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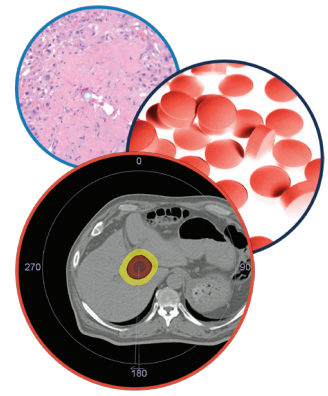

Hepatic Oncology

Mechanisms of doxorubicin resistance in hepatocellular carcinoma

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Hepatocellular carcinoma, one of the most common solid tumors worldwide, is poorly responsive to available chemotherapeutic approaches. While systemic chemotherapy is of limited benefit, intra-arterial delivery of doxorubicin to the tumor frequently produces tumor shrinkage. Its utility is limited, in part, by the frequent emergence of doxorubicin resistance. The mechanisms of this resistance include increased expression of multidrug resistance efflux pumps, alterations of the drug target, topoisomerase, and modulation of programmed cell death pathways. Many of these effects result from changes in miRNA expression and are particularly prominent in tumor cells with a stem cell phenotype. This review will summarize the current knowledge on the mechanisms of doxorubicin resistance of hepatocellular carcinoma and the potential for approaches toward therapeutic chemosensitization.

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KEYWORDS
• apoptosis
• chemoembolization
• FOXO3 • liver • multidrug resistance • sirtuins
• topoisomerase

Practice points

- Doxorubicin-based chemoembolization is a key therapy for hepatocellular carcinoma (HCC), but its utility is limited by pre-existing and acquired tumor resistance.
- The doxorubicin anti-tumor effect primarily involves topoisomerase-mediated double-strand DNA breaks with the subsequent triggering of DNA damage associated cell cycle arrest and apoptosis pathways.
- Clinical resistance of HCC to doxorubicin involves multiple mechanisms largely related to changes in drug accumulation, topoisomerase activity or apoptosis signaling.
- Changes in DNA damage triggered apoptosis are responsible for the major fraction of clinical resistance.
- Specific miRNA increases and decreases are a primary mechanism for effecting these changes.
- Therapeutic approaches to overcome resistance are an important future goal but have not yet reached clinical practice.

Hepatocellular carcinoma (HCC) is one of the major causes of cancer deaths worldwide and its incidence is increasing [1]. The therapy for HCC remains suboptimal and treatment with traditional cytotoxic chemotherapeutic agents such as cisplatin, doxorubicin and 5-FU has been limited by systemic toxicity, poor efficacy, and acquired resistance of the tumors after exposure [2–4]. Sorafenib, a multi-kinase inhibitor, has been shown to produce modest increases in survival of

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selected patients [5,6], but its effects are relatively small and it is not tolerated by patients with more advanced liver disease. Improvements in patient outcome have thus largely resulted from the use of surgical resection, local ablative techniques and liver transplantation [7,8].

One of the more promising developments in HCC treatment has been in targeted delivery of cytotoxic chemotherapy agents directly to the tumor. Selective injection of embolizing agents in combination with doxorubicin into arteries feeding tumors, or trans-arterial chemoembolization (TACE), has been shown to provide a survival benefit in patients with unresectable HCC [9,10] and is now the standard of care for patients with intermediate stage HCC [7]. Embolization of the tumor alone causes ischemia and can produce tumor shrinkage. However, the combination of the embolic effect with the addition of a chemotherapy agent, typically doxorubicin, has been shown in large randomized studies to increase tumor response, decrease progression and improve overall survival [11,12]. The use of embolic drug-eluting microspheres that release doxorubicin (DEBDOX) in a controlled manner has thus improved the TACE technique allowing for higher doses with reduced systemic exposure [13]. Doxorubicin-based TACE now plays an important role in shrinking (downstaging) tumor size and number to allow eligibility for liver transplantation [9].

