

MicroRNAs in Human Embryonic and Cancer Stem Cells

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Received: February 2, 2010

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The authors have no financial conflicts of interest.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate messenger RNAs at the post-transcriptional level. They play an important role in the control of cell physiological functions, and their alterations have been related to cancer, where they can function as oncogenes or tumor suppressor genes. Recently, they have emerged as key regulators of “stemness”, collaborating in the maintenance of pluripotency, control of self-renewal, and differentiation of stem cells. The miRNA pathway has been shown to be crucial in embryonic development and in embryonic stem (ES) cells, as shown by Dicer knockout analysis. Specific patterns of miRNAs have been reported to be expressed only in ES cells and in early phases of embryonic development. Moreover, many cancers present small populations of cells with stem cell characteristics, called cancer stem cells (CSCs). CSCs are responsible for relapse and treatment failure in many cancer patients, and the comparative analysis of expression patterns between ES cells and tumors can lead to the identification of a miRNA signature to define CSCs. Most of the key miRNAs identified to date in ES cells have been shown to play a role in tumor diagnosis or prognosis, and may well prove to be essential in cancer therapy in the foreseeable future.

Key Words: miRNA, microRNA, embryonic stem cell, cancer stem cell

INTRODUCTION

When a stem cell divides, it can produce a copy of itself as well as differentiated cell progeny. Stem cells allow the generation of embryonic tissues (embryonic stem cells) and the maintenance of adult tissue (adult stem cells). Stem cells play a key role in embryonic development. A complex organism derives from a single cell, the fertilized egg. During embryogenesis, cells are initially proliferative and pluripotent; they only gradually become restricted to different cell fates. The first evidence of pluripotent stem cells' existence in the embryo comes from the 1960-1970s, when it was seen that early mouse embryos transplanted to ectopic sites produced teratocarcinomas, tumors that contain a variety of differentiated cell types as well as embryonal carcinoma (EC) cells (undifferentiated cells).¹ A tumor containing both EC cells and differentiated cells can be generated from one EC cell.² In the 1970s, it was shown that when EC cells were injected back into early embryos, they could reveal their pluripotency and participate in the formation of chimeras.^{3,4} But it was not until 1981 that Martin⁵ and Evans and Kaufman⁶

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discovered embryonic stem (ES) cells, permanent pluripotent cell lines which could be derived directly from an embryo at blastocyst stage.

ES cells may participate in normal embryonic development when they are injected into the blastocyst. In contrast, ES cells develop into tumors if injected in ectopic sites in adult mice. Moreover, the role of microenvironment in the growth control of embryonic stem cells has also been examined. When ES cells were injected in pregnant mouse uteri, no tumor formation was observed; in contrast, the ES cells injected into pseudopregnant uteri often developed into tumors, and those injected into non-pregnant uteri always developed into teratocarcinomas. These results showed that the pregnant-uterine microenvironment may participate in the control of ES cell growth.⁷ Now we know that stem cells are controlled by extrinsic signals from their regulatory niche and also by intrinsic factors like the hyperdynamic plasticity of chromatin proteins.⁸ In summary, ES cells can readily be shown to differentiate into essentially all cell phenotypes, whereas most isolates of adult stem cells have more limited potential for differentiation. Both retain the capacity to divide indefinitely, and this division must be finely regulated because few divisions can alter tissue homeostasis and many divisions can drive to cancer initiation. Many cancers present small populations of cells with stem cell characteristics, called cancer stem cells (CSCs). Recently, novel elements, microRNAs (miRNAs), have emerged as crucial regulators for proper stem cell maintenance and function (Fig. 1).

miRNA characteristics

miRNAs are small non-coding RNAs (22-24 nucleotides in length) that negatively regulate messenger RNA translation to protein by binding to 3'UTRs of target messenger

