

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD,
CONSUMER PRODUCTS AND THE ENVIRONMENT**

RNA Related Effects as Mechanism of Carcinogenicity

During the Horizon scanning exercise in November 2008, the Committee was presented with a review by Scholzová *et al.* (2007, Cancer Lett. 246(1-2):12-23) on RNA regulation and cancer development. Members considered this to be an area of interest and it to be an appropriate time for review due to the substantial amount of emerging data in the literature. The Department of Health Toxicology Unit has produced the attached paper which presents some of the studies which describe a role for RNA in carcinogenicity.

The committee is asked to consider the attached paper and to answer the following questions:

1. What is members' opinion of the mechanisms which have been proposed to explain the role RNA may play in carcinogenicity?
2. Do members consider that any other mechanisms may be of importance?

Secretariat
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RNA Related Effects as Mechanism of Carcinogenicity

Introduction

RNA, which is made up of nucleic acids, has a variety of functions in a cell and is found in many organisms including plants, animals, viruses, and bacteria. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) differ functionally. DNA primarily serves as the storage material for genetic information. RNAs are versatile molecules capable of an array of functions. One of the primary functions of RNA is to facilitate the translation of DNA into protein. RNAs also function as information-carrying intermediaries in gene expression, and they act as key catalytic, structural, and regulatory elements in the cell.

In recent years many new small functional RNAs have been found. RNA is usually thought of as messenger RNA that serves as a template for translation of genes into proteins. In contrast, functional and non-coding RNA molecules are transcribed from a DNA sequence, but not translated into proteins. The encoding DNA sequence is often referred to as an RNA gene. Functional RNA genes in the human genome include transfer RNA (tRNA), ribosomal RNA (rRNA) and various other small non-coding RNAs. Several hundred genes in our genome encode small functional RNA molecules collectively called microRNAs (miRNAs).

In this paper, we discuss RNA editing, alternative splicing, non-sense mediated decay (NMD), RNA binding proteins and RNA interference and the role of miRNAs, as mechanisms that potentially influence tumourigenesis. Deregulation of gene expression is a hallmark of the cancer cell. Modifications of mRNA stability and/or translational efficiency are increasingly reported in cancer. mRNA stability and translation are controlled through a complex network of RNA/protein interactions involving recognition of specific target mRNAs by RNA binding proteins. Mutations in cis-regulatory elements, aberrant expression of RNA binding proteins or modulation of signalling pathways that post-translationally modify RNA-BPs or other associated proteins can modify the regulation of a given mRNA. These alterations can occur in diverse cancer types and are often correlated with advanced stage and grade of tumours, resulting in mis-regulation of genes involved in cancer progression. In eukaryotes mRNA transcripts are also extensively processed by other post-transcriptional events such as alternative splicing and RNA editing in order to generate many different mRNAs from the same gene, increasing the transcriptome and then the proteome diversity. Various cell types and developmental stages regulate alternative splicing patterns differently in their generation of specific gene functions. In cancers, splicing is significantly altered, and understanding the underlying mechanisms and patterns in cancer will shed new light onto cancer biology. Alteration in RNA editing as well as expression level of the RNA editing enzymes has been connected to tumour progression and is discussed in this paper.

RNA interference (RNAi)

In recent years RNA interference (RNAi) has emerged as a major mechanism in eukaryotic gene expression. RNAi is a process of sequence specific post-

transcriptional gene silencing initiated by double stranded RNA (dsRNA). The two main types of short RNAs that target complementary messengers in animals are 1) small interfering RNA (siRNA) and 2) miRNA.

Small interfering RNAs

Small interfering RNAs (siRNA) comprises an RNA duplex with perfect homology to a target mRNA, resulting predominantly in its degradation. siRNA was first identified in plants but has now been identified in higher organisms. SiRNAs have been shown to repress genes eliminating the corresponding messenger RNA transcripts and thus preventing protein synthesis (Sassen et al., 2008). Introduction of synthetic siRNA or short hairpin RNAs (shRNA), which are processed to siRNA intracellularly, has emerged as a new tool for regulating gene expression. This tool is being used to investigate the properties of the known or suspected factors that play a role in oncogenesis, to discover the new modulators of this process and is considered as an option for the therapy of the disease.

A dsRNA-specific endoribonuclease III, known as DICER, is responsible for the processing of dsRNA to siRNA. The siRNA's are incorporated into RISC (RNA-inducing Silencing Complex). There is a strict requirement for siRNA to be phosphorylated to enter RISC. Duplex siRNA is unwound by an ATP-dependent RNA helicase, leaving the antisense strand exposed to guide RISC to its homologous target mRNA for endonucleolytic cleavage. The target mRNA is cleaved at a single site in the centre of the duplex region between the guide siRNA and the target mRNA, 10 nt from the 5' end of the siRNA, further mRNA degradation by endonucleases and exonucleases follows.

MicroRNAs (miRNAs)

RNAi regulation of endogenous genes in mammalian cells occurs via production of miRNAs. MiRNAs are a class of small non-coding RNAs whose mature products are ~22 nucleotides long. MiRNAs are pleiotropic regulators of gene expression at the post-transcriptional and/or translational level and play a role in the processes of tumour suppression and oncogenic transformation.

MiRNAs are encoded in the genome and transcribed by RNA polymerase II as primary transcripts known as pri-miRNAs (primary-miRNAs). Pri-miRNAs are typically 3 to 4 kilobases long single-stranded RNAs with 5'cap, 3'poly(A) tail and complicated secondary structure. The pri-miRNAs are processed in the nucleus into one or more precursor-miRNA by the Drosha RNAase III endonuclease to give a 60-70 nt stem loop intermediate, having a 5' phosphate and ~2nt 3' overhang. This pre-miRNA is actively transported from the nucleus to the cytoplasm by Ran-GTP (RAs-related Nuclear protein-GTP) and the export receptor, Exportin 5. The cuts made by the RNase III enzyme Drosha in the nucleus define one end of the mature miRNA and the other is defined in the cytoplasm by DICER, which recognises the double stranded portion of the pre-miRNA and cuts the two helical turns away from the base of the stem loop. This eliminates the loop itself and leaves the 5' phosphate and the ~2nt 3' overhang characteristic of the RNAase III products. Once loaded onto RISC, there is no substantial difference between miRNA and siRNA

towards the target mRNA. The final short RNA duplex possesses a strand, which is highly but not perfectly complementary to one or more RNA target mRNA. This complementarity drives the assembly of an RNA-protein complex on the target mRNAs, which, in turn prevents the latter from being translated.

