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Review

The *miR-34* family in cancer and apoptosis

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Recently, the transcription factor encoded by tumor suppressor gene *p53* was shown to regulate the expression of microRNAs. The most significant induction by p53 was observed for the microRNAs *miR-34a* and *miR-34b/c,* which turned out to be direct p53 target genes. Ectopic *miR-34* expression induces apoptosis, cell-cycle arrest or senescence. In many tumor types the promoters of the *miR-34a* and the *miR-34b/c* genes are subject to inactivation by CpG methylation. *MiR-34a* resides on 1p36 and is commonly deleted in neuroblastomas. Furthermore, the loss of miR-34 expression has been linked to resistance against apoptosis induced by p53 activating agents used in chemotherapy. In this review, the evidence for a role of *miR-34a* and *miR-34b/c* in the apoptotic response of normal and tumor cells is surveyed.
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Mutations in the p53 tumor suppressor gene are found in nearly all types of cancers.[1,2] The transcription factor encoded by the *p53* tumor suppressor gene is post-transcriptionally activated by DNA damaging agents/radiation, oxidative stress or activation of oncogenes.[3–5] The critical signals induced by these events are presumably DNA double-strand (ds) breaks, which activate ATM kinase that in turn phosphorylates p53. A number of additional modifications promote accumulation and increased transcriptional activity of p53, which directly regulates numerous target genes that mediate its diverse tumor suppressive effects.[6] The induction of cell-cycle arrest, which can be transient or permanent (senescence), and the promotion of apoptosis in cases in which the damage is too severe are considered to be important for p53-mediated tumor suppression.[5] The decision between these outcomes is determined by the level of p53 protein accumulation, with lower levels favoring arrest and higher levels promoting apoptosis.[7] This effect is presumably because of differential affinities of p53-binding sites in the vicinity of target genes with pro arrest or cell death functions. Furthermore, proteins associating with p53 at specific promoters may influence this decision.[7] Traditionally, the cellular effects of p53 are thought to be mediated by its ability to transactivate genes, which encode effector proteins that induce cellular processes: examples (and the phenotypic end points) are *p21* (G_1-arrest), *14-3-3σ* (G_2-arrest) and *Puma* (apoptosis).[8–10] However, p53 has also been reported to induce the downregulation of specific proteins: for example, the p53-mediated loss of cyclin-dependent kinases (CDK4) and cyclins (Cyclin E2) may contribute to p53-induced cell-cycle arrest.[11] Direct repressive effects of p53 on gene expression are mediated by binding to response elements that overlap with activating sites, by squelching of transcriptional activators and by recruitment of histone deacetylases.[6,12] Furthermore, p53 indirectly represses genes through activation of p21 that leads to association of pRB with E2F and therefore silencing of E2F target genes.[13] For example, the survival factor, Bcl-2, is transcriptionally repressed by p53 through steric interference with DNA binding of the POU4F1 transcription factor at the *Bcl-2* promoter.[14]

The discovery of microRNAs (miRNAs) suggested that the p53-mediated induction of miRNAs might contribute to the downregulation of proteins observed after p53 activation. miRNAs form a class of endogenously expressed, small noncoding RNAs that mediate post-transcriptional regulation of gene expression (reviewed in Ref.[15–19]). miRNA-encoding genes are transcribed by RNA polymerases II to yield primary transcripts (pri-miRNAs), which are processed by the nuclear RNase III enzyme, Drosha, to form stem-loop-structured miRNA precursor molecules. These resulting pre-miRNAs are transported to the cytoplasm in which the RNase III enzyme, Dicer, cleaves off the ds portion of the hairpin and generates a short-lived dsRNA of about 20–25 nucleotides in size. The duplex is subsequently unwound and only one strand gives rise to the mature miRNA, which is incorporated into miRNA–protein complexes (miRNPs). miRNAs guide miRNPs to partially complementary binding sites located in the 3′ untranslated region (3′-UTR) of the target mRNAs, which match to the seed sequences of the miRNAs. The bound miRNPs inhibit translation or destabilize the target mRNAs.[20,21] Both processes result in the downregulation of the protein encoded by the mRNA. Estimates based on