RNA.⁹ These molecules were described for the first time in 1993 by Victor Ambros and colleagues in *Caenorhabditis elegans*, and to date, more than 700 miRNAs have been identified in humans. They are encoded in the entire genome, including the exonic, intronic, and intergenic regions, but 90% are found in intronic regions.¹⁰ They are transcribed from these regions through RNA polymerase II action - first as large molecules, called pri-miRNAs, that can exceed 1 Kb and that could be a polycistronic transcript containing more than one miRNA. In the same nucleus they are processed by RNase complexes (Drosha and DGCR8) into ~70 nucleotide fragments with stem-loop structures called pre-miRNAs. The pre-miRNA is exported to the cytoplasm by Exportin-5 and RAN is the official full name action. There it is further cleaved by Dicer action to generate a duplex molecule of ~25 nucleotides in length. These duplex molecules bind a protein complex called the RNA induced silencing complex (RISC), where one of the two chains is selected, known as mature miRNA, to guide this complex to the target mRNA. In general, when a miRNA and a mRNA exhibit total complementarities, RISC is capable of degrading target mRNA, whereas if base pairing complementarity is incomplete, translational silencing of the target occurs. Through these mechanisms, miRNAs decrease translation of human genes (Fig. 2).^{9,10}

Since their discovery, miRNAs have been shown as powerful key regulator elements, since a single miRNA can silence several hundreds of genes and one gene can be targeted by several miRNAs, producing different grades of repression.⁹ miRNAs target a large number of genes in relation to a great variety of biological processes, including regulation of proliferation and apoptosis and stem cell self-renewal and differentiation. Moreover, miRNA expression is regulated in a tissue- and developmental stage-specific

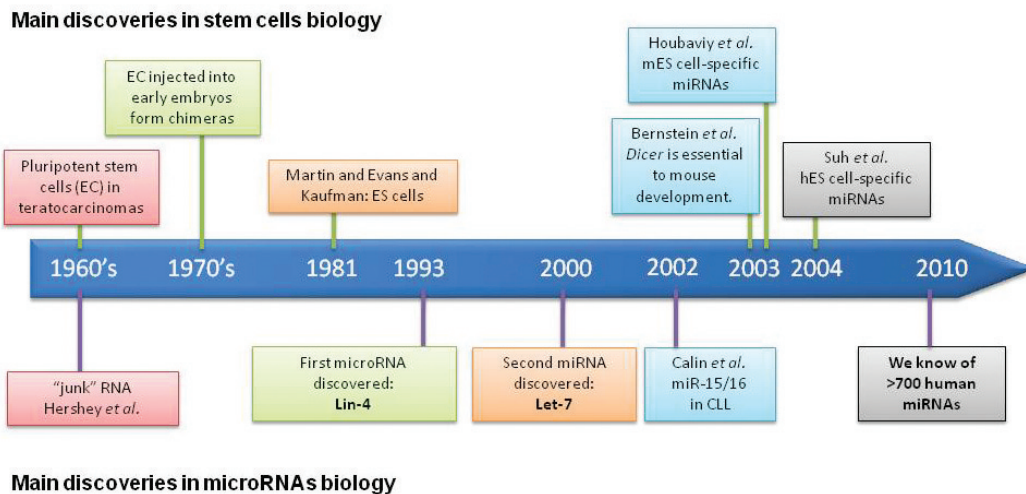


Fig. 1. Timeline showing the main discoveries in stem cell biology and miRNA research. miRNA, microRNA.

