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Further understanding of mechanisms involved in liver cancer chemoresistance

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ABSTRACT

An important limitation for the success of chemotherapy in the treatment of primary liver cancer (hepatocellular carcinoma, hepatoblastoma and cholangiocarcinoma) is the marked efficacy of mechanisms of chemoresistance (MOC). These have been previously classified into five groups depending on whether they result in: a reduced drug uptake or enhanced drug export (MOC-1); poor intracellular activation of prodrugs or higher inactivation of active drugs (MOC-2); changes in the molecular targets that impairs the action of the drug by increasing the activity of the metabolic route to be inhibited or stimulating alternative routes (MOC-3); ability of tumor cells to repair drug-induced modifications in the target molecule, usually DNA (MOC-4); and the activation or inhibition of intracellular signaling pathways that lead to a change in the balance between pro- and anti-apoptotic factors favoring tumor cell survival (MOC-5). Nevertheless, novel information appeared over the last few years has recommended to consider two additional groups, MOC-6 and MOC-7, based on changes in tumor microenvironment, mainly hypoxia and acidity, and epithelial-mesenchymal transition, respectively. These contribute to the defensive armamentaria developed or enhanced in liver cancer cells to resist the pharmacological attack, which accounts for a negligible beneficial effect of commonly used antitumor drugs and only a modest response to novel targeted therapies based on tyrosine kinase inhibitors, such as sorafenib. Therefore, further advances are urgently needed to better understand the molecular and cellular bases of the chemoresistant barrier and help scientists in this field to develop new tools able to overcome cancer cell defenses.

INTRODUCTION

An important limitation for the success of chemotherapy in the treatment of primary liver cancer [hepatocellular carcinoma (HCC), hepatoblastoma (HPB) or cholangiocarcinoma (CCA)] is the marked efficacy of mechanisms of chemoresistance (MOC) that have previously been classified into five groups[1] depending on whether they result in: reduced drug uptake or enhanced drug export (MOC-1); poor intracellular activation of pro-drugs or higher inactivation of active drugs (MOC-2); changes in the molecular targets that impairs the action of the drug by increasing the activity of the target route to be inhibited, or the appearance

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or stimulation of alternative routes (MOC-3); ability of tumor cells to repair drug-induced modifications in the target molecule, usually DNA (MOC-4); and the activation or inhibition of intracellular signaling pathways that lead to a change in the balance between pro- and anti-apoptotic factors favoring tumor cell survival (MOC-5). Nevertheless, novel information on the role of several adaptive mechanisms involved in liver cancer chemoresistance has emerged in the last few years. These regard the existence of cancer stem cells with particularly poor sensitivity to anticancer drugs, the interference with the inflammatory processes and cytokine expression, cellular autophagy status, changes in tumor microenvironment and phenotipic transition of cancer. This situation recommends considering at least two additional MOC that we propose to be classified into MOC-6 and MOC7.

CHEMORESISTANCE DUE TO CHANGES IN TUMOR MICROENVIRONMENT (MOC-6)