There are estimated to be at least 700 miRNAs in the human genome (Liang and Li, 2009), comprising 1-4% of all expressed human genes, which makes miRNAs one of the largest classes of gene regulators. Most human miRNAs are found within introns of either protein-coding or non-coding mRNA transcripts (Rodriguez et al., 2004). The remaining miRNAs are either located far from the other transcripts in the genome, within the exons of noncoding mRNA genes or within 3' untranslated regions (UTRs) of mRNA genes, or they are clustered with other miRNA genes (Bartel, 2004).

They negatively regulate their targets in one of two ways depending on the degree of complementarity between the miRNA and the target. The first mechanism involves miRNA that bind with perfect or nearly perfect complementarity to protein-coding mRNA sequences inducing the RNA-mediated interference (RNAi) pathway. This involves mRNA transcript cleavage by ribonucleases in the miRNA associated, multi-protein RNA-induced silencing complex (miRISC) which results in the degradation of target mRNAs. The second mechanism does not involve cleavage of their mRNA targets. These miRNAs exert their regulatory effects by binding to imperfect complementary sites within the 3' UTR of their mRNA targets and they repress target-gene expression post-transcriptionally, apparently at the level of translation, through a RISC complex that is similar to, or possibly identical with, the one that is used for the RNAi pathway.

MicroRNAs and Cancer

A large amount of evidence has been presented in the literature indicating a role for miRNAs in many human diseases, including cancer. This evidence includes but is not limited to 1) a unique set of miRNAs exists in a specific disease; 2) unique expression of miRNAs in a certain human disease and 3) aberrant expression of miRNAs in human disease (Zhang and Farwell, 2008). According to Sassen et al. (2008), three important observations early in the history of miRNAs suggested a role in human cancer. Firstly, the earliest miRNAs discovered in the nematode worm *C. elegans* and the fruit fly *Drosophila* were shown to control cell proliferation and apoptosis by Lee et al. (1993) and Brennecke et al. (2003). Their deregulation may therefore contribute to proliferative diseases such as cancer. Secondly, when human miRNAs were discovered, it was noticed that many miRNA genes were located at fragile sites in the genome or regions that are commonly amplified or deleted in human cancer (Calin et al. 2004). Thirdly, malignant tumours and tumour cell lines were found to have widespread deregulated miRNA expression compared to normal tissues (Calin and Croce, 2006; Gaur et al., 2007; Lu et al. 2005).

The first evidence of a link between miRNAs and human cancer came from an observation in patients diagnosed with a common form of adult leukaemia, B cell chronic lymphocytic leukaemia (CLL), that two miRNA

genes miR-15a and miR-16-1 located in a 30kb region on chromosome 13 were deleted or reduced in CLL cases (Calin et al., 2002). Deletions within the 13q14 locus occur in more than 65% of all CLL cases, as well as in 50% of mantle cell lymphomas, 16-40% of multiple myelomas and 60% of prostate cancers. The miR-15a/16-1 cluster is also reported to be down-regulated in pituitary adenoma (Bottoni et al., 2005) and prostate carcinoma (Porrka et al., 2007). In these cancers, miR-15a/16-1 expression is preferentially down-regulated to favour cancer development by inhibiting apoptosis. Calin et al (2008), using microarray analysis, studied the down regulation of miRNA in CLL patients with high levels of miR15a/16-1 with respect to CLL patients with low levels of these two miRNAs. They identified a signature of 60 genes in common between CLLs and MEG-01 cells transfected with miR15a/16-1. Genes, such as MCL1, ETS1 and JUN, which play a role in apoptosis and cell cycle control, were found to be significantly differentially expressed in these cells (Calin et al., 2008). Cimmino et al (2005) demonstrating that miR-15a and miR-16-1 expression is inversely correlated to Bcl2 expression in CLL and that both miR-15a and miR-16-1 negatively regulate Bcl2, which is an anti-apoptotic gene that is often over-expressed in many types of human cancers, including leukaemias and lymphomas. The tumour suppressor role of miR-16-1 was further supported by the identification of a germ line mutation (C to T mutation, 7bp downstream of the miR-16-1 precursor) which causes low levels of miRNA expression in vitro and in vivo and which was associated with deletions of the normal allele.

Michael et al. (2003) identified many miRNAs that are expressed in human colorectal epithelium. Characterisation of these miRNAs has shown an association between reduced levels of two specific miRNAs (miR-143 and miR-145) and precancerous and neoplastic colorectal tissue.

Iorio et al. (2005) identified miRNAs where expression is significantly down-regulated in cancer versus normal breast tissues. MiR-106, miR-125b and miR-145 and miR-21 and miR-155 were up-regulated suggesting they potentially act as tumour suppressor genes and oncogenes, respectively (Iorio et al., 2005). The expression of miRNAs was correlated with specific breast cancer pathological features such as oestrogen-receptor status, tumour stage, vascular invasion and proliferative index.

Glioblastomas multiforme is the most frequent occurrence and malignant form of primary brain tumours. Ciafre et al. (2005) found that miR-21 was strongly upregulated and miR-181a, miR-181b and miR-181c were downregulated in glioblastoma compared to normal brain controls, using a microarray analysis. It has been shown also that the malignant human brain tumour, glioblastoma, strongly over-expresses miR-21 (Chan et al., 2005). Its expression was increased 5 to 100 fold in human glioblastoma multiforme tissue compared with control non-neoplastic brain tissues. Inhibition of miR-21 expression led to caspase activation and associated apoptotic cell death in multiple glioblastoma cell lines. These findings suggest that overexpression of miR-21 may function as an oncogene in glioblastomas by blocking key apoptosis-enabling genes.

MiR-21 is the most consistently up-regulated miRNA across many cancer types of solid tissues (lung, breast, stomach, prostate, colon, brain, head and neck, oesophagus and pancreas) (Chan et al., 2005; Iorio et al., 2005; Diederichs and Haber, 2006; Roldo et al., 2006). It is also overexpressed in diffuse large B-cell lymphomas (Lawrie et al., 2007), in chronic lymphocytic leukaemia (Calin et al., 2005), in uterine leiomyomas (Wang et al., 2007), in human malignant hepatocytes and in hepatocellular carcinoma (Meng et al., 2007). Volinia et al. (2006) performed a large scale miRNome analysis on 540 samples including breast, stomach, prostate, colon and pancreatic cancers and identified a solid-cancer signature comprising a large proportion of overexpressed miRNAs. These include miR-155, miR-17-5p, miR-20c, miR-92 and miR-106a as well as miR-21.

