Small is the new big – interplay of miRNAs in cancer

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MicroRNAs (miRNAs) are small non-coding regulatory RNAs that post-transcriptionally regulate gene expression. About 1500 miRNAs have been discovered in humans and these can target and regulate up to 60% of coding genes. MicroRNAs play a key role in all cellular functions and are implicated in most diseases, including cancer. Deregulation of microRNA expression can affect various metabolic and signalling pathways and results in cancer development and progression. This review provides a comprehensive view of various cancer types and their associated microRNAs.

Keywords: Cancer subtypes, deregulation, gene expression, microRNAs.

Introduction

MicroRNAs (miRNAs) are a group of small non-coding RNAs that post-transcriptionally regulate gene expression1. miRNAs can be located in introns, exons of coding genes, non-coding genes and intergenic regions. The biogenesis of miRNAs is a complex process. RNA polymerase II (RNA Pol II), or less frequently Pol III, generates a primary miRNA transcript (pri-miRNA). The pri-miRNA is then processed by a microprocessor complex to yield a ~70 nt precursor miRNA (pre-miRNA). The pre-miRNA is then exported to cytoplasm via exportin-5, where it is further processed into unstable 19–25 nt miRNA duplex structures by RNase III protein called Dicer. The less stable of the two strands (guide strand) is incorporated into a ribonucleotide complex to form miRNA-induced silencing complex (miRISC). Based on the complementarities between the guide miRNA and target mRNA, miRNA can either cleaves target RNAs with the help of Ago2, or induce a translational suppression (Figure 1). miRNA-mediated gene regulation is crucial in the biological system as it can regulate hundreds to thousands of different mRNAs that play essential roles in various metabolic, signalling or developmental pathways. Therefore, any alteration of miRNA expression can result in various human disease conditions, including cancer2.

Cancer is considered a complex genetic disease that involves long-term accumulation of various mutations in coding as well as non-coding genes3. With the increasing awareness of small RNAs and long non-coding RNAs, it is crucial to understand the role of these regulatory RNAs on various cellular pathways and how their deregulation can transform a normal cell into a cancer cell. miRNAs can bind and deregulate tumour suppressor genes such as p53 (ref. 4). p53 plays a crucial role in regulating various key processes, such as cell-cycle progression, migration, epithelial–mesenchymal transition, stemness, metabolism, differentiation and cell survival4. Thus, targeting such miRNA levels in the cancer patients can be a potential therapeutic.

miRNAs that are altered in various cancers can act as potential biomarkers that will help us understand the disease state and progression. There are various reports that support the evidence of miRNAs having important diagnostic utilities, the advantage being that a low quantity of tissue samples or body fluids is needed to assess miRNA levels. Additionally, miRNA expression profiles can easily distinguish normal tissue from tumour tissue and also different subtypes of cancer5. Moreover, assessing miRNAs is a stable and less invasive diagnostic tool and miRNA levels can help in predicting the recurrence and metastasis of cancer6. Together, miRNAs can function as potential diagnostic and prognostic markers and further aid in therapeutics7,8. In the present review article, we have focused on microRNAs and their crucial role in tumourigenesis and metastasis in various types of cancer.

miRNAs in common types of carcinomas

Oral cancer

Oral cancer arises from different regions of the oral cavity9. Oral cancer is heterogeneous in nature and shows dismal survival rate of ~50% that has not changed for decades. It is the sixth most common cancer occurring globally and accounts for 30% of all cancers in India10. Thus, a better understanding of the molecular basis of tumourigenesis is needed to facilitate the development of drugs and strategies that will lead to improved clinical outcomes.
Soga et al.\textsuperscript{11} were the first to perform a comprehensive miRNA profiling study of oral cancer through an exhaustive bioinformatics analysis. Using TaqMan miRNA Array 2.0, they found 12 miRNAs (miR-31*, miR-31, miR135b, miR-193a-5p, miR-103, miR-224, miR-93, miR-200c, miR-183, miR-203, miR-21 and miR-223) were upregulated more than four fold in oral cancer compared to the normal subjects. miRNAs such as miR-21, miR-203, miR-31 and miR-31* have already been functionally validated to play a crucial role in the oncogenicity of various malignancies\textsuperscript{12–16} and miR-31 and miR31* have been shown to contribute to oral cancer\textsuperscript{17}.

Lu et al.\textsuperscript{18} discovered that 23 miRNAs are differentially expressed between six oral cancer cell lines and five lines of normal oral keratinocytes. Of the 23 miRNAs, they found that miR-10-b was the most upregulated in cancer cell lines and xenograft oral cancer mouse model (20-fold). miR-10b is crucial for cell migration and invasion in many cancers\textsuperscript{19,20}. miR-10b was found in the plasma of cancer patients but was absent in normal subjects, indicating its diagnostic potential. The other miRNAs such as miR-196a, miR-196b, miR-582-5p, miR-15b, miR-301 and miR-148b were also found to be upregulated in oral cancer patients. On the other hand, miR-128a, miR-503 and miR-31 were downregulated in patients compared to normal subjects.

