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Review

MicroRNAs: Predictors and modifiers of chemo- and radiotherapy in different tumour types

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ABSTRACT

MicroRNAs (miRNAs) represent a class of naturally occurring small non-coding RNA molecules. They regulate gene expression at the post-transcriptional level and control thereby cellular mechanisms including developmental transitions, organ morphology, apoptosis and cell proliferation. As might be expected from molecules with these roles, miRNAs are involved in cancer development, and deregulation of several miRNAs has been found in various cancer types. Some miRNAs modulate expression of known oncogenes or tumour suppressor genes whereas others function as so called onco-miRs or tumour-suppressor-miRs. Recently, miRNAs have been studied as potential diagnostic or therapeutic targets in cancer treatment. There is increasing interest in an association between miRNA expression in tumours and chemo- and radiosensitivity, both with regards to predicting or modulating sensitivity. And indeed, different miRNAs have been found to predict sensitivity to anticancer treatment: miR-30c, miR-130a and miR-335 are downregulated in various chemoresistant cell lines, hsa-Let-7g and hsa-miR-181b are strongly associated with response to 5-fluorouracil-based antimetabolite S-1. In addition, several miRNAs were shown to influence sensitivity to chemo- or radiotherapy: miRNAs of the Let-7 family induced radiosensitivity in vitro/in vivo, inhibition of miR-21 and miR-200b increased sensitivity to gemcitabine in cholangiocarcinoma cell lines, and restoration of miR-34 in *p53*-deficient human gastric cancer cells induced chemosensitisation. This article summarises the current literature describing the impact of miRNAs on prediction and modification of anticancer treatment including the possible intracellular pathways involved in these processes.

1. Introduction

1.1. *Epigenetics and microRNAs*

Epigenetic changes are defined as heritable changes in gene expression that occur without alteration of DNA sequences. The two widely recognised epigenetic modifications are DNA cytosine methylation and the post-translational modification of histones. These changes play key roles in biological processes such as gene regulation, development and carcinogenesis.[1–7]

MicroRNAs (miRNAs, miRs) represent a class of naturally occurring, small (19 to 25-nucleotides), non-coding RNA molecules. They can be included among the components of the

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'epigenetic machinery' with a profound effect on the modulation of gene expression.[8] Today more than 1500 miRNA genes in over 50 species have been identified, over 600 of these miRNAs were identified in humans (for further detailed information see miRBase (http://microrna.sanger.ac.uk/)).[9] However, these numbers are rapidly increasing with improvements in miRNA detection and prediction approaches.

MiRNAs are encoded in the genome. Their biogenesis has been investigated by many authors.[6,10–12] The final step of this biogenesis involves incorporation of the mature miRNA into an effector complex, called RNA-induced silencing complex (RISC). In animals, single-stranded miRNA binds through partial or complete sequence homology to the 3′ untranslated region of target mRNAs and causes either block of translation or, less frequently, mRNA degradation.

MiRNAs can regulate gene expression at the post-transcriptional level and thereby control fundamental cellular aspects as developmental transitions, organ morphology, bilateral asymmetry, stress response, metabolism, cell proliferation and apoptosis.[13] Their major impact is the direct interaction with the mRNA via the described RISC. It is postulated that each miRNA regulates up to 100 different mRNAs and that more than 10,000 mRNAs appear to be directly regulated by miRNAs.[14–17] Furthermore, miRNAs seems to affect gene expression in close cooperation with epigenetic changes. There is an increasing understanding of the interaction between miRNAs and DNA cytosine methylation or the post-translational modification of histones: miRNAs are involved in establishing DNA methylation and can probably also affect histone modifications. Furthermore, epigenetic mechanisms have been shown to influence miRNA expression.[6,18,19]

1.2. MicroRNAs and their role in carcinogenesis

Calin and colleagues described in 2004 that probably more than 50% of miRNA genes are located in cancer associated genomic regions or in fragile sites.[20] Subsequently, recent research has revealed strong evidence for the role of miRNAs in the initiation and progression of cancer. Alterations in miRNA levels are associated with dysplasia and cancer, and the expression of miRNAs has been clearly linked to cancer development.[10,11,21–23] MiRNA profiles can be used to classify human cancers. In different tumour tissues, miRNA expression profiling has been shown to provide more accurate classification of tissue and tumour types than global messenger RNA expression profiles.[6,24] In addition to these findings, evidence for regulation of angiogenesis by miRNAs has been obtained for several miRNAs (e.g. miR-221, miR-222, miR-17-92 and miR-27b) including anti-angiogenic as well as pro-angiogenic effects.[25] Distinct miRNA expression profiles are also associated with prognosis/disease progression and with well defined clinico-pathological features of human cancer[24] and are able to predict outcome.[10,26]

1.3. Onco-miRs and suppressor-miRs

Recent studies have focused on the intracellular mechanisms by which miRNAs are involved in the initiation and progression of cancer, especially on possible functions and pathways regulated by miRNAs. Different miRNAs were demonstrated to regulate known oncogenes.[27,28] Recently, Lu and colleagues demonstrated that the translation of the tumour suppressor gene *PDCD4* is negatively regulated by the miRNA miR-21 in different tumour cell lines.[29] Furthermore, several pathways which modulate cell proliferation and apoptosis have been directly linked to miRNA alterations. So, miRNAs can either regulate known oncogenes or tumour suppressor genes at the post-transcriptional level or act themselves as oncogenes or tumour suppressor genes, so called onco-miRs and suppressor-miRs.[10,11,14,23]

1.4. Therapeutic approaches using 'miRNA-techniques'

With increasing knowledge about the function of miRNAs, their possible diagnostic and, more important, therapeutic associations gained in attention. Due to the interaction of miRNAs with different oncogenes and tumour suppressor genes one potential aim could be to modulate these oncogenes and tumour suppressor genes by up- or downregulation of the involved miRNA. Another feasible approach would be to address intracellular pathways which are directly controlled by onco-miRs and suppressor-miRs in the same manner.

Hence, the most promising application of miRNAs might lie in estimation of outcome and modification of response in known and well established anti-tumour therapies such as radiation and chemotherapy. For example, alterations in miRNA expression profiles could provide information about sensitivity or resistance of certain tumour types to different treatments before starting any therapy ('response prediction'); alternatively or in addition, changes in expression during a therapy might offer a tool for control of success of treatment ('response control'). Last but not least, modification of miRNA expression by up- or downregulation may possibly enhance sensitivity to the applied chemo- or radiotherapy ('response modulation'). In fact, Blower and colleagues demonstrated significant correlations between microRNA expression patterns and compound potency patterns, suggesting that miRNAs may play a role in chemoresistance.[30]

This review article summarises the results of current literature regarding the impact of miRNAs on prediction and modification of anticancer treatment including the possible intracellular pathways involved in these processes.

