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MicroRNAs in Mutagenesis, Genomic Instability and DNA Repair

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Abstract

MicroRNA (miRNA) are aiding our understanding of cancer biology, and are now coming close to therapeutic use as well. Here, we focus specifically on the interaction between miRNAs and genomic instability. .MiRNA regulation is essential to many cellular processes, and escape from this regulatory network seems to be a common characteristic of malignant transformation. Genomic instability may preferentially target miRNAs either because of selective pressure or because of inherent vulnerability related to their location near fragile sites. Furthermore, disruption of miRNA processing elements affords a more global release from miRNA regulation. Finally, we review how miRNAs function as both effectors and modulators of the DNA damage response, intricately weaved with traditional elements such as ATM, P53 and MMR. Thus, miRNAs are both an important substrate for genomic instability, as well as having a crucial role in cellular DNA sensing and repair mechanisms.

1.0 Introduction

The elucidation of microRNA (miRNA) function is an important achievement in modern biology. In the span of just over a decade, a novel biological concept was discovered, studied in great detail, incorporated into larger biological schemes and now stands close to clinical application. Specifically, the role of miRNAs in cancer biology is the most extensively studied. Indeed a recent review updated the landmark “hallmarks of cancer” blueprint¹ to include miRNAs in every part of the tumorigenic process². Of note, in the “hallmarks” article by Hanahan and Weinberg, the characteristic of genomic instability was placed apart from the acquired capabilities associated with tumor physiology, as it represents the means by which evolving tumor populations reach these six biological endpoints¹. Mutation of specific genes is an inefficient process, reflecting the maintenance of genomic integrity by DNA monitoring and repair mechanisms. These systems ensure that mutations remain a rare event, and thus genomic instability is not a mere hallmark of cancer but perhaps *the* hallmark of cancer, allowing for hyper-accelerated evolutionary processes. Although the story of miRNAs in cancer physiology is just beginning to unfold, with only approximately one fourth of over 3400 estimated miRNAs identified experimentally (data

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from Mirbase; http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=hsa)³, in this review we aim to describe what is known not only about how miRNAs form a prime target for genomic instability and mutagenesis, but also how miRNAs play an important role in regulating genomic stability and repair.

2.0 MicroRNA alterations in carcinogenesis

Evidence from multiple tumor samples reveal a marked change in miRNA expression profiles, indicating widespread alterations in miRNA networks in tumorigenesis⁴. As miRNAs regulate key processes involved in tumor evolution such as transcriptional regulation, differentiation, proliferation and apoptosis, such alteration may be of crucial importance to cancer formation. The first detailed example of this link came from the study of the minimally deleted region of a common chromosomal 13q14 abnormality in chronic lymphocytic leukemia, showing that deletion of miR-15a and miR-16-1 is associated with this lymphoproliferative disorder⁵. The causative role and the mechanism of this miRNA deletion were also confirmed and elucidated in a mouse model⁶, and it was also suggested that these alterations may be acquired early in the disease process.

Following this report, numerous studies have revealed the details of deregulated miRNA expression in various human malignancies, and the list of the potential and recognized tumor-suppressive miRNAs and oncogenic miRNAs is growing⁷. Moreover, it is now evident that some characteristics of cancer-related biological processes; tumor angiogenesis, maintenance of cancer stem cell, and metastasis, are associated with miRNA switches^{8–11}. The complexity by which different cancer cells acquire miRNA variation is ever expanding. For example, miR-223 regulation in leukemia demonstrates how gross genomic alterations may be involved in the deregulation of miRNA function. It was shown that the t(8;21) translocation product AML1/ETO recruits chromatin remodeling enzymes at an AML1-binding site on the pre-miR-223 gene, and induces heterochromatic silencing of miR-223¹². A high frequency of genomic alterations was also confirmed in a study of genomic hybridization on 227 human ovarian, breast and melanoma cancer cells, with a strong correlation between genomic changes such as copy number alteration, and miRNA expression^{13,14}. Thus, it appears that miRNAs form a lucrative and commonly affected target for genomic instability, leading to significant perturbations of principal regulatory systems, and allowing tumor progression.

