Chapter

Hepatocarcinoma Angiogenesis and DNA Damage Repair Response: An Update

Xi-Dai Long, Zhou-Ximao Long, Xiao-Ying Huang, Jin-Guang Yao, Qiang Xia and Yan Deng

Abstract

Hepatocarcinoma is one of the most common lethal human malignant tumors, mainly because of active angiogenesis. This kind of high angiogenesis often accounts for early metastasis, rapid recurrence, and poor survival. Growing evidence has proved that hepatocarcinoma angiogenesis is closely associated with multiple risk factors, such as DNA damages resulting from hepatitis B and C virus infection, aflatoxin B1 exposure, ethanol intake, and obesity. Genetic alterations and genomic instability, probably resulting from low DNA damage repair response (DRR) and the following unrepaired DNA lesions, are also increasingly recognized as important risk factors of hepatocarcinoma angiogenesis. Dysregulation of DRRs and signaling to cell cycle checkpoints involving in DRR pathways may accelerate the accumulation of DNA damages and trigger the dysregulation of angiogenesis-related genes and the progression of hepatocarcinoma. In this review, we discussed DNA damages/DRRs and angiogenesis during hepatocarcinogenesis and their interactive regulations. Hopefully, the review will also remind the medical researchers and clinic doctors of further understanding and validating the values of DNA damages/DRRs in hepatocarcinoma angiogenesis.

Keywords: hepatocarcinoma, angiogenesis, DNA damage, DNA damage repair response

1. Introduction

Hepatocellular carcinoma, also termed as hepatocarcinoma, is one of the most common malignant tumors, with more than 500,000 new cases per year [1]. Until recently, it has been frequent to consider hepatocarcinoma as a tumor with low incidence in the western world but with high incidence in the eastern countries [1]. However, increasing data exhibit that the incidence of this tumor has increased in both western and eastern countries. Etiologically, several risk factors, including hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxin B1 (AFB1), and alcohol, have been identified for increasing disease incidence worldwide [2]. Although molecular mechanisms of hepatocarcinoma caused by these risk factors have not still been clear, chronic and permanent liver damage and damage response may play a vital role. Macrocosmically, liver damage consists of a series of pathological changes, such as chronic hepatitis, liver cirrhosis, nodular hyperplasia, and

dysplasia [3]. Microcosmically, chronic DNA damage, including the formation of DNA adducts, DNA strand break and bulk, gene mutations, and genomic instability, is the most important type [4].

Because of early blood metastasis and high death rate of this malignancy, it has become the third most common cause of cancer-associated deaths worldwide. This death risk could be explained by high angiogenesis capacities of hepatocarcinoma [1, 2]. Increasing evidence has exhibited that hepatocarcinoma patients with high microvessel density (MVD) in tumor tissues would feature a poor prognosis, and angiogenesis has been regarded as an important marker predicting the risk of invasiveness and metastasis [5]. This chapter summarizes the latest findings in hepatocarcinoma angiogenesis, DNA damage, and damage repair response (DRR). We also try to shed light on the effects of DNA damage and dysregulation of DRR on tumor angiogenesis.

2. Angiogenesis and regulation in hepatocarcinoma

2.1 Angiogenesis process in hepatocarcinoma

Several previous reviews have summarized the angiogenesis in hepatocarcinoma [5–7]. In brief, angiogenesis is a kind of crucial biological function and survival potential for normal organism development, growth, and adaptation to new environment. The dynamic balance between increasing and decreasing potential of angiogenesis is essential in the different physiological and pathological conditions, such as injury cure, damage repair, inflammatory procession, tumor progression, blindness, and ischemia. Hepatocarcinoma angiogenesis was extensively studied via cell models, experimental animal models, and human tumor samples [5–7]. Accumulating data have proved that local hypoxia in tumor tissues and the change in genome resulting from genetic or environmental risk factors will lead to the secretion and synthetics of angiogenetic regulative factors and triggering angiogenesis [8–10]. In hepatocarcinoma tissues, the process of angiogenesis consists of the following several stages: sprouting, extracellular matrix component (ECMs) reconstruction, endothelial cell (EC) migration and proliferation, lumen formation, and stabilization of newborn vessels (**Figure 1**) [11].

The establishment of conditions allowing ECs proliferation and migration, which often results from local hypoxia, first facilitates endothelial sprouting and budding. During this stage, hypoxia induces the secretion and synthetics of angiogenetic factors, such as nitric oxide (NO), vascular endothelial growth factor (VEGF), CD31, angiopoietin-1, and so on [11]. The NO-induced vasodilation and VEGF-caused high permeability result in the extravasation of plasma components (including fibrinogen and fibrin). Together with ECMs, these plasma components lay down and form provisional scaffolds for migrating ECs. The basement membranes and ECMs (mainly consisting of collagen I and IV and laminin) are next degraded, and subsequently, ECs migrate into local sites and proliferate. Increasing proliferation of ECs in the local hypoxia tissues leads to the formation of nascent vessels with lumen. After that, nascent vessels are recruited and structurally stabilized under the conditions of physical forces and a series of molecules such as platelet-derived growth factor β (PDFG-β), angiopoietin-1, angiopoietin-2, VEGF, and transforming growth factor β1 (TGF-β1) [7, 11, 12].

