Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Human haematological and epithelial tumor-derived cell lines express distinct patterns of onco-microRNAs

Flavia Biamonte[#*], Fabiana Zolea[#], Gianluca Santamaria, Anna M. Battaglia, Giovanni Cuda, Francesco Costanzo

Research Center of Biochemistry and Advanced Molecular Biology, Department of Experimental and Clinical Medicine, "Magna Græcia" University of Catanzaro, Italy

Correspondence to: flavia.biamonte.fb@gmail.com

[#]These authors equally contributed

Received June 28, 2017; **Accepted** November 6, 2017; **Published** November 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.11.14

Abstract: MicroRNAs post-transcriptionally regulate gene expression thus playing a critical role in a wide range of physiological and pathological processes, including cancer initiation and progression. Moreover, a growing number of evidences highlights that miRNAs themselves are differentially expressed between normal and malignant tissues. In this study, we analysed differences in miRNA expression profile between haematological and epithelial tumor-derived cell lines and explored their role in definying different cancer cells phenotypes. Cancer Focus microRNA PCR Panel was used to analyze eighty-four oncomiRNAs in two human haematological (K562 and HL-60) and in two epithelial (H460 and MCF-7) cancer cell lines. Bioinformatic tools were used to identify miRNA-specific signatures and to discover potentially deregulated pathways. Our analysis led to the identification of i) a large repertoire of miRNAs commonly expressed in the four cell lines, including two equally highly expressed (UPmiRs) and four equally low expressed (DNmiRs); ii) two miRNAs signatures, one associated with the haematological and one with the epithelial cell lines; iii) miRNA signatures specific for the acute or for the chronic myeloid leukemic cells; iv) miRNA signatures specific for the lung or for the breast carcinoma cells. As a whole, these results strengthen the significance of miRNAs profiling in human cancer subtyping, providing the ground for the identification of novel potential biomarkers for specific cancer cell phenotypes.

Key words: OncomiRNAs; Epithelial cells; Haematological cells; K562; HL-60; H460; MCF-7.

Introduction

During the last two decades, the rapid development of new experimental tools and an increasing scientific interest in non-coding RNAs has led to an explosion of knowledge regarding microRNAs and their role in physiological and pathological conditions (1).

Since their initial identification, more than 2,500 mature human miRNAs have been annotated in miRBase Release 2.0 and an even greater number of predicted target mRNAs has been discovered in the human genome (2). Noteworthy, a single miRNA can post-transcriptionally regulate the expression of hundreds of mRNAs (1), thus contributing to many essential cellular functions and processes (3) such as differentiation (miR-223) (4), metabolism (miR-133, miR-29a/b and miR-27a) (5), proliferation (miR-17 and let-7) (6), apoptosis (miR-34 and miR-21) (6) and cell response to different stress stimuli (miR-210) (7). Thus, aberrant expression or mutations of these key regulators often result in a failure in orchestrating normal development and cell functions, thereby leading to the onset of various diseases (3). In human haematological and epithelial cancers it has been shown that the deregulated microRNA expression may affect either the early stages of cancer progression or the acquisition of metastatic potential (8, 9). The role of miRNAs in cancer pathogenesis is accomplished through the regulation of several oncogenes such as E2F, RAS, and MYC, as well as tumor suppressor genes, whereof p53 is the best known example. In other words, a single microRNA may act either as an oncogene or a tumour suppressor, depending on its tissue specific expression and its ability to regulate multiple targets (10, 11).

Human cancer cell lines represent excellent experimental models and renewable resources to characterize the expression profiles of oncomiRNAs (12). Here, we evaluate the expression profile of 84 oncomicroRNAs in two haematological (K562 and HL-60) and two epithelial (MCF-7 and H460) tumor-derived human cancer cell lines. Through this analysis, we found a repertoire of miRNAs commonly expressed in all the cell lines and, more importantly, we provide a catalog of miRNAs that might help in highlighting differences among individual cancer types and to better understand their underlying biology.

Materials and Methods

Cell lines

Four cancer cell lines (K562, HL60, MCF-7 and H460) were used to evaluate oncomicroRNA patterns. The epithelial cancer cell lines were derived from non-small cell lung cancer (H460, ATCC® HTB177™) and breast cancer (MCF-7, ATCC® HTB22™); the haematological cancer cell lines where obtained from chronic

(K562, ATCC® CCL243™) and acute (HL-60, ATCC ® CCL-240™) myeloid leukemia. MCF-7 and HL-60 were cultured respectively in DMEM (Sigma Aldrich, St. Louis, MO) and IMDM (Sigma Aldrich, St. Louis, MO); K562 and H460 were maintained in RPMI 1640 (Sigma Aldrich, St. Louis, MO) as recommended by ATCC. All media were supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS; Sigma Aldrich, St. Louis, MO). Cells were incubated in a humidified incubator at 37°C supplied with 5% CO_2.

miRNA extraction and quantitative real-time PCR (RT-PCR)

MiRNA-enriched total RNA was extracted from K562, HL-60, H460 and MCF-7 cells by using miRCURY™ RNA Isolation Kit Cell and Plant (Exiqon, Woburn, USA) following the manufacturer's protocol. The concentration, the purity and the integrity of the RNA were checked spectroscopically (260/280 and 260/230 absorbance ratios) through Nanodrop Instrument (Thermo Scientific, Waltham, MA, USA). Cancer Focus microRNA PCR Panel (EXIQON, Woburn, USA) was used to assess the expression levels of 84 onco-miRNAs by quantitative real-time PCR (qRT-PCR). Each sample was assayed in duplicate, and the experimental data were normalized to the expression levels of the housekeeping small nuclear RNA, SNORD49. Relative expression was calculated as previously described (13).

Functional analysis of microRNAs

Functional analysis of miRNAs was performed using Ingenuity Pathway Analysis (IPA) (14). IPA maps each miRNA within a molecular network and defines it as "focus molecule". The identified networks are shown as a graph where genes are represented as nodes with various shapes representing the functional class of the gene product. The biological relationships between two nodes are represented as lines, displayed with various labels describing the nature of the relationship. Pathway enrichment analysis highlighted the most significant (threshold: -log p value=1.30) canonical pathways obtained by IPA.

KEGG analysis

Target genes of miRNAs obtained were used as input for the KEGG pathway database. KEGG is a collection of manually drawn pathway maps representing the knowledge on the molecular interaction and reaction networks for metabolism, genetic information processes, environmental information processing, cellular processes and human diseases. KEGG was used for pathway enrichment analysis using as threshold a p value ≤ 0.05.

Statistical analysis

Student's t testing for comparison of two groups was used to analyze miRNA expression levels between the different samples. We selected only miRNAs with a Log_2 (fold change) above +1 or below -1; those differences with a $p \leq 0.05$ were considered statistically significant.

Venn diagrams were generated to identify miRNAs commonly or exclusively expressed as well as to study the overlap between miRNAs up- or down-regulated in all the experimental models.

The analysis of the more representative miRNAs among haematological and epithelial cancer cell lines was represented through a Volcano plot using $-Log_{10}$ (p values) and the Log_2 (fold change). We selected only miRNAs differentially expressed with a Log_2 (fold change) above +1 with a $p \leq 0.05$.

