MicroRNA Signatures of Tumor Hypoxia

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Abstract

Micro RNAs (miRNAs) are regulatory RNA molecules that can act as oncogenes or tumor suppressors. Over the years, many studies have documented altered expression of miRNAs in different types of tumors in response to hypoxic conditions. The hypoxic microenvironment favors the selection of cancer stem-like cells that show features like indefinite self-renewal, chromosomal abnormalities, highly dysregulated differentiation, and tumor development. These factors favor the aggressiveness of tumors. So, the role of miRNAs in diagnosis, prognostication, and therapeutics of cancer is being explored by many researchers. In cancer therapeutics, different approaches like viral vectors, artificial biomolecules, locked nucleic acid, antisense anti-miR oligonucleotides, miRNA antagomirs, and miRNA sponges are being tried to revert the expression of miRNA back to its physiological state.

Keywords

Biomarkers · Cancer · Hypoxia · microRNA · Signature · Tumor

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7.1 Introduction

Around 70–80% of the human genes are transcribed to RNA. Out of this, only around 2% are coded to proteins. The remaining genome is made up of noncoding genes that get transcribed to noncoding RNA (ncRNA). ncRNAs comprise "long noncoding RNAs, microRNAs (miRNAs), PIWI-interacting RNAs, small interfering RNAs, and others." miRNAs are nonprotein-coding molecules containing about 24 nucleotides. They are not expressed to proteins. However, they are important as regulators of gene expression. These act as regulators in a number of cell processes, e.g., cell differentiation, proliferation, cell death, and development of cancer (Shen et al. 2013). miRNAs were initially discovered as small ncRNAs that regulate the timing of the development of larvae in Caenorhabditis elegans (Lee et al. 1993).

7.1.1 MicroRNA Biogenesis

The formation of miRNAs is a complex process and involves multiple steps. Long primary transcripts are processed by successive cleavage and maturation steps to form miRNAs. The major pathways of miRNA biogenesis are canonical pathway and noncanonical ones.

Canonical Pathway The formation of pri-miRNA by transcription of the noncoding region is the first step of miRNA synthesis. RNA polymerase II catalyzes this step. In the next step, the hairpin structure of pri-miRNA is cut by microprocessor complex that results in the formation of pre-miRNA. The microprocessor complex in turn is formed by the interaction of Drosha (RNase III enzyme) with the "Di George Syndrome Critical region 8" (DGCR8) gene. Further processing of pre-miRNA occurs after its transport to the cytoplasm by Exportin 5. There "RNase III Dicer" cuts the stem-loop structure of pre-miRNAs to form a double-stranded miRNA moiety, containing 18–24 nucleotides. "RNA-induced silencing complex" (RISC) is a molecular complex that is formed by Argonaute 2, Dicer, and other proteins. One strand of duplex miRNA remains incorporated into RISC. This strand is called guide or active strand. The other strand of the miRNA is subjected to degradation (Yoda et al. 2010). RISC binds to the target sequence on the mRNA in the 3' untranslated region. If miRNA base pairs perfectly with the mRNA target sequence, it promotes the degradation of mRNA. Imperfect pairing causes translational repression (Wightman et al. 2018). In various cancers, mutations are commonly found in genes coding for enzymes like Drosha, Dicer, Exportin-5, and Argonaute (Adams et al. 2014).

Noncanonical Pathway In this pathway, the proteins of canonical pathway like Dicer, Drosha are involved but combinations are different. There are two types of noncanonical pathways: Drosha/DGCR8-independent and Dicer-independent. miRtrons biogenesis is not dependent on Drosha/DGCR8 (Berezikov et al. 2007).

These pri-miRNAs are processed by splicing to form pre-miRNAs (Ruby et al. 2007). Pre-miRNAs are transported to the cytoplasm by Exportin-5. In cytoplasm, Dicer processes them to form mature miRNAs (Havens et al. 2012; Goymer 2007). The Dicer-independent pathway involves the cleaving of pri-miRNA to pre-miRNA by Drosha protein. Maturation of this miRNA is completed by loading it into Ago2 in the cytoplasm. For example, the maturation of miR-451 is not dependent on Dicer protein (Cheloufi et al. 2010). The biological roles of noncanonical miRNAs and their contribution to the development of cancer are not clearly understood.

7.1.2 Role of miRNA in Tumor Angiogenesis

The formation of new blood vessels, i.e., angiogenesis is important for physiological processes like the development of embryos and reproduction. It also plays an important part in pathological processes like the healing of wounds, tumor development, and progression. The various modes of development of new blood vessels in tumors are sprouting angiogenesis, intussusception, vasculogenesis, vascular mimicry, vessel co-option, or cancer stem-like cells differentiation into endothelial cells. The various factors regulating angiogenesis are growth factors, vascular genes, epigenetic mechanisms, and the expression of miRNAs. miRNAs regulating the various angiogenic mechanisms are called angiomiRNAs (angiomiRs) (Annese et al. 2020).

