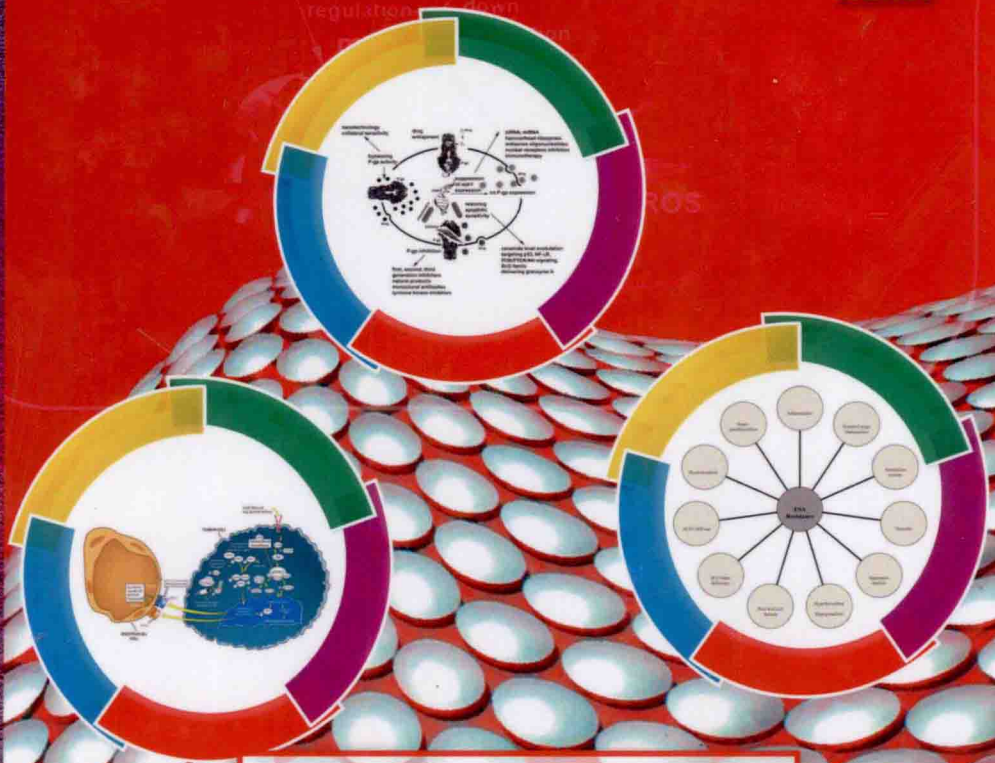
Advances in Drug Resistance Research

Christudas Morais
Editor



Public Health in the 21st Century
Michael W. Popejoy (Series Editor)

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Preface

Advances in the understanding of the molecular mechanisms of various diseases have helped the development and implementation of novel therapeutics in clinical practice. Unfortunately patients develop resistance to chemotherapeutics. There are two types of resistance to drug treatment, intrinsic and extrinsic. Intrinsic resistance commonly refers to the innate insensitivity of cells to drug treatment. In contrast, in extrinsic or acquired resistance, the cells are initially sensitive to treatment, but progressively become insensitive. Extrinsic resistance often correlates with time. This book examines the biochemical and molecular mechanisms of innate and extrinsic drug resistance and their clinical relevance. The nine chapters in this book, written by a group of scientists and clinicians, provide an excellent synopsis of the advances in our understanding of drug resistance.

Chapter 1 gives an in-depth view of mechanisms of resistance to cisplatin. In particular, this chapter focuses on the role of deregulated DNA damage repair process, suppressed apoptotic signalling, and clonality as means of developing cisplatin resistance.

Chapter 2 discusses the role of p-glycoprotein and multi-drug resistance molecules, especially the ABC transporters, in drug resistance. It also focuses on the advances made in the field of natural products as effective chemotherapeutics and how they can be used to overcome drug resistance.