Results

OncomiRNA profiles of K562, HL-60, H460 and MCF-7 cell lines

We used the Cancer Focus microRNA PCR panel to analyze the expression profile of 84 different oncomiRNAs in the chronic K562 and the acute HL-60 myeloid leukemia cell lines and in the lung H460 and the breast MCF-7 carcinoma cell lines. Through a duplicate set of experiments, we found that all miRNAs were detected in at least one of the four cell lines. In particular, 76 miRNAs were expressed in K562 and in HL-60 cells, and a total of 80 and 81 miRNAs were detectable in the MCF-7 and H460 cells, respectively. The oncomiRNAs of each cell line, sorted in a decreasing order of expression according to their relative raw threshold cycle (Ct) value (including those below the limit of detection, Ct>45) are listed in Table 1.

From a global analysis of the four profiles, it appears that the vast majority of miRNAs was detected with a Ct value between 26 and 30, while the group of the most highly expressed ones (Ct≤25) was less than 13% in each cell type, with the exclusion of HL-60, where they reached the 40% (Figure 1).

Commonly high expressed (UPmiRs) and low expressed (DNmiRs) miRNAs in the four cell lines

As expected, we found that the largest category of the microRNAs analyzed (69 out of 84) was ubiquitously expressed in all four cell types. Among them, miR-16-5p and miR-21-5p were expressed with a Ct≤25 in all cell lines (UPmiRs) (Venn diagram in Figure 2A); similarly, miR-206 and miR-149-3p were expressed with 36≤Ct≥45 in the four cell lines (DNmiRs) (Venn

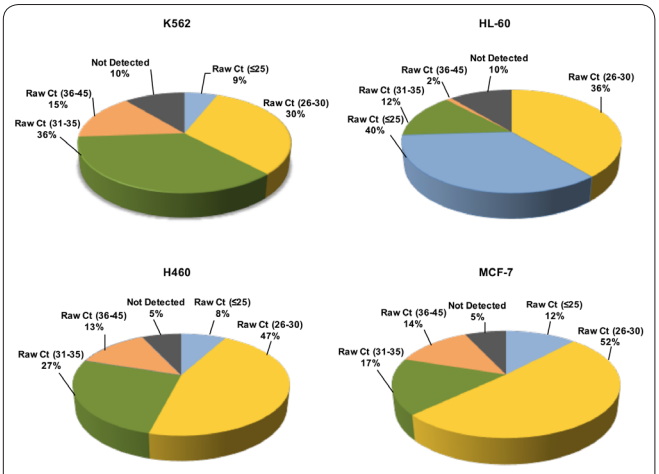

Figure 1. Global analysis of the four oncomiRNA profiles. Pie charts illustrating the global expression of the 84 oncomiRNAs in K562, HL-60, H460 and MCF-7 cells. The pie size corresponds to the percentage composition of miRNAs identified in each cell line clustered according to their Ct ranges.

Table 1. The 84 oncomiRNAs analyzed in each cell line and their relative raw threshold cycle (Ct).

	Global miRNA expression profiles			
	K562	**HL60**	**H460**	**MCF-7**
Raw Ct ≤ 25	hsa-miR-20a-5p, hsa-miR-106a-5p, hsa-miR-19b-3p, hsa-miR-19a-3p, hsa-miR-126-3p, hsa-miR-16-5p, hsa-miR-21-5p.	hsa-miR-20a-5p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-106a-5p, hsa-miR-16-5p, hsa-let-7e-5p, hsa-let-7a-5p, hsa-miR-21-5p, hsa-miR-93-5p, hsa-miR-223-3p, hsa-let-7f-5p, hsa-miR-18a-5p, hsa-miR-25-3p, hsa-miR-23a-3p, hsa-let-7g-5p, hsa-miR-26a-5p, hsa-miR-106b-5p, hsa-miR-15a-5p, hsa-miR-181a-5p, hsa-miR-24-3p, hsa-miR-221-3p, hsa-let-7i-5p, hsa-miR-191-5p, hsa-let-7d-5p, hsa-miR-99a-5p, hsa-miR-103a-3p, hsa-miR-30c-5p, hsa-miR-155-5p, hsa-miR-17-5p, hsa-miR-146a-5p, hsa-miR-15b-5p, hsa-miR-125b-5p, hsa-miR-101-3p, hsa-miR-27b-3p	hsa-let-7i-5p, hsa-let-7a-5p, hsa-miR-16-5p, hsa-miR-21-5p, hsa-miR-24-3p, hsa-miR-23a-3p, hsa-let-7b-5p	hsa-miR-21-5p, hsa-miR-200c-3p, hsa-miR-93-5p, hsa-let-7a-5p, hsa-miR-103a-3p, hsa-miR-141-3p, hsa-miR-16-5p, hsa-miR-24-3p, hsa-miR-23a-3p, hsa-let-7b-5p
Raw Ct 26-30	hsa-miR-93-5p, hsa-miR-223-3p, hsa-miR-103a-3p, hsa-miR-18a-5p, hsa-miR-191-5p, hsa-miR-23a-3p, hsa-miR-25-3p, hsa-miR-107, hsa-miR-210, hsa-miR-423-5p, hsa-let-7a-5p, hsa-miR-24-3p, hsa-miR-106b-5p, hsa-miR-26a-5p, hsa-miR-92b-3p, hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-23b-3p, hsa-miR-15a-5p, hsa-miR-15b-5p, hsa-miR-27b-3p, hsa-miR-17-5p, hsa-miR-22-3p, hsa-let-7e-5p, hsa-miR-148a-3p	hsa-miR-181b-5p, hsa-miR-107, hsa-miR-26b-5p, hsa-miR-27a-3p, hsa-let-7c, hsa-miR-222-3p, hsa-miR-30b-5p, hsa-miR-148a-3p, hsa-miR-210, hsa-miR-29a-3p, hsa-miR-423-5p, hsa-miR-29c-3p, hsa-let-7b-5p, hsa-miR-23b-3p, hsa-miR-9-5p, hsa-miR-92b-3p, hsa-miR-7-5p, hsa-miR-31-5p, hsa-miR-34a-5p, hsa-miR-29b-3p, hsa-miR-186-5p, hsa-miR-192-5p, hsa-miR-182-5p, hsa-miR-30d-5p, hsa-miR-100-5p, hsa-miR-215, hsa-miR-150-5p, hsa-miR-145-5p, hsa-miR-10b-5p	hsa-miR-103a-3p, hsa-miR-19b-3p, hsa-miR-20a-5p, hsa-miR-125b-5p, hsa-miR-181a-5p, hsa-miR-31-5p, hsa-let-7g-5p, hsa-miR-29a-3p, hsa-miR-106a-5p, hsa-miR-93-5p, hsa-let-7f-5p, hsa-miR-34a-5p, hsa-let-7d-5p, hsa-miR-107, hsa-miR-423-5p, hsa-miR-29b-3p, hsa-miR-19a-3p, hsa-miR-23b-3p, hsa-miR-222-3p, hsa-miR-30c-5p, hsa-miR-26a-5p, hsa-miR-191-5p, hsa-let-7c, hsa-miR-25-3p, hsa-miR-99a-5p, hsa-miR-22-3p, hsa-miR-106b-5p, hsa-let-7e-5p, hsa-miR-181b-5p, hsa-miR-27b-3p, hsa-miR-15a-5p, hsa-miR-29c-3p, hsa-miR-30b-5p, hsa-miR-100-5p, hsa-miR-9-5p, hsa-miR-210, hsa-miR-126-3p, hsa-miR-130a-3p, hsa-miR-18a-5p	hsa-miR-25-3p, hsa-miR-27b-3p, hsa-miR-107, hsa-miR-106b-5p, hsa-let-7f-5p, hsa-miR-23b-3p, hsa-let-7e-5p, hsa-miR-20a-5p, hsa-miR-181a-5p, hsa-miR-106a-5p, hsa-miR-19b-3p, hsa-let-7g-5p, hsa-let-7d-5p, hsa-miR-181b-5p, hsa-miR-191-5p, hsa-miR-205-5p, hsa-miR-26a-5p, hsa-miR-34a-5p, hsa-miR-19a-3p, hsa-miR-196a-5p, hsa-miR-30b-5p, hsa-let-7c, hsa-miR-92b-3p, hsa-miR-200a-3p, hsa-miR-27a-3p, hsa-miR-200b-3p, hsa-miR-30c-5p, hsa-miR-15b-5p, hsa-miR-423-5p, hsa-miR-15a-5p, hsa-miR-7-5p, hsa-miR-210, hsa-miR-148a-3p, hsa-miR-182-5p, hsa-miR-18a-5p, hsa-miR-101-3p, hsa-miR-125b-5p, hsa-miR-126-3p, hsa-miR-26b-5p, hsa-let-7i-5p, hsa-miR-192-5p, hsa-miR-29c-3p, hsa-miR-29b-3p