7.2 MicroRNAs

7.2.1 MicroRNAs in Cancer

Progressive DNA alterations play an important part in the development of cancers (Hanahan and Weinberg 2011). The initiation and growth of tumor are favored by angiogenesis, inflammatory mechanisms, and capacity of the tumor cells to evade immune response. Cancer is a disease of multifactorial etiology and involves both coding and noncoding parts of DNA. DNA mutations and epigenetic deregulation of the noncoding RNAs have been described in tumor progression. miRNAs have quickly evolved as a key molecular component. The role of noncoding genome in cancer development was first elaborated in chronic lymphocytic leukemia. The most common chromosomal abnormality in CLL (chromosome 13q14 deletion) was found to target miR-15 and miR-16 (Calin et al. 2005).

7.2.2 Biomarkers and Their Usefulness in Cancer Diagnosis

Biomarkers are very important for diagnosing and managing cancer patients. Each type of tumor has a specific microRNA signature that helps in its differentiation from the surrounding normal tissues. Based on these signatures, cancers can be

Table 7.1 Current clinical trials (open) using miRNA components in cancer diagnosis (ClinicalTrials.gov)

miRNA	Trial reference no.	Disease	Observation/intervention
Circulating	NCT04906330	Breast cancer	miRNAs released from the tumor cells into the bloodstream can be detected even when the tumor is undetectable by other methods
Circulating	NCT04965259	Liver cancer	Validating a panel of miRNAs for detecting hepatocellular carcinoma at an earlier stage in high-risk patients
miRNA-10b	NCT01849952	Glioma	Evaluating the expression levels of miRNA-10b in tumor, blood, and cerebrospinal fluid samples in patients with glioma
10 miRNAs	NCT04285476	Thyroid cancer	Validating a signature of 10 miRNAs to allow the stratification of the cytology of indeterminate type
Numerous	NCT04305366	Head and neck cancer	Investigating the presence of miRNA biomarkers in saliva, blood, fine needle aspirate, and tissue samples in patients with carcinoma of head and neck region and control group
miR_CPMRC	NCT04662996	Prostatic cancer	Studying the miRNA (MiR_CPMRC) expression to find the factors predicting the risk of resistance to treatment in metastasized castration-resistant prostate cancer
Circulating	NCT05146505	Ovarian cancer	Investigating miRNAs as probable biomarkers for diagnosing and assessing prognosis in high-grade serous ovarian cancer
miR200b	NCT03776630	Ovarian cancer and Endometrial cancer	Validation of the 5-miR index to assess the risk of metastasis to lymph nodes in ovarian cancer Prognostic value of the pre-/post-treatment changes in plasma concentration of miR200b with regard to progression-free survival

categorized into different prognostic groups. The circulating miRNAs are used as biomarkers for different cancers and this field is expanding rapidly. Currently, many observational studies and clinical trials are studying the importance of miRNA in diagnosing and assessing the prognosis of different cancer types. Some of these clinical trials are briefly summarized in Table 7.1.

7.2.3 Functions

miRNAs perform fine adjustments in the translation process according to cellular requirements and thus regulate gene expression. miRNAs strengthen the cellular processes by regulating the transcriptional processes (Ebert and Sharp 2012). miRNAs thus allow the cells to adapt to sudden, temporary changes in their microenvironment like in response to stress. e.g., in glioblastoma, a decrease in the level of miR-451 is found when glucose levels are low. This activates the "adenosine monophosphate-activated protein kinase" (AMPK) pathway leading to decreased cell proliferation and increased cell survival. The reverse occurs when energy levels are high. High miR-451 levels cause suppression of AMPK signaling and activate cell proliferation. Thus, miR-451 level in glioma correlated with the rapid growth of tumor and poor prognosis (Godlewski et al. 2010).

7.2.4 Therapy

Besides the role of miRNA as a biomarker, the miRNAs and anti-miRNA constructs are being investigated as potential therapeutics against cancer. Many clinical trials have evaluated the roles of OncomiRs or tumor suppressor miRNAs in regulating various cellular processes (Table 7.2). Currently, both nonviral (lipid-based, polymeric, nanoparticles) and viral miRNA delivery systems are being used. The miRNA-based therapies are a newly emerging field, and the adverse effects of these treatments need to be assessed.

