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A Comprehensive Review on the Genetic Regulation of Cisplatin-induced Nephrotoxicity

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Abstract: Cisplatin (CDDP) is a well-known antineoplastic drug which has been extensively utilized over the last decades in the treatment of numerous kinds of tumors. However, CDDP induces a wide range of toxicities in a dose-dependent manner, among which nephrotoxicity is of particular importance. Still, the mechanism of CDDP-induced renal damage is not completely understood; moreover, the knowledge about the role of microRNAs (miRNAs) in the nephrotoxic response is still unknown. miRNAs are known to interact with the representative members of a diverse range of regulatory pathways (including postnatal development, proliferation, inflammation and fibrosis) and pathological conditions, including kidney diseases: polycystic kidney diseases (PKDs), diabetic nephropathy (DN), kidney cancer, and drug-induced kidney injury. In this review, we shed light on the following important aspects: (i) information on genes/proteins and their interactions with previously known pathways engaged with CDDP-induced nephrotoxicity, (ii) information on newly discovered biomarkers, especially, miRNAs for detecting CDDP-induced nephrotoxicity and (iii) information to improve our understanding on CDDP. This information will not only help the researchers belonging to nephrotoxicity field, but also supply an indisputable help for oncologists to better understand and manage the side effects induced by CDDP during cancer treatment. Moreover, we provide up-to-date information about different *in vivo* and *in vitro* models that have been utilized over the last decades to study CDDP-induced renal injury. Taken together, this review offers a comprehensive network on genes, miRNAs, pathways and animal models which will serve as a useful resource to understand the molecular mechanism of CDDP-induced nephrotoxicity.

Keywords: Apoptosis, Cisplatin, microRNAs, miRWalk/miRWalk 2.0, Nephrotoxicity, Pathways, Tubular injury.

1. INTRODUCTION

Nowadays cisplatin (CDDP) is a well-known and widely used chemotherapeutic drug. Its antineoplastic properties were accidentally discovered almost fifty years ago and, with one of the highest cure rate and effectiveness in the treatment of some malignancies, the use of CDDP opened a new era in neoplasms treatment. Although, CDDP was synthetized for the first time in 1845 and its structure was deduced in 1893, its chemotherapeutic properties were unknown until the 1960s. In 1971, CDDP was used for the first time in the treatment of a cancer patient and, seven years later, was approved by the U.S. Food and Drug Administration (FDA database), becoming available for clinical practice as Platinol (Bristol-Myers Squibb) [1].

With its clinical use, CDDP has demonstrated to be a potent chemotherapeutic drug, with approximately 90% of efficiency in the treatment of testicular cancers [2]. Moreover, CDDP shows a broad spectrum of antitumor activity and currently has been used in the treatment of numerous and different types of cancers, such as ovarian [3, 4], cervical [5], bladder [6], non-small cell lung cancers [7], head and neck [8] and testicular cancer [9-12], among many others.

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Despite its effectiveness, the use of CDDP is limited due to its severe side effects in normal and therefore healthy tissues. The occurrence and severity of these side effects often show a dose-dependence, limiting the dosages in which CDDP can be administered and, therefore, compromising the success of the chemotherapeutic treatment.

Emetogenesis, ototoxicity or neurotoxicity are some of the possible and diverse consequences caused by this antineoplastic drug. However, the major limiting factor in CDDP treatment is, undoubtedly, nephrotoxicity. With a renal excretion, CDDP is accumulated in the kidneys in a greater manner than in any other organ. Although, CDDP itself is not harmful for the kidneys, however, its conversion into a potent nephrotoxin in the renal tubular cells turns it into a threat for the organ and triggers the CDDP-induced renal pathology.

The specific number of pathways activated in the kidney by this nephrotoxin is still unknown, but the involved pathways lead to renal damage and impairment of both, function and morphology.

Although, it does not affect all cancer patients undergoing CDDP treatment, approximately 20-35% of them show renal impairment and related complications after CDDP administration [1, 13-17]. In these patients, it is very common to observe a decrease in glomerular filtration rate (GFR) along with an increase of blood urea nitrogen (BUN) and serum creatinine (sCr), as well as a reduction of serum po-

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tassium and magnesium levels [14, 15, 18, 19]. CDDP may also induce damage to the renal vasculature resulting in a diminished blood flow and ischemic injury of the organ, which contributes to further reduce the filtration capacity of the organ [2]. Long-term effects of CDDP on kidney function are not fully understood, but many patients develop some degree of renal impairment with permanently reduced GFR, sometimes subclinical, from which they never fully recover [20, 21]. Although, acute manifestations and renal failure are very important aspects, subclinical reduced kidney function is also a relevant issue as, in association with pre-existing or future pathologies, or simply with ageing, this can lead to complications ending in morbidity and mortality promoted, in first instance, or enhanced by CDDP.

Deepen the knowledge of CDDP-induced nephrotoxicity is, therefore, essential in many aspects of different scopes, including clinical medicine, biomedical research or veterinary practice, but especially in the fields of oncology and nephrology. With this review we aim to provide an integrative view to facilitate a better understanding of this complex and multifactorial pathology. Moreover, we intend to describe and highlight the importance of microRNAs (miRNAs) in CDDP-induced nephrotoxicity. miRNAs constitute a rapidly expanding field which is gaining attention as a possible resource of biomarkers, prevention and better understanding of many disorders. Gathering the knowledge acquired to date in relation with CDDP could help to understand many aspects of this particular pathology. In short, a comprehensive knowledge of the basis of CDDP nephrotoxicity and the involved molecular pathways, together with latest *in vivo* and *in vitro* findings is needed to deal with CDDP-nephrotoxicity progression, prevention and possible interactions with pre-existing or future additional pathologies in the patients, as well as better management of the side effects during chemotherapy regimens.

2. CISPLATIN CYTOTOXIC FEATURES

CDDP toxicity has been attributed to a number of molecular processes: DNA damage, mitochondrial damage, caspase activation, formation of reactive oxygen species (ROS), apoptosis and necrosis, as well as inflammation. Unlike most chemotherapeutic drugs, CDDP is a simple inorganic molecule, with a chemical structure consisting of a central platinum ion linked to 2 chloride ions and 2 ammonia molecules. The cytotoxicity induced by this platinum compound does not result from the heavy metal itself but is due to highly reactive metabolites into which CDDP is converted after entering the intracellular environment. The formation of CDDP reactive form is determined by the concentration of chloride ions, which promotes or not an aquation of the drug. Under high concentration of chloride the aquation reaction does not take place, and CDDP remains neutral. This is what happens in the extracellular fluid and this is why CDDP remains unaltered in the bloodstream. However, chloride concentration is lower in the intracellular environment and therefore as soon as CDDP enters into the cell it is converted into a highly reactive form in which one or two of its chloride ligands are replaced by water molecules or hydroxyl ligands.