2.1 Fragile sites may contribute to genomic instability-derived microRNA alterations

Chromosomal fragile sites are specific loci prone to breakage and rearrangements when cells are exposed to partial inhibition of DNA synthesis. Many genes involved in cancer-specific recurrent translocations are located within fragile sites (reviewed in Drukin and Glover¹⁵). In 2004, Calin *et al.*, performed the first systematic analysis of miRNAs in fragile sites, based on the known or predicted miRNAs at the time¹⁶. They mapped 186 miRNAs and compared their location to the location of known nonrandom genetic alterations, with the observation that miRNA genes and miRNA clusters are frequently located at or around fragile sites, as well as in regions of loss of heterozygosity, amplification, or common breakpoint regions. Overall, 98 of 186 (52.5%) of miRNA genes are in cancer-associated genomic regions or in fragile sites, which correlated with the lower measured expression of miRNAs. It may be hypothesized that this association is a result of a detection bias or that the use of fragile sites as integration sites for viral genomes and/or transposable elements may contribute to this intriguing finding. Although the evolutionary basis for this finding is still perplexing, this first report suggested that genomic instability may preferentially target miRNAs. Additional studies demonstrated a similar association between miRNAs and fragile sites or cancer susceptibility loci, in murine cancer models^{17,18}. Furthermore, based on computational models to predict miRNA promoters (either host gene promoter for

intragenic miRNAs or independent promoters), it was also hypothesized that in addition to direct hits to the miRNA genes, promoter deletion can also affect miRNA expression¹⁷. In a more recent report, utilizing current miRNA libraries Lagana *et al*¹⁹ have found that fragile sites are particularly enriched in miRNAs but also in protein coding genes. The mapping of human miRNA genes revealed that 242 of 715 miRNAs (33.8%) were located in chromosome fragile sites, considering that fragile sites account for about 25.8% of the length of all sites considered, with a similar finding for protein coding genes (33.9% are located in fragile sites). They have also shown that there is a significant chromosomal variability, for example chromosome 19 has the highest number of miRNAs in fragile regions, while no miRNAs have been yet found in the fragile sites of chromosome 20.

2.2 Epigenetic instability and microRNAs

Changes in miRNA expression can also be achieved by epigenetic modification, indeed, many miRNAs are found near CpG islands²⁰. In a landmark report by Saito *et al*, miR-127, a tumor suppressor miRNA targeting *BCL-6*, was found to be embedded in a CpG island and silenced by DNA methylation²¹. Furthermore, the same group also demonstrated that therapy with chromatin modifying agents successfully reactivated the silenced miRNA in a bladder cancer cell line²². This work led to the identification of multiple other examples of epigenetic silencing of miRNAs in cancer^{23–27}. Specific examples include the silencing of miR-9-1 through hypermethylation as a frequent and early event in breast cancer, and the role of miR-1 silencing in hepatocellular carcinoma demonstrated convincingly in DNMT1 knock out mice²⁸. More systematic methods of observing epigenetic modification of miRNAs were attempted by differential profiling of miRNA expression patterns in *DNMT1* and *DNMT3b* double-gene knockouts in HCT116 cells²⁹, and in metastatic lymph node cancer cells after 5-aza-2'-dC-induced demethylation²⁶. Conversely, in a study of miRNAs in ovarian cancer, three miRNAs (miR21, miR-203, miR-205) were found to be over-expressed, suggesting that at times epigenetic activation through hypomethylation may lead to miRNA over-expression *in vivo*³⁰. Thus epigenetic changes may also play a key role in miRNA perturbation secondary to genomic instability.