Vessels in hepatocarcinoma differ from other liver diseases or normal vessels [5, 11, 13]. First, tumor vessels typically appear as irregular diameter and abnormal branching patterns [5]. Second, pericytes of vessels are often incompletely covered or lost; furthermore, their basement membranes are also incomplete [11]. Third, tumor vessels sometimes form irregular channels and the walls of these channels are

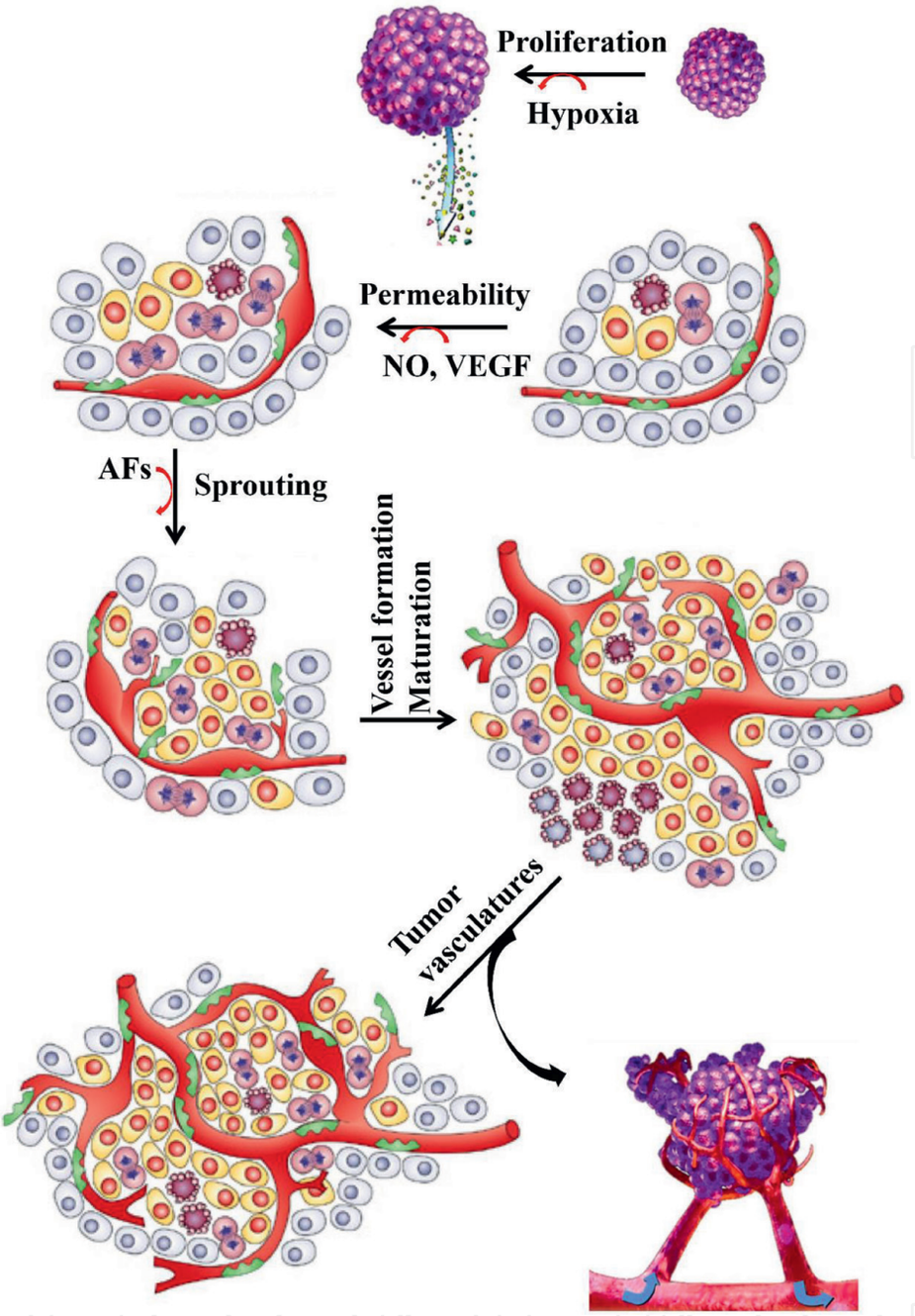

Figure 1.
Angiogenesis procession in hepatocarcinoma. The procession of angiogenesis consists of: (1) sprouting and budding; (2) ECM remodeling; (3) EC proliferation and migration; (4) lumen formation and three-D organization; and (5) stabilization of nascent vessels.

comprised of cancer cells. Moreover, the endothelial cells may be replaced by cancer cells partially or completely. Finally, angiogenesis in hepatocarcinoma not only appears abnormal architecture but also accompanies abnormal molecular expression and regulation [6, 14]. These characteristics result in abnormal structures and function for hepatocarcinoma; however, they can provide some important cues for early diagnosis and therapeutic strategies for cases with hepatocarcinoma.

2.2 Angiogenesis regulation in hepatocarcinoma

A series of angiogenic and antiangiogenic factors (**Tables 1** and **2**) regulate the angiogenesis process in hepatocarcinoma [5]. During the process of hepatocarcinoma angiogenesis, hypoxia and VEGF family play a vital role. Hypoxia in local

No.	Active factors	Effects	Process involved in hepatocarcinoma
AF01	NO	Stimulating vasodilation	Increasing vessel permeably
AF02	VEGF family members	(1) Increasing vascular permeability (2) inducing EC proliferation (3) Progressing leukocyte adhesion (4) Regulating neovascular lumen diameter	(1) Sprouting and budding (2) Vessel growth 3-D organization
AF03	VEGF-R	Integrate angiogenic and survival signals	Vessel growth
AF04	NRP-1	Integrate angiogenic and survival signals	Vessel growth
	Angiopoietins	Inducing EC proliferation	Vessel growth
	IL-4	Inducing EC proliferation	Vessel growth
	IL-8	Inducing EC proliferation	Vessel growth
	Hepatocyte growth factor	Inducing EC proliferation	Vessel growth
	Tissue factor	Inducing EC proliferation	Vessel growth
	Fibronectin	Progressing ECM remodeling	
AF05	Integrins avb3	(1) ECM receptors, intercellular communication (2) Mobilized during EC migration (3) Regulating neovascular lumen diameter	ECM remodeling and EC migration Newborn vessel stabilization
AF06	Integrins avb5	(1) ECM receptors, intercellular communication (2) Mobilized during EC migration (3) Regulating neovascular lumen diameter	ECM remodeling and EC migration Newborn vessel stabilization
AF07	Integrins a6b1	(1) ECM receptors, intercellular communication (2) Mobilized during EC migration (3) Regulating neovascular lumen diameter	ECM remodeling and EC migration Newborn vessel stabilization
AF08	uPA	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF09	Plasminogen activators	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF10	MMPs	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF11	Heparinases	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF12	chymases	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF13	Tryptases	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization
AF14	Cathepsins	(1) Remodeling ECM (2) Releasing and activating growth factors	ECM remodeling and EC migration Newborn vessel stabilization