CDDP aquated forms are positively charged molecules and can easily react with a number of molecular targets, modifying the structure and correct functioning of the same. Among its molecular targets are included the nucleophilic sites of intracellular macromolecules with which CDDP interacts to form DNA, RNA and protein adducts [22]. The interaction of CDDP (in its highly reactive form) with the genomic DNA leads to the formation of inter- and intra-strand crosslinks which distort the duplex structure and replication and transcription processes with the consequent genotoxic stress, cell cycle arrest and cell death induction [2, 23, 24]. Although highly detrimental when it occurs in healthy cells, the ability of CDDP to induce damage of nuclear DNA and generate genotoxic stress is actually crucial for its therapeutic properties in cancer treatment [23-25]. In fact, proliferating tumor cells are especially susceptible to DNA damage and, given the effectiveness of CDDP, this is one of the reasons why, during many years, its chemotherapeutic effects have been mainly attributed to the formation of strand crosslinks [26]. Moreover, this trend has been supported by findings that show cells with deficient DNA repairing processes to be more sensitive to CDDP cytotoxic effects [27]. However, other investigations have suggested that mitochondrial DNA and other mitochondrial targets might be even a more usual location of CDDP binding due to its lower repairing ability [28-30]. This idea is also supported by the small amount of cellular platinum (<1%) that is found binding to genomic DNA, with a poor correlation between the degree of DNA platination and cells sensitivity to CDDP-induced cell death [31]. Moreover, the supremacy of DNA damage as the main cause of CDDP cytotoxicity was further challenged with the results of Mandic *et al.* who demonstrated that CDDP-induced apoptosis occurs independently of genomic DNA damage by using enucleated cells [32]. The mitochondria are a negatively charged organelles, thus the positively charged CDDP reactive form would preferentially accumulate within it, an idea supported by the correlation between CDDP sensitivity and the density of mitochondria [33] as well as the organelle membrane potential [34].

In any case, many different signaling pathways are activated by CDDP, not only in the kidneys, but also in other organs, inducing tissue damage through diverse mechanisms. Although nephrotoxicity is the most important side effect, CDDP is related to a number of systemic toxicities. Among them are stand out the induction of neurotoxicity, ototoxicity, myelosuppression or gastrointestinal toxicity. These toxicities are dose-dependent and, therefore, CDDP dosage during the treatment of cancer patients is challenge minimizing side effects without compromising the effectiveness of the therapy.

3. CDDP-INDUCED NEPHROTOXICITY

Nephrotoxicity is an unconventional side effect of chemotherapy in general, as most of the used drugs are intended to target proliferating cell pathways. Renal tubular cells are quiescent cells, but despite this, kidneys are especially affected by CDDP.

By definition, nephrotoxicity is the development of functional and/or morphological kidney damage after exposure to certain treatments, specific drugs or exogenous toxins. Among the functional consequences of a nephrotoxic insult commonly are found tubular or glomerular dysfunction, with

a clear impact on GFR, loss of blood pressure control, and/or impairment in the renal endocrine function.

Since the introduction of CDDP in the clinical field, its nephrotoxic effects showed up as a very important issue. Many efforts have been invested over the ensuing years to try to find out equally effective but less toxic compounds that could replace CDDP. However, at present no suitable substitutes have been found and, therefore, CDDP remains being broadly used.

CDDP-induced nephrotoxicity is a complex multifactorial process which occurs in a number of different species including dogs, rats, mice and humans. The first evidence of kidney damage in animal models due to CDDP exposure, with characteristic morphological changes in the renal tubules, was found in 1971 [35]. Since then, lots of studies have tried to decode the complexity of CDDP nephrotoxicity and, although the details have not been fully elucidated, the research of the last decades has shed light on many aspects of CDDP mode of action in the renal tissue. (Table **1**) depicts the commonly used *in vitro* and *in vivo* models used to study CDDP-induced nephrotoxicity.

CDDP is primarily excreted by the kidneys where it is accumulated during the excretion process to a greater degree than any other organ. Its accumulation takes place preferentially in the proximal tubular cells (PTCs) of the S3 segment of the renal proximal tubules (PTs). As a consequence, this is the most affected part of the kidney. It also accumulates in the distal collecting tubule and the S1 segment of the PT in a lesser degree [36]. This accumulation of CDDP by proximal tubular epithelial cells is so disproportionate that it exceeds approximately 5 times the serum concentration [37, 38] and, therefore, even non-toxic CDDP levels in blood can reach toxic levels in the kidneys [39, 40].

This accumulation capacity has caught the attention of the scientific community for many years and led to numerous studies focused on unraveling the uptake mechanisms into PTCs. Two different membrane transporters have been identified in CDDP uptake processes: Ctr1 and OCT2.

More than 10 years ago, Ishida *et al.* [41] showed that deletion of the high-affinity cooper transporter Ctr1 in yeast leads to a decrease in CDDP cellular accumulation, which relates to a higher resistance against CDDP toxic effects. They further confirmed the role of Crt1 in mammalian cells, specifically in mouse cell lines lacking one or both mCtr1 alleles, obtaining similar results as in yeast [41]. Although *in vitro* down-regulation of Ctr1 in renal cells leads to the same observations, its *in vivo* effects are still unknown.

Organic cation transporters (OCTs) have been considered as the other major transport system in CDDP. Specifically, OCT2, which is mainly expressed in kidneys in contrast to the transporter OCT1, which is more commonly expressed in liver [2]. This differential expression of OCTs among tissues might explain, at least partially, the distinct specificity and sensitivity to CDDP between different organs. The critical role of OCT2 in renal CDDP uptake was revealed by Ciarimboli *et al.* [42] in isolated human PTs by studying the uptake of the fluorescent organic cation 4-[4-(dimethylamino)styryl]-*N*-methylpyridinium during CDDP exposure. It has been also demonstrated that CDDP uptake by renal tubular cells in culture is reduced by cimetidine, an OCT2 inhibitor [2, 42]. These findings are further supported by the decreased CDDP uptake found in isolated PTs from human diabetic kidney, which are known to have a lower OCT2 expression due to diabetes [2] , and the increased uptake and CDDP toxicity in human PTCs overexpressing OCT2 [42].

Moreover, *in vivo* experiments revealed that OCT1/OCT2-deficient mice were protected against CDDP-induced tubular injury [43, 44].

The morphological changes induced by nephrotoxic events range from microscopic lesions, such as tubular dilatation and glomerular abnormalities, to macroscopic changes, as cysts development. CDDP effect is mainly seen, at histological level, as tubular damage. This includes tubular dilatation and degeneration/necrosis, formation of hyaline casts, karyomegaly, basophilia, and loss of brush border of epithelial cells as well as sloughed necrotic cells and debris in the tubular lumen.