2.3 MicroRNAs, beyond gene copy number

Layers of post transcriptional regulation of astonishing complexity are being unraveled in miRNA biology, all demonstrating that miRNA function is dictated only in part by the actual transcription of the miRNA gene. Although far less well studied, these layers of complexity may play an important part of miRNA alteration by genomic instability in cancer, far beyond copy number changes. One of the most intriguing examples of miRNA single base alteration leading to decreased processing is in the case of the SNP rs2910164 in miR-146a which contributes to the mispairing in the hairpin of the precursor associated with a predisposition for thyroid carcinoma³¹. A striking aspect of this finding is that heterozygosity carried a higher susceptibility than homozygosity, an unusual occurrence in cancer genes, with further demonstration of homozygous to heterozygous somatic mutations at rs2910164 in several patients with papillary thyroid tumors. rs2910164 was also associated with a younger age of breast cancer diagnosis in familial breast cancer, which in this case is thought to involve increased binding affinity to the target mRNA, BRCA1³². Another example is an SNP in the terminal loop of pre-miR-27a, rs895819, which confers a reduced risk of developing breast cancer in families with a history of BRCA and non-BRCA-related disease^{33,34}, caused perhaps by changes in miRNA maturation. The location of the rs895819 SNP in the centre of the terminal loop of mir-27a may act to decrease the size of the loop and affect the binding of DROSHA or alternatively, affect the binding affinity of several DROSHA inhibitors (e.g lin28) (Reviewed in Ryan *et al*²⁰).

In addition to mutations in the miRNA, alterations of the 3' UTR of a gene may create or abolish, a miRNA binding site. This has been explored in detail in the case of *let-7* binding to the 3' UTR of the *KRAS* oncogene, where a SNP (rs61764370) was correlated with an increase risk of developing lung and oral cancer^{35,36}, with the possible presence of a negative feedback mechanism leading to further decrease of *let-7* family members³⁷.

In addition, miRNAs may have target sites in the 5' UTR and open reading frames. This is further complicated by occasional intron interruption which requires splicing for effective binding³⁸, or removal of binding sites by splicing affecting relative abundance of splice variants³⁹. Another aspect of miRNA heterogeneity has been recently identified and termed isomiRs⁴⁰. These variations are thought to arise from variable DROSHA and DICER cleavage sites in the hairpin loop, leading to 3' deletion/addition, 5' deletion/addition and internal modifications. An additional layer of complexity of miRNA transcriptional regulation was recently discovered, whereby pseudogenes participate in the regulatory interaction between miRNAs and coding mRNAs by competing with their sister functional genes over binding with miRNA⁴¹. The magnitude of this effect on miRNA biology in cancer remains to be fully explored, but this first publication provided valuable insights with regards to pseudogene contribution to miRNA regulation of key players such as the PTEN and KRAS proteins.

Thus in addition to the more crude copy deletion and amplification already demonstrated in miRNA deregulation in cancer, it is plausible that genomic instability may lead to mutations that can affect miRNA regulation by altering processing, target binding sites and pseudogene binding in a highly complex fashion.

2.4 Global modification of microRNA processing

In addition to hits to specific miRNAs, more global changes to miRNA processing can also contribute to tumorigenesis. While miRNA maturation is a tightly regulated event with biogenesis of mature miRNAs involving several enzymatic steps, experimental observation of cancer cells showed widespread alterations in miRNA expression. While some miRNAs are elevated, most miRNAs have significantly reduced expression⁴. Furthermore, The global repression of miRNA maturation promotes cellular transformation and tumorigenesis⁴². While the mechanisms remain to be fully elucidated, it suggests that miRNAs might have intrinsic function in tumor suppression.

This global suppression may be a result of a block in intranuclear processing by Drosha as recently suggested⁴³ and demonstrated in ovarian cancer to be associated with poor survival⁴⁴. Thomson et al further studied this by comparing expression maps of mature miRNA and of their primary transcript, where they demonstrated the loss of correlation between pri-miRNA and mature expression in the tumor samples⁴³. Alternatively, global miRNA decreases may result from disruption of pre-miRNA's cytoplasmic export after Drosha processing. XPO5 is responsible for miRNA nuclear export, and was found to be down-regulated in lung cancer⁴⁵.

