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Review

Molecular Cancer Therapeutics

Tumor-Related Molecular Mechanisms of Oxaliplatin Resistance

Eva Martinez-Balibrea[1,2], Anna Martínez-Cardús[3], Alba Ginés[2], Vicenç Ruiz de Porras[2], Catia Moutinho[3], Laura Layos[1], José Luis Manzano[1], Cristina Bugés, Sara Bystrup[2], Manel Esteller[3,4,5], and Albert Abad[1,2,6]

Abstract

Oxaliplatin was the first platinum drug with proven activity against colorectal tumors, becoming a standard in the management of this malignancy. It is also considered for the treatment of pancreatic and gastric cancers. However, a major reason for treatment failure still is the existence of tumor intrinsic or acquired resistance. Consequently, it is important to understand the molecular mechanisms underlying the appearance of this phenomenon to find ways of circumventing it and to improve and optimize treatments. This review will be focused on recent discoveries about oxaliplatin tumor-related resistance mechanisms, including alterations in transport, detoxification, DNA damage response and repair, cell death (apoptotic and nonapoptotic), and epigenetic mechanisms. *Mol Cancer Ther; 14(8); 1767–76. ©2015 AACR.*

Introduction

One of the major challenges in modern oncology relies on the finding of predictive molecular factors of response to treatment. Oncologists are facing a difficult moment in which complexity as well as high cost of treatments are constantly growing. This leads to the necessity of improving the way patients are selected to receive treatment, thus avoiding inefficacy and harm in resistant patients and optimizing schedules in those who are sensitive to a given drug.

Oxaliplatin is a third-generation platinum drug that is used for treatment of colorectal, gastric, and pancreatic cancers and is undergoing clinical trials in ovarian, breast, and non–small cell lung cancer, among others. Remarkably, its introduction in the year 2000 in the treatment of metastatic colorectal cancer, in which cisplatin and carboplatin had been demonstrated to be inactive, led to an important increase not only in objective response rates, improving percentage of metastasis resection, but also in overall survival (OS). Thus, schedules combining oxaliplatin plus 5-fluorouracil (5FU) were demonstrated to increase objective responses to first-line therapy up to 50% compared with 15% for 5-FU monotherapy (1). Thanks to that, colorectal cancer treatment has improved significantly in the last decade, in which the median OS rate has increased to 24 months (2) and the relapse-free survival rate is beyond 10 years in a quarter of patients that, after a response to an oxaliplatin-containing regimen, have a successful metastases resection (3). Unfortunately, intrinsic or acquired resistance to oxaliplatin-based combinations still is the major cause of treatment failure. For this reason, it is of paramount importance to elucidate causes underlying this phenomenon in order to circumvent it, and to uncover better ways of fighting cancer. In this review, we will address the molecular mechanisms associated with oxaliplatin resistance, frequently activated at the same time (multifactoriality), such as intracellular transport and detoxification, alterations in DNA repair mechanisms, epigenetic, and cell death mechanisms, among others. A summary of the described mechanisms is depicted in Fig. 1.

Oxaliplatin Mechanism of Action

To better understand the mechanisms underlying oxaliplatin resistance, it is important to know how this platinum drug exerts its antitumor effect. Oxaliplatin {[oxalate(2-)-*O*,*O*′][1*R*,2*R*-cyclohexanediamine-*N*,*N*′]platinum-(II)} is a member of the family of platinum-containing chemotherapeutic agents that also include cisplatin and carboplatin. In oxaliplatin, the two ammine ligands have been replaced by a single bidentate ligand, (1*R*,2*R*)-cyclohexane-1,2-diamine (*R*,*R*-dach). This structural difference confers it a different spectrum of activity and activates different cellular damage recognition mechanisms as compared with its analogues (4). Oxaliplatin is administered intravenously. Pharmacokinetically, it is characterized by a short initial phase of distribution and a long final phase of drug removal, which mainly takes place in the kidneys, 48 hours after drug administration (5). The main dose-limiting toxicity caused by this drug is peripheral sensorial neuropathy. Although passive diffusion was considered to be the principal process involved in its cellular uptake, more

[1]Medical Oncology Service, Catalan Institute of Oncology (ICO), Hospital Germans Trias i Pujol, Badalona, Barcelona, Catalonia, Spain. [2]Health Sciences Research Institute of the Germans Trias i Pujol Foundation (IGTP). Badalona, Catalonia, Spain. [3]Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet, Barcelona, Catalonia, Spain. [4]Department of Physiological Sciences II, School of Medicine, University of Barcelona, Barcelona, Catalonia, Spain. [5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain. [6]Oncology Unit, Hospital CIMA Sanitas, Barcelona, Catalonia, Spain.