7.3 miRNAs Responsible for Cancer Aggressiveness

Cancer aggressiveness term is used for highly invasive, end-stage cancer that is resistant to therapy and bears a poor prognosis. Depending on the target transcripts, miRNAs are classified into oncogenic (oncomiRs) and tumor suppressors (antioncomiRs). The cellular processes like proliferation, migration, invasion, and cell death are regulated by changes in miRNA expression. Besides this, regulation of miRNAs also affects angiogenesis, epithelial–mesenchymal transformation, sensitivity to radiotherapy and chemotherapy that favors tumor aggressiveness. Various studies focusing on the function/expression of miRNA in carcinoma prostate, carcinoma cervix, breast cancer, and glioblastoma have proven that miRNAs affect different aspects of tumorigenesis (Macharia et al. 2019). A study found the correlation of miR-10b expression in carcinoma breast patients with staging, survival, and size of tumor. Further, involvement of lymph nodes was associated with a higher miR-10b expression (Zhang et al. 2018a). So, it has been proposed that miRNAs may be used for the diagnosis and assessment of prognosis in many types of cancers.

Table 7.2 Clinical trials evaluating miRNA-based therapies in human cancers (Adapted from (Balacescu et al. 2019), ClinicalTrials.gov)

miRNA	Trial reference no.	Cancer type	Delivery system	Trial status
miR-16 mimic	NCT02369198	Non-small cell lung cancer Malignant pleural mesothelioma	EDVs (EnGeneIC Delivery Vehicle nanocell)	Phase I complete Likely to enter phase II
miR-34 mimic	NCT01829971	Small cell lung cancer Lymphoma Multiple myeloma Non-small cell lung cancer Melanoma Renal cell carcinoma Primary liver cancer	Lipid nanoparticles	Phase I terminated (serious adverse reactions related to immune system)
Anti-miR-255	NCT02580552	Chronic lymphocytic leukemia Cutaneous T-cell lymphoma Adult T-cell leukemia/ lymphoma Mycosis fungoides Diffuse large B-Cell lymphoma, ABC subtype	Locked Nucleic acid-modified antisense inhibitor	Phase I (active, recruitment not started)
Anti-miR-255	NCT03713320	Mycosis fungoides Cutaneous T-cell lymphoma	Locked nucleic acid (LNA)-modified antisense inhibitor	Phase II terminated (business reasons, no issues regarding efficacy/safety

7.4 miRNAs, Epigenetic Mechanisms, and Cancer Aggressiveness

Studies have found dysregulation of miRNA in a number of malignancies, yet the mechanisms underlying this phenomenon are not clear. miRNA dysregulation in cancer has been found to be due to abnormal DNA methylation and modifications in histone proteins mainly (Suzuki et al. 2013).

DNA is methylated by the enzyme DNA methyltransferase (DNMT). There are various types of DNMTs (DNMT1, DNMT3A, and DNMT3B). Out of these, DNMT1 is found to be most abundant in mammalian cells. In general, methyltransferases convert cytosines of CpG (cytosine-phosphate-guanine) dinucleotide sequences to 5-methylcytosine using S-adenosyl methionine as a methyl donor. Studies have reported both hyper and hypomethylation in different types of cancers. DNA hypomethylation is known to upregulate oncogenic miRNAs while tumor suppressors are downregulated by DNA hypermethylation, this facilitates the start and progression of cancer (Macharia et al. 2019).

Many types of histone modifications occur, acetylation and methylation being the common ones. Histone acetyltransferases (HAT) mediate lysine acetylation, which usually leads to the uncoiling of the chromatin and enhanced transcription. Removal of acetyl group mediated by histone deacetylases (HDAC) provokes chromatin condensation and leads to gene inactivation. The effect of methylation of lysine residues on gene expression is site-dependent and varies with the extent of methylation. Gene expression is activated by methylation at K36, H3K4, and K79 while at H3K9, H3K27, or H4K20 it leads to transcriptional repression.

The downregulation of miR-101, a tumor suppressor, is found in many tumors like glioblastoma. It regulates the histones H3K27me3, H3K9me3, H3K4me2, and H4K20me3 and reverses the methylation status of "cytoplasmic polyadenylation element-binding protein 1" (histone-modified hypomethylated gene) promoter (Li and Wu 2017). The breast cancer susceptibility gene (*BRCA1)* associates with HDAC2 and causes repression of miR-155 expression. The in vivo growth of tumor cell lines is accelerated by overexpression of miR-155 while its knockdown attenuates tumor growth *(*Chang et al. 2011*)*. miR-615 is epigenetically activated through the H3K9 acetylation and loss of DNA methylation in prostate cancer cells (Hulf et al. 2011).

