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REVIEW ARTICLE

MicroRNAs Determining Carcinogenesis by Regulating Oncogenes and Tumor Suppressor Genes During Cell Cycle

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Abstract: ***Aim*:** To provide a review considering microRNAs regulating oncogenes and tumor suppressor genes during the different stages of cell cycle, controlling carcinogenesis.

***Methods*:** The role of microRNAs involved as oncogenes' and tumor suppressor genes' regulators in cancer was searched in the relevant available literature in MEDLINE, including terms such as "microRNA", "oncogenes", "tumor suppressor genes", "metastasis", "cancer" and others.

***Results*:** MicroRNAs determine the expression levels of multiple cell cycle regulators, such as cyclins, cyclin dependent kinases and other major cell cycle activators including retinoblastoma 1 (RB-1) and p53, resulting in alteration and promotion/inhibition of the cell cycle.

***Conclusion*:** MicroRNAs are proven to have a key role in cancer pathophysiology by altering the expression profile of different regulator proteins during cell division cycle and DNA replication. Thus, by acting as oncogenes and tumor suppressor genes, they can either promote or inhibit cancer development and formation, revealing their innovative role as biomarkers and therapeutic tools.

Keywords: Cancer, metastasis, microRNA, oncogene, tumor suppressor gene, segregation.

1. INTRODUCTION

Cancer constitutes one of the three main causes of death in the United States and represents heterogeneous masses of cells with aggressive migratory characteristics and multiple patterns and routes of dissemination. The appearance, growth, and spread of tumor are mainly related to genetic and environmental factors with metastasis being the main causative parameter in cancer-related morbidities and mortality [1].

The human genome consists of approximately 25000 genes, each one being responsible for the regulation and function of the different - even neoplastic - cells [2]. Many cellular elements are involved in the process of gene expression. DNA, pre and posttranscriptional RNA and translational proteins, which are also the final products of genomic expression, are the most important factors in the whole process. Recent data have proven that the different mRNAs produced during gene expression have different stability levels while the newly discovered microRNAs (miRNAs) are capable of controlling and altering the expression of the mRNAs. Moreover, microRNAs have proven to be able to regulate different molecular pathways and checkpoints of the cell division cycle. Thus, microRNAs may play an important role in cell proliferation and apoptosis and possibly a key participatory role in cancer development [3].

The aim of this review is to summarize the latest data considering the role of microRNAs in relation to oncogenes, tumor suppressor genes and cancer development, through direct or indirect regulation of the cell cycle.

2. CELL CYCLE REGULATORS

Proto-oncogenes encode for proteins responsible for the cell cycle phases, including DNA replication, chromosome segregation, transcription and translation. The cell division cycle has three states-quiescent, interphase (G1, S, G2) and mitosis (p, m, a, t) - and four distinct phases named G0, G1, S, G2 and mitosis (Fig. **1**). Mitosis consists of prophase, metaphase, anaphase, and telophase [4, 5]. The Transcription Factors (TF) scan the DNA and the binding of the TF on the altered nucleotide sequence allows Tumor Suppressor Genes

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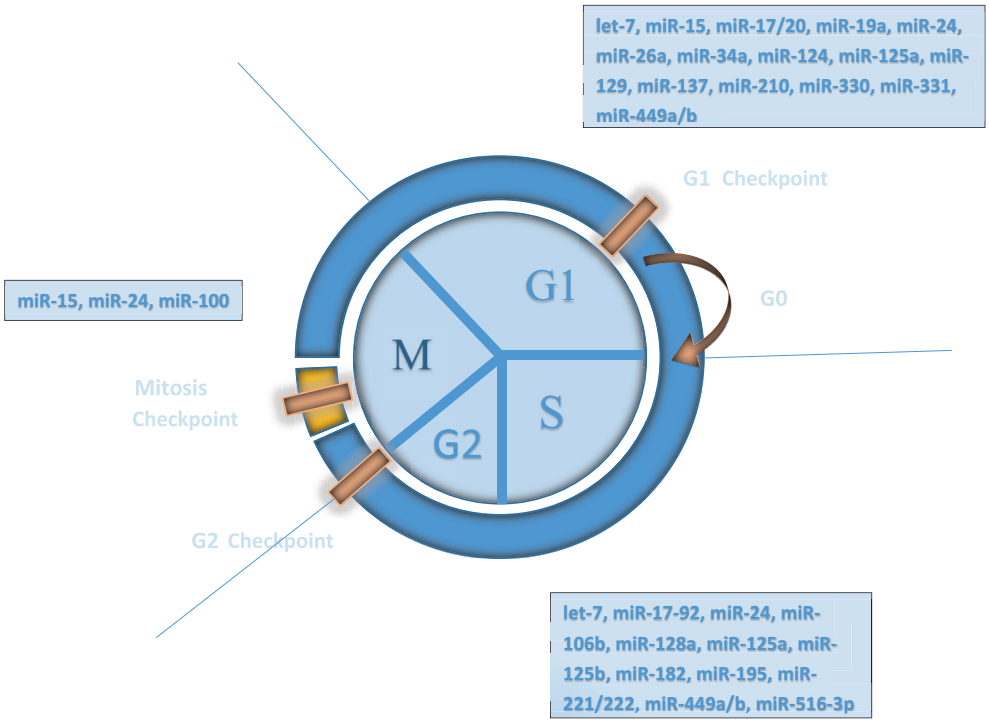

