The role of microRNA in the response to cisplatin treatment

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Abstract
Resistance to the cytotoxic effects of cisplatin can be mediated through changes in a wide variety of cellular processes and signalling pathways. The discovery of microRNAs as regulators of protein expression through the targeting of mRNA has led to a number of studies on the effect of cisplatin treatment on microRNA expression, and the ability of microRNAs to modulate cisplatin resistance.

Introduction
Cisplatin is a DNA-damaging cytotoxic agent. The biological activity of cisplatin was first described in 1965 [1], and it has subsequently been licensed as a chemotherapeutic agent for the treatment of a wide variety of tumours (http://www.cancer.gov/cancertopics/druginfo/cisplatin). Since the discovery of the anti-tumour effects of cisplatin, many novel platinum-based compounds (most notably carboplatin and oxaloplatin) have been developed in an attempt to increase efficacy and reduce side effects [2]. Nevertheless, cisplatin remains widely used. Resistance to the cytotoxic effects of cisplatin are frequently observed, with many patients displaying either innate resistance to the drug or a cisplatin-resistant recurrence of the disease following an initial period of treatment [3]. Resistance to cisplatin can be brought about by alterations to a huge number of intracellular pathways (for comprehensive reviews, see [3,4]).

microRNAs (miRNAs)
Since their initial discovery in Caenorhabditis elegans [5], and subsequent identification in plants [6] and higher animals [7], miRNAs have been implicated in contributing to the regulation of numerous cellular processes. In vertebrates, miRNAs are able to target and down-regulate mRNA. Target specificity is regulated by a requirement for a small region of sequence complementarity between bases 2 and 8 of the miRNA and a corresponding seven nucleotide sequence in the 3′-UTR (untranslated region) of the target mRNA [8]. The relatively small amount of complementarity required between miRNA and target mRNA means that each miRNA is able to target many different mRNAs [9]. The ability of a single miRNA to affect the expression of a wide variety of proteins has led to increased interest in miRNAs as mediators of the cellular response to DNA-damaging agents.

Regulation of miRNA expression by cisplatin.

Transcription
Modulation of the transcription of miRNA primary transcripts (pri-miRNAs) represents a major site at which the expression of mature miRNAs can be regulated. Most intergenic miRNAs are transcribed by RNA polymerase II [10], and form distinct transcriptional units with their own promoters [11]. Thus transcription of pri-miRNAs can be subject to regulation by elements of the DDR (DNA damage response) pathway in the same manner as protein-coding genes following cisplatin treatment.

A study on the nephrotoxic effects of cisplatin identified p53-dependent regulation of miR-34a occurring following cisplatin treatment in kidney cells [12]. Induction of the DDR and activation of p53 with other cytotoxic agents (such as doxorubicin) in various cell lines has identified miR-192, miR-194 and miR-215 as being regulated by p53 following DNA damage [13].

The oncogenic p63 isoform ΔNp63α, which is commonly overexpressed in SCC (squamous cell carcinoma) [14], has been shown to be phosphorylated by ATM (ataxia telangiectasia mutated) following cisplatin treatment of HNSCC (head and neck squamous cell carcinoma) cells, leading to altered transcription levels of miR-181a, miR-519a, miR-374a (all down-regulated) and miR-630 (up-regulated) [15]. These miRNAs were shown to target various apoptotic proteins (including bcl-2, caspase 3 and PARP1 [poly(ADP-ribose) polymerase 1]), suggesting that this is a pathway by which miRNAs contribute to the cisplatin-induced DDR in certain cancers.

Epigenetic regulation
Alteration of gene expression by DNA methylation has been shown to play an important role in the development of cisplatin resistance in several cell culture models [16]. Epigenetic drugs such as 5-azacytidine have been shown to reverse cisplatin resistance by restoring the expression of certain resistance-causing proteins [17,18]. There is now evidence to suggest that epigenetic regulation of miRNA expression can contribute to the development of a
cisplatin-resistant phenotype. For example, a study in ovarian cancer tissue and cell lines has highlighted increased methylation of the miR-130b promoter and an associated down-regulation of miR-130 as modulating resistance to cisplatin through regulation of the CSF1 (colony-stimulating factor 1) gene [19].