Resistance to doxorubicin has thus emerged as a central problem limiting treatment of patients with HCC. While TACE is highly effective in many patients, approximately 50% of tumors treated with DEBDOX show no response and only 27% show a complete response [12,13]. One of the important goals of therapy for HCC is to better understand the mechanism of doxorubicin resistance so that new or adjunct approaches can improve the effectiveness of treatment. This review will summarize our knowledge of the mechanisms of doxorubicin resistance in HCC with an eye toward possible development of chemosensitization approaches.

Mechanisms of doxorubicin antitumor effects

Doxorubicin is an anthracycline antibiotic that is widely used as a human antitumor therapeutic agent. Doxorubicin sensitivity is the result of diffusion of the drug to the nucleus and a series of signaling events that are initiated by doxorubicin's interaction with DNA. This ultimately leads to a series of programmed responses culminating in cell apoptosis. It appears to have multiple antitumor effects but the best understood of these involves its interaction with topoisomerase IIα (TOP2A) [14]. This enzyme is involved in separating entangled DNA strands and as part of its function it transiently generates and then repairs protein-bound double-strand DNA breaks (DSBs) [15]. Doxorubicin stabilizes the cleaved-strand intermediate, suppressing the completion of the process resulting in numerous protein-bound DSBs [14]. DSBs have numerous negative consequences for cells and notably trigger caspase-dependent apoptosis programs that involve the activation of master regulators p53 and FOXO3, and suppression of pro-growth signaling pathways, that lead to changes in the ratio of anti/pro-apoptotic Bcl-2 family proteins [16]. This DNA damage response is the primary factor accounting for the antitumor effect of doxorubicin and blocking just this downstream response to DNA damage is sufficient to attenuate doxorubicin toxicity [17]. Multiple other mechanisms have been observed to be involved in doxorubicin cytotoxicity as well and these include the formation of TOP2A-independent DNA adducts [18], inhibition of DNA and RNA synthesis and mitochondrial ROS production triggering apoptosis [19].

Molecular mechanisms of doxorubicin resistance

Doxorubicin resistance results from reduction in the ability of the drug to accumulate in the nucleus, decreased DNA damage and suppression of the downstream events that transduce the DNA damage signal into apoptosis. The general mechanisms involved are illustrated in Figure 1.

• Multidrug resistance transporters

Doxorubicin is a hydrophobic molecule that passes through cellular membranes independently of specific transporters. However, cells can fail to accumulate the drug through active drug efflux via ATP-dependent efflux transporters. This phenomenon, first described in a number of cancers and labeled 'multidrug resistance', results from the expression of a group of multidrug resistance efflux pumps. These proteins, members of the ATP-binding cassette (ABC) transporter family, were initially identified for their pathological role in tumors before their normal physiological functions were understood. They are now known to be important

components of transport in a number of tissues. Hepatocytes use multiple different ABC transporters for the transport of organic ions such as bile acids and conjugated bilirubin [20]. Since these pumps are highly abundant in hepatocytes, it is not surprising that they are expressed in HCC as well where increased expression results in chemotherapy resistance.

The basal expression of ABC proteins is controlled by multiple transcription factors including NF-Y and members of the Sp family [21]. Additionally, p53 has been shown to repress transcription of ABC family proteins [22] while several transcription factors including both AP-1 [23] and NF-κB [24] are capable of upregulating their expression. Activity of the enzyme COX–2 has also been implicated in the control of MDR1 expression as the COX-2 inhibitor, celecoxib, decreases MDR1 expression in multidrug-resistant HCC cells [25,26]. In HCC, three ABC subfamilies, ABCB (the MDR proteins), ABCC (MRP proteins) and ABCG (BCRP proteins) may contribute to doxorubicin resistance. Although they have different substrate specificities during function in normal physiologic conditions, they have all shown an ability to transport doxorubicin [27–29]. MDR1, MRP1, MRP2 and MRP3 are all expressed in HCC at the transcriptional level. MDR1 protein expression is found in 80–90% of HCC cases [27]. MDR family proteins have also been found to be expressed and functionally active on mitochondrial membranes, perhaps protecting mitochondrial DNA from drug-induced damage