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Keywords: p53; *miR-34a*; *miR-34b/c*; *miR-34*; microRNA; SIRT1
Abbreviations: ATM, ataxia teleangiectasia mutated; CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukemia; CpG, cytosine–guanosine dinucleotide; HDAC, histone deacetylase; LNA, locked nucleic acids; LOH, loss of heterozygosity; miRNA, microRNA; miRNP, miRNA–protein complexes; NSCLC, non-small cell lung cancer; siRNA, small interfering RNA; SIRT1, silent information regulator 1; UTR, untranslated region of mRNA
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bioinformatics as well as microarray analyses suggest that ~30% of all genes are subject to regulation by multiple miRNAs.[22]

Roles of miRNAs in cancer

miRNAs have been implicated in the regulation of processes that are deregulated in cancer cells, as proliferation, differentiation and apoptosis.[18] Alterations in miRNA expression in cancer have been documented in numerous studies and suggest that miRNAs critically contribute to the characteristics of cancer cells (for reviews see Refs.[23–27]). Furthermore, some miRNA-encoding genes have been classified as oncogenic or tumor suppressive genes according to their function in cellular transformation and altered expression in tumors. Tumor suppressive miRNAs may function by downregulating the products of proto-oncogenes. For example, the miRNA family *let-7* targets expression of the oncogenes *KRAS, NRAS* and *HMGA2* and its expression is diminished in lung tumors.[28–30] Furthermore, the region encoding *miR-15* and *miR-16* is deleted in 65% of chronic lymphocytic leukemia (CLL) and in other tumors.[26] As *miR-15/16* targets the anti-apoptotic factor, Bcl-2, the loss of *miR-15/16* may explain the upregulation of Bcl-2 in these tumors.

Connecting p53 and the *miR-34* family

In 2007, reports from several laboratories showed that members of the *miR-34* family are direct p53 targets, and their upregulation induces apoptosis and cell-cycle arrest (Figure 1).[31–36] In mammalians, the *miR-34* family comprises three processed miRNAs that are encoded by two different genes: *miR-34a* is encoded by its own transcript, whereas *miR-34b* and *miR-34c* share a common primary transcript (Figure 2a). In mice, *miR-34a* is ubiquitously expressed with the highest expression in brain,[38] whereas *miR-34b/c* is mainly expressed in lung tissues.[35] These analyses also showed that *miR-34a* is expressed at higher levels than *miR-34b/c*, with the exception of the lung, in which *miR-34b/c* is dominantly expressed. Therefore, the two *miR-34* genes presumably have tissue-specific functions. Similar to other p53-target genes, *miR-34* genes may be the important targets for other signaling pathways involved in normal development. Whether this is the case, remains to be determined by genetic analysis in mice.

Functions of p53-induced *miR-34* genes

Unexpectedly, the ectopic expression of *miR-34* genes had rather drastic effects on cell proliferation and survival. Ectopic *miR-34a* and *miR-34b/c* caused a cell-cycle arrest in the G_1 phase.[32,34,35] In addition, *miR-34b/c* inhibited proliferation and colony formation in soft agar.[36] Interestingly, the introduction of *miR-34a* and *miR-34b/c* into primary human diploid fibroblasts induced cellular senescence,[32] a permanent form of cell-cycle arrest, which is presumably also relevant for