Fig. (1). Main microRNAs implicated in the different stages of the cell cycle. The cell cycle consists of the interphase and the mitotic phase. Checkpoints during the cell cycle are regulated by cyclins, CDKs and tumor suppressors, with defects on these checkpoints leading to uncontrolled cell duplication.

(TSG) expression and prevents the cell from a state of relentless duplication. The inability to recognize the altered nucleotide sequence by the TF, leaves the TSG inactive and the neoplastic cells continue on being in an endless proliferation state, disregarding normal apoptotic mechanisms. The main groups of cancer regulating genes are the oncogenes and the tumor suppressor genes. A mutation can be defined as the "interceptor" in normal cell division [6].

The cell division cycle is regulated by multiple and complex pathways. A path consisting of coordinated protein phosphorylation reactions has a key role in controlling cell's fate, since they participate and possibly affect it in many different phases [7].

Cyclins (G1 cyclins, G1/S cyclins, S cyclins, and M cyclins) are considered among the most important cell cycle regulators representing a group of related proteins, with four basic types found in humans and most eukaryotes organisms [8]. In addition to cyclins, specific enzymes called CDK are activated by binding of a cyclin. When binding to a CDK (CDK 1-4), phosphorylation takes place and the CDK is activated as a kinase. This procedure directs the CDK to a specific cell cycle target protein, appropriate to the cell cycle period controlled by the cyclin. The initial processes of DNA replication and actions which are essential for the completion of the cell cycle division are controlled and regulated by the CDK - cyclin complex. Many different cyclins have been reported that are active during the different stages of the cell cycle. Cyclin A partners with CDK2 during the S and G2 phase, cyclin B acts on CDK1 at M phase, cyclin C and D bind to CDK3 and CDK4 respectively at G1 phase while Cyclin E partners and activates CDK2 during the G1/S transition [9, 10].

Other important molecules responsible for the regulation of cell cycle are Cell Division Cycles (CDCs). CDC25 and CDC27 are dual-specificity phosphatases. Their first role is to activate cyclin dependent kinases by abstracting phosphate from residues in the CDK active site, while the second role is to interact with major mitotic protein elements, including Mad2, p55CDC and BUBR1, which are involved in the regulation of mitosis [11-13].

Retinoblastoma 1 (RB-1) and p53 genes belong to the tumor suppressor family (APC, WT1 & 2, NF1 & 2, BRCA1 & 2) whilst MYC and RAS are part of the oncogenic group (KRAS, HRAS, NRAS). These genes participate in the pathways that are responsible for cell cycle progression and cell death. P53 is located in almost all the normal tissues. The expression of this gene represents a cell regulator, participating in DNA repair and promoting apoptosis. Despite the fact that its gene expression product is unstable and is characterized by fast degradation inside the cell, p53 is able to detect DNA sequence alterations. After attaching to the strand, it is capable of being activated and thus, it produces proteins that may terminate cell completion. Retinoblastoma can occur in hereditary and sporadic forms. The RB1 gene found on chromosome 13 is responsible for the production of the Retinoblastoma (pRb) protein that prevents uncontrolled cell multiplication by controlling cell progression. If pRb is phosphorylated by the transcription factor E2F, it becomes inactive and results in the formation of retinoblastoma cancer [14, 15]. The E2F transcription factor family is an important

regulator of the cell cycle progression and proliferation while polo-like kinases (Plk) control many cell cycle features including centrosome maturation, checkpoint recovery, spindle assembly, cytokinesis, and apoptosis. Anaphase-Promoting Complex (APC/C) marks target cell cycle proteins for degradation by the 26S proteasome and the Aurora B kinase functions in the attachment of the mitotic spindle to the centromere [16].

