Cancer Cell International

REVIEW

CrossMark

Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight

K. Lohitesh[†], Rajdeep Chowdhury[†] and Sudeshna Mukherjee[*] ![ORCID]

Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer mortality, accounting for almost 90% of total liver cancer burden. Surgical resection followed by adjuvant and systemic chemotherapy are the most meticulously followed treatment procedures but the complex etiology and high metastatic potential of the disease renders surgical treatment futile in majority of the cases. Another hindrance to the scenario is the acquired resistance to drugs resulting in relapse of the disease. Hence, to provide insights into development of novel therapeutic targets and diagnostic biomarkers, this review focuses on the various molecular mechanisms underlying chemoresistance in HCC. We have provided a comprehensive summary of the various strategies adopted by HCC cells, extending from apoptosis evasion, autophagy activation, drug expulsion to epigenetic transformation as modes of therapy resistance. The role of stem cells in imparting chemoresistance is also discussed. Furthermore, the review also focuses on how this knowledge might be exploited for the development of an effective, prospective therapy against HCC.

Keywords: Cancer, HCC, Chemoresistance, Metastasis, Apoptosis, Autophagy

Introduction

Hepatocellular carcinoma is currently considered as a rapidly evolving disease. According to the Globocan report, an estimated 782,451 new liver cancer cases and 745,517 cancer deaths have occurred worldwide in 2012. Also, as per the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) the relative 5-year survival rate of HCC between 2002 and 2008 has been as low as 15%. The main causative factors contributing to the disease have been chronic alcohol abuse, infection with hepatitis B or hepatitis C virus and food contaminations [1]. As a consequence of such varied etiologies, HCC is a heterogeneous malignancy with complex carcinogenesis. Also despite advances in development of early detection methodologies, the ineffective and expensive procedures available for treatment of HCC pose a challenge for the disease management.

In fact, 80% of HCC patients are currently diagnosed at an advanced stage of the disease with a median survival of 6–8 months only. Surgical resection followed by chemotherapy is the most established curative treatment for HCC. However, operating on the liver can be both complicated and unachievable due to size and distribution of the tumor in the liver, blood vessels and other vital organs. Also, complete surgical removal is mostly not possible for more than two-third of HCC patients where the disease have already metastasized and the patients are at an advanced stage [2–4]. Current treatment procedures mainly include cryosurgery, radiofrequency ablation and embolization but they are mostly palliative approaches without much success rate [5]. Moreover, post-surgery recurrence of the tumor has been a major issue for more than 90% of HCC patients. This has forced to shift the treatment regime towards systemic chemotherapy. Drugs that are used in HCC as monotherapy are listed in Table 1. But currently the use of single agents in therapy is practically non-existent because of their low response. For example, in a large study of doxorubicin,

*Correspondence: sudeshna@pilani.bits-pilani.ac.in
[†]K. Lohitesh and Rajdeep Chowdhury contributed equally to this work
Department of Biological-Sciences, Birla Institute of Technology and Sciences (BITS), Campus, VidyaVihar, Pilani, Rajasthan 333031, India

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 2 of 15

Table 1 List of drugs and their targets used against HCC

S. No.	Name of the drug	Target molecule	Mechanism	Limitation	Year and references
1	Tamoxifen	Antagonist of estrogen receptor	To inhibit P-glycoprotein-mediated drug resistance	Minimum effect	2000 [88]
2	5-Fluorouracil	Thymidylate synthase	Incorporated its metabolites into RNA and DNA	Requires co-treatment with leucovorin and methotrexate, to increase the anti-cancer activity of 5-Fu	2003 [89]
3	Thalidomide	VEGF, inhibits TNF-α synthesis, inhibition of Ikβ kinase activity	Anti-angiogenic activity and immune-modulatory	Fatigue, somnolence, constipation	2003, 2004 [90, 91]
4	Octreotide	Analogue of somatostatin receptors	Anti-tumor effect.	Somatostatin receptor type 2 (SSTR2) was found in some but not all patients with HCC	2004, 2006 [92, 93]
5	Sorafenib	Raf, VEGFR2, VEGFR3, PDGFRs	Inhibits tumor angiogenesis by blocking the activation of the tyrosine kinase receptors	Hypertension, diarrhea, proteinuria, skin-related toxicities, an increased risk for thromboembolism and bleeding events	2006, 2008 [10, 11]
6	Sunitinib	PDGFRs, KIT, RET, and FLT3	Inhibits tumor angiogenesis by blocking the activation of the tyrosine kinase receptors	Modest clinical efficacy	2009 [94]
7	Bevacizumab	VEGF	Blocks VEGF binding to its receptor	Low rate of response, gastrointestinal bleeding, including variceal bleeding	2009 [95]
8	Erlotinib, gefitinib and cetuximab	EGFR	Tyrosine kinase inhibitor, acts on the epi-dermal growth factor receptor (EGFR)	Minimum effect	2009 [96]
9	Doxorubicin	DNA topoisomerase II inhibitors	Induce histone eviction	The use of single agents in therapy is prac-tically non-existent currently because of its erratic and low response	2013 [97]
10	Cisplatin	DNA	Cross link with purine causes DNA damage and ultimately induces apoptosis	Allergic reactions, gastrointestinal disor-ders, decrease immunity to infections, kidney problems, hemorrhage	2014 [98]
11	Oxaliplatin	DNA	Binds to guanine and cytosine leading to cross-linking of DNA	Increase autophagy level results in a tumor resistance Reduction of DYRK2 promotes cell prolif-eration, and resistance to Oxaliplatin	2016 [99]

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 3 of 15

no responses were noted among 109 patients; also among 475 patients who received doxorubicin in various studies, only 16% response rate was documented, with a median survival of 3–4 months only [6]. This led to the evolution of combined regimen drugs. A combination of capecitabine + oxaliplatin + cetuximab showed modest activity only [7]. Among cisplatin-based regimens, the best response rate was obtained with the treatment of PIAF (cisplatin + adriamycin + 5-FU + INF) [8]. More recently, GEMOX (gemcitabine + oxaliplatin) has also been evaluated in a phase-II study, with promising results [9]. Other chemotherapeutic drugs like, sorafenib are also often used to attenuate HCC tumor [10, 11]. But, acquisition of chemoresistance continues to be a major constraint in chemotherapy-based treatment of the disease. An alternative strategy adopted was the administration of chemodrugs like cisplatin, mitomycin C and doxorubicin through hepatic artery infusion [12]. However, surgical catheter insertion into the hepatic artery and inoperable conditions of the tumor owing to HCC's high metastatic potential became a limiting factor. Thus despite recent advancements in chemotherapy, HCC still remain a fatal disease. Hence, focus should be reoriented more on unraveling the molecular mechanisms behind chemoresistance with an objective to develop novel therapeutic targets and diagnostic biomarkers.

Various mechanism of chemoresistance in HCC cells

Reduced drug uptake, efflux of drugs and drug metabolism
A poor response to pharmacological regimens in the treatment of liver cancer can be due to complex mechanisms like, reduced drug uptake, enhanced drug efflux, intracellular drug metabolism leading to reduced concentrations of active agents, changes in the molecular targets of the drugs and enhanced repair of drug-induced modifications. In some cases, the conjoint enhanced expression of drug efflux pumps like, both MDR1 and MRP2 seems to be a contributing factor towards resistance [13]. Thus in patients with non-surgical HCC the response to chemotherapy has been found to be inversely proportional to the expression of multi-drug transport system. In this context, different transporters with their mode of action are listed in Table 2. Hence, recent therapeutic strategies must involve drug pump antagonist against the resistant cells. Alternate mechanisms employed by the tumor cells to inactivate anti-cancer drugs are over-expression of detoxifying enzymes like phase-I (cytochrome P450) and phase-II enzymes in conjugation with glucuronic acid or with glutathione. Cytochrome P450 enzymes and glutathione S-transferases, along with other enzymes like epoxide hydrolase, phase II xenobiotic metabolizing enzymes are reported to increase cells' resistance to alkylating agents or drugs like, cisplatin in HCC [14, 15]. Enhanced drug in-activation by uridine glucuronosyltransferase-1A has also been found to be associated with drug resistance in HCC [14, 16]. Thus, a vivid understanding of the intra-cellular biology of chemoresistance is desirable to develop new therapies, which would help to override the resistance acquired by the conventional cytotoxic drugs.