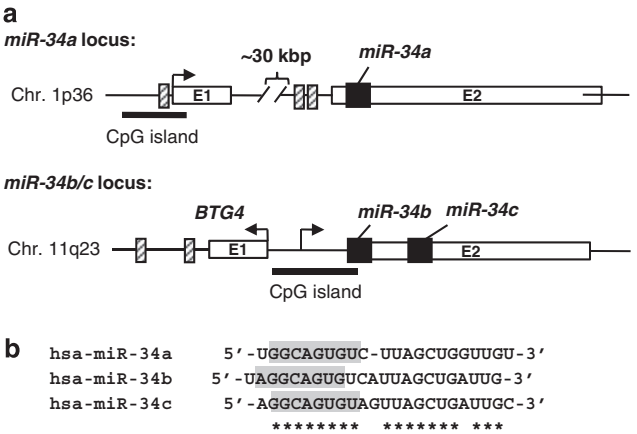

Figure 2 Comparison of the *miR-34* family members, *miR-34a* and *miR-34b/c*. (**a**) Structure of genomic loci of the human *miR-34a* and *miR-34b/c* genes. White and black boxes represent exons and miRNA hairpins, respectively. Hatched boxes indicate p53-binding sites; CpG islands are represented by thick black lines. The model is not shown to scale. Chromosomal (Chr) locations of the genes are provided. (**b**) Sequence alignment of the mature *miR-34a*, *miR-34b* and *miR-34c* molecules. The seed sequences are highlighted by gray shading. Asterisks indicate identical nucleotides

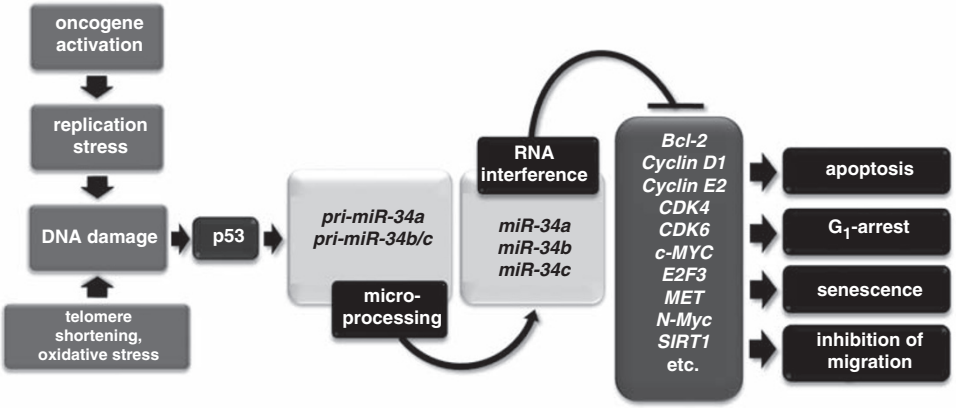

Figure 1 The *miR-34* family as mediator of tumor suppression by p53. After the generation of double-strand breaks p53 is activated through ATM-kinases and transactivates target genes through consensus binding sites. The primary transcripts of the induced *miR-34* genes are processed by DROSHA and DICER complexes. The mature miRNA is incorporated in the RISC complex and mediates inhibition of translation or RNA degradation of the indicated validated and presumably many others not yet confirmed targets. Exemplary cellular outcomes are indicated. Adapted with modifications from Ref[37]

organismal aging.[39] Furthermore, re-expression of *miR-34a* induced apoptosis.[31,33,34,40] As cell-cycle arrest and apoptosis are common end points of p53 activation, *miR-34* genes may be the potent mediators of tumor suppression by p53. Microarray analyses after ectopic introduction of different members of the *miR-34* family into various cell lines revealed hundreds of putative, downregulated *miR-34* targets.[31,32,35] Interestingly, mRNAs with functions in the cell-cycle control and the DNA damage response were overrepresented among the transcripts downregulated by *miR-34*. Furthermore, the downregulated mRNAs showed an enrichment of *miR-34* seed-matching sequences in their 3′-UTRs. Examples include CDK4/6, Cyclin E2, MET and Bcl-2.[32,35] For a survey of currently confirmed targets see Table 1. The observed downregulation of these proteins by *miR-34a* is presumably direct, because reporters carrying the 3′-UTR of the respective genes were inhibited by co-transfection of *miR-34a* and/or *miR-34b/c* in a manner dependent on the presence of an intact seed-sequence matching motif (Table 1). The high similarity among the three processed *miR-34* family members (Figure 2b) suggested that they may have the same targets. Indeed, an expression analysis after separate transfection of *miR-34a*, *miR-34b* and *miR-34c* showed that the affected mRNAs were almost identical.[32] However, differences in the affinities for targets may exist between the three *miR-34* members, as perfect matches are only possible between certain *miR-34* species and the 3′-UTR. An example is *c-MYC*, which seems to be regulated mainly by *miR-34b/c*.[53,54,46] This can presumably be explained by the enhanced complementarity between the *miR-34b* seed sequence and the seed-matching sequence in the *c-MYC* 3′-UTR, when compared with *miR-34a* (Table 1; Figure 2b).

