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Review

MicroRNA and ovarian cancer

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Summary. Ovarian cancer remains a leading cause of morbidity and mortality, with little change in survival rates over the past 30 years. Research in the molecular biology underlying the disease demonstrates frequent mutation in the *p53/Rb/p16* tumor suppressor pathways and activation of *c-myc*, *K-ras* and *Akt* oncogenic signaling. Recently, miRNAs have been demonstrated to play an important role in controlling proliferation, apoptosis and many other processes altered in the cancer state. In this review we discuss a number of recent publications that implicate a role for microRNAs in ovarian cancer and assess how this new field may improve our fundamental understanding of the disease and provide improved diagnostic and therapeutic approaches.

Key words: Carcinogenesis, Epithelial ovarian cancer, MicroRNAs, Mouse models

Ovarian cancer

It is predicted that over 22,000 women will be diagnosed with ovarian cancer and over 15,000 will die from the disease in the US during 2007 (Jemal et al., 2007). Like cancers at many other sites, 5-year survival is good if diagnosed at an early stage when the cancer is confined to the ovary. However, almost 70% of women are diagnosed at an advanced stage, at which point 5-year survival is 30%. Obviously, improved screening programs to detect cancers at an early stage and better therapeutics to treat advanced disease are urgently required. Unfortunately, asymptomatic development in humans, combined with a scarcity of accurate animal models, has resulted in a marked lack of knowledge of the pathogenesis of ovarian cancer. However, risk factors include persistent ovulation, age and family history, the latter being largely attributed to carriers of hereditary *BRCA1* mutations, which may account for up to 10% of epithelial ovarian cancers (EOC) (Risch et al., 2001).

EOC is the most prevalent type of ovarian cancer, far outnumbering the sex cord-stromal tumors and germ cell tumors. The presumed origin of EOC is the ovarian surface epithelium (OSE), a single layer of cuboidal cells coating the ovary (Auersperg et al., 2001; Nikitin et al., 2004). Early in life, the OSE is generally smooth but with age becomes increasingly convoluted, resulting in an increase in number of inclusion cysts. Such cysts, which may form by pinching off of invaginations or by trapping of epithelial cells during postovulatory wound healing, may be a precursor lesion for progression to carcinoma. Importantly, however, a study by Horiuchi et al. demonstrated that while almost 50% of tumors developed from pre-existing lesions, in the remaining patients no such pre-existing lesions had been present 12 months prior to diagnosis. Furthermore, cancers arising from cysts were categorized as low-grade by pathological analysis, while those with no evidence of a precursor lesion were mostly of a high-grade serous carcinoma (Horiuchi et al., 2003). This study gives significant weight to the hypothesis that high-grade serous carcinomas are not derived step-wise from low-grade tumors but instead arise de novo from the OSE (Shih Ie and Kurman, 2004), which, if true, has implications for screening processes, given that serous carcinoma is the most common histological subtype of EOC.

MicroRNAs

It had been known for many years that the *Caenorhabditis elegans* heterochronic gene *lin-4* is required for correct developmental timing and that its loss of function results in reiterations of early fates at inappropriate developmental stages (Chalfie et al., 1981). Conversely, mutations in *lin-14* results in an opposite phenotype and is completely epistatic to lin-4 loss of function, suggesting that *lin-4* may negatively regulate *lin-14*. Despite much effort, however, no LIN-4 protein could be identified and it was not until 1993,

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when *lin-4* was cloned in the Ambros lab, that it was realized that the gene is non-coding (Lee et al., 1993). Rather, two transcripts of 61 nt and 22 nt in length, the later which is partially antisense complementary to the 3' untranslated region (UTR) of *lin-14* (Wightman et al., 1993), were the only detectable products – the first miRNA. However, not until the identification of a second small RNA, *let-7* (Reinhart et al., 2000), and the observation that it is widely conserved (Pasquinelli et al., 2000), did miRNAs receive attention by the wider scientific community.