DNA repair pathway aberrations and chemoresistance in HCC
HCC develops in a milieu of underlying chronic inflammation, which promotes DNA damage and chromosomal aberration. It has been observed that HCC deploys DNA repair mechanisms, like stalled DNA replication fork by homologous recombination (HR), base mismatches by mismatch repair (MMR), double-strand break (DSB), non homologous end joining (NHEJ) to become chemoresistant. They can upregulate various enzymes involved in DNA repair mechanisms for acquisition of chemoresistance [17]. However, it is not necessary that the repair pathways are always upregulated in chemoresistant models. Defect in MMR pathway genes can also induce resistance to certain therapeutic agents. In HCC cells, over-expression of MDR-1 is associated with patients having defective MMR genes contributing to drug resistance [18]. A better understanding of the role of DDR

Table 2 List of drug transport systems and their status in HCC

S. No.	Transporter	Mode of action	Up-regulated or down-regulated	References
1	MDR1 (ABCB1 or P-glycoprotein), MRP1 (ABCC1), MRP2 (ABCC2), MRP3(ABCC3) and ABCG2	Extrudes unrelated anti-tumoral agents like anthracyclines, taxanes, vinca alkaloids etc. from the cell	Up regulated	[18, 100]
2	OATP1B1 and OATP1B3	Transports anti-tumor drugs inside the cells	Down regulated	[101, 102]
3	SLC28 and SLC29	Uptake of nucleoside derived anticancer drugs	Down regulated	[103]
4	Organic cationic transporter-1 (OCT1)	Uptake of tyrosine kinase inhibitors	Down regulated; hyper-methylation of the SLC22A1 promoter	[104]

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 4 of 15

pathways in HCC may help us to develop novel strategies for treatment or prevention of HCC. Various DNA repair enzymes, which are involved in chemoresistance, are listed in Table 3; these can be exploited in a judicious way to develop novel therapeutic strategies.

Impairment of the apoptotic machinery and associated chemoresistance in HCC

Apoptosis is a highly conserved phenomenon contributing to tissue homeostasis by targeted elimination of single cells without disrupting the functionality of the respective tissue. Cancer strikes by developing resistance against cell death signaling pathways, like apoptosis upon failure of immune surveillance. A similar aspect underlies the poor responsiveness of HCC towards chemotherapy. Upon exposure to cytotoxic agents, HCC cells down regulate death receptor CD95 and upregulate CD95-ligand (FasL) [19–22]. The defect in down-stream Fas-associated death domain (FADD) signaling also potentially contributes to the chemoresistance in HCC [23, 24]. It has further been observed that the anti-apoptotic protein-brain and reproductive organ-expressed protein (BRE), can bind to tumor necrosis factor (TNF) receptor-1 and Fas conferring the ability of chemoresistance to HCC cells by inhibiting death receptor induced apoptosis [25, 26]. FLICE inhibitory protein (c-FLIP), a recently identified intracellular inhibitor of caspase-8 activation that can potentially inhibit death signaling mediated by various death receptors, like Fas, TNF-receptor (TNF-R), and TNF-related apoptosis-inducing ligand receptors (TRAIL-Rs) and by NFκB activation, was found to be constitutively expressed in HCC cell lines and over-expressed in human HCC tissues. An over-expression of c-FLIP was associated with shorter recurrence free survival time in HCC patients [27, 28]. A resistance towards TRAIL induced apoptosis is also reported in HCC by virtue of loss of TRAIL receptor and over activation of NF-κB. Activation of NF-κB, and nuclear localization of NF-κB, p65, p50 and p52 subunits is often found to be associated with cell proliferation and resistance in HCC [29]. Furthermore, Mcl-1, an anti-apoptotic member of the Bcl-2 family, is over-expressed in HCC, which contributes to the malignant phenotype and resistance towards apoptosis and chemotherapeutics. On a similar note, over-expression of Bcl-2 and Bcl-xL has been found in HCC cell lines and is associated with resistance to paclitaxel [30]. An over-expression of a specific set of microRNA, like, miR23a that allow the escape from TGF-β-induced apoptosis has also been observed in HCC [31]. Additionally, the presence of abnormally functioning p53 is also a common finding in many drug-resistant tumor cells and HCC is no exception. It is reported that p53 contributes to cell survival and chemoresistance in HCC under nutrient-deprived conditions by enhancing autophagy [32]. Also, gain of function (GOF) mutations in p53 are reported to impart resistance to doxorubicin and paclitaxel in HCC cells [33]. Overall apoptosis resistance in HCC is the outcome of varied molecular alterations and further research needs to be channelized in this direction to trace the links to individual pathways regulating apoptosis in HCC.

Activation of cell survival signaling and evasion of chemoresistance in HCC

In conjunction with de-regulation of apoptosis, HCC cells simultaneously trigger multiple survival signaling pathways to evade the physicians armory and maintain neoplastic progression. For example constitutive activation of Insulin-like growth factor (IGF) signaling pathway is an important contributor to drug resistance in HCC against sunitinib [34]. Various other growth receptors like epidermal growth factor receptor (EGFR) and connective tissue growth factor (CTGF) are also reported to be upregulated and involved in proliferation and drug resistance of HCC [35, 36]. Also, over-expression of Forkhead box M1 (FoxM1) transcription factor that act as a master regulator of HCC cell growth through regulation of cell cycle, glycolysis and EMT contributes significantly to the development of chemoresistance in HCC [37]. The multiple proliferation signaling pathways that enhance angiogenesis, drug resistance and cell proliferation facilitating HCC cell growth are summarized in Table 4. A

Table 3 List of DNA repair enzymes de-regulated in HCC

S .No.	DNA repair enzyme	Therapy given	Repair mechanism involved	Reference
1	ERCC-1	Platinum based anti-cancer agents	Nucleotide excision repair (NER)	[105]
2	Flap endonucleases (FENs)	Cisplatin	Nucleotide excision repair (NER) and Base excision repair (BER)	[106]
3	Chk2	Paclitaxel	DNA damage checkpoint	[107]
4	ATM signaling	Sorafenib	DNA damage checkpoint	[108]
5	Apurinic/apyrimidinic endonuclease (APE1)	Irradiation	Base excision repair (BER)	[109]

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 5 of 15

Table 4 Molecular signaling pathways altered in HCC

S. No.	Altered signaling pathways	Relevant molecule	Alteration	Targeted therapies	References
1	Hedgehog	SMO	Activating overexpression	GDC-0449, cyclopomine	2006 [110]
7	Hippo	MST1/2	Down-regulated	–	2009 [111]
8	Hippo	pYAP	Down-regulated	–	2009 [111]
3	Notch	NOTCH1	Overexpression	Gamma secretase	2009 [112]
2	Wnt/beta-catenin	APC	Inactivating mutation	–	2012 [113]
6	PI3K/AKT/mTOR	MTORC1	Up-regulated	Everolimus, rapamycin	2012 [114]

targeted therapy by specific inhibition of alternated signaling cascades can therefore be thought upon as one of the options to decelerate HCC progression.

Autophagy as defense to chemotherapeutic stress

Autophagy is an evolutionarily conserved process through which lysosomal degradation of damaged and superfluous cell components are achieved and recycled back into basic bio-molecules in the cytosol. HCC cells can use autophagy as a cellular defense mechanism upon facing stress conditions like, nutrient deprivation, hypoxia and drug insult. Reports of autophagy activation post oxaliplatin treatment and enhanced oxaliplatin induced cell-death upon autophagy inhibition in HCC, supports the pro-survival role of autophagy in HCC cells [38]. Similar to above, the anticancer effects of two other drugs, like, bevacizumab and sorafenib were enhanced upon autophagy inhibition in HCC [39, 40]. Our study, also shows that autophagy plays a pro-tumorigenic role facilitating EMT in HCC cells [41]. Inhibition of autophagy by late stage autophagy inhibitors like, chloroquine showed a marked suppression of liver tumor in vivo studies as well [39]. Furthermore, cisplatin treatment is known to trigger unfolded protein response (UPR) in HCC cells which subsequently inhibits cell apoptosis through activation of autophagy [42]. Accumulation of phosphorylated p62, a protein that is generally degraded through autophagy, was found to stimulate hepatitis C virus induced tumors in HCC. Concurrently, inhibition of p62 phosphorylation suppressed proliferation and anticancer drug tolerance in HCC [43]. In spite of a spur in research articles demonstrating the role of autophagy in cancer, the exact role of autophagy on tumor cells is still controversial and remains to be further elucidated in hepatocellular carcinoma.

Cancer stem cells in HCC chemoresistance

The 'stem cell model' of carcinogenesis suggests that cancer originates and is maintained by a rare fraction of cells called the cancer stem cells (CSCs) [44, 45]. They are responsible for the growth of neoplastic tissues and are naturally resistant to chemotherapy. In HCC, CSC markers include epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD44, CD24, CD13, and oval cell marker OV6, some of which confer chemoresistance property to them. CD133+ HCC cells are reported to confer chemoresistance via the preferential activation of the Akt and Bcl-2 survival pathway [46]. The EpCAM+ CSCs in HCC also show chemo-resistance against genotoxic agents like, 5-FU [47]. HCC tumor growth and metastasis are thus undoubtedly driven by CSCs leading to transient effects of the current chemotherapies against HCC.