The induction of *miR-34* genes allows p53 to regulate the expression of a large number of proteins, even after their transcripts have already been synthesized. This type of regulation may be advantageous in situations of cellular stress as it does not require the translation of additional effector proteins that would presumably take too long to allow time for repair. Furthermore, it facilitates the simultaneous regulation of numerous processes by p53. Interestingly, the mechanism of RNA interference has been implicated in other forms of stress response (reviewed in Ref.[58]). Furthermore, targeting of p53-induced mRNAs by *miR-34* may contribute to the fine tuning of the p53 response and prevent an uncontrolled, irreversible response to p53 activation.[59] The several modes of regulation exerted by the miRNAs were recently summarized:[19] besides acting as an on/off switch the transcriptional induction of miRNAs may allow to fine tune protein levels to make cells more responsive to external signals. Furthermore, mRNAs that are transcriptionally repressed may be simultaneously targeted by miRNAs. This dual regulation allows an accelerated transition to the off-state. This may be the case for *Bcl-2*, because its transcription is directly repressed by p53, as discussed above,[14] and its 3′-UTR is targeted by *miR-34a*.[35]

Inactivation of *miR-34* in cancer

As cell-cycle arrest, senescence and apoptosis are tumor suppressive mechanisms, the permanent inactivation of members of the *miR-34* family, which induce these cellular responses, may be a selective advantage for cancer cells. Besides decreased expression of *miR-34* because of the inactivating mutations of *p53* or the expression of viral inhibitors of p53, the *miR-34*-encoding genes themselves may be targets for the mutational or the epigenetic inactivation in cancer. Interestingly, *miR-34a* resides on the chromosomal locus 1p36, which has been proposed to harbor a tumor suppressor gene because it displays homozygous deletions in neuroblastoma and in other tumor types. An unbiased screen for genes with tumor suppressive function on 1p36 also revealed *miR-34a* as a candidate tumor suppressor gene.[41] Interestingly, *N-MYC*, which is deregulated in the neuroblastoma, is a direct target of *miR-34a*.[55] The correlation between 1p36 loss and *miR-34a* downregulation in the neuroblastoma was confirmed in this study. Furthermore, the expression of *miR-34a* was low or undetectable in 11 of 15 pancreatic cancer cell lines[31] and the expression level of *miR-34b* was decreased by more than 90% in 6 out of 14 non-small cell lung cancer.[35] However, this loss of expression did not strictly correlate with LOH, *p53* mutation or increased CpG methylation.[35] More recently, the epigenetic inactivation of *miR-34a* was identified in cell lines derived from some of the most common tumors (breast, lung, colon, kidney, bladder, pancreatic cancer and melanoma) and also in primary melanoma.[38] In addition, CpG methylation of *miR-34b/c* was found in colorectal cancer,[45] in oral squamous cell carcinoma[60] and in malignant melanoma in which it correlated with metastatic potential.[46] Furthermore, experimental animal models of liver carcinogenesis showed downregulation of *miR-34a*.[61] Taken together, inactivation of the *miR-34a* and *miR-34b/c* genes presumably is a common event during tumorigenesis.