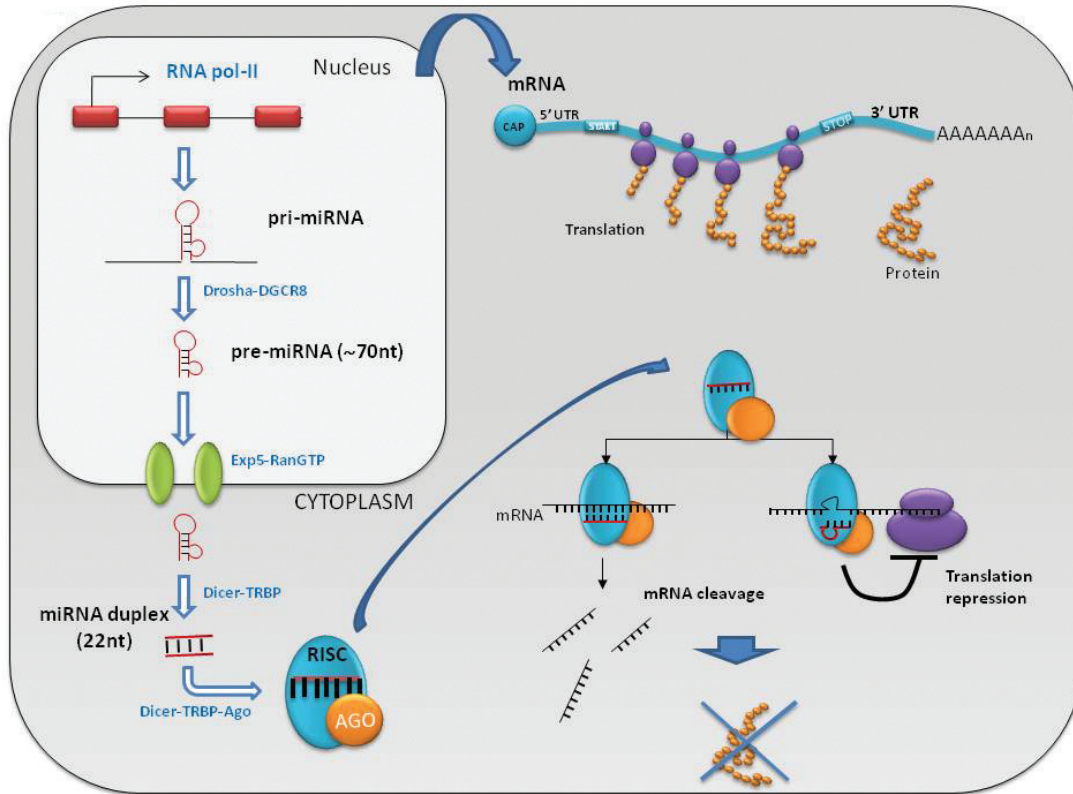


Fig. 2. miRNA biogenesis pathway.

manner.¹¹⁻¹⁵

miRNA deregulation is thus related to an increasing number of diseases, including cancer. All the mechanisms that can alter the normal expression of a gene can affect the expression of a miRNA, including chromosomal translocations, amplifications, and deletions or mutations.¹⁶ High-throughput analyses have shown that miRNA expression is commonly deregulated in several types of cancer.¹⁷

miRNAs in stem cell regulation

ES cell regulation by miRNAs

Human ES cells are cell lines established from the explanted inner cell mass of human blastocysts. Experiments with Dicer and DGCR8 knockouts have provided evidence that miRNAs play a crucial role in development in general and specifically in ES cells. Dicer deletion is lethal in the early stages of development in *Drosophila*¹⁸ and mice.¹⁹ Dicer conditional knockouts showed defects in proliferation, miRNA maturation, and differentiation,^{20,21} and DGCR8 conditional knockout showed defects in proliferation and cell cycle progression,²² demonstrating that the miRNA pathway is closely related with the regulation of ES cells.

Houbaviy, et al.²³ were the first to analyze ES cell-specific miRNAs. They identified a signature of overexpressed miRNAs in mouse ES cells that were repressed as they

differentiated into embryoid bodies and were absent in the adult organ tissues. From the set of miRNAs identified, the authors distinguished three groups of miRNAs: miRNAs with ES cell-specific functions (the miR-290-295 cluster and miR-296); miRNAs also found in adult tissues, which could thus regulate general cell physiology (miR-15a, miR-16, miR-19b, miR-92, miR-93, miR-96, miR-130 and miR-130b); and miRNAs whose expression increases upon differentiation (miR-21 and miR-22).

One year later, Suh, et al.²⁴ reported the first miRNA signature in human ES (hES) cells. As in the previous work, the miRNAs identified are related to each other in clusters (polycistronic primary transcripts). They identified 36 miRNAs and grouped them into four categories: ES cell-specific miRNAs (miR-200c, 368, 154*, 371, 372, 373*, 373); miRNAs found in both ES cells and their malignant counterpart the EC cells (miR-302a, 302a*, 302b, 302b*, 302c, 302c*, 302d and 367); miRNAs that are rare in ES cells but abundant in HeLa and STO cell lines (let-7a, miR-301, 374, 21, 29b, 29); and miRNAs expressed in the majority of the cell lines tested (miR-16, 17-5p, 19b, 26a, 92, 103, 130a and 222).

