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REVIEW ARTICLE

Functional Role of MicroRNA-23b-3p in Cancer Biology

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Abstract: MicroRNAs (miRNAs) constitute a class of short non coding RNAs that have crucial biological roles by acting mainly as negative regulators of gene expression. The alteration of miRNAs expression has been frequently demonstrated in cancer. Furthermore, miRNAs expression data clearly revealed their possible use as diagnostic, prognostic and predictive biomarkers. In this review, we focus on the biological role of human miR-23b-3p in cancer. Several data demonstrated that miR-23b-3p targeted different genes involved in cancer aggressive properties such as proliferation, migration, invasion, and metastasis. In this context, it is known that miR-23b-3p, as other miRNAs, can target either tumor-suppressor genes or oncogenes in different types of tumors. Therefore, its net biological effect can be tumor-specific, mainly depending on the consequent alterations on the downstream effects of the altered pathways. MiR-23b-3p has been found down-regulated or up-regulated in primary tumors and dysregulated in plasma and serum of cancer patients. Its expression levels correlate with the overall survival, disease-free survival and prognosis in several malignancies, thus assuming a remarkable role as molecular biomarker with clinical relevance. Finally, miR-23b-3p is generally considered a responsive molecular therapeutic target as reported in several *in vitro* and *in vivo* studies. This suggests that the ectopic modulation of its expression may potentially be important for translational medicine approaches.

Keywords: Biomarker, cancer, cell-free miRNA, exosome, microRNA, miR-23b.

1. INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression mainly at post-transcriptional level. To date, 2,694 human mature miRNAs have been identified and registered in miRBase (Release 22, March 2018), the major database of published miRNA sequences and annotation [1]. It is known from computational analysis that more than 60% of protein coding genes contain at least one conserved miRNA-binding site suggesting that their expression is regulated by miRNAs. Because they are key regulators of a large number of genes, miRNAs play an important role both in physiological processes, like differentiation and embryonic development, and in pathological conditions, including cancer onset.

In this review, we focused on miRNA-23b-3p (miR-23b-3p) that has been largely described as an important modulator of several pathways in cellular physiology. MiR-23b (MIMAT0000418) is located on chromosome 9 at position q22.32 and is a member of the intronic miR-23b~27b~24-1 cluster placed in the intron 14 of the host gene C9orf3, an M1 zinc aminopeptidase. The pre-miR-23b generates both mature miR-23b-3p and its complementary strand miR-23b-5p also recently proved to be a functional miRNA [2]. Further, it is necessary to distinguish the miR-23b~27b~24-1 cluster from the miR-23a~27a~24-2 cluster. The latter is located on chromosome 19p13.12 and its coding sequence generates a primary transcript of 2,159 nt in length. Quan *et al.* have reported that miR-23a, -27a and 24-2 act generally as onco-miR in several human tumors. By meta-analysis, they have found high expression levels of miR-23a, miR-27a and miR-24-2 in cancer and their expression levels were associated with worse overall survival and unfavorable prognosis [3]. In the physiological context, miR-23b-3p has been implicated in differentiation of multiple cell lines. For instance, consistently with its ability to reduce cell growth, high level of miR-23b-3p is pivotal during the differentiation of keratinocytes [4], chondrocytes [5], and skeletal muscle [6]. In addition, miR-23b-3p has a relevant role in the fine regulation of cardiovascular system since it promotes angiogenesis [7], reduces proliferation and migration of vascular smooth muscle cells [8] and blocks the cycle progression of endothelial cells [9]. Recently, Wang *et al.* discussed in detail the functional roles of miR-23b-3p in different types of diseases, including virus and parasite infections, autoimmune diseases and thyroid dysfunctions [10].

In addition, as occurring for many miRNAs, miR-23b-3p is strongly associated with cancer. Considering the increas-

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ing amount of data in the literature, an extensive evaluation of its role in cancer is required. Therefore, in this review we aim to highlight the biological functions of human miR-23b-3p in tumor biology focusing on cellular signaling pathways and gene regulatory network. Furthermore, we will explore the role of circulating miR-23b-3p contained in extracellular vesicles, including exosomes, to understand its functions in cell-cell communication. Finally, we will highlight the promising role of miR-23b-3p as cancer-related diagnostic and prognostic biomarker by analyzing its expression both at tissue and at circulating levels.

2. THE DUAL ROLE OF MIR-23B-3P IN CANCER

In this part of the review, we will describe the biological mechanisms through which miR-23b-3p is involved in carcinogenesis as revealed by insightful publications on this topic. The majority of evidences have shown that miR-23b-3p impaired tumor cell aggressive properties by targeting oncogenes and thus it may act as a tumor suppressor miRNA (ts-miR). However, other data have shown that miR-23b-3p also negatively regulated tumor suppressor proteins suggesting a possible oncogenic function (onco-miR) for this miRNA. The algorithm TargetScan (Release 7.1, June 2016) predicted a total of 1,332 transcripts as miR-23b-3p targets in human [11]. Here, we considered the validated targets of miR-23b-3p and their functions in order to comprehend and summarize the dual role of miR-23b-3p in cancer biology as either a tumor suppressor or onco-miR. Table **1** shows the protein coding genes targeted by miR-23b-3p that have been experimentally validated using standard approaches (including reporter gene assay, qPCR, and western blotting) in different human cell lines. By considering all the evidence available, miR-23b-3p has been described as a pleiotropic modulator in different tumors and its fundamental function in cancer development and metastasis has been explored in depth. For the dual role of miR-23b-3p in cancer, it is known that also other miRs can target either tumor-suppressor genes or oncogenes in different types of cancer. In this context, it is common opinion that the net biological effect of a miR can be tumor specific, mainly depending on the consequent alterations on the downstream effects of the altered pathways [12-40].