Raw Ct 31-35	hsa-miR-196a-5p, hsa-miR-101-3p, hsa-miR-186-5p, hsa-miR-27a-3p, hsa-miR-200c-3p, hsa-miR-29a-3p, hsa-miR-182-5p, hsa-miR-132-3p, hsa-miR-30d-5p, hsa-let-7c, hsa-miR-26b-5p, hsa-miR-194-5p, hsa-miR-10a-5p, hsa-miR-29c-3p, hsa-miR-200a-3p, hsa-let-7f-5p, hsa-miR-29b-3p, hsa-let-7b-5p, hsa-miR-215, hsa-miR-192-5p, hsa-miR-145-5p, hsa-miR-181a-5p, hsa-miR-7-5p, hsa-miR-141-3p, hsa-miR-221-3p, hsa-let-7g-5p, hsa-miR-125b-5p, hsa-let-7d-5p, hsa-miR-181b-5p, hsa-miR-222-3p	hsa-miR-132-3p, hsa-miR-200c-3p, hsa-miR-141-3p, hsa-miR-126-3p, hsa-miR-194-5p, hsa-miR-195-5p, hsa-miR-10a-5p, hsa-miR-214-3p, hsa-miR-22-3p, hsa-miR-196a-5p,	hsa-miR-148a-3p, hsa-miR-200c-3p, hsa-miR-196a-5p, hsa-miR-186-5p, hsa-miR-26b-5p, hsa-miR-27a-3p, hsa-miR-15b-5p, hsa-miR-182-5p, hsa-miR-221-3p, hsa-miR-101-3p, hsa-miR-132-3p, hsa-miR-10a-5p, hsa-miR-92b-3p, hsa-miR-141-3p, hsa-miR-17-5p, hsa-miR-192-5p, hsa-miR-194-5p, hsa-miR-30d-5p, hsa-miR-215, hsa-miR-7-5p, hsa-miR-1, hsa-miR-155-5p	hsa-miR-194-5p, hsa-miR-29a-3p, hsa-miR-215, hsa-miR-22-3p, hsa-miR-132-3p, hsa-miR-99a-5p, hsa-miR-30d-5p, hsa-miR-186-5p, hsa-miR-195-5p, hsa-miR-17-5p, hsa-miR-31-5p, hsa-miR-222-3p, hsa-miR-221-3p, hsa-miR-143-3p
Raw Ct 36-45	hsa-miR-31-5p, hsa-miR-195-5p, hsa-miR-146a-5p, hsa-miR-141-3p, hsa-miR-206, hsa-miR-214-3p, hsa-miR-9-5p, hsa-miR-150-5p, hsa-miR-149-3p, hsa-miR-130a-3p, hsa-miR-10b-5p, hsa-miR-133a, hsa-miR-143-3p, hsa-miR-202-3p	hsa-miR-149-3p, hsa-miR-206.	hsa-miR-195-5p, hsa-miR-214-3p, hsa-miR-146a-5p, hsa-miR-10b-5p, hsa-miR-149-3p, hsa-miR-200b-3p, hsa-miR-206, hsa-miR-145-5p, hsa-miR-150-5p, hsa-miR-202-3p, hsa-miR-133a,hsa-miR-205-5p	hsa-miR-214-3p, hsa-miR-9-5p, hsa-miR-10a-5p, hsa-miR-145-5p, hsa-miR-130a-3p, hsa-miR-206, hsa-miR-149-3p, hsa-miR-150-5p, hsa-miR-202-3p, hsa-miR-133a, hsa-miR-146a-5p, hsa-miR-1.
Undetected Ct>45	hsa-let-7i-5p, hsa-miR-1, hsa-miR-100-5p, hsa-miR-155-5p, hsa-miR-200b-3p, hsa-miR-205-5p, hsa-miR-34a-5p, hsa-miR-99a-5p	hsa-miR-202-3p, hsa-miR-1, hsa-miR-200a-3p, hsa-miR-143-3p, hsa-miR-200b-3p, hsa-miR-205-5p, hsa-miR-133a , hsa-miR-130a-3p	hsa-miR-200a-3p, hsa-miR-143-3p, hsa-miR-223-3p	hsa-miR-10b-5p, hsa-miR-100-5p, hsa-miR-155-5p, hsa-miR-223-3p

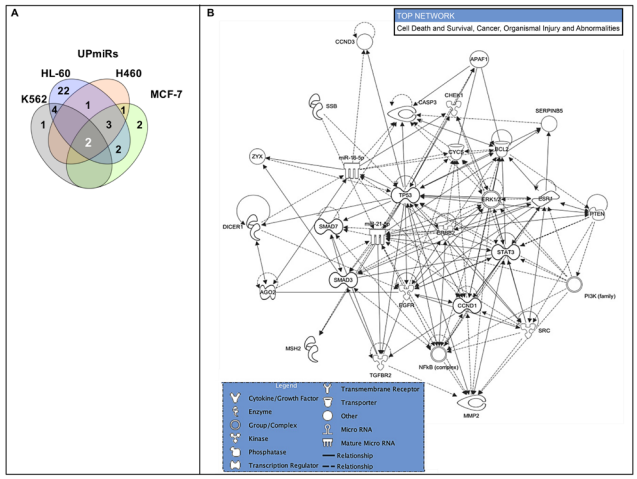

Figure 2. UPmiRs in the haematological and the epithelial cancer cell lines. (A) Venn diagram of the commonly highly expressed miRNAs (UPmiRs) in K562, HL-60, H460 and MCF-7 cell lines (B) Ingenuity Pathway Analysis of miR-16-5p and miR-21-5p. The analysis revealed one significant network "*Cell Death and Survival, Cancer, Organismal Injury and Abnormalities*". A solid line represents a direct interaction between two genes, while a dotted line indicates an indirect interaction.