The presence and activity of these regulator proteins during the cell cycle determine the progression of the cell cycle's fate. The overexpression of specific regulators, such as the cyclins, or the elimination of CDK inhibitors or pRB has been proven to be present in human cancer proving:. firstly, the levels of these proteins are of major importance during the different stages of cell cycle division and secondly, altering their levels could be a key factor for cancer development [17, 18].

3. MICRORNAs ALTERING THE STAGES OF CELL CYCLE

MicroRNAs are small endogenous non-coding RNAs of short length (21-23 nucleotides) that act as negative regulators of gene expression in eukaryotic organisms. Their role is to control stability, transcription and translation of protein-coding DNA and mRNAs. It is estimated that more than 30-60% of the human DNA is regulated by microRNAs [19].

MicroRNAs are transcribed by RNA polymerases II. The initial forms of microRNAs-precursor mollecules are produced and after cleavage, the mature microRNA product is formed. More specifically, the formation pathway of microRNA is initiated through long primary transcripts (pri-microRNAs) which are transcribed by the nuclear RNase III Drosha. This leads to the formation of an intermediate microRNA (pre-miRNA) of 70 nucleotides. Exportin-5 is then responsible for exporting pre-miRNAs in the cytoplasm that is then cleaved forming the double-stranded microRNA. One strand is degraded and the other one is then incorporated into the RNA-Induced Silencing Complex (RISC), forming the final microRNA product. The strand is integrated into Argonaute (Ago) protein and interacts with target microRNA transcripts. The inhibition of gene expression occurs only when microRNAs bind in 3'UTR or 5' UTR of target mRNAs. MicroRNAs have been identified as regulators in different eukaryotic organisms with each individual microRNA being able to affect many genes. Therefore, microRNAs control gene expression and possibly its inhibition, indirectly by inactivating the transcripted mRNAs that are responsible for converting the genetic information into proteins (gene expression) [20].

MicroRNAs are detected among many body fluids and tissues and it has been reported that they may be implicated in the initiation and progression of many cancers such as breast cancer [21]. Many studies have revealed miR-124-3p's main role as a tumor suppressor molecule, while miR-203 is up-regulated in breast cancer samples. Moreover, low levels of miR-125a have been associated with poor breast cancer prognosis. The tumor-suppressive role of miR-199a-sp in triple-negative breast cancer has been demonstrated, however, the final step in neoplasia development results from the joint action of tumor inhibitors and cancer inducers [22].

Studies have proven that microRNAs displaying antiproliferative properties, can function as tumor suppressors. Conversely, when carcinogenesis takes place, these microRNAs are inactivated or their levels are deregulated. Moreover, they are able to alter the levels of multiple cell cycle regulators and control cell proliferation, differentiation and apoptosis (including the formation of cancer) by controlling the expression profile of different proteins of specific target oncogenes and tumor suppressor genes [23]. Current data suggest that miR-137, miR-449, miR-15a, miR-16, miR-24, miR-129, miR-34a, miR-124, miR-125b, miR-195, and let-7 family members regulate the expression of major kinase complexes inducing cell cycle arrest, including CDK1, CDK2, CDK6, cyclin D1, D3 and E1, during the G1 phase [24-33] (Table **1**).