2. Methods

We performed a PubMed search with various combinations of the following keywords: chemoresistance, chemosensitivity, radioresistance, radiosensitivity, chemotherapy, radiotherapy, exosomal, circulating, miRNA, microRNA. The following number of articles (in brackets) were identified to meet the criteria: miRNA and radiotherapy (9), microRNA and radiotherapy (10), miRNA and radioresistance (1), microRNA and radioresistance (1), miRNA and radiosensitivity (3), microRNA and radiosensitivity (3), miRNA and chemotherapy (146), microRNA and chemotherapy (150), miRNA and chemoresistance (6), microRNA and chemoresistance (7), miRNA and chemosensitivity (6), microRNA and chemosensitivity (6),

*exosomal and miRNA (7), *exosomal and microRNA (7), *circulating and miRNA (33), *circulating and microRNA (34). From those articles relevant publications dealing with miRNAs and their impact on chemo- and radiosensitivity were obtained by screening the abstracts or, if necessary, the entire article. Further, relevant articles were extracted by screening the references of these papers. In case of non-availability of the whole article the abstract was taken into consideration despite the limited data provided. This procedure was conducted twice until beginning of March 2009 (*until 10th October 2009) to avoid missing of any relevant contribution.

3. MicroRNAs and anticancer treatment

3.1. *Response prediction*

Advances in detection techniques of miRNAs led to the development of microarrays. By enabling screening of tissue samples for several miRNAs simultaneously this approach revealed convincing evidence that a large number of miRNAs are deregulated in drug resistant or sensitive cancer cell lines. For example, Kovalchuk and colleagues[31] found 137 differentially regulated miRNA genes (63 upregulated and 75 downregulated) when comparing doxorubicine-resistant and -sensitive breast cancer cell lines. Furthermore, they demonstrated Dicer and Argonaute-2, two enzymes involved in the biogenesis and function of miRNAs, to be downregulated in doxorubicine-resistant cells. Another microarray study in 16 different ovarian cancer cell lines demonstrated 27 out of 335 miRNAs to be significantly associated with responsiveness to one or more chemotherapeutic agents (cisplatin, doxorubicine, topotecan, paclitaxel, docetaxel and gemcitabine). 18 miRNAs were positively correlated with increasing resistance to individual drugs (miR-213, miR-181b, miR-181a, Let-7e, miR-520f, miR-21, miR-502, miR-514, miR-371, miR-99b, miR-518c-AS, miR-515-5p, miR-34b, miR-431, miR-126, miR-23b, miR-381, miR-340) whereas 9 miRNAs (miR-518c, miR-132, miR-330, miR-339, miR-142-5p, miR-29c, miR-331, miR-185, miR-106a) showed decreased expression with increasing resistance. Seven miRNAs showed an association with response to more than one chemotherapy (miR-213: doxorubicine, gemcitabine; miR-181a: doxorubicine, gemcitabine; miR-181b: doxorubicine, gemcitabine; miR-99b: docetaxel, paclitaxel; miR-514: docetaxel, paclitaxel; miR-518c-AS: docetaxel, topotecan; miR-520f: doxorubicine, cisplatin). The authors identified via a literature and data base search 52 possible targets for these miRNAs.[32]

Xia and colleagues[33] reported that 10 out of 342 human miRNAs (Let-7a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-23b, miR-106a, miR-106b, miR-196a, miR-320) were downregulated more than 2-fold in a multidrug-resistant gastric cancer cell line, another 2 miRNAs were upregulated more than 2-fold (miR-302b, miR-492). In a tamoxifen-resistant breast cancer cell line 8 miRNAs (miR-221, miR-222, miR-181, miR-375, miR-32, miR-171, miR-213, miR-203) were significantly (1.5-fold) overexpressed and another 7 miRNAs (miR-342, miR-489, miR-21, miR-24, miR-27, miR-23, miR-200) were over 50% downregulated.[34] In complementation to these data Yang and colleagues[35] described similar findings in human patient samples: 34 out of 326 miRNAs were differently expressed between responders and non-responders to platinum-based (platinum, platinum-cyclophosphamide or platinum-paclitaxel) chemotherapy in epithelial ovarian cancer.

Additional and more detailed investigations on specific miRNAs provided verification of their utility as indicators of sensitivity to a chemotherapeutic anticancer treatment. Furthermore, these articles highlighted possible targets of the miRNAs. These studies comprised various anti-tumour drugs including commonly used chemotherapeutic agents (e.g. 5-fluorouracil (5-FU), cisplatin and others), immuno-response modulating interferon, the selective oestrogen receptor modulator tamoxifen and recently developed, modern anti-tumour drugs such as gefitinib which affects anti-apoptotic pathways. The following table (Table 1) provides an overview about the most important findings in current literature.

3.2. *Response control*

Changes in miRNA expression profiles during an anticancer treatment could potentially provide a new tool to estimate the success of a certain therapy. Currently there are only limited data available regarding alterations in miRNA expression profiles during chemo- and radiotherapy.

3.2.1. *Response control during radiotherapy*

Four authors investigated the effect of irradiation on miRNA expression. To understand basic genetic principles arising during irradiation Koturbash and colleagues[36] examined the so called bystander effect in spleen and plasma samples of rats. The bystander effect describes the development of secondary malignancies in non-irradiated tissue during radiotherapy. Using *a rat model* with whole body and only-head irradiation epigenetic changes and miRNA expression in spleen and blood were investigated. The authors could show that a local irradiation of the head resulted in a systemic genetic response: a significant and persistent global DNA hypomethylation combined with hypomethylation and reactivation of long interspersed nucleotide element-1 (*LINE-1*) retrotransposons was found in spleen until 7 months after cranial irradiation. On the search for the mechanism of these findings de novo methyltransferase DNMT3a and methyl-binding protein MeCP2, which are well known as key regulators of DNA methylation and epigenetic alterations, were identified to be downregulated. By investigating miRNA profiles using microarray significantly elevated levels of miR-194 were found in non-irradiated plasma and spleen. These findings were evident from 24 h after irradiation up to 7 months after exposure. Interestingly, *DNMT3a* and *MeCP2* were found to be possible targets of miR-194 indicating that the correlation between upregulation of miR-194 and downregulation of DNMT3a and MeCP2 may provide an important long-term influence of miR-194 on this phenomenon. Interestingly, *in mice* investigation of miRNA changes in spleen and skin tissue after acute or fractionated irradiation revealed similar findings. In the spleen of only-head irradiated mice four miRNAs (miR-350, miR-546, miR-489, miR-194) were differentially expressed 6 h after radiotherapy. miR-194 expression was enhanced as in the previous experiments with rats indicating that upregulation of miR-194 in bystander spleen is a cross-species phenomenon.[37] Further cell culture

Table 1 – Specific miRNAs and their meaning as indicator of sensitivity to an anticancer treatment. Regarding to possible response prediction of radiotherapy there are no data available in any tumour type.