Following export from the nucleus, Dicer is responsible for the cleavage of pre-miRNA into mature miRNA. Conflicting results have been reported regarding its expression in cancer cells with some indicating up-regulation or amplification as in the case of prostate, lung and ovarian cancer^{14,45,46}, while others have shown that reduced expression of Dicer is associated with a poor prognosis in lung cancer⁴⁷. Kumar et al demonstrated in a mouse knock out model that DICER functions as a haploinsufficient tumor suppressor in cancer⁴⁸. Frameshift mutations in TRBP, a regulator of Dicer stability, that introduce premature stop codons, resulted in reduced TRBP expression, reduced DICER expression and lower miRNA production as well as higher cellular proliferation levels⁴⁹.

An exciting study by Suzuki et al, demonstrated an important link between genomic instability and global miRNA regulation through the role of the most frequently mutated protein p53 in regulating miRNA processing⁵⁰. The authors revealed that DNA damage and p53 activation increased expression of not only a known p53 target, miR-34a, but also a broad set of mature miRNAs, and their accumulation is mediated transcriptionally and post-transcriptionally. This was found to be a result of a direct interaction between p53 and endogenous Drosha/DGCR8, modulated through the p68/p72 RNA helicase. Collectively, their results suggested that the p53 pathway promotes the recruitment of the p68–Drosha complex to the target pri-miRNAs, enhancing Drosha-mediated processing of several miRNAs. Furthermore, mutant p53 proteins with oncogenic activities hindered miRNA processing in a transcription-independent manner, through interference with miRNA biogenesis by negative titration of Drosha microprocessor components and interference with Drosha/p68 accessibility to pri-miRNAs.

In summary, avoidance of tight gene regulation by miRNAs might be a general feature of cancer cells. Genomic instability may contribute to global miRNA escape through mutations affecting different parts of the processing and maturation machinery either directly or indirectly as in the case of p53. The loss of a miRNA-controlled gene regulatory layer may serve as a global program for cellular proliferation as it may hold an intrinsic tumor-suppressive function.

3.0 MicroRNAs affecting genomic instability and DNA repair

3.1 MicroRNA alterations in models of DNA damage

The DNA damage response requires the carefully orchestrated action of a number of distinct pathways including those that detect the damage, halt the cell cycle, and mediate DNA repair (Figure 1). Though these pathways may be in large part transcriptionally regulated, it has become apparent that proteins involved in this response are also post-transcriptionally regulated. Thus miRNAs were shown in several models of DNA damage to serve both as an effector arm of the DDR as well as important regulators of its evolution (Figure 1).

Galluzzi et al have shown that cisplatin induced DNA damage results in the specific up-regulation of miRNAs, with a differential up-regulation in response to other proapoptotic agents such as C₂-ceramide and cadmium dichloride⁵¹. They identified a role for specific miRNAs (miR-181a and miR-630) in regulating DNA damage induced apoptosis. miR-181a sensitized cells to cisplatin induced DNA damage by stimulating Bax oligomerization and the activation of proapoptotic caspases, While miR-630 conferred cytoprotection resulting from decreased proliferation coupled to upstream inhibition of the signaling cascades that emanate from damaged DNA such as H2AX and ATM activation.

When UV mediated DNA damage was studied in Dicer-depleted cells a dramatic depletion of G1-phase cells and increase in S-phase cells was observed, suggesting that miRNAs have a role in cell-cycle checkpoints and/or DNA repair⁵². The authors showed that Ago2 translocates to stress granules after UV damage, in an ATM/ATR independent fashion, but requiring CDK signaling. In addition, UV exposure resulted in miRNA expression with different temporal patterns including early expression as in the case of miR-221 that regulates the UV-responsive cell-cycle control gene p27/kip1 or late expression as in the case of miR-34a, which is a direct p53 target (Figure 1). Finally, the interaction of miR-16 and the G1–S checkpoint CDC25a was also shown to have an essential role in UV induced cell cycle arrest⁵².

Radiation induced DDR dramatically down-regulated *let-7* miRNA in human lung cells⁵³, supporting the role of the *let-7* family miRNAs in a cancer-related DDR network. In this

model too, DNA damage induced miR-34 expression, dependent on p53, followed by induction of cell cycle arrest and promotion of apoptosis⁵⁴. The critical role of miR-34 in the radiation response was also demonstrated in vivo in *C. elegans* and in vitro in breast cancer cell lines⁵⁵.