Corresponding Author: Eva Martinez-Balibrea, Institut Català d'Oncologia, Hospital Germans Trias i Pujol and Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona 08916, Barcelona, Spain. Phone: 34-4978684; Fax: 34-4978654; E-mail: embalibrea@iconcologia.net

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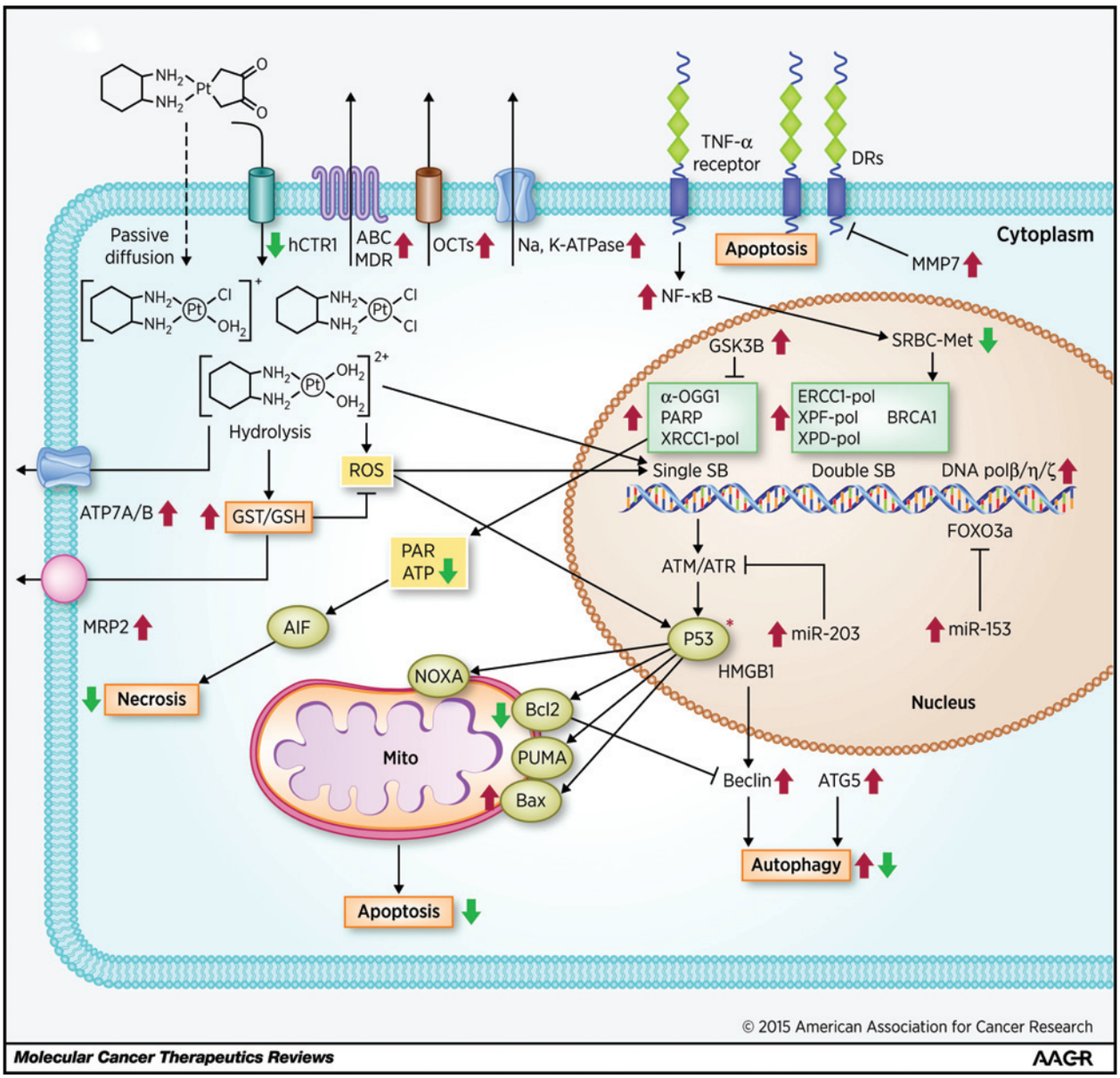

Figure 1.
Summary of oxaliplatin-associated resistance mechanisms. Arrows and line-ended arrows mean activation and inhibition, respectively. Green and red block arrows are for increased and decreased expression, respectively. –pol, polymorphism; -Met, methylated; Mito, mitochondria, DRs, death receptors; SB, strand breaks.

recently it has been shown that facilitated or active transport are also important (6). Once inside the cell, it binds to nucleophilic molecules, mainly DNA but also RNA and proteins (5). As a DNA-interacting agent, it mainly forms intrastrand adducts between two adjacent guanine residues or guanine and adenine disrupting DNA replication and transcription. Indeed, of its greater structure, oxaliplatin produces fewer DNA adducts than cisplatin at equimolar concentrations but causes higher cytotoxicity (7). The DACH–Pt complex can present three isomeric conformations that interact with DNA in different manners, the TRANS-L being the most effective isomeric form (4). The nucleotide excision repair (NER) pathway has been described to be the main oxaliplatin-induced damage repair system.

Cellular Influx/Efflux and Detoxification of Oxaliplatin

For many years, it has been assumed that platinum drugs were passively incorporated into the cells. However, evidence about the role of facilitated or active transport systems has grown up in the last years. The most important cellular transport and detoxification systems associated with oxaliplatin resistance will be explained below.