ES cell identity can be formed by the transcription factors Nanog, OCT4, and Sox2.²⁵⁻³¹ These genes can be regulated by miR-134, miR-296, and miR-470.³² Moreover, Marson, et al.³³ report that these genes regulate the expression of

miRNAs related to the pluripotency state by binding to the promoter region. The miR-302-367 cluster is one of the miRNAs regulated by these transcription factors and its expression is restricted to the ES cell compartment. This cluster regulates the cell cycle by promoting self-renewal and pluripotency.³⁴

Analyses of the role of miRNAs in the differentiation of hES cell lines have identified two groups of miRNAs: those that are expressed in the undifferentiated state and are markers of pluripotency (miR-200c, 371, 372, 302a, 320d, 373, 302c, 21, 222, 296, 494, 367), and miRNAs that act to regulate the differentiation of cells into one of the different embryonic lineages (miR-17, 92 and 93, which are overexpressed in differentiated cells, and miR-154, 29a, 143, 29c, and let-7a, which are underexpressed in differentiated cells).³⁵ In an analysis of miRNAs that change during hES cell differentiation into embryoid bodies, a set of 30 miRNAs was identified, some of which had previously been identified as let-7a.³⁶ The majority of the miRNAs that characterize hES cells are expressed from chromosomes 19 and X.³⁶

Other investigators examined the target related to hES cell-specific miRNAs, and identified TRPS1 and KLF13 transcription factors and MBNL2 a RNA binding protein as targets of miR-302d and miR-372.³⁷ The same authors identified in these hES cell lines a set of miRNAs upregulated (miR-372, 302d, 367, 200c, 199a, 19a and 217) or downregulated (miR-19b, 221, 222, let-7b, and let-7c) by Activin A, an important gene that participates in the maintenance of pluripotency.³⁸

Another work reported that the miR-302 cluster on chromosome 4 and the miR-520 cluster on chromosome 19 were highly expressed in undifferentiated hES cells. These miRNAs negatively correlate with target genes with chromatin structure modification function and play a role in the maintenance of chromatin structure.³⁹

Epithelial-mesenchymal transition

During embryogenesis, epithelial-mesenchymal transition (EMT) plays a role in the formation of various tissues and organs. EMT consists in the conversions of epithelial cells to mesenchymal cells; the reverse process is called mesenchymal to epithelial transition (MET). Epithelial cells establish close contacts with their neighbors and an apicobasal axis of polarity through the sequential arrangement of adherens junctions, desmosomes, and tight junctions. The epithelial cell layer maintains global communication through gap junctional complexes, and it remains separated from adjacent tissues by a basal lamina. Epithelia have the capacity to function as barriers or in absorption. Conversely, mesenchymal or stromal cells are loosely organized in a three-dimensional extracellular matrix and comprise

connective tissues adjacent to epithelia. The conversion of epithelial cells to mesenchymal cells is fundamental for embryonic development and involves profound phenotypic changes that include the loss of cell-cell adhesion, the loss of cell polarity, and the acquisition of migratory and invasive properties.⁴⁰ Most adult tissues arise from a series of EMT and MET. However, during one's adult life, only a certain subset of cells retains the ability to undergo EMT. Epithelial tumor cells often activate EMT, a latent embryonic program, to acquire tumoral properties, including enhanced motility and an increased invasiveness capacity.⁴⁰

Recently, miRNAs have been related to the control of EMT. Gregory, et al.⁴¹ showed that the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 were key regulators of EMT. These miRNAs were markedly downregulated in cells that had undergone EMT in response to TGF- β , and their enforced expression was enough to prevent EMT. These miRNAs act through their targets ZEB1 and ZEB2⁴¹ and TGF- β 2,⁴² which are E-cadherin transcriptional repressors. These miRNAs are thus able to enforce the epithelial phenotype through post-transcriptional repression of these genes, allowing the expression of E-cadherin and of polarity factors which are integral in forming cell-cell junctions (Fig. 2). The miR-200 family and miR-205 have been reported to be expressed in an epithelial-specific manner in the adult (in tissues that contain a high proportion of epithelial cells)⁴³ and during embryogenesis.⁴⁴⁻⁴⁶ Recent findings have also connected the let-7 family with the miR-200 family in EMT and stem cell formation (reviewed in⁴⁷).

