
Norahayu Othman¹ and Noor Hasima Nagoor¹,²*

¹ Institute of Biological Sciences, Division of Genetics and Molecular Biology, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.
² Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603, Kuala Lumpur, Malaysia.

Email addresses:
NH: hasima@um.edu.my (Corresponding Author *)
NO: norahayu_o@siswa.um.edu.my

Abstract
Lung cancer remains to be one of the most common and serious type of cancer worldwide. While treatment is available, the survival rate of this cancer is still critically low due to late stage diagnosis and high frequency of drug resistance; thus highlighting the pressing need for a greater understanding of the molecular mechanisms involved in lung carcinogenesis. Studies in the past years have evidenced that microRNAs (miRNAs) are critical players in the regulation of various biological functions, including apoptosis, which is a process frequently evaded in cancer progression. Recently, miRNAs were demonstrated to possess pro-apoptotic or anti-apoptotic abilities through the targeting of oncogenes or tumor suppressor genes. This review examines the involvement of miRNAs in the apoptotic process of lung cancer, and will also touch on the promising evidence supporting the role of miRNAs in regulating sensitivity to anti-cancer treatment.
1. Introduction

Lung cancer remains a major health problem worldwide. In 2012 lung cancer was the most commonly diagnosed cancer worldwide making up 13.0% of the total incidence of cancer. It was also the most common cause of death from cancer worldwide, accounting for nearly one in five cancer deaths (19.4% of the total) [1]. Lung cancer are clinically divided into two main groups based upon the size and appearance of malignant cells: small cell lung cancer (SCLC) (16.8%) and non-small cell lung cancer (NSCLC) (80.4%) [2]. The most effective option for treatment of lung cancer is surgical resection, when feasible [3]. However, majority of patients are diagnosed at an advanced or metastatic stage of disease in which case chemotherapy and/or concurrent administration of chemotherapy and radiation is the most beneficial form of treatment [4]. Nevertheless, even with treatment, the 5-year survival rate in patients is only 16.6% [5], with poor survival rates mainly being attributed to late stage diagnosis and high frequency of drug resistance. Obtaining a better understanding regarding the molecular mechanisms involved in lung carcinogenesis is of utmost importance in the aim to identify the diagnostic and prognostic markers for early detection and targeted treatment of lung cancer.

Apoptosis plays an important role during development and in the maintenance of multicellular organisms through the removal of damaged, aged or autoimmune cells [6]. The apoptotic process can be divided into the extrinsic and intrinsic pathway. Each pathway will ultimately result in the activation of cell death proteases, which in turn initiates a cascade of proteolysis involving effector caspases that carries out the completion of the apoptotic process [7]. In contrast to normal cells, cancer cells have the ability to evade apoptosis to promote cell survival under the conditions of environmental stress. There are a number of mechanisms by which cancer cells are able to suppress apoptosis. For example, the tumor suppressor gene p53 is a widely mutated gene in human tumorigenesis [8]. p53 mutation will inhibit the activation of DNA repair proteins leading to a decrease in the initiation of apoptosis [7], allowing for cells to divide and grow uncontrollably, forming malignant tumors. Furthermore, cancer cells are able to disrupt the balance between pro- (BCL-2, BCL-XL) and anti-apoptotic factors (BAX, BIM, PUMA) [9]. Increased expression of pro-apoptotic Bcl-2 protein contributes not only to the development of cancer but also to resistance against a wide variety of anti-cancer agents, such as cisplatin (DDP) and paclitaxel [10-12].

MicroRNAs (miRNAs) are a subset of non-coding RNAs of about 20 to 25 nucleotides long which post-transcriptionally regulate gene expression via inhibition of mRNA translation, by binding to specific target sites in their 3’-untranslated region (3’UTR),
or inducing degradation of target mRNA through cleavage [13]. An individual miRNA is able to modulate the expression of multiple genes; correspondingly, a single target can be modulated by many miRNAs [14]. MiRNAs were reported to be involved in a vast range of biological processes, including apoptosis [15-22]. As miRNAs play a key role in an assortment of biological processes, an altered miRNA expression is likely to contribute to human diseases including cancer [23]. Previous studies have shown that compared to normal tissues, malignant tumors and tumor cell lines were found to have widespread deregulated miRNA expression [24-28]. MiRNAs are critical apoptosis regulators in tumorigenesis and cancer cells are able to manipulate miRNAs to regulate cell survival in oncogenesis. Many studies carried out in the past several years are aimed at elucidating the specific miRNAs associated with apoptosis in cancer and their related target genes. In this review we will examine the recent progress of research on miRNA-mediated regulation of apoptosis in lung cancer and its future therapeutic applications.

2. Anti-apoptotic miRNAs

Evasion of apoptosis is a significant hallmark of tumor progression, and one mechanism by which miRNAs influence development of cancer is through the regulation of the apoptotic process as shown in various studies [29-32]. MiRNA expression can be either up-regulated or down-regulated and evidence have shown dysregulated miRNAs can behave as oncogenes or tumor suppressor genes in lung cancers [18, 28, 33]. Amplification of miRNAs can lead to the down-regulation of tumor suppressors or other genes that are involved in apoptosis [34].

miR-197: For example, the expression of miR-197 is increased in cancer tissues in comparison to normal specimens. Fiori et al. (2014) demonstrated that knockdown of miR-197 in NIH-H460 and A549 cells promoted induction of apoptosis, evident by the observation of caspase 3-7 activation and increased apoptotic population by Annexin staining. Furthermore, the direct interaction of miR-197 with the 3’UTR of BMF and NOXA was demonstrated by the luciferase reporter assay [35]. When activated by intra- or extracellular stimuli, pro-apoptotic Bmf binds to and neutralizes anti-apoptotic Bcl-2 family members on the mitochondrial membrane, thus allowing pro-apoptotic proteins Bak and Bax to dimerize and promote the release of cytochrome c, ultimately leading to cell death [36]. Noxa is a BH-3 only pro-apoptotic protein transcriptionally activated by p53. Collectively, miR-197 is able to act upon different levels of the p53 pathway to counteract the induction of apoptosis, thus allowing cells to proliferate uncontrollably [35].
miR-21: miR-21 is found to be frequently up-regulated in a number of cancers; however its potential role in tumorigenesis in vivo are not fully explored. Using transgenic mice with loss-of-function and gain-of-function miR-21 alleles, Hatley and colleagues elucidated the role of miR-21 in NSCLC pathogenesis in vivo [37]. It was determined that miR-21 regulates tumor proliferation and survival, which are two integral components of NSCLC pathogenesis, by targeting negative regulators of the RAS pathway as well as by targeting pro-apoptotic genes [37]. In regards to the apoptotic pathway, over-expression of miR-21 in vivo leads to decreased protein levels of Apaf-1, an important component of the intrinsic mitochondrial apoptotic pathway, as well as decreased expression of FasL, a key initiator of the extrinsic apoptotic pathway. Furthermore, RHOB, with a tumor suppressor role, is a target of miR-21 and its dysregulation leads to an increase in cell growth and inhibition of apoptosis [38]. Together these results suggest that relieving miR-21 down-regulation of these pro-apoptotic and tumor suppressor genes could provide a means to enhance the effect of current chemotherapy.