The damage to renal tubules results in defective reabsorption, which ultimately translates into changes of classical blood parameters, with increased levels of sCr and BUN in serum, impaired renal function, as reflected by a lower GFR, as well as other physiological alterations such as hypomagnesemia and hypokalemia.

4. SIGNALING PATHWAYS IN CDDP-INDUCED NEPHROTOXICITY

When renal tubular cells are exposed to CDDP, several complex pathways are activated and lead to damage. In addition, due to the nephrotoxic insult induced by CDDP, a strong inflammatory response takes place and exacerbates the injury in the renal tissue. Many signaling cascades have been suggested to play critical roles in CDDP-induced nephrotoxicity. These signaling pathways include the following:

4.1. Cell Death Mechanisms

Tubular cell death is a common histopathological hallmark of renal tissue damage in CDDP-induced nephrotoxicity. During CDDP exposure, both, apoptosis and necrosis mechanisms are found to occur in the renal tissue [45]. It has been suggested that the cell death pathway, apoptotic or necrotic, followed by renal tubular cells depends primarily upon the extension and severity of the nephrotoxic insult. A possible relationship between cell death mechanisms and the dose of CDDP has previously been reported [46]. It is thought that an extensive renal injury due to high CDDP dosages can lead to necrosis of PTCs, whereas, lower doses are found to be associated with programmed or apoptotic cell death and lesser renal damage. Both mechanisms have been found to be activated in several animal models of CDDP-induced nephrotoxicity [47-49]. Moreover, a reduction in the tubular cell apoptosis and necrosis during CDDP-induced damage has previously been observed by knocking out the apoptosis genes [50].

Accumulating evidence favors the use of PTCs for the systematic investigation of CDDP-induced cell death, as it is in PTs where apoptosis mainly takes place [50]. In the past

Table 1. Overview of *in vitro* and *in vivo* models used to study the key factors in CDDP-induced nephrotoxicity. 'PTs', 'PTCs' and 'CDDP' denote proximal tubules, proximal tubular cells and Cisplatin, respectively.

Experimental Models	Approaches	Observations	References
In vitro models use to study CDDP-induced nephrotoxicity			
Yeast	Deletion of Ctr1 transporter to track changes in CDDP uptake	Increased CDDP resistance and reduced intracellular accumulation	[41]
Mouse cell lines	Lack of Ctr1 alleles to study changes in CDDP uptake	Increased CDDP resistance and reduced intracellular accumulation	[41]
Human PTs from healthy kidneys	Cimetidine OCT2 inhibition to track changes in CDDP uptake	Decreased CDDP uptake	[43]
Human PTs from diabetic kidneys	Reduced OCT2 expression due to diabetic condition	Decreased CDDP uptake	[2, 43]
TNFR1-deficient cells	Ablation of TNFR1 gene	Increased resistance to CDDP-induced cell death	[53]
Fas-mutant cells	Effect of Fas gene ablation on the Fas-mediated apoptotic pathway	Increased resistance to CDDP-induced cell death	[53]
Rabbit PTCs	p53 inhibition	Decreased CDDP-induced apoptosis	[58, 68, 71]
In vivo models use to study CDDP-induced nephrotoxicity			
TNFR1-deficient mice TNFR2-deficient mice	Ablation of TNFR1 or TNFR2 gene to study changes in CDDP pathogenesis	Amelioration of CDDP-induced renal failure	[53, 86]
TNF-α-deficient mice	Effect of TNF-α deficiency in the activation of cytokines due to CDDP	Resistance to CDDP-nephrotoxicity	[2, 52]
Fas-mutant mice	Ablation of Fas gene to reveal its involvement on CDDP-induced cell death	Diminished CDDP-induced cell death and renal dysfunction	[53]
Bax-deficient mice	Bax gene knocked out to determine the pathological role of Bax	Decreased apoptosis and tissue damage induced by CDDP	[50]
JNK inhibition rat model	Inhibition of JNK using SP600125	Reduction of the apoptotic cell death and inflammation due to CDDP	[88]
OCT1/OCT2-deficient mice	Role of OCTs in CDDP oto- and nephrontoxicity	Reduced CDDP toxicity	[43, 44]
nu/nu mice (T-cell deficient)	Possible protective effects of CD+4CD25+Treg cells	Attenuation of CDDP-induced renal dysfunction and tubular injury, and increased survival	[9]
CD4- or CD8-T-cell-deficient mice	Role for T-lymphocytes on CDDP-induced AKI	Attenuation of renal dysfunction after CDDP administration	[101]

few years several studies have focused on programmed cell death, characterized by nuclear and cytosolic shrinkage of renal tubular cells during CDDP exposure. This has led to the identification of numerous apoptotic pathways involved during CDDP nephrotoxicity. Of special importance in this pathology are the extrinsic pathway, the intrinsic or mitochondrial pathway and the endoplasmic reticulum (ER) stress pathway.

The extrinsic pathway for programmed cell death is headed by the members of death receptor family, which includes Fas, TNFR1, TNFR2, DR3 and DR4/5, among others. The interaction between these transmembrane receptors and their ligands leads to the transduction of intracellular signals directing ultimately to cell death. The signal transduction in this pathway involves the activation of several caspase cascades, such as caspase-8, to induce apoptosis [51]. A number of studies demonstrated that CDDP contributes to the up-regulation of death receptors and their ligands, increasing therefore the proportion of cells that die through apoptosis during its nephrotoxic insult. More than 10 years ago, Ramesh and Reeves [52] revealed that CDDP induces TNF-α *in vivo*. This cytokine plays an important role during CDDP nephrotoxicity, not only in terms of cell death induction, but also in relation to inflammation.

The role of TNF-α in CDDP-induced damage was revealed by using pharmacological and genetic TNF-α inhibi-

tors, an approach that produced a reduction in some cytokines and chemokines [2]. The diminished production of cytokines and chemokines results in an amelioration of the inflammatory response and, ultimately, a reduction of CDDP-induced damage.

As already mentioned, CDDP is able not only to up-regulate ligands as TNF-α but also its corresponding receptors. Regarding TNF-α receptors, both, TNFR-1 (p55) and TNFR-2 (p75), are up-regulated after CDDP exposure [49], situating these transmembrane receptors as important mediators of CDDP-induced kidney injury. In fact, renal tubular cell apoptosis and renal failure due to CDDP is ameliorated in TNFR1-deficient cells and mice [53], suggesting a key role of the signaling pathways driven by TNFR-1. Further *in vivo* studies showed that TNFR2-deficient mice experienced a lower severity of CDDP effects when compared to those mice with a deficient expression of TNFR1 [49]. Therefore, TNFR2 might have a more important role than TNFR1 in CDDP-induced renal dysfunction. As for TNF-α and its receptors, the elevated expression of Fas and its ligand has also been found to take part in the cellular death through apoptosis in cultured human PTCs exposed to CDDP [54].

