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MicroRNAs and their relevance to ABC transporters

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MicroRNAs (miRNAs) are small noncoding RNAs, which regulate the expression of their target genes post-transcriptionally by RNA interference. They are involved in almost all cellular processes, including proliferation, differentiation, apoptosis, cell survival and the maintenance of tissue specificity. Recent findings also suggest that efflux pumps of the ABC (ATP-binding cassette) transporter family are subject to miRNA-mediated gene regulation. Moreover, it seems that ABC transporters are embedded in a concerted and miRNA-guided network of concurrently regulated proteins that mediate altered drug transport and cell survival in changing environmental conditions. In this review, we summarize recent findings of miRNAs interacting with ABC transporters, which have been connected with drug distribution as well as with drug resistance. Additionally, we specify findings of complex miRNA–protein pathways conferring increased drug export and cell survival.

Introduction

The living organism is constantly exposed to various amounts of exogenous substances of different toxicity as well as endogenous products of metabolism. In order not to completely cede the access, distribution and elimination of these compounds exclusively to the physical process of diffusion, evolution developed a filter system consisting of transport proteins, which can transport substrates against their concentration gradient. These membrane-bound transport pumps build a functional border between the body and its environment or between different tissue compartments. In particular, transporter proteins of the ABC (ATP-binding cassette) family play a major 'doorman' function [1–3]. It is essential for the organism that the expression of these proteins is tightly regulated and can be instantly adapted to changing environmental conditions entailing altered substrate concentrations. Despite transcriptional regulation [4, 5] or 'on-demand' trafficking of transporter proteins from intracellular storage to the membrane [6, 7], it is becoming more obvious that epigenetic mechanisms, such as DNA methylation and histone modifications, modify the expression of ABC transporters and contribute to increased drug efflux and cell survival [8]. In the concert of transcriptional and epigenetic gene expression control, microRNAs (miRNAs) play an important linking part, contributing to adaptive regulation of ABC transporters. By RNA interference, miRNAs lead to inhibition of translational processes or messenger RNA (mRNA) degradation of their target genes.

In this review, we provide a brief overview of recent findings about the role of miRNAs in the regulation of human ABC transporters and how this interaction affects intracellular drug levels and drug resistance.

Processing, function and regulation of miRNA transcription

MicroRNAs are small noncoding RNA molecules of 20–24 nucleotides, initially cleaved from transcripts of several

hundred nucleotides with multiple hairpin loop structures. Sequential processing, involving the RNase III Drosha, first generates precursor miRNAs (pre-miRs) exhibiting a single hairpin structure and a length of 70–100 nucleotides (nts). These pre-miRNAS are exported from the nucleus into the cytoplasm and are cleaved by RNase III endonuclease dicer into miRNA/miRNA* duplexes. After degradation of one of the RNA strands, the mature single-stranded miRNA guides the RNA-induced silencing complex (RISC) to the 3′-untranslated region (3′-UTR) of the target mRNA. Consequently, the target mRNA is degraded or the translational process inhibited [9–12]. It is noteworthy that one single miRNA can target dozens of target genes and one gene can be regulated by dozens of different miRNAs, emphasizing the complexity of the interplay between miRNAs and their target genes.

The miRNA expression profile varies during development as well as between distinct tissue types. Moreover, miRNAs are regulated in a similar manner to protein-coding genes [13, 14]. There is increasing evidence that miRNAs are regulated by autoregulatory, double-negative or reciprocal negative feedback loops, involving transcription factors and their active downstream cell pathways [15]. Transcription factors bind within a 1 kb region upstream of the miRNA genes [16], interacting with *cis*-regulatory motifs found upstream of the predicted transcription start site, thereby repressing or activating miRNA gene expression.

MicroRNAs targeting ABCB1

The most prominent and so far most intensely investigated member of the ABC transporter family is P-glycoprotein (P-gp), encoded by the *ABCB1* gene, also known as multidrug-resistance gene (*MDR1*). It is apically expressed and exhibits two nucleotide-binding domains (NBDs) and two membrane-spanning domains (MSDs). P-glycoprotein limits the uptake of xenobiotics across membrane barriers, e.g. of the intestine or brain. In the liver and the kidney, P-gp contributes to the elimination of its substrates. In chemotherapeutic-resistant cancer cell lines, P-gp is often observed to be upregulated and is suggested to contribute to the phenomenon of drug resistance [17].