No.	Active factors	Effects	Process involved in hepatocarcinoma
AF15	PlGF	Inducing EC proliferation	Vessel growth
AF16	aFGF	Inducing EC proliferation	Vessel growth
AF17	bFGF	Inducing EC proliferation	Vessel growth
	FGF-R1	Receptor for aFGF	Vessel growth
	FGF-R2	Receptor for bFGF	Vessel growth
AF18	HGF	Inducing EC proliferation	Vessel growth
	c-Met	Receptor for HGF	Vessel growth
AF19	TGF-a	Inducing EC proliferation	Vessel growth
AF20	TGF-b	Inducing EC proliferation	Vessel growth
	EGF-R	Receptor for TGF-a and TGF-b	Vessel growth
AF21	MCP-1 and other chemokines	Pleiotropic role in angiogenesis	Newborn vessel stabilization
AF22	MEF2C	Regulating neovascular lumen diameter	Newborn vessel stabilization
AF23	Ephrin's	Determining branching and arterial/venous specification	Newborn vessel stabilization
AF24	PDGF-B and receptors	Recruiting pericytes	Newborn vessel stabilization
AF25	Ang-1	(1) Stabilizing intercellular contacts (2) Inhibiting permeability	Newborn vessel stabilization
AF26	Ang-2	Ang-1 antagonist (destabilizes vessels; causes EC death)	Vessel regression
AF27	Tie-2	Receptor for Ang-1 and Ang-2	Newborn vessel stabilization
AF28	TGF- 1	(1) Promoting vessel maturation (2) stimulating ECM generation (3) Inducing differentiation of mesenchymal cells to pericytes	ECM remodeling and EC migration
AF29	Endoglin	(1) Promoting vessel maturation (2) stimulating ECM generation (3) Inducing differentiation of mesenchymal cells to pericytes	ECM remodeling and EC migration
AF30	Cyr61	(1) Stimulating directed migration of EC through an AVB integrin-dependent pathway (2) Acting as ECM modifiers (3) Promoting EC survival	ECM remodeling and EC migration
AF31	Fisp12	(1) Stimulating directed migration of EC through an AVB integrin-dependent pathway (2) Acting as ECM modifiers (3) Promoting EC survival	ECM remodeling and EC migration

Abbreviations: *VEGF, vascular endothelial growth factor; ECM, extracellular matrix component; EC, endothelial cell; PEDF, Pigment epithelium-derived factor; platelet and endothelial cell adhesion molecule 1; TIMPs, Tissue inhibitor of metalloproteases; IFN, interferon; MMPs, matrix metalloproteinases; Ang, angiopoietin; IL, interleukin; PIGF, placenta growth factor; HGF, hepatocyte growth factor; TGF, transforming growth factor; EGF, epidermal growth factor.*

Table 1.
Angiogenesis active regulative factors in hepatocarcinoma.

No.	Active factors	Effects	Process involved in hepatocarcinoma
IF01	Arrestin	Suppressing VEGF-regulating vessel growth	Vessel growth
IF02	Canstatin	(1) Interruption of stable cell-ECM connections (2) Inducing EC apoptosis	Vessel regression
IF03	Interleukin 12	Suppressing EC cell proliferation	Vessel growth
IF04	PEDF	Suppressing EC cell proliferation	Vessel growth
IF05	VE-cadherin	(1) Adhering junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF06	PECAM-1	(1) Adhering junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF07	Plakoglobin	(1) Adhering junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF08	b-Catenin	(1) Adhering junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF09	Claudins	(1) Tightening junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF10	Occludin	(1) Tightening junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF11	JAM-1	(1) Tightening junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF12	JAM-2	(1) Tightening junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF13	JAM-3	(1) Tightening junction molecules (2) Intercellular adhesion (3) Providing vessel tightness	Newborn vessel stabilization
IF14	Connexins	(1) Gap junction molecules (2) Facilitating intercellular communication	Newborn vessel stabilization
IF15	Integrins avb3	Suppressing VEGF- and Flk-1-mediated EC survival	Vessel growth
IF16	Integrins avb5	Suppressing VEGF- and Flk-1-mediated EC survival	Vessel growth
IF17	PAI-1	(1) Inhibiting ECM degradation by MMPs (2) Inhibiting EC proliferation	ECM remodeling and EC migration
IF18	TIMPs	(1) Inhibiting ECM degradation by MMPs (2) Inhibiting EC proliferation	ECM remodeling and EC migration
IF19	Angiostatin and related plasminogen fragments	Suppressing tumor angiogenesis	Vessel growth
IF20	Endostatin	Suppressing EC cell proliferation	Vessel growth

No.	Active factors	Effects	Process involved in hepatocarcinoma
IF21	Antithrombin III	Suppressing EC cell proliferation	Vessel growth
IF22	IFN-a	Suppressing EC cell proliferation	Vessel growth
IF23	IFN-b	Suppressing EC cell proliferation	Vessel growth
IF24	LIF	Suppressing EC cell proliferation	Vessel growth
IF25	PF4	Suppressing EC cell proliferation	Vessel growth
IF26	TSP-1	Inhibiting lumen formation	Vessel regression
IF27	Ang-1 (excess)	Making vessels too tight and inhibiting sprouting	Newborn vessel stabilization
IF28	Ang-2	Facilitating sprouting in the presence of VEGF	Vessel regression
IF29	sTie-2	Inhibitor for Ang-1 and Ang-2	Vessel regression
IF30	sFlt-1	Inhibitor for VEGF family	(1) Sprouting and budding (2) Vessel growth 3-D organization
IF31	Thrombospondin-1	Suppressing EC cell proliferation	Vessel growth
IF32	Thrombospondin-2	Suppressing EC cell proliferation	Vessel growth
IF33	Tumstatin	Suppressing EC cell proliferation	Vessel growth
IF34	Vasostatin	Suppressing EC cell proliferation	Vessel growth