The intrinsic pathway involves non-receptor-mediated signaling and occurs mainly through mitochondrial paths. The activation of this pathway leads to the loss of mitochondrial membrane potential and the release of apoptogenic factors into the cytosol. The activated proapoptotic proteins translocate to the nucleus, promoting DNA fragmentation, and activate caspase cascades, leading to cell death through several pathways.

In CDDP-nephrotoxicity, the involvement of the intrinsic cell death pathway was demonstrated by Lee *et al.* [55] and Park *et al.* [56] in cultured renal epithelial cells that, after exposure to the nephrotoxicant, showed Bax activation together with cytochrome *C* release, caspase-9 activation and, ultimately, apoptosis. These observations were also reported in further studies [57, 58]. Bcl-2 family is composed of several members that prevent or promote cell death, such as Bcl-2 and Bcl-xL, or Bax and Bak, respectively [59]. The exposure of rat PTCs to CDDP leads to changes in the expression of various members of the Bcl-2 family. Specifically, Jiang *et al.* demonstrated that CDDP suppresses the antiapoptotic gene Bcl-xL while it induces the proapoptotic members Bak and PUMA-α, and confirmed the activation of Bax, with the consequent cytochrome c releasing, during the cytotoxic insult [58]. Such deregulation in the expression patterns of apoptotic genes indeed favors the activation of Bax, leading to mitochondrial injury and apoptosis.

To unmask the role of Bax in CDDP-induced cell death in *in vivo*, the use of knockout models has been crucial. Genetic disruption of Bax gene in mice generates a Bax-deficient mouse model with neither morphological nor functional differences with respect to wild-type (WT) animals [50]. Wei *et al.* confirmed the activation of Bax in the kidneys of WT animals after CDDP administration and they demonstrated a higher resistance to CDDP damage in Bax-deficient mice [50]. In the same study, it was highlighted that the attenuation of tubular apoptosis and cytochrome *C* release in primary cultures of PTCs isolated from both types of animals, WT and Bax-deficient model, after CDDP administration.

Besides changes in Bcl-2 family member expression at mitochondrial level, CDDP also induces *in vitro* the release of the apoptosis-inducing factor (AIF), an intermembrane flavoprotein [60], as well as morphological changes [61].

Caspase-12, an initiator caspase, is ER-specific [62]. Liu and Baliga carried out an investigation using proximal tubule LLC-PK1 cells in which capase-12 was activated following CDDP treatment and a significant decrease was observed in apoptosis when LLC-PK1 cells were transfected with an anticaspase-12 antibody [63]. Recently, the ER stress and associated signaling, such as caspase-12 cleavage, were reported in a rat model of CDDP nephrotoxicity [64]. A novel ER-related protein (iPLA2) which is expressed in rabbit renal PTCs has been implicated in CDDP injury and its inhibition led to an amelioration of CDDP-mediated apoptosis.

Based on these and other findings, ER-stress pathway has been suggested to contribute to the tubular cell apoptosis signaling during CDDP nephrotoxicity. However, more investigations are required to further explore the mechanism of ER-stress activation following tubular cell apoptosis during CDDP-induced nephrotoxicity.

4.2. p53 Pathway

p53, which is a tumor suppressor protein, it is activated in response to several stress signals, such as hypoxia, DNA damage and alterations of the cell cycle [65]. It shows translocation to the nucleus and overexpression in cells entering apoptosis [66]. Apoptosis can be induced by p53 by directly interacting and activating pre-existing proapoptotic molecules or by promoting the production of these proapoctotic factors through transcription of the corresponding genes [16].

In the past years, p53 has gained importance as a key factor in CDDP-induced cell death. In fact, it has already been shown that p53 is activated *in vivo* [16] and *in vitro* [60, 67, 68] after CDDP administration.

The first evidence of p53 being involved in CDDP nephrotoxicity was provided by Cummings and Schnellmann, who showed a partial suppression of CDDP-induced apoptosis in rabbit PTCs by the p53 inhibitor pifithrin-α [68]. Nowadays, it has been demonstrated that both pharmacological and genetic inactivation of p53, implies a reduction in the activation of caspase cascades and a decrease of PTCs apoptosis *in vitro* [60, 67, 68]. Similarly, it has been shown that inhibition of p53 reduces the degree of apoptotic cells and renal injury *in vivo* [16, 69]. These findings are also supported by a recent study using human renal proximal tubular epithelial cells in which it is shown how the reduction of p53 activation, induced by the flavonoid Apigenin, leads to CDDP-induced apoptosis [70].

Although, the precise mechanisms through which p53 is activated during CDDP nephrotoxicity are not fully understood, it is generally accepted that its activation may be promoted by DNA damage or genotoxic stress [2] and oxidative stress [71, 72].

4.3. Cell Cycle Pathway

A successful cell cycle progression depends upon the activation of cyclins and cyclin-dependent kinases (CDKs).

These proteins work together in G1 to initiate S phase and in G2 to initiate mitosis. During different transition phases, cell cycle inhibitors (INK4 and CIP/KIP family) are required to prevent the abnormal regulation of cyclin-CDK complexes. These inhibitors are known to be associated in controlling of G1 phase, inhibition of DNA replication as well as growth arrest [73, 74]. Previous studies have shown the role of CDKs and their inhibitors (such as p21, a well-known inhibitor of CIP/KIP family) in CDDP nephrotoxicity [48, 75, 76]. Moreover, the cross-talk between p21 and CDKs plays a vital role in deciding whether renal tubular cells are able to survive or are going into cell death. Additionally, during CDDP-induced nephrotoxicity, the quiescent cells have been found to enter cell cycle [48] and at the same time, p21 is also induced via p53-dependent and p53-independent signaling [77]. Importantly, during a comparative investigation using p21-null and WT mice, it was observed that p21 null mice are highly prone to CDDP-mediated acute renal failure [48] which further indicates that the activation of p21 mediates a renoprotective response during CDDP nephrotoxicity.