miR-34 and apoptosis

Before *miR-34a* was identified as a p53 target Welch *et al.*[40] reported that ectopic *miR-34a* induces apoptosis when re-introduced into the neuroblastoma cell lines, which show decreased expression of *miR-34a*. In connection with the identification of *miR-34a* as a p53 target this finding was confirmed and extended. In cancer, *miR-34*-mediated apoptosis may be suppressed by inactivation of p53 and/or *miR-34* genes (Figure 3). Chang *et al.*[31] showed that *miR-34a*-induced apoptosis is at least, in part, dependent on the presence of wild-type *p53* indicating that *miR-34a* may feed back to p53 (see below). Furthermore, locked nucleic acids directed against *miR-34a* protect cells to some extent from the DNA damage-induced apoptosis in wild-type p53-expressing cells.[33] Bommer *et al.*[35] showed that *Bcl-2* is targeted by *miR-34a* and that *miR-34a*-defective MEFs show a decrease in spontaneous apoptosis. Whether *miR-34* induces apoptosis presumably depends on the cellular context and therefore the expression levels of the respective *miR-34* target proteins involved in the regulation of apoptosis. For example, we observed apoptosis after induction of a conditional *miR-34a* allele in H1299 lung cancer cells, whereas the same construct induces a G_1-arrest in U-2OS osteosarcoma cells (Hermeking *et al.*, unpublished results and Ref.[34]). In addition, the level of

Table 1 Survey of current *miR-34* targets

Gene name	Sequence alignment between the target mRNA (upper sequence) and *miR-34* (lower sequence)	*miR-34* species[a]	Expected biological effect after *miR-34*-mediated repression	References
Direct miR-34 targets[b]				
Bcl-2	5′-UCGAAUCAGCUAUUU*ACUGCC*A -3 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Apoptosis	35,41–43
CCND1	5′-UUUACAAUGUCAUAU*ACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	G_1-arrest	42,44
CCNE2	5′-CAAUUCACAAGUUA*CACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a* (*miR-34b*, *miR-34c*)	G_1-arrest	32,35,45
CDK4	5′-GUGAGCAAUGGAGUGG*CUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a* (*miR-34b*, *miR-34c*)	G_1-arrest	32,45
CDK6	5′-UAUAACUACAUAUUG*ACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-GCAGUGUGGAAAUU*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a* *miR-34b*	G_1-arrest	35,38,46,42,44
CREB	5′-UUUUCUAUGCGCAAA*ACUGCCU*G-3′ 3′-GUUAGUCGAUUACUG**UGACGGA**U-5′	*miR-34b*	Inhibition of proliferation	47
DLL1	5′-CCGGCCGCCUGCGG*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-AUUAGAAACACAAA*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Influence on Notch signaling	35,48
E2F3	5′-AAUUAAUUUGUAAA*CACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a* *miR-34c*	Inhibition of proliferation, senescence	40,46,41,42,49
MET	5′-UCCAAUGGUUUUUU*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-AAUUAGAUACUUGU*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a* *miR-34b* *miR-34c*	G_1-arrest, inhibition of invasion and migration	32,45,50–52
c-MYC	5′-UUAGCCAUAAUGUAA*ACUGCCU*C-3′ 3′-GUUAGUCGAUUACUG**UGACGGA**U-5′	*miR-34b* *miR-34c*	G_1-arrest	53,54,46
N-MYC	5′-CAAAACUGGACAGU*CACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-UGUACUAAUUCUUA*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	G_1-arrest	41,55
SIRT1	5′-CCAGCUAGGACCAUU*ACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Increased p53 acetylation and activation (positive feedback loop)	56,42
Genes downregulated after ectopic miR-34 expression[c]				
CAV1	5′-GAACCAACAACCUC*AACUGCCU*A-3′ 3′-GUUAGUCGAUUACUG**UGACGGA**U-5′	*miR-34b*, *miR-34c*	Inhibition of migration	45
CDC25C	5′-AAGUCACCAAAAGA*CACUGC*AG-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	G_2-arrest	32,49