miR-212: Acetylcholinesterase (AChE), a component of the cholinergic system, has the ability to influence apoptotic sensitivity both in vitro and in vivo [39-41]. In NSCLC tissues AChE levels are low and are associated with tumor aggressiveness, increased risk of post-operative recurrence and low survival rate [42]. Lu et al. (2013) determined that AChE expression in NSCLC was post-transcriptionally modulated by miR-212 binding to its 3’UTR. Interestingly, alterations in neither AChE nor miR-212 expression significantly affected cell survival; however it was observed that during DDP–induced apoptosis miR-212 levels were reduced with a concurrent increase in AChE protein levels. This suggests that miR-212 plays a role in DDP resistance by directly inhibiting AChE and preventing apoptosis. Therefore, interference against miR-212 may potentially be a means to improve the pharmaco-toxicological profile of DDP in NSCLC [43].

miR-17-5p and miR-20a: The miR-17-92 cluster, which is composed of seven miRNAs and resides in intron 3 of the C13orf25 gene at 13q31.3, is frequently over-expressed in lung cancers [44]. Matsubara et al. (2007) demonstrated that inhibition of two components of the miR-17-92 cluster, miR-17-5p and miR-20a, with antisense oligonucleotides can induce apoptosis selectively in lung cancer cells that over-express miR-17-92 [45]. Previously, miR-17-5p and miR-20a have been shown to directly target E2F1 [46], thus inhibition of these miRNAs may cause the induction of apoptosis in part through the induction of E2F1 and subsequent cell cycle progression into S phase [47]. However
additional studies would have to be carried out to determine the actual targets for the miR-17-92 cluster to gain a better understanding of the development of this cancer.

3. Pro-apoptotic miRNAs

MiRNAs that are down-regulated are considered tumor suppressor genes. Tumor suppressor miRNAs usually prevent tumor development by negatively regulating oncogenes and/or genes that control cell differentiation or apoptosis [48]. MiRNAs that act as tumor suppressors can be down-regulated as a result of deletions, epigenetic silencing, or loss of expression of transcription factors [49].

3.1. B-cell lymphocyte 2 (BCL-2) family related miRNAs

Members of the evolutionarily conserved BCL-2 family are thought to be the central regulators of apoptosis. The expression level of BCL-2 differs for different cell types, however high levels and aberrant patterns of BCL-2 expression were reported in a wide variety of human cancers, including lung cancer [50]. Elevation of Bcl-2 protein expression contributes not only to the development of cancer but also to resistance against a wide variety of anti-cancer agents [10-12].

miR-7: Xiong et al. showed that miR-7 was down-regulated in NSCLC cells and BCL-2 was identified as a direct target [51]. Transfection of miR-7 in A549 cells led to a significant reduction in endogenous BCL-2 mRNA and protein levels; and correspondingly led to increase in the activities of caspase-3 and caspase-7 in cells with apoptotic nuclei [51]. These results thus provide evidence that BCL-2 may be involved in miR-7 mediated apoptosis induction in A549 cells.

miR-335: BCL-W, another anti-apoptotic member of the BCL-2 family, was found to be a direct target of miR-335 [52]. miR-335 was down-regulated in A549 and NCI-H1299 cells, and up-regulation of this miRNA via transfection of miR-335 mimics, led to a suppression of cell invasiveness and promotion of apoptosis. Furthermore Wang et al. (2013) discovered that miR-335 directly targeted SPI gene, a member of the family of Sp/Kruppel-like transfection factors [53], which can enhance the activity of promoters of numerous genes involved in cell proliferation, apoptosis, differentiation, cell cycle, progression and oncogenesis thus regulating these genes’ expression [54].

miR-608: Studies in our lab identified a BCL-XL-induced miRNA, miR-608, involved in the regulation of cell death in A549 and SK-LU-1 cells [55]. BCL-XL, a major prototype of the anti-apoptotic BCL-2 gene, was found to be over-expressed in NSCLCs [56]. Silencing of
BCL-XL in A549 and SK-LU-1 led to the significant dysregulation of a number of miRNAs, as determined through miRNA microarray; with miR-608 being the most up-regulated miRNA. Up-regulation of miR-608 in A549 and SK-LU-1 via miR-608 mimics led to an increase in apoptotic population, as determined by Annexin-V FITC apoptotic assay, in comparison to NP-69 cells (normal human nasopharyngeal epithelial cell line) [55]. Bioinformatics analysis determined that miR-608 may be associated with various signaling pathways, primarily the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), wingless-type MMTV integration site family (WNT), transforming growth factor (TGF-β), mitogen activated protein kinase (MAPK) and the intrinsic pathway. However the true targets of miR-608 and its direct effects on the apoptotic process is yet to be determined.

3.2. Protein kinase C (PKC) family related miRNAs

PKC is a serine/threonine kinase that is involved in various signal transduction pathways including those related to cellular proliferation, differentiation and apoptosis [57-59]. PKC plays a role in lung cancer and levels of PKC proteins were found to be increased in various cell lines (A549, NCI-H1355, NCI-H1703, NCI-H157 and NCI-H1155) in comparison to primary normal human bronchial epithelial cells (NHBE) [60].

**miR-203**: To determine the role that miR-203 can play in the influence of cellular function, putative target prediction was carried out and PKC-α was determined to be a target [61]. Luciferase reporter assay further revealed miRNA-203 direct binding of the 3’UTR of PKC-α mRNA transcript. miR-203 negatively regulated proliferation and migration through the repression of PKC-α, and miR-203 was also able to modulate cell apoptosis. However, siRNA silencing of PKC-α resulted in a less significant apoptotic phenotype in comparison to that observed by miR-203 over-expression, thus suggesting that miR-203 may modulate multiple apoptotic genes that work together to regulate cell apoptosis [61]. Further studies must therefore be carried out to determine the additional apoptosis related targets of miR-203.

**miR-143**: miR-143 expression was reported to be down-regulated in cancer tissues and inhibition of miR-143 promotes cell proliferation but hinders cell apoptosis. To determine the role that miR-143 plays in the apoptotic process, Zhang et al. (2013) investigated the possible targets of miR-143 and found that PKC-ε, a crucial enzyme in various cellular signaling pathways [62], was a putative target. Using the luciferase reporter assay it was determined that miR-143 specifically targets PKC-ε, and over-expression of miR-143 increases the cell apoptosis in A549 cells [63]. PKC-ε was suggested to play a role in regulating the anti-apoptotic signaling pathway through the up-regulation of Bcl-2 with a
concurrent suppression of pro-apoptotic Bid [64-66]. Furthermore, PKC-ε is able to activate Akt to apply its pro-survival effects [67, 68]. Therefore, the targeting of PKC-ε could potentially be a valuable therapeutic strategy for lung cancer.

3.3. Other miRNAs

**miR-198**: miR-198 is down-regulated in NSCLC cell lines and over-expression of this miRNA inhibits cell viability and enhances apoptosis in A549 cells. Over-expression of miR-198 induces the expression of poly (ADP-ribose) polymerase (PARP) and of cleaved caspase-3. miR-198 was also able to inhibit growth of tumor grafts in nude mouse. *FGFR1*, a lung cancer oncogene, which is a membrane-bound receptor tyrosine kinase that regulates proliferation via the MAPK and PI3K pathway, much like EGFR, was found to be a direct target of miR-198 [69].