Copper transporters

Copper influx and efflux transporters have been shown to have a role in the accumulation of platinum drugs (reviewed in ref. 8).

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Table 1. Most relevant *in vitro* demonstrated oxaliplatin resistance mechanisms

General mechanism	Specific mechanism	Methodology	Ref.
Cellular transport	ATP7A upregulation	Knockdown of Sec61β	10
		Resistance acquisition model	11
	OCT2 overexpression[c]	Gene introduction	13[a]
	MRP4 overexpression and alteration in N-glycosylation	Resistance acquisition model	16
	N,K-ATPase reduced expression	Resistance acquisition model and gene introduction	20
Detoxification	Increased intracellular GSH	Primary leukemia cells isolated from CLL patients	23[a]
DNA repair	Increased ERCC1 and XPF levels	Resistance acquisition model—genetic intervention	36[a]
	Increased ERCC1 levels	Resistance acquisition model	35
	Increased expression of DNA polymerases β, η, ζ, REV1	Resistance acquisition model—genetic intervention	45, 46[a], 47
Cell death	Increased levels of Survivin	Resistance acquisition model and colonospheres culture	67, 68
	Loss of Bax expression	Resistance acquisition model—knockdown of Bax	69, 70
	Overexpression of MMP7	Resistance acquisition model	71, 72
	Enhanced autophagy	Genetic intervention	80, 81[a,b], 83[b]
Epigenetic alteration	SRBC epigenetic inactivation	Resistance acquisition model—genetic intervention	89[a]
	miR-153, -203, -143 overexpression	Functional analyses	93[a], 94[a], 95[a,b]
NF-κB signaling pathway	Increased activation of NF-κB	Resistance acquisition model and pharmacologic inhibition	100, 103

NOTE: This table lists all the in-text referenced *in vitro*-based studies that have demonstrated the involvement of a given mechanism on oxaliplatin resistance by using either more than one cell line or an acquired resistance model, additional *in vivo* experiments, or clinical data.
[a]This work contains patient-associated clinical data.
[b]This study contains *in vivo* results.
[c]This feature was demonstrated to be associated with sensitivity.

The human copper transporter 1 (hCTR1) participates in the uptake of oxaliplatin, although its role in resistance acquisition is not as clear as it is for other platinum drugs. For instance, two independent works reported that an upregulation of hCTR1 was clearly involved in cisplatin and carboplatin resistance, whereas this effect was not so evident in the case of oxaliplatin (8). This suggests that other transporters are also responsible for its cellular uptake, thus reflecting the different spectrum of activity among these platinum drugs. Two intracellular p-type ATPases, ATP7A and ATP7B, involved in the sequestration and extrusion of copper have also been shown to have a role in resistance to platinum drugs. Interestingly, Stephen B. Howells' group reported that ATP7A and B have the ability to sequester cisplatin, carboplatin, and oxaliplatin into subcellular compartments, thus limiting their cytotoxicity. However, all three of the platinum drugs failed to trigger the trafficking of ATP7A to the plasma membrane, which seems to be essential for its ability to actually export copper from the cell. Moreover, transporter-proficient cells were resistant to the cytotoxic effect of copper, cisplatin, and carboplatin but were hypersensitive to oxaliplatin as compared with transporter-deficient cells. This fact was associated with increased levels of platinum adducts in DNA in the case of oxaliplatin but not in cisplatin- or carboplatin-treated cells (9). Recently, the same group reported that Sec61β, a subunit of Sec61 protein translocon, affects cytotoxicity of platinum drugs through the upregulation of ATP7A and its distribution but does not affect other copper transporters such as hCTR1, hCTR2, ATP7B, or antioxidant 1 copper chaperone (ATOX1; ref. 10). In our own experience, we found that resistance acquisition to oxaliplatin was accompanied by a cross-resistance to copper and a downregulation in hCTR1 expression. When parent- and resistant-derived cells were exposed to oxaliplatin, a significant upregulation of *ATP7A* was only observed in sensitive cells (11). Although an extensive bibliography exists at the preclinical or *in vitro* level, data about clinical influence of copper transporters on patients treated with oxaliplatin are limited. We studied the expression levels of ATP7A and ATP7B in tumors from patients with colorectal cancer treated with oxaliplatin-based first-line chemotherapy and we found that low levels of ATP7B were associated with a better outcome (12). The lack of larger clinical studies in this sense makes it difficult to reach conclusions about the applicability of testing the levels of copper transporters as surrogate markers of oxaliplatin resistance.

The most relevant *in vitro* or clinically demonstrated resistance mechanisms described in this and in the following sections are summarized in Tables 1 and 2, respectively.