Evidences for epigenetic regulation of chemoresistance in HCC

The role of epigenetics, has surfaced as an emerging topic in regulation of chemoresistance in the last decade. Amongst the epigenetic modifications, DNA methylation has garnered a great deal of attention recently in terms of development of chemoresistance. HCC tumors exhibit specific DNA methylation signatures that can be associated with tumor progression implying the potential benefit for their identification and subsequent targeting. Hernandez-Vargas et al. were able to identify a set of hypermethylated gene promoters (APC, RASSFIA, CDKN2A and FZD7) which could differentiate HCC tumors from paired surrounding control liver tissues. Specifically, promoter methylation of DNMT1was also found to be significantly associated with poor prognosis [48]. Song et al. further analyzed DNA methylation pattern in HCC tissues from 27 HCC patients, and observed significant enrichment of promoter methylation in various genes involved in cell death and cancer; the top five of them were genes like, BMP4, CDKN2A, GSTP1, and NFATC1 [49]. Also, another study showed that transcriptional repression of miR-193a-3p promoter through hyper methylation is involved in 5-FU resistance in HCC cells [50]. Hence, suppression of DNA methylation was capable of enhancing the sensitivity of HCC cells to 5-fluorouracil (5-FU). Though, 5-aza-2′-deoxycytidine (5-aza-dC), a specific DNA methylation inhibitor alone, did not induce cell death in vitro; however, a combination

Lohitesh *et al. Cancer Cell Int (2018) 18:44*

Page 6 of 15

of 5-aza-dC with 5-FU showed a reduction in cell viability and induction of apoptosis to a greater degree than with 5-FU only [51]. However, in a clinical study with low dose decitabine (5-Aza-2′-deoxycytidine), substantial remission and favorable toxicity profiles were obtained in patients with advanced HCC [52]. Evaluation of another epigenetic aspect- histone methylation has been less explored and correlated with clinico-pathlogical features in HCC. However, high levels of trimethylated histone H3 lysine 4 (H3K4me3), a transcriptional suppressive signature was found to be associated with poor survival, prognosis and aggressive tumor features in HCC [53]. Also, subunits of polycomb repressive complexes-2 (PRC-2)-SUZ12 and EZH2 were found to impart distinct roles during HCC pathogenesis. Over-expression of EZH2 was consistently observed in advanced HCC [54] while in, chronic HBV infection, the HBx protein was found to modulate SUZ12 protein levels, that maintained "stemness" of a sub-population of hepatocyte cells thus contributing to drug refractoriness [55]. Similar to altered DNA methylation and histone modification patterns, aberrant miRNA expression profiles are also linked to drug resistance and liver progression. A list of miRNAs commonly de-regulated in HCC is shown in Table 5. Another sub-population of non-coding RNAs (ncRNAs), the long non-coding RNAs (lncRNAs) have emerged as critical regulators of HCC drug sensitivity thus offering a novel exciting opportunity to treat HCC. LncRNA HOTAIR (HOX antisense intergenic RNA) and MALAT-1 (metastasis-associated lung adenocarcinoma transcript 1) were found to be upregulated in large cohorts of HCC patients and their suppression increased the chemotherapeutic sensitivity of HCC cells to cisplatin and doxorubicin [56, 57]. Alongside DNA methylation inhibitors, HDAC inhibitors have also been investigated in preclinical and clinical studies in HCC. Belinostat, a deacetylase inhibitor was found to stabilize un-resectable advanced HCC [58, 59]. However, more in-depth understanding of epigenetic alterations is required to gain more insights into the in vivo determinants of responses to epigenetic drugs in HCC.

Ribosome biogenesis as HCC resistance mechanism

The cancer cells for prolonged survival can employ excessive ribosome biogenesis and translation initiation. Recently, RACK1, the receptor for activated C-kinase 1, a component of the 40S subunit of ribosome, was found to be upregulated in HCC and contribute to chemoresistance in vitro and in vivo as well. The preferential translation involved in growth and survival was promoted by ribosomal RACK1 coupled with PKCβII by phosphorylating eIF4E. Inhibition of PKCβII or depletion of eIF4E abolished RACK1-mediated resistance in HCC [60]. These results imply that RACK1 may function as a factor promoting chemoresistance in HCC and targeting RACK1 can be an efficacious strategy for HCC cure.

Role of telomerase in HCC chemoresistance

The enzyme telomerase is activated in many malignant tumors; i It is known to bestow anti-apoptotic and chemoresistant properties to cancer cells. In accordance to above, it was observed that siRNA against human telomerase reverse transcriptase (hTERT) and cisplatin therapy act synergistically in suppression of HCC progression compared to individual therapy [61]. Low-dose of cisplatin also activated telomerase activity in SMMC7721 human HCC cell line. NF-κB has been reported to be responsible for cisplatin-induced activation of the telomerase in a dose dependent manner. It was thus observed that upregulation of hTERT expression by cisplatin is

Table 5 List of miRNAs de-regulated in HCC

S. No.	MiRNAs deregulated in HCC	Mechanism	Expression Level	Reference
1	miR-21	Potential biomarker for early stage HCC diagnosis	Up-regulated	[115]
2	miR-338-3p	Suppresses HCC cell invasion by inhibiting metalloproteinase (MMP-9)	Down-regulated	[116]
3	miR-122	Inhibits cycle cyclins & reduces MDR expression	Down-regulated	[117]
4	miR-181& let-7	IL-6 and twist-regulated miRNA expression	Up-regulated	[118]
5	miR-193a-3p	Affects DNA methylation state	Up-regulated	[119]
6	miR-199a/b-3p	Targets mTOR and c-met	Down-regulated	[120]
7	miR-210	Targets apoptosis-inducing factor, mitochondrion-associated, 3 (AIFM3) in hypoxic HCC	Down-regulated	[121]
8	miR-494	Reduces the expression of PTEN but increases PI3 K and p-Akt expression	Up-regulated	[122]
9	miR-1180	Activates NF-κB pathway by downregulating its negative regulators	Up-regulated	[123]
10	miR-122	Up-regulates IGF-1R that contribute to activation of RAS/RAF/ERK signaling which is associated with drug resistance	Down-regulated	[124]

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 7 of 15

NF-κB-dependent which contributes to chemotherapy resistance in HCC cells [62]. Additionally, translocation of telomerase into mitochondria can prevent intrinsic pathway of apoptosis under chemotherapeutic stress. Cisplatin-resistant SK-Hep1 cells showed increased translocation of hTERT to mitochondria resulting in decrease of apoptosis. Mitochondrial translocation of hTERT reduced mtDNA damage though the telomere length of chemoresistant cells was shortened. Drug-resistant HCC cells can thus escape from apoptosis through hTERT-mediated mitochondrial protection [63].

Topoisomerases and HCC chemoresistance

Human DNA topoisomerases are essential enzymes that play a central role in DNA duplex maintenance. In many cancers, aberrant expression of topoisomerase2A (TOP2A) has been reported and is considered to be a valuable prognostic marker for tumor advancements, recurrences and predictor of poor survival [64, 65]. Elevated protein score of TOP2A has also been correlated with non-responsiveness to chemotherapy in in vitro doxorubicin resistant HCC models [66]. Another report further shows a copy number gain in TOP2A locus in doxorubicin resistant HCC cells [67]. Strikingly, the topoisomerase transfected cell lines were around five–tenfold more resistant to cisplatin and other alkylating drugs endorsing its role in drug resistance. As expected, hence combination of Tirapazamine (TPZ), a new anti-cancer drug with Topoisomerase I inhibitors exhibited synergistic cytotoxicity and induced significant apoptosis in several HCC cell types [68]. Targeting topoisomerases can thus be an appropriate strategy for HCC patients who are resistant to conventional cytotoxic therapy.

