **B**. Organization of snoRNA genes in representative eukaryotic genomes **C.** Small nucleolar RNAs (snoRNAs) in vertebrate are predominantly located in introns. Following splicing, debranching and trimming, mature snoRNAs are either exported, in which case they function in ribosomal RNA (rRNA) processing, or remain in the nucleus, where they are involved in alternative splicing and additional yet unknown functions.

SnoRNA42 (SNORA42) is located on chromosome 1q22 which is a commonly frequent amplified genomic region in lung cancer and overexpression of SNORA42 is frequently and remarkably found in NSCLC cells [141]. In addition, SNORA42 exhibited close correlations between its increases of copy number and expression level, suggesting that SNORA42

overexpression could be activated through its amplification. Importantly, engineered repression of SNORA42 caused marked repression of lung cancer growth in vitro and in vivo and it is associated with increased apoptosis by a p53-dependent pathway. Although not exhibiting apoptosis, p53 null and mutant p53 cancer cells with reduced levels of SNORA42 also show inhibited proliferation and growth,   suggesting that SNORA42 knockdown can inhibit cell proliferation in p53-dependent or -independent manner. These independent studies on U50 and SNORA42 provide evidence for the functional importance of snoRNAs in cancer, and they show that snoRNAs can promote, as well as suppress, tumour development. In 2002, Wu and coworkers demonstrated that the expression of snoRNAs 5S was differentially displayed in different tissues and noticeably was highly expressed in normal brain, but its expression drastically decreased in meningioma [142]. Recently, genome-wide approaches identified six snoRNAs (SNORD33, SNORD66, SNORD73B, SNORD76, SNORD78, and SNORA42) that were statistically differently expressed between the non small cell lung cancer tumor and paired noncancerous samples [143]. Specifically, all these snoRNAs displayed a strong up-regulation in lung tumor specimens and the majority of them is located in commonly frequent genomic amplified regions in lung cancer: SNORD33 is located in chromosome 19q13.3 that contain potential oncogenes in lung cancer, while SNORD66 and SNORD76 are situated in chromosomal regions 3q27.1 and 1q25.1, respectively 3q27.1 and 1q25.1 are two of the most frequently amplified chromosomal segments in solid tumors, particularly NSCLC [143].

As well as the initial evidence that snoRNAs are involved in cancer development, there are some preliminary data showing that the genes that host snoRNAs might also contribute to the aetiology of this disease. A research screening for potential tumor-suppressor genes identified that Growth arrest-specific transcript 5 (gas5) gene as almost undetectable in actively growing cells but highly expressed in cells undergoing serum starvation or density arrest [144-145]. Gas5 is a multi-snoRNA host gene which encodes 9 (in mouse) or 10 (in human) snoRNAs and like all known snoRNA host genes exhibit characteristics which belong to the class of genes encoding 5' terminal oligopyrimidine (5'TOP) mRNAs [146]. The first and stronger evidence that GAS5 is related to cancer is the identification that GAS5 transcript levels are significantly reduced in breast cancer samples relative to adjacent unaffected normal breast epithelial tissues and some, but not all, GAS5 transcripts sensitize mammalian cells to apoptosis inducers [147]. Other studies have also showed that GAS5 reduced expression is associated with poor prognosis in both breast cancer and head and neck squamous cell carcinoma [148]. Of note, GAS5 has been also identified as a novel partner of the BCL6 in a patient with diffuse large B-cell lymphoma, harboring the t(1;3)(q25;q27) [149]. Another example of a mature spliced transcript that harbors C/D-box snoRNAs and can function independently of the snoRNAs is represented by the transcript Zfas1 [150]. This gene intronically hosts three C/D box snoRNAs (Snord12, Snord12b, and Snord12c) and has been identified as one of the most differentially expressed gene during mouse mammary development. siRNA-mediated downregulation of Zfas1 mRNA in a mouse mammary cell line increased proliferation and differentiation without substantially affecting the levels of the snoRNA hosted within its intron. The human homologue, ZFAS1 (also known as ZNFX1-AS1), which is predicted to share secondary structural features with

mouse Zfas1, is expressed at high levels in the mammary gland and is downregulated in breast cancer. Taken together, these findings indicates that snoRNA host genes might have important functions in regulating cellular homeostasis and, potentially, cancer biology but more studies are needed to understand their involvement in molecular basis of disease and classify them as sources of potential biomarkers and therapeutic targets.