Solute carrier superfamily of membrane transporters

The solute carrier (SLC) transporters play a role in the physiologic absorption and/or excretion of drugs and xenobiotics in the intestine, liver, and kidney (6). One of the 55 existing subfamilies, the human SLC22, has been shown to participate in detoxification of xenobiotics of different nature. Among them, the subgroup of organic cation transporters (OCT), which consists of SLC22A1 (OCT1), SLC22A2 (OCT2), and SLC22A3 (OCT3), is involved in the transport of platinum drugs, OCT2 being most clearly associated with cisplatin and oxaliplatin uptake and cytotoxicity (6). Human embryonic kidney (HEK) 293 cells stably expressing the hSLC22A2 gene (OCT2) were more sensitive to oxaliplatin and, to a lesser extent, to cisplatin. However, in human ovarian cancer, positive mRNA expression of this transporter was only found in 15% of the cases and did not show a statistically significant association with clinical outcome. Of note, in nine human colorectal cancer cell lines, OCT2 mRNA expression was not detected (13). Thus, although this transporter seems to be able to introduce oxaliplatin into the cells in an experimental setting, the low expression found in ovarian tumors and cell lines suggests a very limited relevance to transport these drugs into them. Other authors have reported 50% of positivity in human colorectal cancer tumors (14), indicating that further clinical studies are needed to validate its usefulness as a tumor-associated predictive marker.

ABC transporters

The ABC family of drug efflux transporters has a major role in pumping out of tumor cells more than 80% of currently used

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Table 2. Proposed clinically relevant resistance mechanisms

Biomarker	Feature associated with resistance	Sample type	*n*	Ref.
ATP7B expression	High levels protein and mRNA	FFPE tumors	50	12
GSTP1 Ile105Val polymorphism	Ile/Ile genotype; Ile allele	Blood PBLs	107	30
			102	31
ERCC1 expression	High mRNA expression	FFPE tumors	50	41
			91	42
	ERCC1-positive protein staining		160	43
ERCC1 C118T polymorphism	C/C genotype	Blood PBLs	447	50
			126	51
	T allele		126	49
			168	48
XRCC1 Arg399Gln polymorphism	Arg/Arg genotype	Blood PBLs	126	49
			289	57
	Gln/Gln genotype		432	58
XPD Lys751Gln polymorphism	Gln Allele	Blood PBLs	165	61
	Lys/Gln genotype		188	60
	Lys allele		289	57
FoxM1 expression	High mRNA levels	Frozen tumors	49	86
SRBC methylation	Methylated SRBC	FFPE tumors	189	89
miR-27b, -148a, -326	High expression	Plasma	150	96

NOTE: This table lists all the in-text referenced clinical studies that have demonstrated the involvement of a given molecular marker on oxaliplatin resistance. When more than one study exists, we have included only those in which a relevant (>100) number of patients were analyzed.
Abbreviations: FFPE, formalin-fixed, paraffin-embedded; PBL, peripheral blood lymphocytes.

chemotherapeutic drugs. Specifically, the ABCC subfamily, which comprises the multidrug resistance–associated proteins (MRP), has been shown to be involved in the development of the resistance phenomena associated to platinum drugs (15). A role of MRP1 and MRP4 has been pointed out in oxaliplatin resistance, as an increased expression and an alteration in *N*-linked glycosylation of these transporters were associated with a decrease in drug accumulation and an increased oxaliplatin resistance in an ovarian carcinoma *in vitro* model (16). The association between oxaliplatin resistance and the ABCB1 (MDR1) expression has also been studied showing unconvincing results. For example, Ekblad and colleagues described an overexpression of this membrane transporter as a consequence of oxaliplatin resistance acquisition *in vitro*, although functional tests did not show any increase in ABCB1 transport activity in the oxaliplatin-resistant compared with the parental cell lines (17). Other authors have reported no association between these transporters and the sensitivity to oxaliplatin in neither human colorectal cancer cell lines nor clinical samples (18, 19). These results highlight the necessity of further investigation about the role of MDR1 in oxaliplatin transport and resistance.

Besides the ABC transporters, the reduced expression of the sodium pump Na,K-ATPase β1 subunit from the Na,K-ATPase, which not only maintains intracellular ion homeostasis but also is critical for the maintenance of polarized phenotype of epithelial cells, has been found to be associated with oxaliplatin resistance *in vitro* in an Na,K-ATPase enzyme activity–independent manner (20).

The glutathione system

A decrease in intracellular platinum drugs, including oxaliplatin, due to drug efflux through the glutathione (GSH)-mediated export, which is in turn mediated by the ABCC family of transporters (21), has been postulated as an important mechanism of resistance. Once the cytoplasm is reached, oxaliplatin becomes hydrated, which facilitates its reaction with thiol-containing molecules such as GSH or metallothioneins. Contradictory results have been reported concerning the association between the levels/activity of GSH and oxaliplatin resistance *in vitro* (22–24). Of interest is the work from Zhang and colleagues that underscores the importance of the microenvironment in mediating chemoresistance. They demonstrated an increase in intracellular GSH leads to oxaliplatin resistance in chronic lymphocytic leukemia (CLL) cells. This increase is due to the release of cysteine into the microenvironment by bone marrow stromal cells, which effectively import cysteine and convert it to cysteine, which is in turn taken up by CLL cells to promote GSH synthesis.