Biogenesis of miRNAs starts with transcription of their primary transcript (pri-miRNA), largely by RNA polymerase II, but polymerase III for some miRNAs within Alu repeats (Lee et al., 2004; Borchert et al., 2006). The pri-miRNA, which may be several kilobases in length, is then subjected to step-wise processing by two RNase III endonucleases. Firstly, in the nucleus, Drosha together with DGCR8/Pasha processes the pri-miRNA to a ~70 nt double stranded precursor miRNA (pre-miRNA) (Lee et al., 2003; Landthaler et al., 2004). After Exportin-5 mediated export to the cytoplasm (Yi et al., 2003; Lund et al., 2004), pre-miRNA molecules are subjected to processing by Dicer resulting in a ~22 nt RNA duplex: one stand being the mature miRNA and the other being the passenger strand (denoted in the literature as miRNA*). The miRNA duplex is unwound with the miRNA*, generally the stand with the highest stability at the 5' end, being degraded, while the mature miRNA is loaded into the RNA induced silencing complex (RISC) (Khvorova et al., 2003, Schwarz et al., 2003). Although the mechanism of miRNA unwinding is unclear, the unwinding of *let-7* can be attributed to the P68 RNA helicase (Salzman et al., 2007). In addition to Drosha-mediated processing to generate pre-miRNAs, a Drosha-independent biogenesis pathway has recently been reported (Berezikov et al., 2007; Okamura et al., 2007; Ruby et al., 2007). Mirtrons, short introns that, after splicing and de-branching, form pre-miRNA-like molecules, are a second source of pre-miRNA molecules. Once exported to the cytoplasm by Exportin-5, they are processed by Dicer to mature miRNAs and seem to function identically to canonically synthesized miRNAs. The number of identified mirtrons is far lower than that of miRNAs, however, and it would appear that Drosha-dependent miRNAs are by far more common.

There are 2 possible outcomes upon binding of the miRNA-loaded RISC to a target 3' UTR (Fig. 1). The first possibility, and by far the most common in animals, is translational repression. For this to occur, perfect base pairing between miRNA/mRNA is not required and mismatches and bulges are tolerated, so long as perfect base pairing is achieved at the seed region of the miRNA, which encompasses nucleotides 2-8. The actual mechanism of repression is currently controversial. However, a number of recent publications give significant weight to the hypothesis that initiation of translation is inhibited (Petersen et al., 2006; Wang et al., 2006; Thermann and Hentze, 2007; Wakiyama et al., 2007). Additionally, another paper demonstrates that an increase in the cap binding protein eIF4e attenuates miRNA-mediated repression in a cell-free system (Mathonnet et al., 2007). These data are consistent with the presence of a cap binding domain within RISC component Ago2 (Kiriakidou et al., 2007), suggesting that repression occurs by Ago2 competitively inhibiting eIF4e binding to the cap structure. This may then lead to decapping, deadenylation and sequestering of the mRNA target from translation machinery by the localization to processing bodies (reviewed by Standart and Jackson, 2007). Through this mechanism, a substantial decrease in protein concentration with little or no change in mRNA levels can be achieved. In contrast, a reduction of both mRNA and protein is observed if perfect complementarity is achieved between the entire miRNA