7.5 Hypoxia Microenvironment

The tumor microenvironment (TME) is constituted of malignant cells, blood vessels, lymphatics, fibroblasts, immune cells, pericytes, adipocytes, and chemical and physical components (Balkwill et al., 2012). The interaction of TME with tumor cells is the predominant factor affecting clinical outcomes. During the development of tumor, cancer cells often have limited access to oxygen. Aberrant blood vessel formation and restricted blood supply in most of the solid tumors lead to hypoxic environment. This causes release of "hypoxia-inducible factors" (HIFs) that mediate cell response to hypoxia. The adaptation, selection, and propagation of cancer and stromal cells are dependent upon HIF signaling pathways, thus favoring the cancer progression (Petrova et al. 2018).

HIF helps in the transcription of various factors involved in normal homeostasis. Additionally, hypoxic tumor cells overexpress the factors like vascular endothelial growth factor (VEGF), platelet-derived growth factor-B (PDGF-B), epidermal growth factor, transforming growth factor-beta (TGF-β), enzymes of the glycolytic

pathway, and anti-apoptotic factors that lead to increased cell survival, growth, and metastasis. (Vaupel and Harrison 2004).

HIFs (HIF1, HIF2, and HIF3) are dimeric proteins containing alpha and beta subunits. The alpha subunit is cytoplasmic and is regulated in response to oxygen levels, whereas beta subunit is nuclear and is constitutively expressed. HIF1α and HIF2α, in association with HIF-1β, facilitate the major HIF transcriptional activity. In normoxic conditions, the two conserved proline residues of alpha subunit get hydroxylated. Von Hippel–Lindau tumor suppressor binds to hydroxylated HIF1α and gets degraded by the proteasomal system. During hypoxic conditions, HIF-1α is not hydroxylated and thus is not degraded; instead, it forms a heterodimer after binding with HIF-1β subunit in the nucleus. This active form of HIF-1 binds to specific areas on genes called hypoxia response elements of the target genes and regulates transcription (Emami Nejad et al. 2021). Under different oxygen tension, HIF1α and HIF2α display different stabilization patterns with respect to different oxygen concentrations. HIF-1α is mainly active in severe hypoxic condition (1% O_2) while HIF-2α is strongly expressed in cancer areas with good vascular supply (5% O_2) (Holmquist-Mengelbier et al. 2006).

7.6 Hypoxia and Cancer Aggressiveness

Pathological hypoxia in tumors favors the survival of cells and tumor growth. Hypoxia leads to upregulation of expression of HIF-1α and HIF-2α and its signaling molecules. This promotes angiogenesis, makes the tumor chemo- and radioresistant, and increases cancer aggressiveness.

7.6.1 Blood Vessel Formation

During embryogenesis, blood vessel formation takes place by vasculogenesis involving precursor cells that get differentiated into endothelial cells. This is followed by angiogenesis, i.e., the process of development of new blood vessels from the already present vessels. Then, the process of maturation occurs when the vessels interact with smooth muscles and other cells of connective tissue. In pathological conditions like tumor progression, abnormal angiogenesis is noted where hyperproliferating cancer cells become hypoxic due to a gap in oxygen supply and demand. The transcription factors HIF-1α and HIF-2α take part in various steps of blood vessel formation. They cause the endothelial progenitors to differentiate to mature endothelial cells. Matrix metalloproteinase expression is also enhanced, which leads to the sprouting and splitting of the already existing vessels. Lastly, HIF-α also helps in vessel maturation by inducing PDGF and TGF-β.

The new vessels formed in tumors are often immature and leaky, thus unable to meet the oxygen and nutrient requirements of the growing tumor cells. As the tumor grows, oxygen demand is further escalated. This aggravates the hypoxia, which in turn acts as a stimulus for angiogenesis. This culminates in a vicious circle. As a

result, the tumor tissue has abnormal, excessive vasculature that is unable to meet its oxygen demand (Muz et al. 2015).

7.6.2 Metastasis

Highly permeable and heterogeneous vasculature promotes the spread of tumor by the movement of tumor cells to different parts of the body. Hypoxic cells have better abilities to metastasize. Hypoxia affects the invasiveness and metastatic behavior of cancer cells by "epithelial–mesenchymal transitions" (EMT). EMT is an important phenomenon whereby the epithelial cells acquire mesenchymal phenotype (Thiery and Sleeman 2006). The phenomenon of EMT is observed during the development of embryos, tissue regeneration, and in many malignancies. There is a decrease in adherence of cells to each other and thus cell motility increases. There is downregulation of the expression of epithelial-associated genes like β-catenin and E-cad, and an increase in expression of vimentin, SMA, N-cad, and CXCR4 (mesenchymal-like genes). Hypoxia increases the concentration of TGF-β, a master regulator, that increases EMT by upregulating the synthesis of transcription factors like Snail and Slug (Muz et al. 2015).