**miR-146a**: Expression of miR-146a is low in malignant tissues in comparison to corresponding adjacent normal lung tissues. Functionally, miR-146a suppresses cell growth, inhibits cell migration and increases cellular apoptosis [70]. Up-regulation of miR-146a expression via miR-146a mimic transfection resulted in the down-regulation of EGFR as well as phosphorylated EGFR, both at the mRNA and protein levels. Furthermore, downstream pathways (ERK-1/2, AKT and STAT) were also down-regulated in response to miR-146a mimic transfection, albeit with a weaker effect as that seen by cells transfected with *EGFR* specific siRNA. miR-146a mimic also led to the decrease of phosphorylation of the NF-κB inhibitor IκBα, but not total IκBα. Levels of phospho-NFκB, total NF-κB and the total immune-modulating kinase, IRAK-1 were also found to be decreased following miR-146a mimic transfection, suggesting that miR-146a regulates NF-κB and IRAK-1 signaling [70].

**miR-26a**: miR-26a expression is down-regulated in lung cancer tissues relative to normal tissues. Transfection of miR-26a into A549 cells was able to decrease cell proliferation, block the G1/S phase transition of cell cycle, and induce apoptosis [71]. The chromatin regulator enzyme *EZH2*, which regulates survival and metastasis of cancer cells [72], was found to be a direct target of miR-26a. Down-regulation of *EZH2* expression, caused by over-expression of miR-26a will transactivate downstream tumor suppressor genes *DAB21P* and *RUNX3*. *DAB21P* is a potent growth inhibitor that induces G0/G1 phase cell cycle arrest and could lead to apoptosis [73], while *RUNX3* leads to cell cycle arrest, apoptosis and significant decrease of tumor growth and abrogation of metastasis [74].

**miR-451**: Poor tumor differentiation, advance pathological state, lymph node metastasis and poor prognosis are associated with down-regulation of miR-451, which occurs
in lung cancer [75]. To observe the functions of miR-451, Wang et al. (2011) up-regulated miR-451 expression via mimics and observed suppressed in vitro proliferation, chromatin condensation and nuclear fragmentation upon 4’,6-diamidino-2-phenylindole (DAPI) staining, and significant caspase-3 activity. These results suggested that ectopic expression of miR-451 was able to induce an increase in apoptosis in a caspase-3 dependent manner. In addition, the RAB14 gene was identified as a direct target of miR-451. Inhibition of RAB14 led to a decrease in phosphorylation of Akt, which subsequently decreased levels of Bcl-2 protein expression and increased pro-apoptotic Bax or Bad protein expression. As the expression levels of RAB14 protein were inversely correlated with the expression levels of miR-451 in NSCLC tissues it was concluded that down-regulation of RAB14 may be the mechanism by which miR-451 carries out its tumor suppressor functions [75].

miR-192: miR-192 was found to be down-regulated in A549, NCI-H460 and 95D cell lines [76]. Cell viability was greatly decreased following miR-192 up-regulation, while levels of apoptosis were elevated with induced expression of PARP protein and cleaved caspase-7, thus suggesting that miR-192 induces apoptosis through the caspase pathway. Using bioinformatics analysis, RB1 gene was determined to be a putative target of miR-192 and luciferase reporter assays confirmed direct binding of miR-192 to the 3’-UTR of this gene [76]. Since RB1 plays a vital role in regulating cell apoptosis, its down-regulation was shown to induce γ-H2AX foci formation, a marker of DNA damage, and to promote apoptosis in A549 cells [77].

4. MiRNA and response to cancer therapy
Many cancer therapies available today aim to induce tumor-selective cell death, however resistance to chemotherapeutics is a significant obstacle to the long-term treatment and survival of NSCLC patients [78]. Presently, there are various chemotherapeutics that are being utilized in the treatment of lung cancer, including FDA approved drugs (DDP, paclitaxel, docetaxel, gemcitabine and EGFR-TKIs), natural compounds (curcumin) and small organic compounds (PRIMA-1). The association of miRNAs as regulators of malignancy and apoptosis has been widely reported, thus it is reasonable to assume that miRNAs play significant roles in sensitivity/resistance to common cancer treatments [79]. Indeed, recent studies have demonstrated miRNAs as potential agents involved in the sensitivity of lung cancer cells to cytotoxic therapy.
4.1. Cisplatin (DDP)-related miRNAs

DDP is a platinum-coordinated complex that is the most widely used chemotherapy for human NSCLC in the past two decades [80-82]. However, multiple administration of DDP results in the development of drug resistance leading to failure of treatment, as demonstrated by tumor growth or tumor relapse [78, 83]. Therefore, to overcome the treatment plateau of DDP on NSCLC, the biological mechanisms by which DDP action is enforced must be further elucidated. As miRNAs act as critical regulators in the development of drug resistance, it would be interesting to research the mechanism through which oncogenic miRNAs modulates DDP-induced apoptosis in NSCLC.

miR-451: miR-451 was down-regulated in NSCLC tissues in comparison to normal lung tissues, and up-regulation of miR-451 enhances DDP chemosensitivity in A549 cells by inhibiting cell growth and inducing apoptosis enhancement [84]. Bian et al. (2013) demonstrated in their study that up-regulation of miR-451 enhanced caspase-3-dependent apoptosis through the inactivation of the Akt signaling pathway, which in turn decreased Bcl-2 while increasing expression of Bax protein levels. Furthermore, results of Annexin V-FITC apoptosis assay indicated that in miR-451 transfected A549 cells (A549/miR-451) a higher percentage of apoptosis was observed in comparison to mock A549 cells. Caspase-3 activity in A549/miR-451 treated with DDP was significantly increased against the control, thus suggesting that miR-451 up-regulation increases chemosensitivity of A549 cells by enhancing DDP-induced apoptosis. Together these results suggest a possible strategy for treatment of human NSCLC through the combined application of DDP treatment with miR-451 up-regulation [84].

miR-31: On the other hand, miR-31 is up-regulated in NSCLC cell lines and was demonstrated to induce DDP resistance. To demonstrate this, Dong et al. (2014) transfected miR-31 mimics into DDP-sensitive SPC-A-1 cells which led to a marked increase in the resistance of SPC-A-1 cells, while transfection of miR-31 inhibitors increased sensitivity of resistant NCI-H1299 to DDP treatment. To elucidate the mechanism by which DDP resistance is induced by miR-31, bioinformatics analysis was carried out and ABCB9, a membrane transporter involved in drug uptake [85], was predicted to be a target gene. The luciferase reporter assay then confirmed direct miR-31 regulation of ABCB9 by binding to its 3’UTR [86]. Over-expression/knockdown studies indicated a significant decrease in the percentage of DDP-induced apoptotic cells when miR-31 was increased via mimics and a marked increase in DDP-induced apoptotic cells when miR-31 inhibitors were introduced;
thus suggesting that miR-31 exerts an anti-apoptotic effect in DDP-induced apoptosis through the inhibition of ABCB9.