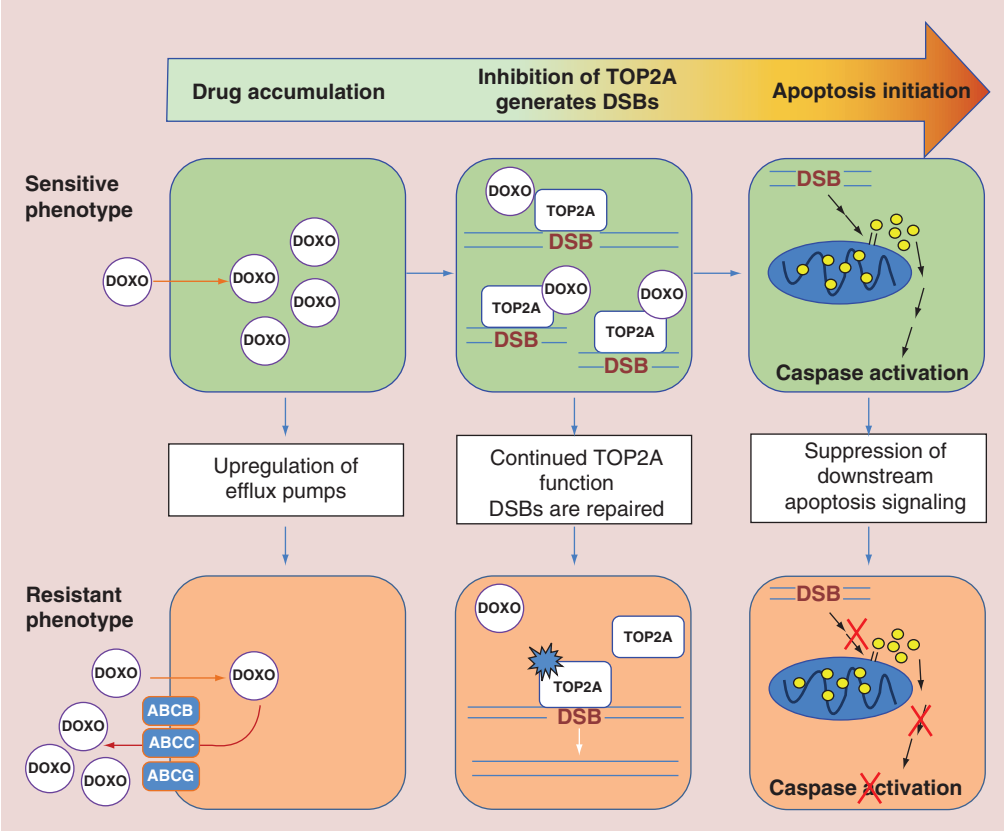

Figure 1. General mechanisms of doxorubicin resistance in hepatocellular carcinoma. Doxorubicin first must accumulate within the cell but this process is inhibited by the upregulation of ABC family efflux pumps in resistant cells. Doxorubicin then prevents the repair of TOP2A-generated DSBs in DNA, increasing TOP2A-bound DSBs. Overexpression and mutations in TOP2A allow continued TOP2A function in resistant cells. Finally, DNA damage induces apoptotic signaling pathways causing cytochorome C release from mitochondria which leads to caspase activation and cell death. Downregulation of the effectors of apoptosis and upregulation of anti-apoptotic proteins prevents the completion of apoptosis in resistant cells.
ABC: ATP-binding cassette; DSB: Double-strand DNA break.

by keeping the drug out of mitochondria, or suppressing apoptosis by altering mitochondrial outer membrane permeability [30].