Another important aspect of the association between snoRNAs and tumorigenesis is represented by the involvement of their associated proteins in cancer. A point mutations in the DKC1 gene is the cause of a rare X-linked recessive disease, the dyskeratosis congenita (DC) [151-152]. Individuals with DC display features of premature aging, as well as nail dystrophy, mucosal leukoplakia, interstitial fibrosis of the lung, and increased susceptibility to cancer. DKC1 codes for dyskerin, a putative pseudouridine synthase, which carries out two separate functions, both fundamental for proliferating cells. One function is the pseudo-uridylation of ribosomal RNA (rRNA) molecules as a part of the H/ACA ribonucleoprotein complex, and the other is the stabilization of the telomerase RNA component necessary for telomerase activity. Dkc1 mutant mice recapitulate the major features of DC, including an increased susceptibility to tumor formation. Early generation (G1 and G2) of Dkc1 mutant mice showed a full spectrum of DC and presented alterations in rRNA modification, whereas defects in telomere length were not evident until G4 mice, suggesting that deregulated ribosome function is important for the initiation of DC and that impairment in telomerase activity in Dkc1 mutant mice may modify and/or exacerbate the disease in later generations. To this regard, DKC1 was identified as one of only seventy genes that, collectively, constitute a gene expression profile that strongly correlates with the development of aneuploidy and is associated with poor clinical prognosis in a variety of human cancers. Therefore, one hypothesis is that an alteration of physiologic dyskerin function, irrespective of the mechanism, may perturb mitosis and contribute to tumorigenesis but this idea will require more detailed investigation. Another possibility is related to the strong effect of dyskerin loss on H/ACA accumulation. Recent finding in fact have shown that some H/ACA box and C/D box can be processed to produce small RNAs, at least some of which can function like miRNAs [153]. Such processing may be of crucial importance, as miRNAs have important roles in the development of many cancers as previously discussed. To date, Xiao and colleagues have recently reported that an H/ACA box snoRNA- derived miRNA, miR-605, has a key role in stress-induced stabilization of the p53 tumour suppressor protein [154]. p53 transcriptionally activates its negative regulator, MDM2, in addition to miR-605. miR-605 counteracts MDM2 through post-transcriptional repression; under conditions of stress, this snoRNA-derived miRNA offsets the MDM2 negative-feedback loop, generating a positive-feedback loop to enable the rapid accumulation of p53. However, whether this regulation of p53 by miR-605 is relevant to cancer biology has not yet been addressed. Like dyskerin, NHP2 and NOP10 proteins, both components of the H/ACA snoRNPs, are also significantly up-regulated in sporadic cancers and high levels may be associated with poor clinical prognosis. Moreover, germline NHP2 and NOP10 mutations give rise to autosomal recessive forms of dyskeratosis congenita, and cancer susceptibility is also a feature of these genetic forms of the disease. Since the functions of several snoRNAs have not yet been identified (orphan snoRNAs), it is possible

that disruption of snoRNP biogenesis by any mechanism may affect an array of important cellular processes, and could potentiate cancer development and/or progression.

7.2. piRNAs: Guardians of the genome

Piwi-interacting RNAs (piRNAs) are germline-specific small silencing RNAs of 24–30 nt in length, that suppress transposable elements (TE) activity and maintain genome integrity during germline development, a role highly conserved across animal species [155-156]. TEs are genomic parasites that threaten the genomic integrity of the host genome: they are able to move to new sites by insertion or transposition and thereby disrupt genes and alter the genome [157]. In animals, endogenous siRNAs also silence TEs, but the piRNA pathway is at the forefront of defense against transposons in germ cells [158]. piRNAs specifically associate with PIWI proteins, which are germline-specific members of the AGO protein family, AGO3, Aubergine (Aub) and Piwi, and form a piRNA-induced silencing complex (piRISC) which will guide the TE silencing [159-162]. Any mutations in each of the three members of the PIWI family lead to transposon derepression in the germline, indicating that they act non-redundantly during TE silencing. Initial screening of piRNA sequences revealed that there are hundreds of thousands, if not millions, of individual piRNA sequences [163-165]. Furthermore, they are characterized by the absence of specific sequence motifs or secondary structures such as miRNA precursors. Despite their large diversity, most piRNAs can be mapped to a relatively small number of genomic regions called piRNA clusters. Each cluster extends from several to more than 200 kilobases, it contains multiple sequences that generate piRNAs and some piRNAs map to both genomic strands, suggesting bidirectional transcription [163-165] Indeed, analysis of piRNA clusters in different *Drosophila* species has shown that, although the clusters locations are conserved, their sequence content has evolved very quickly suggesting adjustments in the piRNAs patrimony in order to suppress new active transposons invading the species. Therefore, piRNA clusters may be considered as repositories of information, enabling production of many mature piRNAs that target diverse TEs. Two main pathways, highly conserved in many animal species, have been discovered to be responsible for the biogenesis of the piRNAs: the primary pathways and the Ping-Pong amplification (**Figure 3**) [166-168]. First, the primary piRNA biogenesis pathway provides an initial pool of piRNAs that target multiple TEs. Next, the Ping-Pong cycle further shapes the piRNA population by amplifying sequences that target active transposons. It is currently unclear how primary piRNAs are produced from piRNA clusters but it is likely that piRNA precursors are single-stranded and therefore do not require Dicer for their processing. Interestingly, piRNAs that associate with each member of the PIWI protein family have a distinct size, suggesting that PIWI proteins can act as 'rulers' that define the size of mature piRNAs. Several additional proteins (e.s. Zucchini, Armitage and Yb) have also been identified that are involved in primary piRNA biogenesis and mutations in and/or depletion of any of these three proteins eliminates primary piRNAs associated with PIWI proteins. In some cell types, such as somatic follicle cells of the *D. melanogaster* ovary, primary piRNA biogenesis is the only mechanism that generates piRNAs. However, in germline cells of the *D. melanogaster* ovary and in the pre-meiotic spermatogonia in mice, there is another mechanism called the Ping-Pong cycle that amplifies specific sequences generated by the primary biogenesis pathway