miRNA processing
pri-miRNAs are first processed into pre-miRNAs (precursor miRNAs) in the nucleus by the Drosha–DGCR8 (DiGeorge syndrome critical region gene 8) complex. While this core complex is sufficient for the generation of pre-miRNAs from pri-miRNAs, several proteins have been identified, which are able to interact with this processing machinery in order to regulate the biogenesis of specific subsets of miRNAs. One such protein is the KHSP (KH-type splicing regulatory protein), which has been shown to interact with Drosha and increase the binding and subsequent processing of specific pri-miRNAs such as pri-let7a-1 and pri-miR-21 [20]. Recently, this protein has been found to be up-regulated in the nucleus of cisplatin-treated HeLa cells [21]. This suggests that expression of some miRNAs might be regulated in response to cisplatin treatment by this mechanism.

Dicer is an RNase III family endonuclease, which controls the maturation of pre-miRNAs into miRNAs in the cytoplasm [22]. Dicer has been found to be up-regulated in several cancer types including adenocarcinoma of the prostate [23] and lung [24]. It has been shown in breast cancer cells that siRNA (small interfering RNA)-mediated knockdown of Dicer sensitizes cells to the cytotoxic effects of cisplatin [25], suggesting that in this cancer type at least, global miRNA expression is a net contributor to resistance. Nevertheless, alterations in the expression of individual miRNAs have been shown to both sensitize and protect various cancer cells from cisplatin treatment.

Direct interaction between miRNAs and cisplatin
Cisplatin is able to platinate RNA in a very similar mechanism to that by which it platinates DNA. Although the overwhelming majority of the cytotoxic effect of cisplatin treatment is due to its interaction with DNA, there is some evidence from work with siRNA that platination of small RNAs can affect their processing and biological activity [26]. This leads to speculation that platinum-based drugs might be able to modulate expression of certain genes via interacting with miRNAs that normally regulate them.

Cisplatin-related targets of miRNAs
The ability of individual miRNAs to target many mRNAs, and the fact that resistance to the cytotoxic effects of cisplatin can be achieved through alterations in many cellular processes [3,4] has resulted in many attempts to identify proteins which might be subject to miRNA regulation as a response to cisplatin treatment, or which might serve as useful biomarkers for patients and tumours likely to benefit from cisplatin-based chemotherapy. Studies in which resistance-mediating target mRNAs have been identified for dysregulated miRNAs are summarized in Table 1.

Influx/efflux of cisplatin
Reduction in protein levels of CTR1, a copper transporter which is also able to pump cisplatin into a cell, can lead to decreased effectiveness of the drug [27]. Despite down-regulation of this protein being a contributing factor to many cases of cisplatin resistance, the CTR1 mRNA containing a 4 kb 3′-UTR with numerous predicted sites of miRNA interaction (http://www.targetscan.org), there are to date no reports of miRNAs contributing to this process. Cisplatin resistance can also be achieved through up-regulation of the cisplatin-exporting proteins ATP7A [28] and ATP7B [29]. As with CTR1, there is currently no evidence of miRNA involvement in their regulation during the development of cisplatin resistance.

Intracellular detoxification
Whereas cisplatin is not known to be a substrate of MDR1 (multidrug-resistance protein 1), MDR1 is thought to be able to contribute to cisplatin resistance by assisting with the efflux of cisplatin/glutathione conjugates [30]. The MDR1 mRNA is a target of both miR-7 and miR-345, and the down-regulation of these two miRNAs in breast cancer cells contributes to cisplatin resistance through the up-regulation of this protein [31]. MDR1 up-regulation and increased cisplatin resistance have also been identified as a consequence of miR-134 down-regulation in tongue SCC [32]. Autophagy, a process by which cells digest their own damaged organelles, has been shown to contribute to cisplatin resistance in some cell types, and a recent study implicated an increase in miR-30a expression in the regulation of this resistance [33].

Growth factor signalling
In a model of cisplatin-resistant ovarian cancer, miR-130a was found to be down-regulated, and the ability of this miRNA to target the mRNA of CSF1 was confirmed [34]. High CSF1 expression in ovarian cancer is a well-established predictor of poor response to cisplatin treatment [35]. ALK7, a key negative regulator of the TGFβ (transforming growth factor β) signalling pathway has been shown to be a target of miR-376c in ovarian cancer. An associated reduction of ALK7 expression has been shown to correlate with increased cisplatin resistance in cells, and a poorer prognostic outcome in patients [36].