MicroRNA	Possible targets	Up-/ downregulation	Correlation with sensitivity to chemotherapy	Chemotherapeutic agent	Cancer type	Author
Dicer[a] and Drosha[a]	miRNAs in general	↓(↑)	↔	No details given	Ovarian cancer	Merritt et al.[75]
Dicer[a], Argonaute-2[a]	miRNAs in general	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
Let-7	*K-RAS*, *H-RAS*, *HMGA2*, *c-Myc*, *E2F*, *NF2*, *cyclin D2*	↓	↑	Cyclophosphamide	NCI-60 panel of cell lines	Salter et al.[48]
Let-7g	*K-RAS*, *H-RAS*, *HMGA2*, *c-Myc*, *E2F*, *NF2*, *cyclin D3*	↓	↑	5-FU-based antimetabolite S-1	Colorectal cancer	Nakajima et al.[76]
Let-7i	*K-RAS*, *H-RAS*, *HMGA2*, *c-Myc*, *E2F*, *NF2*, *cyclin D4*	↓	↓	Platinum, platinum-cyclophosphamide, or platinum-paclitaxel	Ovarian cancer	Yang et al.[35]
Let-7	*K-RAS*	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-17	*Myc*	↓	↑	Adriamycin and cyclophosphamide	NCI-60 panel of cell lines	Salter et al.[48]
miR-17-5p	*p21*, *BIM*	↑	↓	No details given	Neuroblastoma	Fontana et al.[57]
miR-21	*Bcl-2*, *PTEN*, *TPM1*, *function as oncogene*	↑	↓	Fluorouracil based	Colon adenocarcinoma	Schetter et al.[77]
miR-21	*PTEN*	↑	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-27b	*CYP1B1*	↑	-	CYP1B1 ↓ (no chemotherapy)	Uterine cervix adenocarcinoma, breast adenocarcinoma	Tsuchiya et al.[65]
miR-27b	*CYP1B1*	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-28	*BRCA1*	↑	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-30c	*RASA1*, *ERG*, *SEMA6D*, *SEMA3A*	↓	↓	Paclitaxel, cisplatin	Ovarian cancer	Sorrentino et al.[78]
miR-34	*p53*	↓	↑	5-Fluorouracil and paclitaxe	NCI-60 panel of cell lines	Salter et al.[48]
miR-34a	*E2F3*, *NOTCH1*	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-106a	*RB1*	↑	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-122	miR-122 = target of IFN-α	↓	↓	IFN-α	Chronic hepatitis C	Sarasin-Filipowicz et al.[79]
miR-127	*BCL6*	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-128b	*EGFR*	↓	↑	Gefitinib	Lung cancer	Weiss et al.[80]
miR-130a	*M-CSF*	↓	↓	Paclitaxel, cisplatin	Ovarian cancer	Sorrentino et al.[78]
miR-181b	cytochrome C, *ECIP-1*, *MAPPKKK1*, *TEM6*, *E2F5*, *GATA6*, *PP2B*, *eIF5A*	↓	↑	5-Fluorouracil-based antimetabolite S-1	Colorectal cancer	Nakajima et al.[76]
miR-200b	*PTPN12*	↓	↑	Adriamycin	NCI-60 panel of cell lines	Salter et al.[48]
miR-200c	*TCF8*	↓	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-206	*ER alpha*	↑	↓	Doxorubicine	Breast cancer	Kovalchuk et al.[31]
miR-214	*PTEN*, *Akt pathway*	↓	↑	Cisplatin	Ovarian cancer	Yang et al.[61]
miR-335	*CALU*, *MAX*, *MAP2*, *PGF*	↓	↓	Paclitaxel, cisplatin	Ovarian cancer	Sorrentino et al.[78]

[a] miRNA processing/function enzymes.

experiments with cancer cell lines in therapeutic radiotherapy settings revealed that irradiation causes a wide range of alterations in miRNA expression during therapy. In lung cancer and normal lung cell lines levels of 81 out of 440 miRNAs were shown to differ significantly after irradiation with 23 miRNAs being downregulated after treatment. Most of these changes were normalised after 24 h. Interestingly, all members of the Let-7 family ($n = 7$) except Let-7g decreased significantly by 2–8 h after irradiation in both cancerous and normal lung epithelium. Let-7g was elevated after radiotherapy. These findings were also observed in another two lung cancer cell lines. Furthermore, *Let-60/RAS* and genes in the DNA damage response pathway were identified as possible mechanisms affected by Let-7. The authors conclude that a global miRNA response might exist in lung cells after irradiation and that miRNAs might be components of the cellular response to cytotoxic insult.[38] In addition to these results, radiotherapy of two different prostate cancer cell lines resulted in differential expression of a high number of miRNAs. Josson and colleagues used a microarray with 330 miRNAs of which only 55% were detected in the two tested prostate cancer cell lines. Expression of 48 miRNAs altered significantly after application of radiotherapy, 22 of them presented even a more than threefold change in levels (investigated in a clonogenic assay). Of those 22 miRNAs hsa-miR-521, hsa-miR-196a and hsa-miR-133b were shown to be decreased and hsa-miR-34c was shown to be increased in both cell lines after radiation. For further analysis the authors focused on miR-521. Levels of two possible targets, Cockayne syndrome protein A (CSA, a DNA repair protein) and Manganese Superoxide Dismutase (MnSOD, an anti-apoptotic and antioxidant protein), could be shown to correlate inversely with the levels of miR-521.[39] (see Supplementary material A)

3.2.2. *Response control during chemotherapy*

Similar findings regarding the extent of changes in the global miRNA expression were reported after anticancer treatment with various chemotherapeutic drugs in different cancer cell lines and patient samples. In an assay comparing genetic alterations after cisplatin treatment between testicular germ cell tumour (TGCT) cells and somatic tumour cells (colon carcinoma) Dicer 1 was identified to be downregulated in testicular germ cell tumour and upregulated in colon cancer. While screening the literature, authors hypothesised that cisplatin-induced downregulation of Dicer 1 might impact oncogenic miRNAs as hsa-miR-372/373 (which are overexpressed in TGCT). These two miRNAs have been reported to regulate pathways controlled by classic tumour suppressors and oncogenes including p53, MYC and RAS.[40] An assay of 153 miRNAs was conducted in two colorectal cancer cell lines treated with the antimetabolite 5-fluorouracil (5-FU).[41] A high number of miRNAs were affected by the drug in both cell lines but 22 miRNAs were found to be differentially expressed more than twofold after chemotherapy in C22.20 cells (17 miRNAs (miR-19a, miR-20, miR-21, miR-23a, miR-25, miR-27a, miR-27b, miR-29a, miR-30e, miR-124b, miR-132, miR-133a, miR-141, miR-147, miR-151, miR-182, miR-185) were upregulated whereas 3 miRNAs (miR-200b, miR-210 and miR-224) were downregulated). In HC.21 cells the same 22 miRNAs presented significant alterations as described above but miR-20, miR-21, miR-151, miR-182 and mi-210 did not reach a twofold change in expression after therapy. By screening the literature authors identified the majority of these 22 miRNAs to be deregulated in various cancers. Furthermore, potential targets for some of these miRNAs were extracted from literature: polycistronic miR-17-92 cluster and miR-21 were linked to anti-apoptotic and oncogenic activity through *Bcl-2*, *c-Myc* and *E2F-3*.[11,41–45] Another two miRNAs, miR-124b and miR-135b, were identified as components of the mismatch repair system (i.e. *MLH1* and *MSH2*), and miR-141 has been found to target *MSH2* and *APC*.[46] MiR-200b has been identified to inhibit the tumour suppressor gene *PTPN12* and its suppression of such oncogenes as *Src* or *Ras*.[47,48] In another article, Sun and colleagues[49] investigated the impact of pure (as it can be administered orally) or liposomal (as it can be administered via i.v. infusion) administered curcumin on pancreatic cancer cells. They found that treatment with curcumin altered expression of 29 miRNAs significantly: 11 miRNAs were upregulated (miR-103, miR-181a, miR-181b, miR-181d, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-27a, miR-34a) whereas 18 miRNAs were downregulated (miR-140, miR-146b, miR-148a, miR-15b, miR-195, miR-196a, miR-199a*, miR-19a, miR-204, miR-20a, miR-25, miR-26a, miR-374, miR-510, miR-7, miR-92, miR-93, miR-98) after 72 h of incubation with curcumin. Treatment with liposomal curcumin significantly upregulated the expression of only 5 miRNAs (miR-193b, miR-34a, miR-22, miR-92, miR-21) and downregulated 10 miRNAs (miR-199b, miR-199a*, miR-25, miR-15b, miR-15a, miR-31, miR-16, miR-24, Let-7i, miR-20b). The authors pointed out that especially miR-22 (upregulated by 65.5% after curcumin and by 68% after liposomal curcumin treatment) and miR-199a* (downregulated by 54.2% after curcumin and by 71% after liposomal curcumin treatment) presented consistent and substantial alterations in expression. ESR1 (which plays a potentially important role in the oestrogen nuclear pathway) and SP1 (may have critical roles in growth and metastases) proteins were shown to be inversely correlated to expression of miR-22. Zhao and colleagues[50] found expression levels of miR-17–92 cluster's primary transcript to be decreased in human colon cancer cells treated with 5-FU. Furthermore, they demonstrated that the expression of *c-Myc* mRNA was decreased and the expression of *TSP-1* mRNA was increased by 5-FU treatment. Additional experiments revealed that c-Myc does not act as a transcription factor for the expression of *TSP-1*. Due to the fact that the miR-17-92 cluster was recently reported to be upregulated by *c-Myc* and the cluster was shown to downregulate *TSP-1* expression, authors concluded that *TSP-1* expression might be controlled via *c-Myc* mediated miR-17-92 expression. In addition, alpha-mangostin which is a chemotherapeutic agent with cytotoxic effect mainly used for the treatment of skin infections and wounds was demonstrated to enhance expression of miR-143 and decrease expression of Erk5. *Erk 5* is a member of the *MAP* kinase family which are involved in proliferation. As reviewed, miR-143 inhibits the translation of *ERK5* mRNA.[51] The only publication looking at miRNA expression in patient samples under therapy was performed using a TaqMan miRNA assay analysing expression of miR-10a, miR-21, miR-31, miR-125b, miR-137, miR-145, miR-212, miR-339 and miR-361 in patients with rectal cancer undergoing chemoradiotherapy