Primary thyroid carcinoma (PTC) is the most common malignancy in thyroid tissue accounting for 80% of all thyroid cancers (He et al., 2005). Pairwise significance analysis of microarray data indicated that six miRNA genes were significantly overexpressed in tumour tissue from PTC with fold changes >1.5 in at least seven patients. These miRNAs were as follows: miR-146, miR-221, miR-222, miR-21, miR-155, and miR-181a. Prediction analysis using the microarray data indicated that five of the over-expressed miRNAs (miR-221, miR-222, miR-146, miR-21, and miR-181a) were sufficient to successfully predict cancer status (He et al., 2005). Three of the miRNAs (miR-146, miR-221, and miR-222) which showed substantial overexpression (11- to 19-fold higher level in PTC tumours) showed a loss of KIT transcript and Kit protein. KIT is an important tyrosine kinase receptor in cell differentiation and growth. He et al. (2005) also showed polymorphisms in 5 out of 10 primary thyroid cancers in the KIT 3'UTR that corresponds to the site of interaction of miR-221, miR-222 and miR-146, suggesting an altered miRNA-target interaction.

PTEN

PTEN (Phosphatase and tensin homolog) is a tumour suppressor gene encoding a phosphatase that regulates cell cycle, akt and p53 activity (Li and Ross, 2007). MiR-21 directly targets PTEN whose down regulation will release its inhibition on protein kinase B resulting in significantly reduced apoptosis in cancer cells. Meng et al. (2007) showed that inhibition of miR-21 expression increased PTEN expression in human hepatocellular carcinomas. Focal Adhesion Kinase (FAK), an established downstream target of PTEN, is a protein tyrosine kinase involved in the regulation of cell-cycle progression, cell survival and cell migration. Meng et al. (2007) demonstrated that inhibition of miR-21 in HCC cell lines significantly reduced the phosphorylation of FAK and Akt and significantly reduced the expression of matrix metalloproteases 2 and 9, downstream targets of PTEN and key mediators of tumour cell survival, cell migration and invasion. Interestingly, Frankel et al. (2008), investigating the role of PTEN-miR-21 interaction in breast cancer cells, showed that inhibition of miR-21 caused only subtle changes in PTEN protein levels, suggesting that cell and tissue specific differences may result in different functional miR-21 targets.

Pdcd4

Pdcd4 (programmed cell death 4) is a tumour suppressor known to be up-regulated in apoptosis (Yang et al., 2003) and down-regulated in several cancer forms (Chen et al., 2003; Gao et al., 2007; and Zhang et al., 2006). Recently, two independent reports revealed that Pdcd4 is a target of miR-21 in colon cancer (Asangani et al., 2008) and MCF-7 human breast cancer cells (Frankel et al., 2008). Asangani et al. (2008) showed that Pdcd4 is negatively regulated by miR-21 at the post-transcriptional level, via a specific target site (nt 228-249) within the 3'UTR. They also demonstrated that miR-21 induces invasion/intra-vasation/metastasis in colorectal cancer cells. Lu et al. (2008) demonstrated that the translation of Pdcd4 is negatively regulated by miR-21 in HEK-293T, MCF-7 and JB6 cell lines.

Si et al. (2007) found that miR-21 was highly expressed in breast tumours and found that the anti-miR-21-mediated cell growth inhibition was associated with increased apoptosis and decreased cell proliferation. In a study by the same laboratory, Zhu et al. (2007a) found that the tumour suppressor gene tropomyosin 1 (TPM1), which carries a putative miR-21 binding site, was down-regulated by miR-21, whereas anti-miR-21 up-regulated TPM1 expression through the miR-21 binding site at the 3'-UTR region.

MYC

Pathologically activated expression of MYC is one of the most common oncogenic events in human cancers (Nesbit et al., 1999). The MYC oncogene encodes the transcription factor c-Myc that regulates cell proliferation, growth and apoptosis. There appears to be a close relationship between miRNAs and the increased expression of MYC, leading to the development of B-cell malignancies.

An aggressive B-cell leukaemia occurs when MYC is translocated into the miR-142 locus (Gauwerky et al., 1989). The MYC gene translocated only four nucleotides downstream of the miR-142 3'-end and alignment of mouse and human miR-142 containing EST sequences indicates a ~20 nt conserved sequence element downstream of the miR-142 hairpin, which is lost in the translocation (Lagos-Quintana et al., 2003). It was suggested that the absence of this conserved downstream sequence element in the putative miR-142/mRNA fusion prevented the recognition of the transcript as a miRNA precursor to be properly processed, and therefore may have caused accumulation of fusion transcripts and overexpression of myc.

Another miRNA that has been linked with MYC overexpression and B-cell cancers is miR-155. This miRNA is encoded by nucleotides 241-262 of B-cell integration cluster (BIC). Metzler et al. (2004) found that the only phylogenetically conserved region within BIC spans 138 nucleotides and encodes the hairpin region of miR-155. High expression of miR-155 and its host gene BIC have been reported in paediatric Burkitt lymphoma (BL) (Metzler et al., 2004), human B cell lymphomas (Eis et al., 2005) and Hodgkin's lymphoma (Kluiver et al., 2005). A 100 fold upregulation of the miR-155 precursor has been reported in paediatric BL (Metzler et al., 2004). Yanaihara et al (2006) found that high expression of miR-155 has a significantly worse impact on patients with lung adenocarcinoma as an

independent risk factor. MiR-155 has also been reported to be upregulated in breast cancer (Iorio et al., 2005).

Two recent articles by He et al. (2005) and O'Donnell et al. (2005) have indicated a clear association between miRNAs and cancer. A polycistronic miRNA cluster known as miR-17-92 comprises seven miRNAs (miR-17-5p, miR-18a, miR-19a, miR-196-1 and miR-92-1) and resides in intron 3 of the *C13orf25* gene at 13q31.3. It is markedly overexpressed in lung cancers, especially with small-cell lung cancer histology (Hayashita et al., 2005) and upregulated in 65% of B-cell lymphoma samples (He et al., 2005). Expression profiling studies have revealed widespread overexpression of these miRNAs in diverse tumour subtypes including such as those derived from breast, colon, lung, pancreas, prostate and stomach (Petrocca et al., 2008; Volinia et al., 2006). Using the well-studied E μ -myc transgenic mouse model of B cell lymphoma, He et al. (2005) provided direct evidence that the miR-17-92 cluster has oncogenic activity. Enforced expression of the miR-17-92 miRNAs dramatically accelerated disease onset and progression. O'Donnell et al. (2005) reported that transcription of the miR-17-92 cluster is directly transactivated by c-MYC. In particular, the miR-17-92 cluster seems to be tightly linked to the functions of the E2F family of transcription factors, which are critical regulators of the cell cycle and apoptosis. O'Donnell et al. (2005) found that the expression of E2F1 was negatively regulated by miR-17-5p and miR-20a (2 miRNAs in the miR-17-92 cluster) in HeLa cells. Their findings revealed a mechanism through which the c-MYC protein simultaneously activates E2F1 transcription and limited its translation, resulting in a tightly controlled proliferative signal.