diagram in Figure 3A). In order to explore the molecular pathways affected by UPmiRs and DNmiRs, we performed Ingenuity Pathway Analysis (IPA). In this analysis we also included, in the DNmiRs subset, miR-133a and miR-202-3p since they are low expressed in 3 out of the 4 cell lines (K562, H460 and MCF-7) and even undetected in HL-60 cells. Interestingly, IPA highlighted that both the UPmiRs and the DNmiRs insist on the same network "*Cell Death and Survival, Cancer, Organismal Injury and Abnormalities*" composed of genes notoriously related to cancerogenesis such as *p53, STAT3, ERK1/2, AKT, ERBB2, CCND1* and *ESR1* (15, 16) (Figure 2B and Figure 3B). Furthermore, the two UPmiRs resulted also to be involved in the "*Cell cycle"* and "*Cell-to-cell signaling and interaction"* pathways

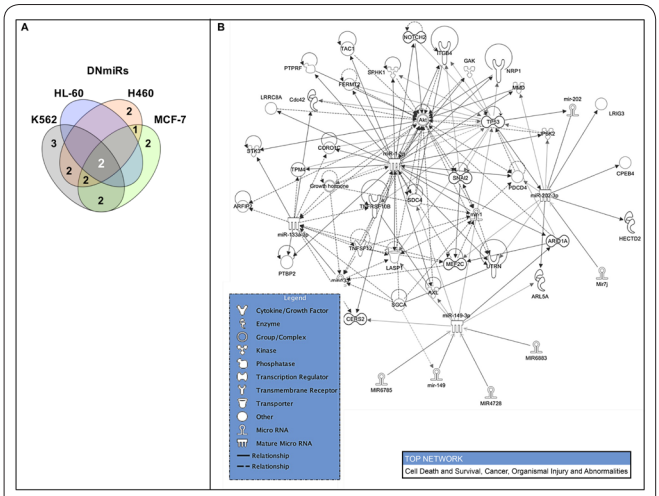

Figure 3. DNmiRs in haematological and epithelial cancer cell lines. (A) Venn diagram of the commonly low expressed miRNAs (DNmiRs) in K562, HL-60, H460 and MCF-7 cell lines. (B) Ingenuity Pathway Analysis of miR-206, miR-149-3p, miR-133a and miR-202-3p. The analysis revealed three significant networks among which the most enriched was "*Cell Death and Survival, Cancer, Organismal Injury and Abnormalities*". A solid line represents a direct interaction between two genes, while a dotted line indicates an indirect interaction.

while the four DNmiRs also affected the "*Cellular development"*, "*Cell death and survival"* and the "*Cell-to-cell signaling and interaction"* pathways (data not shown).

miRNA expression signatures discriminate haematological and epithelial cancer cell lines

The mirRNome analysis also led to the identification of two microRNA signatures, one associated with the haematological cancer cell lines and one with the epithelial ones. Each signature comprised both *tissue specific miRNAs* and *tissue-enriched miRNAs*. In the *tissue specific miRNAs* category we included the ones expressed in the haematological cells and undetected in the epithelial ones, and viceversa; on the other hand, as *tissue-enriched miRNAs* we considered the microRNAs over-expressed in the haematological cell lines compared to the epithelial ones (FC>2), and viceversa. As shown in Figure 4A, miR-223-3p is the only haematological-specific miRNA, while miR-200b-3p, miR-205-5p and miR-1 represent the epithelial-specific ones. The Volcano Plot reported in Figure 4B shows that, among the remaining miRNAs, 14 (miR-17-5p, miR-18a-5p, miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-25-3p, miR-106a-5p, miR-210, miR-191-5p, miR-148a-3p, miR-15a, miR-16, miR-30b and miR-30d) were significantly over-expressed in K562 and HL-60 (red dots) and two (miR-34a-5p and miR-130a-3p) were over-expressed in H460 and MCF-7 (green dots). The *haematological-* and the *epithelial-enriched miRNAs*, together with their relative expression levels, are listed in Table 2. Notably, 11 out of 14 of the *haematological-enriched miRNAs* belong to genomic clusters (Figure 4C).

As shown in Figure 5A, IPA analysis of the *haematological-specific* miR-223-3p highlighted the existence of nine direct gene targets, of which almost

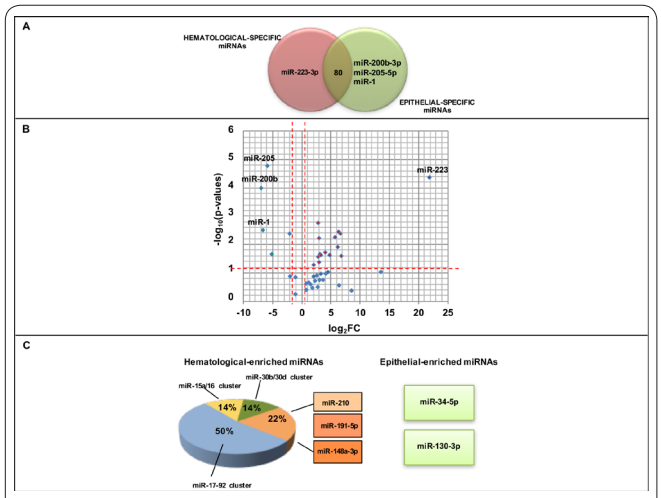

Figure 4. MiRNA signatures distinguish haematological from epithelial cancer cell lines. (A) Venn diagram shows the 80 common and the 4 specific miRNAs expressed in haematological and epithelial cancer cell lines. (B) Volcano plot shows the differentially-expressed miRNAs between the haematological and the epithelial cancer cell lines. Fold changes ($\log_2$FC ≥1) and *p* values ($-\log_{10} p$ value). The haematological- and the epithelial-enriched miRNAs are shown in red and green respectively; miRNAs differentially expressed without statistical significance are blue colored. (C) On the left, the pie chart describes the percentage of composition of the haematological-enriched miRNAs. On the right, the two epithelial enriched miRNAs are shown.

Table 2. Haematological and epithelial enriched miRNAs.

Tissue-Enriched MiRNAs	
Haematological	**Epithelial**
hsa-miR-17-5p	miR-34a-5p
hsa-miR-18a-5p	miR-130a-3p
hsa-miR-19a-3p	
hsa-miR-19b-3p	
hsa-miR-20a-5p	
hsa-miR-25-3p	
hsa-miR-106a-5p	
hsa-miR-210	
hsa-miR-191-5p	
hsa-miR-148a-3p	
hsa-miR-15a	
hsa-miR-16	
hsa-miR-30b	
hsa-miR-30d	

50% are linked to haematopoiesis-related processes as transcription factors (*NF1A* and *LMO2),* GTP-binding proteins (*RHOB*) and signalling transducers (*STMN1*) (17-20). IPA analysis of the *epitelial-specific miRNAs* (miR-200b-3p, miR-205-5p and miR-1) revealed their involvement in three top molecular networks "*Cellular Assembly and Organization, Connective Tissue Disorders, Developmental Disorder"*, *"Cellular Development, Cellular Growth and Proliferation, Cell death and Survival"* and *"Cellular Movement, Cancer, Gastrointestinal Disease"* pathways (Figure 5B).

