miRNA in Molecular Diagnostics

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miRNAs influence the expression of numerous proteins, including the expression of tumor suppressors and protooncogenes, thus becoming oncogenes and tumor suppressors themselves. As the same miRNA can have different targets in different tissues, its function will also be different in different types of tumors, depending on the intracellular milieu and the set of proteins for which its translation is modulated. Therefore, the same miRNA can act as a tumor suppressor and an oncogene in different tumors. Even in the same tumor, the same miRNA can be involved in regulation circles with feedback loops and potentially affect both tumor suppressors and oncogenes. As 50% of miRNA genes are located in regions associated with cancers, their expression is found to be deregulated in tumors. miRNAs were found to be members of signaling circuits, often involving also long non-coding RNAs (lnc RNAs) and circular RNAs (cRNAs).

miRNA malignant tumors viral infections

1. Introduction

As oncomirs and tumor suppressors, miRNAs mostly influence processes of cell proliferation and apoptosis. Examples of most investigated miRNAs in various malignant tumors and their targets are listed [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69][70][71][72][73][74][75][76][77][78][79][80][81][82][83][84][85][86][87][88][89][90][91][92][93][94][95][96][97][98][99][100][101][102][103][104][105][106][107][108][109][110][111][112][113][114][115][116][117][118][119][120][121][122][123][124][125][126][127][128][129][130][131][132][133][134][135][136][137][138][139][140][141][142][143][144][145][146][147][148][149][150][151][152][153][154][155][156][157][158][159][160][161][162][163][164][165][166][167][168].

As illustrated on the example of osteosarcoma [169], miRNA can influence all hallmarks of cancer: cell cycle, proliferation control mechanisms, cell migration, metastasis and invasion, autophagy, apoptosis, senescence, and differentiation. They also affect resistance to chemotherapeutics, metabolism, and immune surveillance [47][111][133][141].

Specific changes in miRNA regulation can be found in all types of tumors, but detecting those that could be used as diagnostics markers is challenging, as they are cell- or tumor-specific. Currently, besides detecting one specific miRNA, often, a set of deregulated miRNAs is correlated with a certain type of tumor. For example, Sharma and Gupta analyzed miRNAs as potential biomarkers for the diagnosis and prognosis of different types of cancer and detected 723 dysregulated miRNAs in 16 types of tumors. Forty-three miRNAs were differentially expressed in six or more types of tumors [170].

2. miRNAs in Leukemia and Lymphoma

First miRNAs found to be directly involved in tumor development were detected in leukemia patients. The involvement in proliferation and apoptosis and their roles in the signaling loops in B cell differentiation are probably the best known and understood in hematopoietic cells and their malignancies [171]. Calin discovered that the deletion of 13q14 in chronic lymphocytic leukemia (CLL) coincided with a loss of miRNA15a and miRNA16-1 (miR15/16), and in nearly 70% of CLL patients, these miRNAs were found to be either absent or epigenetically downregulated [1]. Their deletion influenced the expression of antiapoptotic proteins BCL2 and MCL1, leading to survival and resistance to chemotherapeutics, as well as cyclin D1 expression and cell proliferation [1][2][3]. As their target is also p53, their deletion leads to the development of a specific CLL subtype. miR15/miR16 cluster downregulation was also found to be involved in other types of leukemia [4].

Another miRNA gene cluster for which its expression correlated with development of hematopoietic disorders was miR17-miR92 cluster, containing six miRNAs, produced from a polycistronic transcript [5]. Its amplification was found in B cell lymphoma, T cell leukemia, and some solid tumors [5][172]. Its locus is regulated by c-Myc, and its main targets are BIM, PTEN, p21, and p57 [173]. Its deregulation compromises apoptosis and increases proliferation, cell survival, resistance to chemotherapy, and BCR signaling [7][8][9].

Numerous miRNAs were further found to be involved in normal development and leukemogenesis in both B and T lymphocytes. One of them is miR34a, which acts as a tumor suppressor and is regulated by p53. Its main targets are *FOXP1*, *ZAP-70,* genes involved in apoptosis, such as *BCL2*, and genes regulating cell cycle progression and proliferation, such as *BCL6*, *B MYB*, *CDK6*, and *AXL* [10].