Chapter 3 summarizes the different types and origins of fibroblast population that exist within solid tumors, their roles in dictating the response of tumors to therapies and the underlying mechanisms by which the dynamic tumor-fibroblast cell interaction provide a protective niche for tumor cells to escape from cytotoxic effects of chemotherapy.

Chapter 4 gives a comprehensive review of resistance to erythropoiesis stimulating agents (ESA), especially erythropoietin (EPO). EPO is administered to correct anemia in patients with chronic kidney disease. This chapter discusses the causes, epidemiology, clinical sequelae and management of ESA resistance.

Chapters 5 and 6 address the challenges of drug resistance in prostate cancer. Because prostate cancer cells depend on androgen and its receptor for their growth and survival, targeting the androgen signalling has been the major avenue of treatment. However, resistance to therapy continues to be one of the major hurdles for the successful treatment of prostate cancer. These chapters discuss the findings and limitations from ongoing and completed clinical trials, potential mechanisms of therapy resistance, approaches to overcome resistance and future challenges.

Chapters 7-9 cover a number of issues related to drug resistance in renal cell carcinoma. Until the introduction of targeted therapy, immunotherapy used to be the mainstay of treatment for metastatic renal cell carcinoma. Chapter 7 presents an overview of chemotherapy and immunotherapy resistance in metastatic renal cell carcinoma. Despite the initial clinical benefits of targeted therapies, resistance develops after a median of 5–11 months of treatment. Chapter 8 summarizes the current challenges of drug resistance in the effective treatment of metastatic RCC. Chapter 9 specifically focuses on the extrinsic and intrinsic mechanisms of sunitinib resistance in renal cell carcinoma.

It has been a pleasure working with the authors. I am grateful to all the authors for their generosity in sharing their expertise and experience to the scientific community through this book. I am confident this book will be an important contribution to science, and an invaluable tool for researchers who are interested in drug resistance.

Christudas Morais, MSc, MPhil, PhD

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Chapter 1

Mechanisms of Cisplatin Resistance: DNA Repair and Cellular Implications

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Abstract

Cisplatin (cis-diamminedichloroplatinum (II)), is a platinum based chemotherapeutic employed in the clinic to treat patients with lung, ovarian, colorectal or head and neck cancers. Cisplatin acts to induce tumor cell death via multiple mechanisms. The best characterized mode of action is through irreversible DNA cross-links which activate DNA damage signals leading to cell death via the intrinsic mitochondrial apoptosis pathway. However, the primary issue with cisplatin is that while patients initially respond favorably, sustained cisplatin therapy often yields chemoresistance resulting in therapeutic failure. In this

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chapter, we review the DNA damage and repair pathways that contribute to cisplatin resistance. We also examine the cellular implications of cisplatin resistance that may lead to selection of subpopulations of cells within a tumor. In better understanding the mechanisms conferring cisplatin resistance, novel targets may be identified to restore drug sensitivity.

Keywords: Cisplatin resistance, interstrand cross-links, DNA repair, tumor-initiating cell

Introduction

Cisplatin, (cis-diamminedichloroplatinum (II), or CDDP) was first approved for clinical use in 1978 for treatment of testicular and bladder cancers [1]. The observed anti-tumor effects have led to the use of cisplatin as a frontline chemotherapeutic in the treatment regimens for patients with lung, ovarian, colorectal or head and neck cancers [1-3]. While still clinically relevant today, cisplatin has also spawned the platinum-based drug derivatives carboplatin and oxaliplatin. Patients treated with these platinum-based drugs display early therapeutic success with disease regression or a halt in further progression. As with many chemotherapeutic options, adverse events exist for cisplatin with observed kidney, peripheral nerve and inner ear toxicity [4, 5]. Yet the primary issue with cisplatin therapy is that despite initial therapy successes, many patients eventually develop resistant disease [6-8]. Notably, cisplatin refractory disease is also cross-resistant to carboplatin [9]. Given that cisplatin based therapies remain the sole therapeutic option in certain circumstances [10], it is key that the underlying mechanisms potentiating resistance are uncovered.