Altered lipid metabolism

Cancer cells are able to synthesize lipids in a manner similar to embryonic tissues, and an altered lipid metabolism has often been linked to cancer pathogenesis. For example, stearoyl-coA de-saturase (SCD), a rate limiting enzyme and an essential regulator of lipid homeostasis in the liver is strongly over expressed in HCC. Interestingly, administration of 5-FU also resulted in a time dependent, upregulation of SCD through PI3K and JNK-mediated pathways [69]. While, suppression of SCD by genetic or pharmacologic strategies resulted in increased sensitivity to chemotherapy-induced apoptosis suggesting that SCD play a pro-survival role in HCC [69]. Similarly, another enzyme involved in lipid metabolism- carbonyl reductase1 (CBR1), known to protect cells against lipid peroxidation, promoted chemoresistance in HCC through induction of the master transcription regulator and angiogenesis promoter-HIF-1α [70]. Furthermore, isolated mitochondria from HCC with increased cholesterol
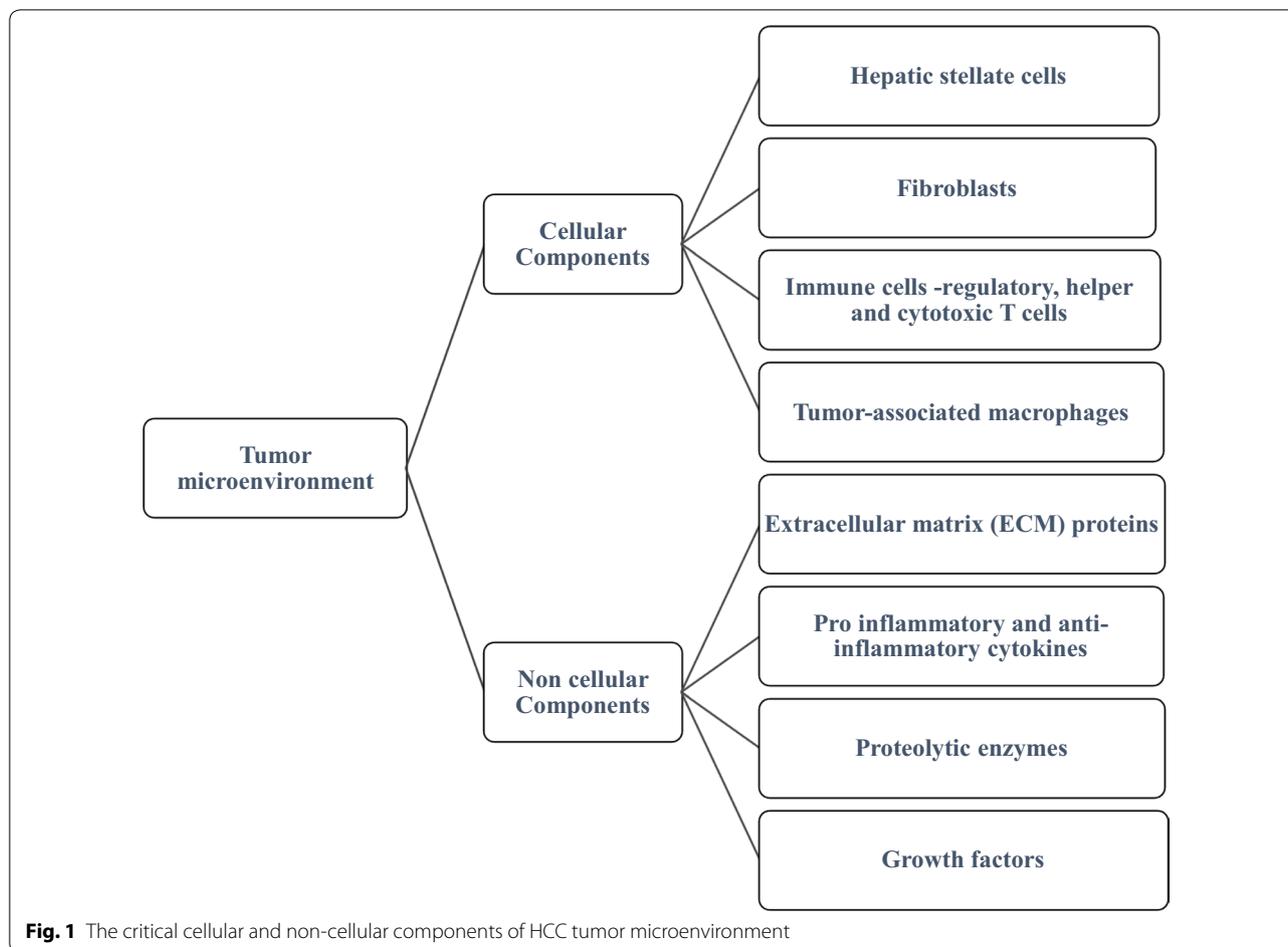
levels were resistant to release of cytochrome c or Smac/DIABLO in response to various apoptotic stimuli [71]. Thus, altered lipid metabolism can emerge as a novel therapeutic niche in HCC therapy.

Tumor microenvironment

Tumor microenvironment (TME) plays a crucial role in HCC development and maintenance. The critical cellular and non-cellular components of HCC tumor microenvironment are showed in Fig. 1 and their functions are summarized in Table 6 [72]. Unlike normal fibroblasts, cancer-associated fibroblasts (CAFs) are the most abundant type of connective tissue present in the TME of multiple cancer cell types [73, 74]. CAFs play an important role in the HCC microenvironment as most liver cancers are derived from fibrosis and cirrhosis. Chuang et al. in 2012 and 2013 showed that co-culture of CAFs with HCC can enhance proliferation, migration, and invasion by altering the transcriptome of HCC cells [75, 76]. The CAF cells can specifically upregulate pro-inflammatory cytokines like CCL2, CCL26, IL6, and LOXL2, which are correlated with proliferation, invasion and angiogenesis of HCC cells [77]. Another key component of HCC tumor microenvironment is the hepatic stellate cells (HSCs), which are generally involved in the process of liver regeneration mostly in case of injuries [78]. However, in addition to its regenerative property, HSCs can exhibit liver carcinogenesis promoting functions as well. They can secrete growth factors and cytokines like, HGF and IL-6 and can engage in a reciprocal crosstalk with HCC cells [79]. Activated HSCs can promote the migration, proliferation and resistance in HCC cells through modulation of TGF-β signaling [80]. Since HSCs are actively involved in tumor progression, targeting HSCs may represent a prospective therapeutic strategy in HCC. Furthermore, immune cells like, CD4 T cells (Treg) and myeloid-derived suppressor cells (MDSCs), which are also part of TME have been implicated in promoting HCC tumorigenesis [81]. Tumor-associated macrophages (TAMs) can also affect HCC tumor progression through NF-κB, STAT-3, and HIF-1 signaling [81]. Further studies are however required to better understand the function of immune cells in HCC TME for enhancement of immunotherapeutic strategies. Table 7 shows some of the tumor microenvironment targeting drugs that are now under investigation for HCC treatment.

Targeted therapy against HCC

For many years, the foundation of HCC treatment has been surgery and non-targeted chemotherapy with conventional drugs like, cisplatin, as discussed earlier. However, over the last decade, partly because of advancements in genomic technologies, targeted therapies with

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 8 of 15

Fig. 1 The critical cellular and non-cellular components of HCC tumor microenvironment

drugs that home into specific cancer cell types and bring about molecular changes have cemented their place as standard treatment for many cancers, HCC is no exception. Sorafenib, a molecular multi-kinase inhibitor has currently been the drug of choice for HCC and is the first molecular targeted agent that has shown survival benefits in HCC patients. However, use of sorafenib has also been hindered by acquisition of resistance [82]; Gao et al. found that elevated fibroblast growth factor 19 (FGF19) expression or hyper-activation of FGF19/FGFR4 signaling was essential for sorafenib resistance in HCC [83]. Hence blocking of FGF19/FGFR4 axis by ponatinib, a third-generation tyrosine–kinase inhibitor, could overcome the resistance of HCC cells to sorafenib [83]. Further, anti-angiogenic drugs like, sunitinib, linifanib and brivanib were tried as independent therapy in HCC but they failed to prove their non-inferiority over sorafenib [84]. Also, HCC patients who are intolerant to sorafenib or have high expression of cellular mesenchymal–epithelial transition factor (c-MET) were found to benefit from Tivantinib therapy, a highly selective inhibitor of

c-MET receptor tyrosine kinase [85, 86]. Furthermore, progress in immunotherapy has provided new dimensions to HCC therapy as well. Immune tolerance in HCC is mediated through decreased co-stimulatory signaling that results in immuno-suppression. Hence, anti-human cytotoxic T-lymphocyte antigen 4 (CTLA-4) and anti-programmed death 1 (PD-1) monotherapy have been utilized in HCC treatment but with limited success. Some of the immunomodulators now under investigation for HCC treatment listed in Table 8. Another rapidly emerging immunotherapy approach has been chimeric antigen receptor-engineered (CAR)-T cell therapies that have already demonstrated efficacy against hematologic malignancies. CART technology utilizes the antitumor activity of "domesticated" T cells that have been engineered to express cancer specific antigen-targeted-receptor to treat malignant tumors [87]. However, the major challenge for CAR-T cell therapy in HCC has been the selection of specific antigen to differentiate tumor from normal tissue to prevent off-target effects. The antigens that have till date been used as targets in CAR-T therapy for HCC are

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 9 of 15

Table 6 List of various components of HCC tumor microenvironment and their role

S. No.	Cellular and non cellular components	Role in tumor microenvironment	Effect of various components	References
1	Tumor associated macrophages (TAMs)	Tumor development by impairing cytotoxic CD8+ T cell mediated immune responses	Chemo resistance in HCC.	[125]
2	Cancer associated fibroblasts (CAFs)	Produces growth factors like hepatocyte growth factor (HGF), members of the epidermal growth factor (EGF), fibroblast growth factor (FGF) and members of Wnt families, and cytokines, such as stromal-derived factor (SDF)-1α and IL-6	Chemo resistance in HCC	[126, 127]
3	Matrix metalloproteinases (MMPs)	Causes tissue remodeling, inflammation, tumor cell growth and metastasis in many cancers. MMP-2,-9, and -14 activate TGF-β1, which reciprocally activates MMP. Upregulation of MMP-9 is connected with provocation of PI3 K/PTEN/AKT/mTOR pathways in human HCCs. MMPs also inhibit apoptosis signaling in cancer cells	Chemo resistance in HCC	[128, 129]
4	Immune cells	CD8+ T cells, NK cells are fooled by cancer cells as an immune evasion mechanism	Poor prognosis in HCC patients	[130, 131]
5	Kupffer cells	Upon activation secrete excessive level of osteopontin	Chemo resistance of cisplatin in small cell lung cancer. It can be postulated to play a role in Hepatocellular carcinoma too	[132]
6	Hepatic stellate cells (HSCs)	Produce collagen in the liver. They get activated upon liver damage and undergo phenotypic changes leading liver fibrosis. They secrete hepatocyte growth factor	Chemo resistance in HCC	[133]