In summary, studies have shown that miRNAs play a diagnostic and/or prognostic potential as biomarkers for oral cancer. However, many of these miRNAs need to be functionally validated in relation to oral cancer.

**Esophageal cancer**

Esophageal cancer (EC) is the eighth most common cancer worldwide, with 481,000 new cases (3.8% of the total) estimated in 2008, and the sixth most common cause of death from disease with 406,000 deaths (5.4% of the total)\textsuperscript{21}. Despite being a commonly diagnosed cancer, the prognosis of EC is poor\textsuperscript{22}. Thus it becomes urgent to find novel diagnostic and prognostic markers in the clinic and for treatment of the disease\textsuperscript{23}. Guo et al.\textsuperscript{23} identified seven miRNAs (upregulation of miR-25, -424 and -151; downregulation of miR-100, -99a, -29c and -140) that can have a potential function as biomarkers to distinguish malignant EC from normal tissue. Additionally, several studies have shown that miRNAs are consistently identified in systemic circulation in patients diagnosed with EC compared to healthy individuals\textsuperscript{24}. For instance, miR-21 that is overexpressed in various types of EC was found upregulated in the plasma of esophageal squamous cell carcinoma (ESCC) patients compared to healthy individuals. Similarly, a panel of serum miRNAs (miR-10a, -22, -100, -148b, -223, -133a and -127-3p) was found upregulated in ESCC, which could distinguish stage I/II ESCC patients from the control group\textsuperscript{24}.
Recently, miRNAs have been established to regulate invasion and metastasis that are known to cause mortality due to cancer\(^{22}\). In EC, miRNAs miR-143, -145 and -133a/b were identified that significantly inhibited cell growth and invasion by targeting FSCN1 that promotes cell motility and growth\(^{25}\). Moreover, a significant correlation was found between miR-10b and suppression of a tumour suppressor gene, KL4, which resulted in enhanced invasiveness\(^{26}\). miR-92a was another crucial miRNA that was highly overexpressed in various tumour tissues and significantly correlated with the lymph node metastasis status and TNM staging that led to the suppression of cadherin1 (CDH1) expression\(^{27}\). Consequently, inhibition of miR-17-92 cluster member miR-19a by antisense oligonucleotides induced apoptosis and inhibited tumour growth in vivo, probably through targeting tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\))\(^{28}\). Therefore, miRNAs seem to have a significant potential role in understanding and identifying EC in various stages, including metastasis.

**Colon cancer**

Colon cancer (CC) is one of the leading causes of death worldwide and third most common cancer that affects both men and women\(^{29}\). Deregulation of signalling pathways such as WNT, RAS–MAPK, PI3K, TGF\(\beta\), p53 and DNA mismatch-repair pathways leads to initiation and progression of CC\(^{30}\). Most of the studies classify CC on the basis of microsatellite instability (MSI), which is frequently associated with CpG island methylator phenotype (CIMP and hyper-mutation). The other classification is based on the patients that are microsatellite stable but chromosomally unstable\(^{31}\). The Cancer Genome Atlas Project has taken leaps to profile genomic changes in 20 different cancer types and has so far published results on ovarian cancer, glioblastoma and CC\(^{30,32,33}\). A comprehensive integrative analysis was performed on 224 colorectal tumour and normal pairs, and the tumours were identified on the basis of possible biological differences in colon and rectum tumours. The analysis revealed that in majority (94\%) of the non-hypermethylated tumour samples, irrespective of copy number, anatomical origin had a mutation in one or more members of the WNT signalling pathway, predominantly in APC\(^{30}\). Recently, our group identified differential expression of 39 miRNAs, including miR-135b, -96, -182, -1 and -133a depending on mismatch-repair status of CC tissues relative to normal colon tissues\(^{34}\). Further, we have examined 52 normal colonic mucosa, 41 adenomas (polyps), 158 adenocarcinoma with proficient DNA mismatch repair (pMMR), and 64 adenocarcinoma with defective mismatch repair (dMMR) that were selected on the basis of sporadic (\(n = 53\)) and inherited CC (\(n = 11\))\(^{35}\). We observed that all the sporadic dMMR had inactivated MLH1 due to promoter hypermethylation\(^{35}\). Based on unsupervised PCA and cluster analysis, we were able to demonstrate that miRNAs could easily distinguish normal colon tissue, adenomas, pMMR carcinomas and dMMR carcinomas\(^{35}\).