MicroRNAs in CSCs

Many cancers have small populations of CSCs, cells with stem cell characteristics, which are a likely cause of relapse in cancer patients. The early phases of carcinogenesis resemble embryonic development, often involving the re-expression of embryonic mesenchymal genes. Undifferentiated stem cells display miRNA expression profiles reminiscent of cancer cells. Numerous known oncogenic miRNAs are expressed in early stages of development in undifferentiated cells; however, their expression decreases in differentiated tissue, whereas the opposite holds true for tumor suppressive miRNAs. In a previous study, we established what was to our knowledge the first connection between hES cells and tumor tissues through miRNA expression.⁴⁸ We carried out a comparative study of miRNA expression in human colon mucosa in early embryonic stages (7-8 weeks) versus late embryonic stages (9-12 weeks). Likewise, we compared miRNA expression in normal colon tissue versus colorectal cancer stages I and II. Overlapping miRNA expression was detected between embryonic colonic mucosa and colorectal.⁴⁸

Table 1. Main microRNAs Related to Pluripotency, Self-Renewal and Differentiation in Stem Cells

	Chromosome location	Validated targets	Transcription regulated by	Posttranscription regulated by
Pluripotency				
Mmu-miR-290-295	7	Rbl2	c-MYC OCT4 SOX2 NANOG TCF3	
Hsa-miR-371-372 (human homolog of miR-290-295)	19	TRPS1 KLF13 MBNL2	OCT4 SOX2 NANOG TCF3	
Hsa-miR-302-367 cluster	4	Cyclin D1 CDK4	NANOG OCT 3/4 SOX2 REX1	
Hsa-let-7 family:		HMGA2 IMP-1	c-MYC (-)	
let-7a-1, let-7d, let-7-f-1	9	LIN28		
let-7a-2		LIN28B		
let-7a-3, let-7b	11	RAS		
let-7c	22			
let-7e	21			
let-7f-2, mir-98	19			
let-7g	X			LIN28 (let-7g)
let-7i	3			
miR-202	12			
	10			
Mmu-miR-134	12	OCT4		
Mmu-miR-296	2	NANOG		
Mmu-miR-470	X	SOX2		
Self-renewal				
miR-17-92	13	E2F1	c-MYC	E2F1-3
miR-15a	13	BCL2		
miR-16-1		MCL1 CCND1 WNT3A		
Hsa-miR-21	17	PTEN PDC4 RECK MASPIN NFIB TPM1 SPRY2	AP-1 STAT3 REST (-)	
Hsa-miR-34 family:		CDK4	P53	
Hsa-miR-34a	1	CDK6		
Hsa-miR-34b, c	11	Cyclin E2, D1 E2Fs Bcl2 SIRT1 N-MYC Notch-1/2		
Differentiation				
miR-200 family and miR-205		ZEB1 ZEB2 TGFB1	ZEB1 (-) ZEB2 (-) TGFB1	

To date, no specific profiles of miRNAs in CSCs have been described. However, alterations in stem cell miRNAs have been reported in some tumors, where they can play a role as tumor suppressors or oncogenes.

Let-7/miR-98 family

Let-7 was the first human miRNA to be discovered. This miRNA family is highly conserved in invertebrates and mammals and is required for the timing of cell fate determination in *C. elegans*.^{49,50} Many human let-7 genes map to regions altered or deleted in human tumors,¹⁶ indicating that these genes function as tumor suppressors. Let-7 plays a role in lung cancer progression, where it is downregulated and where the postoperative survival time in lung cancer patients directly correlated with let-7 expression levels.^{51,52} Let-7 may directly control cellular proliferation by negatively regulating the human RAS genes,⁵³ known lung cancer oncogenes.

Moreover, let-7 is a marker for less advanced cancer and its expression inversely correlated with HMGA2, an early embryonic gene, but not with classical epithelial or mesenchymal markers, such as E-cadherin or vimentin, in SC1 and SC2 cells.⁵⁴ The expression of let-7 and HMGA2 acts as prognostic markers in ovarian cancer.⁵⁴ The loss of let-7 in cancer occurs early during the transformation process and results in reverse embryogenesis and dedifferentiation.⁵⁵ Along these lines, we investigated whether there are similar patterns of the let-7 family miRNA expression in pseudoglandular human embryonic lungs and in human lung tumors and found they were downregulated both in embryonic lung tissue and in lung tumors. Our findings support the model of cancer as an alteration of normal development, as many miRNAs were similarly expressed in early human lung development and stage I-II of lung cancer development.⁵⁶ Moreover, let-7 has been shown to be strikingly downregulated in mouse ES cells, where LIN28 is highly expressed but decreases during differentiation.³³ These two factors (let-7 and LIN28) form a tight feed-back loop that is fundamental to maintaining the precise levels of each to maintain the “stemness”, and so their dysregulation may lead to cancer.

