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MICRORNAS AS DOUBLE EDGED SWORD IN CANCER

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ABSTRACT

Cancer is the leading causes of death world wide. Basically it can be defined as the disease of the cell. One among the mechanism of transfer of a normal cell to cancerous is the alteration in the genetic material DNA. Treatment for cancer remains as a biggest challenge. Here comes the importance of miRNAs. They are endogenous RNA which regulates a wide range of cellular processes such as proliferation, differentiation, development and apoptosis by suppressing the expression of target mRNA thus playing a central role in various human diseases including cancer. Hence advancement in miRNA research is necessary to develop it as a powerful therapeutic tool in cancer. Oncomirs are miRNAs acts as double edged sword in cancer because up-regulation and down-regulation of miRNAs are observed in cancerous cells and hence acts as oncogenes and tumor suppressors respectively. miRNAs can act as potential biomarkers. Studies shows that the change in level of miRNA are directly associated with cancer. Hence till-date reviewing as onco-miRs is necessary which further the researcher to develop it as a specific and potential target in cancer treatment. miRNAs are promising therapeutic tools for cancer because in humans, 50% of miRNA genes were localized in cancer-associated genomic regions which include minimal regions of amplification, loss of heterozygosity, fragile sites and common breakpoint regions. miRNA are found to be up-regulated and down-regulated in almost all cancer cells. Hence, in this article an attempt is made to review the evidence of microRNAs in cancer as both oncogenes and tumor suppressors.

Keywords: MicroRNA, Double Edged Sword Tumor Suppressors, Onco-miRs, Gene Regulation

1. INTRODUCTION

MicroRNAs are small ~22 nucleotides (nt) long, non protein coding, single standed RNAs found in both plants and animals (Bartel, 2004). Lee *et al*. (1993) in the Victor Ambros lab first discovered miRNA, *lin-4* in *Caenorhabditis elegans* through a genetic screen for defects in the temporal control of post-embryonic development. The term microRNA was coined (Ruvkun, 2001). Unlike siRNA, they are found endogenous (Bartel, 2004; Ambros, 2001; Carrington and Ambros, 2003). Many miRNAs are found to be highly conserved molecules (Pasquinelli *et al*., 2000). The complete mature miRNA sequence of *let-7*, isolated primarily in *Caenorhabditis elegans*, has been evolutionarily conserved from worms to humans (Lee and Ambros, 2001). Currently, thousands of miRNAs have been identified in nematodes, amphibians, fishes, plants, mammals and viruses (Zhang *et al*., 2006a) using different approaches including experimental methods, computational approaches, EST and Genomic Survey Sequence analysis (Lee and Ambros, 2001; Brown and Sanseau, 2005; Zhang *et al*., 2005; 2006b). Out of hundreds of miRNAs that have recently been identified, only about 200-300 miRNAs have been currently identified in humans (Griffiths-Jones, 2004; Griffiths-Jones *et al*., 2006). Studies prove that miRNAs could also cause gene silencing (Lee *et al*., 1993; Pasquinelli *et al*., 2000;

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Wightman *et al*., 1993; Olsen and Ambros, 1999; Reinhart *et al*., 2000). Recent evidence indicates that miRNAs exhibit important regulatory roles in development, cell proliferation, cell survival and apoptosis and thus play a central role in gene regulation in health and disease (Bentwich *et al*., 2005; He and Hannon, 2004; Meltzer, 2005). In this review, we brief gene regulation of miRNA, its association with cancer, furthermore with instances to prove it as tumor suppressors and oncogenes.