Next, we explored the pathways potentially regulated by the *haematological-* and the *epithelial-enriched miRNAs* by using the Pathway Enrichment Analysis (Figure 6). Taking into account that the haematological-enriched miRNAs were numerically more abundant than the epithelial ones (14 *vs* 2), this analysis highlighted i) pathways on which both the *haematological-* and *epithelial-enriched miRNAs* insist with an equivalent significance (*Tissue development*, *Cellular development*, *Cellular morphology*), ii) pathways more significantly affected by the *haematological-enriched miRNAs* (*Haematological disease*, *Immunological disease, Inflammatory disease)* and iii) pathways more significantly affected by the *epithelial-enriched miRNAs* (*Hereditary disorders* and *Respiratory system development and function*).

miRNA signatures distinguish between K562 and HL-60 leukemia cell lines

Next, we focused on the identification of specific miRNA signatures able to distinguish between K562 and HL-60 leukemia cell lines. K562 is a *Bcr-Abl* positive chronic leukemic cell line that can spontaneously differentiate toward erythroid and megakaryocytic lineages; HL-60 is a model of promyeloblast acute myeloid leukemia that can be induced in vitro to differentiate into different cell types, including granulocytes and monocytes (21). From the K562 *vs* HL-60 comparison we found that only K562 cells express, albeit at low levels (33≤Ct≥42), miR-200a, miR-miR-202, miR-130, miR-143 and miR-133a. KEGG analysis, performed on the target genes of these miRNAs, revealed their involvement in 20 pathways. Noteworthy, as shown in Table 3,

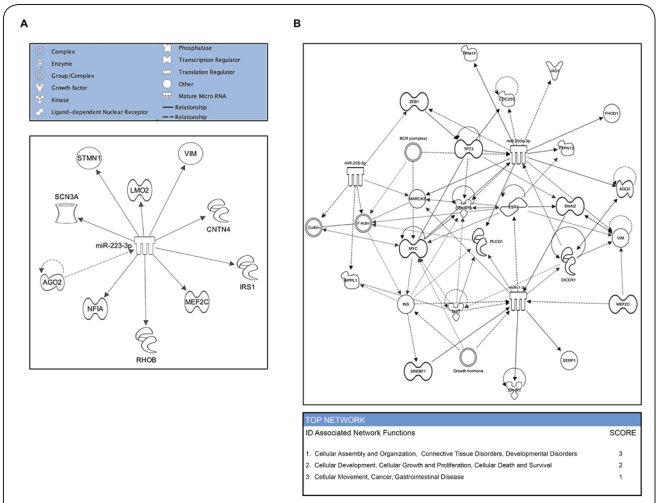

Figure 5. IPA of the haematological- and the epithelial- specific miRNAs. (A) Ingenuity Pathway Analysis of the haematological-specific miR-223-3p shows the existence of nine experimentally observed direct targets. (B) IPA analysis of the epithelial-specific miR-200b-3p, miR-205-5p and miR-1 revealed their involvement in three top molecular networks "*Cellular Assembly and Organization, Connective Tissue Disorders, Developmental Disorder", "Cellular Development, Cellular Growth and Proliferation, Cell death and Survival", "Cellular Movement, Cancer, Gastrointestinal Disease".*

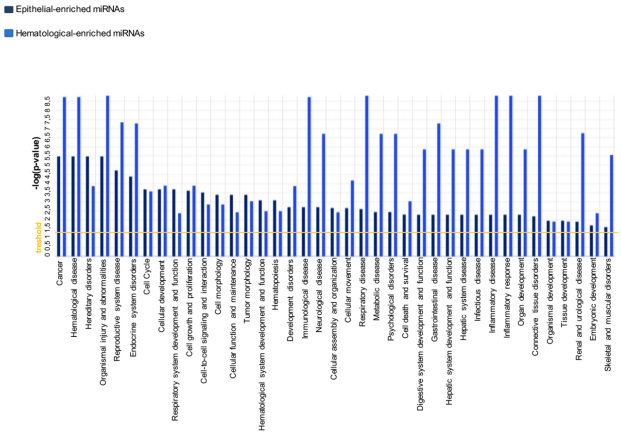

Figure 6. Pathway enrichment analysis of the haematological- and epithelial-enriched miRNAs. The canonical pathways included in this analysis are shown along the x-axis of the bar chart. The y-axis indicates the statistical significance. The yellow threshold line represents the default significance cutoff at p=0.05

"*Chronic Myeloid Leukemia*" stood out among the three pathways involving the highest number of target genes. In HL-60 acute myeloid leukemia cells the specific miRNAs were represented by let-7i, miR-100-5p, miR-155-5p, miR-34a-5p and miR-99a-5p. KEGG analysis, on their target genes, highlighted their implication in 38 pathways. In particular, more than fifty putative target genes impacted on the *RAS*, *MAPK* and *PI3K/AKT* signalling pathways (Table 3).

miRNA signatures distinguish between H460 and MCF-7 cell lines

Finally, from the comparison between the lung H460 and the breast MCF-7 carcinoma-derived cell lines we identified miR-100-5p, miR-10b-5p and miR-155-5p as expressed only in H460 cells while the panel of the MCF-7 specific miRNAs included miR-143-3p and

Table 3. KEGG analysis of genes targeted by miRNAs exclusively expressed in the chronic K562 or in the acute HL-60 leukemia cells.

Exclusively expressed miRNAs	Pathways	No.target genes	p value
HL-60: -let-7i-5p, miR-100-5p, miR-155-5p, miR-34a-5p, miR-99a-5p	Mucintype O-Glycan biosynthesis	8	1,14E-10
	Glioma	19	2,93E-05
	Ras signaling pathway	49	5,46E-05
	Prolactin signaling pathway	22	8,35E-05
	FoxO signaling pathway	36	0,0001737
	Neurotrophin signaling pathway	32	0,0001737
	Colorectal cancer	19	0,0001737
	Signaling pathways regulating of pluripotent stem cells	33	0,0001737
	Melanoma	21	0,0004254
	Pancreatic cancer	19	0,0004963
	Chronic myeloid leukemia	20	0,0008876
	Proteoglycans in cancer	42	0,0010531
	MAPK signaling pathway	55	0,0016506
	Central carbon metabolism in cancer	17	0,0041454
	Non-small cell lung cancer	15	0,0048607
	Pathways in cancer	69	0,0051777
	Prostate cancer	24	0,0051777
	Rap1 signaling pathway	43	0,0053726
	Sphingolipid signaling pathway	25	0,0082498
	T cell receptor signaling pathway	26	0,0082498
	TGF-beta signaling pathway	15	0,0100743
	Estrogen signaling pathway	20	0,0103145
	Renal cell carcinoma	18	0,0103337
	Dorso-ventral axis formation	10	0,0115874
	Endometrial cancer	14	0,0115874
	ErbB signaling pathway	22	0,0140543
	Phosphatidylinositol signaling system	15	0,0218019
	cGMP-PKG signaling pathway	33	0,0241969
	PI3K-Akt signaling pathway	57	0,0254969
	Apoptosis	17	0,0277163
	Glycosaminoglycan biosynthesis - keratansulfate	4	0,0288184
	Adrenergic signaling in cardiomyocytes	31	0,0288184
	Focal adhesion	40	0,0288184
	p53 signaling pathway	17	0,0311321
	Other types of O-glycanbiosynthesis	9	0,0347753
	B cell receptor signaling pathway	17	0,0347753
	Type II diabetes mellitus	13	0,0347753
	Thyroid hormone signaling pathway	24	0,0434189
K562: miR-200a, miR-miR-202, miR-130, miR-143, miR-133a	Colorectal cancer	5	7,54E-07
	Pathways in cancer	11	2,67E-06
	Chronic myeloid leukemia	6	2,76E-05
	Prostate cancer	5	6,32E-05
	MAPK signaling pathway	8	6,01E-04
	HTLV-I infection	8	0,00384
	Neurotrophin signaling pathway	5	0,00494
	Pancreatic cancer	4	0,00522
	Adherens junction	4	0,00636
	VEGF signaling pathway	5	0,00636
	Wnt signaling pathway	4	0,00844
	Progesterone-mediated oocyte maturation	4	0,00844
	TGF-beta signaling pathway	4	0,009633
	GnRH signaling pathway	4	0,0122
	T cell receptor signaling pathway	3	0,0186
	Endometrial cancer	4	0,0190
	Cell cycle	3	0,0231
	Amyotrophic lateral sclerosis (ALS)	3	0,0295
	Glioma	3	0,0306
	Melanoma	5	0,0374
	Tuberculosis	4	0,0416