MDR1 expression was found to inversely correlate with response to systemic chemotherapy in one study [27], but the precise extent to which expression of ABC proteins accounts for clinical drug resistance is less clear. The situation is somewhat clearer in cell culture models of HCC. One method of generating doxorubicin-resistant cultured HCC cells for study is to select for resistant HCC cells *in vitro* after exposing them to incrementally increasing doses of doxorubicin. This method consistently induces the expression of MDR1 and other ABC family members [31,32], and the upregulation of these transporters can be shown to cause drug resistance since inhibitors of ABC proteins such as verapamil and cyclosporine A are able to restore doxorubicin sensitivity [33]. However, verapamil has not proven to be useful as a doxorubicin sensitizing agent in patients, perhaps due to the presence of other efflux transporters, pharmacological interactions between the drugs [34], the loss of important normal physiological functions [35], or the presence of unrelated resistance mechanisms. Therefore, while overexpression of MDR efflux pumps may be an important cause of drug resistance in deliberately selected HCC cell lines *in vitro*, other mechanisms appear likely to be important in human disease.

• Topoisomerase II α

The primary means by which doxorubicin causes cellular toxicity is by targeting the alpha isoform of topoisomerase II (TOP2A) [14], resulting in numerous protein-bound DSBs and the subsequent triggering of apoptosis [36]. It has been hypothesized that one mechanism of resistance to doxorubicin might be through the reduction in TOP2A expression and increased reliance on the beta isoform of topoisomerase II that is less sensitive to doxorubicin [36]. Supporting this hypothesis is the finding that breast cancers with co-amplified HER2 and TOP2A genes have increased sensitivity to doxorubicin, while tumors with a TOP2A deletion have increased resistance [37,38]. Additionally, this mechanism of resistance to doxorubicin has been seen in several cancer cell lines [39]. The situation in HCC, however, appears to be different where TOP2A is increased rather than decreased. In HCC, TOP2A protein level has been shown to be increased independently of gene amplification in 73% of human HCC tumors compared with adjacent non-tumor tissue [40]. It is also overexpressed in several HCC cell lines with acquired doxorubicin resistance [41]. Furthermore, TOP2A expression was found to be positively correlated with histological grade, vascular invasion and early age of onset in a tissue microarray of 172 HCC tumors, and it positively correlated with doxorubicin resistance and shorter survival in 148 patients in a prospective randomized study [42]. TOP2A overexpression has been found to be associated with several indices of tumor aggressiveness in many other types of cancer as well, presumably due to the role of TOP2A in facilitating DNA replication and transcription. While the association of increased TOP2A with tumor growth seems logical, it is not understood why it is also associated with doxorubicin resistance. One hypothesis is that the high expression levels are associated with the development of mutations in TOP2A that lead to its insensitivity to doxorubicin [43]. Another possibility is that in order for cells to survive the high levels of TOP2A they must simultaneously suppress the downstream apoptosis programs normally triggered by DNA strand breaks and it is the acquisition of this adaptive characteristic that confers doxorubicin resistance. At the present time, this issue remains unresolved.

• p53

The tumor suppressor p53 is a frequently altered target in doxorubicin-resistant HCC. It is one of the key DNA damage sensors and acts as a transcriptional activator of pro-apoptotic factors including Bax, Bak, CD95 and TRAIL receptors [44,45]. In addition, it transcriptionally represses anti-apoptotic factors including Bcl-2 and Survivin [46,47]. Doxorubicin upregulates p53 [48], which occurs through its phosphorylation by DDR kinases, which inhibit its binding to and phosphorylation by MDM2, part of the pathway of constitutive ubiquitination and proteosomal degradation that normally leads to low steady-state levels of p53 [49]. An inhibitor of MDM2–p53 binding, Nutlin-3 has been shown to enhance p53 stabilization and activation, and increases doxorubicin sensitivity in HCC cells with wild-type p53 [50]. Mutation or deletion of p53, or disruption of p53 activation pathways are frequent events in HCC tumorigenesis, providing a possible mechanism for intrinsic resistance to doxorubicin [51]. The specific role of p53 in doxorubicin resistance has been illustrated

by experiments showing that restoring p53 expression in HCC cells promotes doxorubicin-induced apoptosis [52].