Abbreviations: *VEGF, vascular endothelial growth factor; ECM, extracellular matrix component; EC, endothelial cell; PEDF, Pigment epithelium-derived factor; platelet and endothelial cell adhesion molecule 1; TIMPs, Tissue inhibitor of metalloproteases; IFN, interferon; MMPs, matrix metalloproteinases; Ang, angiopoietin.*

Table 2.
Angiogenesis inhibitive regulative factors in hepatocarcinoma.

tumor tissues, an important pathophysiological phenomenon caused by rapid growth of tumor, leads to the expression of hypoxia-inducible factor (HIF)-1α, which is a key inducible factor for angiogenesis in hypoxia tissues [7, 14]. On the one hand, HIF-1α can induce the expression of hypoxia-response-related genes like NO, VEGF, transforming growth factor (TGF) α and β, adrenomedullin (ADM), LDL-receptor-related protein 1 (LRP1), and leptin; on the other hand, local hypoxia status in tumor tissues also downregulates the expression of antiangiogenic factors such as thrombospondin-1 (TS1) and -2 (TS2) [15–17]. Additionally, growing literature has shown that lots of factors, including genetic or acquired alterations in the oncogenes (i.e., Ras, c-Jun, and Myc) and tumor suppressor genes (i.e., TP53), Hepatitis B Virus X (HBx) protein, chromobox 4, and DNA damage induced by chronic inflammation and AFB1 exposure, can increase the expression proangiogenic factors [18–23]. For example, HBx protein has a potential for increasing HIF-1α expression via promoting transcriptional and translational activity and therefore accelerating angiogenesis during carcinogenesis process of hepatocarcinoma [24]. Recent studies have reported that chromobox 4 (a known transcriptional regulator and also a SUMO E3 enzyme) can promote angiogenesis via stabilizing HIF-1 in hepatocarcinoma [18, 19]. VEGF (including its glycoprotein family members VEGF-A, -B, -C, and -D) is another important angiogenic factor that always upregulates in most cases with hepatocarcinoma [5]. The upregulation of VEGF in hepatocarcinoma is proved not only to increase tumor neovascularization but also to accelerate tumor growth via in vitro cell experiments and animal

models. The role of VEGF is mediated mainly by two receptors: VEGF-R1 (also called Flt-1) and VEGF-R2 (also termed as KDR/Flk-1). Both VEGF-R1 and VEGF-R2 have tyrosine kinase activity and are normally expressed in hepatic parenchyma cells including endothelial cells of portal and sinusoidal tracts [5, 6]. In hepatocarcinoma, both mRNA and protein amount of them are increasing noticeably in the tumor tissues compared to peri-tumor tissues [25]. Some other factors, such as angiopoietin 1 and 2, involve in the regulation of angiogenesis in hepatocarcinoma (**Tables 1** and **2**) [5, 6, 13]. Together, increasing angiogenic potential but decreasing antiangiogenic potential facilitates hepatocarcinoma angiogenesis.

2.3 Angiogenesis biomarkers in hepatocarcinoma

In the past decades, several biomarkers, such as VEGF, angiogenin, and MVD, have been selected for elucidating angiogenic potential of hepatocarcinoma. **Table 3** summarized the potential of these biomarkers for hepatocarcinoma angiogenesis and angiogenesis-related tumor biological actions. Among these biomarkers, VEGF is concerned especially because of its clinic significance. For example, a hospital-based clinic samples analyses (including 7 cases with liver low-grade dysplastic nodule [DN], 8 cases with liver high-grade DN, 11 cases with early hepatocarcinoma, 17 cases with small hepatocarcinoma, and 21 cases with advanced hepatocarcinoma) by Park et al. [26] showed that the amount of VEGF increased gradually from low-grade DN to early hepatocarcinoma. Furthermore, this increasing expression of VEGF is significantly associated with neoangiogenesis (marked by MVD with CD34 staining) and cancer cell proliferation. Collectively, we can conclude that increasing VEGF expression and MVD are positively associated with tumor vascularization and the following tumor progression and poor survival of tumor cases. Furthermore, increasing evidence has exhibited that serum levels of VEGF are not only parallel with the amount in tumor tissues but also can predict therapy response of patients with hepatocarcinoma [29–32]. Thus, VEGF may be useful for improving therapeutic strategies of hepatocarcinoma based on the angiogenesis thesis.

No.	Study design	Samples	Results	Ref#
1	Hospital-based sample study	LGDs (n = 7), HGDs (n = 8), eHCCs (n = 11), shocks (n = 17), and aHCCs (n = 21)	(1) VEGF expression increases gradually from LGD to eHCC. (2) The sHCCs has an increasing neoangiogenesis and cell proliferation compared to aHCCs. (3) The levels of VEGF expression are positively associated with MVD (marked by CD34 staining).	[26]
2	Hospital-based sample study	HCCs (n = 60)	Amount of VEGF in the serum of patients positively correlates with that in the tumor tissues.	[27]
3	Hospital-based sample study	HCs (n = 20), CHs (n = 36), LCs (n = 77), and HCCs (n = 86)	Plasm VEGF levels are increasing in patients with HCC compared to in non-HCCs and this increase will more noticeable in cases with metastasis HCCs.	[28]
4	Hospital-based sample study	HCs (n = 30), LCs (n = 26), and HCCs (n = 52)	Plasm VEGF levels are increasing in patients with HCC compared to in non-HCCs and this increase will shorten the survival of HCCs.	[29]
5	Prospective study	HCCs (n = 100)	Plasm VEGF levels of HCC cases are related to tumor stage, postoperation recurrence, and blood invasion.	[30]