[163,169]. Mainly the Ping-Pong pathway engages AGO3 and Aubergine, both of which are accumulated in perinuclear structures located at the cytoplasmic face of the nuclear envelope in animal germline cells, named "nuage". The pathway depends on the endoribonuclease or Slicer activity of AGO3 and Aubergine, which act catalytically one after the other, leading to a cleavage of the target RNAs between their tenth and eleventh nucleotides relative to the 'guide' small RNAs. This process results in the generation of repeated rounds of piRNA production having exactly the same sequence of the original primary piRNA. The ping-pong pathway amplifies piRNAs in *D. melanogaster* testes, especially those originating from TEs. Non-TE-derived piRNAs seem to be barely amplified by the amplification loop. This two steps of piRNA biogenesis can be compared with the function of the adaptive immune system in protecting against pathogens. The primary piRNA biogenesis pathway resembles the initial generation of the hypervariable antibody repertoire, whereas the amplification loop is analogous to antigen-directed clonal expansion of antibody-producing lymphocytes during the acute immune response. An emerging number of studies highlight the role of piRNAs or PIWI proteins in the regulation of tumorigenesis. First examples of the piRNA involvement in cancer is represented by the up-regulation of HIWI, one of the four human Piwi homologues, in about 60 % of seminomas [170]. In fact, HIWI maps to a locus known as a germ cell tumor susceptibility locus (12aq24.33). HIWI overexpression has also been found in somatic cells such as soft-tissue sarcomas or ductal pancreas adenocarcinoma, and strongly correlates with bad prognosis and high incidence of tumor-related death, providing an example for a potential tumorigenic role of a piRNA-related protein in somatic cells [171,172]. In some cancers, PIWIL2 overexpression has been suggested to induced resistance in cells to cisplatin, which might arise because of increased chromatin condensation that prevents the normal process of DNA repair [173]. Furthermore, new high-throughput sequencing data revealed the presence of piRNAs in somotic cells, such as HeLa cells. These somatic piRNAs appear located in the nucleolus and in the cytoplasmic area surrounding the nuclear envelope and in contrast with the large population of known piRNAs in male germ cells, this population of piRNAs is dramatically smaller [174]. Another recent study demonstrated that the level of piR-651 is significantly higher in several cancer histotype including lung, mesothelium, breast, liver, and cervical cancer compared to non-cancerous adjacent tissues and inhibition of piR-651 induced block of gastric cancer cells at the G2/M phase [175,176]. Another example is represented by the downregulation of piR-823 in gastric cancer tissues; its enforced expression inhibited gastric cancer cell growth in vitro and in vivo, suggesting a tumor suppressive properties for piR-823 [177]. Interestingly, piRNAs not are only involved in direct regulation by degradation of TE but they have also been linked to DNA methylation of the retrotraspon regions, extending piRNA functions beyond post-transcriptional silencing. In fact, CpG DNA methylation, which is required for efficient transcriptional silencing of LINE and LTR retrotransposons in the genome, is decreased in the male germ line of mice with defective PIWI proteins. Specifically, mice with defective PIWI proteins fail to establish de novo methylation of TE sequences during spermatogenesis, leading to the hypothesis that the piRISC can also guide the *de novo* methylation machinery to TE loci. In this scenerio, piRNAs may present a perfect guide for discriminating TE sequences from normal protein-coding genes and marking them for DNA methylation; however, the biochemical details of how these two mechanisms of piRNA