Glutathione S-transferases (GST) catalyze the conjugation of toxic and carcinogenic electrophilic molecules with GSH protecting cellular macromolecules from damage (25). Among different subclasses of the GST superfamily (Alpha, Pi, Mu, Theta, Zeta), subclass GSTP1 has been shown to be highly overexpressed in colon cancer and in drug-resistant tumors. GSTP1 directly participates in the detoxification of cisplatin and is an important mediator of both intrinsic and acquired resistance to this platinum (26, 27) but a lack of evidence showing a role in oxaliplatin detoxification exists. Thus, although the work of Mathieu and colleagues demonstrated increased levels of GSTP1 in a xenograft model of non–small cell lung cancer (NSCLC) treated with oxaliplatin (28), other authors have shown an absence of association between these protein levels and oxaliplatin sensitivity. For instance, Tozawa and colleagues showed that elevated levels of GSTP1 were associated with resistance to cisplatin but at the same time, with sensitivity to oxaliplatin in a gastric cancer cisplatin–resistant cell line (29). The works from Arnould and Pendyala go in the same direction evidencing a lack of correlation between GST activity and oxaliplatin cytotoxicity (22, 24).

Despite this controversy, a plethora of publications have reported both positive and negative associations between genetic variants of *GSTP1* and outcome to oxaliplatin-based

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chemotherapy. Specifically, the Ile105Val polymorphism has been shown to affect the enzymatic capacity for the conjugation of various cytotoxic drugs subsequently influencing the effect of chemotherapy on tumor cells. Thus, the Val/Val genotype (decreased enzymatic capacity) has been associated with better OS in patients with colorectal cancer and gastric cancer receiving oxaliplatin-based chemotherapy in both adjuvant and metastatic settings (30, 31), although negative studies have also been published (32, 33). It has to be taken into account the heterogeneity of these works regarding the chemotherapy received [first-line oxaliplatin (32); second-line oxaliplatin (30, 33); adjuvant treatment (31)] and other factors such as the cohort size (all of them include about a hundred patients except the study of Le Morvan and colleagues in which only 59 patients treated with first-line oxaliplatin were studied) which leads to the necessity of prospective clinical trials to validate these data.

Oxaliplatin-Induced DNA Adducts Repair

The formation of DNA adducts is an essential step for inducing the anticancer activity by platinum compounds. Therefore, molecular mechanisms involved in recognition and/or repair of such adducts would have an important role in determining their antitumor activity. Cisplatin–DNA adducts can be recognized and repaired by the mismatch repair system (MMR), whereas oxaliplatin–DNA adducts are not (4). For this reason, tumors with defective MMR are intrinsically resistant to cisplatin but are sensitive to oxaliplatin. An example of this is the case of colorectal cancer, frequently deficient in MMR system, in which oxaliplatin has shown to be an active drug, whereas treatment with cisplatin or carboplatin has shown to be ineffective (34).

In contrast, DNA damage induced by cisplatin and oxaliplatin is repaired *in vitro* with similar effectiveness and kinetics by the NER system (4). One of the most important NER mediators, the excision repair cross-complementing group 1 (ERCC1), and its catalytic partner XPF (xeroderma pygmentosum group F, ERCC4) have been demonstrated to be involved in oxaliplatin resistance. Preclinically, intrinsic low levels of ERCC1 or its genetic knock down are generally associated with sensitivity to oxaliplatin in different *in vitro* models (35–37). In a recent article, ERCC1 induction after treatment with oxaliplatin was found to depend on *KRAS* mutations, being the mutated cell lines unable to upregulate ERCC1 expression and leading to an increased sensitivity to the drug (38). Other proteins from the NER system such as XPF and XPG (xeroderma pygmentosum group G, *ERCC5*) have been shown to have a role in oxaliplatin resistance. Thus, their siRNA-mediated gene silencing affects DNA repair efficiency negatively in oxaliplatin-treated cells making them more sensitive to the drug (39). Some negative studies also exist, such as that from Stordal and colleagues, who reported a decrease in ERCC1 in association with oxaliplatin-induced cell-cycle arrest but not with resistance or altered DNA repair capacity (40). Using an *in vitro* model of oxaliplatin-acquired resistance, we showed that parental cells were able to upregulate *XPD* (xeroderma pygmentosum group D, ERCC2) and *ERCC1* gene expression, whereas oxaliplatin-resistant derived cells were not (11). Similarly, several clinical studies have reported an association between tumor expression levels of ERCC1 and clinical outcome in oxaliplatin-treated patients (refs. 41, 42; reviewed in ref. 37). A recently published study reported a shorter 5-year disease-free survival (DFS) and OS rates for those patients with OXA-treated stage III colorectal cancer with positive ERCC1 tumors (43). Although clinical and preclinical data about association between ERCC1 expression and outcome of oxaliplatin-treated patients exists, it is still premature to make definitive conclusions and larger and prospective studies are required to validate the ERCC1 gene expression levels as a useful predictive marker for oxaliplatin treatment.