No.	Study design	Samples	Results	Ref#
6	Hospital-based sample study	HC (n = 15) and HCCs (n = 98)	Serum VEGF is a significant biomarker for HCC survival (including OS and RFS).	[31]
7	Prospective study	HCCs (n = 80)	Serum VEGF levels were correlated with clinical data, tumor response to TACE and survival results.	[32]
8	Hospital-based sample study	HCCs (n = 48)	TACE treatment can upregulate expression and bFGF in HCC tissues possibly due to hypoxia and ischemia.	[33]
9	Hospital-based sample study	HCCs (n = 38)	TACE treatment can upregulate expression and bFGF in HCC tissues possibly due to hypoxia and ischemia.	[34]
10	Hospital-based sample study	HCCs (n = 41)	Angiogenin mRNA in serum and tumor tissues positively associating with MVD and poor prognosis of cases	[35]
11	Hospital-based sample study	HCCs (n = 90)	MMP-2, MMP-9 and VEGF expression is positively correlated to the prognosis of HCC patients.	[36]
12	Hospital-based sample study	HCCs (n = 30)	The serum levels of Ang-2, HGF, IL-8, PDGF-BB, and VEGF were correlated with poor effects of sorafenib treatment in patients with HCC.	[37]
13	Hospital-based sample study	CHs (n = 79) and HCCs (n = 89)	(1) TEMs are involved in HCC angiogenesis. (2) The frequency of circulating TEMs was significantly higher in HCC than non-HCC patients. (3) The TEMs have higher diagnostic value for HCC than AFP, PIVKA-II and ANG-2.	[38]
14	Animal model	/	Mobilized EPCs participate in tumor angiogenesis of HCC	[39]

Abbreviations: *LGDs, patients with low-grade dysplasia; HGD, patients with high-grade dysplasia; eHCCs, patients with early hepatocellular carcinoma; HCC, hepatocellular carcinoma; sHCCs, patients with small HCC; aHCCs, patients with advanced HCC; HCCs, patients with HCC; HCs, healthy controls; LCs, patients with liver cirrhosis; VEGF, vascular endothelial growth factor; MVD, microvessel density; OS, overall survival; RFS, tumor reoccurrence-free survival; TACE, transarterial chemoembolization; bFGF, basic fibroblast growth factor; EPCs, bone marrow-derived endothelial progenitor cells; TEMs, TIE2-expressing monocytes/macrophages; Ang-2, angiopoietin-2; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; IL-8, interleukin-8.*

Table 3.
The potential of biomarkers for hepatocarcinoma angiogenesis and angiogenesis-related tumor biological actions.

3. DNA damage and DRR in hepatocarcinoma

3.1 DNA damage induced by risk factors for hepatocarcinoma

Multiple risk factors, including HBV and HCV infection, AFB1 exposure, ethanol consumption, and obesity, have been reported to correlate with hepatocarcinogenesis (**Figure 2**) [4]. These risk factors can induce multiple types of DNA damage, such as DNA single-stand break (SSB), double-strand break (DSB), base damage, DNA-adduct formation, oxidation damage, gene mutation, chromosomal aberration, and genomic instability [4]. Results from epidemiological and experimental studies show that viral-DNA damage relationship is characterized by:

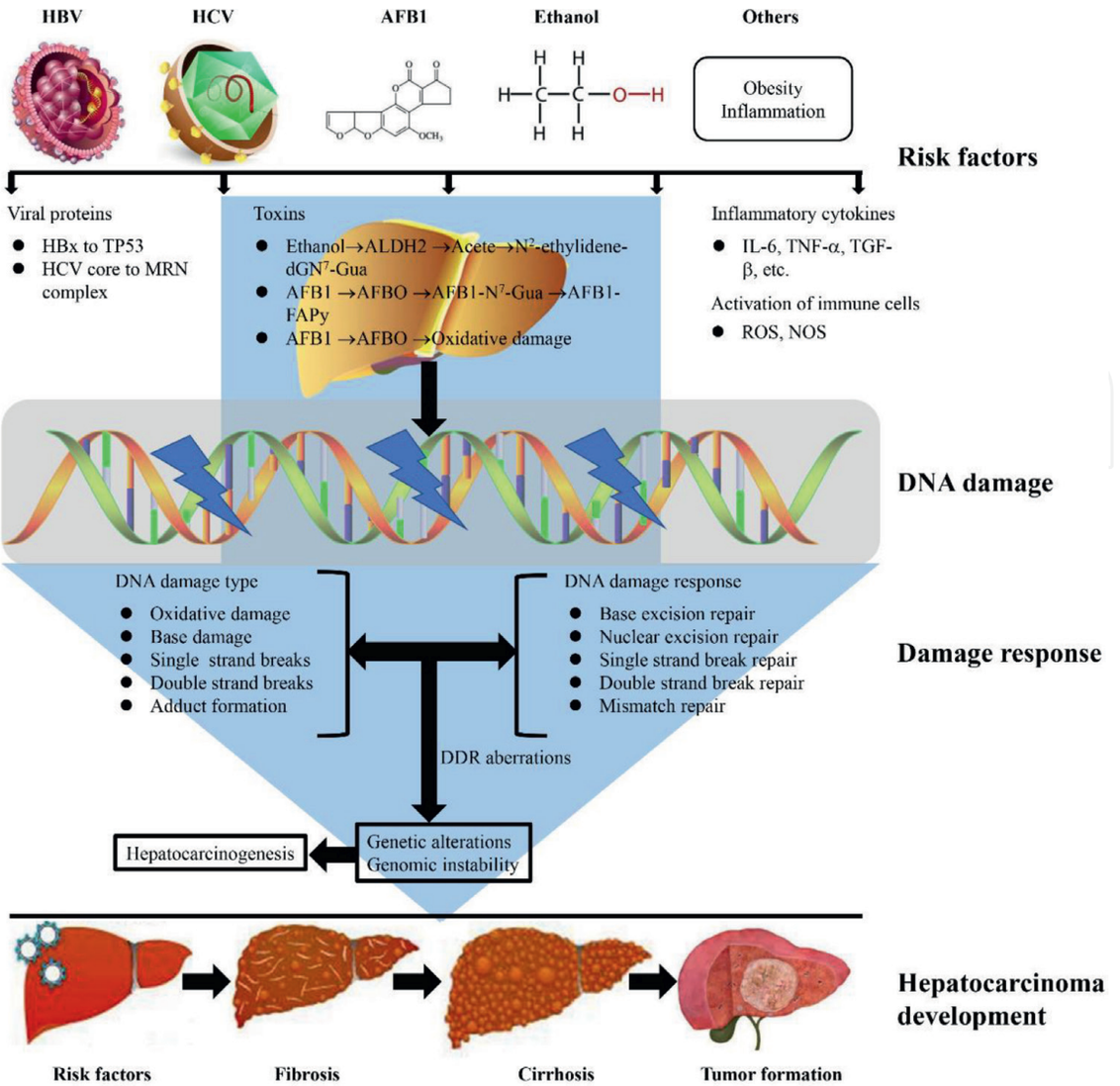

Figure 2.
Risk factors-induced DNA damage and damage repair response during hepatocarcinoma. **Abbreviations**: *HBV, hepatitis B virus; HCV, hepatitis C virus; AFB1, aflatoxin B1; AFBO, AFB1-8,9-epoxide; IL, interleukin; TGF, transforming growth factor.*

(1) the integration of viral gene (such as HBx gene) into the genome of liver cells and resulting genomic instability of host cells [21, 24, 40, 41]; (2) TP53 mutation conducted by HBx integration resulting in abnormal cell response, including DNA repair, cell proliferation and cycle, and apoptosis potential [22]; (3) HCV core interfering the formation of Mre11/Rad50/Nbs1 (MRN) complex through the bind with Nbs1 [5]; (4) the inhibition of such DNA repair proteins as Ataxia telangiectasia mutated kinase (ATM) [42, 43]; and (5) inducing dysregulation of signal pathways, including Wnt/β-catenin pathway, sex steroid pathway, p38MAPK pathway, PI3K/Akt pathway, transforming growth factor β (TGFβ) pathway, NF-κB pathway, and so on [11].

For AFB1-induced DNA damage, adducts formation and gene mutations are concerned especially [44]. AFB1 is a known I-type chemical hepatocarcinogen produced mainly by *A. parasiticus* and *A. flavus* and a suspected risk factor for hepatocarcinoma in some dependent areas such as Sub-Saharan area, the southeast region of Asia, and the coast of southeast China. Results from prospective epidemiological and animal studies have exhibited that AFB1-induced DNA damage plays a vital role in the process of hepatocarcinoma caused by AFB1 exposure [40, 45]. Studies of AFB1 metabolism have further proved that cytochrome P450 (CYP) enzymes

in hepatocytes can facilitate AFB1 into its epoxy compound, also termed as AFB1-8,9-epoxide (AFBe). AFBe can covalently bind to genomic DNA and ultimately induce multiple types of DNA damage [46, 47]. Increasing evidence exhibits that AFB1 can multiplicatively interact with HBV and/or HCV infection during hepatocarcinogenesis, and that, this multiplicative interaction may be associated with more noticeable DNA damage induced by both AFB1 exposure and HBV/HCV infection [23]. Epidemiological studies based on the case-control design with a large sample have proved that patients with chronic virus hepatitis (including B and C type) will feature increasing hepatocarcinoma risk under the conditions of high AFB1 exposure [46]. Furthermore, patients with high AFB1 often companies with chronic virus infection and faces higher frequency of gene mutation like TP53 and ras [47]. Interestingly, the mutation at the codon 249 of TP53 gene, namely G:C > T:A mutation resulting in the change of arginine to serine, has been identified as a relatively specifically change and named AFB1-induced hot-spot mutation [44]. This mutation may lead to the dysfunction of TP53 protein and abnormal cell actions like promoting cell growth, inhibiting cell apoptosis, and inhibiting transcription mediated by TP53 [40].

Other risk factors like alcohol intake also cause malignant transformation of hepatocytes. Chronic ethanol intake will significantly increase hepatocarcinoma risk (about five times) if more than 80 g/day × 10 years. Actually, less than this amount of uptake also increases cancer risk in spite of nonsignificance [48]. Although mechanisms are not still clear, increasing data have shown that chronic hepatic injury, abnormal regeneration, and cirrhosis may act some role in hepatocarcinogenesis [4]. Pathological and molecular biological studies display that acetaldehyde, an important metabolic product of ethanol, can bind to DNA and form DNA adducts. The DNA adduct formation caused could trigger replication errors and/or mutations in tumor suppressor genes and/or oncogene [4]. Additionally, oxidative DNA damage is more noticeable in tissues with hepatocarcinoma than peri-tumor tissues [40, 46]. However, it is unclear whether acetaldehyde-DNA adducts and oxidative damages are true carcinogens and how they trigger hepatocarcinogenesis [4, 49]. Therefore, future studies on DNA damage are needed to better validate these risk factors and detailed molecular mechanisms.

3.2 DRR in hepatocarcinoma

DNA damage will trigger DRR pathways, a kind of prompt signal event which can harmonize whether cells obtain cycle arrest for DNA repair or induce death for eliminating cells with severe DNA damage and genomic instability [4]. In human, cells develop several types of surveillance mechanisms consisting of SSB repair (SSBR), DSB repair (DSBR), base excision repair (BER), base mismatch repair (MMR), and nucleotide excision repair (NER) (**Figure 2**) [4, 40]. Among these DNA repair pathways, BER, MMR, and NER can repair base damage such as base mismatches, AFB1-DNA adducts, DNA pyrimidine dimers, and DNA damage induced by irradiation and anticancer drugs. SSBR can repair SSB that is a severe DNA damage, if not repaired quickly, will disrupt genic transcription and replication and ultimately results in lethal DNA damage [40]. DSBR pathway involves in homologous recombination (HR), single-strand annealing (SSA), and nonhomologous end joining (NHEJ). HR pathway can repair DSBs through an accurate repair method using the undamaged homologous chromosome or sister-chromatid as DNA repair temple; whereas NHEJ and SSA pathways are nonhomologous repair methods and usually lead to essential mutagenesis, so far