action might be linked have not yet been revealed [178,179]. All together, these data revealed that PIWI-associated RNAs and PIWI pathway has a more profound function outside germline cells than was originally thought but many more studies are needed to clarify their specific role in tumorigenesis.
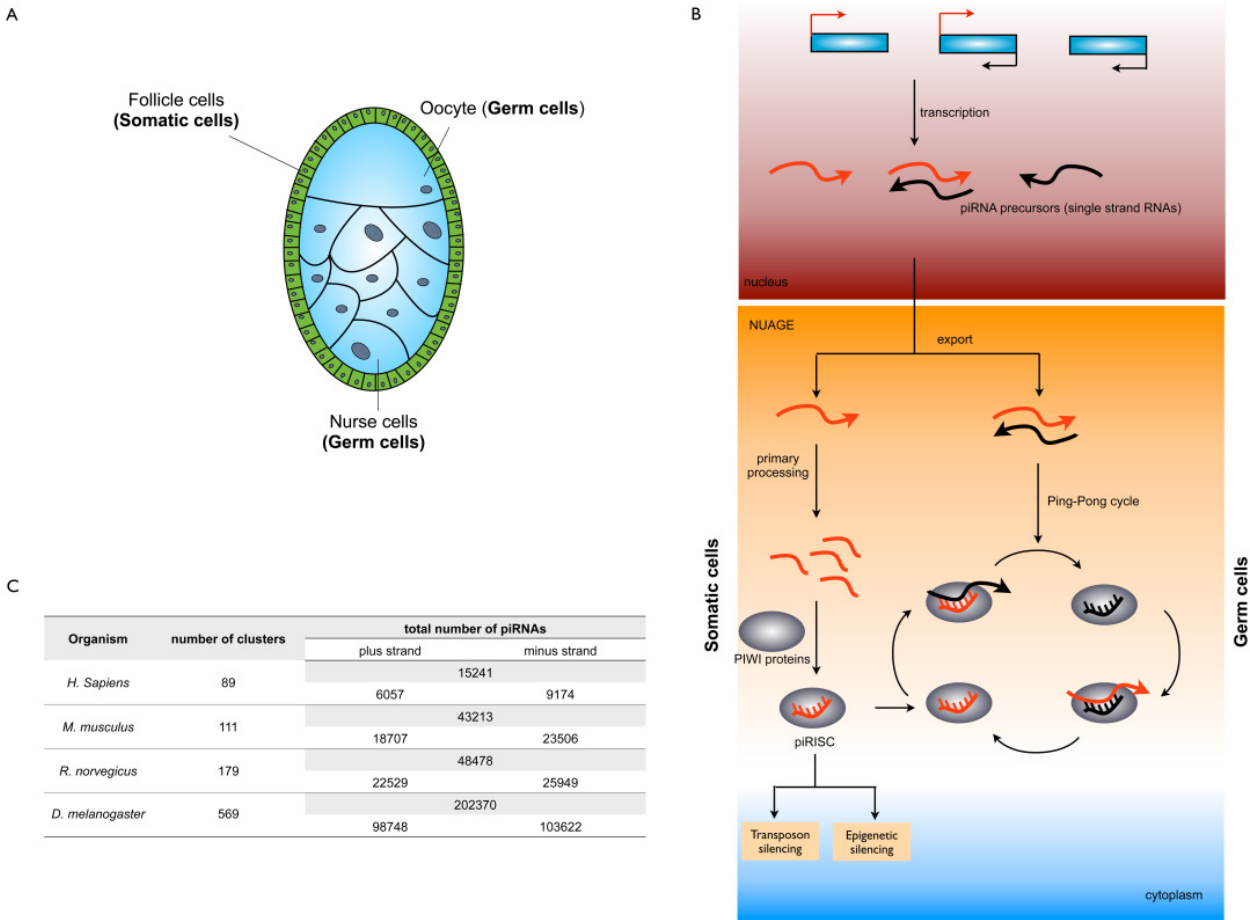
Figure 3. piRNAs. A, schematic representation of the Drosophila egg chamber. **B,**piRNAs (which are 24–32 nt in length) are processed from single-stranded RNA precursors that are transcribed largely from mono- or bidirectional intergenic repetitive elements known as piRNA clusters. Unlike miRNAs and siRNAs, piRNAs do not require Dicer for their processing. First, primary piRNAs are produced through the primary processing pathway and are amplified through the ping-pong pathway, which requires Slicer activity of PIWI proteins. Subsequently, additional piRNAs are produced through a PIWI-protein-catalysed amplification loop (called the 'ping-pong cycle') via sense and antisense intermediates. Primary piRNA processing and loading onto mouse PIWI proteins might occur in the cytoplasm. The PIWI ribonucleoprotein (piRISC) complex functions in transposon repression through target degradation and epigenetic silencing. **C,** total number of piRNA clusters in different animal species according to the piRNA Database (http://pirnabank.ibab.ac.in/).