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 10 of 15

Table 7 Drugs targeting TME in clinical trials for HCC treatment

S. No.	Drug	Molecular targets	Phases of clinical trial	Year and references
1	Sibrotuzumab	FAPs	I	2003 [134]
2	PI-88	HPR	II	2009 [135]
3	Selumetinib	MEK	II	2011 [136]
4	Brivanib	VEGFR, PDGFR	III	2013 [137]
5	Lilifanib	VEGFR	III	2013 [138]
6	Axitinib	VEGFR	II	2015 [139]
7	Galuniserib	TGF-β	I	2015 [140]

Table 8 Immune-modulators in clinical trials for HCC treatment

S. No.	Drug	Molecular targets	Phases of clinical trial	Year and references
1	Tremelimumab	CTLA-4	II	2013 [141]
2	Icaritin	IL-6/Jak2/Stat3	II	2015 [142]
3	Lenalidomide	TNF-α, interferon γ, IL-6, IL-10, and IL-12.	II	2015 [143]
4	Codrituzumab	Glypican-3	II	2016 [144]
5	Nivolumab	PD-1	I	2016 [145]
6	Ipilimumab	CTLA-4	II	2017 [146]
7	Tasquinimod	Protein S100A9	II	2017 [147]

Table 9 Application of CAR-T cells for HCC

S. No.	Antigen	Gene transfer vehicle	Phases of clinical trial	Year (Clinicaltrials.gov identifier or reference)
1	Epidermal growth factor receptor	Lentivirus	I/II	2013 [148]
2	Mucin-1	–	I/II	2015 (NCT02587689)
3	CD133	Retrovirus	I	2015 (NCT02541370)
4	Carcinoembryonic antigen	Retrovirus	Preclinical	2016 [149]
5	Epithelial cell adhesion molecule	–	I/II	2016 (NCT02729493)
6	Glypican-3	–	I/II	2016 (NCT02723942)

mentioned in Table 9. While therapeutic benefit has been achieved in early clinical trials with CAR-T therapy, the use of this therapy is still at its infancy and is limited due to either unique properties of HCC, off-target effects, lack of specific tumor antigens in HCC or cost of production [87].

Conclusion

We believe that drug companies are currently fighting a losing battle against advanced HCC tumors. The various chemotherapeutic drugs currently in use against HCC have shown strong chemoresistance providing a massive setback to therapeutic regimens targeting HCC. Also, liver cancer patients generally have a poor tolerance to chemotherapy due to liver dysfunction. Characterization of molecular strategies underlying chemoresistance

is hence essentially needed to identify appropriate targets to effectively sensitize these resistant cells. However, designing such strategies are by no means easy. Till date development of targeted drugs has not yet improved the outcome much significantly. This might be attributed to various factors extending from apoptosis evasion, stem cell activation, enhanced DNA repair, topoisomerase activation, lack of proper targets for immunotherapy to dynamic changes in TME and others, as discussed in this literature (Fig. 2). These factors either independently or in unison contribute to emergence of refractoriness to drug therapy in HCC. To compound the scenario, therapeutic effectiveness may also vary depending on patient properties and at various stages of tumor development. Hence, formulating a unified molecular targeted therapeutic strategy for all HCC patients is unlikely to succeed.

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 11 of 15

Fig. 2 Potential molecular mechanisms that regulate chemoresistance in Hepatocellular Carcinoma
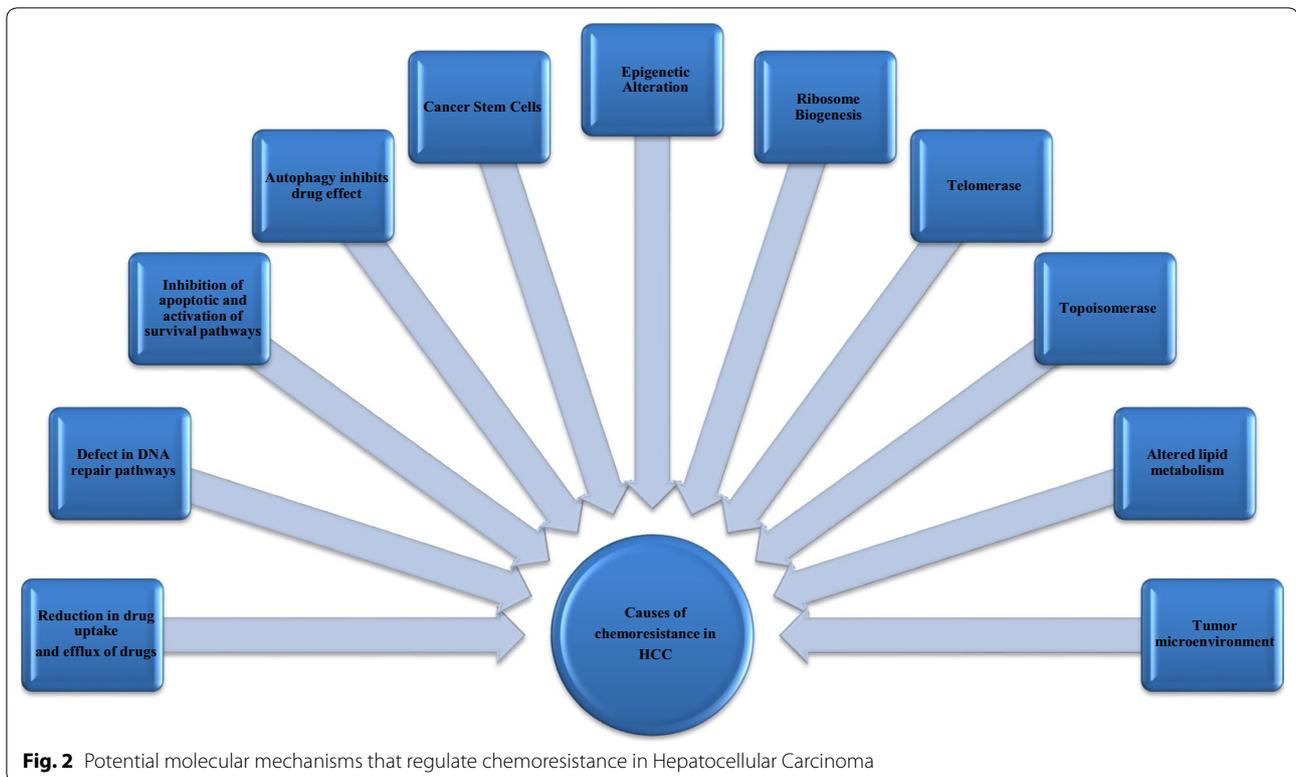
Therefore, future therapies targeting HCC should be based on combination of context and stage dependent molecular targeted drugs against resistance with or without conventional drugs to successfully treat HCC.

References
1. Raoul JL. Natural history of hepatocellular carcinoma and current treatment options. Semin Nucl Med. 2008;37(2):S13–8.
2. Chuma M, Terashita K, Sakamoto N. New molecularly targeted therapies against advanced hepatocellular carcinoma: from molecular pathogenesis to clinical trials and future directions. Hepatol Res. 2015; 45(10):E1–11.
3. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. Nat Rev Clin Oncol. 2015;12(7):408–24.
4. Belghiti J, Kianmanesh R. Surgical treatment of hepatocellular carcinoma. Hpb. 2005;7(1):42–9.
5. Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, et al. Early-Stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation 1. Radiology. 2005;234(3):961–7.
6. Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. Cancer Treat Rev. 1988;15(1):1–31.
7. Sanoff HK, Bernard S, Goldberg RM, Morse MA, Garcia R, Woods L, et al. Phase II study of capecitabine, oxaliplatin, and cetuximab for advanced hepatocellular carcinoma. Gastrointest Cancer Res GCR. 2011;4(3):78.
8. Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, et al. A randomized phase III study of doxorubicin versus cisplatin/interferon α-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst. 2005;97(20):1532–8.

