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Comments and Critique

The Molecular Basis of Cisplatin Sensitivity/Resistance

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IT IS well established that, while some tumour types respond well to platinum-based chemotherapy, other types exhibit intrinsic resistance. Notable examples of responding tumours are testicular and, to a lesser extent, ovarian cancers. Intriguingly, it has been known for some years that *in vitro* continuous cell lines and xenografts derived from testicular tumours also show hypersensitivity to both cisplatin and carboplatin, thus suggesting an inherent cellular sensitivity of this tumour to platinum [1–3].

These complementary clinical and laboratory observations have led to studies attempting to understand the underlying cellular and molecular basis of how platinum drugs succeed in cancers of the testis, since this might lead to improved strategies for the circumvention of cisplatin resistance in currently unresponsive tumour types. Investigations at the cellular level, using a variety of tumour cell lines possessing acquired resistance to cisplatin, have shown resistance to be due to one or more of decreased drug accumulation, increased cytoplasmic detoxification (through increased levels of glutathione and/or metallothioneins) and increased DNA repair/tolerance of platinum adducts on DNA (see [4] for a recent review).

Recent evidence suggests that the hypersensitivity of testicular tumours might be related to defective removal of platinum–DNA adducts. In a human testicular cancer cell line (GCT27) and a variant with 5.6-fold acquired resistance to cisplatin, atomic absorption studies (which primarily assay the most frequent intrastrand crosslinks on adjacent deoxyguanosines; Pt–GG) showed that the resistant line removed DNA–platinum adducts at a rate comparable to most other mammalian cell lines (removal half time of 32 h). However, the removal half time for the parent line was much slower at 67 h [5]. Hence, the parent line (derived from a previously untreated patient) appeared to possess a defective ability to remove platinum adducts from its DNA and, therefore, acquired resistance in this case could more accurately be described as acquired loss of sensitivity.

In this issue, Hill and colleagues (pp. 832–837) present a comprehensive cytotoxicity and DNA repair based study involving six human testicular tumour cell lines, five being established from previously untreated patients [6]. Notably, following exposure to cisplatin, the levels of the major Pt–GG adducts (measured immunochemically) were not significantly reduced in any of the five lines established from previously untreated patients whereas the remaining line established from a drug-treated patient had earlier been identified as being proficient in the removal of Pt–GG adducts. Thus, a clear picture is emerging suggestive of a general deficiency in the removal of the major platinum–DNA intrastrand crosslink in testicular tumours, and being responsible for their hypersensitivity to cisplatin/carboplatin.

Of possible additional significance, however, is that the studies of Hill and colleagues did not measure removal rates for cisplatin-induced interstrand cross-links (ICL;these represent up to 2% of cisplatin-induced DNA adducts). Perhaps, surprisingly, there remains debate as to whether the major Pt–GG intrastrand crosslink or the ICL is responsible for the cytotoxic effects of cisplatin. On the one hand, the evidence presented in the study by Hill and colleagues emphasises the possible importance of the intrastrand crosslink [6]. Moreover, studies using human cell extracts (from HeLa cells), have shown that the major Pt–GG intrastrand crosslink in duplex M13 DNA is refractory to repair [7]. Alternatively, studies determining repair at the level of individual genes (primarily the dihydrofolate reductase gene) in two human ovarian carcinoma cell lines and sublines with derived resistance to cisplatin, have shown a marked and consistent increase in the gene-specific repair of ICL in the resistant lines but no difference between the lines in the repair of intrastrand adducts [8]. In addition, in an earlier study using the SuSa testicular teratoma cell line, a deficiency in the removal of ICL (measured in the overall genome) was observed [6]. Interestingly, a recent study has shown that the *trans* isomer of cisplatin (*trans*DDP), which is inactive as an antitumour agent and incapable of forming the major cisplatin-induced GG intrastrand crosslink, preferentially forms ICL between guanine and complementary cytosine residues rather than between guanines as formed by cisplatin [9].