In a comparison between pMMR and dMMR tumours, we identified four miRNAs (miR-31, -552, -592 and -224) that were statistically different (≥ two fold change)\(^{35}\)

We have identified common miRNAs that are perturbed in multiple tumour types, including colon cancer, rhabdomyosarcoma (both ARMS and ERMS types) and synovial sarcoma\(^{36}\). The analysis revealed that miR-183 was significantly upregulated in CC, ERMS, ARMS and synovial sarcoma. miR-135b was upregulated in three cancer types, excluding ERMS. In concurrence with the analysis, we found that miR-183 cluster members (miR-96 and miR-182) were also upregulated related in CC. Furthermore, we identified that miR-183 regulates EGR1, a tumour suppressor gene that in turn regulates PTEN, another important tumour suppressor gene. On knockdown of miR-183 in CC cell lines, we found a significant deregulation of a miRNA network composed of miR-183–EGR1–PTEN\(^{36}\). Thus, miR-183 has a potential oncogenic role through the regulation of tumour suppressor genes such as EGR1 and PTEN. The deregulation of this miRNA regulatory network is conserved in many cancer types.

**Hepatocellular carcinoma**

Hepatocellular carcinoma (HCC), one of the most common cancers, results from an array of deregulation of multiple intracellular and extracellular signalling pathways. miRNAs have been shown to play a major role in such deregulation of signalling pathways\(^{37}\). For instance, miR-199a/a* family and miR-1 are downregulated in HCC that post-transcriptionally regulates MET, which is overexpressed in 40–70% of HCCs\(^{38}\). It has been shown that miR-199a/a* and miR-1 are methylated in HCCs, especially in primary tumours\(^{38}\). Additionally, RAS family present downstream of such RTK receptors contains multiple complementary sites for binding of let-7 members, and overexpression of let-7 in various cancer cells has shown reduced RAS protein levels\(^{39}\). Thus, there is a potential role of let-7 downregulation in HCC.

Another study showed a direct role of miR-221 that is upregulated in various cancers, including HCC, suggesting its oncogenic role\(^{36}\). Its oncogenic function was further confirmed by its ability to modulate the expression of the cyclin-dependent kinase inhibitor CDKN1B/p27, a key controller of cell cycle progression\(^{40}\). More recently, miR-221 has been shown to target another cyclin-dependent kinase inhibitor, CDKN1C/p57 BH3-only protein BIM\(^{41,42}\). Through this mechanism, miR-221 can protect cancer cells from anoikis, leading to metastasis.

Also, miR-122 is downregulated in more than 70% of HCCs\(^{43}\). Studies revealed that miR-122 targets cyclin G1,
which is a negative regulator on p53 that activates MDM2, thus leading to p53 degradation\textsuperscript{44}. As a result, cyclin G1 overexpression can result in cell proliferation\textsuperscript{44}. Recently, it was shown that the absence of cyclin G1 was associated with a lower susceptibility to liver tumours, which was associated with an increased p53 tumour suppressor activity in a mouse model\textsuperscript{46}. Therefore, the upregulation of cyclin G1 due to miR-122 downregulation in human HCC may lead to p53 downregulation and promote tumourigenesis. Further, miRNAs can sense the changes in cancer microenvironment compared to healthy tissue. For example, interferon treatment is not effective in all hepatic cancer patients, but levels of miR-26 in patients suffering from hepatic cancer can help them stratify for interferon treatment\textsuperscript{47}.

**Pancreatic ductal adenocarcinoma**

Pancreatic ductal adenocarcinoma (PDAC) is considered the 13th most common cancer-related death in the world\textsuperscript{48}. Aberrant miRNA expression patterns have been identified in PDAC. The miRNAs that were found to be upregulated were miR-221, -424, -301, -100, -376a, -125b-1, -21, -16-1, -181a/c, -92-1, -15b, -155, let-7f-1, -212, -107, -24 and let-7d. The significant downregulated miRNAs include miR-345, -142-3p and -139 (ref. 49). Another group did similar miRNA array profiling studies and found that only two miRNAs, miR-217 and miR-196a, could distinguish PDAC from normal pancreas and pancreatitis\textsuperscript{50}. Additionally, various miRNAs have been identified that promote invasion and metastasis in PDAC\textsuperscript{51}. For instance, EP300, a histone acetyl transferase that plays an important role in cell growth and division, is regulated by a group of miRNAs (miR-194, -200b, -200c, and -429) in PDAC\textsuperscript{52}. miR-27a is also upregulated in various PDACs and in other malignancies\textsuperscript{53}. One of the main targets of miR-27a is SPRY2, which plays a crucial role in inhibiting tumour growth and metastases through Ras/MAPK pathway inactivation\textsuperscript{54}.

Further, there are various miRNAs that function as tumour suppressors. For instance, miR-96 is a potential tumour suppressor as it directly targets and inhibits KRAS activation\textsuperscript{55}. In PDAC, miR-96 is downregulated compared to normal pancreatic tissues\textsuperscript{54}. Thus, it is possible that miR-96 holds potential therapeutic use in KRAS-driven PDAC. miR-20a is another crucial miRNA that has potential metastasis-suppressing effects by negative regulation of STAT3, proliferation inhibition and invasion of PDAC cells in vivo as well as in vitro. Therefore, controlled expression of miR-20a expression may be an important factor in therapeutics in human PDAC\textsuperscript{56}.

**Cervical cancer**

With over 528,000 cases occurring every year, cervical cancer is the fourth most commonly occurring cancer in women after breast, colorectal and lung cancers\textsuperscript{57,58}. It is also the fourth leading cause of death in women and one-fifth of all new cases are diagnosed in India\textsuperscript{57}.

