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Review

Chemoresistance and chemosensitization in cholangiocarcinoma☆

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ABSTRACT

One of the main difficulties in the management of patients with advanced cholangiocarcinoma (CCA) is their poor response to available chemotherapy. This is the result of powerful mechanisms of chemoresistance (MOC) of quite diverse nature that usually act synergistically. The problem is often worsened by altered MOC gene expression in response to pharmacological treatment. Since CCA includes a heterogeneous group of cancers their genetic signature coding for MOC genes is also diverse; however, several shared traits have been defined. Some of these characteristics are shared with other types of liver cancer, namely hepatocellular carcinoma and hepatoblastoma. An important goal in modern oncologic pharmacology is to develop novel strategies to overcome CCA chemoresistance either by increasing drug specificity, such as in targeted therapies aimed to inhibit receptors with tyrosine kinase activity, or to increase the amounts of active agents inside CCA cells by enhancing drug uptake or reducing efflux through export pumps. This article is part of a Special Issue entitled: Cholangiocytes in Health and Diseaseedited by Jesus Banales, Marco Marzioni, Nicholas LaRusso and Peter Jansen.

1. Genetic signature of chemoresistance in CCA

Chemotherapy is a common option in the management of patients suffering from cholangiocarcinoma (CCA), because due to the frequent late diagnosis of this tumor and hence its advanced stage, surgical alternatives are not recommended in approximately 70% of patients [1]. Unfortunately, the 5-year survival rate for these patients is lower than 10%, which is due in part to the negligible degree of success of available chemotherapeutic regimens [2]. The reason for the poor response of CCA to anticancer agents is based on the existence of complex mechanisms of chemoresistance (MOC) that usually act synergistically to help cancer cells escape from the deleterious effects of cytostatic drugs. MOC characterize the multidrug resistance (MDR) phenotype, which is accounted for by the so-called "resistome", defined as the entire set of MOC-associated proteins that are expressed at each moment of the tumor life.

More than one hundred genes are involved in the resistome of different tumors, including CCA [3]. Several attempts to classify resistome-associated genes have been performed to better understand this complex situation. The nomenclature suggested by us some years ago, initially comprised five groups of MOC [4] that have recently been extended to seven [5] this increase in number is in view of the novel advances in the knowledge regarding the role of tumor microenvironment and phenotypic transition in the ability of tumor cells to avoid drug-induced cell death (Fig. 1). Genes involved in MOC-6 and MOC-7 are still poorly defined in CCA, and some information concerning these MOC has been included in other chapters of this issue, although their actual relevance in CCA chemoresistance still remains to be established. In the present review article we will focus our attention on MOC-1, MOC-2 and MOC3, whereas MOC-4 and MOC-5 will be briefly commented on below.

The importance of MOC-4 in CCA, which is associated with DNA repair, is high because DNA-damaging agents, such as platinum-derived drugs are used in the standard treatment of palliative chemotherapy for

Abbreviations: ABC, ATP-binding cassette; ASBT, apical sodium-dependent bile acid transporter; Bamet-UD2, cisplatin-ursodeoxycholic acid derivative; CCA, cholangiocarcinoma; DHMEQ, dehydroxymethyl epoxyquinomicin; EGFR, epidermal growth factor receptor; 5-FU, 5-fluorouracil; GSTP1, glutathione-S-transferase P1; HPB, hepatoblastoma; HCC, hepatocellular carcinoma; MDR, multidrug resistance; MOC, mechanism of chemoresistance; MTs, metallothioneins; MRP, multidrug resistance-associated protein; MRP2pr, MRP2 promoter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PEG, polyethylene glycol; TKI, tyrosine kinase inhibitor; TKR, tyrosine kinase receptor