MicroRNAs leading to downregulation of P-glycoprotein in malignant cells

Table 1 and Figure 1 summarize recent findings about miRNAs leading to a downregulation of P-gp expression. In 2008, by comparison of parental MCF-7 breast cancer cells with their doxorubicin-resistant counterpart (MCF-7/DOX), an increased expression of P-gp and anti-apoptotic proteins B-cell CLL/lymphoma 6 (BCL6) and NOTCH1 was observed. In the resistant cell line, the expression level of miR-451 was below the limit of detection. Using 3′-UTR reporter gene assays, the authors could confirm the negatively regulating effect of miRNA-451 on P-gp expression. In turn, transfection of miR-451 re-established the sensitivity of the MCF-7/DOX cells to doxorubicin [18]. Also, colon cancer spheres exhibited a decreased expression of miR-144/451 in comparison to parental colorectal cancer cells [19]. Transfection of precursor miR-451 conferred a decrease of tumorigenicity and self-renewal to colon spheres. Additionally, macrophage migration inhibitory factor, cyclo-oxygenase-2 and P-gp were found to be downregulated. Macrophage migration inhibitory factor is another confirmed target of miR-451 [20] and was shown to increase the expression of cyclo-oxygenase-2 [19].

miR-331-5p and miR-27a were inversely correlated with doxorubicin resistance and P-gp expression in K562 chronic myelogenous leukaemia cells [21]. Sensitivity to doxorubicin was synergistically increased after transfection of miRNA 27a and miR-331-5p. Moreover, in bone marrow samples obtained from acute myeloid leukaemia and acute lymphoid leukaemia patients with complete remission compared with samples obtained from relapsed patients, both miRNAs showed a lower expression pattern [21].

In doxorubicin-resistant MCF-7 cells, miR-137 was also found to be downregulated and inversely expressed with Y-box binding protein-1 (YB1) and P-gp. miR-137 was confirmed to interact with YB1 by RNA interference. Accordingly, restoration of miR-137 caused a downregulation of YB1 and P-gp, leading to increased sensitivity to doxorubicin, vincristine and paclitaxel. It is noteworthy that YB1 is a transcription factor for ABCB1 and binds to the ABCB1 promoter in a stress response manner [22].

MicroRNA array-based comparison of doxorubicin-resistant human breast cancer cells (MDA-MB-231-R) with their doxorubicin-sensitive counterpart (MDA-MB-231-S) revealed a downregulation of miR-298. At the same time, the MDA-MB-231-R cells showed a higher expression of P-gp. 3′-UTR reporter gene assays identified binding sites for miR-298 and for miR-1253, which both synergistically impair the translation of P-gp. Transfection of miR-298 and miR-1253 caused downregulation of P-gp and increased doxorubicin sensitivity. In turn, transfection of miR-298 inhibitor into MDA-MB-231-S cells conferred doxorubicin resistance and increased P-gp expression. Northern blot experiments showed that the resistant cells expressed pri-miR-298, but compared with sensitive cells, no further processing to mature miR-298 was detected. Although the resistant cells exhibited no difference in Drosha expression, the authors suggested an impaired miR-298 processing as a potential reason for the