2.1. MiR-23b-3p Generally Impairs Cancer Cell Proliferation

Cell cycle dysregulation is a common feature in human cancer cells that frequently display uncontrolled proliferation, increased DNA mutations and chromosomal aberrations [41]. In this context, miRNAs may either potentiate or limit the expression of cell cycle proteins and contribute to this regulatory network in cell cycle. Regarding miR-23b-3p, it was found to negatively regulate cyclin H in endothelial cells. In detail, under the pulsatile shear flow, miR-23b-3p expression was increased and this impaired the activity of cyclin-dependent kinase-activating kinase (CAK) complex through the repression of cyclin H. This inhibitory effect on CAK suppressed cell cycle progression and reduced basal transcription by deactivating RNA polymerase II [9]. miR-23b-3p has a role in the control of cell cycle progression also in cancer. In ovarian cancer, miR-23b-3p has been identified as a negative regulator of cyclin G1 and its over-expression was able to inhibit tumor growth *in vitro* and in human ovarian cancer xenograft models [19]. In recent years, novel insights gained in the field of cancer research supported the importance of cross talk between cell cycle and metabolism. Since cancer cells have uncontrolled cellular growth and proliferation, they require high amount of energy to drive cell cycle by modifying their metabolic activity. For this reason, cancer cells have a sustained mitochondrial activity that derived mainly from glutaminolysis, the catabolism of glutamine to generate ATP and lactate. In prostate cancer cells, miR-23b-3p, which is silenced by c-Myc, directly targeted mitochondrial glutaminase resulting in the increase of glutamine catabolism and with consequent gain of energy and homeostasis of reactive oxygen species [22].

Several studies have demonstrated that some miR-23b-3p targets are part of important pathways involved in regulating cell growth in a variety of different tumors. Among these, the oncogene MET (a tyrosine kinase receptor) is overexpressed in several types of cancer and activates some signal pathways, including PI3K-AKT, RAS-MAPK, and STAT, sustaining cell survival, migration, and transformation [42, 43]. miR-23b-3p is a negative regulator of MET in different cancer cells, including cell lines of hepatocellular carcinoma (HCC) [25], oral squamous cell cancer [26], bladder cancer [27], and cervical cancer [28].

Fulciniti *et al.* reported that, in multiple myeloma cells, miR-23b-3p targeted Sp1, a zinc finger transcription factor that binds to GC-rich motifs of many promoters and triggers the nuclear factor-κB (NF-κB) pathway. In these cells, the down-regulation of miR-23b-3p determined the activation of Sp1 and NF-κB and sustained cell growth. Both lentivirus- or *mimics* molecules-mediated over-expression indicated that miR-23b-3p suppressed cancer cell proliferation and survival both *in vitro* and *in vivo* [44]. Finally, an interesting study performed in prostate cancer revealed that: i) Src kinase and Akt were direct targets of miR-23b-3p; ii) miR-23b was frequently silenced through DNA methylation in cancer tissues and cells; iii) miR-23b-3p decreased proliferation, colony formation, migration, and induced G_0-G_1 cell-cycle arrest and apoptosis [13]. Moreover, the same authors identified the oncogene Zeb1 as a target for miR-23b-3p in bladder cancer cells and the comparable biological effects of this miR on proliferation, cell cycle and apoptosis were also reported [40]. In conclusion, miR-23b-3p has a key role in controlling the proliferation rate of several tumor cells and it is a responsive molecular target able to impair cell proliferation.

2.2. MiR-23b-3p Plays a Key Role in Cell Migration, Invasion and Cancer Metastasis

Metastasis formation represents the main cause of mortality in cancer patients. It is a highly coordinated process in which cancer cells acquire the abilities to locally invade, penetrate and survive into circulation, and, after the extravasation, to establish in a different tissue to produce a secondary tumor. Important evidence has highlighted the roles of miRNAs in these complex processes. Interestingly, miR-23b-3p has been defined as an "anti-metastatic human miRNA" by Zhang *et al.* These authors demonstrated that miR-23b-3p directly regulated a group of prometastatic genes in colon cancer cells including FZD7, MAP3K1, PAK2,

Table 1. Summary of human genes targeted by miR-23b-3p in different cancer cell lines.