As p21 harbors several functional domains, many proteins (e.g., cyclins, CDKs, caspase-3, PCNA and c-Myc) can interact with it. Recently, a cdk2-binding domain has been identified at the amino terminal end of p21, which is responsible for a cytoprotective action. In addition, during CDDP exposure, activation of cdk2 was noted and this induction was attenuated by p21. Also, the tubular cells are protected from CDDP-mediated apoptosis via inhibiting cdk2 [78]. This suggests that cdk2 could be responsible for apoptosis of tubular cells during CDDP nephrotoxicity. These results were confirmed by Price *et al.* [75] and Yu *et al.* [76]. Notably, the embryonic fibroblasts of cdk2-null mice were found to be protected against CDDP-induced apoptosis and sensitivity could be achieved by using cdk2 transfection [75, 76]. In addition to these observations, E2F1 was identified as an important regulator of cdk2 [76]. Collectively, this evidence clearly suggests that p21 protects renal cells from CDDP-induced apoptosis by inhibiting cdk2.

4.4. MAPK Pathway

The mitogen-activated protein kinase (MAPK) signaling cascade comprises several highly conservative serine/threonine protein kinases, inducing the production of p38, ERKs (extracellular signal-regulated kinases) and JNKs (c-Jun N-terminal kinases) or SAPK (stress activated protein kinase). After activation, the MAPKs-signaling contributes in the regulation of cell regulatory processes encompassing proliferation, differentiation, migration, apoptosis, and survival [79, 80]. Using *in vitro* and *in vivo* models of CDDP nephrotoxicity, many studies have elucidated different regulatory patterns involved in the activation of ERK, JNK/SAPK and p38 pathways [2, 81]. Two ERK isoforms (ERK 1 and 2) out of 8 are usually expressed and have been extensively investigated [2, 81]. These two isoforms are stimulated by MEK1 and MEK2. The treatment of primary cultures of renal tubular cells with CDDP showed an elevation in the expression of ERK1/ERK2 and their agglomeration in mitochondria [82]. Moreover, CDDP-mediated apoptosis and mitochondrial dysfunction could be reduced by inhibiting ERK1/2 with the help of MEK inhibitors (PD98059 and U0126). It has been shown that these inhibitors abrogate caspase activation, but do not help in blocking the release of cytochrome C from mitochondria [83, 84]. Temporary transfection of MEK1 in turn resulted in increased apoptosis, while dominant-negative MEK1 reduced apoptosis in renal tubular cells induced by CDDP [84]. Moreover, an early activation of ERK, p38 and JNK/SAPK by CDDP induced the development of acute renal injury and renal failure [83]. Recently, activation of ERK in CDDP nephrotoxicity was confirmed using MEK inhibitor [85].

So far only a few studies have deciphered the role of p38 and JNK/SAPK in CDDP nephrotoxicity. Ramesh *et al.* [86] and Mishima *et al.* [87] demonstrated that p38 is not directly involved in the regulation of renal tubular cell injury and death; however it may mediate the expression of TNF-α in tubular cells which further may result in an inflammatory response during CDDP nephrotoxicity. The authors also revealed a renoprotective role of p38 inhibitors in both *in vitro* and *in vivo* models. On the other hand, in renal tubular cells and kidney tissues, it has previously been reported that CDDP induces JNK/SAPK activation [83, 88]. By treating an *in vivo* rat model with the SP600125 (JNK inhibitor), Francescato *et al.* [88] suggested that SP600125 could play a role in the reduction of renal apoptosis and inflammation during CDDP nephrotoxicity.

Recently, two independent studies have shown the renoprotective effect of stem cells [89] and Ginseng, as well as its active Ginsenosides [90] during CDDP-induced nephrotoxicity.

To date, there is no single study available which has explored the spatiotemporal activation of these three major tiers of MAPK signaling during CDDP nephrotoxicity. It is therefore of utmost importance to carry out a systematic study to analyze the spatiotemporal activation of ERK, p38, and JNK/SAPK all together in the same samples during CDDP treatment and also to find out specificity of known and novel inhibitors utilized to inhibit these three major tiers.

4.5. Oxidative Stress

Oxidative stress is also an important issue in CDDP-induced kidney damage. Reactive oxygen species (ROS) are able to interact and destroy the structure of different cellular components, such as DNA, proteins and lipids, and many of them have been found to be increased in cultured renal tubules, kidney slices, and also in *in vivo* experiments during CDDP exposure [2].

Under the pathological conditions promoted by CDDP, three different mechanisms have been considered to intervene in ROS increased production: shift of the cellular redox status due to depletion or inactivation of antioxidants such as glutathione; mitochondrial dysfunction; and, due to ROS production in microsomes via cytochrome P450.

It has been suggested that ROS play a role in mediating CDDP-induced apoptosis, but not in necrosis [91] and that superoxide radicals are involved in the acute cellular damage induced by this chemotherapeutic drug in rabbit renal cortical slices [92].

However, using porcine renal PTCs, Kruidering *et al.* [93] showed that ROS formation takes place during CDDP toxic insult but that it is not the direct cause of cell death.

More recent studies have suggested that the formation of ROS induced by CDDP depends on the concentration and duration of exposure to the drug [94]. Therefore, ROS might play an important role with respect to the severity of CDDP side effects, including nephrotoxicity, and the use of antioxidants could help to reduce the induced damage. Future investigations need to be carried out to understand the relationship between the cellular redox system and the expression of anti- and pro-apoptotic factors in CDDP-induced nephrotoxicity.

4.6. Inflammatory Response

Inflammation has been recognized as an important factor in the pathogenesis of CDDP nephrotoxicity, contributing to exacerbation of renal damage and the development of kidney failure. Initially most of the studies were focused on the direct cytotoxicity exerted by CDDP to the renal tubular cells, in the past 15 years the inflammatory response of the renal tissue started to gain more attention. Since then, several cytokines and chemokines have been found to be up-regulated in the kidney during CDDP-induced acute renal failure and many of the mediators that take part in these inflammatory events have been identified. Among them, TNF-α has been identified and well documented as a key upstream regulator [2] and as the main factor of the pro-inflammatory response due to its enhanced expression after CDDP exposure [49, 52, 86, 95].

TNF-α is a pleiotropic cytokine which activates pro-inflammatory cytokines and chemokines and recruits leukocytes during an inflammatory response. As a result, TNF-α causes oxidative stress and amplifies the damage in the renal tissue.

The mRNA expression of TNF-α has been found to be increased during CDDP-induced nephrotoxicity [52, 96] and, as a consequence, TNF-α up-regulates many other cytokines which may promote the migration of inflammatory cells to the renal tissue [52].

The role of TNF-α has been partially elucidated by inhibiting its release or activity. With these experimental approaches, it has been shown that the inhibition TNF-α notably suppresses the induction of other cytokines and provides protection against CDDP nephrotoxicity both *in vivo* and *in vitro* [52, 97-100]. The role of TNF-α in CDDP pathogenesis has been further investigated *in vivo* using TNF-α-deficient mice [2, 52]. In both cases, inhibition and TNF-α deficiency, diminished renal injury and dysfunction as well as a reduction of the histological changes due to CDDP has been reported.