Table 1

MicroRNAs interfering with ABCB1 expression

MicroRNA	Effect of miRNA on gene expression	Mechanism	Tissue	Reference
MicroRNAs associated with decreased P-glycoprotein expression				
miR-27a	P-gp↓;	*	K562 chronic myelogenous leukaemia cell line, bone marrow of acute myeloid leukaemia and acute lymphoid leukaemia patients	[21]
miR-137	P-gp↓	Indirect, probably via Y-box binding protein-1* [22]	Human breast cancer cell line MCF-7	[22]
miR-145	P-gp↓	*	Human colon carcinoma cell line Caco-2	[66]
miR-200c	P-gp↓	Direct (*in silico* predicted binding site) or via BMI-1* [25] and ZEB1* [51]	Human breast cancer cell line MCF-7, breast cancer tissue	[24]
miR-298	P-gp↓	*	Human breast cancer cell line MDA-MB-231	[23]
miR-331-5p	P-gp↓	*	Chronic myelogenous leukaemia cell line K562, bone marrow of acute myeloid leukaemia and acute lymphoid leukaemia patients	[21]
miR-451	P-gp↓	*	Human breast cancer cell line MCF-7	[18]
		Macrophage migration inhibitory factor* [20]/cyclo-oxygenase-2/P-gp	Spheres of colorectal cancer cell lines DLD1, HT29, LS513 and SW620	[19]
miR-1253	P-gp↓	*	Human breast cancer cell line MDA-MB-231	[23]
MicroRNAs associated with increased P-glycoprotein expression				
miR-27a	P-gp↑	++	Human oesophageal squamous cell lines ECA109 and TE-13	[32]
		++	Human gastric cancer cell line MKN45	[33]
		++	Ovarian cancer cell line A2780	[31]
		Indirect, maybe via repressing HIPK2	Ovarian cancer cell line A2780	[34]
miR-138	P-gp↑	++	Human leukaemia cell line HL-60	[30]
miR-296	P-gp↑	++	Human oesophageal squamous cell line ECA109	[35]
miR-451	P-gp↑	++	Ovarian cancer cell line A2780	[31]

Symbols are as follows: ++, indirect, exact mechanism unknown; and P-gp, P-glycoprotein. *Confirmed mRNA/miRNA interference by 3′-untranslated region (3′-UTR) reporter gene assay.

observed doxorubicin resistance due to a lower expression of Dicer [23].

Breast cancer patients who exhibited a poor response to epirubicin treatment showed lower miR-200c expression compared with patients who showed a good response. In line with these *in vivo* findings, parental MCF-7 cells revealed a strong downregulation of miR-200c in contrast to their doxorubicin-resistant counterpart, MCF-7/ADR. Restoration of miR-200c induced accumulation and cytotoxicity of doxorubicin in MCF-7/ADR cells, which was linked to a decrease of P-gp expression. Although *in vitro* confirmation experiments were not performed, the authors reported an *in silico* predicted miR-200c binding site in the 3′-UTR of *ABCB1* [24]. Because breast cancer stem cells were associated with low expression of the miR-200 family [25] and with increased invasiveness [26–29], these findings suggest P-gp as a further puzzle piece in a miR-200 family-coordinated acquisition of chemoresistance in cancer stem cells and in cancer cell lines.

MicroRNAs leading to an upregulation of P-glycoprotein in malignant cells

Table 1 and Figure 1 summarize recent findings about miRNAs leading to an upregulation of P-gp expression. Transfection of miR-138 in vincristine-resistant human leukaemia cell line HL-60/VCR led to a decreased expression of P-gp and the anti-apoptotic protein B-cell CLL/lymphoma 2 (BCL-2). Additionally, the apoptotic protein BCL2-associated X protein (BAX) showed an increased expression pattern. Cotransfection of ABCB1 promoter gene vector construct with miR-138 inhibitor led to a decreased reporter gene activity, suggesting an indirect influence of miR-138 on ABCB1 expression, maybe by RNA interference with a repressive acting transcription factor [30].

Another experiment in doxorubicin-resistant ovarian cancer cell lines (A2780DX5) showed downregulation of P-gp mediated by antagonizing miR-451 and miR-27a [31]. Downregulation of miR-27a resulted in a decrease of P-gp