4.2. Paclitaxel-related miRNAs

Paclitaxel was the first identified member of taxanes in the list of FDA-approved anticancer drugs. This compound has been shown to have significant single-agent activity against various solid tumors [87, 88] including NSCLC [89]. However, combination of this compound with DDP or carboplatin showed superior response and improved survival rates [90].

miR-133a/b and miR-361-3p: High-throughput screening (HTS) approach was performed by Du and colleagues in 2013 to identify miRNAs that modulate lung cancer cell survival and response to paclitaxel treatment [91]. Using three NSCLC cell lines that have distinct genetic backgrounds (NCI-H1155, NCI-H1993 and NCI-H358), inhibition of two miRNAs (miR-133a/b and miR-361-3p) were found to potentially decrease cell viability; although cytotoxicity of the two miRNAs vary greatly, which may be due to different endogenous expression levels of the miRNAs in each cell line. Interestingly, the inhibitors of miR-133a/b and miR-361-3p were found to reduce cell survival through different mechanisms. miR-133a/b inhibitor was able to dramatically increase apoptotic events as seen by increased percentage of cells undergoing apoptosis and increased levels of activated caspase-3. However miR-361-3p only showed a modest effect on caspase-3 activation thus suggesting additional mechanisms are involved in the cytotoxicity of this miRNA. The effect of miRNA inhibitors on cell cycle distribution was then evaluated and results indicated that S phase arrest contributes to cytotoxicity induced by miR-133a/b and miR-361-3p inhibitors. Together these results suggest that miR-133a/b and miR-361-3p may function as oncogenes in cancer cells by regulating tumor suppressor genes.

miR-101: Increasing evidence has revealed that EZH2 has oncogenic properties, as an increased expression of EZH2 augments proliferation and invasion of cancer cells [92-94], while depletion leads to a decline in cell proliferation, increased apoptosis and inhibition of metastatic tumor growth in vivo [95, 96]. Over-expression of EZH2 has been associated with tumor progression and cancer aggressiveness in NSCLC [97]. In a study by Zhang and colleagues (2011), it was discovered that a decreased expression of miR-101 was associated with EZH2 over-expression in NSCLC tissues [98]. Luciferase reporter assay revealed that miR-101 regulates EZH2 expression through the binding its 3'UTR mRNA. Over-expression of miR-101 led to a decrease in EZH2 protein levels with subsequent decrease in the
proliferation and invasive ability of NSCLC cells. Furthermore, over-expression of miR-101 led to a sensitization of NSCLC cells to paclitaxel.

4.3. Docetaxel-related miRNAs

Docetaxel, a semi-synthetic analog of paclitaxel, is one of the first-line chemotherapy regimens for advanced NSCLC, with genotoxic effects caused by microtubule stabilizing, apoptotic induction through microtubule bundling and Bcl-2 blocking [99, 100].

miR-100: In a miRNA microarray profiling carried out by Rui and colleagues in 2010, miR-100 was significantly down-regulated in docetaxel-resistant SPC-A1/DTX cells relative to SPC-A1 parental cells [101]. To elucidate the role that miR-100 plays in the formation of docetaxel resistance, the authors’ transfected miR-100 mimics into SPC-A1/DTX cells [102]. Results suggested that restoration of miR-100 expression chemosensitizes cells to docetaxel in vitro, complemented with a suppression of cell proliferation, enhancement of apoptosis, and cell cycle arrest in the G2/M phase of cell cycle. Ectopic miR-100 expression was also able to down-regulate in vivo cell proliferating ability. Moreover, PLK1 gene was identified to be a direct target of miR-100. PLK1 plays a role in promotion of cell proliferation and over-expression of this gene has been observed in various human cancers [103] including NSCLC [104]. Knockdown of Plk1 protein expression by miR-100 led to a significant suppression of cell proliferation of SPC-A1/DTX, dramatic increase of early apoptosis rate, G2/M arresting population, and an increase in the response of SPC-A1/DTX cells to doxetaxel both in vitro and in vivo. miR-100 was therefore concluded to function as a chemosensitizer restorer to docetaxel by targeting PLK1 and inducing the suppression of cell proliferation, enhancement of apoptosis and mitotic arrest.

miR-650: High expression of miR-650 can be found in lung cancer tissues, and its dysregulation is correlated with advance clinical stage as a poor prognostic factor for these patients [105]. Furthermore, Huang et al. (2013) determined that the expression of miR-650 is negatively correlated with patients’ response to docetaxel. Using two docetaxel-resistant cell lines (SPC-A1/DTX and H1299/DTX), the authors demonstrated that down-regulation of miR-650 was able to reverse the resistance. ING4, a novel tumor suppressor gene, was then identified as the functional target of miR-650 and results from flow cytometry and Hoechst staining assays indicated that miR-650 inhibitor was able to induce an increase in caspase-3-dependent apoptosis. Cells transfected miR-650 inhibitors exhibited decreased expression of Bcl-2 protein, with an increased expression of Bax protein, led to the progression of
apoptosis. The findings of this study confirmed that miR-650 was able to confer doxetaxel chemoresistance through the regulation of Bcl-2/Bax expression by targeting of ING4 [105].

4.4. Gemcitabine-related miRNA
Gemcitabine, a pyrimidine nucleoside anti-metabolite, has been shown to be an effective agent most particularly when administered in combination regimes [106]. Due to its theoretical ability of interfering with the inhibition of repair of platinum-induced DNA damage, gemcitabine is the perfect partner for platinum compounds. Gemcitabine in combination with DDP represents a common first-line treatment for patients with advanced NSCLC, especially in Europe [80, 107-110].

miR-133b: miR-133b is greatly reduced in cancer tissue in comparison to adjacent normal lung tissue [111]. Prediction programs identified two common predicted targets of miR-133b, the anti-apoptotic MCL-1 and BCL-W, both of which are members of the anti-apoptotic BCL-2 family [112] and has previously been reported to be increased in both solid and hematological malignancies including lung cancer [113, 114]. Transfection of miR-133b using pre-miR-133b resulted in a decrease in Bcl-W and Mcl-1 protein expression with a moderate increase of apoptosis. However combination treatment of miR-133b over-expression with 24 hours treatment of gemcitabine resulted in a greater degree of cleaved PARP expression as well as apoptosis. This concludes that miR-133b is able to target pro-survival molecules and induce apoptosis in the setting of chemotherapeutic agents [111].

4.5. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs)-related miRNAs
EGFR is a plasma membrane glycoprotein that belongs to a family of four different tyrosine kinase receptors (EGFR (ErbB1), HER2/neu (ErbB2), HER3 (ErbB3) HER4 (ErbB4)) [115]. Dimerization of EGFR may result in cancer cell proliferation, inhibition of apoptosis, invasion, metastasis and tumor induced neovascularization [116]. Mutations and subsequent over-expression of EGFR can be found in all histologic subtypes of NSCLC [117]. Deletion in exon 19, which removes the conserved sequence LREA, and a single point mutation in exon 21, which leads to the substitution of arginine for leucine at position 858 (L858R), are the most clinically relevant and extensively studied drug-sensitive mutations [118]. Studies have shown that these mutations preferentially bind to first generation EGFR-TKIs, gefitinib and erlotinib [119, 120]. First generation EGFR-TKIs function by selectively targeting the receptor via a competitive, reversible binding at the tyrosine kinase domain, thus leading to
the inhibition of ATP binding and subsequent signal transduction and downstream functions [121]. However, acquired resistance to EGFR-TKIs in the metastatic setting is unavoidable. While the average progression-free survival (PFS) is between 10 to 16 months, treatment duration can last as short as 1 month [122]. Drug resistance therefore still remains a problem and new therapies and strategies must be developed to overcome such resistance.