Cervical cancer commonly occurs due to chronic infection with human papillomavirus (HPV). HPV invades the basal cells and is maintained as episomes\textsuperscript{59}. The oncogenic transformation begins once the viral DNA is incorporated into host DNA. The E6 and E7 genes of the viral DNA inactivate common tumour suppressor genes, including p53 and RB, which induce neoplastic transformation. E6 also inhibits cellular apoptosis, and the E7 protein degrades Rb family proteins necessary for cell-cycle progression\textsuperscript{60}.

Large-scale miRNA microarray analysis has shown that cervical cancer and normal tissue samples have distinct miRNA profiles. Among the differentially expressed miRNAs, miR-21 is highly overexpressed in tumour tissue. miR-21 is a negative regulator of programmed cell death 4 (PDCD4), which is a tumour suppressor with apoptotic function that blocks translation and tumour growth\textsuperscript{61}. Another oncogenic miRNA highly expressed in cervical cancer is miR-10a, which causes tumour growth, metastasis and invasion by suppressing CHL1, a tumour suppressor gene\textsuperscript{62}. miR-19a and miR-19b target Cullin-5 (CUL5)\textsuperscript{63}. The CUL5 gene is a core component of E3 ubiquitin ligase that forms the proteasomal degradation complex\textsuperscript{64}. miR-20a, another miRNA from the same cluster, positively regulates oncogene tyrosine kinase, nonreceptor 2 (TNKKS2), causing enhanced cellular invasion and metastasis\textsuperscript{65}.

Many studies have examined various tumour suppressor miRNAs that play a crucial role in suppressing cervical cancer. For instance, miR-138 expression is lower in cervical cancer cells than in normal tissue. miR-138 is known to suppress telomerase activation and cellular immortalization\textsuperscript{66}. In addition, miR-7 was identified as a likely tumour suppressor because it decreased cellular growth and increased cellular apoptosis in cancerous tissue. Similar function was observed by Cui et al.\textsuperscript{57} when they overexpressed miR-125b in cervical cells, which could inhibit cell growth, induce apoptosis and decrease tumourigenicity by suppression of the phosphoinositide 3-kinase catalytic subunit delta (PIK3CD) through targeting of the PI3 K/Akt/mTOR signalling pathway\textsuperscript{67}.

It is clear that miRNAs play a critical role in cervical cancer. Many of these miRNAs such as miR-21, miR-27a, miR-34, miR-34a, miR-146a, miR-155, miR-196a, miR-203 and miR-221 are overexpressed and present at high levels in the serum of cancer patients\textsuperscript{68,69}. This indicates that miRNAs could likely be used as diagnostic markers for cervical cancer. miRNAs are also believed to have therapeutic potential, as researchers are currently developing drugs to target specific miRNAs (e.g. miR-21) that are overexpressed in cervical cancer\textsuperscript{70}. In addition, strategies are being developed to deliver miRNAs such as miR-143 that are expressed in low levels in

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cancer and are known to negatively regulate oncogenes \(^ {71}\). These findings highlight the potential medicinal value of miRNAs in cervical cancer and suggest that miRNAs may have a future role in personalized medicine.

**Ovarian cancer**

New cases of ovarian cancer have been estimated to be 225,000 worldwide in 2008, accounting for around 4% of all cancers diagnosed in women \(^ {12}\). The incidence rates vary considerably across the world, with developed countries being nearly twice as high as those in less developed countries \(^ {8}\). Moreover, there is preliminary evidence that screening can improve survival, but the impact of screening on mortality from ovarian cancer is still unclear \(^ {72}\). In such type of cancer, understanding the role of regulatory RNAs becomes essential.

One of the recent studies by Zhang et al. \(^ {74}\) used an integrative genomic approach in human epithelial ovarian cancer. The group compared miRNA expression levels in 18 ovarian cancer cell lines, non-neoplastic cell lines with normal ovarian epithelial cells. They identified four miRNAs, miR-26b, -182, -103 and -26a, that were upregulated and additionally found downregulation of known tumour suppressor miRNAs let-7d and miR-127. Overexpression of miR-26b, -182, -103 and -26a was due to an amplified chromosomal region. Downregulation of let-7d and miR-127 was due to epigenetic regulation that silenced the tumour suppressor miRNAs \(^ {74}\). In addition to this study, there are other miRNA expression studies that look into miRNA levels in different subtypes of ovarian cancer \(^ {75}\). For instance, miR-519a was significantly upregulated in serous and clear cell carcinoma compared to mucinous subtype \(^ {76}\). Overexpression of miR-519a was positively correlated with poor survival outcomes \(^ {76}\). Similarly, downregulation of miR-153 and miR-485-5p had a positive correlation with advanced clinical stage FIGO (International Federation of Gynecology and Obstetrics) grade 3. miR-519a was found to be high in clinical stages III and IV (advanced clinical stages) compared to stages I and II (early clinical stages) \(^ {76}\). Additionally, miR-100 has been shown to be downregulated in clear cell ovarian carcinoma cell lines and, when over-expressed, it enhanced sensitivity to rapamycin analog RAD001 (everolimus) by inhibiting mTOR pathway \(^ {77}\). Such differential miRNA profiles are helpful to distinguish different subtypes, and improve diagnostics and prognosis of the cancer. For instance, patients with low let-7a methylation had overall a poor survival outcome compared to those with high methylation. The miRNA-200 family also plays a crucial role in ovarian cancer, and the miR-200 family cluster that includes miR-200a, -200b and -429, when downregulated, is correlated with poor survival \(^ {78}\). Yang et al. \(^ {79}\), confirmed that miR-214, -199* and -200a were associated with high-grade and late stage tumours.