While *FOXP1* is regulated by miR34a in pro/pre-B cells, miR150 regulates this gene in mature B cells. In pro-B cells, miR150 regulates Myb [11][171]. Its deletion increases BCR signaling and survival pathways involving PIK3AP1 and AKT2 and influences telomerase expression. By targeting *CXCR4* it regulates mobilization and migration of mononuclear cells [12]. Its expression is downregulated in one CLL subtype and in different types of lymphomas.

miR155 can act as both an oncogene and a tumor suppressor in different steps of B cell development, and it is another example of different types of negative feedback loops present in the signaling regulations of miRNAs [171]. It is regulated by BCR activation, its downstream targets are transcription factor Pu.1 and AID, which are involved in immunoglobulin somatic hypermutation [13], and it regulates Akt signaling, proliferation, motility, and the modulation of TGFβ pathways [14]. miR181b also targets AID, in addition to Bcl2, MCL1 and TCL1, and Akt kinases coactivator, thus influencing apoptosis, cell survival, and differentiation. This miRNA is downregulated in CLL, and its levels can correlate with disease progression in the samples of the same patient [15][16].

3. miRNA in Brain Tumors

miRNA in glioblastoma and other brain tumors were analyzed by several research groups, detecting characteristic sets of 5–10 up- and downregulated miRNAs as specific signatures that correlated with patients' survival. These miRNAs influence MAPK, PI3K/Akt, mTOR, and Wnt signaling pathways and deregulate apoptosis and the control

of proliferation [20][29]. Among miRNAs, the most analyzed miRNA in these tumor types is miR7, and it is involved in neural cell differentiation and acts as a tumor suppressor. It mainly influences targets in Akt and MAP kinase pathways, and its downregulation increases proliferation, survival, and inhibits apoptosis [21][174]. Other miRNAs often involved in glioblastoma development are miR21, miR221, and miR181 [22][23][29]. miR21 overexpression leads to the inhibition of apoptosis and increases in cell proliferation [22][23][175]. Other mentioned miRNAs are involved in PI3K/Akt regulation, Notch and p53 signaling, and DNA repair [27][29][35].

4. miRNA in Lung Cancer

miR21 [37], miR148 [50], and miR205 [38] are among the most investigated miRNAs in lung cancer, but numerous other miRNAs were also found to be deregulated in this type of tumor. Several sets of miRNAs with altered expression were also identified in lung adenocarcinoma, in addition to observing differentially expressed miRNAs in different types of lung cancer [176][177][178][179]. It was found that EGFR mutation in lung cancer cells leads to changes in the expression of 17 miRNAs, including the miR17-92 cluster [52]. These miRNAs influence proliferation, survival, resistance to chemotherapy and apoptosis, and migration and cancer cell stemness [46][180]. The main targets of deregulated miRNAs in lung tumors are Ras and Myc pathways, PTEN and PI3K/Akt signaling leading to cell proliferation, the p53 pathway, and others influencing resistance to chemotherapeutics and apoptosis [38][42][43][44]. Cell migration, proliferation, and resistance to chemotherapeutics are influenced by the interaction of miRNA with HIF and TGFβ pathway elements [36][49][131]. Other processes regulated by miRNAs in lungs are metabolism and glycolysis, as well as epithelial–mesenchymal transition (EMT) [44][46][49][181][182].

5. miRNA in Breast Carcinoma

Numerous miRNAs and signatures of deregulated miRNA were detected in breast cancer [183][184]. Among those, the most investigated are miR125b, miR145, miR21, miR155, and miR205 [54][55][185]. The main targets of deregulated miRNAs are molecules involved in MAP/AKT/STAT3 signaling pathways and those regulating cell proliferation, epithelial–mesenchymal transition, angiogenesis by targeting VEGFA, cell stemness, and resistance to chemotherapy [34][53][56][57][58][59][186].

6. miRNA in Bladder and Renal Carcinoma

In bladder cancer, deregulated miRNAs often include miR34a, miR21, and miR222, and several miRNAs are linked to migration and invasion [69][70][71]. Their targets are β catenin, CDK2, E cadherin, as well as integrin α5, influencing resistance to chemotherapy [67][73][74].

In renal cancer tissues, the main targets of deregulated miRNAs include proteins participating in proliferation, such as those in Akt and Wnt signaling, migration, invasion, and EMT [72][77][78][80][187].

7. miRNA in Colon, Hepatocellular and Gastric Carcinoma

In colon cancer, deregulated miRNAs, including miR200c, miR145, miR181, miR101, and miR21, mainly interfere with cell proliferation and migration, apoptosis, Wnt/β-catenin, and MAPK pathways [81][83][84][87][88]. In this entity, specific sets of miRNAs with prognostic and diagnostic potential were detected [188].