Moreover, Woods et al. (2007) demonstrated that both E2F1 and E2F3 can directly activate transcription of the miR-17-92 cluster. Woods et al. (2007) proposed a model whereby miR-17-92 promoted cell proliferation by shifting the E2F transcriptional balance away from the pro-apoptotic E2F1 and towards the proliferative E2F3 transcriptional network.

In mammals, a paralog of the miR-17-92 exists. The miR-106b-25 has been shown to be differentially expressed in a number of different cancers. Li et al. (2009) demonstrated the upregulation of the miRNAs encoded by the miR-106b-25 cluster in hepatocellular carcinoma. Other studies have also shown that miR-106b-25 is overexpressed in HCC and in human liver tumour cell lines (Meng et al. 2007; Li et al. 2009). Ambros et al. (2008) determined genome-wide expression of miRNAs and mRNAs in primary prostate tumours and non tumour prostate tissues. They reported that components of miRNA processing and several miRNA host genes (MCM7 and C9orf5) were significantly upregulated in prostate tumours and that tumours expressed the miR-106b-25 cluster at significantly higher levels than non-tumour tissues. Knockdown studies for the miR-106b-25 cluster showed that inhibition of each member of the miR-106b-25 cluster inhibited cell proliferation (Li et al. 2009). They also showed that the entire miR-106b-25 cluster resulted in more effective inhibition of cell proliferation and also suppressed anchorage-independent growth. The role of the miR-106b-25 cluster in cell growth and

proliferation has also been described by Ivonovska et al. (2008) and Petrocca et al. (2008).

Let-7

Another putative tumour suppressor in human cancers is the let-7 miRNA. The *C.elegans* let-7 miRNA was identified by genetic analysis of the developmental timing defects of mutants and the human let-7 is abundantly expressed in normal lungs. The let-7 family contains miRNAs that have been shown to regulate expression of a proto-oncogene the rat sarcoma viral oncogene homolog (RAS) through post-transcriptional repression (Johnson et al., 2005). RAS proteins are membrane associated signalling proteins that regulate cell growth and differentiation. Almost 15-30% of human tumours possess mutations in RAS genes. The miRNAs that are encoded by the let-7 family were implicated as tumour suppressors because they map to fragile sites associated with lung, breast, urothelial and cervical cancers (Calin et al., 2004). Johnson et al. (2005) showed that over-expression of RAS proteins in lung cancer tissue correlated with reduced expression of let-7 miRNA. Johnson et al. (2005) also demonstrated that 3'UTRs of the human RAS gene contain let-7 complementary sites, allowing let-7 to regulate RAS expression. Takamizawa et al. (2004) demonstrated that transcripts of certain let-7 homologues were significantly down-regulated in human lung cancer and found that this was correlated with a poor prognosis. Takamizawa et al. (2004) demonstrated that over-expression of the let-7 miRNA in an A549 adenocarcinoma cell line resulted in inhibition of lung cancer cell growth in vitro.

Shah et al. (2007) demonstrated that activated PPAR α was a major regulator of hepatic miRNA expression. Wild-type (WT) mice were fed either a control diet or a diet containing Wy-14,643, a specific PPAR α agonist and microarray expression profiling demonstrated that expression of 27 miRNAs was significantly changed following Wy-14,643 treatment. Shah et al. found that the PPAR α agonist WY-14,643 suppressed let-7C expression in WT mice but not in PPAR α -null mice, indicating that the repression was PPAR α -dependent. Basal levels of expression of let-7C inhibit c-MYC leading to inhibition of cell growth and cell cycle arrest. The overexpression of the c-MYC gene has been demonstrated to increase hepatic proliferation and increase the incidence of liver cancers. Let-7C was shown by Shah et al. (2007) to target c-MYC via interaction with the 3' untranslated region of c-MYC mRNA. The PPAR α -mediated induction of c-MYC via let-7C subsequently increased expression of the oncogenic miR-17-92 cluster. The authors suggest that collectively this pathway may lead to increased hepatic proliferation and tumourgenesis. Interestingly, these effects of Wy-14,643 were not apparent in PPAR α -null mice expressing the human PPAR α receptor transgene (knocked in).

MicroRNAs and their role in metastasis

Metastasis is a complex, multi-step process where primary tumour cells invade adjacent tissue, enter the systemic circulation and lymphoid system.

The tumour cells are then carried through the vasculature, penetrate distant tissue parenchyma and finally proliferate from microscopic growths (micro-metastases) into macro secondary tumours (Filder et al., 2003). The precise role played by expressed microRNA in specific steps of malignant progression including metastasis, are still unknown. However, a number of studies have investigated the association of specific miRNAs with specific stages of malignant progression.

EMT

Epithelial to mesenchymal transition (EMT) has been considered an essential early step in metastasis of epithelial-derived tumours. Gregory et al. (2008) found that all five members of the microRNA-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 were markedly downregulated in cells that had undergone EMT in response to transforming growth factor (TGF)-beta or to ectopic expression of the protein tyrosine phosphatase (Pez). Enforced expression of the miR-200 family alone was sufficient to prevent TGF-beta-induced EMT. Together, these microRNAs cooperatively regulate expression of the E-cadherin transcriptional repressors ZEB1 (also known as δ -EF1) and SIP1 (also known as ZEB2), factors previously implicated in EMT and tumour metastasis. These findings were confirmed by four other independent studies (Bracken et al., 2008; Korpala et al., 2008; Park et al., 2008 and Burk et al., 2008).

Breast Cancer

Using the 29 identified miRNAs that were differentially expressed between primary breast carcinomas and normal mammary tissues, reported by Iorio et al. (2005), Ma et al. (2007) found that miR-106 was highly expressed only in metastatic breast cancer cells. They reported that the expression level of miR-106 was 50 fold higher in cells of the MDA-MB-231 line which are capable of metastasizing than in cells of the MCF-7 human breast cancer cell line, which have little of any metastatic capabilities (Ma et al. 2007). They also found that miR-106 expression levels were lower in all of the breast carcinomas from metastasis-free patients. However, they found 50% of metastatic-positive patients had elevated miR-106 levels in primary tumours. Ma et al. (2007) also demonstrated that miR-106 positively regulates cell migration and invasion in vitro and can initiate cell invasion in vivo. They also identified that the transcription factor Twist (a metastasis-promoting gene) directly regulates miR-106, which in turn inhibits synthesis of the HOXD10 protein, permitting the expression of the pro-metastatic gene product, RHOC. This gene product favours cancer cell migration and invasion. Conversely, silencing of miR-106 inhibits Twist-mediated cell migration and invasion.

