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Review

# MicroRNA regulation of core apoptosis pathways in cancer

*Raquel T. Lima* [a,b], *Sara Busacca* [c], *Gabriela M. Almeida* [a], *Giovanni Gaudino* [d], *Dean A. Fennell* [c], *M. Helena Vasconcelos* [a,b,*]

[a] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal*
[b] *Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal*
[c] *Centre for Cancer Research and Cell Biology, Queen's University of Belfast, Belfast, Northern Ireland*
[d] *University of Hawaii Cancer Center, Honolulu, HI, USA*

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ABSTRACT

Recent research has demonstrated that microRNAs (miRNAs) are key regulators of many cell processes often deregulated in cancer, including apoptosis. Indeed, it is becoming clear that many miRNAs are anti-apoptotic and mediate this effect by targeting pro-apoptotic mRNAs or positive regulators of pro-apoptotic mRNAs. Conversely, many pro-apoptotic miRNAs target anti-apoptotic mRNAs or their positive regulators. We have reviewed the current knowledge in this area including evidence of miRNA involvement in cancer drug resistance.



## 1. Introduction

### 1.1. *Drug resistance and apoptosis*

Overcoming drug resistance to conventional and targeted therapies remains a key challenge in the fight against cancer.[1] Acquired drug resistance is of major clinical significance as the majority of solid cancers is diagnosed at the advanced stage where treatment intent is palliative. Drug resistance manifests typically with very low response rates to conventional chemotherapy. For example in non-small cell lung cancer or mesothelioma, where the initial response rate to chemotherapy is around 30%, in the relapsed setting this falls to less than 10%. Evasion of apoptosis is both a hallmark of cancer and is involved in tumourigenesis and drug resistance. Where apoptosis can be effectively induced, *e.g.* via targeting activating mutations of receptor tyrosine kinases such as the epidermal growth factor receptor,[2–4] this is associated with dramatic clinical responses.[5,6] This reinforces the basic concept that achieving efficient apoptosis is essential for optimising therapeutic responses and, therefore, clinical outcome.

Predicting apoptosis resistance to drug based cancer therapies should enable not only personalisation of treatment, minimising futile administration and unnecessary morbidity, but also reduce health economic burden. MicroRNAs (miRNAs or miRs) have recently been shown to regulate apoptosis and may therefore represent a novel class of biomarkers for facilitating personalised treatment.

* *Corresponding author at:* IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal. Tel.: +351 22 5570700; fax: +351 22 5570799.
E-mail address: hvasconcelos@ipatimup.pt (M.H. Vasconcelos).
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### 1.2. MicroRNAs

MicroRNAs play a major role in tumourigenesis and are frequently located at cancer-associated genomic regions or in fragile sites.[7] The miRBase database already has more than 1000 entries for mature human miRNAs (http://www.mirbase.org accessed on 13 October 2010). miRNAs are known to control gene expression at the mRNA level through RNA interference (RNAi).[8] A schematic representation of miRNA mediated RNAi is shown in Fig. 1. In summary, miRNAs are synthesised from non-coding DNA in the nucleus as long precursors called primary-miRNA (pri-miR) by RNA polymerase II. They are then processed by the RNase III enzyme called Drosha and the dsRNA-binding protein, Pasha, into pre-miRNA. The pre-miRNA is then exported into the cytoplasm by Exportin-5, where it is further processed by an RNase III enzyme, Dicer, to give origin to the miR–miR* duplex. This duplex is incorporated into RISC, it is unwound by a helicase and the mature miRNA strand guides RISC to complete or partial complementary target mRNAs. Depending on the degree of complementarity to the target mRNA, the mechanism of silencing target mRNA expression will be one of the following:

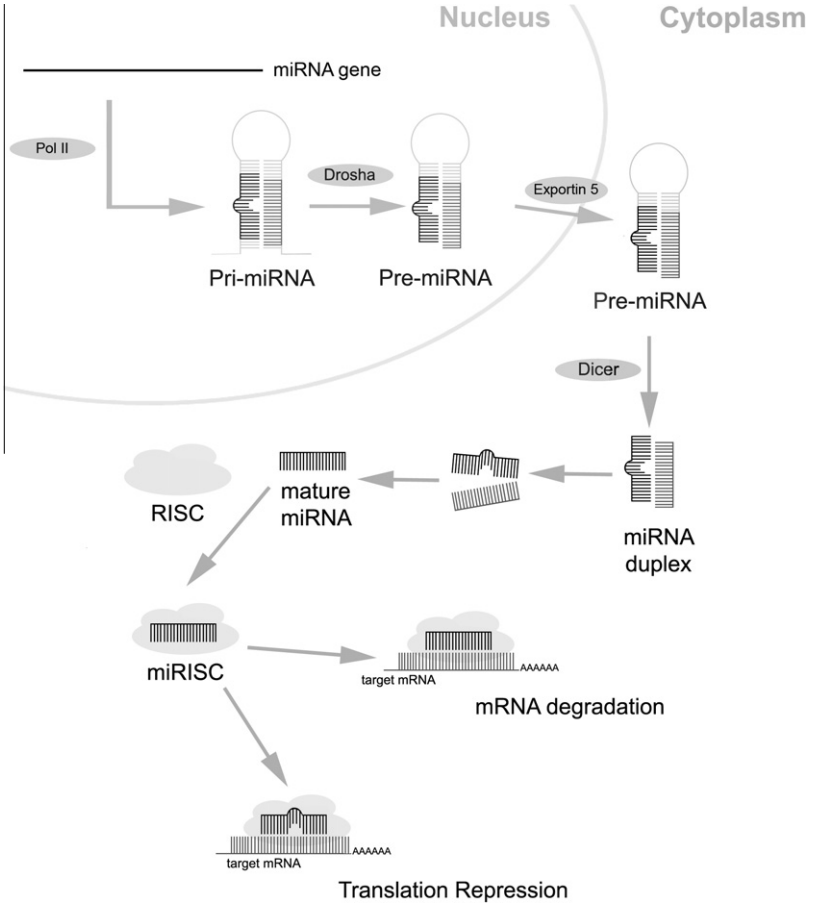


**Fig. 1 – miRNA-mediated RNAi mechanism. The biogenesis of miRNA starts in the nucleus and involves a vast group of protein complexes. The miRNAs are generally transcribed in the nucleus from 'non-coding' or 'non-messenger' DNA regions as primary-miRNA (pri-miR), by RNA polymerase II.[10,111] These large RNA precursors, which are several hundred base pairs long, will be further processed by the RNase III enzyme, Drosha, and the dsRNA-binding protein, Pasha, into hairpin-shaped stem-loop structures of approximately 60–70 nt with a 5′phosphate and a 3′-2 nt overhang named pre-miRNA.[10,112,113] Pre-miRNA will then be actively transported into the cytoplasm by Exportin-5, a Ran-GTP dependent transporter. Once in the cytoplasm, the pre-miRNA will be further processed by a second RNase III enzyme, named Dicer, giving rise to the small RNA duplex molecule, the miR–miR* duplex, with 2 nucleotides (nt) 3′ overhangs. This duplex will then be incorporated into a protein/RNA complex, referred to as RNA-induced silencing complex (RISC). Once in the complex (miRISC complex), the miRNA duplex is unwound by a helicase and the mature miRNA strand will be used to guide RISC to the 3′ UTR of target mRNAs with either complete or incomplete homology. Depending on the degree of complementary of the miRNA with the target mRNA, the mechanism of gene silencing will differ. When there is perfect homology with the target mRNA there will be target mRNA cleavage.[8–12] If incomplete complementarity is the case, there will be translation inhibition or alteration of mRNA stability namely, by deadenylation.[8,117,118] Usually, the complementarity is restricted to the nucleotides 2–8 in the 5′ end of the miR, the 'seed' sequence.[119,120] Once associated to an incomplete complementar target mRNA, the miR-RISC complex leads to the blockage of translation as it directs the mRNA into the P-bodies,[121,122] specialised cytoplasmic compartments where translational repression and mRNA turnover occurs.**

(i) if full complementarity to a target mRNA is met, the target mRNA will be cleaved and (ii) if only partial complementarity is met, then translation repression or alteration of mRNA stability will follow.[8–12]

There is currently intensive research aimed at identifying all miRNAs, their target mRNAs and their biological functions. It is known that, due to the fact that only partial complementarity to the target mRNA is required, one miRNA may block the translation of many target mRNAs. This explains the current estimation that, although the miRNAs identified to date are approximately a thousand, miRNAs control about one-third of all human mRNAs.[8] Nevertheless, it is possible that single miRNAs target single pathways by targeting multiple mRNAs of the same cellular pathway. In fact, it is accepted that one miRNA may be simultaneously targeting a complexity of mRNAs, which function towards the same end in the cell, by being involved in the same cellular signalling pathways or in the crosstalk between those pathways. In addition, the current understanding of the miRNA mediated RNAi mechanism of gene silencing is even more complex due to the fact that the expression of a single mRNA may be regulated by many miRNAs.

Indeed, miRNAs are involved in several (if not all) critical cellular processes altered in cancer, such as proliferation, differentiation and apoptosis.[13–19] This makes them attractive candidates as cancer biomarkers. Furthermore, given their involvement in the regulation of cellular apoptosis and the understanding that most chemotherapeutic drugs kill cells by this mechanism (schematically represented in Fig. 2), we have reviewed the available evidence regarding known anti-apoptotic and pro-apoptotic miRNAs, and have related this to their involvement in drug resistance and their potential as predictors of anti-cancer drug efficacy.

## 2. Anti-apoptotic miRNAs

### 2.1. *Extrinsic cell death pathway*

The extrinsic cell death pathway results from activation of cell surface death receptors, through the binding of specific ligands (such as TNF, TRAIL or FasL – Fig. 2), which causes recruitment and oligomerisation of FADD within the Death-Inducing Signaling Complex (DISC). The oligomerised FADD then binds Caspases-8 and -10, promoting their activation.[20–22] Apoptosis induced by the chemopreventive agent curcumin, in a non-small cell lung cancer cell line, was attributed to down-regulation of miR-186* causing the consequent increased expression of its direct target, *Caspase-10*.[23]

Several miRNAs were identified, through an RNAi screening approach, as putative direct and indirect regulators of TRAIL-induced apoptosis pathway. The screening has led to the discovery and characterisation of several genes involved in this pathway. Interestingly, the expression of many of the genes known to be involved in apoptosis seems to be regulated by miRNAs identified using this approach.[24] For example, miR-221 and miR-222 are up-regulated in TRAIL resistant cells and down-regulated in sensitive cells. These miRNAs display marked effects on TRAIL signalling through their activity on the cell cycle regulator $p27^{kip1}$. Indeed, transfection with anti-miRNAs rendered cells sensitive to TRAIL while treatment with pre-miRNAs increased resistance.[25] A further report also indicates that miR-221 and miR-222 target $p27^{kip1}$ in melanoma.[26] miR-21 has been recently shown to be positively regulated by an AKT-dependent pathway and to exert its anti-apoptotic effects by direct inhibition of *FasL*.[27]