The most significant miRNAs involved in signaling circuits in hepatocellular carcinoma are those regulating the PI3K/Akt pathway, cell proliferation, apoptosis, invasion, EMT, and glucose metabolism [90][92][93][94][95]. They mainly act as tumor suppressors [99][189][190].

In gastric carcinoma, miRNAs also regulate cell proliferation and migration by targeting PTEN and EGFR, as well as MAPK pathways, and EZH2, which participates in chromatin remodeling [99][100][102]. Numerous miRNAs in this disease are related to resistance to apoptosis through the regulation of Bcl2 or other members of its family, angiogenesis, and resistance to chemotherapy [105][106][107][191].

In pancreatic cancer, miRNAs regulate EMT through TGFβ signaling, as well as processes of invasion and the inhibition of apoptosis [108][109].

8. miRNA in Cervical Carcinoma, Testicular Tumors, and Prostate Cancer

In cervical carcinoma, miRNAs promote tumor proliferation, migration, invasion, and influence apoptosis and chemoresistance. Examples are miR21, which influences Akt/mTOR pathway, proliferation, growth, and EMT [113]; miR375, which targets E-cadherin; and miR138, which targets EZH2, influencing chromatin remodeling [114][192]. It was observed that viral proteins E6 and E7 increase the expression of miR18a [110], influencing Hippo signaling, in human papilloma virus (HPV)-associated cervical carcinoma.

In prostate cancer, miRNAs influence proliferation, apoptosis, migration and invasion. The main targets are Akt and MAPK pathways and HIF and VEGF pathways [114][119][123][124].

In different types of testicular germ cell tumors, several miRNAs are differently expressed and vary from low expression in teratoma, medium expression in seminoma, and high expression in embryonal carcinoma. The main deregulated miRNAs are miR199-214, influencing tumor metabolism through epigenetic regulators, miR371-373 influencing p53 pathway, cell cycle regulation, Wnt/β-catenin signaling, and senescence; and miR223, influencing apoptosis and cell growth through FBXW7 [125][127][128][132][133]. Other miRNA targets are cell cycle regulators, members of the p53 pathway involved in apoptosis regulation, DNA damage sensitivity, cell differentiation, and lactate metabolism [127][131][193][194].

9. miRNA in Skin Tumors

An analysis of metastatic melanoma revealed 44 miRNAs acting as tumor suppressors and 23 as oncomirs [195]. Some of those miRNAs control the expression of MITF, transcription factor involved in differentiation, proliferation,

and the survival of melanocytes, cell motility, and invasiveness [196]. Numerous miRNAs control MITF directly, and others control MITF by targeting signaling pathways regulating its expression, such as Wnt and MAP signaling. Furthermore, some miRNAs regulate cell survival and take part in chromatin remodulation [135]. Developments in invasive melanoma are linked to melanoma phenotype switching when a highly proliferative state is exchanged for invasive states characterized by its migration ability. In this state, MITF levels decrease. Phenotypic changes have similarities to EMT, and numerous miRNAs involved in the regulation of migration are deregulated. High MITF expressions also correlate with resistance to chemotherapy, and nearly 20 tumor suppressors and oncomirs are linked to this process, with most of them targeting MAP kinase and PI3K and EMT pathways [138][139]. miRNAs in melanoma also regulate escape from immune surveillance [141].

In cutaneous squamous cell carcinoma, miRNAs influence cell proliferation, invasion, and migration; and inhibit apoptosis and differentiation by targeting PTEN, members of MAP kinase, and cMyc pathways [143][144][146].

10. miRNA in Other Tumors

Aplastic thyroid cancer is a highly invasive thyroid tumor that is fast growing and resistant to chemotherapy. miRNAs influence cell proliferation, invasion and EMT, cell adhesion, differentiation, and cell stemness by targeting PTEN, CDKI, NFκB, TGFβ, Wnt pathway, and ZEB, which are proteins involved in autophagy, apoptosis, and chromatin modulation [152][154][155][158][160][197]. In a rare medullary thyroid carcinoma, specific sets of deregulated miRNAs were detected [155][162].

In osteosarcoma, most deregulated miRNAs are linked to cell proliferation and migration: targeting β-catenin and MAP kinases pathways [164][165][166]. There are miRNAs that act as oncomirs and tumor suppressors depending on the intracellular milieu of different osteosarcomas [167][168][169].

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