## 8. The emergence of long non-coding RNAs

Over the last decade, advances in genome-wide analyses of the eukaryotic transcriptome have revealed that most of the human genome is transcribed, generating a large repertoire of (>200 nt) long non-coding RNAs (lncRNA or lincRNA, for long intergenic ncRNA) that

map to intronic and intergenic regions [181,181]. Given their unexpected abundance, lncRNAs were initially thought to be spurious transcriptional noise resulting from low RNA polymerase fidelity [182]. However, the restricted expression of many long ncRNAs to particular developmental contexts, the often exhibiting precise subcellular localization and the binding of transcription factors to non-coding loci, suggested that a significant portion of ncRNAs fulfills functional roles beyond transcriptional remodelling [183-187]. lncRNA typically refers to a polyadenylated long ncRNA that is transcribed by RNA polymerase II and is associated with epigenetic signatures common to protein-coding genes, such as trimethylation of histone 3 lysine 4 (H3K4me3) at the transcriptional start site (TSS) and trimethylation of histone 3 lysine 36 (H3K36me3) throughout the gene body [188-189]. lncRNAs also commonly exhibit splicing of multiple exons into a mature transcript, and their transcription occurs from an independent gene promoter and is not coupled to the transcription of a nearby or associated parental gene. RNA-Seq studies now suggest that several thousand uncharacterized lncRNAs are present in any given cell type [188-189], and that the human genome may harbor nearly as many lncRNAs as protein-coding genes (perhaps ~15,000 lncRNAs), although only a fraction is expressed in a given cell type. One main characteristic of the lncRNAs is their very low sequence conservation that had fueled the idea that they are not functional. This assertion needs to be carefully considered and takes in consideration several points. First, a recent study identified the presence of 1,600 lncRNAs that show a strong evolutionary conservation and function ranging from from embryonic stem cell pluripotency to cell proliferation [189]. In contrast to the protein coding genes, long ncRNAs can exhibit shorter stretches of sequence that are conserved to maintain functional domains and structures. Indeed, many long ncRNAs with a known function, such as *Xist*, only exhibit high conservation over short sections of their length [190]. Third, rather than being indicative of non-functionality, low sequence conservation can also be explained by high rates of primary sequence evolution if long ncRNAs have, like promoters and other regulatory elements, more plastic structure–function constraints than proteins [190]. The diverse selection pressures acting on long ncRNAs probably reflect the wide range of their functions which can be regrouped in three major subclasses: chromatin remodeling, transcriptional modulation and nuclear architecture/subnuclear localization.

long ncRNAs can mediate epigenetic changes by recruiting chromatin remodelling complexes to specific genomic loci resolving the paradox of how a small repertoire of chromatin remodelling complexes are able To specify the large array of chromatin modifications without any apparent specificity for the genomic loci [191,192]. A recent study found that 20% of 3300 human long non coding RNAs are bound by Polycomb Repressive Complex 2 (PRC2) [193]. Although the specific molecular mechanisms are not defined, there are several examples that can illustrate the silencing potential of lncRNAs (**Figure 4**). The first most known example is represented by the X-chromosome inactivation which is carried out by a number of lncRNAs including Xist and RepA, which bind PRC2 complex, and the antagonist of Xist, Tsix [194]. In pre-X-inactivation cells, Tsix competes with RepA for the binding of PRC2 complex; when the X-inactivation starts Tsix is downregulated and PRC2 becomes available to RepA which can actively induced the transcription of Xist. The up-regulated Xist in turn preferentially binds to PRC2 and spreads across the chromosome X