1.1. miRNA Gene Regulation

In humans, miRNAs target more than 30% of protein coding genes (Berezikov *et al*., 2005; Lewis *et al*., 2005) and the total number of miRNAs by computational analysis indicates there may be more than 1% of the total protein coding genes (Lai *et al*., 2003; Lim *et al*., 2003a; 2003b). MicroRNA biogenesis and mechanism of gene repression are similar to those of exogenous small interfering RNAs (siRNAs) (Izquierdo, 2005). The genes encoding miRNAs are much longer than the processed mature miRNA molecule. MicroRNAs negatively regulate the target gene expression in a variety of ways including translational repression, mRNA cleavage and deadenylation. MicroRNAs can direct RISC to down-regulate gene expression by binding with perfect or nearly perfect complementarity to protein coding mRNA sequences. Most animal miRNAs are thought to use a gene regulation mechanism that does not involve the cleavage of their mRNA targets (Filipowicz *et al*., 2005). RISC is a ribonucleoprotein complex containing members of the Argonaute (Ago) family of proteins. Argonaut is also partially responsible for selection of the guide strand (incorporated strand) on the basis of the stability of the 5' end and destruction of the passenger strand. The strand with lower stability base pairing of the 2-4 nt at the 5' end of the duplex preferentially associates with RISC and thus becomes the active miRNA (Schwarz *et al*., 2003). MiRNA genes can be located in the introns and/or exons of protein-coding genes or in the intergenic regions between protein-coding genes. They can form polycistronic clusters or exist individually (Bartel, 2004; Lagos-Quintana *et al*., 2001; Kim and Nam, 2006). A single miRNA could regulate multiple target genes, while a single gene could be targeted by multiple miRNA (Wu *et al*., 2006). Location of miRNA near protein coding genes and its ability to regulate genes proves its importance in medicine.

1.2. miRNAs and Cancer

Cancer is a complex genetic disease in which oncogene amplication and/or tumor suppressor gene mutation leads to step-wise deregulation of cell proliferation and apoptosis (Wu *et al*., 2006). A number of studies reported that specific microRNA signature had been found in each cancer tissue and microRNA based cancer classification is a very effective and potential tool (Lu *et al*., 2005). The involvement of miRNAs in cancers was confirmed through the observation that miRNAs are frequently located in cancer-associated genomic regions, which include minimal regions of amplification, loss of heterozygosity, fragile sites and common breakpoint regions in or near oncogenes or tumor suppressor genes (Calin *et al*., 2004a). The first report linking miRNAs and cancer involves CLL (B cell lymphocytic leukemia) (Calin *et al*., 2002). miRNAs show globally lower expression in cancer tissues than in normal tissues (Lu *et al*., 2005). Abnormalities in miRNA expression have been implicated in several forms of solid tumors such as cervical (Lee *et al*., 2008), breast (Iorio *et al*., 2005), colorectal (Cummins *et al*., 2006), lung (Hayashita *et al*., 2005) and also in at least two forms of leukemia (Calin *et al*., 2004a; 2004b). Avissar *et al*. (2009) used quantitative RT-PCR to study the expression of miR-375 in Head and Neck Squamous Cell Carcinomas (HNSCCs), found higherexpression of this miRNA in tumors of pharyngeal and laryngeal origin suggesting that alterations in miRNA expression are related to cancers and are useful biomarkers in cancers. This instance shows the role of miRNA in cancer and as potent biomarker.

1.3. miRNAs as Tumor Suppressor

A variety of miRNAs have been identified that appear to have tumor suppressor functions. A recent study has shown that there is a global down-regulation of miRNA expression in various tumor tissues (Lu *et al*., 2005). B Cell Lymphocytic Leukemia (CLL) is characterized by the deletion of miR-15a and miR-16-1, two clustered miRNAs, within the 13q14.3 locus (Calin *et al*., 2002). Deletions at this region also occur in approximately 50% of mantle cell lymphoma, in 16-40% of multiple myeloma and in 60% of prostate cancers, suggesting the location of one or more tumor suppressor genes at this locus (Dong *et al*., 2001). Northern analysis suggested that both miRNAs were down-regulated in the majority of cases (approximately 70%). A predicted target of these miRNAs is B cell lymphoma 2 (Bcl2), an anti-apoptotic

protein. The down-regulation of miR-15 and miR-16 leads to an in increase in Bcl2 expression (Cimmino *et al*., 2005). miR-15a and miR-16-1 were also expressed at lower levels in pituitary adenomas as compared to normal pituitary tissue and their expression inversely correlated with tumor size (Bottoni *et al*., 2005).