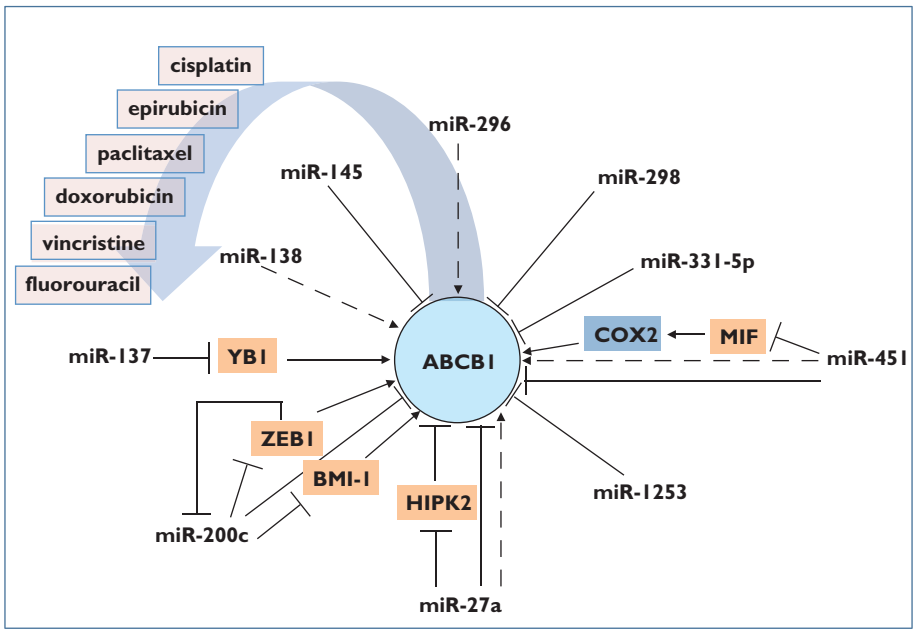

Figure 1

ABCB1 in the complex network of microRNAs (miRNAs) and transcription factors affecting the transport of given drugs. Dashed arrows indicate an indirect upregulating impact of the miRNA on ABCB1 expression, maybe via a repressive transcription factor. Bars indicate direct RNA interference between miRNA and target mRNA. BMI-1, BMI1 polycomb ring finger oncogene; COX2, cyclooxygenase 2; HIPK2, homeodomain interacting protein kinase 2; MIF, macrophage migration inhibitory factor; YB1, Y-box binding protein 1; ZEB1, zinc finger E-box binding homeobox 1

expression in oesophageal squamous carcinoma and in gastric cancer cells [32, 33].

After transfection of miR-27a inhibitor, Li *et al*. reported a downregulation of P-gp and an increased sensitivity to paclitaxel in the paclitaxel-resistant ovarian cancer cell line (A2780/Taxol). As antagonism of miR-27a additionally increased the protein expression of co-repressor homeodomain interacting protein kinase 2 (HIPK2), showing an *in silico* predicted miR-27a binding site, the authors postulated a miRNA-27a/HIPK2/P-gp pathway [34].

However, compared with the reports mentioned above [18, 19, 21], the partly inconsistent results concerning the influence of miR-451 and miR-27a on P-gp expression and the regulating mechanisms, how P-gp is downregulated by antagonizing miR-27a or miR-451, remain to be clarified.

Oesophageal squamous cell cancer was characterized by an increase in miR-296 expression and, vice versa, lower miR-296 expression was associated with prolonged survival. Transfection of miR-296 inhibitor in human oesophageal squamous cells (ECA109) decreased the IC_{50} values of vincristine, adriamycin (doxorubicin), 5-fluorouracil and cisplatin. Also, the tumourigenicity of ECA109 cells transfected with miR-296 inhibitor was reduced in athymic nude mice in comparison to cells transfected with negative control. Antagonizing miR-296 in ECA109 cells caused a downregulation of P-gp, which was accompanied by an upregulation of interferon alpha-inducible protein 27 (IFI27; p27) and a decrease of cyclin D1. Moreover, the pro-apoptotic protein BAX showed an increased expression, whereas the anti-apoptotic protein BCL2 was downregulated. Cotransfection of ABCB1 promoter gene vector construct with increasing concentrations of miR-296 inhibitor led to a decreased reporter gene activity, implying an indirect interaction between P-gp and miR-296, maybe via a repressive transcription factor that has not yet been identified [35].

Regarding all reports about miRNA-mediated inhibition of P-gp expression via direct RNA interference, it has to be critically mentioned that the current version of *ABCB1* cDNA (accession no. NM_000927.4) exhibits a 3′-UTR sequence of 382 nts, whereas the former version (accession no. NM_000927.3) had a length of 611 nts. Based on NM_000927.4, only miR-145, reported by Ikemura *et al*. to target *ABCB1* 3′-UTR [36], may show slight *in silico* predicted overlapping with the currently published ABCB1 3′-UTR. In these circumstances, investigations to elucidate potential tissue-specific differences in lengths of ABCB1 3′-UTR should be considered.