Target Genes	Molecular Functions	Type of Cancer	Cell Lines	Validation Method	Ref.
AKT	Expression of pro-survival genes, cell cycle progression, migration, inhibition of apoptosis	Prostate cancer	PC3, DU145	RA, WB	[13]
ALDH1	Alcohol metabolism	Cervical cancer	Hela, CaSki	RA, WB	[14]
ANXA2	Regulation of cytoskeletal remodeling	Breast cancer	MCF-7, MDA-MB-23	RNA-seq, qPCR, RA	[15]
ARHGEF6	Regulation of cytoskeletal remodeling	Breast cancer	MCF-7, MDA-MB-23	RNA-seq, qPCR, RA	[15]
ATG12	Autophagy	Gastric cancer Pancreatic cancer	SGC7901 BxPC3, PANC-1	RA, WB	[16, 17]
PRDM1	Transcriptional factor, its functions are not understood	Breast cancer	MCF10	RA, WB	[18]
CFL2	Regulation of cytoskeletal remodeling	Breast cancer	MCF-7, MDA-MB-23	RNA-seq, qPCR, RA	[15]
CCNG1	Cell cycle progression	Ovarian cancer	OVCAR3, HO8910-PM, SKOV3	RA, qPCR, WB	[19]
ETS1	Stem cell development, cell senescence and death	Gastric cancer	SC-M1, AZ521, NUGC-3	RA, WB	[20]
FZD7	Receptor for WNT proteins	Colon cancer	HCT 116	RA, WB	[21]
GLS	Catabolism of glutamine	Prostate cancer	PC-3	RA, WB	[22]
HMGA2	Transcription, chromatin remodeling	Pituitary adenoma	HEK293	qPCR, WB, RA	[23]
HMGB2	Transcription, chromatin remodeling	Gastric cancer	SGC7901	RA, WB	[16]
LIMK2	Reorganization of the actin cytoskeleton	Breast cancer	MCF-7, MDA-MB-23	RNA-seq, qPCR, RA	[15]
MAP3K1	Transduction of growth signals	Colon cancer	HCT 116	RA, WB	[21]
MARCKS	Cell motility, phagocytosis, membrane trafficking	Breast cancer	BM2, HEK293	qPCR, WB, RA	[24]
MET	Proliferation, scattering, morphogenesis, survival	HCC Oral SC cancer Bladder cancer Cervical cancer	SkHep1C3 SAS, HSC3 BOY, T24 SiHa	RT-PCR, WB, RA qPCR, WB, RA WB, RA qPCR, RA	[25] [26] [27] [28]
NISCH	Cytoskeletal organization, cell migration	Breast cancer	MDA-MB-231	RA, WB	[29]
NOTCH2	Cell-fate determination, differentiation, proliferation	Gastric cancer	SC-M1, AZ521, NUGC-3	RA, WB	[20]
PAK2	Regulation of cytoskeletal remodeling	Breast cancer Colon cancer	MCF-7, MDA-MB-23 HCT 116	RNA-seq, qPCR, RA RA, WB	[15, 21]
PDCD4	Apoptosis	Gastric cancer	MNK-45, AGS	RA, WB	[30]
PLAU	Cell invasion, migration, proliferation and cancer metastasis	HCC Cervical cancer Breast cancer Colon cancer	SKHep1C3 SiHa MCF-7, MDA-MB-23 HCT 116	RT-PCR, WB, Zymo qPCR, WB, RA RNA-seq, qPCR, RA RA, WB	[25] [28] [15] [21]
PRDX3	Proliferation, differentiation, antioxidant functions	Acute myeloid leukemia	HEK293	WB, RA	[31]
PTEN	Cell cycle progression, cell survival, migration, invasion	Prostate cancer Renal cancer	DU145 A-498	WB, RA WB, RA	[32, 33]

(Table 1) contd….

Target Genes	Molecular Functions	Type of Cancer	Cell Lines	Validation Method	Ref.
Pyk2	Regulation of cytoskeletal remodeling	Glioblastoma HCC	T98G, U87 MHCC97L, HCCLM3	WB WB, RA	[34, 35]
PIK3R3	Regulation of cytoskeletal remodeling	Breast cancer	MCF-7, MDA-MB-23	RNA-seq, qPCR, RA	[15]
RRAS2	Transduction of growth signals	Colon cancer	HCT 116	RA, WB	[21]
RUNX2	Osteoblastic differentiation and skeletal morphogenesis	Epithelial ovarian cancer	SKOV3, OVCAR3	WB, RA	[36]
SP1	Cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, chromatin remodeling	Multiple myeloma	NCI-H929, Primary MM cells	RA, WB	[37]
SRC	Gene transcription, immune response, cell adhesion, cell cycle progression, apoptosis, migration, transformation	Prostate cancer	PC3, DU145	RA, WB	[13]
TGFβR2	Proliferation, differentiation, ECM production	Colon cancer	HCT 116	RA, WB	[21]
VCAN	Cell adhesion, proliferation, migration, angiogenesis, tissue morphogenesis and maintenance	Tongue SC carcinoma	SCC15	RA, WB	[38]
VHL	Ubiquitination and degradation of hypoxia-inducible factor (HIF)	Glioma	U87, LN229	RA, WB	[39]
ZEB1	Transcription factor	Bladder cancer	J82, T24	RA, WB	[40]

RA: reporter gene assay; WB: western blot; Zymo: zymography; SC: squamous cell; MM: multiple myeloma;
ECM: extracellular matrix.

TGFβR2, and RRAS2 involved in critical pathways, such as the JNK, NF-κB, ERK, and PI3k pathways. Furthermore, they reported the ability of this miR to repress cell migration, invasion and angiogenesis, by indirectly suppressing VEGF both *in vitro* and *in vivo* [21].

Among other miR-23b-3p target genes validated in different cancer types, urokinase-type plasminogen activator (uPA) plays a key role in migration of cancer cells [45, 46]. uPA is a serine protease that converts plasminogen into the serine protease plasmin, thus making cancer cells able to degrade basal membrane and extra-cellular matrix. Moreover, uPA interacts with its receptor, uPAR, inducing the activation of intracellular pathways affecting cell proliferation and migration abilities. The overexpression of uPA has been reported in various types of tumor, both at mRNA and protein level suggesting a role as unfavorable prognostic factor in certain types of cancer, including HCC and breast cancer [47, 48]. Salvi *et al.* demonstrated that miR-23b-3p acted as a negative regulator of uPA in HCC cell lines and the high expression of miR-23b-3p by mimics transfection strongly inhibited the expression of uPA and decreased cell migration and proliferation [25]. The role of miR-23b as negative regulator of uPA and its effect on cell migration has also been confirmed in cervical cancer in which the expression of miR-23b is strongly silenced by E6 oncoprotein produced by human papillomavirus subtype -16 (HPV-16) [49].