The first report of microRNAs' role in the cell cycle regulation resulted in an analysis of the miR-15a-16-1 cluster. MiR-15a-16-1, expresses miR-15a and miR-16, both belonging to the same microRNA family (miR-15 family) since they share common sequences. In the first study, which described a connection between microRNAs levels and tumor development, mir-15a-16-1 was reported to be involved and dysregulated when specific chromosome aberrations were present in patients with Chronic Lymphocytic Leukemia (CLL), [34]. MiR-15a-16-1 is down-regulated or even eliminated in about 70% of all patients with CLL and other cancer types, proving it's key role in tumor development [24, 34-36]. The miR-15a-16-1 targets cell cycle regulators CDK1, CDK2, CDK6, CDC27, Cyclin D1, D3, E1, E2F3 and WEE1. Except for miR-15a-16-1, many other microRNAs can target the same cyclin such as miR-16 and miR-34a which are responsible for cyclin E regulation, while CDK6 is targeted by let-7, miR-24, miR-34a, miR-124, miR-125b, miR-129, miR-137, miR-195, and miR-449, cyclin D1, D2 and D3 and downregulated by let-7, miR-15, miR-17, miR-19a, miR-20a, and miR-34, miR-26a [26, 36-41].

MiR-124 and miR-137 silencing have been described in cancer cells, as a result of hypermethylation, leading to CDK6 overexpression [42]. Such an overexpression of a CDK/cyclin complex might cause increased phosphorylation of pRB activating E2F factors leading to G1 progression and S-phase entry. The E2F family is generally split according to function into two groups: one with transcription activators and another one with repressors, while both play a major role during the G1/S transition. O'Donell *et al*., reported that E2F is targeted by microRNAs proving that E2F1 is negatively regulated by miR-17-92, miR-17-5p and miR-20a clusters [43, 44]. The expression of those microRNAs was correlated with E2F1 levels in cancer samples, proving that the microRNAs studied promoted carcinogenesis in many tissues by rendering cells resistant to the apoptosis promoted by E2F1 [45]. Many other microRNAs have proven to play a key role in cancer development and progression by altering E2F factors' expression during cell cycle. E2F1 is proven to be restrained by miR-149, miR-330 and miR-331-3p causing

Table 1. MicroRNAs that alter cell cycle progression.

Cell Cycle Phase	Kinase	Regulator	MicroRNA	Refs.
G1 phase	CDK 2,4,6	Cyclin D	Let-7, miR-15, miR-17/20, miR-19a, miR-26a, miR-34a	[24-33]
		Cyclin E	miR-15, miR- 26a, miR-34a	
		CDC25A	Let-7, miR-125b, miR-322/424, miR-449a/b	
		Direct Regulation	Let-7, miR-15, miR-24, miR-124, miR-125b, miR-129, miR-137, miR-449a/b	
S/G2 phase	CDK 1,2	Cyclin A	Let-7, miR-125a	[40]
		Cyclin B	miR-24	[54-56]
		CDC25A	Let-7, miR-125b, miR-322/424, miR-449a/b	[64, 67]
G2/M phase		CDC27	miR-15	[27, 36]
		APC/C		[13, 16]
		PLK1	miR-100	[62-64]
		Aurora B	miR-24	[62]

a cell cycle restrain in prostate and gastric tumors [46-48]. E2F3 is targeted by miR-125b, miR-210 and miR-195 [46-48]. E2F3 is targeted by miR-125b, miR-210 and miR-195 [32, 49, 50].

MicroRNAs are capable of altering cell cycle and especially G1 progression aiming at the inhibitors of the CDK4/pRB pathway. pRB is targeted by miR-106a. The overexpression of this miR-106a promotes pRB downregulation in tumor cells [51]. Moreover, p130/RBL2 and p107/RBL1 are altered by mir-290 and mir-17-92 clusters [52-54]. The expression of miR-17-5p, promotes proliferation, while the downregulation of the mir-290 cluster promotes RBL2 expression whose activation results in hypomethylation of the genome proving the important role of microRNAs in tumorogenesis [52, 54].