miR-17-92 cluster

The miR-17-92 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1) was one of the first recognized miRNAs with an oncogenic function; the overexpression of the miR-17-92 cluster is known to promote cell proliferation.⁵⁷ miR-17-92 is transcriptionally activated by c-MYC, which in combination with Oct4, Nanog, Sox2, and Klf4, is necessary for the generation of induced pluripotent stem cells.⁵⁸⁻⁶³ The c-Myc-activated miR-17-92 could thus play a role in maintaining pluripotency and self-renewal

in stem cells. In our study of colorectal,⁴⁸ we established the first connection between hES cells and tumor tissue through miRNA expression.⁴⁸ We carried out a comparative study of miRNA expression in colon mucosa in human embryos and colorectal cancer. We focused on the miR-17-92 cluster and specifically on miR-17-5p, which was overexpressed in an early embryonic colon and in stage I and II colorectal tumors. *In situ* hybridization confirmed the high level of expression of miR-17-5p in the crypt progenitor compartment in the normal tissue (stem cell compartment), in the epithelial embryonic tissue and in the tumor.⁴⁸ In a similar study in lung cancer and human lung embryogenesis, we found similar results.⁵⁶ These findings support the potential existence of a relationship between stem cells, cell differentiation, and cancer. Moreover, this miRNA cluster, located in chromosome 13q31, is amplified in a number of B-lymphomas⁵⁷ and is overexpressed in multiple tumors, including lung, lymphoma, myeloid leukemias, hepatocellular carcinomas, and colorectal.⁶⁴

Moreover, a paralog cluster of miR-17-92 - the miR-106a-363 cluster located in chromosome X - has been reported to have oncogenic properties. The miR-106a-363 cluster is overexpressed in 46% of human T-cell leukemias, leading to increased miR-92 and miR-19 levels.⁶⁵

miR-302-367 cluster

This miRNA cluster plays an important role in the maintenance of pluripotency and quickly decreases after cell differentiation and proliferation. It is also expressed in human embryonic carcinoma cells. This miRNA cluster is encoded in chromosome 4 and is comprised of nine miRNAs: miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-367, and miR-67* (polycistronic transcript). There is scarce evidence that this miRNA cluster could play a role in cancer. Recently, however, Lin, et al.⁶⁶ reprogrammed human skin cancer cells into a pluripotent ES-cell-like state with ectopic expression of the miR-302 cluster. Moreover, some members of the cluster are found expressed in certain acute immature leukemia cell lines but not in normal somatic hematopoietic cells.⁶⁷

miR-34 family

Some miRNAs have been implicated in cell cycle control related to p53, a crucial tumor suppressor that responds to cellular stress. Through its DNA binding domain, the p53 protein activates the expression of the miR-34 family in response to DNA damage or oncogenic stress, leading to apoptosis or cell cycle arrest.⁶⁸⁻⁷⁰ The conserved miR-34 family is composed of three miRNAs: miR-34a, miR-34b, and miR-34c. There are two miR-34 loci, one encoding miR-34a (chromosome 1) and the other encoding both

miR-34b and miR-34c (chromosome 11). The promoter regions of both loci contain a p53-binding site.⁶⁸ The miR-34 family has been shown to form part of the p53 network,^{68,71} and its expression is directly induced by p53 in response to DNA damage or oncogenic stress. Moreover, the promoter region of the MIRN34A gene contains CpG islands, and aberrant CpG methylation that reduces miR-34a expression levels has been reported in multiple types of cancer.⁷² The high frequency of miR-34 silencing in tumors suggests that the miR-34 family acts as a tumor suppressor gene, indicating a potential role as a prognostic marker. Recently, our group identified miR-34a as a prognostic marker of relapse in NSCLC patients; patients with both p53 mutations and low miR-34a levels had the highest probability of relapse.⁶⁹ Moreover, miR-34a has been shown to play a role in chemotherapy resistance in CLL.⁷³