Mode of Action

As a platinum based compound, cisplatin is inert but is activated through a series of aquation reactions. The aquation process involves the spontaneous substitution of one or both cis-chloro groups with water molecules in the cytoplasm [11, 12]. This process makes aquated cisplatin highly reactive toward intracellular nucleophiles [13]. It is this process that yields the anticancer effects of cisplatin through reactions within the cytoplasm and

nucleus. Within the cytoplasm, cisplatin reacts with nucleophiles such as methionine, metallothioneins, reduced glutathione (GSH) and proteins via cysteine residues. In this way, cytoplasmic cisplatin may act like a molecular sponge for reduced species and push a cell towards oxidative stress. Recent evidence that enucleated cytoplasts are responsive to cisplatin further highlights the cytotoxic effects of the cytoplasmic cisplatin module [14, 15]. However, these cytoplasmic reactions may also serve to inactivate cisplatin through antioxidant systems [7].

The best characterized mode of action for cisplatin is via its reactivity with DNA. In the nucleus, aquated cisplatin interacts with high affinity toward nucleophilic N7-sites of purine bases to form interstrand and intrastrand DNA cross-links [16]. However, the cytotoxic effects of nuclear cisplatin are largely caused by the intrastrand DNA cross-links which account for 90% of cisplatin-induced DNA lesions [17, 18]. These lesions distort the DNA structure allowing multiple DNA damage signals to be initiated [19]. One of the initiated signals involves the nucleotide excision repair (NER) complex. This system recognises the distorted DNA and attempts to remove adducts to enable cell survival [20]. Other DNA damage signals involve the mismatch repair (MMR) and base excision repair (BER) systems. Herein, the components of these complexes sense the DNA lesions and commence the signals instructing cell fate [21-23]. Hence, the cell will initially attempt to repair the cisplatin-induced DNA lesion through one of the listed DNA repair mechanisms (see above). However, the cytotoxicity of cisplatin relies on overwhelming incidence of the adducts, as well as the high proliferative rate of the tumor. This leaves the repair of DNA largely unattainable. As a result, cell death will ensue predominantly via the apoptotic cascade.

The key link between DNA damage and apoptosis involves the ataxia telangiectasia mutated (ATM)-and Rad3-related (ATR) kinase and the checkpoint kinase 1 (CHK1). Cisplatin-dependent sequential activation of ATR and CHK1 leads to activation and stabilization of tumor suppressor p53 through phosphorylation at serine 20 [24-27]. p53 is the central mediator of cisplatin-induced apoptosis. In cisplatin-sensitive cells, p53 induces apoptosis via both nuclear and cytoplasmic mechanisms. In the nucleus, p53 transcriptionally up-regulates genes involved in cell cycle arrest and DNA repair [28] as well as multiple pro-apoptotic genes including *NOXA* [29] and *Bax* [30]. These pro-apoptotic factors are involved in initiation of the complex intrinsic mitochondrial apoptosis pathway [31]. Cisplatin induces the p53-mediated localization of both NOXA and Bax to the mitochondria yielding the release of cytochrome *c* which activates caspase-dependent apoptosis [32, 33].

In particular, the balance of cell fate is hinged upon the ratio of Bax with the anti-apoptotic Bcl-2. In cisplatin-sensitive cells, Bcl-2 is lost via proteolysis enabling activation of the apoptosis cascade [34, 35]. Interestingly, cisplatin is also reported to induce apoptosis via mitochondria independent mechanisms, but nonetheless utilizing the caspase 8-caspase 3 pathway via p53 [36, 37].