Hypoxia can also promote genetic instability by affecting the DNA repair capacity of tumor cells^{56,57}. Interestingly, two miRNAs, miR-210 and miR-373, are up-regulated in hypoxia in a hypoxia-inducible factor-1 α (HIF-1 α)–dependent manner. miR-210, targets the mRNA of the DNA repair factor, RAD52, while miR-373, targets both RAD52 and the NER damage recognition factor, RAD23B, demonstrating that DNA repair factors may also be regulated in hypoxia by miRNAs, further contributing to overall tumor genetic instability.

3.2 MicroRNAs regulate many specific steps in the DDR

One of the initial signals upon chromatin damage is the phosphorylation of the H2A variant H2AX (γ -H2AX)⁵⁸ (Figure 1). The ATM/ATR-dependent phosphorylation of H2AX is causes the accumulation of MDC1, which is the key regulator for the micro-environment at the vicinity of the damaged DNA. A study of terminally differentiated hematopoietic cells demonstrated that miR-24 affects DNA repair by downregulating H2AX expression, with cells over-expressing miR-24 having twice as many chromosomal breaks and fragments as control cells after exposure to radiation injury⁵⁹. By up-regulating miR-24, terminally differentiated cells shut down protein production of a whole set of genes. The authors hypothesized that this mechanism may serve to sensitize cells to apoptosis by limiting double strand break repair, which may be preferable to error-prone repair via NHEJ. Of note, the miR-24 cluster was reported to be deleted in some poor-prognosis cases of chronic lymphocytic leukemia⁶⁰, and it may be postulated that down modulation of miR-24 in that setting may enhance DNA repair and thereby resistance to cytotoxic therapy.

Ataxia-telangiectasia mutated (ATM) is a serine/threonine kinase that plays a central role in the maintenance of genomic integrity by activating cell cycle checkpoints and promoting repair of DNA double-strand breaks. Here too, a specific miRNA, miR-421, was found to suppresses ATM, resulting in increased sensitivity to ionizing radiation, phenotypically similar to cells of ataxia-telangiectasia patients⁶¹ (Figure 1). Interestingly, miR-421 expression was induced by N-Myc, a frequently amplified oncogene, establishing a signaling loop by which Myc suppresses the DDR. A study of miRNAs in carriers and homozygote patients for the ATM gene revealed miR-125b as an important target of ATM, mediating its suppressive effect on TNFSF4, a disruption which was found in carriers as well, perhaps explaining to some degree the increased risk of breast cancer observed in ATM carriers⁶².

p53 tumor suppressor is the master regulator at the center of a complex molecular network regulating DDR. Abnormalities in the p53 pathway are found in nearly all types of cancers, and p53 mutations are often associated with aggressiveness of tumor and poor prognosis. In DDR, p53 directly or indirectly modulates multiple pathways for tumor suppression, promoting growth arrest and apoptosis. A first advance connecting p53 pathway and miRNA is the identification of the above-mentioned miR-34 family, a direct p53 transcriptional target^{63,64}. The miR-34 family complements p53 through suppression of targets involved in cell cycle and apoptosis, such as cyclin E2, cyclin-dependent kinase 4 (cdk4) and Bcl-2. Further, a positive feedback signal between p53 and the miR34 family is suggested by miR-34a suppression of the silent information regulator 1 (SIRT1), resulting in acetylation of p53 and up-regulation of p21⁶⁵. Additional miRNA targets of p53 include miR-192, miR-194, and miR-215 which may in turn increase the p53 and p21 expression and induce cell cycle arrest (Figure 1). Thus, like miR-34, additional miRNAs function as both effectors and amplifiers of the p53 pathway⁶⁶. In addition, p53 may serve to repress certain miRNA

clusters as has been demonstrated for the miR-17-92 cluster. Interestingly, this p53 induced miRNA suppression is mediated through a competitive block of the recruitment of TATA-binding protein to the miR-17-92 promoter by p53 binding to the promoter since the binding sites of both transcription factors overlap within the miR-17-92 promoter⁶⁷.