The miR-34 family has been related to the Notch pathway, an important pathway during normal development where it acts to regulate many cellular processes. miR-34a targets several mRNAs, such as SIRT1, Bcl-2, N-myc, and Cyclin D1, and has recently been reported to target Notch-1, Notch-2, and CDK6. In pancreatic cancer, stem cells have high levels of Notch-1/2 and loss of miR-34, suggesting that miR-34 is involved in the regulation of pancreatic CSCs via regulation of the Notch pathway.⁷⁴

miR-15, miR-16

miR-15 and miR-16 were two of the first identified miRNAs with a tumor suppressor function. These miRNAs are encoded in 13q14, a region often implicated in cancer, and they are frequently deleted and/or downregulated in chronic lymphocytic leukemia (CLL).⁷⁵ Expression of these miRNAs inhibits cell proliferation, promotes apoptosis, and suppresses tumorigenicity both *in vitro* and *in vivo* (reviewed in⁷⁶) by targeting multiple oncogenes, including BCL2, MCL1, CCND1, and WNT3A. Downregulation of these miRNAs has been reported in CLL,⁷⁵ pituitary adenomas,⁷⁷ prostate carcinoma,⁷⁸ NSCLC,⁷⁹ and ovarian cancer.⁸⁰

These miRNAs play a key role in CSC regulation and in tumor cells, where they help to control self-renewal through cell cycle control.⁸¹ Moreover, in ovarian cancer, these miRNAs regulate Bmi-1. Bmi-1 plays a key role in regulating the proliferative activity of normal stem and progenitor cells.⁸² It is also indispensable for the self-renewal of neural⁸³ and hematopoietic stem cells.⁸⁴ Oncogenic activation of Bmi-1 is found in a wide variety of epithelial malignancies, including ovarian cancer, where it correlates with the downregulation of miR-15/16.⁸¹

Recently, miR-16 has been linked to the p53 pathway. Suzuki, et al.⁸⁵ have shown that p53 interacts with proteins of the Drosha complex to promote processing of a subset of miRNAs, including miR-16-1. We have analyzed the

prognostic implications of miR-16 levels in NSCLC patients and found that miR-16 works as a marker for disease-free and overall survival marker in these patients (unpublished data).

miR-21

miR-21 is encoded in chromosome 17q23 and is overexpressed in a large number of tumors, including breast cancer, Hodgkin lymphoma,⁸⁶ leukemia, glioma, head and neck cholangiocarcinoma,⁸⁷ hepatocellular carcinoma,⁸⁸ colorectal,^{48,89} cervical, ovarian, and pancreatic cancer.⁶⁴ In these tumors, miR-21 promotes proliferation by inhibiting apoptosis and promotes invasion by acting on different targets: PDC4, PTEN, RECK, MASPIN, NFIB, TPM1, or SPRY2 (reviewed in⁹⁰). In contrast, relatively low expression of miR-21 was observed in the HL-60 (promyelocytic leukemia) and the K562 (chronic myelogenous leukemia) cell lines and in a prostatic adenocarcinoma cell line. The transcription of miR-21 has been shown to be activated by AP-1⁹¹ and STAT3,⁹² and the overexpression of miR-21 in multiple types of cancers may reflect an elevated AP-1 or STAT3 activity. In contrast to its high expression in tumors, miR-21 expression was low in ES cells, where miRNAs play a complex role in self-renewal/proliferation, since REST, a gene essential for pluripotency and self-renewal, binds to the miR-21 promoter region, and represses its expression.⁹³

miR-200 family and miR-205

Recent evidence suggests that cells that undergo EMT acquire stem cell-like properties.^{94,95} Moreover, it has been shown that ES cells can adopt a mesenchymal phenotype without losing their pluripotency.^{96,97} Loss of the miR-200 family seems to be a late-stage event in epithelial tumor progression, occurring in de-differentiated tumor cells with a greater metastatic capacity via EMT (Fig. 3). The miR-200 family and miR-205 have been reported to be dysregulated in specific cancers. The miR-200 family is downregulated in benign liver tumors⁹⁸ and upregulated in ovarian⁹⁹ and cholangyocyte¹⁰⁰ tumors. Our group has observed overexpression of the miR-200 family in colorectal cancer (non-published data). miR-205 is downregulated in breast cancer¹⁰¹ and esophageal tumors⁸⁷ and upregulated in ovarian⁹⁹ and bladder tumors.¹⁰² The miR-200 family and miR-205 are also downregulated in poorly differentiated tumors and mesenchymal cancer cell lines.

Microvesicles

Microvesicles (MVs) are plasma membrane-derived vesicles (from -30 nm to 1 μ m), originally considered inert cellular debris, released into the extracellular environment by a variety of cell types, including ES cells and tumor cells.