While it thus might seem attractive to target the regulation of p53, attempts to manipulate p53 by interfering with upstream regulators have produced some unanticipated and paradoxical results. For example, one recent study showed that inhibiting the deubiquitinase USP9x increased p53 ubiquitination and thus decreased p53 levels, yet it still increased doxorubicin sensitivity in HCC cells. This suggests that the effects of ubiquitination inhibitors are more complex than simply causing the degradation of p53 [53]. Furthermore, increasing p53 is clearly not the only way to enhance doxorubicin sensitivity as the three hepatoma cell lines, Huh7, Hep3B, and HepG2 illustrate. HepG2 cells, which have wild-type p53, are the most resistant, and Huh7 and Hep3B which are p53 defective are more doxorubicin sensitive. Clearly, p53, while important, does not account for the complete phenomenon of doxorubicin resistance [54].

• NF-κB

NF-κB is also a transcription factor that has multiple, sometimes opposing functions, such as tumor suppression or promotion depending on the cellular context. In HCC associated with inflammation, such as in HCV or HBV infection, NF-κB tends to have a tumor promoting effect, while in tumors induced by carcinogens such as DEN, NF-κB functions as a tumor suppressor [55]. NF-κB signaling is activated by DNA damage and can have varying effects on subsequent apoptosis primarily through regulation of its target genes, such as Bcl-XL and XIAP [56]. In general, NF-κB has an anti-apoptotic effect in response to drugs that induce DSBs in DNA such as doxorubicin although it may be partially dependent on the cancer cell type [57]. There are few studies investigating the role of NF-κB in resistance to doxorubicin in HCC although it has been shown to be activated in HCC cells in response to doxorubicin [58] and several studies have indicated that activation of NF-κB is a mechanism by which a diverse set of stimuli generate an anti-apoptotic effect. For example, the antiapoptotic gene BAG–1 was found to enhance doxorubicin resistance by potentiating the transcriptional activity of NF-κB [59]. Additionally, the HBV protein HBx has been shown to increase doxorubicin resistance through the activation of NF-κB in HCC cells [60], and reduced expression of miR-26b in HCC promotes doxorubicin resistance due to the loss of its suppression of NF-κB signaling [58].

• FOXO3

FOXO3 is a multifunctional transcription factor involved in the adaptation of cells to a non-proliferating state. It was initially identified as a longevity factor responsible for antioxidant responses, cell cycle arrest, and stem cell survival [61]. Under certain conditions, however, it also promotes apoptosis and some combination of its cell cycle arrest and apoptosis-inducing properties allows it to function as a tumor suppressor [62]. FOXO3 may function as a tumor suppressor in HCC and has been shown to promote apoptosis in HCC cells exposed to various toxic compounds [63–68]. Regulation of FOXO3 is primarily through post-translational modifications including phosphorylation by Akt at three sites that promotes its nuclear export and degradation [61].

FOXO3 mediates doxorubicin-induced apoptosis in a number of different tumor cell types. Doxorubicin increases nuclear accumulation of FOXO3 in breast cancer [69,70], lung cancer, neuroblastoma [71] and osteosarcoma cells [72], and pharmacological approaches that inhibit Akt or otherwise increase FOXO3 nuclear accumulation work synergistically with doxorubicin to enhance apoptosis [73,74]. The mechanisms by which FOXO3 mediates doxorubicin-induced apoptosis include its transcriptional repression of miR-21 which represses translation of Fas-L [71], transcriptional upregulation of Bim, a pro-apoptotic Bcl-2 homolog [75], and transcriptional repression of Bcl-2 [76] and Survivin, an anti-apoptotic Bcl-2 family member [77].

Understanding the role of FOXO3 in doxorubicin resistance is complicated by the fact that FOXO3 can be responsible either for enhanced cell survival or enhanced apoptosis [78]. This ability of FOXO3 to transition between a cell survival factor and a cell death factor may explain the seemingly paradoxical finding that this 'tumor suppressor' is frequently increased in poor prognosis tumors. Increased FOXO3 expression has been observed in breast cancer [69] and certain leukemias that have developed doxorubicin resistance [79], with one potential mechanism being the ability of FOXO3 to transcriptionally activate MDR1 [80].