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 12 of 15

9. Louafi S, Boige V, Ducreux M, Bonyhay L, Mansourbakht T, de Baere T, et al. Gemcitabine plus oxaliplatin (GEMOX) in patients with advanced hepatocellular carcinoma (HCC). Cancer. 2007;109(7):1384–90.
10. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, et al. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Can Res. 2006;66(24):11851–8.
11. Roodhart JM, Langenberg MH, Witteveen E, Voest EE. The molecular basis of class side effects due to treatment with inhibitors of the VEGF/VEGFR pathway. Curr Clin Pharmacol. 2008;3(2):132–43.
12. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1):a001008.
13. Nies AT, Konig J, Pfannschmidt M, Klar E, Hofmann WJ, Keppler D. Expression of the multidrug resistance proteins MRP2 and MRP3 in human hepatocellular carcinoma. Int J Cancer. 2001;94(4):492–9.
14. Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Can Res. 1994;54(16):4313–20.
15. Murray GI, Paterson PJ, Weaver RJ, Ewen SW, Melvin WT, Burke MD. The expression of cytochrome P-450, epoxide hydrolase, and glutathione s-transferase in hepatocellular carcinoma. Cancer. 1993;71(1):36–43.
16. Takano M, Otani Y, Tanda M, Kawami M, Nagai J, Yumoto R. Paclitaxel-resistance conferred by altered expression of efflux and influx transporters for paclitaxel in the human hepatoma cell line, HepG2. Drug Metab Pharmacokinet. 2009;24(5):418–27.
17. Yang SF, Chang CW, Wei RJ, Shiue YL, Wang SN, Yeh YT. Involvement of DNA damage response pathways in hepatocellular carcinoma. Biomed Res Int. 2014;2014:153867.
18. Minemura M, Tanimura H, Tabor E. Overexpression of multidrug resistance genes MDR1 and cMOAT in human hepatocellular carcinoma and hepatoblastoma cell lines. Int J Oncol. 1999;15(3):559–63.
19. Jodo S, Kobayashi S, Nakajima Y, Matsunaga T, Nakayama N, Ogura N, et al. Elevated serum levels of soluble Fas/APO-1 (CD95) in patients with hepatocellular carcinoma. Clin Exp Immunol. 1998;112(2):166–71.
20. Volkmann M, Schiff JH, Hajjar Y, Otto G, Stilgenbauer F, Fiehn W, et al. Loss of CD95 expression is linked to most but not all p53 mutants in European hepatocellular carcinoma. J Mol Med. 2001;79(10):594–600.
21. Ono T, Yamanoi A, Nazmy El Assal O, Kohno H, Nagasue N. Adjuvant chemotherapy after resection of hepatocellular carcinoma causes deterioration of long-term prognosis in cirrhotic patients: metaanalysis of three randomized controlled trials. Cancer. 2001;91(12):2378–85.
22. Liu Z, Liu R, Qiu J, Yin P, Luo F, Su J, et al. Combination of human Fas (CD95/Apo-1) ligand with adriamycin significantly enhances the efficacy of antitumor response. Cell Mol Immunol. 2009;6(3):167–74.
23. Schattenberg JM, Galle PR, Schuchmann M. Apoptosis in liver disease. Liver Int. 2006;26(8):904–11.
24. Witort E, Lulli M, Carloni V, Capaccioli S. Anticancer activity of an antisense oligonucleotide targeting TRADD combined with proteasome inhibitors in chemoresistant hepatocellular carcinoma cells. J Chemother. 2013;25(5):292–7.
25. Chan BC, Ching AK, To KF, Leung JC, Chen S, Li Q, et al. BRE is an antiapoptotic protein in vivo and overexpressed in human hepatocellular carcinoma. Oncogene. 2008;27(9):1208–17.
26. Chui YL, Ching AK, Chen S, Yip FP, Rowlands DK, James AE, et al. BRE over-expression promotes growth of hepatocellular carcinoma. Biochem Biophys Res Commun. 2010;391(3):1522–5.
27. Du X, Bao G, He X, Zhao H, Yu F, Qiao Q, et al. Expression and biological significance of c-FLIP in human hepatocellular carcinomas. J Exp Clin Cancer Res. 2009;28:24.
28. Safa AR, Day TW, Wu C-H. Cellular FLICE-like inhibitory protein (C-FLIP): a novel target for cancer therapy. Curr Cancer Drug Targets. 2008;8(1):37–46.
29. Luedde T, Schwabe RF. NF-κB in the liver—linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2011;8(2):108.
30. Chun E, Lee KY. Bcl-2 and Bcl-xL are important for the induction of paclitaxel resistance in human hepatocellular carcinoma cells. Biochem Biophys Res Commun. 2004;315(3):771–9.
31. Huang S, He X, Ding J, Liang L, Zhao Y, Zhang Z, et al. Upregulation of miR-23a approximately 27a approximately 24 decreases transforming growth factor-beta-induced tumor-suppressive activities in human hepatocellular carcinoma cells. Int J Cancer. 2008;123(4):972–8.
32. Guo XL, Hu F, Zhang SS, Zhao QD, Zong C, Ye F, et al. Inhibition of p53 increases chemosensitivity to 5-FU in nutrient-deprived hepatocarcinoma cells by suppressing autophagy. Cancer Lett. 2014;346(2):278–84.
33. Chan K-T, Lung ML. Mutant p53 expression enhances drug resistance in a hepatocellular carcinoma cell line. Cancer Chemother Pharmacol. 2004;53(6):519–26.
34. Ou DL, Lee BS, Chang YC, Lin LI, Liou JY, Hsu C, et al. Potentiating the efficacy of molecular targeted therapy for hepatocellular carcinoma by inhibiting the insulin-like growth factor pathway. PLoS ONE. 2013;8(6):e66589.
35. Urtasun R, Latasa MU, Demartis MI, Balzani S, Goni S, Garcia-Irigoyen O, et al. Connective tissue growth factor autocriny in human hepatocellular carcinoma: oncogenic role and regulation by epidermal growth factor receptor/yes-associated protein-mediated activation. Hepatology. 2011;54(6):2149–58.
36. Huang P, Xu X, Wang L, Zhu B, Wang X, Xia J. The role of EGF-EGFR signalling pathway in hepatocellular carcinoma inflammatory microenvironment. J Cell Mol Med. 2014;18(2):218–30.
37. Raychaudhuri P, Park HJ. FoxM1: a master regulator of tumor metastasis. Can Res. 2011;71(13):4329–33.
38. Ding ZB, Hui B, Shi YH, Zhou J, Peng YF, Gu CY, et al. Autophagy activation in hepatocellular carcinoma contributes to the tolerance of oxaliplatin via reactive oxygen species modulation. Clin Cancer Res. 2011;17(19):6229–38.
39. Shi YH, Ding ZB, Zhou J, Hui B, Shi GM, Ke AW, et al. Targeting autophagy enhances sorafenib lethality for hepatocellular carcinoma via ER stress-related apoptosis. Autophagy. 2011;7(10):1159–72.
40. Guo XL, Li D, Sun K, Wang J, Liu Y, Song JR, et al. Inhibition of autophagy enhances anticancer effects of bevacizumab in hepatocarcinoma. J Mol Med. 2013;91(4):473–83.
41. Dash S, Sarashetti PM, Rajashekar B, Chowdhury R, Mukherjee S. TGF-β2-induced EMT is dampened by inhibition of autophagy and TNF-α treatment. Oncotarget. 2018;9(5):6433–49.
42. Chen R, Dai RY, Duan CY, Liu YP, Chen SK, Yan DM, et al. Unfolded protein response suppresses cisplatin-induced apoptosis via autophagy regulation in human hepatocellular carcinoma cells. Folia Biol. 2011;57(3):87–95.
43. Saito T, Ichimura Y, Taguchi K, Suzuki T, Mizushima T, Takagi K, et al. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. Nat Commun. 2016;7:12030.
44. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105–11.
45. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer. 2003;3(12):895–902.
46. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene. 2008;27(12):1749–58.
47. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology. 2009;136(3):1012–24.
48. Hernandez-Vargas H, Lambert M-P, Le Calvez-Kelm F, Gouysse G, McKay-Chopin S, Tavtigian SV, et al. Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors. PLoS ONE. 2010;5(3):e9749.
49. Song M-A, Tiirikainen M, Kwee S, Okimoto G, Yu H, Wong LL. Elucidating the landscape of aberrant DNA methylation in hepatocellular carcinoma. PLoS ONE. 2013;8(2):e55761.
50. Ma K, He Y, Zhang H, Fei Q, Niu D, Wang D, et al. DNA methylation-regulated miR-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. J Biol Chem. 2012;287(8):5639–49.
51. Kanda T, Tada M, Imazeki F, Yokosuka O, Nagao K, Saisho H. 5-aza-2′-deoxycytidine sensitizes hepatoma and pancreatic cancer cell lines. Oncol Rep. 2005;14(4):975–9.
52. Mei Q, Chen M, Lu X, Li X, Duan F, Wang M, et al. An open-label, single-arm, phase I/II study of lower-dose decitabine based therapy in patients with advanced hepatocellular carcinoma. Oncotarget. 2015;6(18):16698.

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 13 of 15

53. He C, Xu J, Zhang J, Xie D, Ye H, Xiao Z, et al. High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. Hum Pathol. 2012;43(9):1425–35.

54. Au SLK, Wong CCL, Lee JMF, Fan DNY, Tsang FH, Ng IOL, et al. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. Hepatology. 2012;56(2):622–31.

55. Studach LL, Menne S, Cairo S, Buendia MA, Hullinger RL, Lefrançois L, et al. Subset of Suz12/PRC2 target genes is activated during hepatitis B virus replication and liver carcinogenesis associated with HBV X protein. Hepatology. 2012;56(4):1240–51.

56. Yang Z, Zhou L, Wu L-M, Lai M-C, Xie H-Y, Zhang F, et al. Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol. 2011;18(5):1243–50.

57. M-c Lai, Yang Z, Zhou L, Q-q Zhu, H-y Xie, Zhang F, et al. Long noncoding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. Med Oncol. 2012;29(3):1810–6.

58. Yeo W, Chung HC, Chan SL, Wang LZ, Lim R, Picus J, et al. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. J Clin Oncol. 2012;30(27):3361–7.

59. Ma BB, Sung F, Tao Q, Poon FF, Lui VW, Yeo W, et al. The preclinical activity of the histone deacetylase inhibitor PXD101 (belinostat) in hepatocellular carcinoma cell lines. Invest New Drugs. 2010;28(2):107–14.

60. Ruan Y, Sun L, Hao Y, Wang L, Xu J, Zhang W, et al. Ribosomal RACK1 promotes chemoresistance and growth in human hepatocellular carcinoma. J Clin Investig. 2012;122(7):2554–66.