In addition to the transcriptional regulation of miRNAs by p53, miRNAs can also modify the action of p53 (Figure 1). Direct targeting of p53 by miR-504 through two binding sites in 3'UTR of the *p53* gene was shown to reduce p53-mediated apoptosis and cell-cycle arrest in response to stress⁶⁸, which may explain the tumorigenic effect of the frequently observed amplification of the locus where miR-504 is located (Xq27.1)⁶⁹. miR-125b also directly targets p53 and suppresses apoptosis⁷⁰, findings which are of particular importance considering the fact that p53 function is often reduced in cancer even in the absence of p53 mutations or epigenetic silencing. Diverse indirect mechanisms for p53 modulation by miRNAs have been also found. The miR-29 family targets two negative regulators of p53, Cdc42 and the regulatory subunit of phosphatidylinositol-3 kinase (PI3K), p85 α , thereby enhancing p53 levels⁷¹, while miR-122 positively regulates p53 pathway through suppression of cyclin G1⁷². Finally, downstream miRNA interference with the p53 pathway has been shown for miR-372/miR-373 which neutralize p53-mediated Cdk inhibition, possibly through direct inhibition of tumor suppressor LATS2⁷³.

As is often seen in cellular processes, the termination of the process is contained within the initiated program. This holds true for the DDR as well, with Wildtype p53-induced phosphatase 1 (Wip1) a critical inhibitor in the ATM/ATR -p53 DNA damage signaling pathway. Wip1, induced by p53 early in the DDR, dephosphorylates several key DNA damage-responsive proteins and reverses DNA damage induced cell cycle checkpoints. miR-16 was identified as a specific inhibitor of the mRNA of Wip1 and a negative regulator of its expression. Induced early after DNA damage miR-16 postpones Wip1 activation, thus preventing a premature inactivation of ATM/ATR signaling and allowing a functional completion of the DNA damage response⁷⁴. A significant down-regulation of miR-16 was seen in mammary tumor stem cells, supporting the role of this mechanism in tumorigenesis.

3.3 Additional microRNA modulation of genomic instability

The paucity of mutations in DNA repair caretaker genes in high throughput cancer genome sequencing efforts has yielded further support to the oncogene-induced DNA replication stress model for cancer development over the mutator hypothesis⁷⁵. Within this paradigm, c-Myc may be of cardinal importance. c-Myc functions as an essential regulator of G1-S transition by promoting the transcription of mRNAs encoding proteins that drive cell cycle progression and cell growth. In addition to these well-established functions, Myc has been shown to directly interact with components of the prereplicative complex and to localize to early sites of DNA synthesis. Over-expression of Myc in this context results in checkpoint activation and DNA damage due to replication stress imposed by inappropriate replication origin activity⁷⁶. Myc is repressed in DDR by the induction of miR-34c via a highly conserved target-site within its 3' UTR. As described above, miR-34 induction appears to be primarily p53 mediated, though p38 MAPK signalling to MK2 may serve as an alternate induction pathway⁷⁷. miR-34c-mediated repression of Myc following DNA damage is required to inhibit DNA synthesis and block cells in S-phase to prevent replication of damaged DNA. Further support to the role of miR-34 in tumor suppression is found in the frequent epigenetic silencing of miR-34b/c, observed in cancers²⁶.

MiRNAs not only serve in repressing Myc, but may also serve as downstream effectors of Myc activation, an insight that stemmed from the observation that while there was a strong enrichment for canonical Myc binding sites within the group of genes that were activated by Myc, genes that were repressed by Myc did not exhibit enrichment for Myc binding sites but

rather a substantial enrichment for miRNA binding sites in their 3'-UTR sequences⁷⁸. Thus, For example, the Myc-induced miR-20a, targets *CDKN1A*, a gene encoding a negative regulator of cell cycle progression, and Myc-induced miR-221 and miR-222, target *CDKN1B*, and *CDKN1C*, additional triggers of cell cycle arrest. Myc activation utilizes downstream Myc-induced miRNAs in suppressing the expression of key cell cycle arrest genes. Moreover, Myc induced miRNAs (miR-23b, and miR-193b) were shown to target PTEN, contributing to a proliferation program by blocking the activity of PTEN, thus allowing activation of PI3K and the provision of cell survival signals⁷⁹.