7.6.3 Radiation and Drug Resistance

Hypoxia has also been implicated as a factor responsible for resistance to chemotherapy and radiotherapy in tumors. Hypoxia induces resistance to chemotherapy by (i) causing arrest in cell cycle, (ii) inhibiting cell death, (iii) regulating autophagy and mitochondrial activity, (iv) decreasing drug delivery and cellular uptake through an increase in acidity, and (v) reducing cytotoxicity of a number of drugs due to lack of adequate oxygen concentration.

Similarly, the state of tissue oxygenation is one of the significant factors for the radiation to act on cancer cells. Normoxic cells are irreversibly damaged by the ionizing radiations as oxygen increases the interaction with free radicals. On the contrary, hypoxic conditions confer radioresistance by decreasing the interaction between oxygen and ionizing radiation. Radiosensitivity also depends on phase of the cell cycle. Tumor cells are least sensitive if they are exposed to ionizing radiation at end of the S-phase and intermediate sensitive in the G1 phase. Radiosensitivity is observed maximally in the late G2 and M phases. Therefore, low proliferating, quiescent, hypoxic regions of tumor do not respond to chemo- and radiotherapy while rapidly proliferating normoxic tumor cell are sensitive (Muz et al. 2015)

7.7 microRNAs and Hypoxia Microenvironment

miRNAs act as regulators of many genes including hypoxia-related genes (Macharia et al. 2019). During hypoxia, miR-210 decreases the expression of succinate dehydrogenase cytochrome b small subunit, which is a part of the mitochondrial electron transport chain. This increases the stability of HIF-1, which further results in increased survival of cancer cells (Rupaimoole and Slack 2017). miR-210 has also been implicated in downregulating the apoptosis-inducing factor mitochondria associated 3 (AIFM3), thus supporting cancer cells' survival (Wang et al. 2014) and ephrin A3, an inhibitor of angiogenesis, leading to enhanced angiogenesis (Fasanaro et al. 2008). The von Hippel–Lindau (pVHL) is a major regulator of cell response to hypoxia. Its expression is downregulated by miR-155. pVHL in turn negatively regulates the HIF-1. Thus, the downregulation of pVHL promotes angiogenesis and survival of cancer cells (Kong et al. 2014). The injection of plasmids carrying antisense HIF-1a and VHL into the tumors led to complete regression of large tumors. This therapy promotes cell death by decreasing HIF-1, VEGF, and angiogenesis. These findings indicate that combinational therapy using blockage of HIF-1α and enhancement of VHL may prove to be more effective in the management of cancer patients (Sun et al. 2003). Additionally, some other miRNAs e.g., miR-21-5p, also downregulate VHL, which increases the expression of VEGF-A (Zhang et al. 2014). Similarly, miR-7-5p targets O-linked N-acetylglucosamine transferases and decreases the expression of VEGF receptor 2 (Babae et al. 2014). On the contrary, miR-128 overexpression leads to downregulation of p70S6K1, which reduces the expression of VEGF and HIF, leading to decreased angiogenesis and tumor growth (Shi et al. 2012).

The hypoxic microenvironment affects the synthesis of plenty of miRNAs. For example, tumor hypoxia, through hypoxia-responsive transcription factors, downregulates the expression of Drosha, the enzyme responsible for miRNA processing (Rupaimoole and Slack 2017).

Similarly, miR-103/107, let-7, and miR-630 directly target the DICER 30 untranslated region and downregulates it. Tumor hypoxia is shown to pronounce this effect (Macharia et al. 2019).

7.8 The Stem cells, Cancer Aggressiveness, and Hypoxia

Cancer stem-like cells (CSCs) are a group of cells in the parent tumor that can initiate self-renewal of the tumor cells via differentiation (Chen et al. 2012). They are also called tumor-initiating/propagating cells. CSCs comprise 0.1–0.8% of total tumor cells. CSCs bear similarity to normal stem cells in some features like self-renewal, capacity to divide for long periods, promotion of tumor spread to distant body sites and expression of the cell surface markers of stem cells. But unlike normal stem cells, CSCs show some differences in terms of indefinite self-renewal, growth pattern, chromosomal abnormalities, highly dysregulated differentiation, and

tumor development. Their role has been shown in the origin, progression, spread, and relapse of a number of tumors (Singh et al. 2003; Xie et al. 2016).

Thus, CSCs contribute to tumor aggressiveness as they influence growth of tumors and also have a role in chemo- and radioresistance (Debeb et al. 2009; Li et al. 2008).