The Fas-associated factor 1 (FAF1) is a Fas-binding pro-apoptotic protein and a component of DISC. miR-24 directly regulates FAF1 by binding to the CDS region of its mRNA. In addition, miR-24 was shown to regulate apoptosis of gastric, cervical and prostate cell lines.[28] The Fas associated phosphatase-1 (FAP-1), which inhibits apoptosis induced by Fas, is a direct target of miR-200c. This provides a molecular mechanism to explain both the downregulation of Fas expression and the reduced sensitivity of cells to Fas-mediated apoptosis observed when miR-200c expression is reduced during tumour progression.[29]

*PTEN* (phosphatase and tensin homologue) is a tumour suppressor gene frequently mutated in cancer. It encodes for a protein that inhibits PED, hence interfering with the formation of DISC (Fig. 2). Several miRNAs have been shown to directly down-regulate the expression of PTEN, like miR-17-5p and miR-19[30], miR-21[27] and miR-221 and miR-222.[31] The latter miRNAs were demonstrated to regulate radiosensitivity in gastric carcinoma cells, possibly by direct modulation of PTEN expression and the authors suggest that they could constitute a novel therapeutic strategy for human gastric cancer.[31] These miRNAs that target *PTEN* will most likely contribute to chemo- or radioresistance phenotypes in several other tumour types (particularly when PTEN is not mutated), as their expression is often altered in cancer. Expression of miR-21, for instance, is consistently up-regulated in most tumours studied so far.[32]

### 2.2. *Caspase-9*

During initiation of mitochondrial apoptosis, Cyt *c* released from the mitochondrial intermembrane space after BAX and/or BAK oligomerisation forms a Caspase-9 activating complex or Apoptosome with APAF-1 and Pro-Caspase-9 (Fig. 2). To date, there are two miRNAs known to inhibit expression of Caspase-9, leading to mitochondrial apoptosis block: miR-133 and miR-24a. miR-133 acts as a regulator of survival in cardiac cells, by repressing Caspase-9 expression at both protein and mRNA levels, as demonstrated by luciferase reporter assays.[33] miR-24a has also been recently shown, by reporter assays and loss-of-function experiments, to be capable of repressing apoptosis by directly inhibiting *Caspase-9*[34] and has a pivotal function in controlling the eye size, by preventing apoptosis of the retina during eye morphogenesis in *Xenopus*.

### 2.3. *BH3-only proteins*

BH3-only proteins can work as 'direct activators' of apoptosis or as 'sensitisers' (or 'de-repressors') of anti-apoptotic proteins and are constituted by: BAD, BIK, BID, BIM, PUMA, NOXA among others,[35,36] as represented in Fig. 2.

The miR-32 may act as an oncogene, by targeting the pro-apoptotic function of BIM. BIM interacts with all pro-survival BCL-2 family proteins and plays key roles in apoptosis and re-