Mismatched nucleotides may arise from polymerase misincorporation errors, recombination, or chemical and physical damage to the DNA, and are repaired in high fidelity by mismatch repair (MMR) enzymes. miR-155 was found to play a role in MMR by causing down-modulation of the core MMR heterodimeric proteins MSH2-MSH6 and MLH1-PMS2, resulting in a mutator phenotype⁸⁰. miR-155 altered both the expression and stability of the MMR pathway resulting in a significant increase in mutation rates. In support of this finding, an inverse correlation between miR-155 and MMR protein expression was observed in colorectal cancer cell, though not all tumors with increased miR-155 expression were characterized by Microsatellite instability, a hallmark of defective MMR. Alterations in MMR regulating miRNAs may be complemented by mutations in the target sequences, as was shown with acquired mutations in the 3' UTR of *hMLH1*, linked to disease relapse in patients with acute myeloid leukemia⁸¹.

miRNAs also regulate enzymes that are involved in the methylation of the CpG islands of tumour suppressor genes. For example, miRNAs of the miR-29 family target DNA methyltransferases (DNMTs), DNMT3A and DNMT3B⁸². The introduction of miR-29 into lung cancer cells causes CpG island demethylation in the promoter regions of tumor suppressor genes, resulting in reversion of tumorigenicity. In addition, miR-29s targets indirectly the DNMT1 maintenance DNMT, as shown by the loss of expression of the three DNMTs, in addition to the reactivation of the p16 tumor suppressor after the introduction of miR-26 to AML cells⁸³. This mechanism may underlie the frequently observed 7q deletion chromosomal aberration in AML and MDS⁸⁴.

Finally, Shalgi et al. have made a compelling case for miRNAs as guardians against genomic instability through the repression of transposable elements (TE) in somatic cells⁸⁵. They noted that a variety of genetic studies show that manipulation of miRNAs (e.g Dicer knockout) causes genomic instability, complemented by the observation that tumors have lower global levels of miRNAs. The authors hypothesized that miRNAs in general, and particularly TE-originated miRNAs, mediate global repression of TEs in somatic tissues, promoting genomic stability in a similar way to the function of piRNAs and endo-siRNAs in the germline. Thus, in addition to the heavily explored role of miRNA involvement in cancer as post-transcriptional inhibitors, they propose a different mechanism of involvement of miRNAs in regulation of genomic stability that may provide a more complete understanding of the roles of miRNAs in the regulation of human cancer.

4.0 Concluding remarks

Our growing understanding of the role of miRNAs in cellular regulation has had a profound impact on cancer biology. In recent years, we have learnt that miRNA avoidance contributes to cancer formation not only by enhancing proliferation, but also by directly leading to genomic instability, with increased DNA damage and enhanced mutagenesis. miRNAs seem to play a critical role in the maintenance of genomic stability in normal somatic cells, and thus constitute a regulatory barrier whose elimination might ultimately lead to cancer. MiRNA regulation is of astonishing complexity, and further layers of complexity are

continuously being discovered as we learn more about miRNA processing. It is plausible, that a better understanding of the interaction between this highly complex cellular regulatory machinery and genomic instability may allow for targeted cancer therapies that will use the complexity of the miRNA machinery to fine tune cellular growth and repair in a manner that has thus far eluded us.

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Figure 1. MicroRNAs and the DNA Damage Response

MiRNAs serve as both effectors and modulators of the DNA damage response (DDR). MiRNAs modulate DDR elements from DNA damage sensing through the key ATM/ATR pathway, and also checkpoint elements such as p53 and cdc25a. For a detailed review of miRNA interaction with p53 including mutant p53 please refer to review by Suzuki et al.⁶⁶. Illustrations for cellular components are courtesy of Servier Medical Art (<http://www.servier.com/smart/imagebank.aspx?id=729>).