inducing PCR2-mediated trimethylated histone H3 lysine27. Another important example is represented by the hundreds of long ncRNAs which are sequentially expressed along the temporal and spatial developmental axes of the human homeobox (Hox) loci, where they define chromatin domains of differential histone methylation and RNA polymerase accessibility [195]. One of these ncRNAs, Hox transcript antisense RNA (HOTAIR), originates from the HOXC locus and silences transcription across 40 kb of the HOXD locus in trans by inducing a repressive chromatin state, which is proposed to occur by recruitment of the Polycomb chromatin remodelling complex PRC2 by HOTAIR (**Figure 4**). Recently, it has been proposed that HOTAIR has the ability to bind other histone-modifying enzymes such as the demethylase LSD1 [196]. In fact, knockdown of HOTAIR induces a rapid loss of LSD1 or PRC2 at hundreds of gene loci with the corresponding increase in expression. This model fits other chromatin modifying complexes, such as Mll, PcG, and G9a methyltransferase, which can be similarly directed by their associated ncRNAs [196]. As modulator of epigenetic landmark, it has been shown that HOTAIR has a profound effect on tumorigenesis. In fact, HOTAIR is upregulated in breast carcinoma and colon cancer and its correlates with metastasis and poor prognosis [197] Enforced expression of HOTAIR consistently changed the pattern of occupancy of Polycomb proteins from the typical epithelial mammary cells pattern to that of embryonic fibroblasts [198]. Another important effect of lncRNAs on chromatin modification that can highlight their impact on cancer is the relationship between the lncRNA ANRIL and the INK4b/ARF/INK4a locus, encoding for three tumor-suppressor genes highly deleted or silenced in a large cohort of tumors [199]. ANRIL, which is transcribed antisense to the protein coding genes of the locus, controls the epigenetic status of the locus by interacting with subunits of PRC1 and PRC2. High expression of ANRIL is found in some cancer tissues and is associated to a high levels of PCR-mediated trimethylated histone H3 lysine27. Inhibition of ANRIL releases PRC1 and PRC2 complexes from the locus, decreases the histone methylation status with the following increase of the protein coding gene transcription. Many other tumor suppressor genes that are frequently silenced by epigenetic mechanisms in cancer also have antisense partners, which can affect gene expression with different other mechanism. First, antisense ncRNAs can mask key cis-elements in mRNA by the formation of RNA duplexes, as in the case of the Zeb2 antisense RNA, which complements the 5′ splice site of an intron of Zeb2 mRNA [200]. Expression of the ncRNA prevents the splicing of the intron that contains an internal ribosome entry site required for efficient translation and expression of the ZEB2 protein with a further efficient translation (**Figure 4).** In this context, it has been evaluated that the prevalence of lncRNAs are antisense to introns, hypothesizing their role in the regulation of splicing or capable of generating mRNA duplexes that fuel the RISC machinery to silence gene expression. One major emergent theme is the involvement of the lncRNAs in the assembly or activity of transcription factors functioning as a scaffold for the docking of many proteins, mimicking functional DNA elements or modulation of PolII itself. The first example is represented by the suppression of CCND1 mediated by the lncRNAs through the recruitment and integration of the RNA binding protein TLS into a transcriptional programme. DNA damage signals induce the expression of long ncRNAs associated with the cyclin D1 gene promoter, where they act cooperatively to recruit the RNA binding
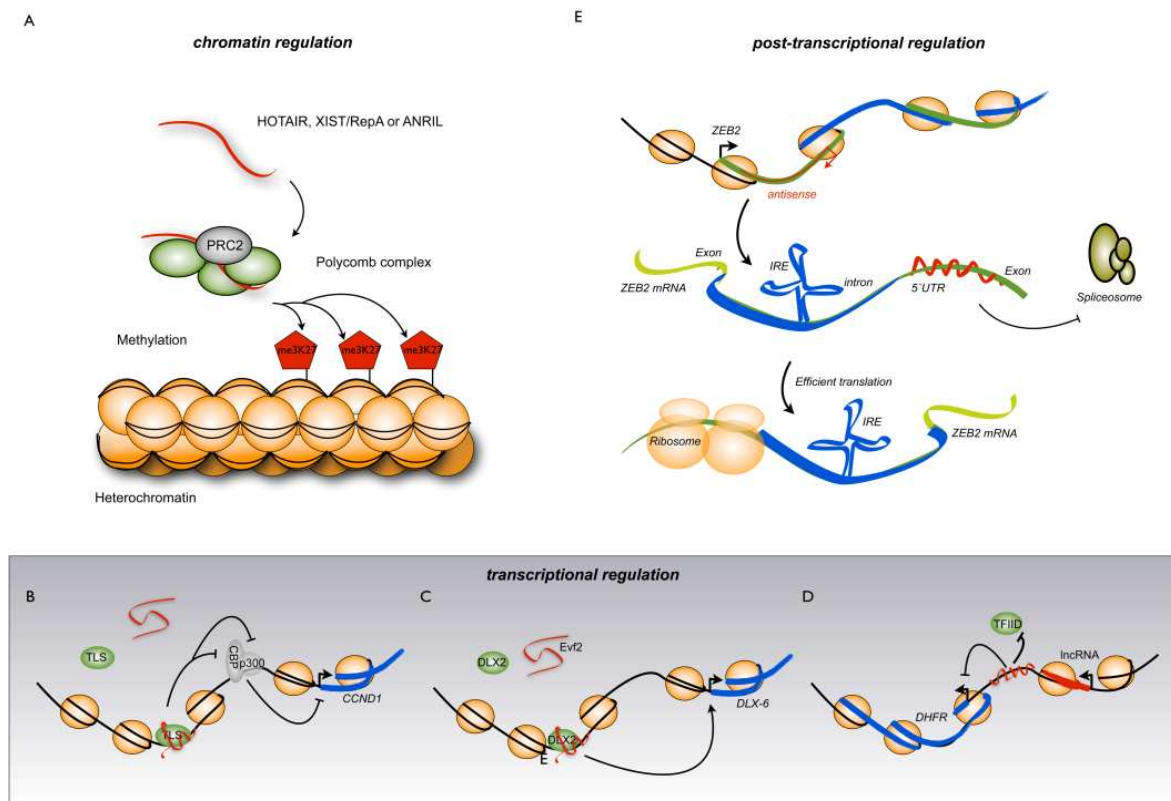
Figure 4. lncRNAs. Schematic representation of the control operated on protein coding gene by the lncRNAs at the level of chromatin remodelling, transcriptional control and post-transcriptional processing. **A**, lncRNAs (Xist, HOTAIR, ANRIL, etc) can recruit chromatin modifying complexes to specific genomic loci to localize their catalytic activity. In this case, the lncRNA recruits the Polycomb complex by inducing trimethylation of the lysine 27 residues (me3K27) of histone H3 to produce heterochromatin formation and repress gene expression. **B, C, D,** lncRNAs can regulate the transcriptional process through a range of mechanisms. First, lncRNAs tethered to the promoter of the cyclin D1 gene recruit the RNA binding protein TLS to modulate the histone acetyltransferase activity of CREB binding protein (CBP) and p300 to repress gene transcription. Second, an ultraconserved enhancer is transcribed as a long ncRNA, Evf2, which subsequently acts as a co-activator to the transcription factor DLX2, to regulate the Dlx6 gene transcription. Third, a lncRNA transcribed from the DHFR minor promoter form a triplex at the major promoter to reduce the access of the general transcription factor TFIID, and thereby suppress DHFR gene expression. **E**, a lncRNA is antisense to Zeb2 mRNA and mask the 5' splice site resulting in intron retention. This retention results in an efficient Zeb2 translation related to the presence of an internal ribosome entry site (IRE) in the retained intron.

