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Mini-review

Potential molecular, cellular and microenvironmental mechanism of sorafenib resistance in hepatocellular carcinoma

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ABSTRACT

Sorafenib, an orally-available kinase inhibitor, is the only standard clinical treatment against advanced hepatocellular carcinoma. However, development of resistance to sorafenib has raised concern in recent years due to the high-level heterogeneity of individual response to sorafenib treatment. The resistance mechanism underlying the impaired sensitivity to sorafenib is still elusive though some researchers have made great efforts. Here, we provide a systemic insight into the potential molecular, cellular and microenvironmental mechanism of sorafenib resistance in hepatocellular carcinoma depending on abundant previous studies and reports.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common primary malignant tumors and the largest cancer-related deaths ranking only second to lung cancer, with a fairly high and increasing incidence, frequently relapse and dismal prognosis [1,2]. Despite the remarkable progress in the prevention, detection, and treatment of cancer over the last five decades, no adequate therapy remains sufficiently effective due to late stage diagnosis and inadequate clinical strategies for inhibiting metastasis and promoting apoptosis [3–6]. Furthermore, multi-drug resistance of cancer cells has been implicated as a major challenge considering the irreplaceable role of chemotherapeutic interventions in anti-cancer treatment [7]. The resistance was postulated to associate with elevated expression of drug efflux transporters, changes in drug kinetics, amplification of drug targets or tumor heterogeneity comprising of genetic variation, the microenvironment, and cell plasticity [8]. For patients with HCC diagnosed at advanced stage, sorafenib is the only choice of systemic therapy when potentially curative treatment, such as resection and liver transplantation, may be merely applicable for patients diagnosed at early stage [9]. Recently, the unstable efficacy of sorafenib has raised concern of more and more researchers and 'sorafenib resistance' has become a hot term used to describe the impaired efficacy of sorafenib, especially for patients with advanced HCC.

Sorafenib, as a multikinase inhibitor, suppresses tumor angiogenesis and proliferation by inhibiting serine/threonine kinases, as well as receptor tyrosine kinases. Intracellular Raf serine/threonine kinase isoforms inhibited by sorafenib include Raf-1 (or C-Raf), wild-type B-Raf and mutant B-Raf. Receptor tyrosine kinases inhibited by sorafenib include vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR)-b, c-KIT, FMS-like tyrosine kinase 3 (FLT-3) and RET [10]. Among these kinases, RAF and VEGFR are presumed to be essential for the anti-proliferative effects evoked by sorafenib. RAF kinases are present at the level of the cancer cells, while the VEGFR is present on the surface of endothelial cells [11]. Inhibition of the VEGFR accounts, at least in part, for the antiangiogenic effects of sorafenib. In majority of patients with advanced HCC, the RAF–MEK–ERK cascade is often activated by autocrine and paracrine loops, involving for example the production of amphiregulin, an agonist of the Epidermal Growth Factor Receptor (EGFR) [12]. The analysis of vitro experiments reported that there was a strong correlation between the inhibition of the RAF–MEK–ERK cascade and the anti-clonogenesis effect of sorafenib [13]. In addition, cytotoxic effect of sorafenib is also a crucial role in antitumor treatment. Generally, apoptosis is the major form of cytotoxicity and it is required for tumor regression and sustained clinical remissions [14]. The pro-apoptotic effect of sorafenib in HCC cells had also been studied extensively. In 2008, the SHARP (Sorafenib HCC Assessment Randomised Protocol)

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trials showed an improved overall survival in Child–Pugh class A patients with advanced HCC upon treatment with the antiangiogenic and antiproliferative agent sorafenib. In addition, Oriental confirmed the efficacy and safety of sorafenib again in treatment of advanced hepatocellular carcinoma by another multicenter, randomized, double-blind, placebo-controlled phase III clinical trials.

However, the promising systemic chemotherapeutic agent sorafenib only demonstrated relatively limited benefits rather than eradicated the microscopic residual and cured the patients with advanced HCC. Sorafenib is beneficial in only around 30% of patients, and acquired resistance often develops within 6 months [15,16], suggesting the existence of primary and acquired sorafenib resistance in hepatocellular carcinoma cells. So far, some researchers have investigated the mechanism underlying resistance to sorafenib from molecular and clinical points of view. Huang et al. have reported that overexpression of both αB-Crystallin and 14-3-3ζ decreased hepatoma cells sensitization to sorafenib in clinical by inducing epithelial–mesenchymal transition (EMT) in HCC cells [17]. Chiou et al. presented evidence that glucose-regulated protein 78 (GRP78) is a positive modifier for sorafenib resistance acquisition in HCC and GRP78 knockdown enhanced the efficacy of sorafenib-mediated cell death [18]. Chen et al. also conducted experiments to investigate sorafenib resistance and the results showed that long-term exposure to sorafenib activated the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and mediate acquired resistance to sorafenib in HCC cell lines [19]. All the insights into the molecular and clinical changes involved in sorafenib treatment provided a greater understanding of the underlying mechanism of primary and acquired resistance to sorafenib. However, a systemic and comprehensive analysis about the mechanism of acquiring resistance to sorafenib was still elusive [20]. Here, in order to clarify the drug resistance mechanism clearly, we reviewed studies on sorafenib resistance in liver cancer from different angles, including molecular markers, signaling pathways, drug resistance principles, regulation systems, therapeutic implications and recent approaches. Increasing evidence suggested that deeper insight into the formation of sorafenib resistance would shed light on the specific drug resistance mechanism and might lead to identification of potential clinical biomarkers for prognosis evaluation and targets for new therapeutic strategies. Therefore, in the present review, by synthesizing the retrospective studies and reports, we provided potential molecular, cellular and microenvironmental mechanism underlying impaired sensitivity to sorafenib in hepatocellular carcinoma, which had profound effect on inhibiting tumor progression, evaluating patient survival and predicting sorafenib treatment response.