The mechanisms underlying TNF-α induction due to CDDP are complex *per se*, and the fact that different cells can produce TNF-α in the kidney, makes it more complicated to understand the complete network involved. On one hand, it has been shown that, during CDDP nephrotoxicity, TNF-α is produced mainly by resident kidney cells rather than by infiltrating inflammatory cells. This fact was demonstrated in a chimeric mice model which underwent ablation of the bone marrow and posterior replacement of the same with donor bone marrow cells from WT or TNF-α knockout mice [99]. However, on the other hand, more recent studies have also demonstrated that T cell-deficient *nu/nu* mice exhibit attenuation of CDDP-induced injury when compared to WT animals [9] and that the reconstitution of T cells in these animals leads to an increase in both renal dysfunction and TNF-α production [101]. Therefore, T cells also play an important role in CDDP-induced renal inflammation.

Together with macrophages and other inflammatory cells of the immune system, T cells infiltrate the damaged renal tissue. They are known to have a pathophysiological role in the development and progression of CDDP-induced injury. Liu *et al.* demonstrated that CD4 T cell-deficient mice exhibit a better renal function than WT after CDDP administration [102]. This finding suggests that $CD4^+$ T cells constitute the main T-cell subset in the mediation of CDDP-induced damage. It also has been shown that $CD4^+$ $CD25^+$ regulatory T cells, which can suppress $CD4^+$ T cell-promoted renal pathology, significantly attenuate CDDP-induced nephrotoxicity *in vivo* [9]. Despite these important findings, the mechanisms underlying the T cells enhancement or protection against CDDP renal damage remains to be determined.

Several other approaches have been studied in order to reduce the nephrotoxic effects by regulating the inflammatory response induced by CDDP. A recent study has demonstrated how the use of C-type natriuretic peptide, which exhibits anti-inflammatory properties, reduces the renal tubular damage and apoptosis due to CDDP administration [103]. Hence, a better understanding and management of the inflammatory response caused by CDDP can help to develop new therapeutic approaches.

5. CDDP RESISTANCE

In general, CDDP resistance is caused due to the alterations of a number of factors which have previously been well categorized by Galluzzi *et al.* [104]. These alterations can mediate pre-, on-, post- and off-target resistance. The pre-target resistance usually occurs from the alterations in transporters (such as Ctr1, ATP7B, MRP2 and ATP11B) which support CDDP uptake into the cells and its export [41, 105-109]. Several studies have already established an association between changes in these transporters and CDDP resistance in cancer patients as well as in preclinical models [41, 105-109].

On-target resistance encompasses those molecular injuries which are directly induced by CDDP; here, the DNA repair system (NER and MMR) is considered to play a crucial role in rectifying CDDP induced DNA damage [110-112]. Genes (e.g., ERCC1, MSH2 and MLH1) encoding for NER and MMR are commonly deregulated or mutated in CDDP resistance [113-115]. Moreover, an elevated CDDP sensitivity has been shown to be associated with TLS polymerases including polymerase, POLH and REV3L [116-119]. Interestingly, molecules engaged with homologous recombination (e.g., BRAC1/2) and cytoplasmic components encompassing cytosolic (myosin, Ila, HSP90), ribosomal (for example, RPL5), reticular (e.g., calreticulin) and mitochondrial components (such as VDAC1) have recently been identified as binding partners of CDDP [120-122]. In addition, CDK2 has been demonstrated to mediate extranuclear CDDP toxicity (via stimulating ER stress, but in some models) and CDK2 knock out cells show CDDP resistance [123, 124].

The post-target resistance may develop because of alterations in the mechanisms which play an important role in recognizing and transforming CDDP induced molecular damage into a lethal signal and in the machinery that directs cell death [125]. As mentioned previously apoptosis and necrosis which are the cell death mechanisms, are supervised by a number of checkpoints and safeguard mechanisms. For example, this class of resistance seems to be significantly affected by both the expression patterns and functions of the representatives of Bcl2 family and caspases which are involved in apoptotic cell death [126], however, not all, but only a few of these candidates have been correlated with CDDP resistance in clinical studies including BCL2, BCL-XL, MCL1, surviving and BIRC protein family [127-129]. Moreover, the post-target resistance of CDDP has been linked with genetic and epigenetic alterations which alter p53 signaling as well as with the defects that occur in many other pro-apoptotic signal transducers such as MAPK14, JNK1 [130-132].

In case of off-target resistance, the abundance of ERBB2 (commonly in breast and ovarian cancers) has been proposed to induce CDDP resistance by providing pro-survival signaling via AKT1 signaling and by temporarily arresting the cell cycle to allow the repairing of CDDP-directed DNA lesions [133-135]. Moreover, MIRK seems to exert CDDP resistance as it supports in the regulation of several antioxidant enzymes [136]. In a recent study, TMEM205 has been found to favor CDDP resistance through a molecular signaling involving RAB8A [137].

Numerous studies have been conducted by adopting different large-scale approaches to cope with the complex issues as well as to get deep insights into CDDP resistance. These large-scale studies are well documented in a review article by Galluzzi *et al.* [104]. For example, Zeller *et al.* [138] applied a system biology approach encompassing a methylomic and a transcriptomic component and identified nine down-regulated genes in A2780 cells and in clinical specimens from ovarian carcinoma patients. The MLH1 out of these 9 genes is often mutated or down-regulated during CDDP resistance [113, 139-141]. Moreover, several microRNAomic profiling investigations among CDDP-sensitive and CDDP-resistant cancer cell lines have recently been performed in which down-regulation of many miRNAs which inhibit the expression of Bcl2-like proteins was identified as an important event in CDDP resistance [142, 143]. In another study, Chavez *et al.* [144] considered a quantitative proteomic approach in combination with network analysis and detected that the level of at least 374 proteins is altered in CDDP-resistant cervical carcinoma HeLa cells. These proteins are the associative members of metabolic pathways, DNA repair and other stress response mechanisms.

6. MICRORNA REGULATION DURING CDDP-INDUCED NEPHROTOXICITY AND ITS USE AS BIOMARKERS

The classical pathology markers are usually insensitive, as many of them only show changes after extensive kidney injury, and are unspecific, since many other physiological processes may modify their basal levels. This lack of specificity and sensitivity can lead to false positive and false negative results, leading to delayed detection of renal injury and/or misleading conclusions.

sCr is one of the most used indicators to detect renal damage. Despite its widespread use, it is well known that sCr is not even close to the optimal renal marker. sCr shows a pronounced insensitivity, showing no significant changes in serum levels until approximately half of the nephrons are lost [145]. Together with sCr, BUN is used also as functional marker and both of them are influenced by several physiological mechanisms and processes, such as synthesis and degradation of proteins [145], dehydration [146] or rhabdomyolysis [147], among many others [145, 148].