protein TLS. The modified and promoter-docked TLS inhibits the histone acetyltransferase activities of CReB binding protein and p300 inducing the silencing of cyclin D1 expression (Figure 4) [201]. A different co-activator activity mediated by lncRNAs is also evident in the regulation of Dlx genes, important modulators of neuronal development and patterning [202]. Dlx5-6 expression is regulated by two ultraconserved enhancers one of which is transcribed in a lncRNA, named Evf-2. Evf2 forms a stable complex with the homodomein protein DLX-2 which in turn acts as a transcriptional enhancer of Dxl5-6 gene (Figure 4). In some cases, lncRNAs can also affect RNA polymerase activity by influencing the initiation complex in the choice of the promoter. For example, in humans, a ncRNA transcribed from an upstream region of the dihydrofolate reductase (DHFR) locus forms a triplex in the major

promoter of DHFR to prevent the binding of the transcriptional co-factor TFIID (Figure 4). This could be a widespread mechanism for controlling promoter usage as thousands of triplex structures exist in eukaryotic chromosomes. Recently, lncRNAs have also shown their tumorigenic potential by modulating the transcriptional program of p53 [203]. An 3kb lncRNAs, linc-RNA-p21, transcriptionally activated by p53, has been shown to collaborate with p53 in order to control the gene expression in response to DNA damage. Specifically, silencing of lincRNA-p21 derepresses the expression of hundred of genes which are also derepressed following p53 knockdown. It has also been discovered that lincRNA-p21 interacts with hnRNPK and this binding is essential for the modulation of p53 activity.