Recent evidence has shown that the pro-death versus prosurvival balance of FOXO3 is

controlled by specific post-translational modifications. Phosphorylation of FOXO3 at serine-7 by p38 causes it to translocate to the nucleus in response to doxorubicin [70] and phosphorylation at serine-574 causes it to selectively bind to pro-apoptotic promoters and induces cell death. In the absence of this phosphorylation, FOXO3 initiates an antioxidant and cell protective transcriptional program [76]. Thus, whether FOXO3 serves as a pro-apoptotic or pro-survival factor likely depends on the state of its modification by upstream enzymes. The situation in HCC has not been studied in as much detail but preliminary studies from our lab show that FOXO3 mediates doxorubicin-induced apoptosis in HCC cells and doxorubicin-resistant human HCCs have higher cytosolic FOXO3 than doxorubicin-sensitive tumors [81].

• PI3K/Akt

Another class of resistance mechanisms is signaling pathways that are drivers of tumor cell proliferation. These frequently inhibit apoptosis during tumorigenesis as well as after chemotherapy exposure. One such signaling pathway is the PI3K/Akt pathway. Akt is activated through phosphorylation by the second messenger PI3K following growth factor stimulation and in response to many cell stressors [82]. It is negatively regulated by the phosphatase, PTEN [83]. Akt then directly and indirectly regulates cell proliferation and apoptosis by phosphorylating and modulating target protein function including FOXO3, Bad, p53, and cyclin-dependent kinase inhibitors [82], as well as by activating parallel pro-growth pathways [83]. This pathway is frequently activated in HCC and is correlated with decreased overall survival [84]. Several studies have shown that inhibiting PI3K/Akt function using pharmacological inhibitors [85,86] or by exogenous overexpression of an upstream inhibitor [87,88] increases HCC cell sensitivity to doxorubicin, while activating PI3K/Akt has the opposite effect [87,89].

• MAP kinases

The MEK/ERK signaling pathway is another important pathway that translates growth signals from the cell surface to transcription factors and other regulatory proteins to promote cell proliferation and inhibit apoptosis [83]. It promotes HCC tumor cell growth and it is frequently activated in HCC. It has also been shown to be activated by doxorubicin [90], serving as a tumor cell response that counters doxorubicin-induced toxicity. Direct inhibition of ERK activity increases doxorubicin sensitivity in HCC cells by inhibiting cell proliferation and promoting apoptosis [90]. Inhibition of EGFR, an upstream activator of the MEK/ERK pathway, also increases doxorubicin sensitivity in HCC cells [91]. In addition, the mechanism of action of the tyrosine kinase inhibitor, Sorafenib, which has been used as a systemic chemotherapeutic treatment for advanced HCC, also involves inhibition of the MEK/ERK pathway [92]. In a randomized controlled trial of patients with advanced stage HCC, sorafenib plus systemic doxorubicin was shown to increase patient overall survival compared with doxorubicin treatment alone [93].

The p38 MAPK pathway is also activated by doxorubicin and may play a role in regulating doxorubicin-induced apoptosis. Its activation is necessary for the phosphorylation of FOXO3 responsible for its nuclear translocation following doxorubicin treatment in breast cancer cells [70]. MK5, a downstream target of p38, is upregulated in HCC cells and downregulated by doxorubicin. Overexpression of MK5 decreased doxorubicin-induced apoptosis [94].

• Sirtuins

The sirtuin family of NAD-dependent deacetylases is also known to play a crucial regulatory role in the cellular response to stress, apoptosis, metabolism and aging [95]. There are seven members of the sirtuin family in humans, SIRT1–7, and the expression of several SIRTs are altered in HCC, some with pro-tumorigenic and some with anti-tumorigenic effects [95–98]. SIRT1 is consistently found to be overexpressed in HCC [99], and was shown to inhibit doxorubicin-induced apoptosis in HCC cells [95]. The mechanism for SIRT1–mediated inhibition of doxorubicin sensitivity in HCC is unknown but it may involve the deacetylation of p53 [100], FOXO3 [101] or YAP2 [102], where deacetylation of each of these factors has been shown to inhibit its apoptotic activity. Additionally, in breast cancer cells with acquired resistance to epirubicin, a doxorubicin homolog, SIRT4, 5, 6 and 7 were found to be upregulated, particularly SIRT6, which was shown to mediate epirubicin resistance by deacetylation and inhibition of FOXO3 [103].