These important observations, predominantly made at the cellular level, inevitably lead one to pose the question, "What are the molecular events controlling the sensitivity of testicular (and some ovarian) tumour cells to cisplatin?" In recent years, a number of genes/proteins have been implicated in determining the sensitivity of cells to cisplatin. A summary of the major ones identified to date is shown in Table 1. Notably, factors have been identified at the cell membrane, cytoplasmic and DNA levels.

In terms of the molecular basis underlying the hypersensitivity of testicular tumours, studies to date have determined levels of possible DNA repair enzymes (e.g. ERCC1 and DNA poly-

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Table 1. Genes/proteins implicated in determining cellular sensitivity to cisplatin

Gene/protein	Comment	Reference
CP^R-200	200 kDa membrane glycoprotein overexpressed in cisplatin-resistant murine lymphoma sublines. Possibly involved in decreased accumulation.	[10]
GSH/MT	Glutathione/metallothionein. Major intracellular non-protein and protein thiols, respectively. Involved in detoxification.	[4] for a review
ERCC1	Human DNA repair gene	[11]
DNA polymerase β	DNA repair associated enzyme	[6]
c-Ha-*ras*	Activation of mutant c-Ha-*ras* oncogene in NIH 3T3 cells increased resistance to cisplatin.	[12]
HSP60	60kDa heat-shock protein. One of "chaperonin" family of mitochondrial proteins.	[13]
CDDP-DRPs	Cisplatin damage recognition proteins. Bind to damage in DNA induced by cisplatin.	[14]
p53	Possible role in p53 dependent apoptosis (plus possible role for *myc*, *bcl2* oncogenes). Elevated wild-type p53 levels in cisplatin-resistant ovarian cell lines	[15, 16]
$p34^{cdc2}$ kinase	Cell cycle regulator. Dephosphorylation inhibited by cisplatin.	[17]

merase β) and cisplatin damage recognition proteins. Interest in the possible role of ERCC1 was largely stimulated by a recent study in 26 ovarian cancer patients [11]. Patients who were clinically resistant to cisplatin- or carboplatin-based chemotherapy had a 2.6-fold higher expression level of ERCC1 (examined using slot blots of RNA) in their tumour biopsy than did patients who responded to that therapy ($P = 0.015$). The study by Hill and colleagues determined levels of both ERCC1 and DNA polymerase β. In contrast to the above study in ovarian cancer patients, none of the five testicular teratoma cell lines studied showed a significant difference in ERCC1 expression (including the repair proficient 833K cell line obtained from a drug-treated patient), whereas DNA polymerase β expression was markedly lower in all the repair deficient lines compared to the 833K line [6].

Proteins, termed DNA damage recognition proteins (DRP), which can bind to damage in DNA induced by cisplatin have recently been identified in human tumour cells (see [14] and references therein). Such proteins are currently thought to either participate in DNA repair pathways (increased levels of DRPs have been observed in some, but not all, cisplatin-resistant cell lines) or, in contrast, block access of repair enzymes to the damage. A cisplatin DRP has recently been shown to share a region of sequence homology to the high mobility group (HMG) chromatin proteins which play a role in the bending or looping of DNA. In addition, HMG1 can bind to platinated DNA. Intriguingly, in a study comparing binding activities of cisplatin DRPs in a panel of testicular versus bladder cell lines, all of the testicular lines showed binding activity of a 75 kDa DRP while the bladder lines showed little or undetectable levels. Further differences between the testicular and bladder panels were also observed for a 25 kDa DRP [14].