61. Zhao J, Chen J, Lu B, Dong L, Wang H, Bi C, et al. TIP30 induces apoptosis under oxidative stress through stabilization of p53 messenger RNA in human hepatocellular carcinoma. Can Res. 2008;68(11):4133–41.

62. Guo XL, Ma NN, Zhou FG, Zhang L, Bu XX, Sun K, et al. Up-regulation of hTERT expression by low-dose cisplatin contributes to chemotherapy resistance in human hepatocellular cancer cells. Oncol Rep. 2009;22(3):549–56.

63. Ling X, Wen L, Zhou Y. Role of mitochondrial translocation of telomerase in hepatocellular carcinoma cells with multidrug resistance. Int J Med Sci. 2012;9(7):545–54.

64. Engstrom MJ, Ytterhus B, Vatten LJ, Opdahl S, Bofin AM. TOP2A gene copy number change in breast cancer. J Clin Pathol. 2014;67(5):420–5.

65. Almeida D, Gerhard R, Leitão D, Davilla C, Damasceno M, Schmitt F. Topoisomerase II-alfa gene as a predictive marker of response to anthracyclines in breast cancer. Pathol-Res Pract. 2014;210(10):675–9.

66. Pang E, Hu Y, Chan KY, Lai PB, Squire JA, Macgregor PF, et al. Karyotypic imbalances and differential gene expressions in the acquired doxorubicin resistance of hepatocellular carcinoma cells. Lab Invest. 2005;85(5):664–74.

67. Wong N, Yeo W, Wong WL, Wong NLY, Chan KYY, Mo FKF, et al. TOP2A overexpression in hepatocellular carcinoma correlates with early age onset, shorter patients survival and chemoresistance. Int J Cancer. 2009;124(3):644–52.

68. Cai TY, Liu XW, Zhu H, Cao J, Zhang J, Ding L, et al. Tirapazamine sensitizes hepatocellular carcinoma cells to topoisomerase I inhibitors via cooperative modulation of hypoxia-inducible factor-1alpha. Mol Cancer Ther. 2014;13(3):630–42.

69. Bansal S, Berk M, Alkhouri N, Partrick DA, Fung JJ, Feldstein A. Stearoyl-CoA desaturase plays an important role in proliferation and chemoresistance in human hepatocellular carcinoma. J Surg Res. 2014;186(1):29–38.

70. Tak E, Lee S, Lee J, Rashid MA, Kim YW, Park JH, et al. Human carbonyl reductase 1 upregulated by hypoxia renders resistance to apoptosis in hepatocellular carcinoma cells. J Hepatol. 2011;54(2):328–39.

71. Montero J, Morales A, Llacuna L, Lluis JM, Terrones O, Basanez G, et al. Mitochondrial cholesterol contributes to chemotherapy resistance in hepatocellular carcinoma. Can Res. 2008;68(13):5246–56.

72. Yang JD, Nakamura I, Roberts LR. The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets. Semin Cancer Biol. 2011;21(1):35–43.

73. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121(3):335–48.

74. Chen L, Qu C, Chen H, Xu L, Qi Q, Luo J, et al. Chinese herbal medicine suppresses invasion-promoting capacity of cancer-associated fibroblasts in pancreatic cancer. PLoS ONE. 2014;9(4):e96177.

75. Lin Z-Y, Chuang Y-H, Chuang W-L. Cancer-associated fibroblasts up-regulate CCL2, CCL26, IL6 and LOXL2 genes related to promotion of cancer progression in hepatocellular carcinoma cells. Biomed Pharmacother. 2012;66(7):525–9.

76. Lin Z-Y, Chuang W-L. Hepatocellular carcinoma cells cause different responses in expressions of cancer-promoting genes in different cancer-associated fibroblasts. Kaohsiung J Med Sci. 2013;29(6):312–8.

77. Jia C-C, Wang T-T, Liu W, Fu B-S, Hua X, Wang G-Y, et al. Cancer-associated fibroblasts from hepatocellular carcinoma promote malignant cell proliferation by HGF secretion. PLoS ONE. 2013;8(5):e63243.

78. Yin C, Evason KJ, Asahina K, Stainier DY. Hepatic stellate cells in liver development, regeneration, and cancer. J Clin Investig. 2013;123(5):1902–10.

79. Sancho-Bru P, Juez E, Moreno M, Khurdayan V, Morales-Ruiz M, Colmenero J, et al. Hepatocarcinoma cells stimulate the growth, migration and expression of pro-angiogenic genes in human hepatic stellate cells. Liver Int. 2010;30(1):31–41.

80. Shapero K, Wylie-Sears J, Levine RA, Mayer JE, Bischoff J. Reciprocal interactions between mitral valve endothelial and interstitial cells reduce endothelial-to-mesenchymal transition and myofibroblastic activation. J Mol Cell Cardiol. 2015;80:175–85.

81. Quatromoni JG, Eruslanov E. Tumor-associated macrophages: function, phenotype, and link to prognosis in human lung cancer. Am J Transl Res. 2012;4(4):376.

82. Cheng A-L, Kang Y-K, Chen Z, Tsao C-J, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34.

83. Gao L, Wang X, Tang Y, Huang S. Hu C-AA, Teng Y. FGF19/FGFR4 signaling contributes to the resistance of hepatocellular carcinoma to sorafenib. J Exp Clin Cancer Res. 2017;36(1):8.

84. Abou-Alfa GK, Venook AP. The antiangiogenic ceiling in hepatocellular carcinoma: does it exist and has it been reached? Lancet Oncol. 2013;14(7):e283–8.

85. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol. 2013;14(1):55–63.

86. Pievsky D, Pyrsopoulos N. Profile of tivantinib and its potential in the treatment of hepatocellular carcinoma: the evidence to date. J Hepatocell Carcinoma. 2016;3:69.

87. Li K, Lan Y, Wang J, Liu L. Chimeric antigen receptor—engineered T cells for liver cancers, progress and obstacles. Tumor Biol. 2017;39(3):1010428317692229.

88. Tan CK, Chow PK, Findlay M, Wong C, Machin D. Use of tamoxifen in hepatocellular carcinoma: a review and paradigm shift. J Gastroenterol Hepatol. 2000;15(7):725–9.

89. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003;3(5):330–8.

90. Hsu C, Chen CN, Chen LT, Wu CY, Yang PM, Lai MY, et al. Low-dose thalidomide treatment for advanced hepatocellular carcinoma. Oncology. 2003;65(3):242–9.

91. Dimopoulos MA, Eleutherakis-Papaiakovou V. Adverse effects of thalidomide administration in patients with neoplastic diseases. Am J Med. 2004;117(7):508–15.

92. Blaker M, Schmitz M, Gocht A, Burghardt S, Schulz M, Broring DC, et al. Differential expression of somatostatin receptor subtypes in hepatocellular carcinomas. J Hepatol. 2004;41(1):112–8.

93. Cebon J, Findlay M, Hargreaves C, Stockler M, Thompson P, Boyer M, et al. Somatostatin receptor expression, tumour response, and quality of life in patients with advanced hepatocellular carcinoma treated with long-acting octreotide. Br J Cancer. 2006;95(7):853–61.

94. Zhu AX, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, et al. Efficacy, safety, and potential biomarkers of sunitinib

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 14 of 15

monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol. 2009;27(18):3027–35.

95. Finn RS, Zhu AX. Targeting angiogenesis in hepatocellular carcinoma: focus on VEGF and bevacizumab. Expert Rev Anticancer Ther. 2009;9(4):503–9.

96. Llovet JM, Bruix J. Testing molecular therapies in hepatocellular carcinoma: the need for randomized phase II trials. J Clin Oncol. 2009;27(6):833–5.

97. Pang B, Qiao X, Janssen L, Velds A, Groothuis T, Kerkhoven R, et al. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nat Commun. 2013;4:1908.

98. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014;740:364–78.

99. Zhang X, Xu P, Ni W, Fan H, Xu J, Chen Y, et al. Downregulated DYRK2 expression is associated with poor prognosis and Oxaliplatin resistance in hepatocellular carcinoma. Pathol-Res Pract. 2016;212(3):162–70.

100. Chenivesse X, Franco D, Brechot C. MDR1 (multidrug resistance) gene expression in human primary liver cancer and cirrhosis. J Hepatol. 1993;18(2):168–72.

101. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflugers Arch. 2004;447(5):653–65.

102. van Montfoort JE, Hagenbuch B, Groothuis GM, Koepsell H, Meier PJ, Meijer DK. Drug uptake systems in liver and kidney. Curr Drug Metab. 2003;4(3):185–211.

103. Zollner G, Wagner M, Fickert P, Silbert D, Fuchsbichler A, Zatloukal K, et al. Hepatobiliary transporter expression in human hepatocellular carcinoma. Liver Int. 2005;25(2):367–79.

104. Goswami S, Gong L, Giacomini K, Altman RB, Klein TE. PharmGKB summary: very important pharmacogene information for SLC22A1. Pharmacogenet Genom. 2014;24(6):324.