In a study of 219 patients, Gee et al (2008) found less miR-106 expression in patients without metastasis (n=114) than in normal breast tissue (n=10). However, unlike Ma et al. (2007) they found lower miR-106 expression in patients with distant relapse and local recurrence. Also, whereas Ma et al. (2007) found that miR-106 over-expression increases tumour size and invasiveness, Gee et al (2008) found its expression correlates inversely and significantly with tumour size and grade.

Zhu et al. (2008) investigated the role of miR-21 in cell invasion and tumour metastasis and found that suppression of miR-21 in metastatic breast cancer MDA-MB-231 cells significantly reduced invasion and lung metastasis. They showed that over-expression of TMP1 (tropomyosin1), which is down-regulated by miR-21, suppresses the invasiveness of MDA-MB-231 cells, and they also identified PDCD4 and maspin as candidate target genes for miR-21. Yan et al. (2008) investigated the global miRNA expression profile in primary breast cancer cells to evaluate their involvement in the malignant progression of the tumour. Their principle finding agrees with that of Zhu et al. (2007) that over-expression of miR-21, one of the most significantly altered microRNA in breast cancer, is associated with progression and poor prognosis of the patient.

Tavazoie et al. (2008) identified a set of 6 microRNAs (miR-335, miR-126, miR-206, miR-122a, miR-199a and miR-489) for which expression is specifically lost as human breast cancer cells develop metastatic potential. Restoration of miR-126, miR-206 and miR-335 expression in malignant cells suppressed lung and bone metastasis by human cancer cells in vivo. MiR-126 significantly suppressed overall tumour growth and proliferation, whereas miR-335 inhibited metastatic cell invasion. In clinical metastasis, Tavazoie et al. (2008) revealed that patients whose primary tumours displayed low expression of miR-335 and miR-126 or miR-206 had a shorter median time to metastatic relapse. It was also reported that miR-335 regulates metastatic gene SRY-box containing transcription factor (SOX4); tenascin C (TNC); c-mer tyrosine kinase (MERTIC) and receptor type tyrosine protein phosphatase (p-TPRN2), whose collective expression in a large cohort of human tumours is associated with risk of distal metastasis.

Huang et al. (2007) demonstrated that miR-373 and miR-520c have metastasis promoting abilities. They showed that miR-373 and miR-520c did not affect cell proliferation but promoted a migratory and invasive phenotype of MCF7 cells in vitro. Furthermore, MCF7 cells over-expressing miR-373 and miR-520c developed metastatic nodules, which were absent in control cells. Elucidating the molecular pathways involved, of nine potential targets CD44 was found to be a direct target of miR-373 and miR-520c. The migration phenotype of miR-373 and miR-520c can be explained by the suppression of CD44 expression. Huang et al (2007) also found significant up-regulation of miR-373 in clinical breast cancer metastasis samples that correlated inversely with CD44 expression.

Breast cancer metastasis suppressor I (BRMSI) inhibits breast melanoma, non-small cell lung and ovarian cancer metastasis in a number of experimental models. BRMSI forms complexes with SIN3, histone deactylases and selected transcription factors that modify metastasis-associated gene expression (EGFR, OPN, PLAU, P14PSKIA). Edmonds et al. (2009) found that BRMSI altered miRNA expression in metastatic breast carcinoma cells, down-regulating miR-106, miR-373 and miR-520c and their downstream targets (for example RohC which is a downstream target of miR-106) and up-regulating miR-146a, miR-146b and miR-335. These results are

in agreement with previous publications of Ma et al., (2007), Hurst et al., 2009, Tavazzoie et al., 2007 and Huang et al., 2007.

Hepatocellular Carcinoma

Budhu et al. (2008) identified a 20 microRNA signature that is associated with hepatocellular carcinoma (HCC) venous metastasis. The signature was capable of predicting survival and recurrence of HCC in patients with multinodular or solitary tumours, including those with early stage disease.

Colorectal cancer

Asangani et al. (2008) demonstrated that miR-21 induces invasion / intravasation / metastasis in colorectal cancer cells and that the tumour suppressor Pcd4 is negatively regulated by miR-21 at the post transcriptional level, via a specific target site (nt 228- nt249) within the 3'-UTR.

Epigenetics and miRNAs.

Cancer has been recognised as both a genetic and epigenetic disease. Genetic lesions alone cannot explain the complexity of the aberrations that arise in a cancer cell (Lujambio and Esteller, 2009). Epigenetics is defined as the inheritance of changes in gene activities that are independent of DNA sequence. The two main epigenetic mechanisms involved in gene regulation, development and carcinogenesis are DNA methylation and histone modification (Lujambio and Esteller, 2009). In cancer cells, the fine control of epigenetic mechanisms is lost and the disruption of epigenetic patterns promotes the expression of the tumoural phenotype. Three main epigenetic alterations occur in human cancer. These are 1) DNA hypermethylation of tumour suppressor genes, 2) global genomic hypomethylation and 3) aberrant histone modification signatures.

DNA hypermethylation of tumour suppressor genes

DNA methylation is a normal process used by mammalian cells in maintaining normal expression patterns; it is involved in the regulation of imprinted gene expression and X-chromosome inactivation among others (Robertson and Jones, 2000). DNA methylation occurs almost exclusively on a cytosine in a CpG dinucleotide and is achieved by the addition of a methyl group to the 5 position of a cytosine ring by the action of three DNA methyltransferases namely Dnmt1, Dnmt3a and Dnmt3b. Dnmt3a and Dnmt3b are de novo methyltransferases and DNMT1 is the maintenance DNMT that ensures that methylation patterns are copied faithfully through each cell division (Klose and Bird, 2006). CpG sites are approximately 80% depleted in the genome and are asymmetrically distributed into CpG poor regions and dense regions known as CpG islands, which are often located in the promoter regions of roughly half of all protein-coding genes (Chuang and Jones, 2007). Methylation of CpG islands in promoter regions is often associated with gene silencing and aberrant DNA methylation occurs in most cancers, leading to the silencing of some tumour suppressor genes (Jones, 2002 and Bird, 2002). Lehmann et al (2008) reported that 61 CpG islands associated with human miRNA genes have been identified, thus far.