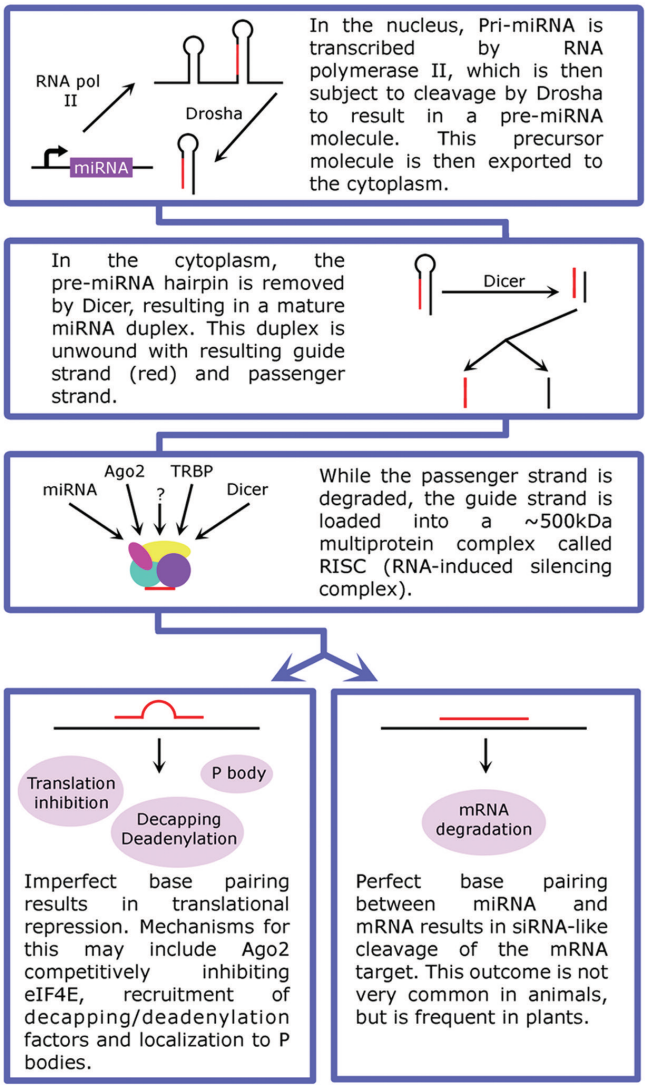

Fig. 1. Flow diagram outlining miRNA biogenesis and function.

and target. The mechanism for this is target cleavage between nucleotides 10 and 11 by Ago2-containing RISC. However, while this outcome is common in plants, it is less so in animals. Computational predictions put the number of targets for many miRNAs in the hundreds to thousands. Moreover, over 30% of the human protein coding genes are predicted to be directly regulated by miRNAs (Lewis et al., 2005) and two or more different miRNAs may regulate a single transcript (Doench and Sharp, 2004), suggesting a highly complex signaling network. Furthermore, miRNA-mediated repression is not limited to coding genes; some transcribed ultraconserved regions (T-UCRs), another class of non-coding RNAs, are also targeted by miRNAs (Calin et al., 2007).

In a highly intriguing twist, it appears that in certain contexts and circumstances, RISC can be "converted" into a translation activating complex. Firstly, Vasudevan et al., demonstrated that tethering AGO2 and fragile-X-mental-retardation-related protein 1 (FXR1) to an AU-rich element (ARE) present in the 3' UTR of *TNFα* was sufficient to increase translation (Vasudevan and Steitz, 2007). In a separate paper, they then went on to show the requirement of *miR-369-3* in directing the AGO/FXR1 complex to the mRNA, resulting in increased translation (Vasudevan et al., 2007). Mutating the seed sequence for the miRNA binding site in the ARE prevents translation upregulation, yet mutating *miR-369-3* to match the mutated binding site restores upregulation. This effect is not a peculiarity of *miR-369-3*, either, since the authors also demonstrate similar effects with artificial miRNA cxcr4 system and the endogenous miRNA *let-7* on its target HMGA2. However, whether activation over repression is observed depends on the stage of the cell cycle – only when cells have been serum starved and have undergone cell cycle arrest is activation observed, while in proliferating cells, repression is seen. While clearly an exciting observation, further work will be required to elucidate the mechanisms responsible and to establish the consequences of miRNA-mediated translation upregulation in vivo in a non-synchronized cell pool.

MicroRNAome alterations in cancer

Given the large number of mRNAs predicted to be directly regulated by miRNAs, it would be expected that a large number of processes be controlled by miRNAs, and indeed this is the case. Processes under miRNA control include hematopoiesis (Chen et al., 2004, Felli et al., 2005), cardiogenesis (Zhao et al., 2007), blastocyst implantation (Chakrabarty et al., 2007) and neuronal development (Schratt et al., 2006). Yet the majority of research has been focused on the possible roles that miRNAs may play in disease and specifically, in cancer.

The first indications of a role for miRNAs in cancer came from the laboratory of Carlo Croce. A bicistronic miRNA cluster containing *miR-15a* and *miR-16* at chromosome 13q14 was observed to be mutated, deleted or have reduced expression in chronic lymphocytic leukemia (Calin et al., 2002, 2005). Later, germline mutations in *miR-15a/-16* were observed and it was shown that these two miRNAs target anti-apoptotic BCL-2 mRNA (Calin et al., 2005; Cimmino et al., 2005). While these reports suggest that *miR-15a/-16* act as tumor suppressors, whether they fit the conventional outline of a tumor suppressor is unclear. There are several highly related members of the *miR-15* family, including a second copy of the *miR-15* and *miR-16* cluster on chromosome 3 and *miR-195* on chromosome 17; therefore even if *miR-15a/-16* is lost at chromosome 13, other family members would still be expressed, suggesting that gene dosage is the most critical factor (Linsley et al., 2007). Importantly, however, miRNAs appear to have cell type specific roles, since while *miR-15a/-16* induces apoptosis by targeting BCL-2 in megakaryoblastic cell line MEG-01, no such induction of apoptosis or change in BCL-2 protein is observed in non-hematopoietic cells analyzed by Linsey et al., (Cimmino et al., 2005, Linsley et al., 2007).