Another interesting ovarian cancer study that profiled miRNAs from tumour-derived exosomes found eight miRNAs, which were previously shown to have diagnostic potential (miR-21, -141, -200a, -200c/b, -203, -205 and -214), had similar expressions between cellular and exosomal miRNAs, with an absence of exosomal miRNAs in control samples \(^ {80}\). Additionally, an early embryonic gene, high-mobility group AT-hook 2 (HMGA2) to let-7 ratio has been identified for prognostic studies \(^ {81}\). HMGA2 is a known target of let-7 family. Higher HMGA2/let-7 ratio signified decreased 5-year progression-free survival (<10%) compared to a lower ratio. Therefore, miRNAs play an important role in identifying different subtypes and exosomes present in ovarian cancer compared to benign/normal ovarian epithelial tissue.

**Breast cancer**

Breast cancer is the most common cancer in women worldwide, affecting approximately 1.5 million women. It is also the principle cause of death from the disease among women \(^ {82}\). Breast cancer is a heterogeneous type of cancer and is generally classified on the basis of presence/absence of estrogen receptor, human epidermal growth factor (HER2) expression, and using gene expression-based classifier or the integrative classification based on genomic and transcriptomic data \(^ {83}\). A recent study performed a miRNA expression pattern in a cohort of breast tumours \((n = 1302)\) with various degrees of heterogeneity as well as adjacent normal breast tissues \((n = 116)\) along with a panel of breast cancer cell lines \((n = 28)\) and matched genomic, miRNA, or long-term survival data \(^ {84}\). The miRNA array demonstrated a global decrease in miRNA in tumours compared to adjacent normal tissues. Additionally, 133 miRNAs were identified in minimal common regions with recurrent copy-number alterations in 5% of samples \(^ {85}\). For instance, they observed gain of miR-17-92 oncogenic family and loss of tumour suppressive miR-31. Interestingly, only 49 out of 227 miRNAs exhibit high correlation with the host mRNA, which includes ER\(^ +\) marker miR-342 (ref. 84). Other miRNAs, such as miR-10a and miR-505, are subtype-specific and are co-transcribed with their host genes, HOXB3 and HOXB4 genes or ATP11C in ER\(^ +\) or ER\(^ -\) samples respectively \(^ {85}\). Additionally, lymphocytic infiltration correlated with five miRNAs (miR-150, -155, -146a, -142-3p and -142-5p) that are known to have a pronounced effect on immune regulation \(^ {86}\). In contrast to clinical covariates, miRNA variation and molecular signature were highly correlated to miRNA expression \(^ {87}\). For instance, there was a high correlation between regulatory components of signalling and development, extracellular matrix, cell adhesion and morphogenesis, and known tumour-suppressive miRNA families such as miR-143/145 (ref. 90), miR-199/214 (ref. 91) and miR-127 (ref. 92).
Additionally, they observed correlation between ER-dependent miR-342 and oncogenic miR-17-92 polycistron with the estrogen and progesterone levels, reflecting their activity in breast cancer. This study was the first detailed systems-level analysis of miRNA expression architecture in a large number of human breast tumours that analysed miRNA levels and integrated them with matched mRNA expression and DNA copy number.

Prostate cancer

Prostate cancer is considered as the second most common cancer leading to death in men over 40 years of age. The major challenge of prostate cancer is the development and acquisition of castrate-resistant prostate cancer phenotype that leads to skeletal metastasis, making it an incurable disease. Therefore, miRNAs become an attractive area of research to find answers to such incurable forms of prostate cancer.