with capecitabine. While a number of these miRNAs showed distinct variation 2 weeks after starting therapy, most miRNAs showed profound intertumoural variability. Only miR-125b and miR-137 demonstrated a significant induction and similar expression trends for most patients two weeks after starting therapy. Furthermore, increased levels of both miRNAs correlated with minor response to therapy and with higher postoperative tumour stage. So authors concluded higher induced levels of miR-125b and miR-137 to be associated with worse response to therapy.[52] (see Supplementary material B)

3.3. *Response modification*

On the basis of the aforementioned results regarding alterations in miRNA expression profiles before or during an anticancer treatment several articles were published addressing a possible modification of these therapies in different cancer types by enhancing or suppressing miRNA expression. In most cases, alterations of miRNA expression were achieved by (transient or stable) transfection of cell lines with either pro-miRs (e.g. synthetic miRNAs, miRNA-mimics or precursors) or anti-miRs (e.g. antisense oligonucleotide). In some cases miRNA-expressing clones or knockdown clones were created. The effects of miRNA modulations on efficiency of various therapies including irradiation, established chemotherapeutic agents (e.g. doxorubicine, 5-fluorouracil, cisplatin, corticoids amongst many) as well as tyrosinkinase-inhibitor imatinib, immune-response modulating interferon gamma and selective oestrogen receptor modulator tamoxifen were evaluated. Partly therapy-resistant or -sensitive cell lines were used for these investigations. Positive and inverse correlations between deregulation of miRNAs and chemo-/radiosensitivity of various tumour types were reported. The following three tables (Tables 2–4) present an overview about the most important findings regarding the effect of up- or downregulation of a specific miRNA on sensitivity to treatment.

3.4. *Effect of up-/downregulation of miRNAs on specific targets*

As pointed out above up- or downregulation of several miRNAs can lead to modified sensitivity to anticancer treatments. The following section of our review highlights the findings about possible targets of and intracellular pathways affected by these miRNAs. Common information about genes and their function were obtained from the Gene Home (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene).

3.4.1. *miRNA-mediated regulation of oncogenes and tumour suppressor genes*

High mobility group AT-hook 2 protein (HMGA2), which is encoded by the *HMGA2* gene, belongs to the *HMGA* family. These

Table 2 – Effect of up-/downregulation of different miRNAs on sensitivity to chemotherapy. The table presents the findings indicating *a positive correlation* between expression of miRNAs and effect on sensitivity to chemotherapy.

MicroRNA	Ectopic up-/downregulation	Chemotherapeutic agent	Effect on sensitivity to chemotherapy	Cancer type	Author
miR-1	↑	Doxorubicine	↑	Lung cancer	Nasser et al.[55]
miR-15b	↑	Vincristine, adriamycin, cisplatin, etoposide	↑	Multidrug-resistant gastric cancer	Xia et al.[33]
miR-15b∼16	↑	–	↑ (increased Glucocorticoid-receptor sensitivity)	–	Rainer et al.[71]
miR-16	↑	Vincristine, adriamycin, cisplatin, etoposide	↑	Multidrug-resistant gastric cancer	Xia et al.[33]
miR-16	↓	NSC 236613, NSC 670550	↓	Lung cancer	Blower et al.[54]
miR-21	↓	NSC 63878, NSC 265450	↓	Lung cancer	Blower et al.[54]
miR-21	↓	NSC 265450	↓	Ovarian cancer	Blower et al.[54]
miR-21	↓	NSC 123127	↓	Glioblastoma	Blower et al.[54]
miR-34a	↑	Camptothecin	↑	Prostate cancer	Fujita et al.[58]
miR-34 (a, b, c)	↑	Doxorubicine, cisplatin, gemcitabine, docetaxel	↑	p53-mutant human gastric cancer with high Bcl-2 levels	Ji et al.[60]
miR-101	↑	Etoposide, curcumin, doxorubicine	↑	hepatocellular carcinoma	Su et al.[64]
miR-451	↑	Doxorubicine	↑	(Doxirubicin resistant) breast cancer	Kovalchuk et al.[31]
miR-451	↑	Imitanib	↑	Glioblastoma	Gal et al.[81,82]
Let-7i	↓	Cisplatin	↓	Ovarian cancer	Yang et al.[35]

Table 3 – Effect of up-/downregulation of different miRNAs on sensitivity to chemotherapy. The table presents the findings indicating *a negative correlation* between expression of miRNAs and effect on sensitivity to chemotherapy.