MiR-24 and miR-31 control the expression of p16INK4a, an inhibitor of CKD4 and 6 thus, being involved in the regulation of cell cycle indirectly by altering the levels of the inhibitor [28, 55]. On the contrary, mir-17-92 and miR-106b reveal a direct regulation of a p53 target -p21Cip1-, while p27Kip1 and p57Kip2 are also regulated by the expression levels of miR-181 and miR-221/222 [56-59]. Mir-221-222 cluster negatively regulates both p27Kip1 and p57Kip2, thus altering the expression of CDK2 and enhancing tumor growth [60]. Most cell-cycle-implicated microRNAs modify cell-cycle outset and transition from G1 to S phase. Let-7, miR-24 and miR-125b can downregulate the outflow of Cyclin A or Cyclin B. MiR195, miR-516-3p and miR-128a can likewise downregulate WEE1, a negative controller of the CDK1-Cyclin B complex during the G2/M progress [61, 62]. The control of Cip/Kip CDK inhibitors by microRNAs may likewise influence mitotic passage by balancing CDK1 movement. Relatively few and different instances of microRNAs exist, which direct mitosis. Polo-Like Kinase 1 (PLK1) is a basic controller of mitosis at a few dimensions. PLK1 phosphorylates CDC25C, which initiates CDK1-Cyclin B1 structures, bringing about its translocation into the core and mitotic passage [63]. MiR-100 is proven to target PLK1, and the downregulation of this microRNA promotes PLK1 overexpression in naso-pharyngeal tumor samples [64]. Lastly, Aurora B kinase, a protein with the capacity of mitotic axle to the centromeres has been lately portrayed to be a target of miR-24, which perceives seedless yet very corresponding successions in the Aurora B-encoding transcript [61-64].

One of the most investigated microRNAs is miR-34 family, due to its ability to regulate tumor suppression. The three molecules belong to the miR-34 family; miR-34a, which is generated from a transcriptional unit on chromosome 1p36, miR-34b and miR-34c, both of which are expressed by processing of a bicistronic transcript from chromosome 11q23. Both genomic loci are considered to be associated with fragile sites of the genome that are frequently modified in cancer. MiR-34a constitutes the prevailing family member type, while miR-34b and mir-34c can mostly be traced in lung, ovary, testes, and trachea [65, 66]. The downregulation of miR-34a has been associated with a wide range of malignancies in human cell lines and mouse tissues, with the most commonly reported cancers of the lung, pancreas and liver. It has been reported that miR-34 levels are directly increased by P53, and that miR-34a can lead to G1/G2 arrest in a parallel fashion to mRNAs that are directly activated by p53 [67, 68].

The important aspects of miR-34a include its function of controlling cell cycle, regulating apoptosis and its ability of inhibiting cancer stem cells. Ji *et al*., reported that miR-34 may be involved in pancreatic cancer stem cell self-renewal, possibly *via* direct downregulation of specific pathways, such as the Notch pathway proteins and Bcl-2 family. Tryndyak *et al*., *via* a rat model of liver carcinogenesis in-

duced by a methyl-deficient diet, showed similar dysregulation of miR-34a expression through the same pathways, indicating a connection of hepatocellular development and progression in humans and micro RNA level alteration [68, 69]. In rat liver cancer cells, miR-34a-mimic downregulates protein expression of the Notch pathway-related Notch-1 (by bounding to the 3' UTR-binding sites of Notch 1 mRNA), Notch-4, and Hes-1, and also influences negatively the apoptosis related Bcl-2 and Bcl-xL. Conversely, it upregulates the expression of cell cycle-related P21 and apoptosis-related Bax. Therefore, it can be concluded that miR-34a may have a key role in liver tumor suppression, as its low expression could result in the inhibition of cell apoptosis [70].

Another known target of miR-34a, which also correlates with the metastatic potential of tumors, is Wnt signaling pathway, specifically c-Met. Decreased c-Met-induced phosphorylation of Extracellular signal-Regulated Kinases 1 and 2 (ERK1/2) has been observed in hepatocellular carcinoma tissue, when enforced miR-34a expression takes place in HepG2 cells. This may be indicative of the suppression role of miR-34a in tumor migration and invasion. Wei *et al*., similarly suggested that cell invasion and metastasis are inhibited when miR-34a is overexpressed in liver cancer cells that were previously depleted [67, 71].

Nonetheless, increased levels of miR-34 have been correlated with Hepatocellular Carcinomas (HCCs). Pok *et al*., found miR-34 a,c to be significantly upregulated in association with cyclin E and p53 in human cirrhotic liver and HCC specimens, compared to dysplastic and normal human liver. Promoted from miR-34 family, this upregulation of the functionally active kinase allows cyclin E-mediated cell-cycle G1/S checkpoint failure, conceivably leading to carcinogenesis [72-74].