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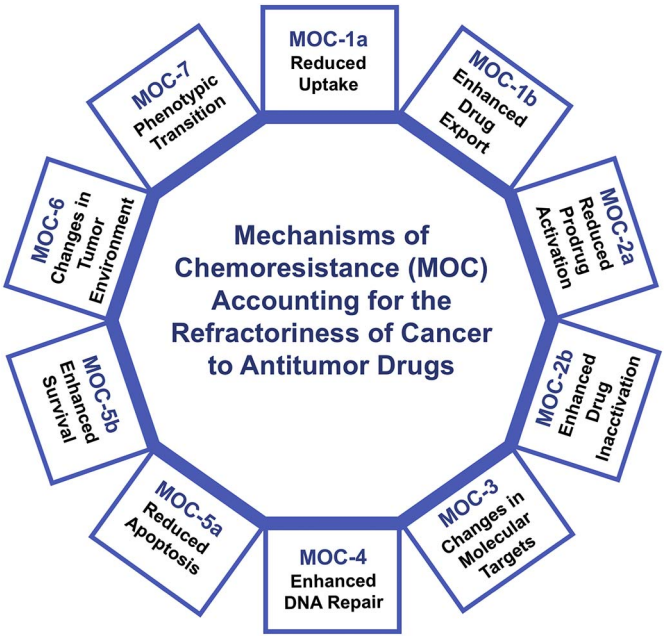

Fig. 1. Classification of the mechanisms of chemoresistance (MOC) involved in the poor response of cholangiocarcinoma (CCA) to available chemotherapy.

patients with inoperable CCA [6]. Only a low rate of mutations in DNA repairing proteins that may influence clinical management have been found in CCA [7], however up-regulation of some MOC-4 genes after drug exposure is a common feature in CCA. For instance, the excision repair cross-complementing protein 1 (ERCC1) is up-regulated in CCA cells after cisplatin treatment [8]. The overexpression of this gene has been suggested as a potential prognostic marker in advanced CCA patients treated with cisplatin [9]. Another example is the more than 10-fold increased expression of "growth arrest and DNA damage inducible alpha" gene (GADD45A), in CCA cells after cisplatin treatment [8]. *In vitro* studies indicated that uracil DNA glycosylase (UNG1) is increased in CCA cells resistant to 5-fluorouracil (5-FU) [10]. No other genes in the MOC-4 group were found to be significantly elevated in CCA as compared with adjacent liver tissue [8].

Since inducing tumor cell death is the goal of most anticancer drugs, changes in the balance between pro-apoptotic (MOC-5a) and pro-survival (MOC-5b) signals markedly affect the effectiveness of the pharmacological treatment. Some changes in MOC-5 genes can be considered as “driver mutations”, *i.e.*, genetic alterations accounting for the induction and maintenance of tumorigenesis (for review see [11]). In this context, a dramatic up-regulation of survivin (gene symbol *BIRC5*) has been reported as a genetic trait shared by hepatocellular carcinoma (HCC, 39-fold increase) hepatoblastoma (HPB, 20-fold increase) and CCA (15-fold increase) [8]. The anti-apoptotic gene *BCL2* has also being found up-regulated (3-fold increase) [8], whereas the pro-apoptotic gene *TP73* is down-regulated (more than 10-fold decrease) in 5-FU resistant CCA cells [10].