Let-7 miRNAs are considered as classical tumor suppressors due to their frequent down-regulation in cancers like lung or colon (Lu *et al*., 2005; Volinia *et al*., 2006). Ras oncogenes were the first target described to be regulated by the *let-7* miRNA family. In both *C. elegans* and human lung cancer cell lines *let-7* negatively regulated RAS (Johnson *et al*., 2005) and it was not expressed in human lung cancer tissues (Johnson *et al*., 2005; Takamizawa *et al*., 2004). Furthermore, overexpression of *let-7* in A549 lung adenocarcinoma cell line inhibited lung cancer cell growth (Takamizawa *et al*., 2004).

Recent studies reported that another target of *let-7* miRNA family is High mobility group a2 (Hmga2) protein, oncogenic in a variety of tumors, including benign mesenchymal tumors and lung cancers. Chromosomal translocations disrupt the repression of Hmga2 by *let-7* miRNA which promotes anchorage-independent growth, a characteristic of oncogenic transformation (Lee and Dutta, 2007; Mayr *et al*., 2007). In lung cancer cell line restoration of the steady state levels of *let-7* inhibited cell replication. Studies in human colon cancer tumors and cell lines show that in addition to Ras, c-Myc might also be a target of *let-7* as its expression reduces levels of RAS and c-MYC proteins (Akao *et al*., 2006). miR-143 and miR-145 has been found to be down-regulated in colorectal cancers (Slaby *et al*., 2007), B-cell lymphomas (Akao *et al*., 2007) and in cervical cancers (Lui *et al*., 2007; Martinez *et al*., 2008). A recent report showed that TCL1, an oncogene that is overexpressed in CLL cells (Herling *et al*., 2006) and Mcl-1, an anti-apoptotic Bcl-2 family member (Mott *et al*., 2007) are targets of miR-29 genes (Pekarsky *et al*., 2006).

Ciafre *et al*. (2005) identified a group of miRNAs: *miR-128*, *miR-181*a, *miR-181*b and *miR-181*c were down-regulated in glioblastoma (Ciafre *et al*., 2005). In primary neuroblastoma tumors, miR-34a on chromosome 1p36.23 was generally expressed at lower levels and it directly targeted the mRNA encoding E2F3 and significantly reduced E2F3 protein levels. This result suggested that miR-34a acted as a tumor suppressor of neuroblastoma tumorigenesis (Welch *et al*., 2007). MiR-34a was frequently absent in pancreatic cancer cells and its responsive genes were highly enriched for those that regulated cell-cycle progression, apoptosis, DNA repair and angiogenesis (Chang *et al*., 2007). Slack's research group demonstrates that *let-7* miRNA inhibits the growth of lung cancer cells in culture and in lung tumors in mice. They also showed that *let-7* can be applied as an intranasal drug to reduce tumor formation in a RAS mouse model lung cancer (Esquela-Kerscher *et al*., 2008).

Bhattacharya *et al*. (2009) identified two miRNAs, miR-15a and miR-16, that are underexpressed in ovarian cell lines and in primary ovarian tissues. Oncogenic activation of Bmi-1 is found in a wide variety of epithelial malignancies including ovarian cancer. Bmi-1 protein levels are down-regulated in response to miR-15a or miR-16 expression and lead to significant reduction in ovarian cancer cell proliferation and clonal growth, suggesting the development of therapeutic strategies by restoring miR-15a and miR-16 expression in ovarian cancer and in other cancersn (Bhattacharya *et al*., 2009).

1.4. miRNA as oncogenes

He and colleagues found the over expression of the miR-17-92 polycistron at 13q31.3 in B-cell lymphomas (He *et al*., 2005). It is also found to be over expressed in several other cancers such as solid cancers (Volinia *et al*., 2006), lung cancer (Hayashita *et al*., 2005) and malignant lymphoma cell lines (Tagawa and Seto, 2005) proving it as potential oncogenes. Recent studies in mouse B-cell lymphoma model reveals miR-19 is a key oncogenic componenet of mir-17-92 cluster (Olive *et al*., 2009). In breast tumors *miR-21* was found to be over expressed when compared to normal breast tissues (Iorio *et al*., 2005; Si *et al*., 2007). Zhu *et al*. (2007) identified down-regulation of tumor suppressor, tropomyosin 1 in breast cancer by *miR-21* could result in tumor growth supporting the notion that tropomyosin 1 as a potential *miR-21* target. It was found to be over expressed in head and neck cancer cell lines (Tran *et al*., 2007), brain tumor and glioblastoma (Chan *et al*., 2005) proving it as an oncogene. The glioblastoma tissues and glioblastoma cell lines analysis shows strong upregulation of miR-221 (Ciafre *et al*., 2005).