MircroRNAs targeting ABCG2

Table 2 and Figure 2 summarize miRNAs associated with post-transcriptional regulation of ABCG2 expression. In contrast to ABC transporters of the B and C family, ABCG2 (breast cancer-resistance protein; BCRP) contains a single

Table 2

MicroRNAs interfering with ABCG2 expression

MicroRNA	Effect of miRNA on gene expression	Mechanism	Tissue	Reference
miR-200c	ABCG2↓ and ABCG5↓	Indirect, probably via BMI-1* [25] and ZEB1* [51]	Primary melanomas and metastatic melanomas, human melanoma cell lines (WM35, WM793, WM115A, WM3523A and 1205Lu)	[50]
miR-212	ABCG2↓	*	Human chronic myelogenous leukaemia cells K562	[47]
miR-328	ABCG2↓	*	Human breast cancer cell line MCF-7	[45, 46]
		*	Human retinoblastoma cell line RB143	[45]
		++	Human chronic myelogenous leukaemia cells K562	[47]
		*	Side population of colorectal cancer cell lines (SW1116, LoVo, HCT116 and SW480) and colorectal cancer tissue	[49]
miR-519c	ABCG2↓	*	Human colon cancer cell line S1	[41, 42]
		*	Human breast cancer cell line MCF-7 and human retinoblastoma cell line RB143	[45]
miR-520h	ABCG2↓	*	$CD34^+CD38^-$ haematopoietic stem cells from human umbilical cord blood	[43]
		*		
		*	Human pancreatic cancer cell line PANC-1	[44]
		*	Human retinoblastoma cell line RB143	[45]

Symbols are as follows: ++, indirect, exact mechanism unknown. *Confirmed mRNA/miRNA interference by 3′-UTR reporter gene assay.

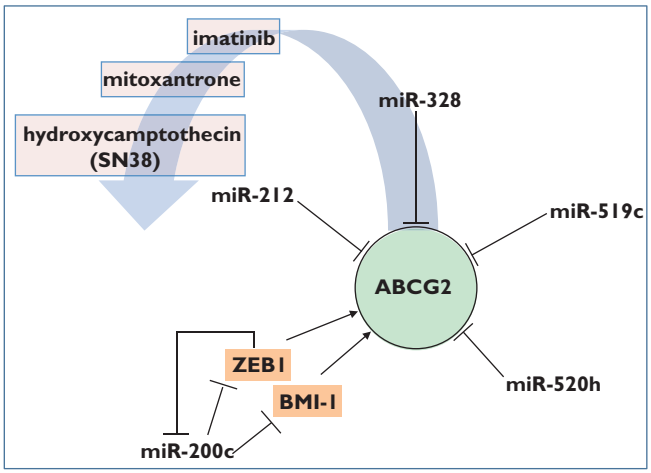

Figure 2

ABCG2 in the network of miRNAs and transcription factors, leading to altered transport of given drugs. Bars indicate direct RNA interference between miRNA and target mRNA. BMI-1, BMI1 polycomb ring finger oncogene; ZEB1, zinc finger E-box binding homeobox 1

NBD and a single MSD and is therefore often termed a 'half transporter'. In order to build a functional transport unit, two ABCG2 proteins have to homodimerize [37]. It is apically expressed at blood–tissue barriers of placenta, brain, intestine, liver and kidney. Moreover, it is expressed in stem cells (therefore, also used as a stem cell marker), as well as in drug-resistant cancer cells, where it limits intracellular bioavailability of chemotherapeutics [38–40].

In 2008, To *et al*. identified a miR-519c binding site in the *ABCG2* 3′-UTR. Interestingly, in comparison to parental S1 colon cancer cells, the mitoxantrone-resistant cell line S1MI80 lost its miR-519c-mediated post-transcriptional control of ABCG2 by expression of shortened *ABCG2* 3′-UTRs. The binding site of miR-520h, located 5′ upstream of the miR-519c binding site, was not affected by the observed 3′-UTR truncation. However, the drug-resistant cells expressed miR-520h at a lower level than the parental cells, implying another mechanism to upregulate ABCG2 expression and to acquire chemoresistance [41, 42]. miR-520h was reported earlier to target ABCG2 mRNA by RNA interference and to be involved in haematopoietic stem cell differentiation [43] and in increased ABCG2 expression, migration and invasion of pancreatic cancer cells [44]. In 2011, Li *et al*. reported a proximally located miR-519c binding site, which overlaps the miR-520h binding site [45], confirming the findings of To *et al*. [41, 42], to have an additive negative regulating effect of miR-519c on ABCG2 expression depending on the number of its binding sites.