It has been demonstrated that platinum compounds, including oxaliplatin, also induce free radical production leading to oxidative DNA damage. The base excision repair (BER) system is the major DNA repair pathway responsible for removal of corrupt DNA bases and repair of DNA single-strand breaks. Therefore, an altered BER capacity would affect the response to platinum agents. Thus, Preston and colleagues demonstrated that ectopic expression of α-OGG1 (oxoguanine glycosylase 1) or its functional homologue, *Escherichia coli* formamidopyrimidine glycosylase (fpg), decreased cell death caused by reactive oxygen species (ROS) initiators and by cisplatin or oxaliplatin (44). The role in bypassing oxaliplatin-induced adducts in human DNA by DNA polymerases β, γ, and η has also been postulated as a resistance mechanism. For example, overexpression of Polβ—the major DNA polymerase involved in BER—or polη has been shown to confer resistance to oxaliplatin in colon and gastric cell lines, respectively (45, 46). More recently, it has been observed that REV1 and Polζ have a role in promoting both translesion DNA synthesis and DNA repair of damaged DNA after exposure to different platinum drugs, including oxaliplatin and in promoting resistance to these agents (47).

Common genetic variants have been described in DNA repair genes. Among them, a silent mutation in codon 118 of the *ERCC1* gene has been widely studied in the clinical setting with respect to the clinical outcome associated with oxaliplatin-based therapies and reporting a variety of results: while we and others have reported a predictive value for oxaliplatin efficacy for the T/T genotype, others have reported a negative effect of the T allele or even a lack of association with outcome (refs. 48–52; reviewed in ref. 37). Moreover, in a recent meta-analysis, the T allele was associated with a reduced response and poor progression-free survival (PFS) and OS in Asians but not in Caucasians (53). A possible explanation for these discrepancies can be found in the work from Gao and colleagues in which they suggest that C118T itself is not related to the phenotypic differences in ERCC1 expression or function but rather this polymorphism may be linked to other causative variants or haplotypes (54). For instance, an SNP in the 3′-untranslated region (UTR) of the gene (C8092A) has recently been shown to predict OS after platinum-based chemotherapy for completely resected patients with NSCLC (55). In addition, the C118T SNP is linked to a haplotype block of 18 kb within *ERCC1* and the adjacent genomic region in European population. Therefore it would be of interest first, to know whether both SNPs are in linkage disequilibrium and second, which is the functional consequence of a C to A change in position 8092 of the gene. Other genetic variants in DNA repair genes have been associated with the outcome to oxaliplatin treatment. Among them, an SNP resulting in an amino acid change (Arg to Gln) in X-ray repair cross-complementing protein 1 (XRCC1) has been shown to correlate with a worse outcome in some tumors (49, 56–58). The *XPD* Lys751Gln polymorphism has also been found to be associated with the outcome after oxaliplatin treatment in colorectal and gastric cancer. Specifically, patients with the Gln/Gln genotype have a worse prognosis as compared with those harboring the Lys/Lys genotype (59–61). In

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view of these results, more efforts are needed to validate them in prospective clinical trials. Two examples can be found in the works of Kim do and Cubillo (62, 63); in the former, patients were randomized to receive either FOLFOX combination or a treatment according to genotypes for certain polymorphisms. In this group, FOLFOX was selected on the basis of *XPD*-751, *GSTP-1*-105, and *XRCC1*-399 genotypes. Response rate was significantly higher in the planned group, mainly due to the high percentage of responses in the FOLFOX-preselected group as compared with nonselected patients. In the work of Cubillo and colleagues, 74 patients were assigned to receive different treatments (including oxaliplatin) according to the expression patterns of toposiomerase I, ERCC1, thymidylate synthase, and thymidine phosphorylase. Results showed no better outcome in these patients as compared with standard results reported elsewhere. These works have some important limitations such as the number of patients or the lack of a control group in the case of the latter and therefore larger and conclusive trials are guaranteed.

Cell Death Mechanisms

It is generally accepted that futile attempts to repair DNA damage generated by oxaliplatin usually finishes in cell death activation and, therefore, alterations in key cell death–related genes and/or tumor suppressors such as p53, often compromise its efficacy. However, whether oxaliplatin efficacy depends on the activation of one or another cell death pathway still is a field of controversy. Main cell death pathways associated with oxaliplatin resistance are described below.

Apoptosis

Oxaliplatin can exert its cytotoxic effect by inducing mainly the intrinsic but also the extrinsic pathway of apoptosis, although it is not clear whether it promotes caspase activation (64). A major player in this scenario is the tumor suppressor protein p53, which can detect DNA damage, activate cell-cycle control checkpoints, and trigger cell death. However, gain-of-function mutations or loss of p53 occur in more than 50% of human tumors, a fact that has been associated with intrinsic resistance to oxaliplatin in cancer cells (65). Although in some clinical studies correlations have been found between TP53 mutations and chemoresistance; in others, the correlation has not been so clear suggesting the involvement of additional genetic changes that have been accumulated in these tumors (66).