Originally characterized from platelets,¹⁰³ MVs are a normal constituent of human plasma, where they play an important role in maintaining hematostasis. MVs contain a variety of elements, including proteins, lipids, and RNA, and recently it has been shown that they are enriched in miRNAs.¹⁰⁴ MVs play a role in stem cell-microenvironment communication, where MVs account for the transfer of genetic information between the microenvironment and stem cells, thus modulating the stem-phenotype.¹⁰⁵ Recently, MVs have been isolated from ES cells. These ES cell microvesicles (ESMV) were capable of reprogramming hematopoietic progenitors.¹⁰⁶ Moreover, ES cells produce microvesicles which contain abundant miRNAs.¹⁰⁷ Tumor cells can also release MVs, which can be detected in the plasma of cancer patients,¹⁰⁸ where they may function as prognostic markers. In ovarian cancer, eight miRNAs (miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214) previously shown to be diagnostic were found more highly expressed in MVs from advanced ovarian cancer patients than in patients with benign ovarian diseases; moreover, these miRNAs were not present in normal controls.¹⁰⁹ These miRNAs could be useful as diagnostic biomarkers in tumors like NSCLC.¹⁰⁹ The detection of miRNAs in plasma/serum MVs in cancer patients opens a new window for tumor screening and therapeutic approaches.

CONCLUSION

miRNAs have emerged as key regulators of “stemness”, collaborating in the maintenance of pluripotency, control of self-renewal, and differentiation of stem cells. Recently, several breakthroughs have increased our knowledge of the role of miRNAs in stem cells. Firstly, miRNAs expressed specifically in ES cells have been identified. Secondly, recent findings indicate that cancer may arise from stem cells (CSCs) and that many cell signaling pathways essential for normal development are also involved in cancer initiation and progression. Moreover, overlapping expression patterns of miRNAs have been identified in embryonic and cancer cells. Taken together, these findings provide hints that a strong link exists between embryonic cells and cancer cells. Thirdly, miRNAs have been linked to the main pathways of stem cells, where some miRNAs regulate or are regulated by stem cell-genes such as Nanog, Oct4, or Sox2. Finally, although miRNAs are not at the top of the stem cell hierarchy, they are crucial intermediate regulators, and the ectopic expression of certain miRNAs, such as the miR-302-367 cluster, can induce pluripotency. As we increase our knowledge of miRNAs in ES cells, we will be able to identify new targets in the fight against

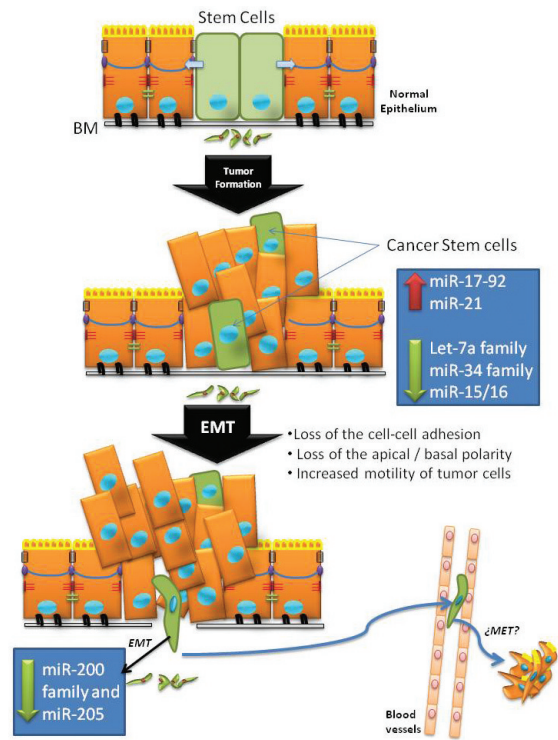


Fig. 3. EMT in tumors.

tumors. CSCs are responsible for relapse and treatment failure in many cancer patients, and the comparative analysis of expression patterns between ES cells and tumors can lead to the identification of a miRNA signature to define CSCs. Most of the key miRNAs identified to date in ES cells have been shown to play a role in tumor diagnosis or prognosis and may well prove to be essential in cancer therapy in the foreseeable future.

ACKNOWLEDGEMENTS

This work was supported by Fondo de Investigaciones Sanitarias de la Seguridad Social [FIS-PI09/00547].

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