105. Youn CK, Kim MH, Cho HJ, Kim HB, Chang IY, Chung MH, et al. Oncogenic H-Ras up-regulates expression of ERCC1 to protect cells from platinum-based anticancer agents. Can Res. 2004;64(14):4849–57.

106. Ding H, Chen D, Zhao C, Shang H, Zhou L, Zhang J, et al. Flap endonuclease-1 plays double roles in cisplatin-based chemoresistance and cell apoptosis resulted from enhanced long patch DNA repair and a gap endonuclease activity. Cancer Res. 2006;66(8 Supplement):1164.

107. Chae S, Kim YB, Lee JS, Cho H. Resistance to paclitaxel in hepatoma cells is related to static JNK activation and prohibition into entry of mitosis. Am J Physiol Gastrointest Liver Physiol. 2012;302(9):G1016–24.

108. Fujimaki S, Matsuda Y, Wakai T, Sanpei A, Kubota M, Takamura M, et al. Blockade of ataxia telangiectasia mutated sensitizes hepatoma cell lines to sorafenib by interfering with Akt signaling. Cancer Lett. 2012;319(1):98–108.

109. Cun Y, Dai N, Xiong C, Li M, Sui J, Qian C, et al. Silencing of APE1 enhances sensitivity of human hepatocellular carcinoma cells to radiotherapy in vitro and in a xenograft model. PLoS ONE. 2013;8(2):e55313.

110. Patil MA, Zhang J, Ho C, Cheung S-T, Fan S-T, Chen X. Hedgehog signaling in human hepatocellular carcinoma. Cancer Biol Ther. 2006;5(1):111–7.

111. Zhou D, Conrad C, Xia F, Park J-S, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell. 2009;16(5):425–38.

112. Giovannini C, Gramantieri L, Chieco P, Minguzzi M, Lago F, Pianetti S, et al. Selective ablation of Notch3 in HCC enhances doxorubicin's death promoting effect by a p53 dependent mechanism. J Hepatol. 2009;50(5):969–79.

113. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet. 2012;44(6):694.

114. Moeini A, Cornellà H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. Liver Cancer. 2012;1(2):83–93.

115. Tomimaru Y, Eguchi H, Nagano H, Wada H, Tomokuni A, Kobayashi S, et al. MicroRNA-21 induces resistance to the anti-tumour effect of interferon-alpha/5-fluorouracil in hepatocellular carcinoma cells. Br J Cancer. 2010;103(10):1617–26.

116. Huang XH, Chen JS, Wang Q, Chen XL, Wen L, Chen LZ, et al. miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. J Pathol. 2011;225(3):463–72.

117. Xu Y, Xia F, Ma L, Shan J, Shen J, Yang Z, et al. MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. Cancer Lett. 2011;310(2):160–9.

118. Meng F, Glaser SS, Francis H, DeMorrow S, Han Y, Passarini JD, et al. Functional analysis of microRNAs in human hepatocellular cancer stem cells. J Cell Mol Med. 2012;16(1):160–73.

119. Ma K, He Y, Zhang H, Fei Q, Niu D, Wang D, et al. DNA methylation-regulated miR-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. J Biol Chem. 2012;287(8):5639–49.

120. Zhang J, Wang Y, Zhen P, Luo X, Zhang C, Zhou L, et al. Genome-wide analysis of miRNA signature differentially expressed in doxorubicin-resistant and parental human hepatocellular carcinoma cell lines. PLoS ONE. 2013;8(1):e54111.

121. Qin Q, Furong W, Baosheng L. Multiple functions of hypoxia-regulated miR-210 in cancer. J Exp Clin Cancer Res. 2014;33(1):50.

122. Liu K, Liu S, Zhang W, Jia B, Tan L, Jin Z, et al. miR-494 promotes cell proliferation, migration and invasion, and increased sorafenib resistance in hepatocellular carcinoma by targeting PTEN. Oncol Rep. 2015;34(2):1003–10.

123. Tan G, Wu L, Tan J, Zhang B, Tai WC, Xiong S, Chen W, Yang J, Li H. MiR-1180 promotes apoptotic resistance to human hepatocellular carcinoma via activation of NF-κB signaling pathway. Sci Rep. 2016;6:22328.

124. Xu Y, Huang J, Ma L, Shan J, Shen J, Yang Z, et al. MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. Cancer Lett. 2016;371(2):171–81.

125. Barajas M, Mazzolini G, Genove G, Bilbao R, Narvaiza I, Schmitz V, et al. Gene therapy of orthotopic hepatocellular carcinoma in rats using adenovirus coding for interleukin 12. Hepatology. 2001;33(1):52–61.

126. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature. 2004;432(7015):332–7.

127. Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res. 2010;316(8):1324–31.

128. Cheng JC, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-κB signal transduction pathway. Oncogene. 2006;25(53):7009–18.

129. Chen JS, Wang Q, Fu XH, Huang XH, Chen XL, Cao LQ, et al. Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: association with MMP-9. Hepatol Res. 2009;39(2):177–86.

130. Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. J Clin Oncol. 2007;25(18):2586–93.

131. Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology. 2007;132(7):2328–39.

132. Gu T, Ohashi R, Cui R, Tajima K, Yoshioka M, Iwakami S, et al. Osteopontin is involved in the development of acquired chemo-resistance of cisplatin in small cell lung cancer. Lung Cancer. 2009;66(2):176–83.

133. Thompson AI, Conroy KP, Henderson NC. Hepatic stellate cells: central modulators of hepatic carcinogenesis. BMC Gastroenterol. 2015;15(1):1.

134. Scott AM, Wiseman G, Welt S, Adjei A, Lee F-T, Hopkins W, et al. A phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res. 2003;9(5):1639–47.

135. Liu C-J, Lee P-H, Lin D-Y, Wu C-C, Jeng L-B, Lin P-W, et al. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. J Hepatol. 2009;50(5):958–68.

136. O'Neil BH, Goff LW, Kauh JSW, Strosberg JR, Bekaii-Saab TS, R-m Lee, et al. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2011;29(17):2350–6.

137. Johnson PJ, Qin S, Park J-W, Poon RT, Raoul J-L, Philip PA, et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable,

Lohitesh *et al. Cancer Cell Int* (2018) 18:44

Page 15 of 15

advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. J Clin Oncol. 2013;31(28):3517–24.

138. Cainap C, Qin S, Huang W-T, Chung I-J, Pan H, Cheng Y, et al. Phase III trial of linifanib versus sorafenib in patients with advanced hepatocellular carcinoma (HCC). Am Soc Clin Oncol. 2013;31:249.

139. McNamara MG, Le LW, Horgan AM, Aspinall A, Burak KW, Dhani N, et al. A phase II trial of second-line axitinib following prior antiangiogenic therapy in advanced hepatocellular carcinoma. Cancer. 2015;121(10):1620–7.

140. Serova M, Tijeras-Raballand A, Dos Santos C, Albuquerque M, Paradis V, Neuzillet C, et al. Effects of TGF-beta signalling inhibition with galunisertib (LY2157299) in hepatocellular carcinoma models and in ex vivo whole tumor tissue samples from patients. Oncotarget. 2015;6(25):21614.

141. Sangro B, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. J Hepatol. 2013;59(1):81–8.

142. Zhao H, Guo Y, Li S, Han R, Ying J, Zhu H, et al. A novel anti-cancer agent Icaritin suppresses hepatocellular carcinoma initiation and malignant growth through the IL-6/Jak2/Stat3 pathway. Oncotarget. 2015;6(31):31927.

143. Safran H, Charpentier KP, Kaubisch A, Mantripragada K, Dubel G, Perez K, et al. Lenalidomide for second-line treatment of advanced hepatocellular cancer: a Brown University Oncology Group Phase II Study. Am J Clin Oncol. 2015;38(1):1–4.

144. Abou-Alfa GK, Puig O, Daniele B, Kudo M, Merle P, Park J-W, et al. Randomized phase II placebo controlled study of codrituzumab in previously treated patients with advanced hepatocellular carcinoma. J Hepatol. 2016;65(2):289–95.

145. El-Khoueiry AB, Sangro B, Yau TC, Crocenzi TS, Welling TH, Yeo W, et al. Phase I/II safety and antitumor activity of nivolumab (nivo) in patients (pts) with advanced hepatocellular carcinoma (HCC): interim analysis of the CheckMate-040 dose escalation study. Am Soc Clin Oncol. 2016;34:4012.

146. Kudo M. Immune checkpoint inhibition in hepatocellular carcinoma: basics and ongoing clinical trials. Oncology. 2017;92(Suppl. 1):50–62.

147. Escudier B, Faivre S, Van Cutsem E, Germann N, Pouget J-C, Plummer R, et al. A phase II multicentre, open-label, proof-of-concept study of tasquinimod in hepatocellular, ovarian, renal cell, and gastric cancers. Target Oncol. 2017;12(5):655–61.

148. K-c Feng, Y-l Guo, Liu Y, H-r Dai, Wang Y, H-y Lv, et al. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. J Hematol Oncol. 2017;10(1):4.

149. Katz S, Point GR, Cunetta M, Thorn M, Guha P, Espat NJ, et al. Regional CAR-T cell infusions for peritoneal carcinomatosis are superior to systemic delivery. Cancer Gene Ther. 2016;23(5):142.