2. The role of changes in intracellular drug concentrations in CCA chemoresistance

Genes accounting for a reduction in the amount of anticancer drugs inside tumor cells were classified into the MOC-1 group, which in turn can be divided into two subgroups: impaired drug uptake (MOC-1a) or enhanced drug efflux (MOC-1b). A good example of the importance of transporters involved in MOC-1a regarding liver tumors is the impaired uptake of sorafenib, the drug of choice for HCC treatment at present. This drug is a member of the family of tyrosine kinase inhibitors (TKIs) able to interfere with the function of several plasma membrane receptors with tyrosine kinase activity (TKR). TKIs need to reach their site of action inside the cell to carry out their pharmacological action. In aqueous solution, sorafenib behaves as a cation and is mainly taken up by the organic cation transporter 1 (OCT1, gene symbol *SLC22A1*) [12]. Impairment in the overall transport function of this carrier protein has been associated with the reduced pharmacological activity of several cationic drugs [13]. Hence, the impaired uptake of sorafenib is known to be involved in the lowered response of HCC and CCA cells to this drug [12]. The measurement of *SLC22A1* mRNA from CCA biopsies and paired adjacent liver tissue revealed that a marked decrease in OCT1 expression was part of the genetic signature of MOC-1a in CCA, and also in HCC and HPB [8]. Other studies have suggested that reduced *SLC22A1* mRNA levels in CCA are associated with tumor progression and reduced patient survival [14] and, together with OCT3 (*SLC22A3*) mRNA levels, could be considered as a prognostic factor for the outcome of HCC patients [15]. In a more recent study, it has been shown that mRNA levels could be misleading because the presence of the functional full transporter at the plasma membrane of tumor cells is actually a more accurate information [16]. This is important because, together with reduced *SLC22A1* mRNA levels, an important proportion of transcribed mRNA is useless due to the appearance of non-functional mutated or truncated variants in CCA [12]. This is a common event affecting many transporters, which often results in significantly altering drug pharmacokinetics [17]. In the case of OCT1, recent studies have shown that impaired functional expression of this transporter in liver tumors is due in part to a high rate of aberrant splicing and to epigenetic factors [18]. The relevance of these findings is enhanced by the fact that novel targeted therapies are often based on the use of cationic organic molecules, which presumably require OCT transporters for entering tumor cells. Nevertheless, the dependence of novel anti-CCA drugs on specific uptake transporters needs to be evaluated in each case in the future. Moreover, it is noteworthy that the expression of other drug transporters, such as some members of the organic anion transporting polypeptide (OATP) family, is lower in CCA than in healthy liver tissue [8,19]. Of this family, OATP1A2 (*SLCO1A2*) is the main transporter in cholangiocytes, whose impairment can affect the uptake of methotrexate, taxanes and imatinib. The uptake of irinotecan through OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) [20], and doxorubicin through probably the three isoforms [21], can depend on the expression/function of theses transporters in CCA. In spite of the cationic charge of sorafenib, OATP1B1 and OATP1B3 can also transport sorafenib and/or sorafenib-glucuronide [22] and thus have an impact on the biodistribution of this drug and its metabolites.

Other transporters and drugs that can be involved in MOC-1a in CCA are: ENT1 (*SLC29A1*) and CNT1 (*SLC28A1*) for gemcitabine and 5-FU; CTR1 (*SLC31A1*) for platinum derivatives; NIS (*SLC5A5*) for ^{131}I; and PEPT1 (*SLC15A1*) for δ-aminolevulinic acid derivatives (for review see [3]).

Among the most common mechanisms accounting for MDR in cancer cells is the presence of transmembrane proteins known as ATP-binding cassette (ABC) transporters. These pumps are responsible for drug efflux from cells, so their overexpression in cancer can reduce the drug bioavailability and favor MDR (MOC-1b) [23]. The prototypic drug export pump is P-glycoprotein or multidrug resistance protein 1 (MDR1, gene symbol *ABCB1*) which is able to transport out of tumor cells a large number of different drugs, such as doxorubicin, etoposide, paclitaxel, vinblastine and probably also sorafenib [24]. MDR1 overexpression in CCA has been found both in cell lines [25] and in clinical samples of biliary cancer [26].

ABC proteins included in the ABCC family of multidrug resistance-associated proteins (MRP) are able to export many drugs commonly used in anti-CCA regimens, such as chlorambucil, cyclophosphamide, daunorubicin, doxorubicin, etoposide, irinotecan, mitoxantrone, topotecan among others, whose success in treating CCA patients has been rather low (for a review see [6]). Studies by Rau et al. [28] have shown that MRP3 (*ABCC3*), whose expression has been correlated with resistance to etoposide, doxorubicin and pararubicin in CCA cell lines