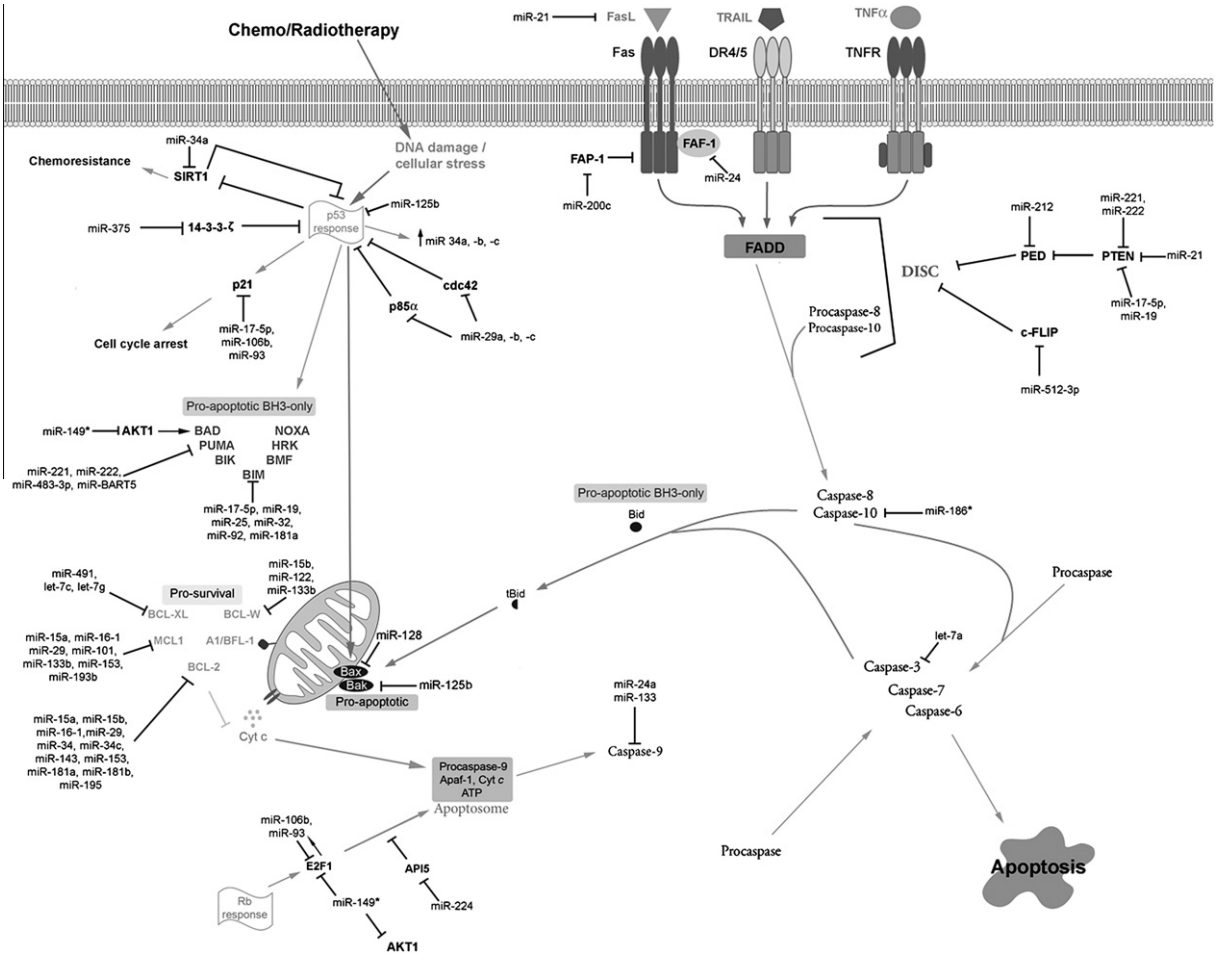


**Fig. 2 – Apoptosis is regulated by miRNAs. Apoptosis is a pivotal process by which anti-cancer drugs mediate cancer cell killing and Caspases are its main executioners. Upon activation, initiator Caspases (including Caspase-8 and -9) cleave and thereby activate executioner Caspases (including Caspase-3). The executioner Caspases are responsible for the cleavage of several critical cellular substrates resulting in cellular destruction.[125,126] Apoptosis may occur via two major pathways: the extrinsic and the intrinsic pathways. The former occurs through the activation of death receptors anchored at the cell membrane, through the binding of their specific ligands (such as FasL, TNF and TRAIL), resulting in Caspase-8 activation.[20–22] The intrinsic, or mitochondrial, pathway has mitochondria as its central regulator[125,128] and is associated with the action of the members of the BCL-2 family of proteins, resulting in the release of several mitochondrial proteins, including Cytochrome c (Cyt c), into the cytosol. Cyt c binds APAF-1 and Pro-Caspase-9, forming a Caspase-9 activating complex or Apoptosome. Following cleavage of Caspase-9, downstream Caspases are activated. Both pathways, intrinsic and extrinsic, converge on downstream Caspases. The BCL-2 protein family is known to regulate apoptosis. This family comprises several proteins that have been related both by sequence homology (each member presenting at least one of four BCL-2 homology domains, BH1–BH4) and by their function in regulating apoptosis.[36,128] The anti-apoptotic BCL-2, BCL-XL, BCL-W, MCL1, A1/BFL-1 are members of this family sharing homology in all four BH domains. On the other hand, BAX and BAK share BH1, BH2 and BH3 domains and promote apoptosis. Another group of pro-apoptotic proteins, the BH3-only proteins, can work as 'direct activators' of apoptosis or as 'sensitisers' (or 'de-repressors') of anti-apoptotic proteins and is constituted by: BAD, BIK, BID, BIM, PUMA, NOXA among others.[35,36] Pro-apoptotic proteins BAX and BAK oligomerise leading to an increase of the mitochondrial outer membrane permeability (MOMP) allowing several mitochondrial proteins to be released from mitochondria into the cytosol. BCL-2 and BCL-XL directly interact with BAX and BAK preventing their oligomerisation and thereby decreasing MOMP[125]. BH3-only proteins seem to act as sensors of the apoptotic stimuli. Some of these proteins, such as BID and BIM, may act as 'direct activators', activating BAX directly (and probably BAK) and can be antagonised by anti-apoptotic BCL-2 family members. The other BH3-only proteins may act as 'de-repressors', by binding to the anti-apoptotic members and antagonising their effect on BAX/BAK, thereby affecting the MOMP.[130–132] BID has another interesting role allowing the cross-talk between the extrinsic and the intrinsic pathways. BID is cleaved to tBID by Caspase-8, after the engagement of death receptors. tBID is then translocated into the mitochondria, leading to Cyt c release into the cytosol and subsequent activation of the Caspases 'belonging' to the intrinsic pathway. BID may also be cleaved by Caspase-3. The tumour suppressor p53 has the ability to decide cellular fate following DNA damage by arresting cell cycle or inducing apoptosis (affecting both intrinsic and extrinsic pathways). p53 activates the transcription of several pro-apoptotic BCL-2**