**miR-30b/c and miR-221/222:** EGF and MET receptors controls gefitinib-induced apoptosis and NSCLC tumorigenesis through the down-regulation of specific oncogenic miRNAs, miR-30b/c and miR-221/222 [123]. Using bioinformatics analysis and luciferase assays, APAF-1 and BIM (previously found to play a role in TKI sensitivity [124, 125]) were determined to be direct targets of miR-221/222 and -30b/c. To investigate the roles these miRNAs play in gefitinib-induced apoptosis, wild-type EGFR expressing NSCLC cells (Calu-1 and A549) and cells with EGFR exon-19 deletions (PC9 and HCC827) were utilized. Upon gefitinib treatment, significant down-regulation of miR-30b/c and miR-221/222 with an increased BIM and APAF-1 protein levels were observed only in PC9 and HCC827 sensitive cells. To further determine the contribution of miR-30b/c and miR-221/222-mediated APAF-1 and BIM down-regulation to cellular TKI response, Garofalo et al. (2012) over-expressed APAF-1 and BIM in A549 resistant cells, which consequently led to gefitinib-induced PARP cleavage. Furthermore, as miR-30b/c and miR-221/222 are regulated by MET, a strong down-regulation was observed of these miRNAs when Calu-1- and A549-MET over-expressing cells were treated with MET inhibitors SU11274. Furthermore an increase in caspase 3/7 activity and decreased cell viability was observed in SU11274-treated Calu-1 cells following exposure to varying gefitinib concentrations. Together, these results suggest that MET inhibition restores gefitinib sensitivity in TKI-resistant Calu-1 through down-regulation of miR-30b/c and miR-221/222 [123].

**miR-214:** miR-214, is significantly up-regulated in gefitinib resistant lung adenocarcinoma cell line, HCC827/GR, in comparison to parental HCC827 lung adenocarcinoma cells. HCC827/GR was obtained by exposing HCC827 cells to increasing concentrations of gefitinib over six months [126]. Using dual-luciferase reporter assay, Wang et al. (2012) confirmed PTEN as a direct functional target of miR-214. PTEN encodes a 403 amino acid dual-specificity lipid and protein phosphatase which functions as a tumor suppressor in many tumors [127, 128]. Knockdown of miR-214 expression resulted in the up-regulation of PTEN protein and inactivation of AKT, which is largely linked to anti-apoptotic function [129, 130]. Furthermore, knockdown of miR-214 re-sensitized HCC827/GR to gefitinib, as demonstrated through MTS assay. miR-214 was thus concluded to potentially
serve as a therapeutic target to reverse the acquired resistance of gefitinib in lung adenocarcinoma cells.

**miR-133b:** Expression of miR-133b is significantly down-regulated in NSCLC tissues in comparison to non-neoplastic lung tissues [131], and the 3’UTR of EGFR was found to be a direct target of this miRNA thus inhibiting its expression. Treatment of EGFR-addicted lung cancer cells, PC-9 and A549 with miR-133b mimic inhibited phosphorylation of EGFR, AKT and extracellular signal-related kinase (ERK)1/2, thus inhibiting their growth and invasion abilities. However in non-EGFR-addicted NSCLC cells NCI-H1650 and NCI-H1975, no significant changes in the expression of phosphorylated EGFR, AKT and ERK1/2 were found. Furthermore, miR-133b was able to restore or enhance EGFR-TKI sensitivity in NSCLC cells, especially in EGFR-addicted cells. These findings reveal that transfection of miR-133b in EGFR-addicted NSCLC has the therapeutic potential for overcoming EGFR-TKI resistance [131].

### 4.6. PRIMA-1-related miRNA

The tumor suppressor p53 gene regulates cell growth through the activation of the transcription of numerous genes specifically those involved in cell cycle regulation, apoptosis and genomic stability [132-134] and has also been implicated in the response to anticancer therapies [133]. p53 has been reported to be frequently mutated in humans cancers with mutations occurring in greater than 50% of lung tumors [135, 136]. Restoration of wild-type p53 function has led to regression of cancers in mice [137, 138], and thus efforts to treat cancers through the reactivation of p53 with a low-molecular-weight compound such as PRIMA-1 (p53-dependent reactivation and induction of massive apoptosis) [139, 140] are widely supported.

**miR-34a:** In a study conducted by Duan *et al.* (2010), the role of miR-34 family members in regulating PRIMA-1 induced apoptosis was investigated. The authors discovered that PRIMA-1 was able to up-regulate miR-34a in p53 mutant cells. Previous studies have shown evidence that the miR-34 family plays a role in the regulation of cell proliferation and apoptosis [141-148]. The results of this study suggests that PRIMA-1 is able to restore wild-type function to mutant p53, which will up-regulate miR-34a to induce apoptosis in lung cancer cells [149].
4.7. Curcumin related miRNA

Curcumin is a compound extracted from the rhizomes of *Curcuma longa* L. and studies carried out exhibited its diverse pharmacological effects which include anti-inflammatory, antioxidant and anti-tumor activities [150]. Previous studies have also shown that curcumin can induce apoptosis in many types of cancer cells [151, 152], through the inhibition of *NF-κB, survivin/BIRC5* and *BCL-2* [153, 154]. However few studies have been carried out to report the importance of miRNA expression modulation in mediating the biological effects of curcumin.

**miRNA-186*: In a study conducted by Zhang *et al.* (2010), curcumin was shown to have the ability to inhibit cell proliferation and induce apoptosis in A549 cells. The authors performed a cluster analysis on the expression profiles on curcumin-treated and dimethyl sulfoxide (DMSO) control-treated samples and found that miR-186* was shown to be significantly down-regulated in response to curcumin treatment; thus suggesting that miR-186* may play an oncogenic role in human lung cancer cells. Inhibition of miR-186* was shown to greatly decrease cell proliferation in A549 cells and increase the induction of apoptosis. Furthermore, caspase-10 was revealed to be a direct target of miR-186* [155]. This study thus provided the first evidence that miR-186* is essential for the anti-cancer effects of curcumin in A549 cells and that caspase-10 may be an important target of miR-186* in preventing apoptosis.