Several data suggested a relevant function of miR-23b-3p in Epithelial Mesenchymal Transition (EMT). During EMT, downregulation of adhesion molecules, such as E-cadherin, and over-expression of mesenchymal markers, such as N-cadherin, impair epithelial cell-cell adhesion and induce invasion and migration. Pellegrino *et al.* have examined in depth the role of this miR in cytoskeletal remodeling and motility in breast cancer cells. Principally, they demonstrated that the over-expression of miR-23b-3p increased cell-cell adhesion, cell spreading, and focal adhesion formation therefore reducing migration and invasion. Using RNA-seq followed by validation with reporter assays, they further identified a set of genes implicated in cytoskeletal remodeling as direct targets of miR-23b-3p, including ANXA2, LIMK2, and CFL2 [15]. Consistent with these data, Sciortino *et al.* showed that miR-23b-3p directly targeted the transcriptional repressor Blimp1 whose function was not fully understood in solid tumors. By using a three-dimensional model generating structures called acini that reproduce the architecture of the ductal lobular unit in mammary gland, they demonstrated that: i) the development of invasive acini was sustained by the activation of p130Cas/ErbB2 that induced Blimp1 overexpression; ii) Blimp1 promoted cell invasion by altering focal adhesion dynamics; iii) miR-23b-3p was down-regulated in invasive acini and the ectopic expression of this miR led to lower invasive and migratory capacity by silencing Blimp1 [18].

In another study, using *in vitro* radial migration assay that allowed obtaining migration restricted cells from the core of cells clump and migrating cells from the rim, miR-23b-3p was found down-regulated in glioma migrating cells. Among the predicted targets of miR-23b-3p, the Proline-rich tyrosin kinase 2 (Pyk2) was identified and validated in this context [35]. Pyk2 is a non-receptor tyrosine kinase that be-

longs to the focal adhesion kinase family and it has been associated with the regulation of gene promoting EMT [50]. In migrating cells, the down-regulation of miR-23b-3p determined the over-expression of Pyk-2 promoting the capacity of cell motility in glioma. Also in HCC, it has been demonstrated that miR-23b-3p negatively regulated Pyk2 and the transfection of miR-23b-3p in HCC cells decreased migration and invasion, and promoted E-cadherin expression together with the expression decrease of mesenchymal markers, including N-cadherin, Vimentin, Slug, Snail, MMP-2, and MMP-9 [34].

2.3. MiR-23b-3p Alters the Stemness in Cancer Cells

In the last decade, significant results have shown that the main characteristics of stem-cell (self-renewal, developing into multiple lineages, and high proliferation rate) are relevant to some forms of human cancer. The results obtained from these studies allowed the identification of a relatively small population of stem cells inhabiting adult tissue called as the Cancer Stem Cells (CSCs) [51]. Their subsequent characterization in different solid tumors defined certain properties of these cells, including deregulated self-renewal pathways, extensive DNA-repair mechanisms, abnormal cellular metabolism, and acquisition of EMT. Nowadays, it is well known that CSCs play a crucial role in initiating cancer and constitute the source of drug resistant cells to conventional chemotherapeutic agents resulting in the tumor recurrence [52]. In the context of CSCs, Wang *et al.* showed that miR-23b-3p was down-regulated in sphere-forming cells derived from cervical cancer cells (these are considered CSCs since they expressed stem cell markers). The authors reported also that miR-23b-3p directly regulated the expression of Aldehyde Dehydrogenase 1 family member (ALDH1), an intracellular enzyme with a detoxifying role that has been defined as a CSCs marker. Interestingly, miR-23b-3p over-expression in CSCs reduced the size and the number of tumor spheres [14]. Similarly, miR-23b-3p negatively regulated Notch2 and Ets1 and it was found down-regulated in gastric cancer cells. In these cells, miR-23b-3p reduced tumor spheres formation, suppressed pluripotency genes expression and affected the tumor spheres microvilli-like ultra-structure on surface of gastric cancer cells by targeting Notch2 and Ets1 [20]. These data support the hypothesis that cancer stem cell-like phenotype is characterized by low level of miR-23b-3p suggesting that altered levels of this miR could modify the stemness of cervical and gastric cancer. However, it was reported that miR-23b-3p was up-regulated in $ALDH^+$ stem cells from ovarian and colorectal cancer cell lines [53, 54]. In colorectal cancer cells, miR-23b-3p promoted colony and sphere formation and EMT affecting CSC phenotype. Furthermore, miR-23b-3p increased the number of $ALDH^+$ CSCs by targeting LGR5 and this event contributed to maintain the stemness of colonic stem cells. Taken together, these results strongly suggest a potential role of miR-23b-3p in the regulation of stem cell phenotype but with different functions depending on cancer type. Further studies and *in vivo* experimentation are of paramount importance to explore miR-23b-3p mechanisms of action in inducing or repressing stemness of cancer cells.