Table 1 *(Continued)*

Gene name	Sequence alignment between the target mRNA (upper sequence) and *miR-34* (lower sequence)	*miR-34* species[a]	Expected biological effect after *miR-34*-mediated repression	References
E2F5	5′-UUGUCUUAUAUUUUU*ACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-UCCAAACAGACGUU*CACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Transcriptional activation	35
HDMX	5′-CCACUGCACUUGGC*CACUGCC*A-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Increased p53 activity	32,57
HMGA2	5′-UUGAAGGGAGAAGA*CACUGC*AG-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Inhibition of proliferation, senescence	43,49
MYB	5′-UAUGACGGUGUACUU*ACUGCCU*U-3′ 3′-GUUAGUCGAUUACUG**UGACGGA**U-5′	*miR-34b, miR-34c*	Inhibition of proliferation	47
Notch1	5′-AUUUUACACAGAAA*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′ 5′-UUUCUGGGGAAAGA*CACUGCC*U-3′ 3′-UGUUGGUCGAUUCU**GUGACGG**U-5′	*miR-34a*	Inhibition of proliferation, apoptosis	43
SFRS2	5′-GUGCAGCCAUUACA*CACAGCCU*A-3′ 3′-GUUAGUCGAUUACUG**UGACGGA**U-5′	*miR-34b, miR-34c*	Influence on miRNA metabolism	45

CAV1, caveolin 1; CCND1, Cyclin D1; CCNE2, Cyclin E2; CREB, cyclic AMP-responsive element-binding protein; MYB, v-myb myeloblastosis viral oncogene homologue; SFRS2, splicing factor arginine/serine-rich 2. The alignments between the 3′-UTR of validated targets with processed *miR-34* are depicted. The *miR-34* species shown to bind or regulate the respective targets are listed in the third column. In general, the alignment with *miR-34a* is provided. If regulation by *miR-34a* has not been validated, the alignment with *miR-34b* is shown. The *miR-34* seed region is highlighted by gray shading and bold letters. The corresponding binding site for *miR-34* in the 3′-UTR of the target mRNA is indicated by underlined, italicized letters. Vertical lines between both sequences indicate perfect Watson–Crick base pairs. For simplicity base pairing outside the seed region, which presumably occurs, is not indicated [a]*MiR-34* family members that have an experimentally validated, inhibitory effect on the indicated target genes. [b]Listed are genes that are presumably inhibited by *miR-34* at the translational level. This was shown by co-expression of *miR-34* and reporter constructs containing the target 3′-UTR with the indicated wild-type *miR-34*-binding site(s) or mutant versions and subsequent luciferase reporter assays. *MiR-34* species in brackets indicate that reporter assays have not been performed at this stage for the respective target mRNA/*miR-34* species pair. [c]Listed are exemplary genes whose expression levels are downregulated on the mRNA and/or protein level as determined by microarray expression, quantitative PCR and/or western blot analyses after expression or transfection of ectopic *miR-34*. A direct regulation of these genes by *miR-34* by the use of reporter assays has not been validated yet. The indicated, exemplary putative *miR-34a*-binding sites were identified by bioinformatic analysis based on the target prediction tools TargetScan (http://www.targetscan.org; release 5.1), PicTar (http://pictar.mdc-berlin.de) and miRanda (http://www.microrna.org)

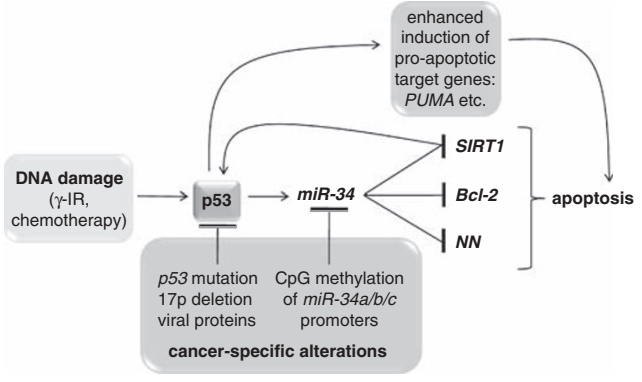

Figure 3 The p53/*miR-34* pathway regulates apoptosis and is altered in cancer. As described in the text, SIRT1 is repressed by *miR-34a*. As a result, p53 deacetylation by SIRT1 is decreased and leads to increased transcription of p53 targets, such as PUMA. Together with the downregulation of Bcl-2 and other anti-apoptotic proteins (NN) *miR-34* activation promotes apoptosis. In cancer, the p53-*miR-34* connection is often targeted by the indicated alterations. As a result, the induction of apoptosis is diminished after the DNA damage induced by chemotherapy

miR-34 expression may affect the decision between apoptosis and cell-cycle arrest.