miR-200a-3p (Table 4). KEGG analysis performed on the target genes of the H460- and MCF-7-specific miRNAs highlighted their involvement in 25 and 14 pathways, respectively. In H460 cells the vast majority of the target genes (about 150) are involved in signalling pathways regulating pluripotency of stem cells, while the MCF-7 specific miRNAs mainly control the Rap1 signalling pathway (Table 4).

Discussion

MicroRNAs play active roles in neoplastic transformation by acting as oncogenes (oncomiRs) or tumor suppressors (3). The present work stems from the observation that aberrant expression profile of miRNAs is actually considered a hallmark of human cancers and that there is a growing interest in analysing their clinical potential as diagnostic biomarkers and innovative therapeutic agents. On the other hand, many facets of the complex miRNA network are still to be elucidated, essentially due to the broad range of genes targeted by each single miRNA (2, 3). Moreover, at least for some miRNAs, it remains to be investigated whether their expression is ubiquitous or associated, in terms of up- or down-regulation, with specific cell types (22). Thus, the identification of miRNA expression patterns and their relative signalling networks, by using human cell lines, might still contribute to cancer classification and to their potential clinical utilization. Here, we profiled the oncomiRNome of four different human cancer cell lines:

Table 4. KEGG analysis of genes targeted by miRNAs exclusively expressed in the lung H460 or in the breast MCF-7 breast cancer cells.

Exclusively expressed miRNAs	Pathways	No.	p value
H460: miR-100-5p, miR-10b-5p, miR-155-5p	Mucintype O-Glycanbiosynthesis	33	4,05E-06
	Proteoglycans in cancer	17	0,000127494
	Prolactin signaling pathway	11	0,00060416
	Glioma	8	0,001876563
	Signaling pathways regulating pluripotency of stem cells	151	0,001876563
	FoxO signaling pathway	15	0,002011015
	Pancreatic cancer	9	0,002011015
	Chronic myeloid leukemia	11	0,002011015
	Non-small cell lung cancer	7	0,002011015
	Colorectal cancer	8	0,002400492
	Ras signaling pathway	17	0,003077376
	TGF-beta signaling pathway	6	0,003210426
	Endometrial cancer	8	0,004571846
	Phosphatidylinositol signaling system	7	0,007152252
	B cell receptor signaling pathway	9	0,008692342
	Cytokine-cytokine receptor interaction	10	0,008692342
	Melanoma	9	0,009553804
	Prostate cancer	11	0,010239142
	Pathways in cancer	26	0,010694288
	Viral carcinogenesis	11	0,016643294
	ErbB signaling pathway	9	0,018865207
	Valine, leucine and isoleucine biosynthesis	1	0,025413762
	Thyroid hormone signaling pathway	9	0,029041142
	Neurotrophin signaling pathway	11	0,037027533
	cAMP signaling pathway	16	0,046533033
MCF-7: miR-143-3p, miR-200a-3p	Hippo signaling pathway	23	9,53E-08
	Glycosphingolipid biosynthesis - lacto and neolacto series	6	3,77E-05
	Proteoglycans in cancer	28	0,000339514
	ErbB signaling pathway	17	0,002335642
	Adherens junction	14	0,005635513
	ECM-receptor interaction	12	0,014671599
	Gap junction	20	0,014671599
	Thyroid hormone signaling pathway	17	0,014671599
	Thyroid cancer	7	0,016573685
	Signaling pathways regulating pluripotency of stem cells	21	0,017992366
	Circadian entrainment	17	0,018583986
	Serotonergic synapse	17	0,018583986
	Rap1 signaling pathway	33	0,038296487
	Long-term depression	11	0,038296487

K562 and HL-60 haematological cells deriving, respectively, from chronic and acute myeloid leukemias, H460 epithelial cell line deriving from non-small cell lung cancer and MCF-7 epithelial cells deriving from the pleural effusion of an invasive breast ductal carcinoma. Next, in order to validate the microRNAs functions as well as their associations and correlations to specific cancer cell types, we used various bioinformatic tools based on free available open genome databases.

This approach provided the framing of the oncomiRNAs in three main categories, as graphically represented in Figure 7.

The 69 miRNAs belonging to the category of the *Commonly expressed miRNAs* show a variable levels of expression among the four cell types, with the notable exception of two broadly highly expressed (UPmiRs: miR-21-5p and miR-16-5p) and four broadly low expressed (DNmiRs: miR-206 and miR-149-3p, miR-133a and miR-202-3p). The specific oncogenic or tumor suppressive role of a given microRNA is correlated to its expression level in cancer (10). In agreement with our findings, the four DNmiRs miR-206, miR-149-3p, miR-133a and miR-202-3p are generally down regulated in many cancer types and are considered tumor suppressors (23-30). Among the UPmiRs, miR-21-5p is already defined as oncogene (31), while the specific role of miR-16-5p is still under debate (32-34). Our data are in line with the few reports describing miR-16-5p as an oncogene (33) and suggest the need of further analyses on its potential dual role in neoplastic transformation.

Next, we identified a group of miRNAs whose combined expression might characterize the phenotype of haematological- *vs* epithelial-derived cells. The two miRNA signatures we propose are composed by *haematological-specific* or *epithelial-specific* miRNAs and by *haematological-enriched* or *epithelial-enriched miRNAs* (Figure 7). The only *haematological-specific* miR-223-3p acts in the fine-tuning of myeloid lineage development and thus it is considered the most discriminatory microRNA between myelod and lymphoid leukemias (35, 36). The majority of the *haematological-enriched* miRNAs belongs to three genomic clusters (miR-17/92, miR-15a/16 and miR-30b/30d); while miR-17/92 and miR-15a/16 have been already reported in haematological malignancies (37, 38), to our knowledge this is one

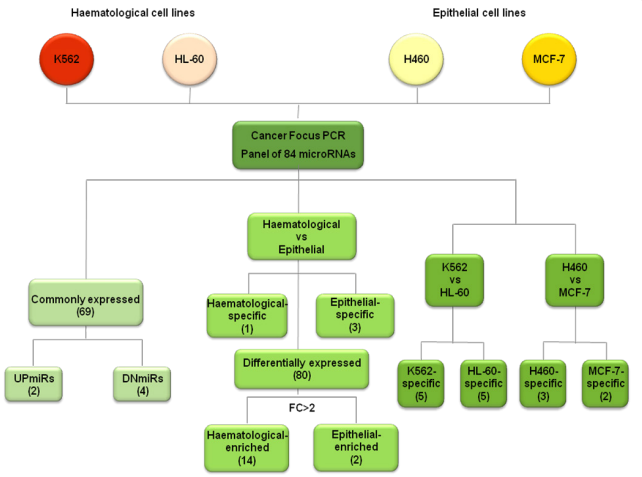

Figure 7. Graphical representation of the main results. Schematic representation of each step of the four oncomiRNoma analyses and their relative main results.

of the first analyses reporting the overexpression of the miR-30b/30d cluster in leukemic cell lines. The three *epithelial-specific* miR-200b-3p, miR-205-5p and miR-1 and the two *epithelial-enriched* miRNAs miR-34-5p and miR-130-3p have been individually described as negative modulators of the epithelial to mesenchymal transition (39-42) but never as members of a specific epithelial signature.