Tumour suppressor miRNAs in prostate cancer include miR-15a, -16, -143, -145, -200 and -488 (ref. 94). miR-15a and -16 are located at 13q14.3 and the deletions at this region have been found in various cancers, including chronic lymphocytic leukaemia (CLL), multiple myeloma, mantle cell lymphoma and prostate cancer. It has been reported that miR-15a and -16 can target oncogenes such as BCL2, CCD1 and WNT3A, which are miRNAs that promote survival, proliferation and invasion of prostate cancer.

miR-143 plays a crucial role in controlling EMT and is considered downregulated in prostate cancer. Xu et al. identified that miR-143 regulates KRAS, pERK1/2 and cyclin D1 that play a role in cell proliferation, migration and chemosensitivity in prostate cancer. Overexpression of miR-143 in prostate cancer cells significantly decreased proliferation and migration, and enhanced sensitivity to docetaxel by affecting EGFR/RAS/MAPK pathway. Additionally, they found that the expressions of miR-143 and -145 were downregulated considerably in metastasis samples. Another key miRNA that controls the EMT process in prostate cancer is miR-200 (ref. 98). It targets zinc-finger E-box binding homeobox 1 (ZEB1), ZEB2 and SNAIL2 expression leading to acquisition of the EMT phenotype. Interestingly, miR-488 has a binding site at the 3′UTR of the AR gene and overexpression of miR-488 reduces expression of AR in both Androgen-dependent (LNCaP) and androgen-independent (C4-2B) prostate cancer cells.

In contrast to tumour suppressor miRNAs, there are oncogenic miRNAs that have been found to be upregulated in prostate cancer. For instance, miR-221 and miR-222 are both considered oncogenic as they were found to be associated with the development and metastasis of prostate cancer. These miRNAs elicit their effect by binding to p27kip1 resulting in its suppression and ultimately tumour growth. It is notable that miR-221 is higher in more invasive prostate cancer cells, e.g. LNCaP-AI cells compared to LNCaP (less invasive). This suggests that miR-221 promotes invasion of prostate cancer cells. Another important miRNA that is upregulated in various cancers (glioma, breast cancer, colorectal cancer, stomach/gastric cancer, hepatocellular carcinoma, pancreas cancer, lung cancer, cholangiocarcinoma, leukemic cancer and prostate cancer, etc.) is miR-21. Major targets of miR-21 that play a role in carcinogenesis include TPM1, PDCD4 and MARCKS. Additionally, studies have also shown that transfection of mature miR-125b causes prostate cancer cell growth that targeted the 3′UTR of BAK1 (a pro-apoptotic member of the BCL-2 gene family that is involved in initiating apoptosis) transcript leading to cell proliferation. These studies suggest that the role of miRNAs could be explored to understand the stratification of prostate cancer.

miRNAs in blood cancers

Lymphoma and leukaemia are the most commonly found blood cancers with different origins. Lymphoma is a type of blood cancer that occurs due to increased proliferation of B and T lymphocytes, whereas leukaemia is a condition caused by an increased number of immature white blood cells. These diseases accounted for nearly 9.5% of the deaths from cancer in 2010, based on the total of 569,490 deaths.

There are two commonly found acute types of leukaemia, acute myeloid leukaemia (AML) and acute lymphocytic leukaemia (ALL). AML consists of cytogenic abnormalities (50%) and chromosomal abnormalities that remain undetectable. A recent study found similar chromosomal alterations in both these leukaemia types; however, expression difference in 27 miRNAs, especially miR-146a distinguished these types when comparing AML patient samples with ALL patient samples. Additionally, the study found that miR-146a was inversely correlated in both AML and ALL. However, this study was not focused on understanding the miRNA abnormalities in these types of cancer, but only mRNA expression profiles.

In a microarray profile study of 122 AML samples that consisted of 60 untreated cases and 50 relapsed or refractory cases with CD34+ normal cells, downregulation of several miRNAs (miR-126, -130a, -93, -125a and -146) was observed. Additional correlation studies of cytogenic abnormalities with observed miRNA expression patterns in AML elicited that there are 14 downregulated and 8 upregulated miRNAs, which were associated with 11q23 transloction versus other AML types. For instance, the overexpression of miR-199a and miR-191 was identified in AML with trisomy 8 and was associated with poor outcome. This was the first study to identify distinct miRNA
profiles between AML patients and normal control, and the subsets of miRNAs related to cytogenetic groups and disease outcome\(^\text{109}\). Also, in remission AML patients, levels of miR15a/16 were upregulated in contrast to relapse patients.

Around the same time, a study showed that nucleo-
 phosphomin (NPMP1) mutations are the most common molecular abnormalities in AML associated with upregula-
tion of miR-10a, -10b, -196a and -196b, located within the homeobox genes \((HOX)^{110}\). Also, miR-21 was upregu-
lated in AML samples when compared to normal CD34\(^+\) cells, thus strengthening importance of miR-21 in AML\(^\text{110}\).