Two peculiar features characterizing tumor microenvironment, i.e. hypoxia and acidity, play an important role in tumor progression, metastasis and response to chemotherapy. Several *in vitro* studies have demonstrated that hypoxia induces enhanced resistance to antitumor drugs, such as cisplatin, doxorubicin, etoposide, melphalan, 5-fluorouracil, gemcitabine, and docetaxel.[2] The family of hypoxia-inducible transcription factors (HIFs) represents the main mediator of the hypoxic response and is widely upregulated in human cancers. HIF-1 and to a lesser extent HIF-2, the oxygen-regulated HIF isoforms, have been associated with chemotherapy failure. Thus, HIF-1 inhibition reverses multidrug resistance in colon cancer cells.[3] Moreover, silencing HIF-1 in tumor cells results in increased sensitivity to anticancer drugs.[4] Several mechanisms and pathways that may underlay HIF-1-mediated chemotherapy resistance in tumor cells under hypoxia have been described. These include: (1) HIF-1-mediated regulation of drug efflux through the activation of transport proteins such as the multidrug resistance 1 (MDR1) gene (*ABCB1*), the multidrug-resistance-associated protein 1 (MRP1, gene symbol *ABCC1*) and the lung resistance protein (LRP) or major vault protein (MVP, gene symbol *MVP*);[5] (2) HIF-1-mediated inhibition of drug-induced DNA damage.[6] This effect is partially mediated via transcriptional down-regulation of topoisomerase II in human tumor cells;[6] (3) HIF-1 functions as a robust suppressor of apoptosis and functional interference with HIF-1 results in enhanced cell death upon treatment with chemotherapeutic agents in tumors of different origins. The molecular nature of this phenomenon was mostly accounted for by anti-apoptotic target genes of HIF-1, which include *Bcl-xL*, *Bcl-2*, *Mcl-1*, *NF-kB* and *BIRC5*;[7] (4) HIF-1-dependent decrease of the DNA-damage response-activated senescence, which is partly accountable for the anti-tumor effect of different chemotherapeutic agents;[7] (5) HIF-1-dependent induction of autophagy which confers a survival advantage to tumor cells and protects them from drug-induced death signals.[7] HIF-1 target genes such as *BNIP3* (Bcl-2/adenovirus E1B 19-kDa interacting protein 3) and *BNIP3L* (Bcl-2/adenovirus E1B 19-kDa interacting protein 3 like), members of the so-called BH3-only subfamily of Bcl-2 family proteins that antagonize the activity of the pro-survival proteins Bcl-2 and Bcl-xL, have suggested to be involved in the hypoxia-induced autophagy.[8] A role of HIF-1 independent mechanisms in hypoxia-induced drug resistance in cancer cells has also been reported. These mechanisms are still poorly understood, but pathways involving phosphoinositol-3-kinase (PI3K), nuclear factor kappa-B (NF-kB), cycloxygenase-2 (COX-2), activator protein-1 (AP-1), c-Jun, Pim-1, and STAT-3 have been reviewed and have been suggested to participate in MOC-6.[2]

Regarding the role of acidic environment in MOC-6, it should be taken into account that, as a result of the active acid production through glycolysis, which occurs in tumor cells even in the presence of oxygen, there is the need of extruding a large amount of H^+ to survive. The mechanisms activated in tumor cells to efficiently eliminate protons include up-regulation of ion pumps, such as vacuolar H^+-ATPase (V-ATPase), and transporters, such as Na^+/H^+ exchanger (NHE), together with an increased turnover of acidic vesicles. The low extracellular pH (pHo) may severely affect drug uptake. For instance, acidic pHo reduces the uptake of chemotherapeutic drugs that behave as weak bases, such as anthracyclines and Vinca alkaloids, and, hence, reduces their cytotoxicity by preventing these compounds from reaching their intracellular targets.[9] Thus, the possibility that basic drugs could be protonated and neutralized in a higher proportion by the acidic pHo of tumor environment has to be considered.[10] It has been demonstrated that compounds able to disrupt tumor pH homeostasis may reverse multidrug resistance phenotype and indirectly inhibit the growth of the tumors. Thus, treatment with sodium bicarbonate induced alkalinization of pHo and tumor growth inhibition in animal models.[9] Moreover, lysosomotropic agents that induce modification of the pHo *vs*. intracellular pH (pHi) gradient and alkalinization of intracellular acidic vesicles may reverse anthracycline resistance in chemoresistant cells.[11] In addition, H^+-pump inhibitors induce drug-resistance reversion in chemoresistant

human melanoma cells and increased sensitivity to cytotoxic drugs in chemoresistant cell lines.[12]

Several strategies are currently being developed to overcome tumor chemoresistance associated to microenvironment acidity including inhibition of deprotonation mechanisms using drugs such as inhibitors of proton transporters NHE-1, carbonic anhydrases, monocarboxylate transporters and proton pumps (PPI).[13,14] A multicentre historically controlled trial has been performed to evaluate the activity of a pre-treatment administration of the PPI esomeprazole as chemosensitizer during neoadjuvant chemotherapy based on methotrexate, cisplatin and adriamycin in patients with osteosarcoma.[15] The analysis of the resected tumors after neoadjuvant therapy revealed that pretreatment with the PPI increases the effectiveness of the polychemotherapy at the tumor level. This was particularly evident in the histological chondroblastic subtype which normally shows poor histological response. This study provides evidences that PPI may be beneficially added to standard regimens in combination to conventional chemotherapy. Other strategies involves the use of induced tumor acidity as an attractant for antitumor drugs such as cyclooxygenase inhibitors and photoactivatable cytotoxic agents such as acridine orange and imidazoacridinones, with tropism for acid environments, where they are activated.[13,14]

CHEMORESISTANCE DUE TO PHENOTYPE TRANSITION OF TUMOR CELLS (MOC-7)