Apoptosis
Weeraratne et al. [37] identified a positive feedback loop involving miR-34a in medulloblastoma, whereby an increase in miR-34a expression resulted in a decrease in the expression of MAGE-A (melanoma antigen gene A) and a subsequent up-regulation of p53 and several key transcriptional targets of p53 including p21Waf1/CIP1 and miR-34a itself. Activation of this feedback mechanism resulted in a cisplatin-sensitized phenotype. miR-128-2, up-regulated as a consequence of a
gain-of-function p53 mutation in NSCLC (non-small-cell lung cancer), has also been shown to up-regulate p21Waf1 through inhibition of the transcriptional repressor EZF5 [38], resulting in a decreased apoptotic response following cisplatin treatment.

The BMI1 Polycomb RING finger protein has been shown to be up-regulated by a decrease in expression of miR-15b and miR-200b in a cell culture model of cisplatin-resistant SCC, with an associated increase in mesenchymal features and drug resistance [39].

The STAT (signal transducer and activator of transcription)-induced STAT inhibitor protein SOCS3 (suppressor of cytokine signalling 3) has been shown to be a direct target of miR-203 [40], an miRNA which is frequently found to be up-regulated in cancer compared with normal tissues [21,26,41]. Up-regulation of this miRNA protects cancer cells against cisplatin-induced apoptosis by suppressing SOCS3, which prevents induction of p53, p21 and Bax.

Activation of the Akt/PKB (protein kinase B) survival signalling pathway generally results in cisplatin resistance, in particular in ovarian cancer [42]. The negative regulator of this pathway, the tumour-suppressor protein PTEN (phosphatase and tensin homologue deleted on chromosome 10), has been shown to be a target of miR-214, an miRNA which is frequently up-regulated in ovarian cancers, where it contributes to cisplatin resistance [43]. PTEN has also been shown to be a target of miR-93, which has also been found to be overexpressed in a cell culture model of cisplatin-resistant ovarian cancer [41]. The PKB phosphatase PPP2RIB, which deactivates Akt, has also shown to be a target of miR-200c in oesophageal cancers, where increased expression of the miRNA results in poorer response to cisplatin treatment [44]. miR-200c has also been associated with poor prognosis and a drug-resistant phenotype in NSCLC [45].

The Bcl-2 family of apoptotic regulatory proteins represents a frequent site for miRNA intervention in cisplatin resistance. The anti-apoptotic protein Bcl-2 has been shown to be up-regulated as a consequence of miR-15b and miR-16 down-regulation in drug-resistant gastric cancer cells [46], whereas miR-181b down-regulation has been shown to contribute to cisplatin resistance by reducing Bcl-2 protein levels in a cisplatin-resistant lung cancer cell line [44]. A separate study in cisplatin-resistant lung cancer cells found that levels of Bcl-2 and another anti-apoptotic Bcl-2 family member, XIAP (X-linked inhibitor of apoptosis), were found to be increased in drug-resistant cells as a consequence of down-regulation of the miR-200bc/429 cluster [47]. Bcl-2 up-regulation and increased sensitivity to cisplatin have also been identified as a consequence of miR-497 down-

<table>
<thead>
<tr>
<th>Cancer</th>
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<th>miRNA</th>
<th>Regulation</th>
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regulation in drug-resistant gastric and lung cancer cell lines [48].

**Discussion**

Alterations to miRNA expression levels are increasingly becoming implicated in the regulation of a wide variety of pathways which contribute to the cellular response to cisplatin. However, the multifactorial nature of cisplatin resistance, combined with the low target specificity of miRNAs and high likelihood of off-target effects make them unpopular for exploration as sites of potential therapeutic regulation at this time. However, they may display more promise as biomarkers with the potential to predict the likely response of a patient to cisplatin-based chemotherapy. One recent study into ovarian cancer identified down-regulation of a cluster of miRNAs on the X chromosome, detected from miRNA array profiling of tissue material, as a strong predictor of early relapsing patients [49].

The relative ease with which miRNA expression levels can be ascertained from tumour material, and in some cases from much less invasive blood and serum samples [50], makes the further validation of these molecules as predictors of patient response to chemotherapy an appealing area of study for the future.

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**References**