Exploiting the pharmacologic manipulation of the epigenome, Saito et al. (2006) demonstrated that a subset of miRNAs are upregulated in bladder transitional cells (T24) by treatment with chromatin modifying drugs (5'aza-2'deoxy-cytidine; 5'aza-CdR) and 4-phenylbutyric acid, PBA, which inhibit DNA methylation and histone deacetylase, respectively). In particular, miR-127, which is located within a CpG island and silenced in human cancer cells, was highly induced after treatment with 5'aza CdR and PBA, with downregulation of its potential target the proto-oncogene BCL6.

A similar approach was recently taken by Toyota et al. (2008), demonstrating that AZA-5CrD treatment of colon cancer cell lines upregulates over 30 miRNAs, including miR-346 and miR-34c. They also reported that downregulation of miR-346/c expression was strongly associated with hypermethylation of its neighbouring CpG island, which notably harbours bidirectional promoter activity and also regulates expression of another candidate tumour suppressor gene, B-cell translocation gene 4 (BTG4). The expression of BTG4 suppressed colony formation of CRC implying that BTG4 as well as miR-346/c are novel tumour suppressors in colon cancer (Toyota et al., 2008).

Lujambio et al. (2007) used a genetic model to compare the miRNA expression profile of the wild-type colon cancer cell line (HCT-116) with the same cell line after genetic disruption by homologous recombination of DNA methyltransferases 1 (DNMT1) and DNMT3b (Double knockout, DKO), using a miRNA microarray profiling method. The double knockout (DKO) cells showed a reduction of DNMT activity and 5-methylcytosine DNA content leading to a release of gene silencing associated with CpG island hypermethylation (Lujambio et al., 2007). Their results demonstrated that DNA hypermethylation contributed to the transcriptional down-regulation of miRNAs in human tumours and that the epigenetic silencing of miR-124a leads to the activation of cyclin D kinase 6 (CDK6), a bona fide oncogenic factor, and the phosphorylation of the retinoblastoma (Rb) tumour suppressor gene.

Aberrant hypermethylation events in the regulatory regions of miRNAs may also play a role in the establishment of human metastasis. Lujambio et al. (2008) used a pharmacological and genomic approach to unmask an aberrant epigenetic silencing program by treating lymph node metastatic cancer cells with a DNA demethylating agent followed by hybridization to an expression microarray. MiR-148a, miR-34B/C and miR-9 were found to undergo specific hypermethylation-associated silencing in cancer cells compared to normal cells. The reintroduction of miR-148a and miR-34b/c in cancer cells with epigenetic inactivation inhibited their motility, reduced tumour growth, and inhibited metastasis formation in xenograft models, with an associated down-regulation of the miRNA oncogenic target genes, such as C-MYC, E2F3, CDK6, and TGIF2. The involvement of miR-148a, miR-34b/c, and miR-9 hypermethylation in metastasis formation was also suggested in human primary malignancies (Lujambio et al., 2008).

In a clinical study of epithelial ovarian cancer, Lu et al. (2007) analysed the methylation of let-7a-3 in tumour tissues using real time methylation specific PCR and evaluated the effect of methylation on let-7a expression, insulin-like growth factor II (IGF-II), a potential target of let-7a and patient survival outcomes. They showed that let-7a-3 was methylated in epithelial ovarian cancer and that low let-7a-3 methylation was associated in ovarian cancer with high IGF-II and low IGFBP-3 expression as well as poor prognosis of epithelial ovarian cancer.

Global Genomic Hypomethylation

Global hypomethylation events can also affect miRNAs. Brueckner et al. (2007) showed that the human let-7a-3 gene on chromosome 22q13.31 is associated with a CpG island. Using bisulfite sequencing analysis, they found that the let-7a-3 gene is heavily methylated in normal human tissues but hypomethylated in some lung adenocarcinomas. They also reported that let-7a-3 hypomethylation facilitated epigenetic reactivation of the gene and elevated expression of let-7a-3 in a human lung cancer cell line resulted in enhanced tumour phenotypes and oncogenic changes in transcriptional profiles (Brueckner et al., 2007).

Using miRNA microarray analysis in hepatocellular carcinoma cells (HCC) treated with 5-AzaC (5-azacytidine) alone or in combination with trichostatin A (TSA), a histone deacetylase inhibitor, Datta et al. (2007) attempted to identify candidate tumour suppressor miRNAs that are silenced by epigenetic mechanisms in HCC cells. They found that miR-1-1 is one such gene that is methylated in human HCC cells and primary HCC, and its activation by epigenetically-acting drugs suppresses tumour cell growth by down-regulating its oncogenic targets MET, FoxP1 and HDAC4.

Iorio et al. (2007) investigated whether aberrant DNA methylation patterns could contribute to the altered miRNA expression characterising human ovarian cancer, using miRNA profiling of the ovarian carcinoma cell line (OVCAR3) before and after treatment with the demethylating agent 5-Aza (5-aza-2'-deoxycytidine). The analysis of miRNA microarray data showed 11 human miRNAs differentially expressed, 9 upregulated and 2 down regulated, with miR-21, miR-203, miR-146b, miR-205, miR-30-5b and miR-30c, the most significantly induced on treatment. They also found that miR-21, miR-203 and miR-205 are overexpressed in ovarian carcinomas compared to normal tissue. The findings that reactivation of the miRNA genes occurs after demethylation treatment suggests that hypomethylation could be the mechanism responsible for the overexpression in vivo.

Alternative splicing

Splicing is a post transcriptional process involved in the maturation of nearly all mRNAs. Alternative splicing is the process whereby identical pre-mRNA molecules are spliced in different ways and is important in normal

development as a means of creating protein diversity in complex organisms (DeKlein et al 1998; Black 2003).

Disruption of the normal splicing code in cancer cells generates alternatively spliced mRNAs that are not produced in normal cells. The aberrant transcripts are part of a surveillance system such as non-sense mediated decay and are degraded before protein expression. However, some aberrant mRNAs are able to produce protein isoforms with tumourigenic properties and some that have been documented include CD44 (Kalnina et al., 2005), tenascin C (Tseleni-Balafouta et al., 2006), human oestrogen (Davies et al., 2004) and progesterone (Nagao et al., 2003) receptors and BRCA1 tumour suppressor gene participating in the pathogenesis of hereditary breast and ovarian cancer (Claes et al., 2002).