DRR pathway gene/ proteins	DRR pathway	Abnormal of DRR	Effects on hepatocarcinoma	Ref#
hOGG1	BER	Ser to Cys at codon 326	Increased hepatocarcinoma risk	[51]
XRCC1	BER and SSBR	Arg to His at codon 280 Arg to Gln at codon 399 Arg to Trp at codon 194	(1) Increasing individuals' susceptibility to HBV infection (2) Increasing individuals' susceptibility to hepatocarcinoma (3) Increasing amount of AFB1-DNA adducts in liver tissues (4) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (5) Increasing the frequency of TP53M (6) Increasing MVD	[52–56]
XRCC3	DSBR	Thr to Met at codon 241 rs1799796 A > G	(1) Increasing individuals' susceptibility to hepatocarcinoma (2) Increasing amount of AFB1-DNA adducts in liver tissues (3) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (4) Increasing the frequency of TP53M (5) Associating with hepatocarcinoma clinicopathological features (6) Increasing MVD	[57–59]
XRCC4	DSBR	rs28383151 G > A Ala to Ser at codon 247	(1) Increasing individuals' susceptibility to hepatocarcinoma (2) Increasing amount of AFB1-DNA adducts in liver tissues (3) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (4) Increasing the frequency of TP53M (5) Associating with hepatocarcinoma clinicopathological features (6) Increasing MVD	[20, 21, 60–63]
XRCC5	DSBR	rs16855458 C > A rs9288516 T > A XRCC5 expression	(1) Increasing individuals' susceptibility to HBV infection (2) Increasing individuals' susceptibility to hepatocarcinoma (3) Associating with biological actions of hepatocarcinoma cells, such as increasing XRCC5 expression inhibiting cancer cells proliferation (4) Functioning as a tumor suppressor by inducing S-phase arrest in a TP53-dependent pathway	[64–69]
XRCC6	DSBR	XRCC6 expression	(1) Increasing individuals' susceptibility to hepatocarcinoma (2) Decreasing Toll-like receptor 4 (TLR4) against hepatocarcinogenesis (3) Increasing DNA damage, and promoting programmed cell death in TLR4-deficient livers (4) Early diagnostic value for hepatocarcinoma	[70–73]

DRR pathway gene/ proteins	DRR pathway	Abnormal of DRR	Effects on hepatocarcinoma	Ref#
XRCC7	DSBR	rs7003908 T > G	(1) Increasing individuals' susceptibility to AFB1 exposure (2) Increasing individuals' susceptibility to hepatocarcinoma (3) Increasing amount of AFB1-DNA adducts in liver tissues (4) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (5) Increasing the frequency of TP53M (6) Interacting with AFB1 exposure during hepatocarcinogenesis (7) Increasing MVD	[21, 74, 75]
DNA-PKcs	DSBR	Amount in liver tissues	Implying hepatocarcinoma-specificity	[76]
TP53	DRR pathway	Genic mutations such as TP53M, Arg to His at codon 273, Arg to His at codon 175, Cys to Tyr at codon 135, and Arg to Trp at codon 248	1. Implying individuals' AFB1 exposure 2. Associating with hepatocarcinoma risk 3. Increasing individuals' susceptibility to hepatocarcinoma 4. Decreasing DRR potential and increasing DNA damage	[40, 45, 77, 78]
XPC	NER	XPC expression Lys to Gln at codon 939	(1) Increasing individuals' susceptibility to hepatocarcinoma (2) Increasing amount of AFB1-DNA adducts in liver tissues (3) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (4) Increasing the frequency of TP53M and decreasing DRR potential (5) Associating with hepatocarcinoma clinicopathological features (6) Increasing XPC expression (7) Increasing MVD	[21, 79–81]
XPD	NER	Lys to Gln at codon 751	(1) Increasing individuals' susceptibility to hepatocarcinoma (2) Increasing amount of AFB1-DNA adducts in liver tissues (3) Increasing amount of adducts (including AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs (4) Increasing the frequency of TP53M and decreasing DRR potential (5) Interacting with gender during hepatocarcinoma (6) Increasing MVD	[21, 82]
Rad50	NER	Rad50 hook domain	Strongly influencing Mre11 complex-dependent DRR signaling, tissue homeostasis, and tumorigenesis	[83]
Nbs1	NER	Rs1805794 C > G Mutations in Nbs1	(1) Increasing hepatocarcinoma risk (2) Associating with TP53 inactivation	[84–87]
PARP-1	BER	DRR potential	(1) Modifying biological actions of hepatocarcinoma cells (2) A novel promising diagnostic marker for hepatocarcinoma	[88–90]

DRR pathway gene/ proteins	DRR pathway	Abnormal of DRR	Effects on hepatocarcinoma	Ref#
Rad10	NER	rs11615 C > T ERCC1–4533 G > A ERCC1–8092 C > A	(1) Increasing hepatocarcinoma risk (2) The amount of ERCC1 expression in tissues with hepatocarcinoma decreases cancer cells' sensitivity on anti-cancer drugs (3) Predicting the outcome of hepatocarcinoma patients receiving TACE treatment	[91–93]
ATM	HR and ENEJ	Ser to Ala at codon 1981 Ser to Ala at codon 1893 Ser to Ala at codon 367 Ser to Ala at codon 2996 Autophosphorylation at codon 1981 Ser	(1) The functional deficiency in radioresistant DNA synthesis and substrate phosphorylation such as TP53, Chk2, Nbs1, and SMCI (2) Increasing cells' sensitivity to risk factors and risk factors-induced DNA damage such as adduct formation and chromosome aberrations (3)The functional dysregulation for G2/M checkpoint (4) Extending activations of DNA damage signaling pathways to reach S phase arrest in hepatocarcinoma cells (5) Leading to ATM unable to be released from other ATM molecules, and increasing gene mutation risk	[94–100]