In Cervical cancer tissues, increased expression of miR-15b, miR-16, miR-146a, miR-155 and miR-223 has been observed. Cimmino *et al*. (2005) reported that miR-15 and miR-16 regulate apoptosis by targeting BCL2. miR-155 was found over expressed in lung cancer (Yanaihara *et al*., 2006), lymphoblastic leukemia/high-grade lymphoma (Calin *et al*., 2005), B-cell

lymphomas, Hodgkin's lymphomas, Burkitt lymphomas and in human breast cancer cells suggesting that it may act as oncogene (Iorio *et al*., 2005; Metzler *et al*., 2004; Eis *et al*., 2005; Kluiver *et al*., 2005).

Studies proved that miRNA expression can be regulated by DNA methylation and it has been suggested that altered miRNA gene methylation might contribute to human tumorigenesis. *Let-7*a-3 was found to be methylated by the DNA methyltransferases DNMT1 and DNMT3B. The gene was heavily methylated in normal human tissues but hypomethylated in some lung adenocarcinomas. Brueckner *et al*. (2007) identified let- 7a-3 as epigenetically regulated miRNA gene with oncogeneic function and suggest that aberrant miRNA gene methylation might contribute to the human cancer epigenome. Analysis of human breast tumors by Wang *et al*. (2009) revealed that miR-27b expression increases during cancer progression, paralleling a decrease in Suppressor of Tumorigenicity 14 (ST14) expression. The 3′-untranslated region of ST14 contains a regulatory element for miR-27b and luciferase experiments indicate that antisense miR-27b enhances ST14 expression in cancer cells which reduces cell proliferation as well as cell migration and invasion. Knockdown or over expression of a specific miRNA allows studying the specific roles of the miRNA in cancer development.

2. CONCLUSION

Oncogenic micro RNAs or Oncomirs are miRNAs with a role in cancer. The first functional evidence of a miRNA, or any non-coding RNA, acting as a mammalian oncogene is about mir-17-92 from studies of Hammond (2006) in B-cell lymphoma. For this reason they refer the host transcript of mir-17-92 as OncomiR-1 (Hammond, 2006). The above discussions clearly reveal microRNas can act both as tumor suppressors and oncogenes. They can be considered as potential therapeutic targets for cancer. Antisense inhibitors could be used to target oncogenic miRNAs and desired therapeutic strategy would increase the function of tumor suppressor miRNAs in cells. The small size and molecular properties of miRNAs makes it agreeable targets and therapeutics in cancer treatment.

2.1. Future Prospective

All non coding RNAs are not miRNAs, hence identifying a common sequence signature or biochemical action helps to overcome this challenge. Little is known about how miRNAs are regulated; much less what polymerase transcribes them. Furthermore, nothing is known about what signals conveys the temporal and/or spatial expression of miRNAs. This can be predicted to become an active area of research that will be highly important in the study of development and disease. Moreover predicting the impact of miRNAs on target proteins is challenging because of their different regulatory effects at the transcriptional and translational levels. Using chromatin modifying drugs to activate tumor suppressor miRNAs can regulate target oncogenes and it may lead to novel cancer therapies in the future. miRNAs can complement other genomic and proteomic biomarkers for cancer diagnosis and prognosis (Cho, 2007; Cho and Cheng, 2007). While hundreds of human microRNAs are known, relatively little is known about their roles and targets. Effective delivery of microRNA in to targeted tissues and maintaining their continuous activity still remains as an obstacle. Once overcoming all these difficulties miRNA remains as a promising cancer therapeutic tool.

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