2.4. MiR-23b-3p is Involved in Cancer Therapy Resistance

Resistance to chemotherapy and radiation is a major problem in limiting the effectiveness of current cancer therapies. Although some biological mechanisms, including mutations of drug target, DNA damage repair, and autophagy, play a role in the acquisition of resistance, the detailed mechanisms by which this process is regulated remain generally undefined. In this context, increasing evidences indicated miRNAs as key regulators of chemo- and radiosensitivity by targeting cancer-related genes. Important results indicated the role of miR-23b-3p in autophagy, an intracellular lysosome-mediated degradation process likely involved in resistance to cancer therapy. Firstly, Wang *et al.* identified the miR-23b-3p target ATG12, an important factor in autophagy vacuole formation. Moreover, the authors observed that radioresistant pancreatic cancer cells (BxPC-3 and PANC1) were characterized by reduced levels of miR-23b-3p and increased autophagy compared with sensible cells. The over-expression of miR-23b-3p inhibited radiation-induced autophagy and sensitized cells to radiation *via* targeting ATG12 [17]. Similarly, An *et al.* showed that the reduced level of miR-23b-3p was associated with chemoresistance in cells and tissues of gastric cancer (GC) and its over-expression reversed the drug resistance both *in vitro* and *in vivo* by reducing the level of ATG12 and HMGB2 expression and consequently inhibiting autophagy [16]. Consistent with these results, a recent study reported that the decrease of miR-23b-3p in chemoresistant GC cells was mediated by the sponge effect of the long noncoding RNA MALAT1. MALAT1 was shown to be over-expressed in GC tissues from chemoresistant patients, competitively able to bind miR-23b-3p. The sequestration of this miR induced the upregulation of its target ATG12 and the consequent activation of autophagy which, in turn, conferred resistance [55]. Salvi *et al.* showed that the combination of miR-23b-3p mimics and sorafenib had additional effects on apoptosis induction in HCC cells suggesting that miR-23b-3p may also play an important role in promoting the sensitivity to sorafenib [56], the only innovative drug used for advanced HCC.

Despite these reliable results, the role of miR-23b-3p is not consistent among different types of cancer. For instance, miR-23b-3p was found significantly upregulated in chemoresistant cancer cells and in tumor samples from resistant patients in endometrial carcinoma and in ovarian cancer [53, 57]. However, the lack of data on the mechanisms of action of miR-23b-3p in these two malignancies requires additional functional studies to define the role of miR-23b-3p in the control of resistance to cancer therapy.

2.5. MiR-23b-3p Functions as an Onco-miR in Different Cancers

Despite the role of miR-23b-3p as ts-miR has been extensively proven, more recent data have demonstrated that it can also act as onco-miR. Data obtained from Chen *et al.* clearly indicated the tumor suppressor VHL as a direct target of miR-23b-3p which resulted up-regulated in glioma samples respect to normal brain tissues and high-grade glioma cells. Interestingly, *in vitro* transfection experiments of anti-miR molecules showed that the down-regulation of miR-23b-3p

suppressed proliferation and invasion and induced apoptosis through targeting VHL which resulted in the inhibition of β-catenin/Tcf-4 and HIF-1α/VEGF pathways [39]. In the same clinical context, the reciprocal regulation and the direct combination between miR-23b-3p and the long non-coding RNA TUSC7 have been reported. It has been demonstrated that miR-23b-3p negatively regulated TUSC7 which acted as a tumor-suppressor gene in glioma cells [58]. In line with the role of miR-23b-3p as onco-miR in glioma, it was further demonstrated that the knockdown of miR-23b-3p mediated by stable lentivirus expression of anti-miRNA reduced angiogenesis, migration and invasion *in vitro* and in nude mouse tumor intracranial model [59].

Recently, Hu *et al.* introduced the hypothesis that miR-23b-3p functions as anti-apoptotic factor in gastric cancer cells by directly targeting Programmed cell death protein 4 (PDCD4), an apoptosis regulatory protein. The authors showed the ability of miR-23b-3p to suppress apoptosis (evaluated as cleaved-CASPs and PARP) by silencing PDCD4 both *in vitro* and in a xenograft mouse model of gastric cancer [30]. Consistent with these findings, miR-23b-3p was found up-regulated in radiation induced thymic lymphoma tissues in a BALB/c mouse model compared to the normal non-irradiated thymus tissue samples. Interestingly, mmu-miR-23b-3p negatively regulated the pro-apoptotic factor Fas and decreased apoptosis in murine thymic lymphoma cells [60].

In breast cancer cells, miR-23b-3p negatively regulates Nischarin, an intracellular protein that acts as tumor suppressor by regulating the metastatic behavior of tumor cells. The silencing of miR-23b-3p strongly inhibited proliferation, anchorage-independent growth, migration, and invasion in highly metastatic 4175 human breast cancer cell line stably expressing anti-miR-23b/27b constructs. The suppression of miR-23b-3p further reduced tumor growth and metastasis formation *in vivo* [29]. Consistent with these results, Ell *et al.* showed that the over-expression of the entire miR-23b cluster increased lung metastasis in a mouse model of breast cancer metastasis [61]. Finally, it has been demonstrated that miR-23b-3p directly targeted PTEN in renal cancer A-498 cells [33], in normal prostate epithelial PNT1B cell line and prostate cancer DU145 cells [32]. Inhibition of miR-23b-3p by anti-miR molecules transfection induced PTEN expression and the concomitant decrease of total Akt in these cell lines. PTEN plays a crucial role as tumor suppressor and it is down-regulated in the majority of cancers. Further investigations in several cell lines and in *in vivo* models may be necessary to highlight the oncogenic activity of miR-23b-3p by targeting important ts-genes decisive for the cancer progression.