Chronic lymphocytic leukaemia (CLL), another com-
monly known leukaemia is characterized by the accumula-
tion of malignant B cells in peripheral lymphoid organs, bone marrow and peripheral blood\(^\text{111}\). CLL cells have genonomic instability, chromosomal alterations (11q23 deletions: ATM; miR-34b/c cluster; trisomy 12 (increased MDM2); 17p deletion (TP53) and 13q14 deletions (miR-15a/16-1)) and other genetic abnormalities\(^\text{112}\). Several miRNAs such as miR-15a/16-1, -34 cluster, -155, -29 and -181b have been implicated in the pathogenesis of CLL\(^\text{112}\). Several miRNAs such as miR-15a/16-1, -34 cluster, -155, -29 and -181b have been implicated in the pathogenesis of CLL\(^\text{112}\). Around 50–60% of CLL patients exhibit deletion of the 13q14 region that encodes miR-15a/16-1 (ref. 114). The down-regulation of miR15a/16-1 is crucial as these target key cell-cycle regulatory and antiapoptotic proteins such as cyclin D1 and BCL2 (ref. 115). Interestingly, spontaneous models of CLL (NZB mice) also exhibit 50% re-
duction of miR-15a/16-1 (ref. 116). Other crucial miRNAs include miR-29 and miR-181 that target Tc1, which is highly expressed in aggressive CLL\(^\text{117}\). miR-34a has also been shown to target EZF2 and B-Myb onco-
genesis in CLL as well as AML. v-Myb is found to be elev-
ated in CLL patients that stimulate the miR-155 host gene. Increased miR-155 levels were associated with en-
hanced \(ZAP70^{\text{118}}\) expression and faster CLL progression\(^\text{118}\). Studies have shown that miR-155 is generally upregu-
lated in most types of lymphomas, which include Burkitt’s lymphoma, diffuse large B cell lymphoma, pri-
mary mediastina large cell lymphoma and Hodgkin’s lymphoma\(^\text{119}\). Recent mice studies have shown that miR-
155 plays a crucial role in B lymphocyte development\(^\text{120}\). Overexpression of miR-155 leads to abnormal prolifera-
tion of polyclonal pre-leukaemic pre-B cell resulting in B cell malignancy\(^\text{120}\). More recently, miR-155 knock-out studies have revealed the presence of defective dendritic cell functions, impaired cytokine secretion and Th2 dif-
ferentiation. In addition to the role of miR-155 in immu-
nity, it has been shown to induce mediators of flogosis and is involved in response to endotoxic shock\(^\text{121}\).

miR-17-92 is a cluster located in 13q31-32 that is highly overexpressed in lymphomas. He et al.\(^\text{122}\) reported that this cluster is commonly amplified in B cell lymphoma patients. They showed that miR-17-92 and cMYC acted together to develop tumours in mice leading to lymphoproliferative disease and autoimmunity and premature death\(^\text{123}\). These studies are in concurrence with our studies done in osteosarcoma emphasizing the func-
tion of miR-17-92 cluster in various chaotic cancers. The mice also showed enhanced proliferation of immature lymphocytes due to downregulation of PTEN and BIM that control apoptosis of B cell lymphocytes\(^\text{122}\). Upon de-
tailed study of this cluster, it was found that MYC binds and activates the expression of 17–92 and simultaneously activates E2F1 enabling a tightly controlled proliferative signal, including lymphomas\(^\text{124}\). Thus, it is evident to study such miRNA clusters that are deregulated in vari-
ous cancers. A controlled regulation of such clusters can help in better prognosis of the disease.

**miRNAs in sarcomas**

Sarcomas are a type of heterogeneous cancer that is meso-
enchymal in origin. They can be broadly stratified into bone and soft tissue sarcomas consisting of more than 50 subtypes. Current treatment strategies fail to be effective against many sarcoma types and ultimately lead to drug resistance. Therefore, a better understanding of pathobi-
ology of the sarcoma is required to potentially enhance development of better diagnostic and prognostic markers and ultimately therapeutics. We have done an extensive review on various types of sarcomas\(^\text{125}\). Recently, deregula-
tions of miRNAs were identified in various types of sarcomas. Our studies show that there are unique miRNA expression signatures that can distinguish various sarco-
mas on the basis of histological types, reflecting differ-
ence in lineages and differentiation status of the tumours\(^\text{125}\). The miRNA expression signatures can help in better diagnosis and prognosis of soft tissue sarcomas. For instance, miR-210, a hypoxia-regulated miRNA is positively correlated with the prognosis and age of a tumour in a gender-specific manner in soft-tissue sarcoma patients\(^\text{89}\). Here, we discuss two major types of sarcomas, i.e. osteosarcoma (OS) and rhabdomyosarcoma, and implications of miRNAs that result in deregulation of various gene regulatory pathways.

OS is the most common bone cancer affecting young adolescents. OS affects 3–5 per million men and 2–4 per million women\(^\text{126}\). OS is genetically chaotic and due to the failure of current diagnosis and therapies, it is urgent to find better therapeutics to prolong the life of a OS patient. Our laboratory has done extensive work in under-
standing the role of miRNAs in OS. Interestingly, we found significant downregulation of miRNAs at 14q32 locus in OS tumours compared to normal bone tissues\(^\text{127,128}\). Additionally, we observed no changes in the DNA copy number in the 14q32 locus, which is sug-
gestive of certain epigenetic mechanisms that led to