• MicroRNAs

Noncoding RNAs have been a recent focus in attempts to understand the mechanisms of chemotherapy resistance. Although noncoding RNAs have diverse functions, much recent focus has been on miRNAs, particularly examining their ability to modulate known mechanisms of resistance through suppression of mRNA translation. By comparing miRNA expression patterns in doxorubicin-sensitive and doxorubicin-resistant tumors a number of resistance-associated miRNA changes have been observed [104,105]. Some of these have been shown to cause resistance while others have a plausible mechanism for inducing resistance that has not yet been proven. There are many other associations for which there is as yet no evidence of a causative connection between the miRNA profile and the resistance phenotype. A summary of miRNA changes that have been shown to have a causative link to doxorubicin resistance is presented in Table 1.

Several miRNA changes are both associated with doxorubicin resistance in HCC and have also been demonstrated to produce resistance in model systems. miR-122, the liver-specific miRNA that represents a large proportion of total miRNAs expressed in the liver, is frequently reduced in HCC [106]. Two separate studies show that restoration of miR-122 in HCC cells increases their sensitivity to doxorubicin due to suppression of cell cycle progression, anti-apoptosis effectors and ABC transporter proteins [106,107]. miR-223 was also shown to increase doxorubicin sensitivity by suppressing MDR1 expression in HCC cells [108]. miR-26b is another miRNA that is downregulated in HCC. It normally suppresses TAK1 and TAB3 which are NF-κB activating proteins. Thus the result of miR-26b suppression is the increased activation of NF-κB, which then promotes resistance. Overexpression of miR-26b in HCC cells inhibits NF-κB activation increasing doxorubicin sensitivity [58]. The downregulation of miR-101 [109], miR-199a–3p [110] and miR-215 [111] is also associated with doxorubicin resistance in HCC and restoring their expression has been shown to increase doxorubicin sensitivity. miR-519d, is overexpressed in HCC and has been shown to inhibit apoptosis and promote tumor cell growth by reducing the expression of several tumor suppressor proteins including p21 and PTEN. Significantly, overexpression of miR-519d in HCC cells was shown to decrease sensitivity to doxorubicin [112].

• Role of cancer stem cells

An important consideration in understanding HCC chemoresistance is to identify whether resistance is primarily a property of the bulk tumor cells or a subpopulation. The cancer stem cell model proposes that there is a limited population of cancer stem cells (CSCs) within a tumor with properties of stem/progenitor cells including self-renewal, and multipotency that are responsible for initiation and maintenance of the tumor [113]. The existence of CSCs in human HCC has been experimentally validated through isolation and xenotransplantation assays in immunodeficient mice, and CSCs have proven to have a pivotal role in the development and progression of HCC [114]. CSCs have been shown to be particularly resistant to chemotherapy [115] and cells surviving in the region of trans-arterial embolization-treated tumors display the CSC marker, CD13 [116], suggesting they are a major source of treatment failure and recurrence. Other markers of CSC expression in human HCC, specifically EpCAM and CD133, were found to be increased in TACE-doxorubicin-treated HCC and associated with tumor recurrence after transplant [117]. The mechanisms for doxorubicin resistance in HCC CSCs appear to be multiple including an increased expression of MDR transporters [118,119] and increased Akt and Bcl-2 cell survival signaling [120], possibly resulting from altered miRNA expression [121]. Treatments specifically targeting proteins and pathways differentially expressed in HCC CSCs, such as anti-CD13 antibodies, have shown promise in increasing the efficacy of doxorubicin treatment [116].