However, even though curcumin has exhibited anti-tumor activity, there has been concern regarding the effects of curcumin on multi-drug resistant cells [156, 157]. To analyze such effects, A549/DDP, the DDP-resistant derivative of parental A549 cells generated by co-culturing parental A549 cells with 6 nm DDP to maintain the drug resistance phenotype, was utilized. In a study conducted by Zhang *et al.* (2010), a comprehensive miRNA profiling of untreated multidrug-resistant cell line (A549/DDP) was performed and compared against results obtained for A549/DDP cells treated with curcumin. Results showed that miR-186* was down-regulated more than 2.5-fold compared to levels in control cells. The anti-apoptotic effects of miR-186* in A549/DDP cells were investigated and it was found that transfection of miR-186* mimics led to an inhibition of apoptosis in comparison to that in the control; thus suggesting that miR-186* plays an oncogenic role in this cell line. To confirm the role miR-186* plays in curcumin-induced A549/DDP apoptosis, flow cytometry was used to detect the rate of apoptosis in A549/DDP cells treated with curcumin, control cells or curcumin combined with miR-186* mimic cells. Results indicated that apoptosis in the combination group was significantly decreased in comparison to cells treated with
curcumin [158]. These findings reveal that curcumin is able to induce apoptosis in the multi-drug resistant cell line by down-regulating miR-186*.

4.8. Multidrug Resistance

miR-200bc/429: In 2012, Zhu et al. reported that the miR-200bc/429 cluster was down-regulated in multidrug-resistant A549/DDP cells, in comparison to parental A549 cell line [159]. Recent studies have suggested that aberrant DNA methylation of the promoter region of the miR-200bc/429 cluster may be a critical mechanism leading to dysregulated expression level of the miR-200 family [160, 161]. While the roles of the two sequence clusters of miR-200 family on the epithelial-to-mesenchymal transition of tumor cells are well studied, the role that this miRNA family plays on apoptosis has been minimally studied. Zhu et al. demonstrated using MTT, that transfection of miR-200bc/429 cluster mimics into A549/DDP greatly enhanced sensitivity of this cell line to various anti-cancer drugs including vincristine (VCR), etoposide (VP-16), adriamycine (ADR), and DDP. It was found that miR-200bc/429 cluster was able to modulate multidrug resistance (MDR) in lung cancer cell lines, at least in part by inhibiting the anti-apoptotic Bcl-2 and XIAP protein expression, thus affecting the mitochondrial release of cytochrome c. Therapeutic methods that target the miR-200bc/429 clusters thus provides a promising method to enhance treatment effect of NSCLC.

miR-181b: In another study by Zhu et al. (2010), miR-181b was also found to be down-regulated in multidrug-resistant A549/DDP cells, in comparison to parental A549 cell line [162]. To determine whether miR-181b has a direct role in MDR development, MTT assay was performed revealing that all A549/DDP cells transfected with miR-181b mimic exhibited a significant increase in sensitivity to a number of anti-cancer drugs including 5-fluorouracil (5-Fu), VCR, DDP, VP-16 and ADR. Bioinformatics analysis predicted anti-apoptotic BCL-2 as a potential target of miR-181, with two conserved target sites in the 3'UT region. Transfection of miR-181a in A549/DDP cells led to a significant decrease in Bcl-2 protein levels, as demonstrated by Western blot. Furthermore, A549/DDP miR-181b transfected cells also led to an increase in apoptosis as detected by flow cytometry. Together these results demonstrate miR-181b’s ability to modulate the development of MDR in lung cancer cell lines, at least in part, by modulation of apoptosis through the targeting of the anti-apoptotic BCL-2.
4.9. TRAIL-related miRNAs

The Apo2L/tumor necrosis factor (TNF)-a-related apoptosis inducing ligand (TRAIL) is a member of the TNF family that is known to induce apoptosis in various cancers [163]. Treatment of transformed cells with TRAIL has been shown to successfully induce apoptosis both in vitro and in vivo [163, 164], however a wide range of human cancer cells are resistant to TRAIL-induced apoptosis [165].

miR-221 and -222: To identify the mechanisms by which miRNAs may play a role in TRAIL resistance, Garofalo et al. (2008) carried out a genome wide profiling of miRNAs in three different lung cancer cell lines (A459, Calu-1 and NCI-H460) and found that miR-221 and miR-222 were markedly up-regulated in TRAIL-resistant cells. In TRAIL sensitive cells NCI-H460, TRAIL was able to induce the activation of the caspase cascade, evaluated by the appearance of cleaved fragments. However, transfection of NCI-H460 cells with pre-miRs-221 and -222 caused a significant reduction of TRAIL-mediated cell death machinery activation. Further experiments deduced that miR-221 and -222 directly targeted p27Kip1, and inhibition of p27Kip1 via pre-miR-221 and -222 transfection led to an increase in cell resistance to TRAIL as assessed by Annexin V staining, and PARP and caspase-8 activation. Taken together, the authors’ results demonstrate that increased levels of miR-221 and -222 may modulate sensitivity of NSCLC cells to TRAIL with important implications in the design of new therapeutic agents.

miR-34a and miR-34c: In another study, miR-34a and miR-34c expression were found to be significantly down-regulated in NSCLC cells and lung tumors in comparison to normal lung tissues. Performing a bioinformatics search, Garofalo et al. (2013) determined that PDGFR-α and PDGFR-β were targets of these miRNAs; both of which have been reported to be over-expressed and associated with poor outcome in lung cancer [166]. Through targeting PDGFR-α and PDGFR-β, miR-34a/c were able to decrease invasiveness as well as increase TRAIL-induced apoptosis. TRAIL resistance is common in lung tumors and it has been reported that PDGFR-α and PDGFR-β regulate the PI3K/Akt and ERK1/2 pathways [167, 168], which play a role in TRAIL-induced apoptosis [169]. Phosphorylation levels of ERKs were found to be decreased following ectopic expression of miR-34a/c; additionally caspase3/7 assay revealed an increase in TRAIL sensitivity. This study demonstrates that inhibition of PDGFR-α and PDGFR-β by miR-34a/c is able to antagonize tumorigenicity and increase sensitivity to TRAIL-induced cell death [170].

miR-212: PED/PEA-15 is a death effector domain (DED) family member, which has been implicated in the processes of cell growth and metabolism [171-173]. Furthermore,
PED/PEA-15 has a broad range of anti-apoptotic ability, being able to inhibit both the intrinsic and extrinsic apoptotic pathways [172, 174]. Zanca and colleagues (2008) reported that PED/PEA-15 over-expression plays a role in TRAIL resistance in NSCLC [175], however the mechanism that regulates its expression is not well known. In further studies, Incoronato et al. (2010) reported that NSCLC-affected lung tissue has an increased expression of PED/PEA-15 with a concurrent down-regulation of miR-212 and decreased response to TRAIL treatment [176]. miR-212 negatively regulates PED/PEA-15 by directly binding to its 3’UTR. miR-212 down-regulation has previously been reported to be involved in lung cancer response to chemotherapy, in particular to docetaxel [177]. In this study, transfection of NSCLC Calu-1 cells with pre-miR-212 led to a decrease in PED/PEA-15 expression with increased caspase-8 activation following treatment with TRAIL, indicating increased sensitivity of Calu-1 cells to TRAIL-mediated cell death. Therefore, the expression of miR-212 could be used to predict therapeutic response to TRAIL in lung cancer.