Mechanisms of Cisplatin Resistance

It is clear that multiple mechanisms are active in sensitizing cells to cisplatin. Alterations to any of these pathways underlie the cisplatin resistant phenotype observed in the clinic. As such, cisplatin resistance can ensue from (i) reduced intracellular cisplatin uptake, (ii) increased efflux of cisplatin from the cell as well as (iii) an enhanced ability of cells to repair cisplatin-induced DNA distortions. Herein, describing the mechanisms relating to cisplatin uptake and drug efflux go largely beyond the scope of this chapter but are adequately reviewed elsewhere [38, 39].

In the following sections we describe three potential mechanisms through which cisplatin resistance may occur, with an especial emphasis on processes downstream of DNA lesion formation. In this sense, we focus on the deregulation of DNA damage repair and suppressed apoptotic signalling as means of developing cisplatin resistance. Also, the role of tumor clonality in cisplatin resistance is discussed.

Deregulation of DNA Damage Repair

As discussed in previous sections, the nucleophilic attack of aquated cisplatin on purine bases can result in the formation of three major lesion types (i) bulky cisplatin adducts on singular purines, (ii) intrastrand cross-links between bases on the same strand and (iii) interstrand cross-links between bases on opposing strands (Figure 1) [40]. In each instance, the cell will initially respond by launching a DNA damage response in an attempt to remove the lesion. This response will vary depending on the nature of the adduct, as well as on the cell cycle phase. For instance, while nucleotide excision repair (NER) is likely the major pathway for removal of cisplatin bulky adducts and intrastrand cross-links outside of S phase of the cell cycle [41], translesion synthesis and/or repair by homologous recombination may represent significant pathways during replication [42]. Furthermore, while

intrastrand cross-links may be processed efficiently by translesion repair alone during replication, the overcoming of interstrand cross-links probably involves proteins of the Fanconi anemiahomologous recombination pathway (see below) [43]. While most likely representing minor repair roles, base excision repair and mismatch repair of cisplatin adducts may represent important mechanisms of 'sensing' cisplatin-mediated DNA damage [44].

Here we describe the known DNA repair pathways involved in the recognition and repair of cisplatin adducts, and discuss some of the recognised mechanisms through which resistance may occur.

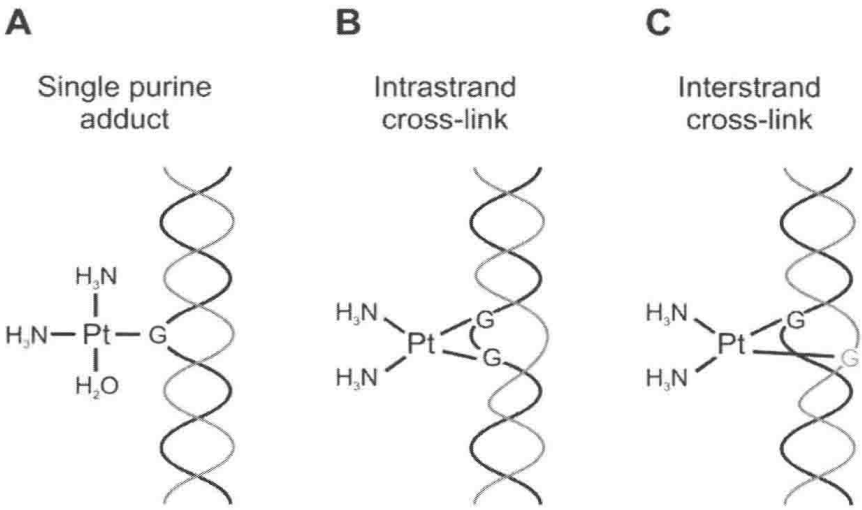

Figure 1. Cisplatin-induced DNA adducts. The predominant DNA adducts caused by cisplatin include (*A*) bulky cisplatin adducts on singular purines, (*B*) intrastrand cross-links between bases on the same strand and (*C*) interstrand cross-links between bases on opposing strands.