In addition to possessing growth inhibitory activities, some miRNAs, such as those in the *miR-17-92* cluster, have been implicated as oncogenes. *miR-17-92* is found in the c13orf25 locus on human chromosome 13q31, a region that is commonly amplified in multiple cancer types (Knuutila et al., 1998). Tumors arising from hematopoietic stem cells expressing both c-myc and a subset of miRNAs from *miR-17-92* demonstrate an absence of apoptosis observed in c-myc-only tumors (He et al., 2005), while Dews et al., demonstrate that this miRNA cluster also results in greater angiogenesis in tumors (Dews et al., 2006). Underlying the importance of Myc in cooperating with *miR-17-92*, Myc directly binds canonical E-box sequences in the *miR-17-92* promoter and members of the *miR-17-92* cluster target E2F1. This suggests an interesting signaling pathway, since Myc and E2F1 are known to induce each others expression, while at the same time Myc represses E2F1 translation through miRNAs (O'Donnell et al., 2005). Complicating the issue further still are reports demonstrating that E2F proteins also bind the *miR-17-92* locus, resulting in a negative feedback loop (Sylvestre et al., 2007; Woods et al., 2007).

The genomic location of miRNAs is not evenly distributed throughout the human genome. Instead, there are some chromosomes, for example chromosome 4, with a lower than expected number, and some with greater than expected miRNAs, for example chromosomes 17 and 19 (Calin et al., 2004). More importantly, however, taking a computational approach, Calin et al. demonstrate that miRNA genes are frequently found at fragile sites, regions commonly amplified or lost, common breakpoint regions and are close to human papilloma virus integration sites (together abbreviated to CAGRs; cancer-associated genomic regions). Of 186 miRNAs studied, 98 (52.5%) were located in CAGRs and a significant number of miRNAs are located either in, or close to, homeobox

clusters, which contain genes that are transcription factors playing important roles in both normal development and carcinogenesis in a variety of cancers, including ovarian cancer (Hennessy and Mills, 2006).

MicroRNA alterations in ovarian cancer

As outlined previously, Calin et al. provided evidence that miRNA expression may be altered by changes in gene copy number by proximity to CAGRs. To gain a more detailed view of miRNA copy number alterations in cancer, Zhang et al. used an array comparative genomic hybridization (aCGH) approach to identify miRNA loci gained/lost in ovarian cancer, breast cancer and melanoma (Zhang et al., 2006). Towards ovarian cancer, 93 primary and 16 cell lines were compared to DNA. A tumor to reference ratio less than 0.8 was considered as a copy number loss, while a value greater than 1.2 was considered as a copy gain. Of 283 miRNA loci analyzed, 105 (37.1%) were determined to be significantly altered in their copy number. Similarly, significant copy changes were observed in 206 of 283 (72.8%) miRNAs in breast cancer and 243 of 283 (85.9%) in melanomas. Quantitative RT-PCR demonstrated that expression of miRNAs was consistent with DNA copy number status for 73.1% of miRNAs, demonstrating that DNA copy number alterations may have an important effect on miRNA expression. Consistent with reports of tissue-specific expression of many miRNAs, the aCGH profile for each cancer type were not the same as revealed by cluster analysis. However, there are a number of copy number gains and losses shared between all three tumor types. Of interest is the loss of the *miR-17-92* locus in all tumor types. In addition, the *miR-15a/16-1* locus is lost in 23.9% of ovarian and 24.7% of breast cancers. Interestingly, Zhang et al. also observed that 24.8% and 51.5% of ovarian tumors exhibited gains in copy number of the *Dicer1* and *Argonaute 2* (*Ago2*) loci. As outlined previously, Dicer and Ago2 proteins are required for efficient miRNA processing and function. Most recently it has been shown that short-hairpin RNA (shRNA)-mediated knockdown of *Dicer1* and *Ago2* increases colony formation in soft agar and tumor formation i*n vivo* (Kumar et al., 2007). Taken together, these data indicate that *Dicer1*/*Ago2* over expression may be a favorable sign for cancer prognosis.