The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose cell-cell interactions and polarity, and acquire a phenotype with mesenchymal characteristics, i.e. enhanced migratory behavior, invasive ability, and resistance to apoptosis activation. Under physiological circumstances during intrauterine life, EMT occurs transiently during embryogenesis and organ development, and after birth in association with wound healing, tissue regeneration and organ fibrogenesis in the context of normal morphogenesis. EMT also takes place in some types of cancer, including HCC and CCA, in cells that have previously undergone genetic changes affecting oncogenes and/or tumor suppressor genes, which favors carcinogenesis.[16] Carcinoma cells that have acquired a mesenchymal phenotype lose E-cadherin expression and express mesenchymal markers, such as N-cadherin, alpha-smooth muscle actin (α-SMA), fibroblast-specific protein 1 (FSP1), vimentin, and desmin. Commonly, carcinoma cells that have lost epithelial phenotype appear in the external layer of primary tumors and they are considered to be the cells that eventually enter into further steps of the invasion-metastasis process.

In liver cancer, a relationship between enhanced chemoresistance and EMT has also been recently described.[17] Poor differentiated liver cancer cell lines, such as HLE, HLF and SK-Hep1, expressing high levels of mesenchymal markers were more invasive and resistant to cisplatin, doxorubicin and sorafenib than other well-differentiated liver cancer cells, such as Hep3B, HepG2 and Huh7. It has been suggested that the development of a more invasive capability and chemoresistance in tumor cells could be attributed to EMT. Clinical observations support the concept that poorer differentiated HCC are more refractory to chemotherapy based on inhibitors of receptors with tyrosine kinase activity (TKI).[18] Moreover, patients with undifferentiated tumors have a worse prognosis.[17]

Although signals triggering EMT in carcinoma cells are not well known, different signaling pathways have been involved in this process.

(1) Transforming growth factor-beta (TGF-β) signaling pathway. TGF-β has been suggested to play an important role in promoting EMT in liver tumor cells.[19] In a study carried out with gemcitabine-resistant MzChA-1 cells from human biliary tract cancer, a relationship between an increase in TGF-β expression, EMT and enhanced invasive activity was found.[20] SMAD proteins are intracellular proteins belonging to the TFG-β pathway. It has been demonstrated that down-regulation of microRNA-145 (miR-145) in human HPB and HCC cells, such as HepG2 and HuH7, respectively, increases resistance to doxorubicin through enhancement of SMAD3 expression.[21] A relationship between overexpression of SMAD2 and SMAD4 and enhanced EMT resulting in mesenchymal phenotype and reduced sensitivity to sorafenib and doxorubicin has been found both *in vitro* and in HCC patients.[22] A down-regulation of miR-125b, a microRNA whose expression is strongly suppressed in HCC, has been suggested to be involved in the acquisition of chemoresistance in this type of tumor cells.

(2) Epidermal growth factor receptor (EGFR) signaling pathways. EMT status in HCC cells is also considered to be a determinant of sensitivity to EGFR inhibitors.[23] Amphiregulin, a ligand of the EGFR, which is not expressed in healthy liver, is up-regulated during chronic liver injury, the background on which most liver tumors develop. Overexpression of amphiregulin in SK-Hep1 cells enhanced their proliferation rate, anchorage-independent growth, drug resistance, and *in vivo* tumorigenic potential.[24] Another signal able

to induce EMT via modulation of EGFR pathways in HCC cells is galectin-1 (Gal-1).[25] Dysregulation of Gal-1 expression in HCC cells leads to an over-activation of FAK/PI3K/AKT and H-Ras/Raf/ERK pathways resulting in enhanced phosphorylation of AKT, mTOR and p70 kinases and up-regulation of the αvβ3 integrin expression. A consequence of the dysregulation of these pathways is EMT induction and higher resistance to sorafenib. Moreover, high levels of Gal-1 in tumors are associated with impaired sorafenib response and reduced overall survival of patients with HCC.[25]

(3) Cell-adhesion proteins involved in intracellular signaling networks. An example is CD44, a stem cell marker that besides being the cell-surface receptor of the hyaluronic acid has been suggested to play functions as a co-receptor for several tyrosine kinase receptors.[26] In a recent study carried out with human liver tumor cell lines in culture and implanted in nude mice, it has been demonstrated that cells showing a mesenchymal-like phenotype and high expression of CD44 were refractory to sorafenib-induced cell death.[19] In contrast, epithelial-like cells were more sensitive to sorafenib-induced apoptosis. The authors of this study have proposed that the appearance of a mesenchymal phenotype in tumor cells could be used as a marker to predict the lack of response of HCC to sorafenib.