Other proteins which may have an involvement in influencing cellular sensitivity/resistance to cisplatin are a 60 kDa heat-shock protein (HSP60) [13], the p53 tumour suppressor [15,16] and p21RAS [12]. Elevated constitutive levels of HSP60 have been reported in an acquired cisplatin-resistant human ovarian carcinoma cell line compared to its parent [13]. The wild-type p53 tumour suppressor gene is thought to be required for efficient activation of at least some pathways of cell death through apoptosis (see [15] and references therein). Notably, testicular tumours rarely exhibit p53 mutations, whereas tumours that are generally unresponsive to chemotherapy commonly acquire p53 mutations. However, much remains to be learned in this area (including the possible interplay between p53 and other oncogenes such as *bcl2* and the interrelationship with its recently identified protein partner BAX), *ras* and *myc* many of which have in themselves been shown to influence sensitivity to cisplatin; see [4] for a review. Furthermore, constitutive levels of wild-type p53 have been reported to be increased in some acquired cisplatin-resistant human ovarian carcinoma cell lines [16] and transfection of a mutant p53 gene construct (codon 143 val to ala) into cisplatin-resistant cells significantly *increased* sensitivity to cisplatin. The interrelationship with other factors regulating the cell cycle (such as $p34^{cdc2}$ protein kinase) [17] may also be of importance.

In conclusion, resistance to cisplatin has generally been studied using *in vitro* cell lines, is often multifactorial, and is probably determined by the individual phenotype/genotype of the cell. Recently, various genes and proteins have been implicated in determining cellular sensitivity to cisplatin. Their individual role in determining a patient's response to platinum-based chemotherapy may now be studied through the usage of appropriate molecular probes. Such studies may shed further light on the reasons underlying large differences in clinical responsiveness to cisplatin/carboplatin and, moreover, should pave the way for improved platinum-based chemotherapy, either through selective modulation of resistance mechanisms or better, more broad spectrum, new platinum-based drugs.

1. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. *Br J Cancer* 1983, **47**, 1–13.
2. Pera MF, Friedlos, F, Mills J, Roberts JJ. Inherent sensitivity of cultured human embryonal carcinoma cell to adducts of *cis*-diamminedichloroplatinum (II) on DNA. *Cancer Res* 1987, **47**, 6810–6813.
3. Bedford P, Fichtinger-Schepman AMJ, Shellard SA, Walker MC, Masters JRW, Hill BT. Differential repair of platinum–DNA adducts in human bladder and testicular tumor continuous cell lines. *Cancer Res* 1988, **48**, 3019–3024.
4. Kelland LR. New platinum antitumor complexes. *Crit Rev Oncol/Hematol* 1993, **15**, 191–219.
5. Kelland LR, Mistry P, Abel G, *et al.* Establishment and characterization of an *in vitro* model of acquired resistance to cisplatin in a human testicular nonseminomatous germ cell line. *Cancer Res* 1992, **52**, 1710–1716.
6. Hill BT, Scanlon KJ, Hansson J, *et al.* Deficient repair of cisplatin-DNA adducts identified in human testicular teratoma cell lines established from tumours from untreated patients. *Eur J Cancer* 1994, in press.
7. Szymkowski DE, Yarema K, Essigmann JM, Lippard SJ, Wood RD. An intrastrand d(GpG) platinum crosslink in duplex M13 DNA is refractory to repair by human cell extracts. *Proc Natl Acad Sci USA* 1992, **89**, 10772–10776.