Given the relatively large number of ovarian cancer samples analyzed by Zhang et al., it is disappointing that no data was presented regarding the histological characteristics of each sample, since particular alterations may be correlated with particular EOC subtypes. However, this issue has been addressed, to some extent, at the transcriptional level by microarray profiling experiments (Iorio et al., 2007). A total of 69 primary EOC tissue, including 31 serous, 8 endometroid and 4 clear cell tumors, was analyzed and compared to total normal ovarian tissue. The *miR-200* family, which is comprised of 3 members (*miR-200a, -200b and -200c*) was among those miRNAs significantly over expressed, while those down regulated included *miR-199a, miR-140 and miR-145*. Only 2 miRNAs were down regulated in serous, endometrioid and clear cell carcinomas, *miR-200a* and *miR-200c*, while 19 were over expressed in all three tumor types, including *miR-140* and *let-7d*. Notably, the expression of the miR-200 family, *miR-140 and let-7d* is concordant with DNA copy number previously reported (Zhang et al., 2006). In addition to miRNAs conserved between EOC histological subtypes, there are also pools of miRNAs that are specifically down regulated in just one subtype, an observation that may have some implications for differential diagnosis in the future. However, while this report may be useful for diagnosis, the authors' choice of total normal ovarian tissue, rather than OSE, as normal control, limits what information can be used towards our basic understanding of miRNAome alterations in the OSE.

As outlined previously, changes in miRNA expression can occur at both the DNA and RNA level. Alterations in *Dicer1*/*Ago2* expression result in global changes in miRNA expression. However, alteration of individual miRNAs in cancer is also common. This may occur via one of several mechanisms, such as a germline mutation, deletion (Calin et al., 2005) or promoter methylation (Saito et al., 2006).

Given that mutation of the transcription factor *p53* is one of the most common genetic alterations observed in EOC, particularly in high-grade serous tumors (reviewed by Corney et al., 2008), we decided to determine whether inactivation of p53 would result in miRNAome alterations (Corney et al., 2007). Towards this aim we took advantage of mice carrying conditional alleles of *p53*, such that *p53* is inactivated by Cre-mediated recombination. Previously, we demonstrated that adenovirus mediated delivery of Cre (AdCre) to the OSE of mice carrying conditional alleles of both *p53* and *Rb* results in frequent serous adenocarcinomas (Flesken-Nikitin et al., 2003). To identify alterations in miRNA expression immediately after acute *p53* inactivation, we decided to use a cell culture approach, whereby the ovary from $p53^{loxP/loxP}$ mice is enzymatically digested to

Table 1. miRNAs altered in ovarian cancer.

miRNA	Reference
miR-105 *miR-143* *miR-203* *miR-373*	Iorio et al., 2007
let-7 *miR-9* *miR-34* *miR-140*	Iorio et al., 2007; Zhang et al., 2006
miR-15/-16 *miR-29a/b* *miR-30b/d* *miR-181b*	Zhang et al., 2006

release the OSE which is briefly cultured prior to AdCre treatment. After 2 further passages, miRNA profiling is performed. Compared to non-floxed wild type OSE, 84 miRNAs were significantly up/down regulated after *p53* inactivation, demonstrating that p53 does indeed control miRNA expression, at least indirectly. We observed that *miR-34b* and *miR-34c*, which are located 417 bp apart on human chromosome 11q23 and together form the *miR-34b/c* cluster, were two of the most down regulated miRNAs, with a 12-fold reduction in their expression as determined by quantitative RT-PCR. Through a bioinformatics approach, a conserved p53 responsive element (p53RE) was identified 2.4 kb upstream of mouse *miR-34b/c* and 3.8 kb upstream of human *miR-34b/c*. We observed that expression of both miRNAs after treatment with the DNA damaging agent doxorubicin was p53-dependent and taken together with ChIP-PET data (Wei et al., 2006) and ChIP-PCR in recent studies (Bommer et al., 2007; He et al., 2007), demonstrates that p53 directly transactivates *miR-34b* and *miR-34c*. In addition, several other groups concomitantly identified another *miR-34* family member, *miR-34a*, as being directly regulated by p53 through the presence of a p53RE 30 kb upstream of the miRNA mature sequence in several other cell types (Bommer et al., 2007; Chang et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tarasov et al., 2007).