sponse to chemotherapy (for example, mediated by EGFR and BCR-ABL tyrosine kinase inhibitors[3,37,38]). Accordingly, down-regulation of BIM by miR-32 may contribute to the resistance of prostatic cancer cells to apoptotic stimuli.[39] miR-25 binds *BIM* at its 3′-untranslated region (UTR) causing inhibition of gene expression, involving a post-transcriptional regulatory mechanism.[40] Recently, a study to elucidate the biological effects of the miR-106b-25 polycistron showed that miR-25 did not alter *BIM* mRNA, suggesting that it works by translational inhibition rather than mRNA degradation.[41] Follicular dendritic cells regulate and support B lymphocyte differentiation, survival and progression, and it has been recently shown that these cells protect B-cell lymphoma cells against apoptosis, hence leading to drug resistance, via activation of a miR-181a-dependent mechanism involving down-regulation of BIM. miR-181a directly targets *BIM*, making it an upstream effector of the BIM-apoptosis signalling pathway.[42] Another two miRNAs that directly target *BIM* are miR-19 and miR-92 (which belong to the miR-17-92 cluster).[30] Lymphoproliferative disease and autoimmunity in mice with elevated expression of miR-17-92 cluster in lymphocytes was partially attributed to suppressed expression of BIM, as well as PTEN. This same mechanism could contribute to lymphoma development in patients carrying amplifications of the miR-17-92 coding region.[30] Upregulation of miR-17-5p by MYCN transactivation mediates the oncogenic properties of MYCN, through a direct inhibition of *p21* and *BIM* translation at both mRNA and protein level. Knockdown of miR-17-5p by an antagomir (anti-miRNA oligonucleotide) increased BIM expression in neuroblastoma cells and this event is sufficient to promote massive apoptosis. Furthermore, *in vivo* treatment with antagomir-17-5p abolished tumour growth in neuroblastoma resistant to chemotherapy. It was, therefore, suggested that miR-17-5p is a key factor inducing protection from MYCN-primed apoptosis in neuroblastoma.[43] This study was the first demonstrating that antagomirs can inhibit tumour growth *in vivo*, indicating that these molecules may one day have therapeutic utility. Interestingly, recent miRNA profiling of malignant mesothelioma cells and tissues revealed that reduced expression of miR-17-5p in patients of the sarcomatoid histological subtypes of this tumour correlates with longer survival.[44]

An oncogenic function for miR-125b in prostate cancer has been suggested since it is highly expressed in most clinical prostate cancer samples and cell lines relative to benign prostate tissue and cell lines. miR-125b targets the pro-apoptotic *BAK1*, therefore, contributing to disease progression and resistance to treatment in prostate cancer.[45] Interestingly, miR-125b was also found to be up-regulated in taxol-resistant breast cancer cells thus causing, through the suppression of BAK1, strong inhibition of taxol induced cell death and apoptosis.[46]

A very recent study on the liver carcinoma cell line, HepG2, has raised the possibility that miR-483-3p modulates PUMA and that its enforced expression can protect cells from apoptosis.[47] miRs-221/222 have also been shown to directly regulate apoptosis by targeting *PUMA* in glioblastoma cells.[48] In addition, there were already previous indications that PUMA expression could be modulated by miRNAs. Indeed, miR-BART5, an Epstein-Barr Virus (EBV) miRNA, has been shown to have anti-apoptotic activity by targeting PUMA expression in nasopharyngeal carcinoma (NPC) and in EBV-associated gastric carcinoma latently infected with EBV and to protect these cells from apoptosis. In fact, this was the first demonstration that some viruses are able to modulate cellular apoptosis through a miRNA.[49]

The ectopic expression of miR-128 in human embryonic kidney (HEK293T) cells clearly resulted in the induction of mitochondria-mediated apoptosis.[50] miR-128 was found to target and consequently lead to the downregulation of pro-apoptotic BAX. Intriguingly, the expression of miR-128 induced apoptosis in both HEK293T and non-small lung cancer cells (NCI-H460). This apparent contradicting result may be due to the also conflicting indications of the role exerted by BAX in apoptosis. In fact, while some studies indicate BAX as an inducer of apoptosis, others have already pointed into the other direction in which BAX acts as an inhibitor of apoptosis. The role of BAX in apoptosis is most probably dependent on cell-specific factors.[51]

### 2.4. *Executioner caspases*

Both the extrinsic and intrinsic apoptosis pathways converge on the effector caspases (Caspase-3, -6 and -7, Fig. 2). The executioner Caspase-3 is inhibited by the miR let-7a, which antagonises drug-induced apoptosis. let-7a plays a role in modifying the sensitivity of cells to therapeutic drugs including doxorubicin, paclitaxel and interferon-gamma. Ectopic let-7a expression decreased the extent of drug-induced apoptosis as well as the apoptotic cell population.[52]

## 3. Pro-apoptotic miRNAs

### 3.1. *miRNAs affecting p53 expression*

p53 is a pleiotropic regulator of cell fate in response to DNA damage, which may induce cell cycle arrest, DNA repair, senescence or apoptosis (Fig. 2).[53,54] p53 may also regulate metabolic pathways, cell growth and autophagy.[55] p53 can facilitate apoptosis by several mechanisms which include: (i) transactivation of BCL-2 family members (BAX, BID, PUMA, NOXA), of the apoptotic effector machinery (Apaf-1, Caspase-8, Caspase-6), of cell death receptors (DR5, FAS) and of cell death ligands (TNFSF10, TNFS6) and other factors; (ii) transrepression of Bcl-2, Survivin, ARC and Gelactin-3; and (iii) direct effects on mitochondria by facilitating oligomerisation of BAX and BAK and by interacting with BCL-2, BCL-XL and MCL1 proteins.[56] miRs regulate important components in the p53 transcriptional network pathway by controlling the upstream regulation of p53 and its pro-apoptotic function (Fig. 2). Recently, in a screening for miRNAs that modulate p53 activity,

**family members, including BAX and the BH3-only proteins PUMA, NOXA and BID[135,136] leading to MOMP and, therefore, to apoptosis.[137,138] p53 may also activate the 'death receptors' (belonging to the TNF R family) as well as Caspase-8[136], members of the extrinsic apoptotic pathway.**

the miR-29 family members – miR-29a, miR-29b and miR-29c – were identified as positive regulators of p53. These miRNAs target CDC42 and p85α, leading to p53 upregulation and apoptosis induction.[57]