Epigenetic modifications are important for the generation of CSCs (Shukla and Meeran 2014; Tao et al. 2018)). The reprogramming causes downregulation of the genes associated with differentiated state, whereas genes specific to stem cells are upregulated (Shukla and Meeran 2014). The Wnt/b-catenin signaling is crucial for cell processes like apoptosis and renewal of stem cell state. The abnormalities of this pathway have been implicated in numerous cancers. Abnormal activation of Wnt/b-catenin signaling due to DNA methylation leads to the silencing of various Wnt inhibitors in breast tumor (Klarmann et al. 2008). In addition, activation of EMT can induce tumor-initiating properties in cells. The defining characteristic of EMT is the loss of adherens junction protein, E-cadherin. Transcription gene silencing of E cadherin occurs due to histone deacetylation. Methylation of the E-cadherin promoter causes the recruitment of histone deacetylases (HDAC) to the site. Deacetylation compacts the chromatin by increasing the ionic interactions among histones and DNA and thus represses transcription (Wang and Shang 2013).

Thus, as is clear from the examples given above, abnormal epigenetic modifications may convert normal stem cells into CSCs and these acquire stem-like phenotypes. This stem-like phenotype helps to support malignant growth.

In addition, different cancer stem cell state markers are being explored to find their importance in cancer development and prediction of prognosis. Cancer stem cell-state markers CD24, CD44, CD133, and aldehyde dehydrogenase 1 (ALDH1) have been associated with carcinoma ovary. The presence of these markers has been linked with worse prognosis (Tao et al. 2018). Similarly, the CSCs markers CD44 and ALDH1 predict poor prognosis in carcinoma breast patients (Kong et al. 2018).

The tumor suppressor protein $p16^{Ink4a}$ limits the G1-S progression and thus regulates cell cycle. The stem cells with reduced expression of $p16^{Ink4a}$ and increased expression of ALDH1 have been found in cervical cancer patients. The level of these markers correlates with poor response to radiotherapy (Fu et al. 2018). On the contrary, cervical cancer patients with a higher level of $p16^{Ink4a}$ showed a better prognosis (Lin et al. 2014). Some other examples of the association of cancer stem markers with the prognosis of different cancers are the negative correlation of CD133 and CD44 with survival rate in gall bladder cancer (Pietras et al. 2014), and overexpression of OCT-4 in cervical cancer tissue in comparison to the normal tissue (Yang et al. 2014). The expression of Nanog protein is found to vary with the stage of cervical cancer. Increased expression is also found in cervical dysplasia in comparison to the normal epithelium (Ye et al. 2008). miRNAs have been studied in the context of hypoxia and stem-like state in some common aggressive tumors. For example, miR-210 has been associated with hypoxia in cervical and breast cancers while miR-23b and miR-125b have been associated with stem-like state in cancer cervix (Macharia et al. 2019).

7.9 miRNAs, Hypoxia, Stem-Like State, and Their Role in Therapeutics

The basis of miRNA therapeutics in the treatment of cancer is the fact that such molecules can be designed that mimic or inhibit mature miRNA. Based on the roles of miRNAs studied so far, it seems pertinent to think that overexpressed miRNAs can be targeted in cancer. In addition, reexpressing those miRNAs, which are downregulated in cancer, also represents a valid and logical approach. Similarly, the cancer stem cells and hypoxia microenvironment may be targeted too.

During prolonged hypoxic conditions, a process of HIF switch occurs that is a "shift of signaling from HIF-1 to HIF-2 and then to HIF-3." This promotes angiogenesis and prolongs cell survival. If the cell is unable to decrease the HIF-1 level, it initiates apoptosis. This switch can be targeted in cancer therapeutics. The role of miRNAs in regulating HIF switch during low oxygen conditions has been well documented, thus making them important therapeutic targets (Serocki et al. 2018). Upregulation of miR-18a after exposure to hypoxia for 24 hours has been documented. This miRNA directly targets HIF1A mRNA. This suggests that miRNA can induce a HIF switch (Han et al. 2015). Additionally, a different HIF signal regulation has also been elucidated. Here miRNA-103/107 mediates the suppression of beta subunits, thus limiting HIF-1 activity and inhibiting angiogenesis (Deng et al. 2016). Although the role of HIF-3 is not very clear, it mainly inhibits the function of HIF-1. miR-147a reduces its level in human cervical cancer (HeLa) cells, leading to stabilization and accumulation of HIF-1 (Wang et al. 2016). HIF-1α is known to be present in cancer stem cells as well as in normal progenitors, which precludes its use as a therapeutic target. Unlike HIF-1α, HIF-2α is not found in non-glioma stem-like cells but increased in glioblastoma stem cells, making it a target in terms of chemotherapeutic (Li et al. 2009). Although many HIF inhibitors have been discovered, none of them is specific inhibitors of HIF-1 (Rodriguez et al. 2016). Efforts are also being focused on developing selective HIF-2 inhibitors. In 2021, an oral HIF-2α inhibitor has been approved for management of von Hippel–Lindau (VHL) disease in adults. Currently, it is indicated for those patients of VHL, who also have associated tumors of kidney, CNS, and pancreas. This HIF-2α inhibitor decreases the transcription and expression of HIF-2α target genes, leading to decreased tumor growth and angiogenesis (FDA 2022).