Data from both our lab and of others demonstrates that members of the *miR-34* family control important processes that are altered in cancer, including proliferation, apoptosis and senescence in various cell types (Chang et al., 2007; Corney et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tarasov et al., 2007). Importantly, however, we have demonstrated that *miR-34b* and *miR-34c* cooperate in their suppression of proliferation and adhesion-independent growth in soft agar and that maximal suppression is achieved when both miRNAs are expressed (Corney et al., 2007). This observation may be due to the fact that each miRNA is predicted to target a separate pool of mRNAs in addition to a conserved pool (Fig. 2). Predicted targets include *Cdk4, Met, Ezh2, Cyclin D1, Myc and Bcl2*, and a number of these have been validated as genuine targets. Given the frequent mutation of p53 in not only in EOC but also in the majority of carcinomas, *miR-34b/c* may be useful therapeutic agents. Towards this, studies towards the therapeutic potential of *miR-34b/c* are currently underway.

It has therefore become clear that transcription factors controlling mRNA expression likewise control miRNA expression. It is therefore of much interest to elucidate the role of hormonal effects on miRNA expression. Hormones such as estrogen, progesterone, androgens and gonadotrophins have all been implicated in the etiology of EOC and mediate changes in cell physiology by initiating and repressing transcription of key genes (reviewed by Lukanova and Kaaks, 2005; Wong and Leung, 2007). Recently, it has been shown that *miR-20a* and *miR-21* are estrogen-responsive in endometrial stromal cells (Pan et al., 2007). Given that both of these miRNAs are expressed in the OSE (Iorio et al., 2007 and our unpublished observations), these miRNAs may be similarly regulated in the OSE.

Use of microRNAs in diagnosis

The *let-7* miRNA family is known to repress expression of the RAS and HMGA2 oncogenes and its decreased expression is associated with a poorer prognosis in non-small cell lung cancer (NSCLC) (Takamizawa et al., 2004; Johnson et al., 2005; Mayr et al., 2007). Shell et al. performed miRNA profiling of the NCI60 panel of human tumor cell lines (Shell et al., 2007). Members of the NCI60 panel can be grouped into 1 of 2 superclusters (SC) based upon their gene expression, with SC1 cell lines having mesenchymal and SC2 cell lines having epithelial properties. Interestingly expression of 5 of 7 *let-7* family members was reduced in SC1 whereas SC2 expressed high levels. Noticing that the mesenchymal-like SC1 cell lines share similarities with EOC, which commonly expresses mesenchymal markers (Rosano et al., 2005), the authors looked at the expression of *let-7* and *HMGA2* in 6 ovarian cancer cell lines. The expression of *let-7* and HMGA2 was inversely correlated, as one might expect given that *let-7* represses HMGA2 expression via 7 binding sites in the *HMGA2* 3' UTR. The authors then went on to analyze HMGA2 expression by performing immunohistochemistry on a tissue microarray containing 100 EOC samples. While no difference in HMGA2 staining intensity was observed between primary tumor sites and metastatic tumors, high HMGA2 expression significantly correlated with poor overall survival. In clinical samples, the *let-7*/HMGA2 relationship was conserved. Importantly, *let-7* and HMGA2 expression was sufficient to group patients into 1 of 2 groups. The first group with a high HMGA2/*let-7*

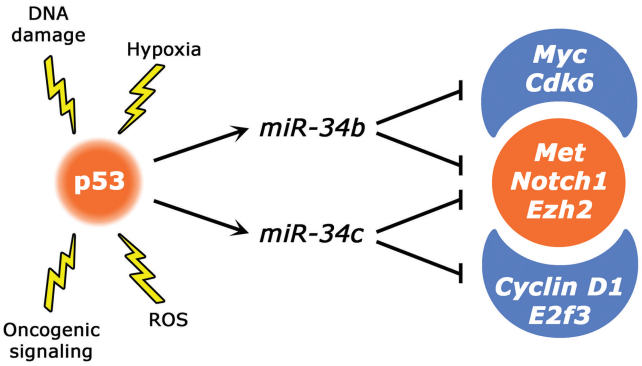

Fig. 2. p53-dependent miRNA-mediated gene silencing. Stimuli such as DNA damage, hypoxia, oncogenic signaling and reactive oxidative species (ROS) posttranscriptionally stabilize and activate p53 protein leading to transactivation of *miR-34b* and *-34c*. A selection of predicted targets are shown for each miRNA. Targets are both overlapping and discrete due to sequence similarity of these miRNAs.

ratio has poor prognosis and estimated 5-year progression-free survival under 10%. In contrast, the second group with a lower HMGA2/*let-7* ratio has a higher 5-year progression survival of about 40%.