The final category of lncRNAs is represented by those molecules capable to generate the formation of compartmentalized nuclear organelles, subnuclear membraneless nuclear bodies whose funtion is relative unknown. One of them is represented by cell-cycle regulated nuclear foci, named paraspeckles. In addition to protein components, two lncRNAs, NEAT1 and Men epsilon, have been detected as essential part of the paraspeckles. While depletion of NEAT or Men epsilon disrupts the paraspeckles, their overexpression strongly increases their number. There is a number of different lncRNAs that localize to different nuclear regions [204]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) localizes to the splicing speckles, Xist and Kcnq1ot1 both, localize to the perinucleolar region during the S phase of the cell cycle, a class of repeat-associated lncRNAs (es SatIII) are associated to nuclear stress bodies which are produced on specifc pericentromeric heterochromatic domains containing SatIII gene itself.

9. Conclusions

Alterations in microRNAs and other short or long non-coding RNA (ncRNA) are involved in the initiation, progression, and metastasis of human cancer. Over the last decade, a growing number of non-coding transcripts have been found to have roles in gene regulation and RNA processing. The most well known small non-coding RNAs are the microRNAs, but the network of long and short non-coding transcripts is complex and is likely to contain as yet unidentified classes of molecules that form transcriptional regulatory networks. The field of small and long non coding RNAs is rapidly advancing toward in vivo delivery for therapeutic purposes. Advanced molecular therapies aimed at downmodulating or upmodulating the level of a given miRNA in model organisms have been successfully established. RNA-based gene therapy can be used to treat cancer by using RNA or DNA molecules as therapy against the mRNA of genes involved in cancer pathogenesis or by directly targeting the ncRNAs that participate in pathogenesis. The use of miRNAs is still being evaluated preclinically; no clinical or toxicologic studies have been published but the future is promising. Kota and collegues reported that systemic administration of this miRNA in a mouse model of HCC using adeno-associated virus (AAV) results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and dramatic protection from disease progression without toxicity (116). Recently, Pineau et al. (117) identified DNA damage-inducible transcript 4 (DDIT4), a modulator of the mTor pathway, as a bona fide target of miR-221. They introduced into liver cancer cells, by lipofection, LNA-modified oligonucleotides specifically designed for miR-221

(antimiR-221) and miR-222 (antimiR-222) knockdown. Treatment by antagomiRs, but not scrambled oligonucleotide, reduced cell growth in liver cancer cell lines that overexpressed miR-221 and miR-222 by 35% and 22%, respectively. Thus the use of synthetic inhibitors of miR-221 may prove to be a promising approach to liver cancer treatment (117). Despite recent progress in silencing of miRNAs in rodents, the development of effective and safe approaches for sequence-specific antagonism of miRNAs in vivo remains a significant scientific and therapeutic challenge. Recently, Elmen and collaborators (118) showed for the first time, that the simple systemic delivery of an unconjugated, PBS-formulated LNA-antimiR effectively antagonizes the liver-expressed miR-122 in nonhuman primates. Administration by intravenous injections of LNA-antimiR into African green monkeys resulted in the formation of stable heteroduplexes between the LNA-antimiR and miR-122, accompanied by depletion of mature miR-122 and dose-dependent lowering of plasma cholesterol. These findings demonstrate the utility of systemically administered LNA-antimiRs in exploring miRNA functions in primates and show the impressive potential of this strategy to overcome a major hurdle for clinical miRNA therapy. In conclusion, the discovery of small RNAs and their functions has revitalized the prospect of controlling expression of specific genes in vivo, with the ultimate hope of building a new class of gene-specific medical therapies. Just how significant are the ncRNAs? They appear to be doing something important and highly sophisticated; there are so many of them, their sequences are so highly conserved, their expression is tissue specific, and they have recognition sites on more than 30% of the entire transcriptome. It seems that ncRNAs were overlooked in the past simply because researchers were specifically looking for RNAs that code proteins. The above discussed data highlight that the complexity of genomic control operated by the ncRNAs is somewhat greater than previously imagined, and that they could represent a total new order of genomic control. In this scenario, understanding the precise roles of ncRNAs is a key challenge. The targeting of other ncRNAs, in addition to miRNAs, is still in its infancy, but new important developments are expected in this area. Therefore, small RNAs could become powerful therapeutic tools in the near future.

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