Furthermore, latest data have demonstrated that miR-34a is closely related to E2F1, E2F2 and CASP3 expression in primary Hepatocellular Carcinoma (HCC). In HCC, the overexpression of E2F1 and E2F3 has been observed, the enforced expression of miR-34a leads to an increased activity of CASP3, and a downregulation of E2F1 and E2F2. In an analysis by Han *et al*., it was shown that the survival rates of HCC patients were increased when high miR-34a, or low E2F1/ E2F3 expression were observed. Hence, it could be suggested that the aggressiveness of HCC may be suppressed by expediting apoptosis in cancerous cells through miR-34a's higher expression [72-74]. However, the practical importance of the main control of these proteins by microRNAs remains for the most part unexplored.

4. *IN VIVO* ALTERATION OF AN EXPRESSED MICRORNA TARGET

The expression profile status of microRNAs in cancer tissues has been proven feasible to identify. Because of this, recent data have revealed a novel therapeutic approach consisting of direct or indirect alteration of the expressed microRNAs regulating tumor carcinogenesis. As the activation of oncogenes is connected to a possible cancer formation, their silencing can be proven essential in preventing tumor development. Nowadays, artificial antisense microRNAs can be synthesized and administered to patients in order to regulate, control and finally block their targets; in this case, the oncogenes are responsible for cancer formation.

There are two main strategies to alter the profile of the targeted microRNAs, which are dependent on whether their expression should be inhibited or to retrieve the lost function of the targeted microRNA. The Antisense Oligonucleotide (AMO), also called antagomir, represents the most commonly used microRNA inhibitor since it has demonstrated the most promising results [75-77]. It consists of oligonucleotides that contain complementary sequences of endogenous microRNAs, with stronger affinity to altering microRNAs targets,, less sensitivity to nucleases, and with lower toxicity [78]. The Peptide Nucleic Acid (PNA) are uncharged Oligonucleotide (ON) analogues in which the deoxyribose phosphate backbone of DNA/RNA has been replaced by a pseudo-peptide and it is similar to DNA and RNA. PNA ONs show high affinity and sequence specificity for complementary RNA and DNA, bind the targeted nucleotide more effectively than the nucleotide - nucleotide binding, possess antisense biological activities *in vivo* with low toxicity levels, and can be administered systematically [79, 80]. MicroRNA sponge and microRNA masking represent another novel therapeutic approach for altering the microRNA expression. These RNAs are produced from transgenes within cells and contain multiple binding sites specific to a microRNA seed region that allows them to block a whole family of related miRNAs. The microRNA sponge downregulates the targeted microRNA and possesses multiple complementary sites on the targeted microRNA, while microRNA masking has complimentary binding site in the 3′ UTR of the target mRNA to completely inhibit the miRNA target [81].

In order to achieve an overexpression of a targeted microRNA, the transfection of double-stranded microRNAs (microRNA mimics) has also been used with remarkable results [82]. For example, the overexpression of downregulated miR-26a in Hepatocellular Carcinoma (HCC) in mouse led to the inhibition of cancer proliferation and the initiation of apoptosis. In addition, the downregulated miR-34a level was increased by delivering artificial miR-34a with NOV340 liposome in an orthotopic model of HCC. This resulted in significant tumor reduction, prolonged survival, and disease protection in animals [65]. To achieve these results, an artificial double-stranded microRNA is administered *via* intravenous and subcutaneous injection or infusion. However, after the administration of the microRNA mimic, the *in vivo* conditions create limitations that lead to loss of microRNA efficiency. In order to enhance the *in vivo* stability and overcome the loss of mRNA silencing ability, encapsulating the microRNA mimic into nanoparticles seems inevitable. Thus, viral vectors (adenoviral vectors that encode small RNA molecules), poly(lactide-co-glycolide) particles, neutral lipid emulsions, neutral liposome 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine, EnGeneIC Delivery vehicle nanocells, synthetic polyethylenimine, dendrimers, cyclodextrin, poly(ethylene glycol), chitosan and N-acetyl-D-galactosamine are all now used as microRNA mimics mediators. Thus, the de-

livery system limiations are overcome, as a result of the similarity between microRNA mimics and small interfering RNA (siRNA) structures [83].