5. Conclusions

In terms of molecular events occurring in tumors, evasion of apoptosis is an important hallmark of tumor progression. Recent evidence has exhibited deregulated miRNAs to play a role in the apoptotic process. In lung cancer, up-regulated miRNAs have been shown to serve as oncogenes, targeting tumor suppressor and/or pro-apoptotic genes; while down-regulated miRNAs can function as tumor suppressors, targeting oncogenic and/or anti-apoptotic genes. Additionally, studies have also indicated that miRNAs play a significant role in altering sensitivity and resistance to cytotoxic treatment. Targeting of specific miRNAs could therefore potentially be used as valuable therapeutics for lung cancer. Together, these studies have illustrated the importance for further studies and validation of miRNAs and their targets. Furthermore, there is a serious shortage in research being carried out in miRNA-regulated apoptosis in SCLC. As SCLC accounts for 16.8% of lung cancer incidence and is a highly aggressive form of lung cancer it would be of great interest to determine the functions of miRNAs in regulation of apoptosis in this lung cancer subtype.

Acknowledgements

This work was supported by the High Impact Research Grant (HIR) (H-21001-F000036) and the University of Malaya Postgraduate Research Fund (PPP) (PV058-2011B). All funders had no role in decision to publish or preparation of the manuscript.
Abbreviations