8. Zhen W, Link CJ, O'Connor PM *et al.* Increased gene-specific repair of cisplatin interstrand cross-links in cisplatin-resistant human ovarian cancer cell lines. *Mol Cell Biol* 1992, **12**, 3689–3698.
9. Brabec V, Leng M. DNA interstrand cross-links of *trans*-diammine-dichloro platinum(II) are preferentially formed between guanine and complementary cytotsine residues. *Proc Natl Acad Sci USA* 1993, **90**, 5345–5349.
10. Kawai K, Kamatani N, Georges E, Ling V. Identification of a membrane glycoprotein overexpressed in murine lymphoma sub-lines resistant to *cis*-diamminedichloro platinum(II). *J Biol Chem* 1990, **265**, 13137–13142.
11. Dabholkar M, Bostick-Bruton F, Weber C, Bohr VA, Egwuagu C, Reed E. ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. *J Natl Cancer Inst* 1992, **84**, 1512–1517.
12. Isonishi S, Hom DK, Thiebaut FB, *et al.* Expression of the c-Ha-*ras* oncogene in mouse NIH 3T3 cells induces resistance to cisplatin. *Cancer Res* 1991, **51**, 5903–5909.
13. Kimura E, Enns RE, Thiebaut F, Howell SB. Regulation of HSP60 and mRNA expression in a human ovarian carcinoma cell line. *Cancer Chemother Pharmacol* 1993, **32**, 279–285.
14. McLaughlin K, Coren G, Masters J, Brown R. Binding activities of *cis*-platin-damage recognition proteins in human tumour cell lines. *Int J Cancer* 1993, **53**, 662–666.
15. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993, **74**, 957–967.
16. Brown R, Clugston C, Burns P, *et al.* Increased accumulation of p53 protein in cisplatin-resistant ovarian cell lines. *Int J Cancer* 1993, **55**, 678–684.
17. Nishio K, Fujiwara Y, Miyahara Y, *et al.* *Cis*-diamminedichloroplatinum (II) inhibits $p34^{cdc2}$ protein kinase in human lung-cancer cells. *Int J Cancer* 1993, **55**, 616–622.

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Chemoprevention of Second Primary Tumours: A Model for Intervention Trials

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CANCERS OF the head and neck and lung remain a worldwide threat to public health. Widespread use of tobacco guarantees that a sharp drop in incidence rates is not likely. For clinicians, treatment of these cancers has remained frustrating. There has been no recent significant progress in the treatment of advanced disease. In addition, these patients, identified by an initial head and neck or lung cancer, have a tremendously increased risk for developing second primary tumours. These second primary tumours develop predominantly in the upper aerodigestive tract and lungs. Following a head and neck cancer, the lifetime risk of developing a second primary tumour is between 20 and 40% [1–3]. The risk of primary disease, local-regional recurrence or distant metastases declines after 2 years, but the risk of developing a second primary tumour remains constant and becomes the major threat to survival for these patients [4]. Chemoprevention, the use of drugs to block or reverse carcinogenesis, before the development of invasive cancer, is now being actively studied as a strategy to prevent second primary tumours. It is hoped that if the strategy is effective in this setting, the findings could be used in developing primary prevention regimens as well. Bolla and colleagues, reporting for the French Study Group on Head and Neck Tumours, have studied this approach, and present here the findings of their randomised trial using the synthetic retinoid etretinate (pp. 767–772).

The concepts which have guided the development of intervention strategies for these patients are the presence of a field defect and the multi-step nature of epithelial carcinogenesis. The hypothesis of field carcinogenesis asserts that as a result of diffuse carcinogen exposure, such as in tobacco smoke, the entire epithelial lining of the upper aerodigestive tract is damaged and is consequently at risk for the development of invasive cancer. The initial evidence taken to support this idea was the occurence of histological changes in resected head and neck cancer specimens [5]. Synchronous multiple primary tumours as well as dysplastic epithelium distinct from the tumour were frequently observed. Similar findings have been described in resected lung cancer specimens [6, 7]. Research is now being directed towards identifying the biological characteristics of the carcinogen-exposed epithelium and the resulting field defect. Biomarkers such as chromosomal polysomy and activation of p53 expression have been identified in histologically normal and premalignant upper aerodigestive tract tissue taken from high-risk patients [8–10]. The concept of multi-step carcinogenesis has been well established in animal models. For human studies, this suggests that high-risk patients could potentially be identified by evidence of some of the earlier changes, and then enrolled in intensive early detection or intervention trials.

The tremendous risk of developing a second primary cancer following a head and neck or lung cancer has been used as a model to develop chemoprevention approaches. In this issue of the journal, Bolla and colleagues present the results of a randomised chemoprevention trial performed among patients with a history of head and neck cancer. Patients identified by an initial cancer have demonstrated evidence of both carcinogen exposure and susceptibility. Presumably, other sites in the epithelium have also been genetically damaged, but have not yet progressed into an invasive cancer. Patients who have already received cancer treatment require further close follow-up and

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