MicroRNA	Ectopic up-/downregulation	Chemotherapeutic agent	Effect on sensitivity to chemotherapy	Cancer type	Author
Dicer[a]	↓	Cisplatin	↑	Breast cancer	Bu et al.[56]
miR-17-5p	↓	Doxycycline	↑	Neuroblastoma	Fontana et al.[57]
miR-18	↑	–	↓ (Repressed glucocorticoid-receptor)	–	Vreugdenhil et al.[72]
miR-124a	↑	–	↓ (Repressed glucocorticoid-receptor)	–	Vreugdenhil et al.[72]
miR-21	↓	Topotecan	↑	Breast cancer	Si et al.[45]
miR-21	↓	Gemcitabine	↑	Malignant cholangiocytes	Meng et al.[44]
miR-21	↓	NSC 621888, NSC 622700, NSC 670550, NSC 122750	↑	Lung cancer	Blower et al.[54]
miR-21	↓	NSC 670550, NSC 122750	↑	Glioblastoma	Blower et al.[54]
miR-27b	↓	–	↑ (CYP1B1)	Breast cancer	Tsuchiya et al.[65]
miR-98	↑	Cisplatin, doxorubicine	↓	Head and neck squamous cell carcinoma	Hebert et al.[53]
miR-200b	↓	Gemcitabine	↑	Malignant cholangiocytes	Meng et al.[44]
miR-206	↑	–	↓ (repressed oestrogen receptor α)	Breast cancer	Kondo et al.[74]
miR-214	↓	Cisplatin	↑	Ovarian cancer	Yang et al.[61]
miR-221 and/or miR-222	↑		↓ (repressed oestrogen receptor α)	Breast cancer	Zhao et al.[73]
miR-221 and/or miR-222	↓	Tamoxifen	↑	Breast cancer	Zhao et al.[73]
miR-222/miR-222	↑	Tamoxifen	↓	Breast cancer	Miller et al.[34]
Let-7i	↓	NSC 670550	↑	Lung cancer	Blower et al.[54]
Let-7a	↓	Gemcitabine, 5-fluorouracil, camptothecin	↑	IL-6-overexpressing malignant cholangiocytes	Meng et al.[47]
Let-7a	↑	Interferon-gamma, doxorubicine, paclitaxel	↓	Squamous cell carcinoma, hepatocellular carcinoma	Tsang and Kwok[63]
BART cluster 1 miRNAs	↑	Cisplatin	↓	LMP1-expressing EBV-positive epithelial cell line	Lo et al.[62]

[a] miRNA processing/function enzyme.

Table 4 – Effect of up-/downregulation of different miRNAs on sensitivity to radiotherapy.

MicroRNA	Ectopic up-/downregulation	Effect on sensitivity to radiotherapy	Cancer type	Author
miR-521	↑	↑	Prostate cancer	Josson et al.[39]
Let-7g	↑	↓	Lung cancer	Weidhaas et al.[38]
Let-7a	↑	↑	Lung cancer, C. elegans	Weidhaas et al.[38]
Let-7b	↑	↑	Lung cancer, C. elegans	Weidhaas et al.[38]

proteins are thought to play a role in the regulation of transcription, differentiation and neoplastic transformation and are therefore suggested to be oncogenes. Expression of *HMGA2* is commonly associated with both malignant and benign tumour formation in adult tissues. Transfection with pre-miR-98 led to a decrease of protein and mRNA levels of

HMGA2 in different hypoxic and normoxic squamous cell carcinoma cell lines. Under normoxic conditions transfection with pre-miR-98 enhanced resistance to cisplatin and doxorubicine, under hypoxia ectopic miR-98 expression was found to only slightly augment chemoresistance to doxorubicine. In order to possibly correlate miR-98 and *HMGA2* effects authors investigated downregulation of HMGA2. And in fact, it could be shown that as well hypoxia as diminished HMGA2 expression caused resistance to doxorubicine. Interestingly, knockdown of miR-98 especially in hypoxia resulted in only minor changes of HMGA2 levels implicating that other miRNAs (for examples members of the Let-7family) might be involved in *HMGA2* regulation.[53] And indeed, another publication pointed out the regulation of *HMGA2* by one member of the Let-7 family. Yang and colleagues demonstrated an overexpression of Let-7i to remarkably downregulate *HMGA2* in epithelial ovarian cancer cell lines.[35]

The *Ras* superfamily of small GTPases includes the *Ras*, *Rho*, *Arf*, *Rab*, and *Ran* families. Ras members are signal transduction proteins, which communicate signals from outside the cell to the nucleus. Three human *RAS* genes have been identified (*H-Ras*, *N-Ras* and *K-Ras*). They function as proto-oncogenes, by regulating proliferation, differentiation and apoptosis, and are involved in the development of a number of cancers. In lung cancer cells for example, transfection with a miR-16 precursor induced a reduction of RAS proteins and especially of K-RAS-2A protein. RT-PCR revealed that at the mRNA level only *K-RAS-2A* mRNA was decreased, but mRNA levels of *N-RAS*, *H-RAS* and *R-RAS* remained unchanged. These authors pointed out that this was the first article identifying *RAS* as putative target of miR-16. Interestingly, in the same cell line modification of Let-7i did not affect protein levels of RAS which is widely accepted as target of Let-7i.[54] In contrast to these findings regarding the Let-7 Family, Yang and colleagues' study supported the well known relationship between Let-7 expression and its target *H-Ras*. In epithelial ovarian cancer cell lines they observed an overexpression of Let-7i to remarkably downregulate H-RAS.[35] In addition, examinations on a C. elegans-based *in vivo* model of radiation-induced reproductive cell death revealed further evidence for Let-7 mediated *RAS* modification. Different members of the Let-7 family were shown to impact radiosensitivity. The authors formulated the hypothesis that radioresistance in mutant animals harbouring a deletion of a *Let-7* family gene was induced by overexpression of some Let-7 targets, including the *Let-60/RAS* oncogene and genes of the DNA damage repair pathway (*rad-51/RAD51*, *coh-1/RAD21*, *fcd-2/FANCD2*, and *cdc-25.3/CDC25*). They confirmed this using RNAi in the mutant model against the targets of interest and showed that all of these genes significantly suppressed radioresistance.[38]

Another study revealed a broad variety of oncogenes and tumour suppressor genes to be regulated by a single miRNA in lung cancer cells: miR-1. A decrease in miR-1 expression led to an increase in MET, Pim-1, FoxP1 and HDAC4. MET (mesenchymal-epithelial transition factor) is a receptor-type tyrosine kinase which is overexpressed in various human cancers. It is well known to be a proto-oncogene which regulates invasive growth including tumour growth, formation of metastasis and angiogenesis by activating several signal transduction pathways including RAS, PI3K, beta catenin and Notch pathway. *Pim-1* is a proto-oncogene which encodes a protein kinase upregulated in prostate cancer. HDAC4 (Histone deacetylase 4) alters chromosome structure and affects transcription factor access to DNA. FoxP1 which is a member of the large *FOX* family is an essential transcription factor required for mammalian development. Higher nuclear *FoxP1* expression is associated with poor prognosis in patients with diffuse large B-cell lymphoma. This gene may act as a tumour suppressor as it is lost in several tumour types.[55]