Epithelial–mesenchymal transitions (EMT)

Epithelial–mesenchymal transitions (EMT) are mainly marked by the loss of cell–cell interactions and of epithelial apico-basal polarity with the concomitant acquisition of mesenchymal markers and enhanced migratory behavior [21,22]. As is well known, EMT is of paramount relevance for various developmental processes, including gastrulation, neural crest formation and carcinoma progression. In the last decades, EMT has been extensively studied and validated in the progression of various carcinomas such as HCC [23,24]. A large body of researches has claimed the important role of EMT in facilitating tumor development and progression by driving metastasis, through the acquisition of enhanced migratory and invasive potential [25–30]. Metastasis, induced by EMT, deconvoluted into several steps including intravasation, circulation, margination, extravasation and colonization, is a crucial cause of deaths in cancer therapy [31,32]. It had been reported that intrahepatic metastases were observed in about 30% of cases after surgical removal of small HCC nodules and in 80% of HCC autopsy cases [33]. Via EMT, epithelial cells dissolve intercellular connections and acquire mesenchymal properties and metastasize to the native or distant sites. Once cancer cells seed the metastatic site, a mesenchymal to epithelial transition (MET) occurs, inducing colonization and growth of the metastatic foci with re-expression of cell adhesion molecule E-cadherin, facilitating tumor cells to seed in the metastatic sites [34–40]. E-cadherin re-expression accompanied by a partial MET in the metastatic sites increases post-extravasation survival of the cancer cells and resistance to multi-drugs [35,37]. Additionally, further study found that a variety of biological molecules such as HIF, TGF and miRNAs involved in the process of MET through different signaling pathways respectively accelerated the formation of distant tumor metastasis [41–43]. In epithelial cancers, EMT resulted in the loss of E-cadherin and in turn, tumor cells attain enhanced migratory and invasive potential. E-cadherin, whose down-regulated expression is a main biomarker for activation of EMT, shows reverse relationship with drug resistance [44]. By undergoing EMT, the tumor cells acquired resistance to a number of chemo and radiotherapies [34,45–48]. The relationship between epithelial–mesenchymal transition (EMT) and drug resistance was first described in connection with cancer stem cells by Mani et al., who inferred that blocking or reversing EMT might cause chemoresistant cells to revert to chemosensitive cells [49]. In addition, in breast cancer therapy, it was reported that epithelial cells acquire some cancer stem cells properties via EMT, such as anti-apoptosis and drug resistance [50]. In the process of EMT in tumor cells, some biological molecules, such as TGF β, Slug and FOXC2, play very crucial role in explaining the sensitivity of tumor cells to chemotherapy drugs. Activation of TGF β or FOXC2 and overexpression of Slug impaired the sensitivity of tumor tissues to drug [49,51,52]. The drug resistance was also reported in correlation with EMT in HCC [17–19]. Various signaling pathways have been involved in regulation of EMT program [27], indicating that these signaling pathways and molecules involved are potentially associated with drug resistance. The Raf/MEK/ERK pathway represents a dominant signaling network promoting proliferation and metastasis and is the main jamming target pathway of sorafenib [53–56]. The genetic and molecular network involved in the signal pathway is very complicated. In fact, some studies have demonstrated that treatment with Raf kinase inhibitors can even paradoxically induce ERK cascade signaling by promoting dimerization of Raf family members [57,58]. The biomarkers for sorafenib efficacy, such as Plasma c-KIT, hepatocyte growth factor and angiopoietin-2, are likely to be downregulated in the sorafenib sensitive cell lines while the phospho-Akt and p85 (a regulatory subunit of PI3K) are upregulated in sorafenib resistant cell lines [19,59,60]. Kunnimalaiyaan et al. hypothesized that Notch signaling pathway is a potential regulator of EMT program in HCC and NICD3 protein increased E-cadherin and activated transcription factors Snail, Slug, and MMPs and induced EMT [27], which might lead to development of sorafenib resistance. Acquired resistance to sorafenib in HCC might also be mediated by the activation of phosphatidylinositol 3-Kinase/Akt signaling pathway [19,61]. Overexpression of both αB-Crystallin (Cryab) and 14-3-3ζ can induce epithelial–mesenchymal transition (EMT) in HCC cells through activation of the extracellular-regulated protein kinase (ERK) cascade, a crucial up-stream factor of ERK1/2/Fra-1/slug signaling pathway, which can counteract the effect of sorafenib [62–67]. Therefore, it is postulated that sorafenib resistance is closely correlated with the dynamic changes of numerous molecules, especially their up- or down-regulated expression, involved in the mutual transition between epithelial and mesenchymal cell phenotypes and various signaling pathways. Particularly, epithelial cells are more susceptible to a Raf kinase inhibitor, sorafenib, whereas mesenchymal cells showed significant resistance [68,69]. In addition, PI3K/Akt showed higher activity and integrin-linked kinase (ILK) exhibit in the mesenchymal cells as compared to epithelial cells [70,71]. Many pathways and molecules involved in these pathways play a crucial

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