5. CLINICAL THERAPEUTIC USAGE OF MICRORNAS IN CANCER

The expression profile of many microRNAs is now proven to be implicated in the development of different cancer types including solid tumors of liver, lung, kidney, pancreas, colon, ovarian and cervical, head and neck, B cell lymphoma, lymphoid leukaemia and breast cancer. In the fields of clinical practice, many microRNAs are used to classify tumors and predict prognosis [84]. The regulation of some of these microRNAs is correlated with hormone receptor status. And the overexpression of these microRNAs, such as let-7, blocks hormone activation of Wnt activity [85]. In anticancer research, some microRNAs are capable of killing tumour cells directly or at least significantly inhibiting cell biological functions in lung cancer and breast cancer, with each injected exosome being capable of targeting specific signaling pathways (EGFR, Notch, Wnt) [86-88]. To date, there are several studies testing specific microRNAs with tumour suppressive functions.

MiR-200 has been tested in preclinical studies for lung cancer in mice. Cortez *et al*, reported that systemic treatment of tumours with miR-200c mimics in DOPC (1,2 dioleoylsn glycero-3 phosphatidylcholine) liposomal carriers increased radiosensitivity and survival. Moreover, the authors confirmed that miR-200 targets the genes that encode oxidative stress response proteins that lead to the generation of increased levels of Reactive Oxygen Species (ROS), resulting in cancer cell apoptosis [89]. In another study, miR-200 targeting interleukins, in orthotopic mouse models of ovarian (miR-200a/b), basal-like breast (miR-141) and lung (miR-200a/b) cancers resulted in decreased tumour nodules and distant metastasis [90].

In a study of 455 patients with hepatocellular cancer, levels of miR-26a were significantly reduced compared with normal tissues, while low levels of miR-26a were correlated with overall poor survival. A murine model of adeno-associated virus-mediated expression of mir-26a resulted in significant tumor regression, which was attributed to the direct targeting of mRNAs encoding the cell cycle controllers cyclin D2 and cyclin E2 [39, 91].

The therapeutic usage of miR-506 and miR-520 is also tested in ovarian cancer orthotopic mouse models. MiR-506 mimics and miR-520 mimics administration resulted in significant tumor regression and in decreased expression of the respective mRNA targets *in vivo* [92, 93]. The same results occurred in a study of miR-630. MiR-630 is an oncomir that is upregulated in response to hypoxia in the tumour environment. Using an antimiR against miR-630 in an orthotopic model of ovarian cancer, a significant reduction in tumour growth and metastasis was observed [94].

MiR-15/16 cluster injected subcutaneously in mice with leukaemia resulted in a significant reduction in tumour volume and growth. Moreover, the delivery of miR-16 using a nanocell delivery system in malignant pleural mesothelioma resulted in tumor-targeted delivery and significant tumour reduction [95].

An early study of the therapeutic potential of Antisense Oligonucleotides (ASOs) in microRNA inhibition demonstrated successful inhibition of miR-10b in a microRNA antagonist (antimiR) in orthotopic model of breast cancer. This antimiR resulted in decreased metastasis, however, the authors reported no reduction in primary tumor growth, suggesting the need for adjuvant treatment with surgery or chemotherapy combinations. Additionally, in an orthotopic glioblastoma mouse model, the administration of miR-10b antagonist (BOX 1) resulted in a significant reduction of tumour growth. A recent study combined locked nucleic acids against miR-10b in mouse models of breast cancer that combined to doxorubicin, that resulted in significantly greater decrease in tumor burden and without any damage of normal tissue being observed [96-98].

MiR-221 is one of the most significantly upregulated microRNAs in hepatocellular cancer, in which miR-221 downregulates key tumour suppressors such as p27KIP1, PTEN and TIMP3. An intravenous administration of a cholesterol-modified form of antimiR-221, showed significant downregulaton of miR-221 and significant shrinkage of tumor with increased survival in hepatocellular mice with cancer [99, 100].

In a mouse model of miR-155-induced lymphoma, controlled by doxycycline, it was demonstrated that doxycycline withdrawal resulted in the shutdown of mir-155 expression and subsequent tumor shrinkage indicating the therapeutic role of this anti microRNA. Because the tumor microenvironment was acidic, a conjugate of a pH Low Insertion Peptide (pHLIP) and antimiR-155 facilitated the specific delivery of antimiR to cancer cells. Mice treated with combined pHLIP antimiR-155 showed a reduction in tumour burden, resulting in prolonged survival with no significant toxicity [101, 102].