3. THE BIOLOGICAL RELEVANCE OF CIRCULATING EXOSOME MIR-23B-3P IN CANCER CELL LINES

Since 2008, miRNAs have been detected in extracellular environment, including different biological fluids and cell culture media. These miRNAs commonly known as circulating miRNAs are very stable under extreme conditions, like ribonuclease digestion, and can be easily detected and quantified by widespread techniques such as RT-qPCR. The origin of circulating miRNAs whether from tumor cell death and lyses or from active secretion by tumor cells is still largely undefined. However, it has been recently observed that mature miRNAs can be coupled with Ago2 protein and subsequently released into extracellular milieu or be selectively incorporated into vesicles and later released in circulation. Extracellular Vesicles (EVs) are secreted from both cancer cells and non-cancer cells and they have a function in the intercellular communications relevant to several physiopathological processes such as angiogenesis, tissue repair, and apoptosis. Among EVs, exosomes are small vesicles (50–150 nm) able to transport and deliver proteins, mRNAs, and ncRNAs including miRs from a donor to recipient cells. Based on these evidences, it has been hypothesized that the extracellular miRNAs (together with other RNAs) may mediate cell-cell signaling *via* paracrine or even endocrine signaling, and may impact on several physiological and pathological processes [62]. Consistent with these findings, Hannafon *et al.* observed the increase of miRNAs level, including miR-23b-3p, in the exosomes released from breast cancer cells treated with Docosahexaenoic acid (DHA), a natural compound contained in fish oil with anti-cancer activity. Interestingly, by co-culturing endothelial cells with DHA-treated breast cancer cells the authors reported that exosomal miR-23b-3p and miR-320b were transferred to endothelial cells in which they repressed the expression of pro-angiogenesis target genes, including uPA, and inhibited tube formation (that was quantified by branch-point count) [63].

Ono *et al.* identified miR-23b-3p also in the exosomes secreted by bone marrow mesenchymal stem cell (BM-MSCs) and investigated the mechanism enabling the dormant state of human metastatic breast cancer stem cells in mouse BM niche. In particular, they generated a bone marrow-metastatic human breast cancer cell line (BM2) by tracking and isolating fluorescent-labeled human MDA-MB-231 cells that disseminated to the mouse BM. By co-culturing BM2 cells with BM-MSCs isolated from human donors, they showed that BM-MSCs-derived exosomes promoted the dormant phenotype in BM2 cells which was characterized by the decrease of stem cell-like surface markers (CD44), proliferation and invasion ability. Importantly, these effects were attributed to the transfer of miRNAs, including miR-23b-3p, from BM-MSCs to breast CSCs through exosomes. Indeed, exosomal miR-23b-3p targeted MARCKS, which promotes cell cycling and motility, and thus representing one of the many mechanisms leading to dormancy and impaired CD44 surface abundance in breast cancer cells [24]. Another important study revealed the presence of miR-23b-3p in exosomes secreted from urothelial carcinoma cell lines with high metastatic capacity (FL3 and SLT4 cells) but not in the ones released from nonmetastatic cells. In addition, the silencing of the exocytotic RAB family members RAB27A and RAB27B attenuated miR-23b-3p exocytosis and led to increased miR-23b-3p activity in FL3 cells. These data seem to suggest that exosome-mediated secretion of ts-miRNAs like miR-23b-3p represented a mechanism to coordinate the activation of metastatic properties during tumor progression [64]. In conclusion, exosomal miR-23b-3p may be released from cancer cells or cancer microenvironment cells resulting in changes of intercellular communication. Additional investigations may further support these conclu-

Table 2. Schematic overview of down- and up-regulation of miR-23b-3p with possible diagnostic role in different primary cancers and relative validated targets.

miR-23b-3p Expression Level in Primary Tumors	Type of Cancer	Validated miR-23b-3p Targets	References
Down-regulation	Colon cancer	*FZD7, MAP3K1, PAK2, TGFβR2, RRAS2*	[21]
	Bladder cancer	*Zeb1, MET*	[27, 40]
	Prostate cancer	*Src, Akt, PRDX3*	[13, 66, 67, 72]
	Hepatocellular carcinoma	*uPA, MET, Pyk2*	[25, 34, 69]
	Epithelial ovarian cancer	*CCNG1, RUNX2*	[19, 36]
	Clear cell renal cell carcinoma (*)	-	[65]
	Lymph node metastases from breast cancer (*)	*PAK2, ANXA2, ARGHGEF6, CFL2, LIMK2, PIK3R3, uPA, PRDM1*	[15, 18]
Up-regulation	Glioma	*VHL,* TUSC7	[39, 58]
	Gastric cancer	*PDCD4*	[30, 70]
	Non-small cell lung cancer	-	[71]
	Breast cancer	*NISCH*	[29]
	Clear cell renal cell carcinoma (*)	*PTEN*	[33]
	Lung metastases from breast cancer (*)	-	[61]

(*) Human tumors with discordant miR-23b-3p expression levels are indicated.

sions and explore additional biological functions of exosomal miR-23b-3p in different cancer types.

4. ROLE OF MIR-23B-3P AS DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN CANCER

Important evidence has demonstrated that the aberrant miRNAs expression in cancer contributes to recognize the role of miRNAs in cancer-associated pathways. In light of this, the analysis of the expression of a certain miRNA could reveal its clinical significance and could be useful in the identification of diagnostic and/or prognostic biomarkers. Regarding miR-23b-3p, important studies showed its dysregulation (down- or up-regulation) both in primary tumors both in liquid biopsies (plasma or serum) from cancer patients. In Table **2**, we reported an overview of the functions of miR-23b-3p acting as a ts-miR or an onco-miR on the basis of validated targets, and the relative level of expression in different primary cancers. In order to clarify the potential clinical role of this miRNA, we summarized the most relevant data indicating the diagnostic and prognostic value of miR-23b-3p expression in cancer.