N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyl transpeptidase (GGT), both urinary enzymes, have also been used as markers of renal toxicity, but, again, they are unspecific, variable, and instable [149].

A number of biomarkers have been proposed as more appropriate markers of renal damage, being more sensitive and specific, with the possibility to be associated with different areas of the kidney depending of the localization of the lesions. Among them are found KIM-1, clusterin, β2-microglobulin, TFF3, cystatin C, RPA-1, NGAL and α-GST [145, 148, 150-153].

Among all the biomarkers for renal injury proposed in the recent years, one of the most promising are the miRNA. The high conservation between different species makes them even more suitable than many other biomarkers in terms of translating the preclinical findings into the clinical field.

miRNAs are representatives of a class of evolutionary conserved short noncoding single-stranded RNA molecules which are largely known to negatively regulate gene expression by annealing to 5' untranslated regions (UTRs) [154, 155], coding sequences [156-160], and/or 3' UTRs of target messenger RNAs (mRNAs), leading to translation inhibition or mRNA degradation [161]. More than 2,580 human miRNAs have been documented in the latest version (Release 21) of miRBase [162]. The possible binding site prediction analysis estimates that approximately 60% of human mRNAs could be modulated by miRNAs [163]. This suggests that each of these tiny regulators (miRNAs) is capable of base-pairing with hundreds of mRNAs [163].

The biogenesis of miRNA takes place in multiple steps and these steps have been thoroughly studied [164-167]. Concisely, the canonical miRNA biogenesis pathway starts with the Drosha protein which processes the long primary miRNA transcript (pri-miRNA which is initially transcribed by RNA polymerase II) into a hairpin stem-loop structure termed precursor miRNAs (pre-miRNAs) in the nucleus [168, 169]. Then, the pre-miRNA is transported into the cytoplasm with the help of Exportin-5 and Ran-GTP, where it transforms into a double-stranded ~22 nucleotides (nt) miRNA by Dicer [168, 169]. One strand of the mature miRNA is loaded into the RISC and base-pairs to the mRNA 3' UTR, whereas, the other strand (miRNA*) is degraded.

Over the past decades significant efforts have been made to improve our knowledge on the miRNA-mediated regulation and to identify miRNAs that are associated with various biological regulatory pathways (e.g., development, differentiation, cell cycle, apoptosis, p53 signaling, MAPK and in-

flammation) and human diseases (such as kidney diseases and cancers). These attempts can be broadly divided into two classes: computational approaches and experimental techniques. A dozen of computational approaches have been developed to generate possible miRNA-target interaction information and these algorithms [170-173]. Only miRWalk [174] and miRWalk2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) [175] have developed to offer several novel and unique features on miRNA interactions. These two databases can be considered as "next generation database of miRNAs" due to their design and aiming to fulfil the current requirements of the miRNA research community. For example, one of the extensively utilized features of these databases is a novel comparative platform of miRNA binding sites predicted by different algorithms within the promoter, cds, 5' and 3' UTRs, and miRNA-miRNA interactions [176-181]. This feature enables users to access miRNA binding sites within the complete sequence of a gene and with other miRNAs. The experimental techniques [81, 158, 182-190] encompassing high-throughput methods (such as microarrays and deep sequencing studies), pre-miRNA transfection, overexpression or inhibition of miRNA and PCR experiments, enable researchers to support the prediction data-sets as well as to elucidate miRNAs that are involved in diverse pathophysiological conditions for example CDDP-induced nephrotoxicity.

In order to collect information on miRNA interactions associated with CDDP, we performed a text-mining search (with the help of a customized query: cisplatin[TIAB] AND (microRNA[TIAB] OR miRNA[TIAB]) on 6th March 2015 in the titles/abstracts of articles documented in PubMed. A total of 204 studies (including 4 review articles) have been found to be associated with CDDP and miRNAs. On further dissecting this information, only 8 out of 204 studies have investigated the role of miRNAs during CDDP-induced nephrotoxicity, whereas, the remaining articles are associated with the miRNA regulation during CDDP-treatment in cancers **(Supplementary file)**. Amongst these 8 articles, 4 studies have been published in the last year (2014), while the remaining 4 investigations are documented in 2010, 2012 and 2013. These observations suggest that the scientific community involved in CDDP-mediated nephrotoxicity has started focusing on the direction of exploring the role of miRNAs and this field is moving slowly but progressing. The next section will therefore summarize the key findings of the 8 aforementioned investigations to improve our understanding in the field of CDDP nephrotoxicity.

In 2007, various studies demonstrated a regulatory connection between miR-34a and p53 during DNA damage and suggested that this miRNA might induce apoptosis [191-194]. The activation of p53 has often been noticed during CDDP nephrotoxicity which in tur contributes to renal cell injury and death [193]. In 2010, Bhatt *et al.* [195] performed an investigation to elucidate the role of miRNA and p53 signaling during CDDP nephrotoxicity. The authors used *in vitro* cell culture (mouse proximal tubular cells i.e. BUMPT-306 cell line) as well as *in vivo* mouse (C57BL/6) models and successfully showed an early induction in the expression of miR-34a during CDDP-induced nephrotoxicity. The up-regulation of miR-34a was found to be p53 dependent and this increased expression could protect renal cells from CDDP-mediated apoptosis. Two years later (in 2012), Zhu *et al.* [196] treated human proximal tubular cells (HK-2 cell line) with CDDP and observed an activation in cell apoptosis due to up-regulation of miR-181a which further targets Bcl2. It has previously been observed that CDDP-activated Bax, a member of Bcl2 family, can alter the function of mitochondrial transmembrane, stimulate cytochrome C release and elicit the mitochondria death pathway [56]. Chen and colleagues found a decrease in CDDP-induced cell apoptosis after the transfection of miR-181a and they validated the interaction among miR-181a and Bcl2 using dual luciferase reporter gene plasmid. These observations suggest that the CDDP-induced up-regulation of miR-181a may result in tubular cell apoptosis via decreasing Bcl2 expression level. Recently, it has been shown that the activation of Nrf2 plays an important role to protect the kidney from CDDP-mediated toxicity by elevating the expression of miR-125b [197]. Clustering analyses (KEGG and GENEMANIA) further revealed a role of AhRR with the molecules engaged with antioxidant enzymes, p53 and its downstream targets. Moreover, it was suggested that miR-125b may control AhRR which could further serve as a key molecule that maintains the expression patterns of two separate gene clusters belonging to metabolism of xenobiotics cytochrome P450 and p53 signaling pathways. In addition, it was proposed that Nrf2 plays a crucial role to maintain a balance between AhR and AhRR expression levels by changing the expression level of miR-125b. These findings establish the missing interaction among Nrf2 and AhR which is necessary for cell survival during oxidative stress.