CONCLUSION

In sum, in addition to the classical MOC-1 to MOC-5, two additional mechanisms of chemoresistance must be included in the defensive armamentarium developed or enhanced in liver cancer cells to overcome the pharmacological attack, MOC-6 and MOC-7, based on changes in tumor microenvironment and EMT, respectively. This accounts for a negligible response to the commonly used antitumor drugs and only a modest response to novel targeted therapies based on TKIs, such as sorafenib. Further advances are urgently needed to better understand the bases of the chemoresistace barrier, which in the future may enlarge the list of MOCs by including for instance autophagy mechanisms.[27] This knowledge is required to develop new tools able to demolish or inactivate cancer cell defenses against chemotherapy.

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Conflicts of interest

There are no conflicts of interest.

Patient consent

There is no patient involved.

Ethics approval

This review paper is waived for ethics approval.

REFERENCES

1. Marin JJ, Romero MR, Briz O. Molecular bases of liver cancer refractoriness to pharmacological treatment. *Curr Med Chem* 2010;17:709-40.
2. Doktorova H, Hrabeta J, Khalil MA, Eckschlager T. Hypoxia-induced chemoresistance in cancer cells: the role of not only HIF-1. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2015;159:166-77.
3. Chen J, Ding Z, Peng Y, Pan F, Li J, Zou L, Zhang Y, Liang H. HIF-1alpha inhibition reverses multidrug resistance in colon cancer cells via downregulation of MDR1/P-glycoprotein. *PLoS One* 2014;9:e98882.
4. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. *Drug Resist Updat* 2011;14:191-201.
5. Zhu H, Chen XP, Luo SF, Guan J, Zhang WG, Zhang BX. Involvement of hypoxia-inducible factor-1-alpha in multidrug resistance induced by hypoxia in HepG2 cells. *J Exp Clin Cancer Res* 2005;24:565-74.
6. Daskalow K, Rohwer N, Raskopf E, Dupuy E, Kuhl A, Loddenkemper C, Wiedenmann B, Schmitz V, Cramer T. Role of hypoxia-inducible transcription factor 1alpha for progression and chemosensitivity of murine hepatocellular carcinoma. *J Mol Med (Berl)* 2010;88:817-27.
7. Sermeus A, Cosse JP, Crespin M, Mainfroid V, de Longueville F, Ninane N, Raes M, Remacle J, Michiels C. Hypoxia induces protection against etoposide-induced apoptosis: molecular profiling of changes in gene expression and transcription factor activity. *Mol Cancer* 2008;7:27.
8. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, Mazure NM. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* 2009;29:2570-81.
9. Raghunand N, He X, van Sluis R, Mahoney B, Baggett B, Taylor CW, Paine-Murrieta G, Roe D, Bhujwalla ZM, Gillies RJ. Enhancement of chemotherapy by manipulation of tumour pH. *Br J Cancer* 1999;80:1005-11.
10. De Milito A, Fais S. Tumor acidity, chemoresistance and proton pump inhibitors. *Future Oncol* 2005;1:779-86.
11. Ouar Z, Bens M, Vignes C, Paulais M, Pringel C, Fleury J, Cluzeaud F, Lacave R, Vandewalle A. Inhibitors of vacuolar H+-ATPase impair the preferential accumulation of daunomycin in lysosomes and reverse the resistance to anthracyclines in drug-resistant renal epithelial cells. *Biochem J* 2003;370:185-93.
12. Luciani F, Spada M, De Milito A, Molinari A, Rivoltini L, Montinaro A, Marra M, Lugini L, Logozzi M, Lozupone F, Federici C, Iessi E, Parmiani G, Arancia G, Belardelli F, Fais S. Effect of proton pump

inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. *J Natl Cancer Inst* 2004;96:1702-13.
13. Fais S, Venturi G, Gatenby B. Microenvironmental acidosis in carcinogenesis and metastases: new strategies in prevention and therapy. *Cancer Metastasis Rev* 2014;33:1095-108.
14. Taylor S, Spugnini EP, Assaraf YG, Azzarito T, Rauch C, Fais S. Microenvironment acidity as a major determinant of tumor chemoresistance: proton pump inhibitors (PPIs) as a novel therapeutic approach. *Drug Resist Updat* 2015;23:69-78.
15. Ferrari S, Perut F, Fagioli F, Brach Del Prever A, Meazza C, Parafioriti A, Picci P, Gambarotti M, Avnet S, Baldini N, Fais S. Proton pump inhibitor chemosensitization in human osteosarcoma: from the bench to the patients' bed. *J Transl Med* 2013;11:268.
16. Brivio S, Cadamuro M, Fabris L, Strazzabosco M. Epithelial-to-mesenchymal transition and cancer invasiveness: what can we learn from cholangiocarcinoma? *J Clin Med* 2015;4:2028-41.
17. Chen X, Lingala S, Khoobyari S, Nolta J, Zern MA, Wu J. Epithelial mesenchymal transition and hedgehog signaling activation are associated with chemoresistance and invasion of hepatoma subpopulations. *J Hepatol* 2011;55:838-45.
18. Huang XY, Ke AW, Shi GM, Zhang X, Zhang C, Shi YH, Wang XY, Ding ZB, Xiao YS, Yan J, Qiu SJ, Fan J, Zhou J. alphaB-crystallin complexes with 14-3-3zeta to induce epithelial-mesenchymal transition and resistance to sorafenib in hepatocellular carcinoma. *Hepatology* 2013;57:2235-47.
19. Fernando J, Malfettone A, Cepeda EB, Vilarrasa-Blasi R, Bertran E, Raimondi G, Fabra A, Alvarez-Barrientos A, Fernandez-Salguero P, Fernandez-Rodriguez CM, Giannelli G, Sancho P, Fabregat I. A mesenchymal-like phenotype and expression of CD44 predict lack of apoptotic response to sorafenib in liver tumor cells. *Int J Cancer* 2015;136:E161-72.
20. Yamada D, Kobayashi S, Wada H, Kawamoto K, Marubashi S, Eguchi H, Ishii H, Nagano H, Doki Y, Mori M. Role of crosstalk between interleukin-6 and transforming growth factor-beta 1 in epithelial-mesenchymal transition and chemoresistance in biliary tract cancer. *Eur J Cancer* 2013;49:1725-40.
21. Ju BL, Chen YB, Zhang WY, Yu CH, Zhu DQ, Jin J. miR-145 regulates chemoresistance in hepatocellular carcinoma via epithelial mesenchymal transition. *Cell Mol Biol (Noisy-le-grand)* 2015;61:12-6.
22. Zhou JN, Zeng Q, Wang HY, Zhang B, Li ST, Nan X, Cao N, Fu CJ, Yan XL, Jia YL, Wang JX, Zhao AH, Li ZW, Li YH, Xie XY, Zhang XM, Dong Y, Xu YC, He LJ, Yue W, Pei XT. MicroRNA-125b attenuates epithelial-mesenchymal transitions and targets stem-like liver cancer cells through small mothers against decapentaplegic 2 and 4. *Hepatology* 2015;62:801-15.
23. Fuchs BC, Fujii T, Dorfman JD, Goodwin JM, Zhu AX, Lanuti M, Tanabe KK. Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. *Cancer Res* 2008;68:2391-9.
24. Castillo J, Erroba E, Perugorria MJ, Santamaria M, Lee DC, Prieto J, Avila MA, Berasain C. Amphiregulin contributes to the transformed phenotype of human hepatocellular carcinoma cells. *Cancer Res* 2006;66:6129-38.
25. Zhang PF, Li KS, Shen YH, Gao PT, Dong ZR, Cai JB, Zhang C, Huang XY, Tian MX, Hu ZQ, Gao DM, Fan J, Ke AW, Shi GM. Galectin-1 induces hepatocellular carcinoma EMT and sorafenib resistance by activating FAK/PI3K/AKT signaling. *Cell Death Dis* 2016;7:e2201.
26. Lee D, Na J, Ryu J, Kim HJ, Nam SH, Kang M, Jung JW, Lee MS, Song HE, Choi J, Lee GH, Kim TY, Chung JK, Park KH, Kim SH, Kim H, Seo H, Kim P, Youn H, Lee JW. Interaction of tetraspan(in) TM4SF5 with CD44 promotes self-renewal and circulating capacities of hepatocarcinoma cells. *Hepatology* 2015;61:1978-97.
27. Shimizu S, Takehara T, Hikita H, Kodama T, Tsunematsu H, Miyagi T, Hosui A, Ishida H, Tatsumi T, Kanto T, Hiramatsu N, Fujita N, Yoshimori T, Hayashi N. Inhibition of autophagy potentiates the antitumor effect of the multikinase inhibitor sorafenib in hepatocellular carcinoma. *Int J Cancer* 2012;131:548-57.