There are two possible causes of such alterations in cancer related to alternative splicing. One is a mutation in the *cis*-splicing regulatory elements of RNA, which may shift production to mRNA with cancer prone potential. The second possible cause is the alteration of RNA binding proteins involved in the regulation of the splicing.

Non-sense-mediated Decay (NMD)

A number of “surveillance” mechanisms that act at several steps of mRNA biogenesis are able to detect and degrade defective and improperly-processed mRNA transcripts. The NMD mechanism detects mRNA harbouring premature termination codons (PTCs) and is responsible for their degradation. The NMD process is functionally and tightly connected with alternative splicing and other RNA-mediated processes such as stimulation of translation and/or telomerase regulation (Lejeune et al., 2003; Wilkinson, 2005 and Fukuhara et al., 2005).

One physiological purpose of the NMD mechanism is to limit the synthesis of C-terminally truncated polypeptides, protecting the cell from its deleterious dominant-negative or gain of function effects (Silva and Romao, 2009). PTC-containing mRNA can either be generated by various types of germline/somatic alterations in the DNA or originate as a result of routine errors during transcription or mRNA processing. Also, PTCs can arise as a consequence of non-faulty processes of mRNA metabolism such as somatic rearrangements in the DNA, alternative splicing or utilisation of the alternative AUG initiation sites following pre-mRNA splicing. NMD targets are recognised via a multiprotein exon-junction complex (EJC) that is deposited 24 nucleotides upstream of each exon-exon junction. It has been established that aberrant mRNAs containing a PTC located within less than 50-55 nucleotides upstream of the last exon-exon junction or in the last exon are not degraded by NMD. The Upf (Up-frameshift) proteins have been considered the conserved core of the NMD machinery (Conti and Izaurralde, 2005). Four Upf's have been identified thus far in humans (hUpf1, hUpf2, hUpf3a, and hUpf3b) (Lykke-Andersen et al., 2000; Mendell et al 2000; Serin et al., 2001). hUpf1 is both an ATP-dependent 5'-3' helicase and RNA dependent ATPase whose activation by phosphorylation is necessary for NMD triggering

(Yamashita et al., 2001). Although UFP1 is crucial for NMD triggering, other pathways for NMD triggering can apparently diverge in their requirement for Upf2, Upf3 and other EJC components (Silva and Romao, 2009). Upf1 is a phosphoprotein (Yamashita et al., 2001) whose activity is regulated by cycles of phosphorylation and dephosphorylation (Conti and Izaurralde, 2005). Four other proteins have been shown to play an important role in mediating the phosphorylation/dephosphorylation cycle (Suppressor with morogenetic effects on genitalia (SMG)1, SMG5, SMG6 and SMG7). SMG1, a phosphatidylinositol-3 kinase-related protein, has been shown to phosphorylate Upf1 at specific serine residues (Yamashita et al., 2001). Dephosphorylation of Upf1 is mediated through SMG5, SMG6 and SMG7 proteins, possibly by mediating the recruitment and activity of protein phosphatase 2A (PP2A), which is thought to be the protein factor responsible for Upf1 dephosphorylation.

It has been speculated that NMD plays a role in cancer development although the precise role is not yet known. A subset of cancers is characterised by mismatch repair (MMR) deficiency arising from the inactivation of mismatch repair genes. These tumours exhibit a particular phenotype called MSI, which is characterised by global instability affecting the microsatellite repeat sequences (Aaltonen et al., 1994 and Thibodeau et al., 1996). Boland et al. (1998) reported that these cancers can occur sporadically in 10-15% of colorectal, gastric and endometrial carcinomas or can occur in the context of hereditary non-polyposis colorectal cancer (HNPCC) syndrome (Bronner et al 1994; Papadopoulos et al. 1994; Fishel et al. 1993 and Leach et al. 1993). The inactivation of the MMR gene is not itself thought to be a transforming event but rather additional genetic changes are believed to be necessary for MSI-H cells to become malignant (Duval and Hamelin, 2002). Most of the mutations are 1 or 2 bp insertions or deletions found within genes containing coding repeat sequences that are particularly prone to alterations in MSI-H cancers (Duval and Hamelin, 2002). The mutations result in frameshifts leading to mutant mRNAs containing premature termination codons (PTC) and encoding for truncated proteins. The NMD system will then target the mRNAs with PTC for rapid degradation.

Analysis of mRNA expression of several target genes in which mutations had previously been reported in a panel of MSI-H and non-MSH-I colorectal cell lines, provided strong evidence for a significant degradation process of frameshift mutation derived mRNAs in MSI-H cancers. Silencing of UFP1 expression in 5 MSI-H cell lines showed that this degradation process was partly dependent on NMD activity. El-Bchiri et al. (2005) provided evidence that frameshift mutation mRNAs decay was mainly dependent on Upf1 activity with Upf2 playing a minor role in the process. El-Bchiri et al. (2005) demonstrated an additive role in the process by using RNA silencing of Upf1, Upf2 or both these factors in HCT116 cell lines. The authors suggest that these data show that NMD is highly involved in target gene mRNA expression levels in MSI-H cancers. El-Bchiri et al. (2008) demonstrated that UFP1 was significantly over-expressed in MSI-primary colorectal cancers (CRCs) compared to normal adjacent mucosa. They also observed a negative effect

of Upf1 and Upf2 expression on the host's immunity against MSI-H cancer cells.

RNA binding proteins and cancer

RNA binding proteins are key components in RNA metabolism, regulating all aspects of RNA biogenesis from RNA maturation, surveillance, nucleocytoplasmic transport to subcellular localisation, translation and RNA degradation. RNAs cannot exist alone in cells and are stably assembled with many RBPs in functional units called ribonucleoprotein (RNP) complexes.

Deregulation of splicing factors might cause alternative splicing of various transcripts in cancer cells. SR proteins are a family of RNA binding proteins that are essential for splicing. An example of an altered SR RBP in cancer is the splicing factor SF2/ASF. In the case of SF2/ASF, there have been several reports of its elevated expression associated with cancer, for example ovarian cancer (Fischer et al., 2004). Karni et al. (2007) found that SF2/ASF acts as a proto-oncogene whose up-regulation results in the modulation of alternative splicing in key target genes. These post-translational changes result in the inactivation of the putative tumour suppressor bridging integrator 1 (BIN1) and the generation of oncogenic isoforms of the gene, ribosomal-protein kinase S6K-1, which is involved in cell death and cell growth.