NF2 (Neurofibromin 2) belongs to the tumour suppressor group of genes. It is thought to link cytoskeletal components with proteins in the cell membrane. There is evidence that NF2 proteins interact with cell-surface proteins and are involved in regulating ion transport. Mutations in this gene are associated with neurofibromatosis type II. Several miRNAs including a number of members of the Let-7 family and miR-21 were shown to be increased in IL-6 overexpressing cholangiocarcinoma cell lines as well as in a xenograft model. In this context inhibition of Let-7a was demonstrated to enhance sensitivity to various chemotherapeutic agents. Accompanying with these findings an increase in caspase-3/7 activity, PARP cleavage and activated caspase-3 was detected indicating the influence of Let-7a on IL-6-mediated anti-apoptotic survival pathways. Searching for mechanism of the Let-7a mediated effect authors found inhibition of Let-7a to directly increase *NF2* expression and consequently decrease basal Stat-3 kinase activity which targets several members of *Bcl-2* family.[47]

p53 which is encoded by the *TP53* gene is well known to regulate the cell cycle and thus function as a tumour suppressor. It plays a central role in the cell's anticancer mechanisms by activating DNA repair proteins, holding the cell cycle at the G_1/S regulation point and initiating apoptosis. Several authors addressed the p53 network to be affected by ectopic changes in miRNA profile. For example, knockdown of *Dicer* in breast cancer cells induced upregulation of cell cycle-dependent kinase inhibitors p21 and p27 and finally resulted in enhanced sensitivity to cisplatin.[56] Likewise, $p27^{Kip1}$ was found to be downregulated in miR-221/222-overexpressing breast cancer cells which led to increased tamoxifen resistance.[34] Both, p21 and p27, were identified as important regulators of cell cycle progression with p53 being the major regulator of these. In addition to these findings *p21* was found to be regulated by miR-17-5p in *p53* knockout and in endogenously *p53*-expressing human neuroblastoma cell lines with different levels of *MYCN* expression. Downregulation of miR-17-5p caused an increase of p21 at both protein and mRNA levels with consecutive enhanced sensitivity to doxycycline therapy without affecting p53 levels. The authors postulated a p53 independent regulation mechanism.[57] Another affection of the p53 network was presented by Fujita and colleagues.[58] They examined *p53*-deficient human prostate cancer cells with low expression of miR-34a which are highly resistant to anticancer drugs. Introduction of *p53* into cells led to increased miR-34a expression. In separate experiments ectopic miR-34a expression was shown to downregulate *SIRT1* (Silent mating type information regulation 2 homolog 1, a histone/protein deacetylase which is involved in apoptosis) at the transcriptional level but not post-transcriptional level,.[59]

Therefore the authors concluded SIRT might be controlled by p53 via miR-34a, suggesting a role for miR-34a in this p53 pathway. These data were supported by findings of Ji and colleagues.[60] In *p53*-mutant human gastric cancer cells which express high levels of *Bcl-2* and very low levels of miR-34, restoration of miR-34 (a, b, c) expression resulted in a downregulation of *Bcl-2*, *Notch1* and *HMGA2* – in slightly differing extents. Furthermore, a significant increase in caspase-3 activity was observed. Mir-34 was thereby demonstrated to be involved in the network and tumour suppressing pathways of p53 as a downstream target of p53.

PTEN (phosphatase and tensin homolog) gene is another well known tumour suppressor gene which acts through the action of its phosphatase protein product and regulates thereby negatively *Akt/PKB* signalling pathway. This mechanism is essential for the regulation of the cell cycle. In various cancer types mutations of *PTEN* had been identified. A number of authors identified different miRNAs to interfere with this *PTEN* pathway. For example, ectopic expression of miR-214 in ovarian cancer cell lines was demonstrated to decrease PTEN protein but not mRNA levels and to elevate phosphorylation levels of Akt (a major target of PTEN as stated above) and Akt substrates glycogen synthase kinase 3β and p70S6K. These findings were also observed in patient cancer samples: among 17 tumours with elevated miR-214, 11 (65%) had decreased PTEN - levels.[61] Besides miR-214 two authors addressed another miRNA to be targeting *PTEN:* miR-21. Whereas one study provided evidence that miR-21 regulated apoptosis and increased sensitivity to gemcitabine in malignant human cholangiocytes by PTEN-dependent activation of PI 3-kinase signalling,[44] the second article described contrary findings in a lung cancer cell line. Western Blot assessment of target protein and mRNA microarrays did not display any alteration in protein or mRNA levels of *PTEN* after transfection with miR-21 precursor, inhibitor or control. The authors concluded that miR-21 may regulate other target mRNAs in this cell line and that miR-21 has multiple downstream effects potentially contributing to its impact on anticancer drug sensitivity.[54]

3.4.2. *miRNA-mediated direct regulation of apoptosis*

Apoptosis as the process of programmed cell death plays a key role in cancer formation and progression. Disturbances in apoptotic pathways lead to uncontrolled cell proliferation which means a crucial step in neoplastic development. Several pathways are involved in the complex mechanism of apoptosis.

EBV latent membrane protein 1 (LMP-1) is a viral protein associated with Epstein-Barr virus (EBV). In nasopharyngeal carcinoma EBV expresses LMP1, which activates the NFκB pathway and thus modulates apoptosis. BART cluster 1 miRNAs were shown to suppress LMP1 protein but not mRNA expression in cervical cancer cells. Furthermore, the authors demonstrated BART cluster 1 miRNAs not only to modulate LMP1 expression but also to affect the LMP1-induced NFκB signalling in EBV-infected epithelial cells. Looking at the effect of BART cluster 1 miRNAs on sensitivity to chemotherapy, a co-expression of the cluster 1 vector with LMP1 in EBV-infected epithelial cell lines protected the cells from cisplatin-induced toxicity and attenuated thereby LMP1-induced cytotoxicity to cisplatin.[62]

Caspase-3 is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in apoptosis. Tsang and Kwog demonstrated this

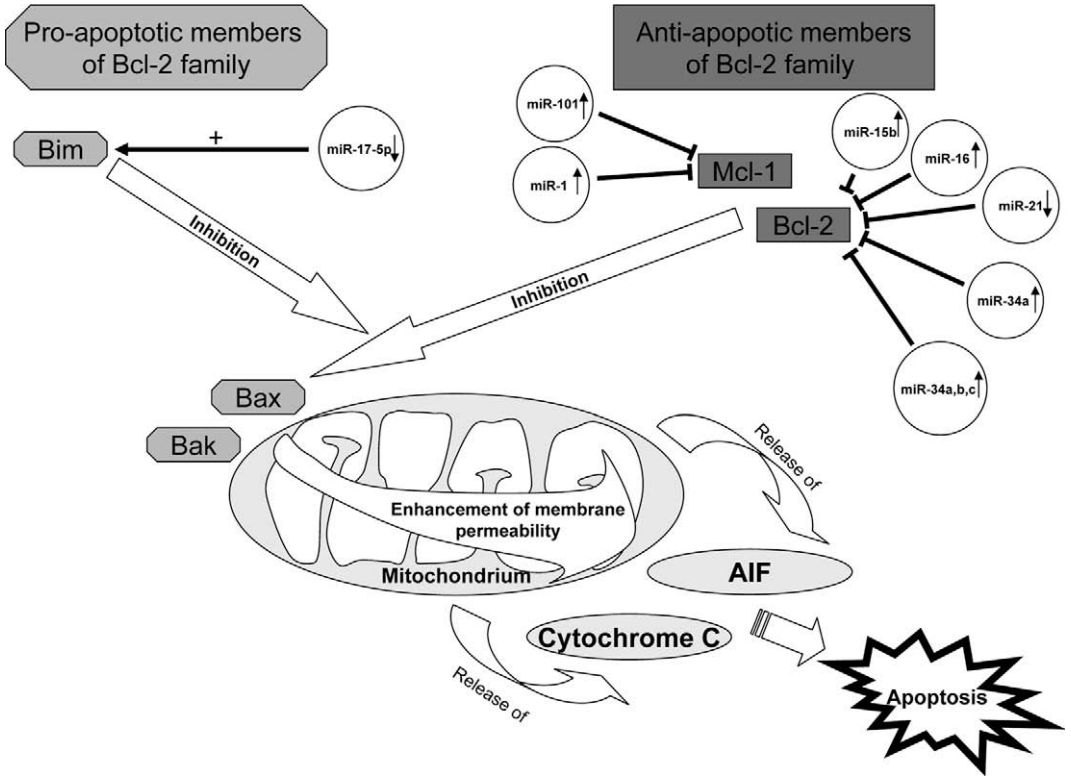

Fig. 1 – Regulation of Bcl-2 pathway by different miRNAs.