Conclusion

Poor response to doxorubicin-based locoregional chemotherapy is a major obstacle to treatment of patients with HCC. Resistance of tumor cells to doxorubicin itself is an important component of clinical treatment failure and recurrence. Multiple mechanisms are responsible for doxorubicin resistance and these include the presence of drug efflux transporters, alterations in the ability of doxorubicin to form DSBs in DNA, and alterations in downstream apoptosis signaling triggered by DNA damage. Many of these changes result from miRNA-mediated changes in protein abundance and are prominent in tumor cells possessing a stem-cell like phenotype.

Table 1. miRNAs associated with doxorubicin resistance.

miRNA	Change in resistant tumor	Cell type	Target gene(s)	Ref.
miR-215	↑	Hepatocyte	DHFR, TS	[111]
miR-26b	↓	Hepatocyte	TAK1, TAB3	[58]
miR-122	↑	Hepatocyte	Cyclin G1	[106]
miR-199a–3p	↓	Hepatocyte	mTOR, c-Met	[110]
miR-519d	↑	Hepatocyte	P21, PTEN, AKT3, TIMP2	[112]
miR-101	↓	Hepatocyte	EZH2	[109]
miR-223	↓	Hepatocyte	ABCB1	[108]
Let–7a	↑	Hepatocyte, SCC	Caspase–3	[125]
miR-138	↓	HNSCC	MDR1	[126]
miR-21	↑	Breast	PTEN	[127]
miR-760	↓	Breast	RHOB, ANGPTL4, ABCA1	[128]
miR-218	↓	Breast	Survivin	[129]
miR-298	↓	Breast	MDR1	[130]
miR-450b–3p	↓	Breast	HER3	[131]
miR-451	↓	Breast	MDR1	[132]
miR-452	↓	Breast	IGF–1R	[133]
miR-200c	↓	Breast	ZEB1	[134]
miR-34a	↓	Breast,	NOTCH1	[135]
miR-34c	↓	Osteosarcoma	NOTCH1, LEF1	[136]
miR-382	↓	Osteosarcoma	HIPK3	[137]
miR-301a	↑	Osteosarcoma	AMPKα1	[138]
miR-708	↓	Ewing sarcoma	EYA3	[139]
miR-125b	↑	Ewing sarcoma	P53, BAK	[140]
miR-522	↓	Colon	ABCB5	[141]
miR-101	↓	Colon	SphK1	[142]
miR-195	↓	Colon	Bcl-w	[143]
miR-103/107	↓	Gastric	Cav–1	[144]
miR-508–5p	↓	Gastric	ABCB1, ZNRD1	[145]
miR-331–5p	↓	Leukemia	MDR1	[146]
miR-27a	↓	Leukemia	MDR1	[146]

DHFR: Dihydrofolate reductase; TS: Thymididylate synthase; Cav–1: Caveolin–1; SCC: Squamous cell carcinoma; HNSCC: Head and neck SCC.

Future perspective

Arterially targeted chemotherapy in the form of TACE-administered doxorubicin is a mainstay of management of HCC patients prior to liver transplantation and although rationally designed targeted therapies for early and intermediate stage HCC continue to be pursued, it is likely to remain an important therapeutic modality for the foreseeable future. Tumor resistance to doxorubicin is the key limitation of this treatment, responsible for treatment failures and recurrences, as well as the need to use high doses of drug, thus limiting the ability to treat patients with the most advanced liver disease. The ability to counteract resistance mechanisms would be a major clinical advance. Attempts have been made to augment doxorubicin sensitivity of HCCs and other tumors by employing inhibitors of MDR transporters [122], PI3K/Akt [86] and sirtuins [99] using small molecule inhibitors or RNAi approaches. None have yet to demonstrate benefit in clinical trials. In experimental models of HCC, doxorubicin chemosensitization has been achieved with PP2A inhibitors [123], CD13 antibodies [116] or different approaches to inhibit MDR transporters including small molecule inhibitors and antisense constructs [33,124]. Further understanding of the mechanisms responsible for doxorubicin resistance will thus be important to make chemosensitization a routine part of the TACE treatment protocol.

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