Inhibitors of apoptosis (IAP) are a family of proteins that act as endogenous inhibitors of programmed cell death. Some of its members have been implicated in resistance to oxaliplatin. For example, in human colorectal cancer cell lines with acquired resistance to oxaliplatin higher levels of survivin were observed as compared to the parental cells (67) and tumors that express BIRC6 show resistance against cisplatin and oxaliplatin (68).

The intrinsic apoptotic pathway is regulated by the Bcl-2 family of proteins. This family includes both proapoptotic (Bad, Bak, and Bax) and antiapoptotic members (Bcl-2, Bcl-xl, and Mcl-1). While loss of proapoptotic Bax decreases sensitivity to oxaliplatin (69), downregulation of the antiapoptotic members Bcl-2 and Bcl-xl increases the sensitivity to oxaliplatin (70).

On the other hand, the extrinsic apoptosis pathway is mediated by the activation of the so called "death receptors" (TNFR1, Fas/CD95, TRAIL, DR4, and DR5) after association of specific ligands. Impairment of this pathway promotes oxaliplatin resistance as it was demonstrated by Almendro and colleagues. In their work, overexpression of MMP7 is associated with oxaliplatin resistance acquisition and its genetic silencing restores oxaliplatin sensitivity by increasing the Fas receptor (71). Later on, they demonstrated how cells with acquired resistance to oxaliplatin displayed mesenchymal characteristics that were enhanced by CD95 triggering, after oxaliplatin treatment, contributing to a metastatic phenotype (72). Another important component of this pathway is the protein Bid. Cells deficient in Bid were dramatically protected from apoptosis when oxaliplatin was combined with subtoxic TRAIL concentrations (73). Besides this, the FLICE-Like inhibitory protein (c-FLIP) is a catalytically inactive caspase-8/−10 homologue whose variants are involved in drug resistance, including oxaliplatin, in a wide range of human tumors (74). Specifically, it has been shown to inhibit oxaliplatin-induced apoptosis through the sustained XIAP protein level and Akt activation (75). Whether these proteins have a role in clinical resistance to oxaliplatin remains to be demonstrated, as most of the published works refer to *in vitro* research.

Regulated necrosis

It has become clear that necrosis can occur in a regulated manner, having a prominent role in multiple physiologic and pathologic settings, including response to genotoxic stress. Alkylating DNA damage and ligation of death receptors, among others, can induce regulated necrosis (reviewed in ref. 76). It has been demonstrated that oxaliplatin can activate both apoptosis and necrosis depending on the cellular model (77). Although there is a lack of literature about it, in a recent work from Grassilli and colleagues, it was demonstrated that glycogen synthase 3 β (GSK3B), a serine–threonine kinase belonging to the glycogen synthase kinase subfamily that is involved in energy metabolism, neuronal cell development, and body pattern formation, was activated in almost 50% of colon carcinomas and in two thirds of drug-resistant ones. Genetic silencing of GSK3B in p53-null cells treated with oxaliplatin induced cell death by caspase-independent necroptotic death (78). It is noteworthy that oxaliplatin effectiveness has been associated with the production of ROS, which in turn is a contributor to the execution of necrosis (76). Then, resistance to regulated necrosis is also possible in cells overtreated with oxaliplatin. Further investigation on key necroptotic factors such as RIPK1, RIPK3, MLKL, or PGAM5 is needed to elucidate the role of this pathway in killing cancer cells treated with oxaliplatin.

Autophagy

Macroautophagy (referred to throughout as autophagy) is a critical catabolic process required for maintaining cellular homeostasis in health and pathologic situations. It is typically observed in response to cellular stress, hypoxia, DNA damage, or endoplasmic reticulum stress. Autophagy is activated in many tumors and its inhibition can lead to either increased cell death or increased survival, depending on several factors (79). Its role in promoting chemoresistance or chemosensitivity is controversial. For instance, reducible HMGB1 (high mobility group box 1) induces Beclin1-dependent autophagy and promotes tumor resistance to oxaliplatin (80). Different authors have reported that oxaliplatin treatment activates autophagy in hepatocellular carcinoma and colon cancer cell lines and xenografts models, contributing to the tolerance of oxaliplatin by decreasing the generation of ROS (81, 82). Downregulation of Beclin1 or ATG5 enhances sensitivity to oxaliplatin indicating that autophagy acts

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as a mechanism of resistance to oxaliplatin (83). In this line, our group has recently reported the PKM2-dependent upregulation at the transcriptional level of the Bcl2-modifying factor (*BMF*), associated with the induction of apoptosis, necroptosis, and autophagy, after oxaliplatin exposure in HT29 parental cell line but not in its oxaliplatin-resistant derived cell line, HTOXA (84). A lack of consensus exists regarding which are the best autophagy markers in human tumors samples and which are the best techniques to determine them and this fact makes difficult to translate this amazing field into the clinical setting. However, the increasing interest of researchers guarantees new advances in the near future.

Senescence

Several researchers have demonstrated that cancer cells derived from solid tumors can undergo senescence when exposed to platinum compounds (85). It recently has been demonstrated that oxaliplatin induces ROS and senescence in hepatocellular carcinoma cells when FoxM1 levels are low. Under these circumstances, patients treated with oxaliplatin were more sensitive to treatment (86).