In mitoxantrone-resistant human breast cancer MCF-7 cells, miR-328 was shown to be inversely regulated with ABCG2. It could also be shown that transfection of miR-328 caused increased sensitivity to mitoxantrone and downregulation of ABCG2 in MCF-7/MX100 cells [45, 46]. After sorting of the human retinoblastoma cells RB143, the expression of miR-519c, miR-520h and miR-328 was observed to be lower in ABCG2-expressing than in ABCG2-non-expressing cancer cells. Direct binding of miR-328 to *ABCG2* 3′-UTR was confirmed by reporter gene assays [45, 46].

By treating K562 human chronic myelogenous leukaemia cells with increasing concentrations of imatinib, we observed an inverse regulation of ABCG2 with both

miR-328 and miR-212 [47]. In a reporter gene assay, miR-212 was shown to inversely regulate ABCG2, whereas miR-328 could not be confirmed as directly targeting ABCG2 mRNA. Imatinib treatment with concentrations lower than 1 μmol l^{-1} elevated ABCG2 and reduced miR-212 and miR-328 expressions, whereas concentrations higher than 1 μmol l^{-1} led to strong recovery of expression of both miRNAs accompanied by declining ABCG2 expression [47]. Considering ABCG2 as stem cell marker, this observation could be of value in avoiding selection of drug-resistant stem-like cancer cells by incorrect dosing of imatinib [48].

A subgroup of colorectal cancer cells known as side population cells exhibited not only stem cell-like properties, such as tumourigenicity, self-renewal, invasiveness and chemoresistance against hydroxycamptothecin, but also significantly lower miR-328 expression levels than their non-side population counterpart. Moreover, ABCG2 and matrix metalloprotease 16 were confirmed to be controlled by miR-328 through RNA interference, underlining the crucial role of miR-328 in the maintenance of stem cell properties and invasiveness of cancer cells [49].

In melanomas, compared with melanocytic naevi, the stem cell factor BMI-1, as well as the ABC transporters ABCG2, ABCG5 and ABCB1, were recently described to be expressed inversely with miR-200c and E-cadherin [50]. While Chen *et al*. reported an *in silico* predicted binding site of miR-200c in ABCB1 3′-UTR [24], it may additionally be speculated that miR-200c mediates an indirect regulation of the efflux transporters ABCG2, ABCG5 and ABCB1, as well as the process of epithelial–mesenchymal transition. This process may be managed via its direct targets, BMI-1 (a member of the polycomb group proteins conferring chromatin modifications and responsible for maintenance of stem cell features by suppressing differentiation and apoptosis) [25] and ZEB1 (zinc finger E-box binding homeobox 1; a transcriptional repressor of E-cadherin) [51]. Moreover, it seems that ZEB1 acts as repressive transcription factor for stem cell properties, inhibiting miRNAs including the miR-200 family, suggesting a reciprocal negative feedback loop between miR-200c and ZEB1 [52, 53]. Taken together, these findings could be summarized as miR-200c/BMI-1/ABCG2, ABCG5, ABCB1 and miR-200c/ZEB1/E-cadherin pathways, both probably conferring in many cancers a progressive, invasive and chemoresistant phenotype.

MicroRNAs targeting members of the ABC C family

Table 3 and Figure 3 summarize recent findings on miRNAs interfering with the expression of members of the ABC C and E family. Further important ABC transporters involved in disposition and efflux of many endo- and xenobiotics, as well as in the phenomenon of drug resistance, belong to the ABC C family. Members of this protein family are comprised of two NBDs and two MSDs (ABCC4, ABCC5, ABCC11 and ABCC12) or three MSDs (ABCC1, ABCC2, ABCC3, ABCC6 and ABCC10) [36].