Interestingly, miR-125b which has been found to target BAK1, also negatively regulates p53. This seems to be physiologically important for cell function and embryonic development. By overexpressing miR-125b, in human neuroblastoma and in lung fibroblasts, the levels of p53 protein were repressed and apoptosis was suppressed. In fact, ectopic expression of miR-125b increased p53 levels and induced apoptosis in human lung. On the other hand, by knocking down miR-125b, an increase in p53 was observed as well as induction of apoptosis.[58] The 14-3-3 protein family can interact with more than 200 proteins, some of which involved in apoptosis. One of its isoforms, 14-3-3ζ, has been shown to decrease p53 protein stability in mammary epithelial cells.[59] Following luciferase reporter assays, 14-3-3ζ has been suggested to be targeted by miR-375. miR-375 was the most downregulated in a miRNA microarray expression profile performed in gastric carcinomas and its ectopic expression reduced cell viability via a Caspase-mediated apoptosis pathway.[60]

### 3.2. *p53-regulated miRNAs*

A recent series of studies have expanded the repertoire of p53-target genes establishing that miR-34a and miR-34b/c are p53 target genes.[61–63] Loss of miR-34 has been linked to resistance to apoptosis induced by chemotherapeutic agents that activate p53.[64] In chemoresistant prostate cancer cells, for example, sensitisation to camptothecin can be achieved by miR-34 up-regulation.[65] Interestingly, a study demonstrating the influence of an HPV oncoprotein in cellular miRNA expression (in cervical cancer tissues and derived cell lines) showed that miR-34a expression was reduced in such cells. This reduction was due to the HPV oncoprotein E6 which destabilises p53, resulting in cellular growth advantage.[66] Moreover, it is known that miR-34a can enhance chemosensitivity by targeting SIRT1.[65,67] Additionally, a positive feedback loop in which p53 induces expression of miR-34a suppressing SIRT1 and consequently increasing p53 activity has been proposed.[68]

Interestingly, it was reported that the expression levels of p21, 14-3-3σ, a downstream target of p53,[69] and miR-34a strongly correlate negatively with Nutlin-3-induced apoptosis. These results led to conclude that as well as acting as a pro-apoptotic miRNA, miR-34a also seems to act as an anti-apoptotic factor in cooperation with p21 and 14-3-3σ and suppresses the p53-dependent apoptotic programme in Nutlin-3 treated cells.[70]

### 3.3. *BCL-2 antagonism*

BCL-2 is a prototypical member of the pro-survival anti-apoptotic family and acts by directly interacting with BAX and BAK to prevent their oligomerisation, thereby inhibiting mitochondrial apoptosis (Fig. 2). Several miRNAs are now known to downregulate BCL-2 expression. miR-34 (a p53 target gene) downregulates BCL-2 protein, which is consistent with a role for miR-34 in p53-mediated apoptosis.[71] In prostate cancer, miR-34c expression was found to be downregulated and to be inversely correlated with tumour aggressiveness. miR-34c was described as negatively regulating BCL-2 and its ectopic expression in prostate cancer cell lines decreased cell growth, due to decrease in cellular proliferation and due to increase in apoptosis.[72]

A study on haematopoietic cancer cells from patients diagnosed with chronic lymphocytic leukaemia (CLL), led to the identification of another regulation mechanism of BCL-2 expression at post-transcriptional level by miR-15a and miR-16-1.[73] In breast cancer cells, the silencing of miR-15a and miR-16, through the use of specific inhibitors, restored the expression of BCL-2.[74] It was demonstrated that the BCL-2 down-regulation, due to miR-15a and miR-16-1 activity, triggers apoptosis.[73] Moreover, the overexpression of miR-15b or miR-16 sensitises the multidrug-resistant human gastric cancer cell line to vincristine-induced apoptosis, and these findings suggest that miR-15b and miR-16 could play a role in the development of multi-drug resistance in gastric cancer cells, at least in part by modulation of apoptosis via targeting *BCL-2*.[75]

miR-153 is a brain tissue specific miRNA and is expressed at a significantly lower level in glioblastoma relatively to non-neoplastic brain tissue. Transfection of this miRNA in cultured cells increased cellular apoptosis, which is blocked by the cognate antagomir. miR-153 was found to target the 3′-UTR of *BCL-2*.[76]

miRNA expression profile analysis of glioblastoma after radiation found miR-181a downregulated. Transient overexpression of this miRNA led to sensitisation of glioblastoma cells to radiotherapy by targeting *BCL-2*.[77] Another miR-181 family member, miR-181b has been suggested to be involved in the development of multidrug resistance in gastric and lung cancer cell lines by modulation of apoptosis also through the targeting of *BCL-2*.[78]

miR-195, which is found downregulated in colorectal cancer tissues and cell lines, was identified to function as a tumour suppressor in this type of tumour. By restoring its expression in colorectal cancer cells, cellular viability was reduced, cellular apoptosis was promoted *in vitro* and tumourigenesis suppressed *in vivo*. The fact that BCL-2 has been found to be one of miR-195 targets suggests that miR-195 probably exerts its role by targeting *BCL-2*.[79] Another miRNA, miR-143, has also been described to directly target *BCL-2*.[80] This miRNA is found down-regulated in osteosarcoma cell lines and primary tumour samples. By restoring miR-143 expression in these cells, cellular viability was reduced, cellular apoptosis was promoted and tumourigenicity was suppressed. In addition, the increased stable expression of miR-143 in colon cancer cells, in which it is also found downregulated, resulted in a decrease in cellular viability and in the increased sensitivity to 5-fluorouracil induced cell death.[81]