Mismatch Repair and Base Excision Repair

Mismatch repair (MMR) is an important cellular pathway in the correction of endogenous polymerase-mediated replication errors [22, 23]. Additionally, MMR may be involved in the repair of small DNA lesions e.g. those caused by alkylating agents [45]. In the earliest instance, base-mismatches are recognized by one of two MutS heterodimers; MutSα (MSH2/MSH6), which binds singular base mismatches or insertions, or MutSβ (MSH2/MSH3), which recognizes larger distortions [46-48]. These complexes are then able to recruit one of three MutL complexes, MutLα (MLH1/PMS2), MutLβ (MLH1/PMS1), or MutLγ (MLH1/MLH3), which, in concert with proliferating cell nuclear

antigen (PCNA) and replication factor C (RFC), are able to unwind the helix at the damage site [49, 50]. Exonuclease 1 (EXO1) may then degrade a section of the damaged strand, including the mismatch/lesion, which is replaced by the concerted efforts of DNA polymerase and DNA ligase [51].

Although MMR is not able to repair cisplatin adducts, the MutSβ heterodimer has been documented to interact with cisplatin intrastrand cross-links [52, 53]. Here it is able to recruit MutL complexes, which, being unable to repair the damage, initiate pro-apoptotic signals; this process represents a key aspect of cisplatin-mediated toxicity [54-56]. It is therefore not surprising that these events are often suppressed in cancer cells which are resistant to cisplatin. In particular, the MutS components MSH2, MSH3, and MSH6, as well as MLH1 of the MutL complex, are often under-expressed or mutated in cancer cells displaying platinum resistance [57-61].

The pathway of base excision repair (BER) has also recently been suggested to impact on cisplatin-lesion repair [62]. This pathway primarily functions in the removal of small base damages, primarily as a result of base oxidation or alkylation [21]. DNA adducts are initially recognized by a specific DNA glycosylase, the identity of which depends on the type of base damage [63]. In the case of cisplatin interstrand cross-links, a new model has suggested that adjacent 'extra-helical' bases that have been disrupted by the crosslink may be subject to glycosylase recognition. This is predicted to initiate a pathway of non-productive processing in which the disrupted base is deaminated by the glycosylase, leaving an abasic residue [62]. The phosphodiester backbone is then most likely cleaved by AP endonuclease 1 (APE1), and the residue replaced by DNA polymerase-mediated strand synthesis; the cisplatin cross-link will however remain. While this pathway does not seem to initiate apoptotic signaling akin to that stimulated by MMR, the localization of BER proteins may preclude other DNA repair proteins from binding the site of damage, preventing the proper clearance of the lesion [44]. In this model, and similarly to MMR, BER seems to function in non-reparative cisplatin adduct recognition; unlike MMR however, BER protein localization is likely to suppress apoptosis induction. In agreement, the increased expression of APE1, DNA polymerase β and human 8-oxoguanine DNA glycosylase (hOGG1) has been attributed with cisplatin resistance, presumably through suppression of this signalling [64-66].

Nucleotide Excision Repair

Nucleotide excision repair (NER) represents an important pathway for the repair of a large number of structurally varied DNA adducts [67]. These

lesions are seemingly recognized as a result of their disruption to base pairing, and the resulting helical distortion, more so than by their specific chemical nature [68]. In keeping, NER has been found to be the major pathway involved in the removal of cisplatin adducts, and as such, is up-regulated in many platinum-resistant tumors [69, 70].

In the initial stages of NER, damage is recognized by a complex composed of the proteins XPC and hHR23B, which appear to initiate unwinding on either side of the lesion [71]. This is further stimulated by the helicase activity of the TFIIH transcription factor, as well as by XPA and replication protein A (RPA) [72], which help expand the bubble. The exposed adduct may then be excised by the concerted efforts of two major nucleases, XPF-ERCC1, which cleaves the phosphodiester backbone 5` to the lesions, and XPG, which cleaves on the 3` side [73, 74]. These events allow for the removal of an approximately 24-32 nucleotide signal stranded oligomer containing the lesion [75]. This gap may then be replaced by the activities of DNA polymerases δ and ε, which use the intact complementary strand as a template, followed by closing of the phosphate backbone by DNA ligase 1-mediated ligation [76].