4.1. Down-Regulation of miR-23b-3p in Cancer Tissues

Consistent with the role of ts-miR, miR-23b-3p was found down-regulated in several primary cancers including colon cancer [21], bladder cancer [40], and clear cell renal cell carcinoma [65]. In prostate cancer, the low expression of miR-23b-3p in tissues obtained from cancer patients was reported by different studies [66-68]. Among these, Majid *et al*. have shown that the miR-23b-3p expression may discriminate between normal and tumor samples using ROC analysis. Moreover, high level of miR-23b-3p was positively correlated with high overall survival (OS) and recurrence-free survival (RFS) of patients according to the Kaplan-Meier survival analysis [13]. In addition, the down-regulation of miR-23b-3p in epithelial ovarian cancer correlated with tumor aggressiveness (low clinical stages, I-II), short OS and progression-free survival of patients pointing to the possible prognostic role of miR-23b-3p in this type of cancer [36]. Grossi *et al.* found that the downregulation of miR-23b-3p in primary HCCs was related to DNA methylation and ROC analysis indicated a good ability in discriminating HCCs from peri-tumoral (PT) counterparts [69]. Finally, the down-regulation of miR-23b-3p was reported in lymph node metastases in a large cohort of breast cancer patients where primary tumors and matching metastases were analyzed [15].

4.2. Over-Expression of miR-23b-3p in Cancer Tissues

As mentioned previously, miR-23b-3p may act as an onco-miR and thus support cancer development. Accordingly, miR-23b-3p has been found up-regulated in cancer tissues compared to normal counterparts in glioma and gastric cancer. By considering qPCR results, miR-23b-3p was highly expressed in high-grade glioma compared to low-grade glioma and normal tissues [39]. In a cohort of 160 patients with gastric cancer and matched normal mucosa, the expression of miR-23b-3p was found dramatically increased in cancer samples and positively correlated with the expression level of miR-23a. Interestingly, the high level of miR-23a and miR-23b-3p (indicated as miR-23a/b) in gastric cancer tissues was significantly associated with the advanced TNM

stage, the presence of lymph node metastasis, and higher degree of invasion. Kaplan-Meier survival analysis indicated that patients with high miR-23a/b levels had a shorter OS respect to patients with low miR-23a/b levels [70].

Interestingly, Begum *et al*. conducted an integrated genetic approach based on microRNAs expression array and qPCR combined with SNP array in order to identify miRNAs that were dysregulated and positioned in allelic imbalance area in non-small cell lung cancer (NSCLC). High levels of miR-23b-3p were found by qPCR in an independent cohort of 114 primary NSCLCs and a low level allelic imbalance for miR-23b locus in tumors was detected by the integrated SNPs data. These authors hypothesized that the up-regulation of miR-23b-3p in NSCLCs was partially due to allelic amplification. Finally, they demonstrated that the over-expression of miR-23b-3p was significantly associated with poor RFS and OS of cancer patients. These data strongly supported the potential role of this miR as an unfavorable prognostic factor in NSCLC [71].

Regarding the expression level of miR-23b-3p in breast cancer, Jin *et al*. showed that the expression level of miR-23b-3p was higher in breast cancer tissues than in normal tissues [72]. By examining breast cancer gene expression datasets deposited in the public functional genomics data repository Gene Expression Omnibus, they found that patients with elevated miR-23b and miR-27b expression had significant shorter RFS times compared to patients with low expression levels [29]. In an additional report, miR-23b-3p was found up-regulated in lung metastatic tissues derived from breast cancer lesions respect to primary tumors [61] making these data incongruent with those previously reporting a down-regulation of miR-23b-3p in lymph node metastases from breast cancer. These discrepancies may be due to the different mechanisms that malignant cell activates when establishes in a certain tissue to generate metastasis, including alteration of miRNAs expression. However, the small sample size is a limitation of these studies and novel investigations involving independent cohorts with a higher number of subjects are needed to confirm these preliminary findings.

4.3. Dysregulation of Circulating miR-23b-3p in Cancer Patients

A large number of studies have shown the clinical relevance of circulating miRNAs, including cell-free miRNAs coupled with Ago2 protein and exosomal miRNAs. Indeed, miRNAs expression profiling studies, using qPCR, microarrays or RNA-seq, have clearly evidenced the differential levels of certain miRNAs in liquid biopsies from cancer patients and their correlation with diagnosis, prognosis and, response to anti-cancer treatment. For these reasons, circulating miRNAs in cancer patients represents promising non-invasive biomarker candidates.

Regarding circulating miR-23b-3p, qPCR experiments were performed to detect the plasmatic level of this miR in a cohort of 138 patients with gastric cancer and 50 healthy subjects. MiR-23b-3p was found to be significantly up-regulated in plasma samples from cancer patients compared to controls. In addition, plasmatic miR-23b-3p levels were associated with T-stage, distant metastasis, and differentiation. Finally, gastric cancer patients with high plasmatic miR levels showed lower DFS and OS than those with low plasmatic levels [73]. On the opposite, miR-23b-3p was significantly decreased in plasma samples from 96 patients with colorectal cancer compared to 48 healthy controls. In this cancer, low plasmatic miR-23b-3p levels were significantly associated with clinicopathological variables (TNM stage, tumor depth, metastasis, and recurrence) and related to poorer OS and RFS of cancer patients [74]. In the same clinical context, Ostenfeld *et al.* developed a method for the isolation of epithelial-derived EV by immunoaffinity-capture using epithelial cell adhesion molecule (EpCAM) as marker. By using a qPCR panel which screens 721 human miRNAs, the authors performed the miRNAs profiling of circulating EpCAM^{+}-EVs. By considering two small patients cohorts, 13 miRNAs were identified significantly more abundant in the EpCAM^{+}-EVs derived from plasma samples of colon cancer patients than in healthy controls. Interestingly, upon surgical tumor removal, the levels of 8 of these miRs were reduced, including miR-23b-3p [75]. Similarly, elevated levels of miR-23b-3p were found in exosomes isolated from plasma samples of a cohort of 196 NSCLC patients and were associated with poor OS by using Kaplan-Meier survival analysis [76]. In accordance with the detection of this miRNA in circulation in lung cancer patients, Zhu *et al.* showed that miR-23b-3p was up-regulated in serum samples of patients with NSCLC or different small cell lung cancer types compared to the benign pulmonary disease and healthy control. Logistic regression and ROC analyses identified a signature of 4 miRs comprising miR-23b-3p, miR-221, miR-148b, and miR-423-3p able to distinguish lung cancer from healthy individuals with a good accuracy (95% CI, 0.824-0.947) [77]. Finally, high levels of miR-23b-3p were found also in whole serum and in exosomes derived from patients with pancreatic cancer [78]. The data regarding the circulating miR-23b-3p levels are summarized in Table **3**. In conclusion, by comparing tissue and circulating miRNA expression levels, miR-23b-3p showed the same over-expression trend in gastric cancer and NSCLC both in tumor specimens and into circulation (plasma, plasmatic exosomes and serum). Instead, miR-23b-3p resulted up-regulated in colon cancer tissues and over-expressed in plasmatic EVs, but down-regulated in plasma of patients with colon cancer. These controversial data published by 3 different research groups can be explained by some factors, such as different cohorts, sample size, and sample type (plasmatic vesicles *versus* whole plasma). Therefore, additional studies in larger homogeneous populations are required to confirm and/or clarify these results. All together, these data provide novel insights on the detection of miR-23b-3p as circulating molecular biomarker in cancer with potential clinical value for diagnosis, prognosis and early detection.