Taken together with a seminal paper which demonstrated that a miRNA profile classifies human cancers of uncertain cellular origin with greater accuracy that a mRNA profile (Lu et al., 2005), these data indicate the potential of miRNA expression analysis in the clinic. The significance of the work performed by Lu et al. is that a miRNA expression profile was able to classify human cancers by their origin, which could be useful given that 2-4% of cases are unable to be diagnosed with any certainty by histopathological means (Pavlidis et al., 2003).

Therapeutic potential of miRNAs

Given the potential of miRNAs to function either as tumor suppressors or oncogenes, a number of groups have used different approaches to increase or decrease expression of different miRNAs in the hope of inducing a therapeutic effect. Several methods to reduce, or knock down miRNA expression have been devised, which may be useful in the case of oncogenic miRNAs. Firstly, 2'-O-methyl modified oligoribonucleotides complementary to the mature miRNA transfected into mammalian cells rapidly and irreversibly binds the miRNA, preventing it from binding its target (Hutvagner et al., 2004; Meister et al., 2004). For *in vivo* knockdown "antagomirs", 2'-O-methyl modified oligoribonucleotides antisense to a miRNA of interest conjugated to a cholesterol molecule, can be injected directly into the mouse (Krutzfeldt et al., 2005). At a dose of 240 mg per kg body weight, an antagomir directed against *miR-122* results in undetectable levels of the mature miRNA for up to 23 days. Due to the relatively high doses required and short lived response, however, Ebert et al. designed an alternative strategy based upon sequestering miRNAs by binding an artificial target expressed at high levels in the cell (Ebert et al., 2007). In this approach, a target is designed that is perfectly complementary to the miRNA with the exception of a bulge at nucleotides 9 to 12 which prevents target cleavage by Ago2. Multiple copies of the target are placed in the 3' UTR of a gene such as green fluorescent protein (GFP) and transfected into cells, generating a "miRNA sponge" transgene. The high expression of the sponge releases miRNA-mediated repression of endogenous targets. Importantly, in principle at least, this technique could be directed to generating transgenic animals with constitutive or conditional knockdown of miRNAs.

As mentioned, some miRNAs have been described as having tumor suppressor properties and ectopic expression of these miRNAs in neoplastic tissues may have therapeutic potential. Numerous groups, including ourselves, have over expressed miRNAs through infection of retrovirus or lentivirus expressing the miRNA of interest. This approach has the advantage of using inducible and/or cell type specific promoters although as with mRNA over expression, there are many safety concerns if this technology is to be used in humans. A second approach is delivery of mature miRNAs to a cell. These molecules are commercially available, can result in increased silencing of a miRNA target and can be delivered to cells through non-viral means (Johnson et al., 2005). However, currently their use has been restricted to transfection of cells in cell culture and while they have worked well in our hands, their usefulness in vivo will depend on efficient delivery to cancerous cells.

Concluding remarks

The emergence of the miRNA field has been rapid yet also widespread, given that they are present in multiple species and are predicted to regulate thousands of human mRNAs. It is plain to see, however, that we are at an early stage in our understanding of these molecules and much remains to be learnt, especially regarding their biogenesis and mechanisms of silencing. Yet it is equally clear that miRNAs possess a large potential to play a role in the clinic, both as diagnostic and therapeutic tools. Such potential is critically required for many cancers, but especially for ovarian cancer given late diagnosis and poor treatment options currently available. In addition, further research in miRNA signaling pathways will increase our knowledge of processes underlying this disease. Mismatches in base pairing tolerated by miRNA-mediated translational repression has resulted in a large and diverse array of predicted targets for each miRNA, such that predicted targets for just one miRNA play roles in multiple aspects of cellular physiology. However, instead of being a disadvantage this is a major advantage of any miRNA-based therapeutic, since function of several pathways could be restored by a single hit.

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