The in silico analysis of the biological pathways potentially targeted by the haematological- and the epithelial- associated miRNAs supported the suitability of their relative attribution to a specific signature. Indeed, pathways more intrinsically related to the haematological (*Haematological* and *Immunological diseases*) or the epithelial (*Cellular movement*) phenotypes have been identified.

The Pathway Enrichment Analysis also highlighted a group of more generic processes and functions related to the development of cells and tissues, cell morphology and cellular functions and maintainance, similarly affected by both the signatures.

Finally, we identified a repertoire of miRNAs able to distinguish the acute from the chronic myeloid leukemia-derived cells as well as the lung cancer from the breast cancer-derived cell lines. The comparison between K562 and HL60 oncomiRNomes led to the identification miRNAs exclusively associated with each of the two cell lines. The K562-signature is composed by miR-200a, miR-miR-202, miR-130, miR-143 and miR-133a, all sporadically reported in this cell line or in chronic myeloid leukemic primary cells (43-46). These miRNAs act as tumor suppressors in solid tumors (47-51). Our results suggest that since they are low expressed also in K562 cells, they might negatively control the leukemogenesis; indeed, KEGG analysis endowed the target genes of these miRNAs in the *Chronic Myeloid Leukemia* pathway. The miRNAs let-7i, miR-100-5p, miR-155-5p, miR-34a-5p and miR-99a-5p constitute the HL-60 signature; of them, let-7i and miR-155 have been previously reported to be strongly down regulated in K562 cells to ensure the overexpression of their erythroid differentiation-related targets (21). The signatures obtained from the comparison of the two epithelial-derived cell lines (H460 vs MCF-7) are mainly composed by miRNAs whose altered expression has been already observed *in vitro*. Notably, for two of them, miR-155 and miR-200a, this observation has been confirmed *in vivo* and these miRNAs are currently used as blood-based biomarkers of lung and breast cancer, respectively (52, 53).

In summary, we suggest that the molecular signatures encompassed by this relatively small number of oncomicroRNAs may reflect the role of these miRNAs in mediating tissue-specific development and/ or tumorigenesis and so contribute in cancer subtyping. However, independent replication of the results reported here using different cancer cell lines would certainly provide further statistical significance.

Acknowledgements

We thank Caterina Alessi for editorial assistance.

This work was supported by Prometeo Grant to GC PON01_02834 and PON03PE_00009_2 ICaRe.

Interest conflict

None

Author's contribution

F.B., F.Z. and AMB: carried out most of the experiments.
G.S.: performed bioinformatic and statistical analysis
F.B., F.Z., FC and GC: planned and supervised most of the experiments; wrote the manuscript and revised the final version.

References

1 Schoof CR, Botelho EL, Izzotti A, Vasques Ldos R. MicroRNAs in cancer treatment and prognosis. AM J Cancer Res 2012; 2: 414-433.
2. Fiorucci G, Chiantore MV, Mangino G, Percario ZA, Affabris E, Romeo G. Cancer regulator microRNA: potential relevance in diagnosis, prognosis and treatment of cancer. Cur Med Chem. 2012; 19: 461-474.
3. Macfarlane LA,Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr. Genomics 2010; 11: 537-561.
4. Shivdasani RA. MicroRNAs. regulators of gene expression and cell differentiation. Blood 2006; 108: 1646-1653.
5. Chen B,Li H, Zeng X, Yang P, Liu X, Zhao X, Liang S. Roles of microRNA on cancer cell metabolism. J Transl Med 2012; 10: 228.
6. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006; 94: 776-780.
7. Magenta A, Greco S, Gaetano C, Martelli F. Oxidative Stress and MicroRNAs in Vascular Diseases. Int J Mol Sci 2013; 14: 17319–17346.
8. Lee YS, Dutta A.MicroRNAs in cancer. Annu Rev Pathol 2009; 4: 199–227.
9. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014; 20:460-469.
10. Shenouda SK, Alahari SK. MicroRNA function in cancer: oncogene or a tumor suppressor ? Cancer Metastasis Rev 2009; 28: 369-378.
11. Zhang B, Pan X, Cobb GP, Anderson TA. MicroRNAs as oncogenes and tumor suppressors. Dev Biol 2007; 302:1-12.
12. Riaz M, TM van Jaarsveld M, Hollestelle A, Prager-van der Smissen W, Heine AAJ, Boersma AWM, Liu J, Helmijr J, Ozturk B, Smid M, Wiemer EA, Foekens JA, Martens JWM. MiRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs. Breast Cancer Res 2013; 15: R33.
13. Zolea F, Biamonte F, Candeloro P, Di Sanzo M, Cozzi A, Di Vito A, Quaresima B, Lobello N, Trecroci F, Di Fabrizio E, Levi S, Cuda G, Costanzo F.H ferritin silencing induces protein misfolding in K562 cells: A Raman analysis.Free Radic Biol Med 2015; 89: 614-623.
14. Biamonte F, Zolea F, Bisognin A, Di Sanzo M, Saccoman C, Scumaci D, Aversa I, Panebianco M, Faniello MC, Bortoluzzi S, Cuda G, Costanzo F. H-Ferritin-Regulated MicroRNAs Modulate Gene Expression in K562 Cells. Plos One 2015; 10:e0122105.
15. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EWT, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA. Roles of the RAF/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 2007; 1773: 1263–1284.
16. Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, Lebeau A, Terracciano L, Al-Kuraya K, Jänicke F, Sauter G, Simon R. Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. Nature Genetics 2007; 39: 655-660.
17. Bernard F, Gelsi-Boyer V, Murati A, Giraudier S, Trouplin V, Adélaïde J, Rey J, Olschwang S, Vainchenker W, Chaffanet M, Vey N, Mozziconacci MJ, Birnbaum D. Alterations of NFIA in chronic malignant myeloid diseases. Leukemia 2009; 23: 583-585.
18. Cleveland SM, Smith S, Tripathi R, Mathias EM, Goodings C, Elliott N, Peng D, El-Rifai W, Yi D, Chen X, Li L, Mullighan C, Downing JR, Love P, Davé UP. Lmo2 induces hematopoietic stem cell-like features in T-cell progenitor cells prior to leukemia. Stem Cells 2013; 31: 882-894.
19. Kent DG, Copley MR, Benz C, Wöhrer S, Dykstra BJ, Ma E, Cheyne J, Zhao Y, Bowie MB, Zhao Y, Gasparetto M, Delaney A, Smith C, Marra M, Eaves CJ. Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. Blood 2009; 113: 6342-6350.
20. Machado-Neto JA, Saad ST, Traina F. Stathmin 1 in normal and malignant hematopoiesis BMB Rep 2014; 47: 660-665.
21. Xiong Q, Yang Y, Wang H, Li J, Wang S, Li Y, Yang Y, Cai K, Ruan X, Yan J, Hu S, Fang X. Characterization of miRNomes in acute and chronic myeloid leukemia cell lines. Genom Proteom Bioin 2014; 12: 79-91.
22. Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, Rheinheimer S, Meder B, Stähler C, Meese E, Keller A. Distribution of miRNA expression across human tissues. Nucleic Acids Res 2016; 44: 3865-3877.
23. Lin F, Yao L, Xiao J, Liu D, Ni Z. MiR-206 functions as a tumor suppressor and directly targets K-Ras in human oral squamous cell carcinoma. Onco Targets Ther. 2014; 7: 1583-1591.
24. Zhang YJ, Xu F, Zhang YJ, Li HB, Han JC, Li L. miR-206 inhibits non small cell lung cancer cell proliferation and invasion by targeting SOX9. Int J Clin Exp Med. 2015; 8: 9107-9113.
25. Ke Y, Zhao W, Xiong J, Cao R. miR-149 Inhibits Non-Small-Cell Lung Cancer Cells EMT by Targeting FOXM1. Biochem Res Int. 2013; 2013: 506731.
26. Bischoff A, Huck B, Keller B, Strotbek M, Schmid S, Boerries M, Busch H, Müller D, Olayioye MA. miR149 functions as a tumor suppressor by controlling breast epithelial cell migration and invasion. Cancer Res. 2014; 74: 5256-5265.
27. Dong Y, Zhao J, Wu CW, Zhang L, Liu X, Kang W, Leung WW, Zhang N, Chan FK, Sung JJ, Ng SS, Yu J. Tumor suppressor functions of miR-133a in colorectal cancer. Mol Cancer Res. 2013; 11: 1051-1060.
28. Gong Y, Ren J, Liu K, Tang LM. Tumor suppressor role of miR-133a in gastric cancer by repressing IGF1R.World J Gastroenterol. 2015; 21: 2949–2958.
29. Zhao Y, Li C, Wang M, Su L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B, Zhu Z. Decrease of miR-202-3p Expression, a Novel Tumor Suppressor, in Gastric Cancer. PLoS One 2013; 8: e69756.
30. Yu J, Qiu X, Shen X, Shi W, Wu X, Gu G, Zhu B, Ju S. miR-202 expression concentration and its clinical significance in the serum of multiple myeloma patients. Ann Clin Biochem 2014; 51: 543-549.
31. Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. J Cell Mol Med 2009; 13: 39-53.
32. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differ 2010; 17: 215-220.
33. Chen D, Li Y, Yu Z, Su Z, Yu W, Li Y, Yang S, Gui Y, Ni L, Lai Y. Upregulated microRNA-16 as an oncogene in renal cell carcinoma. Mol Med Rep 2015; 12: 1399-13404.
34. Zhu Y, Xia Y, Niu H, Chen Y: MiR-16 induced the suppression of cell apoptosis while promote proliferation in esophageal squamous cell carcinoma. Cell Physiol Biochem 2014; 33: 1340-1348.
35. Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD. Regulation of progenitor cell proliferation and granulocyte function by