In contrast to P-gp and ABCG2, ABCC1 was found to be overexpressed in human breast cancer cells resistant to etoposide (MCF-7/VP-16) in comparison to its parental cell line, MCF-7. A microarray study revealed miR-326 to be downregulated in resistant cells. Transfection of miR-326 into MCF-7/VP-16 cells downregulated ABCC1 expression and increased the sensitivity to etoposide and doxorubicin, but not to mitoxantrone. Interestingly,

Table 3

MicroRNAs interfering with the expression of transporters of the ABC C and E family

ABC transporter	MicroRNA	Effect of miRNA on gene expression	Mechanism	Tissue	Reference
ABCC1	miR-326	ABCC1↓	*	MCF-7 human breast cancer cells, breast cancer tissue	[54]
	miR-199a/b and miR-296	ABCC1↓	*	Hepatocellular carcinoma	[58]
ABCC2	miR-297	ABCC2↓	*	Human ileocaecal colorectal adenocarcinoma cell line HCT-8 and human colorectal cancer cell line HCT-116	[55]
	miR-379	ABCC2↓	*	HepG2 human hepatoblastoma cells	[56]
ABCC3	miR-9*	ABCC3↓	Indirect, via ID4 and SOX2*	Glioma cell lines (A172, A1207, LN18 and LN229) and human glioma stem cells derived from patients with glioma	[57]
ABCC4	miR-125a/b	ABCC4↓	*(weak)	Hepatocellular carcinoma	[58]
ABCC5	miR-101, miR-125a and let-7a	ABCC5↓	*	Hepatocellular carcinoma	[58]
ABCC6	miR-9*	ABCC6↓	Indirect, via ID4 and SOX2*	Glioma cell lines (A172, A1207, LN18 and LN229) and human glioma stem cells derived from patients with glioma	[57]
ABCC10	let-7a/e	ABCC10↓	*	Hepatocellular carcinoma	[58]
ABCE1	miR-26a, miR-135b and miR-145	ABCE1↓	*	Hepatocellular carcinoma	[58]

ID4, inhibitor of differentiation 4; SOX2, SRY (sex determining region Y)-box 2. Symbols are as follows: ++, indirect, exact mechanism unknown. *Confirmed mRNA/miRNA interference by 3′-UTR reporter gene assay.

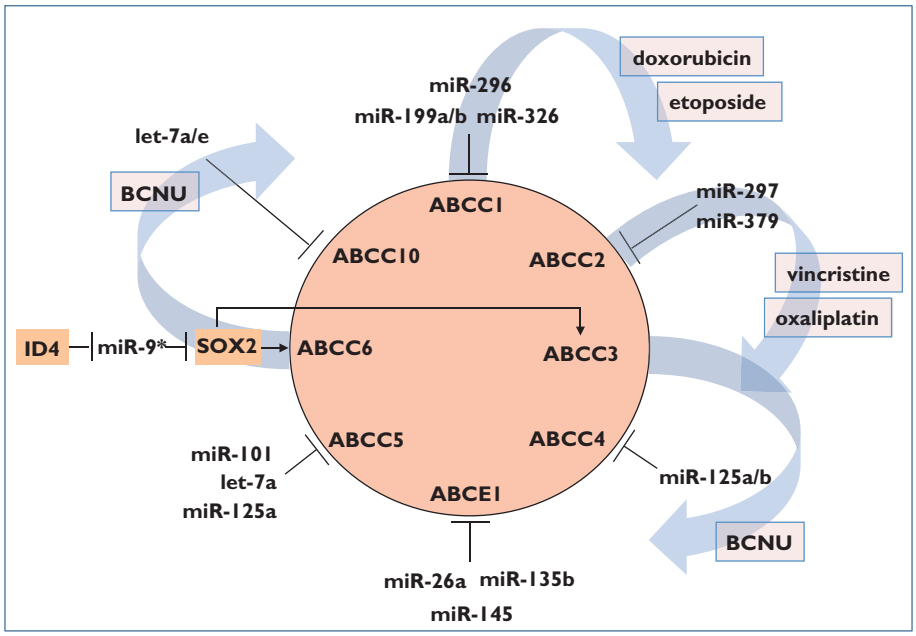

Figure 3

MicroRNAs and transcription factors associated with regulation of transport proteins of the ABC C and E family. Drugs are given, which have been described to be altered in their intracellular accumulation by this network. Bars indicate direct RNA interference between miRNA and target mRNA. ID4, inhibitor of DNA binding 4 (also known as inhibitor of differentiation 4); SOX2, SRY (sex determining region Y)-box 2; BCNU, 1,3-*bis*(2-chloroethyl)-1-nitrosourea