The development of therapies against cancer stem cells poses a significant challenge as both cancer and stem-like cells need to be targeted. Additionally, these therapies may affect the normal organ/tissue function by blocking the regeneration of noncancerous tissue (Huang and Rofstad 2017).

However, a different cell-based delivery system using miRNAs or their inhibitors can be tried. It will affect the CSCs by modifying their functions or by altering the expression of important proteins related to their regulation.

As explained earlier, epigenetic mechanisms might be an important target of therapeutics, as these regulations play an important role in the modulation of miRNA expression. Targeting components of epigenetic pathways can stop the progression of cancer and modify the properties of cancer stem cells (Macharia et al. 2019).

Some of these epigenetic modulators are already approved by FDA while many others are in different stages of clinical trials (Toh et al. 2017).

7.10 Exosomal MicroRNAs in Cancer

Exosomes are extracellular vesicles released from most of the cells in eukaryotic organisms and they are present in all body fluids. Their size varies between 30 and 100 nm. A lipid bilayer forms their outer boundary while a variety of biomolecules like nucleic acids, lipids, and proteins can be their constituents (Abak et al. 2018).

Exosomes regulate cell milieu by the selective release of toxic biomolecules present in cells and immune response stimulation (Desdín-Micó and Mittelbrunn 2017). Their role in tumor development has also been described as they can be transferred between adjacent tumor cells and also from tumor cells into the adjoining milieu (Bullock et al. 2015). Exosomes may increase and maintain tumor cell proliferation and survival. They do so by initiating angiogenesis, remodeling of extracellular matrix, and affecting immune cells function (Iero et al. 2008). Additionally, they have a role to play in imparting drug resistance among tumor cells (Zhao et al. 2015).

miRNAs are an important constituent of exosomes, and their dysfunctional role has been documented in the proliferation of different cancer. For example, it is observed that let-7 miRNAs are found in exosomes released from gastric tumor cells (Ohshima et al. 2010). Additionally, levels of exosomal miRNAs like let-7f, miR-21, etc., are increased in cancer subjects in comparison to the control group (Silva et al. 2011).

Exosomal miRNAs exert significant effects on the control of cancer growth, angiogenesis, and spread to distant sites and impart chemoresistance. They regulate proteins related to cell cycle or signaling pathways. In colorectal cancer (CRC), the transmission of miR-200b is observed between cancer cells and thus their growth is promoted (Zhang et al. 2018b). The growth of hepatocellular carcinoma cells is inhibited by inhibition of the transfer of oncogenic miR-21 into exosomes (Liang et al. 2018).

Exosomal miRNAs also affect EMT. In oral cavity cancer, induction of EMT process occurs due to vimentin and snail's enhanced expression and E-cadherin's reduced expression. These changes are initiated by exosomal miR-21 (Li et al. 2016). While in prostate cancer, inhibition of the activities of N-cadherin and vimentin by exosomal miR-1246 leads to the suppression of the EMT process (Bhagirath et al. 2018). In HCC cells, endothelial proteins are the target of exosomal miR-103, thus increasing blood vessel permeability and advancing the spread of tumor (Fang et al. 2018).

7.10.1 Exosomal miRNAs Affect Chemotherapeutic Resistance in Cancer Cells

Exosomal miRNAs affect chemosensitivity by manipulating cellular signaling pathways. In prostate tumor cells, exosomal miR-34a regulates Bcl-2, leading to increased sensitivity to docetaxel (Corcoran et al. 2014). Gemcitabine sensitivity in pancreatic ductal adenocarcinoma (PDAC) cells is reduced by the macrophage-derived exosomes (MDEs) (Binenbaum et al. 2018). The breast cancer cells that are resistant to tamoxifen are found to have elevated levels of exosomal miR-221/222. This could decrease the target gene expression of p27 and estrogen receptors, hence imparting resistance to tamoxifen (Wei et al. 2014).