caspase to be a direct target of Let-7a. In hepatocellular carcinoma and squamous carcinoma cells caspase-3 protein was downregulated after transfection with Let-7a precursor. The authors suggested this mechanism to be involved in Let-7a modified sensitivity to anticancer treatment.[63]

The *Bcl-2* gene family includes a broad variety of members with pro-apoptotic (including *Bax*, *Bad*, *Bak*, *Bim*, *PUMA*) and anti-apoptotic (including *Bcl-2*, *Bcl-xL*, *Mcl-1*) effects. This family especially seems to be highly involved in miRNA-mediated regulation of apoptosis (Fig. 1). For example, downregulation of miR-21 suppressed both cell growth in vitro in breast cancer cells and tumour growth in a xenograft mouse. In cell culture, apoptosis was increased and anti-apoptotic protein Bcl-2 was downregulated. Furthermore, caspase inhibitor Z-VAD-fmk was able to reverse the growth inhibition caused by anti-miR-21. Interestingly, p53 and PUMA (another pro-apoptotic member of the *Bcl-2* family which is regulated by *p53*) were not found to be differentially expressed compared to control.[45] Further, increased levels of miR-34a, which had been shown to be involved in the p53 network pathway, were demonstrated to decrease levels of Bcl-2 -in prostate cancer cells. Furthermore enhanced miR-34a expression impacted upon expression of additional targets, CDK6, Cyclin D1, E2F3, E2F1-, all involved in cell cycle and apoptosis. Except *E2F1* all of those were identified to be direct targets of miR-34.[58] Similar findings were described in *p53*-mutant human gastric cancer cells expressing high levels of *Bcl-2* -and very low levels of miR-34. After restoration of miR-34 (a, b, c) expression, a downregulation of *Bcl-2*, *Notch1* and *HMGA2* -and an increase in caspase-3 activity was marked.[60] In multidrug-resistant gastric cancer cells overexpression of miR-15b or miR-16 caused a significant increase of apoptosis and apoptosis related enzymes caspase-3 and -7 (key executioners of apoptosis) after vincristine therapy. Furthermore, a decrease in Bcl-2 protein level but not mRNA level was detectable in precursor transfected cells indicating a direct inhibition of *Bcl-2* by miR-15b and miR-16.[33] Knockdown of miR-17-5p in human neuroblastoma cell lines overexpressing MYCN by treatment with antagomiR increased the expression of another pro-apoptotic member of the *Bcl-2* family, *Bim*, at both mRNA and protein level. As stated above, downregulation of miR-17-5p caused an increase of p21 at both protein and mRNA levels with consecutive enhanced sensitivity to doxycycline therapy without affecting p53 levels. Authors postulated therefore miR-17-5p to modulate chemosensitivity and apoptosis via direct downregulation of *p21* and *Bim*.[57] In hepatocellular carcinoma cells miR-101 could be proven to inversely regulate Mcl-1 protein but not mRNA levels. Mcl-1 is an anti-apoptotic member of Bcl-2 family. Besides lower levels of Mcl-1, upregulation of miR-101 caused higher apoptotic rates and an obvious increase in caspase-3/7 activity. This might indicate the possible role of Mcl-1 in miR-101-regulated apoptosis.[64] Nasser and colleagues investigated the effect of ectopic miR-1 expression on doxorubicine treatment in non-expressing lung cancer cells. In miR-1 expressing clones chemotherapy induced a higher apoptosis rate. The authors identified enhanced activation of caspases 3 and 7 and decreasing levels of anti-apoptotic Mcl-1 as probable modulators of sensitivity to doxorubicine. Interestingly, treatment with doxorubicine did not affect levels of p53 and its target gene PUMA or Bcl-2.[55]

3.4.3. *miRNA-mediated regulation of apoptosis-independent mechanisms*

Kovalchuk and colleagues investigated the effect of increased miRNA expression on the *MDR1* gene. This gene is a member of the *MDR/TAP* subfamily which is involved in multidrug resistance. A very important mechanism in drug resistance is an increased energy-dependent efflux of drugs out of cancer cells. This efflux is mediated by the ATP-binding cassette transporter P-gp, which is encoded by the *MDR1* gene. Kovalchuk and colleagues could show that restoration of miR-451 in non-expressing, doxorubicine-resistant breast cancer cells led to downregulation of *MDR1* gene. In accordance with these findings the authors demonstrated that restoration of miR-451 indeed increased sensitivity to doxorubicine treatment.[31]

Treatment with miR-521 inhibitor in prostate cancer cells was shown to enhance levels of CSA and MnSOD. CSA (also known as ERCC8: excision repair cross-complementing rodent repair deficiency, complementation group 8) is a DNA repair protein. Mutations in this gene have been identified in patients with hereditary disease Cockayne syndrome (CS). CS cells are abnormally sensitive to ultraviolet radiation and are defective in the repair of transcriptionally active genes. MnSOD (Superoxide dismutase 2) is a member of the iron/manganese superoxide dismutase family. This protein transforms toxic superoxide into hydrogen peroxide and diatomic oxygen. Mutations in this gene have been associated with cancer. By demonstrating that ectopic expression of miR-521 induced enhanced sensitivity to radiation, authors suggested both mechanisms to be involved in this process.[39]

Tsuchiya and colleagues[65] investigated the role of miR-27b in regulation of cytochrome P450 1B1 (CYP1B1). Cytochrome P450 enzymes are drug-metabolizing enzymes with a wide range of affected substances. CYP1B1 is involved in the activation of various pro-carcinogens and pro-mutagens[66] and in the metabolism of estradiol with consecutive influence on tumour growth.[67,68] The authors found an inverse correlation between expression level of miR-27b and CYP1B1 protein in human breast cancer and showed that inhibition of miR-27b upregulated the protein level and enzymatic activity of CYP1B1. Besides CYP1B1 a number of other cytochrome P450 enzymes (e.g. CYP1A2, CYP2B6, CYP2S1 and CYP3A4) might be regulated by miRNAs indicating the role of miRNAs in drug metabolism controlled by cytochrome P450.[69]

3.4.4. *miRNA-mediated regulation of receptor-dependent mechanisms*

Corticoids are used as chemotherapeutics in the treatment of haematologic malignancies for induction of apoptosis. Furthermore they are often administered in context with chemotherapeutic agents in solid tumours to reduce side effects of chemotherapy and to protect healthy tissue.[70,71] Corticoids mediate their functions via the glucocorticoid receptor (GR) or the glucocorticoid-induced leuzine zipper (GILZ). GILZ appears to play a key role in the anti-inflammatory and immunosuppressive effects and the GR is expressed in several organs and regulates genes controlling development, metabolism and immune response. Upregulation of miR-18 and miR-124a during brain development was shown to significantly repress glucocorticoid receptor (GR) protein activity

and activation of GILZ gene expression.[72] In contrast to these findings, another study presented data showing a positive correlation between miR-15b~16 expression and sensitivity of GR.[71] These results indicate the important role of miRNAs in regulation of corticoid effects and corticoid related alterations in efficiency of anticancer treatment.