microRNA-223. Nature 2008; 451: 1125-1129.
36. Mi S, Lu J, Sun M, Li Z, Zhang H, Neilly MB, Wang Y, Qian Z, Jin J, Zhang Y, Bohlander SK, Le Beau MM, Larson RA, Golub TR, Rowley JD, Chen J. MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci U S A. 2007; 104: 19971-19976.
37. Fuziwara CS, Kimura ET. Insights into Regulation of the miR-17-92 Cluster of miRNAs in Cancer. Front Med (Lausanne) 2015; 2: 64.
38. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differ 2010; 17: 215-220.
39. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008; 10: 593-601.
40. Liu YN, Yin JJ, Abou-Kheir W, Hynes PG, Casey OM, Fang L, Yi M, Stephens RM, Seng V, Sheppard-Tillman H, Martin P, Kelly K. MiR-1 and miR-200 inhibit EMT via Slug-dependent and tumorigenesis via Slug-independent mechanisms. Oncogene. 2013;32:296-306.
41. Siemens H, Jackstadt R, Hünten S, Kaller M, Menssen A, Götz U, Hermeking H.miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. Cell Cycle 2011; 10: 4256-4271.
42. Li BL, Lu C, Lu W, Yang TT, Qu J, Hong X, Wan XP. miR-130b is an EMT-related microRNA that targets DICER1 for aggression in endometrial cancer. Med Oncol. 2013; 30: 484.
43. Lu Y, Li Y, Chai X, Kang Q, Zhao P, Xiong J, Wang J.Long noncoding RNA HULC promotes cell proliferation by regulating PI3K/AKT signaling pathway in chronic myeloid leukemia. Gene 2017; 607:41-46.
44. Li Q, Wu Y, Zhang J, Yi T, Li W.MicroRNA-130a regulates cell malignancy by targeting RECK in chronic myeloid leukemia. Am J Transl Res 2016; 8: 955–967.
45. Flamant S, Ritchie W, Guilhot J, Holst J, Bonnet ML, Chomel JC, Guilhot F, Turhan AG, Rasko JEJ. Micro-RNA response to imatinib mesylate in patients with chronic myeloid leukemia. Haematologica 2010; 95: 1325–1333.
46. Undi RB, Kandi R, Gutti RK. MicroRNAs as Haematopoiesis Regulators. Adv Hematol 2013; 2013: 695754.
47. Su J, Zhang A, Shi Z, Ma F, Pu P, Wang T, Zhang J, Kang C, Zhang Q. MicroRNA-200a suppresses the Wnt/β-catenin signaling pathway by interacting with β-catenin. Int J Oncol 2012; 40: 1162-70.
48. Zhao Y, Li C, Wang M, Su L, Qu Y, Li J, Yu B, Yan M, Yu Y, Liu B, Zhu Z. Decrease of miR-202-3p expression, a novel tumor suppressor, in gastric cancer. PLoS One 2013; 8: e69756.
49. Gong Y,Ren J, Liu K, Tang LM. Tumor suppressor role of miR-133a in gastric cancer by repressing IGF1R. World J Gastroenterol 2015; 21: 2949-2958.
50. Paudel D, Zhou W, Ouyang Y, Dong S, Huang Q, Giri R, Wang J, Tong X. MicroRNA-130b functions as a tumor suppressor by regulating RUNX3 in epithelial ovarian cancer. Gene 2016; 586: 48-55.
51. He Z, Yi J, Liu X, Chen J, Han S, Jin L, Chen L, Song H. MiR-143-3p functions as a tumor suppressor by regulating cell proliferation, invasion and epithelial mesenchymal transition by targeting QKI-5 in esophageal squamous cell carcinoma. Mol Cancer. 2016; 15: 51.
52. Tang D, Shen Y, Wang M, Yang R, Wang Z, Sui A, Jiao W, Wang Y. Identification of plasma microRNAs as novel non invasive biomarkers for early detection of lung cancer. Eur J Cancer Prev. 2013; 22: 540-548.
53. Graveel CR, Calderone HM, Westerhuis JJ, Winn ME, Sempere LF. Critical analysis of the potential for microRNA biomarkers in breast cancer management. Breast Cancer (Dove Med Press) 2015; 7: 59-79.