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downregulation of these sets of miRNAs. In addition to identification of these miRNAs, we identified a subset of 14q32 miRNAs (miR-382, -369-3p, -544 and -134) that targeted cMYC transcript. Furthermore, restoring the 14q32 miRNAs decreased cMYC levels and significantly downregulated miR-17-92 cluster, which is highly upregulated in OS. This regulated network was synergistic to induction of apoptosis in Saos2 cells. In addition to identification of two sets of miRNA, Maires et al. developed a comprehensive molecular genetic map consisting of various miRNA profiles from previously published array and miRNA gene expression profiles from a set of partially overlapping OS tumour samples. Through this study, they functionally validated miR-382 and the cMYC regulatory circuit. Furthermore, they also identified miRNAs that target genes involved in diverse intracellular signalling pathways, including Notch, RAS/p21, MAPK, WNT, and the Jun/FOS pathways. Together, our data suggest a model where the imbalance between the regulatory network involving 14q32 miRNAs, cMYC and miR-17-92 miRNAs could contribute to OS pathogenesis.

Another important type of sarcoma is rhabdomyosarcoma. It is a skeletal muscle-derived tumour and accounts for 6–8% of all pediatric tumours. It consists of two histological subtypes, embryonal (EMRS) and alveolar RMS (ARMS). ARMS is considered to be more aggressive and presents with poorer prognosis compared to EMRS. Most of the studies on RMS focus on myomiR, such as the miR-1/miR-133/miR-206 family. These myomiRs play a crucial role in determining cell fate of myogenic precursors and maintaining muscle tissue homeostasis. All of the above-mentioned myomiRs are significantly downregulated in RMS. We also observed significant downregulation of myomiRs, miR-1 and miR-133, in RMS compared to normal skeletal muscle.

Our data indicate that deregulation of these miRNAs stabilizes the expression of PAX3 transcription factor and cyclin D2 in both ERMS and ARMS types. Interestingly, in ARMS, PAX3 forms a fusion transcript with forkhead homolog 1 (FKHR), and the resultant loss of PAX3 3’UTR in the fusion transcript resulted in an oncogenic mechanism that evades miRNA-mediated regulation of PAX3. In addition to the above miRNAs, miR-29 targets E2F7, which play a crucial role in cell-cycle regulation. Overexpression of miR-29 in RMS cell types decreases the expression of various cell-cycle genes and induces partial G1 arrest, leading to decreased cell proliferation. In summary, our data suggest that the RMS state occurs due to deregulation of multiple miRNAs and their target genes. Moreover, miR-29 can directly target histone deacetylase HDAC4 during osteoblast differentiation causing global downregulation of DNA methylation. Thus, the role of miRNAs in epigenetic regulation in RMS makes it an attractive area of research to understand the RMS biology.

Conclusion

miRNA is one of the many regulatory non-coding RNA types in eukaryotes. The role of miRNAs in cancer is crucial, but the complexity of gene regulation increases as one cannot ignore the presence of other regulatory RNAs. Small interfering RNAs (siRNAs) are one of the known regulatory RNAs commonly generated by the breakdown of viral RNA and function as miRNAs. Another set of regulatory RNAs found in animals is Piwi-interacting RNAs that are usually active in germline cells and play a crucial role in gametogenesis. We have just begun to understand the role of these regulatory RNAs in various biological processes. In addition to our limited knowledge in regulation of the regulatory RNAs, a recent mechanism known as competing endogenous RNAs (ceRNAs) has been discovered that enhances the intricacy of gene regulation. It was found that RNA transcripts could also compete to bind to common miRNAs. A known example is PTEN ceRNA network, where non protein-coding pseudogene of the PTEN known as PTENP1 is able to affect PTEN expression, its downstream PI3K signalling and eventually cell proliferation by directly competing for PTEN-targeting microRNAs. Apart from the various mechanisms mentioned above, there is still a plethora of mechanisms by which these tiny RNAs control cell transformation and tumour progression. We have made an attempt to generate a comprehensive review, but there are other important articles that focus on certain aspects of cancer. For instance, Croce et al. focus on various alterations and mechanisms that are involved in miRNA deregulation in cancer and how such dysregulation is involved in cancer initiation and progression in general. The review article by Garzon et al. discusses the rationale of using miRNAs as anticancer drugs and the strategies and challenges of such therapy. With the attempt and knowledge of such investigations, we have been able to develop miR-34 as a novel therapy in patients suffering from primary liver cancer or metastatic cancer with liver involvement to Phase-I clinical trials. Therefore, despite our limited knowledge on regulatory RNAs, we are certainly making progress toward understanding and revealing each level of complication in gene regulation. It can be said that miRNAs play a crucial role in balancing various biological processes and any imbalance can have a huge impact on the living system.


SPECIAL SECTION: CANCER


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ACKNOWLEDGEMENTS. We thank Jennie Walker Knoot for editing the manuscript. Due to space restrictions we could not cite many significant contributions made by numerous other investigators in this important and rapidly progressing field. This work is supported by grants from Academic Health Center, the Department of Defense (W81XWH01-0-0556), American Cancer Society (RSG-13-381-01), Minnesota Medical Foundation, University of Minnesota, Children’s Cancer Research Fund and The Karen Wykoff Sarcoma Foundation.