CONCLUSION

In the last years, several studies have revealed the importance of miR-23b-3p as a cancer-related miRNA involved in key processes during cancer initiation and progression. The results obtained from *in vitro* and *in vivo* functional studies have clearly demonstrated that miR-23b-3p is implicated in cell cycle control, invasion, migration, proliferation, and stemness of cancer cells. In these different biological processes, miR-23b-3p targets mRNAs of genes promoting

Table 3. Circulating miR-23b-3p levels in different cancer types.

Circulating Levels of miR-23b-3p	Type of Cancer	Sample	Reference
Down-regulation	Colon cancer	Plasma	[74]
Up-regulation	Colon cancer	Plasmatic epithelial-derived EVs	[75]
	Gastric cancer	Plasma	[73]
	Non-small cell lung cancer	Serum	[77]
	Non-small cell lung cancer	Plasmatic exosomes	[76]
	Pancreatic cancer	Serum	[78]
	Pancreatic cancer	Serum exosomes	[78]

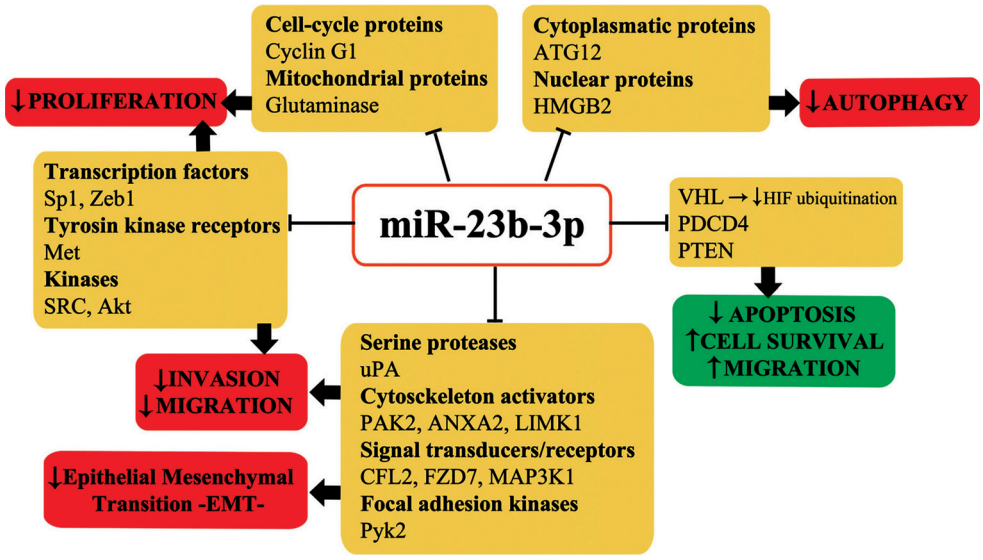

Fig. (1). Schematic model of the most relevant biological roles of miR-23b-3p in cancer. The indicated biological effects are deduced from the functions of the miR-23b-3p targets.

cancer, but also tumor suppressor genes, acting thus as either ts-miR or onco-miR, respectively (Fig. **1**). The hypothesis that miR-23b-3p has a dual role in cancer emerged also from miRNA expression patterns obtained in primary tumor specimens. For example, miR-23b-3p has been found down-regulated in primary tissues of colon cancer and impaired cell invasiveness by targeting several pro-metastatic genes. On the contrary, miR-23b-3p has been found up-regulated in gastric cancer tissues and it is shown to suppress apoptosis by silencing the pro-apoptotic factor PDCD4. This heterogeneity may depend on many factors, including the different subtypes of tumors and the clinical pathological characteristics of cancer patients enrolled in cohorts from different studies. In light of these considerations, more studies may be helpful to characterize the precise role of miR-23b-3p and its clinical implication as tissue biomarker in different tumor contexts.

Regarding the miR-23b-3p levels in liquid biopsies, the detection of this miRNA in body fluids is feasible and has gained increasing interest. Data obtained from miRNA expression profiling strongly highlight the importance of miR-23b-3p as circulating disease biomarker (in EVs or free) with diagnostic, prognostic and predictive value in cancer.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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