7.10.2 Exosomal miRNAs Can Be Used for Diagnosis and Prognostication of Cancers

A lot of research is going on to explore exosomal miRNAs as potential biomarkers for diagnosis and assessment of prognosis of cancer. For example, it is seen that exosomal miRNAs like miR-181b-5p, and miR-361b-5p levels were elevated in subjects suffering from non-small cell lung carcinoma (NSCLC) (Jin et al. 2017). Hence, NSCLC may be detected early by using these miRNA biomarkers. Additionally, in NSCLC patients, exosomal miR-451a, miR-21, and miR-4257 were detected in higher concentrations in comparison to controls and predict poor prognosis (Kanaoka et al. 2018; Dejima et al. 2017).

In esophageal squamous cell carcinoma, exosomal miR-21 and miR-1246 are elevated and are linked to tumor aggressiveness and progression (Tanaka et al. 2013; Takeshita et al. 2013). In glioma, the level of CSF exosomal miR-21 is found to be increased compared to controls and is associated with tumor spread, relapse, and dismal prognosis (Shi et al. 2015).

Epithelial ovarian cancers with elevated exosomal miR-200c and miR-200b show increased growth and bad prognosis (Meng et al. 2016), while, in patients with HCC, a negative correlation of exosomal miR-638 with HCC progression is observed (Shi et al. 2018). Thus, exosomal miR-638 may be studied to explore its role in determining the prognosis of HCC.

Early diagnosis is of paramount importance in starting prompt treatment and thus improving the prognosis in patients living with cancer. Presently, serum biomarkers like alpha-fetoprotein, carcinoembryonic antigen, and prostate-specific antigen are used for detecting some specific tumor, but due to inadequate sensitivity and specificity, their role is limited (Lu et al. 2017). The role of exosomal miRNAs as useful biomarkers for cancer detection seems promising because of their better stability, noninvasive nature and widespread presence in different body fluids.

7.11 MicroRNAs as Potential Therapeutics Against Cancer

miRNA-based gene therapy is being explored as a new approach for cancer treatment owing to its ability in predicting tumor growth and its local and distant spread. The expression of miRNA back to physiological state can be achieved in two ways. If oncomiR is overexpressed, miRNA activity needs to be inhibited, while in the case of suppression of tumor suppressor miRNA, miRNA activity needs to be restored. (Abd-Aziz et al. 2020).

7.11.1 miRNA Inhibition Therapy

In this approach, inhibitors of miRNA are employed to suppress oncomiRs activity in tumor cells. This can be achieved by using various biomolecules such as locked nucleic acid (LNA), antisense anti-miR oligonucleotides (AMO), miRNA antagomirs, and miRNA sponges. miRNA inhibitors lead to the inactivation and removal of the miRNA from RISC (Shah et al. 2016).

AMOs are chemically altered oligo nucleotides. They restore normal translation by inhibiting the binding of miRNAs to specific mRNAs. For example, LNA, a modified AMO inhibits overexpressed miR-21 in glioblastomas (Griveau et al. 2013).

Antagomirs are ssRNA molecules containing 23 nucleotides. They are chemically altered and act as complementary to the targeted miRNAs, thus helping in increasing their stability and preventing it from the breakdown (Meister et al. 2013; Krützfeldt et al. 2005). For example, in tumor cells of mouse mammary glands, metastasis can be inhibited by using miR-10b antagomirs (Ma et al. 2010).

miRNA sponges act as competitive inhibitors of oncomiRs and contain multiple artificial miRNA binding sites (Ebert and Sharp 2010). The circular RNA (circRNA) found naturally in cells acts as endogenous miRNA sponges. With the help of a simple enzymatic ligation technique, a functional and artificial circRNA sponge can be produced. It regulates miRNAs by binding to different miRNA sites. It has been shown that the circRNA can be transfected into miRNA21, leading to inhibition of the proliferation of gastric tumor cells (Liu et al. 2018).

7.11.2 miRNA Restoration Therapy

In this approach, downregulated tumor suppressor miRNAs are restored, leading to apoptosis induction or inhibition of the proliferation of tumor cells. Viral vectors that express miRNAs or artificial biomolecules that mimic miRNAs are employed. miRNA mimics act by restoring the normal functioning of endogenous miRNAs. The chemically altered miRNAs are incorporated into RISC, leading to the target mRNA inhibition (Shah et al. 2016). For example, miR-15, an miRNA mimic, reduced growth in prostatic cancer cell lines by inducing cell death (Bonci et al. 2008).

In another strategy, miRNA expression vectors are used to induce tumor suppression by increasing the expression of the miRNAs. Some examples of vectors are adenoviral, lentiviral, and retroviral vectors. For example, in liver cancers, miR-26 expression is lost. Recombinant adenovirus loaded with the miR-26 injected through intravenous route results in suppression of tumorigenicity by increasing tumor apoptosis and inhibiting cellular proliferation (Kota et al. 2009).

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