### 3.4. *BCL-W antagonism*

BCL-W, another anti-apoptotic BCL-2 family member (Fig. 2), is downregulated by interaction with miR-122. This miRNA may act as an endogenous apoptosis regulator in hepatocellular carcinoma cells. Both mRNA and protein levels of BCL-W were repressed by elevated levels of miR-122, which consequently caused a reduction of cell viability, activation of Cas-

pase-3 and a decrease in the BCL-W/BAX ratio, finally leading to apoptosis.[82]

Furthermore, BCL-W expression was found to be reduced following overexpression of miR-133b in adenocarcinoma cell lines and this miRNA was found to bind to the 3′-UTR of *BCL-W*.[83] The expression of *BCL-W* has also been shown to be targeted by miR-15b in hepatocellular carcinoma, possibly explaining why the expression of this miRNA in hepatocellular tissues was found to be negatively correlated with the risk of recurrence.[84]

### 3.5. *BCL-XL antagonism*

The anti-apoptotic BCL-XL is often overexpressed in cancers and this has been associated with chemotherapy resistance.[85,86] *BCL-XL* has recently been shown, following a gain-of-function screen in a colorectal cancer cell line, to be directly targeted by miR-491.[87] miR-491 induced apoptosis via down-regulation of BCL-XL *in vitro,* and tumours derived from xenografts of miR-491 transfected cells into nude mice were significantly smaller than those in the negative control,[87] suggesting a possible therapeutic potential for miR-491 in the treatment of tumours overexpressing BCL-XL. Moreover, the let-7 family of miRNA was found downregulated in the Huh7 human hepatoma cells and has a putative target site in *BCL-XL* mRNA. let-7c and let7-g overexpression decreased BCL-XL expression in Huh7 and HepG2 cells and expression of let7-c enhanced apoptosis after treatment with the anti-cancer drug targeting MCL1, sorafenib.[88]

### 3.6. *MCL1 antagonism*

MCL1 is another strong inhibitor of apoptosis which is related to chemoresistance. MCL1 is also inhibited by several miRNAs (Fig. 2). Both miR-15a and miR-16-1 function by targeting multiple oncogenes, which may include *MCL1*.[89] Indeed, high-throughput profiling of genes modulated by miR-15a/16-1 in a leukaemic cell line model and in primary CLL samples together with analysis of Gene Ontology database indicate enrichment of *MCL1*.[90]

Furthermore, *in silico* analysis identified a putative target site in the *MCL1* mRNA for the miR-29 family. Accordingly, miR-29b is downregulated in malignant cells, consistent with MCL1 protein upregulation. It was established that the overexpression of miR-29b led to the reduction of MCL1 cellular protein levels and sensitised cancer cells to TRAIL cytotoxicity. Therefore, miR-29 acts as an endogenous regulator of MCL1 protein expression and, thereby, apoptosis.[91] A recent wide survey of malignant mesothelioma tumour samples revealed that miR-29c* is an independent prognostic factor for time to progression as well as survival after surgical cytoreduction. Moreover, overexpression of miR-29c* in mesothelioma cells resulted in significantly decreased proliferation, invasion and colony formation.[92] Besides *MCL1*, miR-29 targets also *BCL-2*. The enhanced expression of miR-29 in hepatocellular carcinoma cells resulted in the loss of mitochondrial potential and release of Cyt *c* to the cytosol, most probably related to MCL1 and BCL-2.[93]

The miR-101 is another negative regulator of MCL1, which is downregulated in hepatocellular carcinoma and has been shown to promote apoptosis. Its transfection into cells increases sensitivity to known chemotherapeutic agents like etoposide and doxorubicin.[94]

A recent study of the expression of miRNAs in lung tumour versus uninvolved tissue identified a significantly reduced expression of miR-133b in lung tumour tissue. Selective overexpression of this miRNA in adenocarcinoma cells reduced MCL1 expression and sensitised cells to gemcitabine.[83]

HCV (Hepatitis C virus), a major cause for hepatocellular cancer has been shown to alter cellular microRNA expression. In fact, miR-193b, which seems to target *MCL1*, is overexpressed in cells stably transfected with HCV genome. Accordingly, these cells show decreased MCL1 expression and increased apoptosis. Furthermore, transfection with miR-193b precursors decreased the IC50 to sorafenib.[95]

Finally, miR-153 targets both *MCL1* and *BCL-2*. By luciferase reporter assays it was confirmed that miR-153 inhibited *BCL-2* and *MCL1* expressions by directly targeting the 3′-UTR regions of those mRNAs.[76]

### 3.7. *PED*

PED/PEA-15 (PED) interferes with both intrinsic and extrinsic apoptotic pathways. Indeed, it inhibits the formation of DISC and Caspase-3 activation triggered by FasL, TNFα and TRAIL. It also inhibits stress-induced apoptosis by simultaneously blocking stress-activated protein kinases and increasing the function of ERK1/2. Finally, it prevents degradation of XIAP.[96] The miR-212 was suggested to be a negative regulator of *PED*. In non-small cell lung cancer, miR-212 expression inversely correlates with PED expression both *in vitro* as *in vivo* and ectopic expression of this miRNA increased TRAIL-induced cell death.[97]

### 3.8. *c-FLIP*

c-FLIP inhibits the recruitment of Caspase-8 and further processing at the DISC. Deregulation of c-FLIP has been associated with several cancers. There is evidence suggesting that both isoforms of c-FLIP prevent taxol-induced apoptosis.[98] The recent finding that miR-512-3p negatively regulates *c-FLIP* expression, and that in HepG2 (hepatocellular cell line) transfection with miR-512-3p promotes taxol-induced apoptosis, may present a novel approach for cancer therapy.[99]