Using an integrative network approach encompassing microarray profiling and bioinformatics analysis, a cross-talk between seven deregulated miRNAs (up-regulated: miR-34a, and let-7g and down-regulated: miR-122, miR-10b*, miR-30e, miR-193 and miR26a) and Foxo3 which cause renal injury during CDDP treatment in mice was discovered [198]. Of these, miR-122 and miR-34a were found to play a critical role in CDDP-directed renal injury by elevating the expression of Foxo3, a key protein which activates the p53 signaling pathway [198]. The elevation of Foxo3 was also detected in the immunohistochemical analysis, suggesting that this gene may be considered as a tentative biomarker candidate of tubular cell injury. Furthermore, these results uncover a comprehensive network of miRNAs, bridging molecules and signaling cascades which regulate CDDP-induced nephrotoxicity.

More recently, two independent investigations conducted by Kanki *et al.* [199] and Pavkovic *et al.* [200] documented a non-invasive method (urine-derived miRNAs) in the CDDP-based nephrotoxicity research. Kanki *et al.* detected 78 miRNAs with significant differences in UVRQ (relative quantity normalized by urine volume) between Sprague-Dawley control groups: fed and fasted rats. On comparing control and CDDP treated rats, 38 (fed) and 49 (fasted) miRNAs were found to have significant differences in UVRQ. In order to measure the expression of potential candidates with TaqMan custom miRNA cards, 30 miRNAs with more than 2-fold changes were selected. 25 miRNAs were found as up-regulated in the urine from CDDP-treated rats. Moreover, the correlation between the elevation of these 25 miRNAs and the severity of necrosis in the proximal tu-

bules was demonstrated. On the other hand, Ellinger-Ziegelbauer and colleagues used male Wistar rats (8 weeks old) and treated them with CDDP once. To measure the expression of urinary miRNAs during CDDP-induced kidney injury using TaqMan cards, urine samples were collected on 3, 5, 8, 15 and 26 days from rats. A total of 136 miRNAs were found to have significant changes in their expressions by TaqMan card profiling and analysis with the modified ΔCt method. Eighteen out of 136 miRNAs were chosen as potential novel biomarker candidates for further examination during renal toxicity. Six (miR-15, miR-16, miR-20a, miR-192, miR-193 and miR-210) out of these 18 miRNAs were identified with a significant increase at 3 days after a lower dose of CDDP. Interestingly, it was suggested that miR-192 may be considered as an additional biomarker to predict proximal tubular necrosis with a similar sensitivity and specificity as KIM-1 (a well-known urinary protein biomarker). Moreover, the expression levels of these 18 miRNAs (except miR-34a, miR-184, miR-21 and miR-327) were slightly lower in the kidney. For example, miR-34a was always up-regulated during the entire investigation (3 mg/kg CDDP) and the expression of miR-184 was activated in kidney on day 3 with a maximum at day 5, while two miRNAs: miR-21, and miR-327 were elevated in kidney on day 8. Furthermore, using microarrays profiling, 274 (162 up- and 112 down-regulated) mRNAs were observed as differentially expressed in the kidney during CDDP administration. Functional annotation analysis revealed that up-regulated mRNAs were mainly associated with apoptosis (Bcl3, Mdm2, Cdki1), cell cycle regulation (Ccng1, Btg2) and stress response, whereas the down-regulated candidates were linked with kidney function. These observations indicate kidney injury. During miRNA-mRNA interactions analysis, 21 mRNAs were predicted as potential targets of 11 out of 18 miRNAs. These interactions were linked with p53 and PI3K/AKT pathways. Several of the differentially expressed mRNAs and miRNAs are known to be linked with acute phase, DNA damage responses, apoptosis, cell cycle and inflammation. Of note, Tp53 is recognized as key regulator of many mRNAs and miRNAs. Interestingly, two potential targets (mRNAs: Capn6 and Snca) of CDDP elevated miR-34a were down-regulated during CDDP administration, indicating the expected inversely correlated relationship between the expression patterns of mRNAs and miRNAs. In summary, these two non-invasive studies have discovered several miRNAs in the urine of CDDP-treated animals which can be utilized as novel biomarkers for predicting the severity of the damage in the kidney.

In another study the miR-155 was associated with several signaling pathways linked with apoptosis and oxidative stress, and their upstream regulators such as c-Fos. Moreover, the c-Fos (harboring two confirmed sites for miR-155) was significantly elevated in miR-155 deficient mice which suggest a novel therapeutic approach to reduce CDDP-induced nephrotoxicity by controlling the expression of this candidate [201]. Taken together, these studies have successfully added an additional regulatory layer of miRNAs and have established the critical role of these tiny regulators (miRNAs) with the previously known signaling pathways. Moreover, these observations enable researchers to carry out further investigations for identifying the role of other miRNAs and indeed provide help through which oncologists can develop novel as well as effective treatments to reduce and/or manage CDDP-induced nephrotoxicity by monitoring the expression of one or more miRNAs.

7. CONCLUDING REMARKS

It is of paramount importance to understand the regulatory mechanism of CDDP-induced nephrotoxicity due to its high prevalence among cancer patients who undergo CDDP treatment. Moreover, a better understanding of the processes induced by CDDP, leading to tubular injury and loss of renal function, can help to prevent this renal pathology.

Over the last decades, many *in vivo* and *in vitro* studies have discovered relevant candidate genes, proteins and their pathways (cell death, cell cycle, apoptosis, MAPK, oxidation and inflammation) whose deregulation leads to CDDP-induced nephrotoxicity. Additionally, the knockout and/or inhibition investigations have been performed using pharmacological inhibitors which reveal the critical role of these candidate genes/proteins and their associated pathways. However, further high-throughput clinical studies are required to explore the spatiotemporal effects of these inhibitors before considering them for the treatment of cancer patients. Interestingly, a novel layer of regulators (miRNAs) is recently integrated in the genetic mediation of CDDP nephrotoxicity. Interestingly, miRWalk and miRWalk2.0 enable the scientific community to collect information on predicted as well as validated miRNA-target interactions.

In two recent studies, a noninvasive approach has already been introduced to discover novel miRNA biomarkers from the urine-derived genetic materials under different conditions (with and without CDDP treatment). Therefore, future miRNA-based studies will positively contribute to broaden the knowledge of CDDP-induced nephrotoxicity, which will provide an indisputable help to oncologists for correlating the renal damage by measuring the expression level of one or more miRNAs and offer a guidelines when to stop CDDP treatment to restore normal renal function and/or to prevent renal tubular damage in cancer patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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