ABCG2-overexpressing and mitoxantrone-resistant MCF-7 cells were not affected in their etoposide sensitivity by miR-326 transfection [54]. Moreover, in normal breast tissue, early breast cancer tissue without metastasis and advanced breast cancer tissue with metastasis, a continuous decline of miR-326 expression could be observed, leading inversely to highest ABCC1 expression in advanced breast cancer tissues. Direct miRNA/mRNA interaction was additionally confirmed by 3′-UTR reporter gene assays [54].

In human colorectal cancer tissue samples, an inverse stage-dependent correlation of miR-297 and ABCC2 mRNA and protein expression was observed. miR-297 is able to downregulate ABCC2 expression by directly targeting ABCC2 3′-UTR. Transfection of miR-297 *in vitro* increases chemosensitivity in colorectal cancer cell lines resistant to vincristine (HCT-8/VCR) or oxaliplatin (HCT-116/L-OHP) as well as *in vivo* in HCT-116/L-OHP cells after being injected in nude mice [55]. ABCC2 mRNA was also identified to be targeted by miR-379 through RNA interference in HepG2 human hepatoblastoma cells. Interestingly, the PXR-ligand rifampicin mediated an upregulation of miR-379, suggesting an initial PXR/miR-379/ABCC2 negative feedback mechanism preventing cells from ABCC2 protein overexpression [56].

Jeon *et al*. reported that ID4 (inhibitor of differentiation 4) inhibits the expression of miR-9* in glioma cells and that miR-9* targets the stem cell marker SRY (sex determining region Y)-box 2 (SOX2) by direct RNA interference. SOX2 in turn transcriptionally induces the ABC transporter ABCC3 and ABCC6, thereby contributing to chemoresistance against 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU) and stem cell maintenance in glioma stem cells. These data suggest a possible ID4/miR-9*/SOX2/ABCC3, ABCC6 pathway to be active [57].

By conducting an mRNA and miRNA expression study in hepatocellular carcinoma and adjacent healthy liver tissues followed by reporter gene-based target gene validation, Borel *et al*. identified ABCC1 as a target of miR-199a/b and miR-296, ABCC4 as a target of miR-125a/b, ABCC5 as a target of miR-101, miR-125a and let-7a, ABCC10 as a target of let-7a/e, and ABCE1 as a target of miR-26a, miR-135b and miR-145 [58]. However, the functional influence of the observed miRNA/mRNA associations in hepatocellular carcinoma remains to be elucidated.

Summary

Efflux pumps of the ABC transporter family are key players in distribution and elimination of endo- and xenobiotics. Several studies describe ABC transporters concurrently regulated with proteins related to apoptosis, cell cycle, stress response, stem-cell-ness, invasiveness and tumourigenicity. There is increasing evidence that miRNAs are crucially involved in co-ordinating and fine-tuning this complex network of proteins mediating increased drug efflux and cell survival. MicroRNAs therefore play an

important role not only in altered drug distribution, but also in the phenomenon of drug resistance [59]. Furthermore, it may be speculated that in reaction to (long-term) treatment almost every drug could generate a distinct cellular miRNA expression pattern, leading to a drug-specific survival mechanism and specific ABC transporter expression. In this context, it also has to be mentioned that genetic mutations or polymorphisms might have an interindividual influence on miRNA-mediated gene regulation by leading to altered seed sequences [60, 61] or mRNA secondary structures. The increasing knowledge about the function of miRNAs makes them interesting candidates as biomarkers of diseases and in the management of drug therapy [62–64]. Moreover, the challenging question is whether miRNAs can be delivered *in vivo* in a targeted manner and therefore, if miRNAs can be used as drugs in the correction of pathogenic deregulated protein networks or in the optimization of classic therapeutic regimens [65, 66].

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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