Epigenetic Mechanisms

A multiple number of studies suggest a direct role of epigenetic mechanisms in cancer chemoresistance, normally due to deregulation of genes involved in DNA damage response, cell-cycle control, apoptosis, and DNA repair pathways. Furthermore, it is proposed that chemotherapy itself can exert a selective pressure on epigenetically silenced drug sensitivity genes present in subpopulations of cells, leading to acquired chemoresistance. Nevertheless, little information exists about epigenetic mechanisms underlying oxaliplatin resistance (87).

DNA methylation and histone modifications

DNA methylation, the addition of a methyl group to the 5-carbon position of cytosine residues, is the most common covalent modification of human DNA and occurs almost exclusively at cytosine residues that are followed immediately by a guanine (so-called CpG dinucleotides). Genes critical to tumor biology are frequently inactivated by hypermethylation of the CpG dinucleotides located in their 5′-CpG island regulatory regions (88). In a recent study, we demonstrated that *SRBC* epigenetic inactivation by promoter CpG island hypermethylation is associated with acquired resistance and poor outcome upon oxaliplatin treatment both *in vitro* and *in vivo* (89). This can be reasonable, as SRBC interacts with BRCA1, a protein important in the repair of DNA double-strand breaks caused by platinum derivatives (90). We hypothesized that in colorectal tumorigenesis, methylation-associated inactivation of *SRBC* can be somehow leading to activation of BRCA1, leading to the opposite effect than the loss of BRCA1, herein the acquisition of resistance to oxaliplatin.

Eukaryotic histones, the scaffold of DNA, can undergo multiple posttranslational modifications that lead to either gene activation or repression (91). Aberrant patterns of histone modifications are a hallmark of cancer. Actually nothing is known about the histone code and its connection with response to oxaliplatin.

MicroRNAs

MicroRNAs (miRNA) are noncoding RNAs that bind to their target messenger RNAs (mRNA) under base complementarily via the miRNAs seed sequence. This induces the target mRNA degradation or translational repression, depending on the complementary level of the binding between miRNA and its target messenger RNAs. Apart from its well-known contribution to various diseases, including cancer, emerging evidence suggests that deregulation of miRNAs is closely associated with the acquired chemoresistance in human neoplasias (92). *In vitro*, overexpression of miR-153, -203, and -143 has been associated with acquired resistance to oxaliplatin through modulation of FOXO3a, ATM kinase, and IGF-1R, respectively (93–95). In the clinical setting, overexpression of miR-27b and -148a in plasma samples from patients with colorectal cancer before receiving oxaliplatin-based first-line chemotherapy was associated with lack of response and worse PFS, whereas overexpression of miR-326 was also associated with worse OS (96). Further studies are necessary to validate these results and confirm the use of assessing miRNAs in the blood of patients treated with oxaliplatin.

In contrast to genetic alterations, epigenetic changes can be modified pharmacologically with the use of DNA (cytosine-5-)-methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors and, as a consequence, the reexpression of epigenetically silenced genes may result in the suppression of tumor growth and an increased sensitivity to anticancer drugs (97). In this sense, vorinostat, an HDAC inhibitor, has been studied in a phase I trial in combination with 5-FU and oxaliplatin achieving 52% of tumor stabilization without a single objective response (98). These results are not very encouraging and highlight the necessity of developing new compounds and/or finding predictive markers that allow us to select candidate patients.

Nuclear Factor κ Light-Chain Enhancer of Activated B Cells

This proinflammatory transcription factor plays an important role in the development and progression of cancer and its aberrant activation has been proposed as the major cause of chemoresistance through the activation of a multitude of mediators, including antiapoptotic genes (99). It has been shown that the sensitivity of colorectal cancer cells to oxaliplatin-induced death is adversely affected by elevated NF-κB activity (100) and that cell lines with acquired resistance to oxaliplatin showed increased activation of NF-κB (p65 subunit) as compared with their matched sensitive parental cells, implicating NF-κB as a potential mediator of oxaliplatin resistance acquisition in colorectal cancer (101, 102). This increase can be due to an epithelial-to-mesenchymal transition (EMT) process in these cells (103). As a consequence, direct targeting of NF-κB activation or its downstream transcriptional targets has been proposed as a strategy to increase oxaliplatin cytotoxicity (104). Clinical trials assessing the influence of NF-κB activation on outcome of patients treated with oxaliplatin-containing regimens are needed to validate its usefulness as predictive marker.

Conclusions

Oxaliplatin has become a very relevant drug in the management of patients suffering mainly from colorectal cancer but also from other tumors. For this reason, it is necessary to elucidate the molecular mechanisms underlying the resistance phenomena, as they are the main cause of treatment failure and tumor progression. Although much work remains to be done, the discovery of

these mechanisms as well as the associated biomarkers will help not only in identifying those patients who are unlikely to benefit from treatment with oxaliplatin but also in developing new treatments designed to overcome such resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Eva Martinez-Balibrea, Anna Martínez-Cardús, Alba Ginés, et al.

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