While the above seems to reflect the NER response to singular cisplatin adducts and intrastrand cross-links [77-79], the response to interstrand cross-links is apparently more complex. This is most likely due to the damage caused to both strands of the helix, such that neither can function as an appropriate repair template. While a large proportion of cisplatin interstrand cross-links are repaired by translesion repair and homologous recombination during S phase [43, 80, 81], there is compounding evidence that an NER-translesion repair pathway may exist during G1/G0 [82-84]. Following recognition of DNA interstrand cross-links, the NER nucleases are suggested to cleave the phosphodiester backbone on either side of the adduct as per above [85], leaving the 'excised' oligomer covalently bound to the opposing strand through the base crosslink. The cell is then able to bridge the gap by action of a specialized class of DNA polymerases known as translesion polymerases (discussed further below), which are able to insert bases opposite the damage site, although with an increased likelihood of introducing base mismatches when compared to those involved in replication [82, 86, 87]. The remaining 'excised' oligomer, and the section of the opposing strand to which it is bound, is then presumably cleaved and resolved by a second NER excision event [88].

Due to the apparent capacity of the NER system to remove each form of cisplatin adduct, it is not surprising that an upregulation of this pathway is

frequently observed as a means of conferring cisplatin resistance. As may be expected, the expression of multiple NER repair proteins has been found to negatively correlate with cisplatin sensitivity, including that of ERCC1 [89-92], XPA [91, 93], and XPC [94]. In the case of XPA, expression levels appear to be regulated by the hypoxia-inducible factor-1α (HIF-1α) [93], an essential transcription factor in the response to hypoxic conditions. Contrasting this, low-level expression of HIF-1α has been correlated with better overall survival in patients with small-cell lung cancer who receive platinum-based chemotherapy, indicating HIF-1 α-mediated NER de-regulation may not be a key mechanism of resistance [95]. In addition, p53-mediated transcriptional regulation of a number of NER proteins also seems to promote resistance to a number of cross-linking agents [96]. For example, acquired cisplatin-resistance in a subset of malignant melanoma cells has recently been attributed to p53-dependent transcriptional up-regulation of XPC [94]. Further, the translational synthesis of XPA, XPC, hHR23B and the RPA subunit RPA32 has also been found to be negatively regulated by eukaryotic initiation factor-3a (eIF3a), the expression of which has been positively correlated with sensitivity to cisplatin [97, 98]. As opposed to deregulated NER protein expression, platinum resistance has also been correlated with gene polymorphic status. Of note, SNPs in *ERCC1* have received recent attention, with a number of studies suggesting an association between some of these variants and cisplatin sensitivity [91, 99-101].

Classical Translesion Repair

Although the NER pathway is able to remove most forms of bulky adduct, in some instances, lesions are able to sustain into S phase. This is especially true of interstrand cross-links, which for this reason, were originally thought only to be processed by homologous recombination. Two possible explanations have been put forward regarding this inefficiency: (i) that interstrand cross-links are not always efficiently recognized when occurring in highly condensed chromatin [43], and (ii) that some of these damage events may be incompletely removed due to their chemical complexity [102].

By any means, the sustainment of bulky DNA adducts into S phase represents a considerable obstacle for the migrating replicative fork, as the processive δ and ε DNA polymerases are not able to insert opposite damaged bases [103]. Additionally, the covalent crosslinking of opposing DNA strands may prevent helicase-mediated unwinding ahead of the fork [104]. In either instance, replication is forced to stall until such time that the adduct is repaired or bypassed.