γ-H2AX  Gamma, H2A histone family, member X
3'UTR  3'-untranslated region
5-Fu  5-fluorouracil
ABC B9  ATP-binding cassette, sub-family B (MDR/TAP), member 9
AChE  Acetylcholinesterase
ADR  Adriamycin
Akt  Protein kinase B
Apaf-1  Apoptotic peptidase activating factor 1
BAK  BCL2-antagonist/killer 1
BAX  BCL2-associated X protein
BCL-2  B-cell CLL/Lymphoma
BCL-W  BCL2-like 2
BCL-XL  BCL2-like 1
BIM  BCL2-like 11 (apoptosis facilitator)
BIRC5  Baculoviral IAP repeat containing 5
BMF  Bcl-2-modifying factor
BTG2  BTG family, member 2
DAB2IP  Disabled homolog 2-interacting protein
DDP  Cisplatin
DMSO  Dimethyl sulfoxide
E2F1  E2F transcription factor 1
EGFR  Epidermal growth factor receptor
ERK1  Mitogen-activated protein kinase 3
ERK2  Mitogen-activated protein kinase 1
EZH2  Histone-lysine N-methyltransferase
FasL  Fas ligand
FDA  Food and drug administration
FGFR1  Fibroblast growth factor receptor 1
FITC  Fluorescein isothiocyanate
HTS  High-throughput screening
IKKα  Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
ING4  Inhibitor of growth family, member 4
IRAK1  Interleukin-1 receptor-associated kinase 1
MAPK  Mitogen activated protein kinase
MCL1  Molecule myeloid leukemia 1
MDR  Multidrug resistance
miRNA  MicroRNA
NF-κB  Nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC  Non-small cell lung cancer
NOXA  Phorbol-12-myristate-13-acetate-induced protein 1
p27Kip1  Cyclin-dependent kinase inhibitor 1B
p53  Tumor protein p53
PDCD4  Programmed cell death 4 (neoplastic transformation inhibitor)
PDGFR-α  Platelet-derived growth factor receptor, alpha polypeptide
PDGFR-β  Platelet-derived growth factor receptor, beta polypeptide
PEA-15  Astrocytic phosphoprotein PEA-15
PED  Preimplantation embryonic development
PI3K/AKT  Phosphatidylinositol 3-kinase/protein kinase B
PKC  Protein kinase C
PKC-α  Protein kinase C, alpha
PKC-ε  Protein kinase C, epsilon
PLK1   Polo-like kinase 1
PRIMA-1 p53-dependent reactivation and induction of massive apoptosis
PFS    progression-free survival
PUMA   BCL2 Binding Component 3
RAB14  RAB14, member RAS oncogene family
RB1    Retinoblastoma 1
RHOB   Ras homolog family member B
RUNX3  Runt-related transcription factor 3
SCLC   Small cell lung cancer
SP1    Specificity Protein 1
SPRY1  Sprouty homolog 1, antagonist of FGF signaling (Drosophila)
SPRY2  Sprouty homolog 2 (Drosophila)
STAT   Signal transducer and activator of transcription
TRAIL  Apo2L/tumor necrosis factor (TNF)-a-related apoptosis inducing ligand
TGF-β  Transforming growth factor, beta
VCR    Vincristine
VP-16  Etoposide
WNT    Wingless-type MMTV integration site family
XIAP   X-linked inhibitor of apoptosis
<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Target genes</th>
<th>Function</th>
<th>Cell lines</th>
<th>In vivo models</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-197</td>
<td>BMF, NOXA</td>
<td>Repress p53-dependent apoptotic cascade, miR inhibition decreases cell viability, miR inhibition impairs cell growth and anchorage-independent colony formation</td>
<td>A549, Calu-1, NIH-H460, NCI-H1299</td>
<td>Nude mice</td>
<td>[35]</td>
</tr>
<tr>
<td>miR-21</td>
<td>SPRY1, SPRY2, BTG2, PDCD4, APAF1, FasL, RHOB</td>
<td>Enhance tumor proliferation and survival, Inhibit apoptosis, miR deletion suppresses Ras-driven transformation</td>
<td>N/T</td>
<td>K-ras&lt;sup&gt;CA2&lt;/sup&gt; mice, CAG-miR-21 transgenic mice, CAG-miR-21;K-ras&lt;sup&gt;CA2&lt;/sup&gt; compound mutant mice</td>
<td>[37]</td>
</tr>
<tr>
<td>miR-212</td>
<td>AChE</td>
<td>Prevent apoptosis, Maintain cell proliferation capacity, Modulate CDDP-induced NSCLC cell apoptosis</td>
<td>HEK-293T, NCI-H520, NCI-H460, SK-MES-1, BEAS-2B</td>
<td>Nude mice</td>
<td>[43]</td>
</tr>
<tr>
<td>miR-17-5p, miR-20a</td>
<td>E2F1</td>
<td>miR inhibition reduces of cell growth, miR inhibition induces apoptosis and increases proportions of sub-G1 populations.</td>
<td>Calu-6, A549, ACC-LC-172</td>
<td>N/T</td>
<td>[45]</td>
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N/D: Not determined, N/T: Not tested
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<th>MicroRNA</th>
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<th>Cell lines</th>
<th>In vivo models</th>
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<tr>
<td>miR-198</td>
<td>FGFR1</td>
<td>Inhibit lung cancer cells proliferation. Enhance cell apoptosis. Inhibits growth of tumor graft in nude mouse.</td>
<td>A549, NCI-H460</td>
<td>Athymic BALB/c nude mice.</td>
<td>[69]</td>
</tr>
<tr>
<td>miR-451</td>
<td>RAB14</td>
<td>Inhibit <em>in vitro</em> proliferation and enhance apoptosis. Decrease phosphorylation of AKT and increased BAX or Bad protein level. Associated with <em>in vivo</em> proliferation capacity.</td>
<td>A549, NCI-H520</td>
<td>Athymic BALB/c nude mice.</td>
<td>[75]</td>
</tr>
<tr>
<td>miR-192</td>
<td>RB1</td>
<td>Inhibit cell proliferation and promotes cell apoptosis. Arrest cell in G1 phase. Inhibit tumorigenesis <em>in vivo</em>.</td>
<td>A549, NCI-H460</td>
<td>Athymic BALB/c nude mice.</td>
<td>[76]</td>
</tr>
<tr>
<td>miR-335</td>
<td>BCL-W, SP1</td>
<td>Suppress proliferation and invasion ability of cells. Induce apoptosis. Suppress metastasis and invasiveness of cells.</td>
<td>A549, NCI-H1299</td>
<td>N/T</td>
<td>[52]</td>
</tr>
<tr>
<td>miR-608</td>
<td>N/D</td>
<td>Increase cell death in Bcl-xL silenced cells.</td>
<td>A549, SK-LU-1</td>
<td>N/T</td>
<td>[55]</td>
</tr>
<tr>
<td>miR-203</td>
<td>PKCα</td>
<td>Decrease cell proliferation. Promote cell apoptosis, but this effect only partially relies on its down-regulation of PKCα.</td>
<td>A549</td>
<td>N/T</td>
<td>[61]</td>
</tr>
<tr>
<td>miR-413</td>
<td>PKCε</td>
<td>Inhibit cell proliferation and enhance apoptosis.</td>
<td>A549, Calu-1</td>
<td>N/T</td>
<td>[63]</td>
</tr>
<tr>
<td>miR-146a</td>
<td>EGFR</td>
<td>Inhibit cell growth and induces cell apoptosis. Suppress motility. Enhance cell proliferation inhibitory effect of TKIs and cetuximab.</td>
<td>NCI-H358, NCI-H1650, NCI-H1975, NCI-H292, HCC827</td>
<td>N/T</td>
<td>[70]</td>
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<tr>
<td>miR-26a</td>
<td>EZH2</td>
<td>Inhibit cell proliferation <em>in vitro</em>. Block G1/S phase transition and induced apoptosis. Decreased metastasis capacity and invasion.</td>
<td>SPC-A1, A549, SK-MES-1</td>
<td>N/T</td>
<td>[71]</td>
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N/D: Not determined, N/T: Not tested
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<th>Function</th>
<th>Cell Lines</th>
<th>In vivo models</th>
<th>Citation</th>
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<tr>
<td>Docetaxel</td>
<td>miR-100</td>
<td>Plk1</td>
<td>Chemosensitize lung adenocarcinoma cells to docetaxel</td>
<td>SPC-A1, A549, NCI-H1299, SPC-A1/DTX</td>
<td>Nude mice</td>
<td>[102]</td>
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<tr>
<td>miR-650</td>
<td>ING4</td>
<td></td>
<td>Confer docetaxel chemoresistance both \textit{in vitro} and \textit{in vivo}</td>
<td>SPC-A1, NCI-H1299</td>
<td>Athymic BALB/c nude mice</td>
<td>[105]</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>miR-451</td>
<td>N/D</td>
<td>Enhance DDP chemosensitivity</td>
<td>A549</td>
<td>BALB/c nude mice</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>miR-31</td>
<td>ABCB9</td>
<td>Induced DDP resistance</td>
<td>SPC-A1, LTEP-A2, NCI-H460, NCI-H1299</td>
<td>N/T</td>
<td>[86]</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>miR-133a/b, miR-361-3p</td>
<td>N/D</td>
<td>Oncogenic miR inhibition reduces cell survival</td>
<td>NCI-H1155, NCI-H1993, NCI-H358</td>
<td>N/T</td>
<td>[91]</td>
</tr>
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<td></td>
<td>miR-101</td>
<td>EZH2</td>
<td>Decrease proliferation and invasive ability of cells</td>
<td>NCI-H226, A549, NCI-H358, 801D</td>
<td>N/T</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gemcitabine sensitivity</td>
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<td></td>
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<td>EGFR-TKI</td>
<td>miR-30b/c, miR-221/222</td>
<td>APAF-1, BIM</td>
<td>Gefitinib-induced PARP cleavage</td>
<td>Calu-1, A549, PC-9, HCC827</td>
<td>N/T</td>
<td>[123]</td>
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<tr>
<td></td>
<td>miR-214</td>
<td>PTEN</td>
<td>Oncogenic Knockdown sensitizes cells to gefitinib</td>
<td>HCC827, HCC827/GR</td>
<td>N/T</td>
<td>[126]</td>
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<tr>
<td></td>
<td>miR-133b</td>
<td>EGFR</td>
<td>Inhibit cell’s growth and invasion abilities</td>
<td>PC-9, A549, NCI-H1650, NCI-H1975</td>
<td>N/T</td>
<td>[131]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>miRNA-186*</td>
<td>Caspase-10</td>
<td>Inhibits cell apoptosis</td>
<td>A549, A549/DDP</td>
<td>N/T</td>
<td>[155, 158]</td>
</tr>
<tr>
<td>TRAIL</td>
<td>miR-221, miR-222</td>
<td>N/D</td>
<td>Impair TRAIL-dependent apoptosis</td>
<td>Calu-1, A549, NCI-H460</td>
<td>N/T</td>
<td>[169]</td>
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<tr>
<td></td>
<td>miR-34a, miR-34c</td>
<td>PDGFR-α, PDGFR-β</td>
<td>Augment TRAIL response</td>
<td>NCI-H460, A549, NCI-H1299, Calu-6 NCI-H1703, NCI-H1703,</td>
<td>N/T</td>
<td>[170]</td>
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<tr>
<td></td>
<td>miR-212</td>
<td>PED</td>
<td>Increase sensitivity to TRAIL</td>
<td>Calu-1, NCI-H460</td>
<td>N/T</td>
<td>[176]</td>
</tr>
<tr>
<td>PRIMA-1</td>
<td>miR-34a</td>
<td>N/D</td>
<td>Induce apoptosis in the lung cancer cells containing mutant p53</td>
<td>A549, NCI-H211, NCI-H1155, NCI-H1299</td>
<td>N/T</td>
<td>[149]</td>
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</tr>
<tr>
<td>Multidrug Resistance</td>
<td>miR-200bc/429</td>
<td>BCL2, XIAP</td>
<td>Enhanced sensitivity to various anti-cancer drugs including VCR, CDDP, VP-16 and ADR</td>
<td>A549, A549/CDDP</td>
<td>N/T</td>
<td>[159]</td>
</tr>
<tr>
<td>miR-181b</td>
<td>BCL2</td>
<td>Increased sensitivity to a number of anti-cancer drugs including VCR, 5-Fu, CDDP, VP-16 and ADR</td>
<td>A549, A549/CDDP</td>
<td>N/T</td>
<td>[162]</td>
<td></td>
</tr>
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</table>

N/D: Not determined, N/T: Not tested
Figure 1. Scheme depicting up- and down-regulated miRNAs and the roles they play in various biological pathways including apoptosis, proliferation and angiogenesis.
Figure 2. Scheme depicting the roles miRNAs play in sensitivity and resistance to common cancer treatments.


paclitaxel (T) but not from the escalation of doxorubicin (A) dose level in the adjuvant chemotherapy of patients (pts) with node-positive primary breast cancer. Proceedings - American Society of Clinical Oncology 17: 101a.