Translation of mRNA is also a regulatory point for the expression of tumour suppressors and oncogenes in cancer cells. The mRNA cap binding protein EIF4-E is a key translational factor downstream of the phosphatidylinositol 3'kinase-Akt-mammalian target of rapamycin (P13K-Akt-mTOR) pathway. EIF4-E has been shown to be highly expressed in different tumour types such as carcinoma of the breast, prostate, head and neck squamous cell carcinomas (HNSCC) (DeBenedetti and Harris, 1999).

RBPs, involved in cell differentiation and proliferation, might also modulate cancer cell development. An example of this is the STAR (Signal transduction and RNA binding) family of RBPs. Their expression is altered in cancer. The best studied of the STAR family is SAM68. It has a KH homology domain that is overexpressed in breast and prostate cancer cells (Lukong and Richard, 2003; Busa et al., 2007). It has been shown that downregulation of SAM68 expression or activity affects prostate cancer cell proliferation and survival (Busa et al., 2007). Decreasing SAM68 expression in prostate cancer cells, using RNAi methodology, reduced the proliferation rates and delayed cell cycle progression of androgen-responsive LNCaP cells.

RNA editing

RNA editing is another mechanism capable of changing the genetic information outside genomic DNA. This mechanism involves the enzymatic alteration of single or multiple nucleotides, either in the non-coding or coding sequence of pre-RNA, which occurs concomitantly with transcription and RNA processes such as splicing, 5' capping and polyadenylation. Two families of RNA-editing enzymes were identified in humans: the adenosine deaminases acting on RNA (ADAR) family, involved in adenosine (A) to inosine (I) RNA editing and apoB mRNA-editing catalytic peptide (APOBEC) family, which

includes the related activation-induced deaminases (AID) family and which induce cytosine (C) to uracil (U) transformation in RNA and DNA.

The heterogeneous nuclear ribonucleoprotein K (hnRNP k) is an RNA binding protein involved in multiple processes involved in gene expression (Bomsztyk et al 2004). K protein contains three K homology (KH) domains that mediate RNA/DNA binding and the K interactive (KI) region that recruits many protein partners. K protein has been shown to be upregulated in malignant cells such as human breast cancer cells (Mandal et al., 2001), in mouse hepatic neoplasms (Ostrowski and Bomsztyk, 2003) and human hepatocellular carcinomas (Li et al., 2004). The K protein also regulates the expression of genes involved in mitogenic responses and tumorigenesis. Klimek-Tomaczak et al. (2006) discovered an editing site in RNA encoding the K protein, where the editing event resulted in a single amino acid change (Ala to Thr) and the consequent appearance of a new K variant protein seen uniquely in large bowel adenocarcinoma cells but not normal cells.

Another form of editing is A to I editing, which involves a site specific modification in stem-loop structures within precursor mRNAs. The splicing and translational machineries recognise inosine (I) as guanosine (G). A number of editing sites occur in coding regions and may result in amino acid substitutions affecting the protein properties and interactions. A well characterised example of site specific editing in a coding region is an A to I change in mRNA encoding a subunit of an ionotropic glutamate receptor (GluR) channel. In the GluR subunit B, a single editing position (the Q/R site) controls the Ca^{++} permeability of the ion channel. The RNA editing almost quantitatively (>99.9%) changes the glutamine (Q) codon CAG to a CIG, specifically, arginine (R). Maas et al. (2001) examined whether the cellular machinery of A to I RNA editing might be involved in the progression of cancerous growth within the human brain. They reported a significant decrease in GluR-b Q/R site editing in human glioma tissues compared to control tissues. They also found that the measured percentage of GluR-b (Q) in tumours (12-31%) corresponded to an increase in the amount of unedited GluR-b of more than 300 fold. They suggest that because the Q/R site of GluR-b is targeted specifically by the ADAR editing enzyme, underediting at this position might be due to the decreased expression of ADAR or downregulation of ADAR enzymatic activity.

Martinez et al. (2008) suggest that RNA editing of androgen receptor gene transcripts may contribute to the etiology of hormone refractory phenotypes in advanced stage of androgen-independent prostate cancer. They reported that RNA editing could explain the AR gene transcript missense mutation as the nucleotide transitions (A to I, C to U, U to C, and G to A) correlated to the substrate specificities of RNA editing enzymes (such as ADAR and APOBEC1).

A to I editing events can also occur in non coding repetitive sequences, mostly Alu elements, and these events tend to undergo multi-editing in tight clusters. Using bioinformatic analysis, Paz et al. (2007) identified reduced A to I editing in human testis, prostate, lung, kidney and brain tumours compared

to their normal counterparts. They identified significant global hypoediting of Alu repetitive elements with the gene encoding the thyroid hormone receptor associated protein 1 (MED13) in brain tumoural tissues compared with normal tissue. Paz et al also demonstrated that gene-specific coding and non-coding region editing differs between normal and malignant tissues. For example, editing levels are elevated in proteins such as bladder cancer-associated protein (BLCAP) and BRCA1 or reduced in proteins such as Filament A actin (FLNA) or cytoplasmic FMR1 interacting protein 2 (CYF1P2) in tumours. Paz et al. (2007) found reduced RNA expression levels of editing enzymes in brain tumours compared to normal brain tissues. Overexpression of ADAR in a glioblastoma cell line resulted in decreased proliferation rate, thus suggesting that A to I editing in brain tumours is involved in the pathogenesis of cancer. However, Zilberman et al. (2009) failed to demonstrate a role for RNA editing in the development of urothelial carcinoma. Examination of 9 genes representing both coding areas and Alu repetitive elements revealed no significant changes in editing levels between normal and urinary bladder tumour tissue.

Conclusions

There is evidence to suggest that RNA plays an important role in the mechanisms of tumourigenesis, although the precise contribution of individual RNA molecules and RNA regulated processes such as alternative splicing, RNA editing, NMD or RNAi on cancer development and progression needs further investigation.

Studies in recent years have suggested that miRNAs play an important role in the pathogenesis of human cancers. Some miRNAs may be directly involved in cancer development by controlling cell differentiation and apoptosis, while others may be involved in cancers by targeting cancer oncogenes and/or tumour suppressors. It is thought that understanding the function of miRNAs is and will provide new insights with regard to the molecular basis of cancers, and new biomarkers for cancer diagnoses and cancer therapy.

This review has also highlighted that several miRNAs can regulate the same gene. The interaction between these miRNAs and what mechanisms control these miRNAs for targeting the same gene are still unclear. Evidence from the studies available in the literature indicates that miRNAs are regulated by epigenetic processes and act as regulators of the epigenome. However, further studies are needed to shed light on the full magnitude of interactions between miRNAs and the epigenetic machinery.

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