Tamoxifen is a selective oestrogen receptor modulator that is widely used in the treatment of breast cancer. Indication for the application of tamoxifen is the oestrogen receptor positive (early and advanced) cancer. Oestrogen receptor α (ERα) acts as a ligand-activated oncogene product in breast tissues. Mir-221 and miR-222 were shown to be overexpressed in tamoxifen-resistant breast cancer cell lines (see above). Two groups addressed the impact of a modification of these miRNAs on sensitivity of cells to tamoxifen. Up- or downregulation of either one or both of the miRNAs could be demonstrated to inversely influence sensitivity to tamoxifen. Knockdown of both miR-221 and miR-222 made tamoxifen-resistant cells more vulnerable to therapy than knockdown of one miRNA alone. Besides the mechanism of downregulation of $p27^{Kip1}$ (see above), Zhao and Lin and colleagues found a suppression of ERα protein but not mRNA by miR-221 and miR-222 to possibly cause tamoxifen resistance. They could show that knockdown of miR-221 and miR-222 partly restored ERα and tamoxifen sensitivity.[34,73] Another miRNA was further demonstrated to affect ERα: miR-206. Transfection of breast cancer cells with a miR-206 precursor led to inhibition of cell growth and repression of ERα mRNA. Furthermore, introduction of pre-miR-206 precursor caused an inhibition of oestrogen-induced growth of MCF-7 cells implicating its role in endocrine therapy via ERα mediated effects.[74]

3.5. *Circulating exosomal miRNAs: emerging disease biomarkers and predictors of outcome*

As clinical analysis of miRNA expression is mainly based on biopsies and tumour samples, the question rose whether miRNAs can be detected for example in blood samples, and if yes, whether these findings could contribute to the identification of diagnostic and prognostic tumour markers. And in deed, in 2007 Valadi and colleagues were the first to report that endocytic exosomes, which were assessed in human and murine mast cell lines for their meaning for cell-cell communication, contain approximately 121 different miRNAs.[83] Shortly thereafter, these findings where confirmed in human samples as microvesicles derived from the plasma of human healthy individuals were shown to carry miRNAs which were differentially expressed compared to those in mononuclear cells.[84] In the meantime, stable circulating miRNAs had been proven to be detectable in exosomes of or directly in body fluids such as serum/plasma,[85–101] urine,[86,99] saliva,[86,99,102] amniotic fluid and pleural fluid.[86,99] MiRNA expression profiles were found to correlate with different benign conditions and diseases as for example pregnancy stage,[85,86] type 2 diabetes[91] or liver injury.[100] More important in the context of this review, circulating miRNAs had been shown to be capable to distinguish between patients with malignancies and healthy controls in various tumour entities (prostate cancer: miR-141,[87] glioblastoma: miR-21,[88] NSCLC: miR-25 and miR-223 amongst 91 miRNAs,[91] colorectal cancer: 69 miRNAs[91], ovarian cancer: miR-21, -92, -93, -126, -29a, -155, -127 and -99b,[92] progesterone receptor positive breast cancer (versus receptor negative tumours): miR-155,[93] diffuse large B-cell lymphoma: miR-155, miR-210 and miR-21,[94] serous papillary ovarian adenocarcinoma: miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 amongst 218 miRNAs.[96] For a better understanding of the basic biologic principles of these observations, particularly the work of three authors has to be highlighted: Rabinowits and co-workers and Taylor & Gercel-Taylor presented first evidence that the expression pattern of miRNAs in circulating exosomes in lung cancer and ovarian cancer patients were similar to the expression profile in the underlying tumour. Rabinowits further demonstrated that lung cancer patients presented increased concentrations of circulating exosomes and miRNAs compared to healthy controls[95,96] In addition, Skog, et al. found that the levels of 11 miRNAs, which were known to be expressed in gliomas, were generally lower expressed in microvesicles in these patients but correlated well with the tumour.[88] These data indicate that miRNA profiles in exosomes might in fact serve as a surrogate for the malignant tumour. Even more interesting regarding a possible clinical use of circulating miRNAs, the profile of these molecules seems to correlate with clinical stages and outcome. Lawrie et al. found high miR-21 levels in sera of diffuse large B-cell lymphoma patients to be associated with an improved relapse-free survival,[94] and Taylor et al. described exosomal miR-200c and miR-214 to be lower expressed in ovarian cancer patients with Stage I compared to Stages II and III.[96] As this exciting field of miRNA research is still very young, there are to date no data available about possible correlations between blood-based miRNA analyses and the prediction of an anticancer treatment.

4. Summary and future expectations

MiRNAs represent a recently identified class of small, non-coding RNA molecules which control gene expression at post-transcriptional levels. The growing knowledge about their impact on several aspects of carcinogenesis and their meaning for therapeutic usage implicates miRNAs to be promising candidates for response prediction, control and modification of conventional and/or new developed anticancer treatments. It has been shown that alterations in miRNA expression profiles can be used to estimate and monitor the success of different therapeutic modalities. More important, several authors demonstrated that miRNAs can successfully modulate sensitivity of cells to well known chemotherapeutic agents. In addition, first preliminary studies showed that miRNAs can be detected in different easily accessible body fluids and might serve as a surrogate for the underlying malignancy. These data point out the immense prospect of using miRNAs in combination with existing therapeutic strategies to maximise the effect of cancer treatment and to improve survival of patients. The currently most promising miRNA targets might be miR-21, miR-34a, miR-200b and members of Let-7 family amongst others.

Despite of these encouraging results there are some limiting aspects which have to be addressed. First, involvement of

single miRNAs in modulation of anticancer treatment has just been discovered. Based on the huge amount of miRNAs in the human genome and the broad variety of mRNAs regulated by these molecules, existing literature provides only a very small insight into the probably extremely complex construct of 'miRNA – chemotherapeutic agent' interactions. Second, there is still a considerable lack of understanding the detailed mechanisms and intracellular pathways involved in the miRNA-mediated effects. Thus, further extensive basic research will be needed to fully lay open the whole number of miRNAs involved in modulation of chemo- or radiotherapies and the way they affect cellular homeostasis. Third, exosomal-derived miRNA profiling has not yet been analysed regarding its potential to predict response to an anticancer treatment. Last but not least, existing studies were conducted in vitro or by using xenograft animal models. Until now there is no proof for a tolerance of this treatment or even similar effects of 'miRNA application' on chemotherapy in humans. Therefore, additional studies have to be carried out in the future to validate the safety and efficiency of such treatment combinations in clinical settings.

Conflict of interest statement

None declared.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2009.10.027.

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