### 3.9. *Apoptosis Inhibitor-5 upregulation*

Apoptosis inhibitor-5 (API-5) is a suppressor of E2F dependent apoptosis involving dArk/Apaf dependent activation of both initiator and effector Caspases as shown in Fig. 2.[100] API-5 is a target of miR-224. miR-224 was found as significantly upregulated in hepatocellular carcinoma, contradicting the conventional idea that apoptosis is reduced during carcinogenesis and cells overexpressing this miRNA displayed lower cell viability when compared to control cells. This is due to the effect of miR-224 in increasing apoptosis by targeting *API-5*.[101] miR-224 may function as a diagnostic and prognostic marker. Indeed, it was shown that this miRNA correlates with acute myeloid leukaemia subtypes, in particular the *t*(15;17) rearrangement,[102] a molecular predictor of favourable clinical

**Table 1 – Pro-apoptotic and anti-apoptotic miRNAs and targets.**

| Apoptosis related gene | miRNAs targeting the genes' mRNA | References |
|---|---|---|
| *Pro-apoptotic genes targeted by anti-apoptotic miRNAs* | | |
| *Caspase-10* | miR-186* | 23 |
| $p27^{kip1}$ | miR-221, miR-222 | 25,26 |
| *Fas L* | miR-21 | 27 |
| *FAF1* | miR-24 | 28 |
| *FAP-1* | miR-200c | 29 |
| *PTEN* | miR-17-5p, miR-19 , miR-21, miR-221, miR-222 | 27,30,31 |
| *Caspase-9* | miR-24a, miR-133 | 33,34 |
| *BIM* | miR-17-5p, miR-19, miR-25, miR-32, miR-92, miR-181a | 30,39–43 |
| *p21* | miR-17-5p, miR-106b, miR-93 | 40,43 |
| *BAK1* | miR-125b | 45,46 |
| *PUMA* | miR-221, miR-222, miR-483-3p, miR-BART5 | 47–49 |
| *BAX* | miR-128 | 50 |
| *Caspase-3* | let-7a | 52 |
| *Anti-apoptotic genes targeted by pro-apoptotic miRNAs* | | |
| *CDC42* and *p85α* (upregulating p53) | miR-29a miR-29b, miR-29c | 57 |
| *14-3-3ζ* | miR-375 | 60 |
| *SIRT1* | miR-34a | 65,67 |
| *BCL-2* | miR-15a, miR-15b, miR-16-1, miR-29 family, miR-34, miR-34c, miR-143, miR-153, miR-181a, miR-181b, miR-195 | 71–80,90,93 |
| *BCL-W* | miR-15b, miR-122, miR-133b | 82–84 |
| *BCL-XL* | miR-491, let-7c, let-7g | 87,88 |
| *MCL1* | miR-15a, miR-16-1, miR-29 family, miR-101, miR-133b, miR-153, miR-193b | 74,76,83,89–91,95 |
| *PED* | miR-212 | 97 |
| *c-FLIP* | miR-512-3p | 99 |
| *API-5* | miR-224 | 101 |
| *E2F1* | miR-93, miR-106b, miR-149* | 40,104 |

outcome. Moreover, miR-224 was found to be overexpressed in follicular cell-derived carcinomas, but at different extent among individual tumours subtypes.[83,103] E2F1 is a member of the E2F family of transcription factors that can mediate cell proliferation as well as p53-dependent and independent apoptosis. E2F1 has been shown to activate miR-106b and miR-93, two miRNAs that are frequently up-regulated in certain gastric tumours, and that, in turn, these miRNAs also directly target the mRNA of *E2F1*, establishing a miRNA-directed negative feedback loop. Up-regulation of these miRNAs was shown to impair TGFβ-dependent cell cycle arrest (via p21 direct inhibition) and apoptosis in gastric cancer and may be involved in TGFβ resistance in this type of cancer.[40] Another miRNA that has been shown to repress expression of E2F1 was miR-149*.[104] Ectopic expression of this miRNA induced apoptosis in human cancer cells by inhibiting E2F1 and Akt1 (a direct activator of the pro-apoptotic BAD).[104]

## 4. Concluding remarks

Chemotherapeutic drug resistance is one of the main factors hampering successful cancer therapy. This drug resistance may be due to resistance to apoptosis. Recently, the study of mechanisms involved in drug resistance has highlighted miRNAs as important regulators of cell death. However, some miRNAs may act as anti-apoptotic while others act as pro-apoptotic (Table 1). miRNAs may target one or simultaneously various mRNAs involved in core apoptotic signalling. Therefore, decreased expression of pro-apoptotic miRNAs and/or increased expression of anti-apoptotic miRNAs in human tumours could be associated with high apoptosis threshold and chemoresistance.[105,106] Analysis of miRNA signatures of tumour biopsies has potential to enable prediction of chemoresistance. Distinct miRNAs (some of them related to apoptosis) and miRNA signatures have recently been associated with drug resistance in ovarian cancer.[107–109] The field of miRNAs based pharmacogenomics is still a relatively new albeit, exciting one. Studies involving large randomised clinical datasets/samples are still lacking. Further research will identify whether core apoptosis pathway targeting miRNAs will have utility as effective predictors of drug response. In addition, modulation of miRNA expression with miRNA mimetics or with antagomirs may be a possible future option.[110]

## Conflict of interest statement

None declared.

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