6. MICRORNAs AND EMBRYONIC STEM CELLS (ESCS)

The unlimited ability of self-renewal and differentiation into multiple cell lines characterize stem cells. ESCs are pluripotent and thus can differentiate into all possible cell types. It is also suggested that the initiation of cancer, is connected to a certain type of Cancer Stem Cells (CSCs). MiR-291a-3p, miR-291b-3p, miR-294, and miR-295 have proven to promote G1/S transition by direct target of Cyclin D, CDK4 and 6, while indirect downregulation of the cyclin E-CDK2 complex by miR-290-295 urges the cells to enter the S phase faster [103-105]. Also, miR-290-295 downregulates various cell cycle inhibitors (such as RB, RBL1, RBL2), altering the distribution of ESC in each cycle phase. In addition, by increasing the expression of important transcription factors, the miR-290-295 cluster enhances somatic reprogramming and by targeting Caspase 2, miR-290-95 is shown to be involved in suppressing apoptosis. This results in a reduced ESCs in phase G1 and a higher fraction of cells in phases S or G2/M [106-108].

Due to the enhanced proliferation, metabolism of ESCs relies mostly on glycolysis. Therefore, glycolysis-associated genes, have been associated with miR-290-295 that is capable of altering epigenetic pathways including histone acetylation, DNA methylation, and Polycomb protein activation, which inactivate differentiation genes [109-113].

MiR-17-92 is a MYC oncogene regulator, which acts by controlling the chromatin stage of cell cycle-related genes [109]. Through miR-17-92, MYC inhibits the expression of chromatin regulatory genes, which demonstrates its crucial role in early mammalian development [114, 115]. Moreover, *via* the same microRNA, MYC participates in the formation of euchromatin responsible for specific protein production, and in the duplication of the DNA, hence promoting a shift in the number of cells in the proliferating state [114]. Likewise, miR-106b, which shares genetic similarities with miR-17 and miR-20a, has been shown to promote transition from G1 to S phase by targeting p21, resulting in a higher proportion of cells in phase S compared to G1 [56].

The miR-302-367, which consists miR-302a/b/c/d, and miR-367, plays a major role in the proliferation of ESCs - that are mostly expressed in the early stages of fetal development. This microRNA target genes involved in epigenetics such as the MECP1-p66 and MECP2 that are downregulated through the act of the miR-302-367 cluster [116], promote transcription of pluripotent genes in mammalian ESCs [59]. Moreover, the miR-302-367 promoters are activated when bound by OCT4 and SOX2, which are the core transcription factors directly participating in the maintenance of ESCs [116, 117]. It has also been shown that by targeting the SMAD-dependent and the PI3K/PKB pathway, this microRNA promotes pluripotency in ESCs. MiR-302 inhibits the Transforming Growth Factor Beta-Receptor 2 (TGFBR2) and homologous RAS gene family member C (RHOC), resulting in reduced epithelial-mesenchymal transition [116, 118, 119]. In addition, the miR-302 cluster has demonstrated a negative regulation of p21 and LATS2 activity both in hESCs and mESCs. These molecular mechanisms reveal the miR-302-367 cluster's important role with regard to pluripotency and alterations of the cell cycle [120, 121].

CONCLUSION

Cancer is the end result of the accumulation of activating somatic mutations that eventually lead to the first stages of carcinogenesis and aberrant cell multiplication. Different types of cancer manifest unique mutations that can be simple alterations that take place on the genome, either leading to cell death and cancer formation. They can even be unnoticed without creating any changes. The cell-cycle-dependent transcription factors, controlled by microRNAs reveal a new level of complexity. Many microRNAs prevent proliferation and this function can be mediated by controlling various mitotic pathways including the pathways leading to the activation of various CDKs. Recent data reveal the innovative therapeutic use of microRNAs *via* local or parenteral administration. In many cases, a few microRNAs induce proliferation by targeting CDK inhibitors or pRB family members. It is now proven that microRNAs are functionally integrated into many critical cell cycle- pathways, promoting alterations that lead to cancer development or even repression, ultimately promoting either oncogenes or tumor suppressor genes.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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