miR-34a feeds back to p53

miR-34a was shown to target *SIRT1* mRNA leading to translational repression of SIRT1 (Figure 3).[56] SIRT1 is an NAD-dependent deacetylase, which has been shown to inhibit several pro-apoptotic proteins.[62] As mentioned above, apoptosis induced by the re-introduction of *miR-34a* is dependent on *p53* to some extent.[31] This observation was confirmed by Yamakuchi *et al*.[56] and linked to the targeting of *SIRT1* mRNA by *miR-34a*. They could show that p53 acetylation on lysine 382 increases after *miR-34a* expression. This was associated with increased transcriptional activity of p53, which led to the induction of *p21* and *PUMA*. The latter presumably mediated apoptosis in the scenario. Ectopic expression of a *miR-34a*-resistant *SIRT1* cDNA partially rescued *miR-34a*-induced apoptosis indicating that additional anti-apoptotic proteins may be targeted by *miR-34a*. In summary, the regulation of SIRT1 by *miR-34a* is part of a positive feedback loop that

leads to further activation of p53, once it has been activated (Figure 3). As SIRT1 activity is NAD-dependent the metabolic state of the cell may also influence the effectiveness of this regulation. Loss of *miR-34* through genetic or epigenetic mechanisms interrupts this feedback resulting in lower p53 activity and thereby provides a selective advantage for cancer cells. Fujita *et al.*[42] also reported regulation of SIRT1 by *miR-34a*, although they exclusively observed the effects of *miR-34a* on the expression of *SIRT1* mRNA and not on the translation. Another feedback loop between p53 and *miR-34a* may involve the downregulation of the p53-inhibitor HDMX by *miR-34a*.[57]

miR-34 in cancer therapy and detection

Given the tumor suppressive functions ascribed to the *miR-34* family, it will be interesting to determine whether the detection of *miR-34* expression also has diagnostic or prognostic potential in other tumor types. When compared with the generation of mRNA expression profiles the detection of miRNAs may have diagnostic advantages. For example Lu *et al.*[63] could show that the expression analysis of 217 miRNAs is superior to genome-wide analysis of mRNAs for the purpose of classifying tumors.

Zenz *et al.*[64] found that the expression of *miR-34a* is decreased in CLL. This was associated with *p53* mutations, chemotherapy (fludarabine)-refractory disease, impaired DNA damage response and decreased apoptosis.[64] Mraz *et al.*[65] also found that *miR-34a* is consistently downregulated in CLL with *p53* mutations. This implies that the detection of *miR-34a* expression may potentially be used as a predictor of therapy response. Furthermore, the restoration of *miR-34a* activity may be useful to prevent chemotherapy resistance. After the administration of the MDM2 inhibitor, Nutlin-3, to human diploid fibroblasts, induction of *miR-34a* and *miR-34b*/c, as well as senescence was observed.[66] As mentioned above, the cellular context may determine the outcome of *miR-34* induction. In the cancer cells expressing wild-type p53 induction of *miR-34* expression by Nutlin-3 presumably leads to apoptosis. The identification of additional *miR-34* targets regulating apoptosis may help to find further points of intervention.

Outlook

siRNAs have been proposed for clinical applications, and the feasibility of this approach is currently being tested.[67] If specific introduction of siRNAs into tumors is successful, it may be possible to restore *miR-34* function for cancer therapeutic purposes in the future. Furthermore, the possibility to detect epigenetic inactivation of *miR-34a* and/or *miR-34b/c* genes using methylation-specific PCR or loss of *miR-34* expression holds cancer diagnostic and prognostic potential for the future.

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