Abbreviations: *hOGG1, human oxoguanine glycosylase 1; XRCC1, X-ray repair cross complementing 1; BER, base excision repair; SSBR, single-strand break repair; HBV, hepatitis B virus; XRCC3, X-ray repair cross complementing 3; AFB1, aflatoxin B1; DSBR, double-strand break repair; WBC, white blood cell; TP53M, hot-spot mutation at codon 249 of TP53 gene; DNA-PKcs, DNA-activated protein kinase catalytic subunit; XRCC4, X-ray repair cross complementing 4; XRCC5, X-ray repair cross complementing 5; XRCC6, X-ray repair cross complementing 6; XRCC7, X-ray repair cross complementing 7; XPC, xeroderma pigmentosum, complementation group C; XPD, xeroderma pigmentosum, complementation group D; NER, nucleotide excision repair; PARP-1, poly(ADP-ribose) polymerase 1; ATM, Ataxia telangiectasia mutated kinase.*

Table 4.
The association between abnormal DRR potential and hepatocarcinogenesis.

as to induce chromosomal aberrations, abnormal cell cycle, and/or uncontrolled cell proliferation [50]. During DRR pathways, DNA repair genes play a central role [4]. Dysregulation of DRR caused by DNA repair genic mutations or low DNA repair capacity will increase hepatocarcinoma risk. **Table 4** summarized the effects of abnormal DRR in hepatocarcinogenesis. This evidence shows that dysregulation of DRR resulting from mutations in DNA repair genes and corresponding dysfunctions may promote hepatocarcinogenesis through the following pathways: (1) increasing individuals' susceptibility to risk factors such as hepatitis virus infection and AFB1 exposure [40, 60, 101]; (2) increasing individuals' susceptibility to cancer [45]; (3) increasing amount of carcinogens-DNA adducts in liver tissues [40]; (4) increasing amount of adducts (such as AFB1-DNA and AFB1-albumin adducts) in the peripheral WBCs and affecting immune reaction [61]; (5) increasing the frequency of tumor suppressor genes or oncogenes like Ras and TP53M [40, 47, 52, 61, 79]; and (6) interacting with risk factors during hepatocarcinogenesis [23]. Thus, the potential of DRR pathways should play an important function for hepatocarcinogenesis.

4. Hepatocarcinoma angiogenesis induced by DRR

Risk factors induced DNA damages and dysregulated DRRs are regarded as molecular events [4]. In human, risk factors for hepatocarcinoma can manifest acute and chronic DNA damage. Acute and noticeable DNA damages often lead to severe chromosome aberration and even cell death, whereas chronic DNA damages are the earliest molecular change in hepatocytes and ultimately result in hepatocarcinoma [40]. In the past decades, angiogenesis induced by dysregulation of DRR pathways may act as a vital role in the process of hepatocarcinoma. Evidence from epidemiological and clinicopathological studies has shown that higher potential of angiogenesis is in the liver of patients with chronic DNA damage and low DRR capacity [40, 102–105]. For example, Pastukh et al. [102] investigated the association between recruitment of DNA repair enzymes involving in BER pathway and VEGF expression via a chromatin immunoprecipitation technique. They found that hypoxia-induced reactive oxygen species (ROS) stress caused promoter base modifications targeted to hypoxic response elements (HREs) and increased VEGF expression. During this modification, 8-oxoguanine (8-oxodG, an oxidative DNA damage product) in VEGF promoter was temporally correlated with binding of human 8-oxodG glycosylase 1 (hOGG1, a BER repair enzyme), HIF-1α, redox effector factor-1, endonuclease one, and breaks in DNA strands. If 8-oxodG was decreased in the promoter region of VEGF, VEGF expression would downregulate [102]. Recent molecular epidemiological studies have further proved that genetic variants in hOGG1 genes increase hepatocarcinoma risk and modify the prognosis of this malignancy [103–105]. Collectively, these data suggest that increasing ROS like 8-oxodG resulting from low DRR capacity may promote angiogenesis.

Studies from high HBV and HCV infection and high AFB1 exposure area also display that the degrees of DNA damages are positively associated with MVD in tumor tissues from hepatocarcinoma [20, 55, 75, 79, 82]. For example, Lu et al. [20] investigated the effects of XRCC4 expression in tumor tissues on clinicopathological features and prognosis of hepatocarcinoma and found that decreasing XRCC4 expression was related to low DRR capacity, causing the formation of DNA adducts and TP53M. The dysregulation of XRCC4 may promote tumor proliferation and increase MVD. Several other studies further show that the low DRR capacity resulting from significant mutations in coding region of DNA repair genes (such as XRCC4, XRCC1, XPC, XPD, and XRCC7) increases MVD (**Table 4**) [21, 40, 52, 55, 59, 61, 62, 79, 80, 82]. Results from Lu et al. [20] and our studies [61, 62] showed that genetic alterations in the coding regions of XRCC4 gene (including Ala to Ser at codon 247 and Thr to Ala at codon 56) can decrease levels of XRCC4 protein expression and cause increasing amount of AFB1-DNA adducts and mutative frequency of TP53 gene in tissues with hepatocarcinoma. They also found that the amount of AFB1-induced DNA adducts, including 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB1 (AFB1-N^7-Gua) and formamidopyridine AFB1 adduct (AFB1-FAPy), was positively associated with the number of microvessels (a biomarker for angiogenesis). Results from our studies [79, 106, 107] furthermore displayed that three low DNA repair markers related to AFB1, including tumor risk, TP53M frequency, and AFB1-FAPy adduct amount, were significantly correlated with the number of microvessels in liver tissues. These individuals with high AFB1-FAPy adduct level in liver tissues had an increasing risk of high MVD than those low adduct level (OR = 1.68, 95% CI = 1.45–2.87) [106]. Liu et al. [108] and Wang et al. [109] further proved that the upregulation of microRNA-429 and microRNA-24 expression in tissues with hepatocarcinoma not only increased the amount of AFB1-DNA adducts

and the number of microvessels but also grew tumor metastasis risk via vessels and shorted patients' survival. Recent evidence has shown that microRNA-24/microRNA-429 can modify the capacity of DDR via controlling Nbs1 (a regulator of DRR) [110, 111] and angiogenesis via regulating the crosstalk between the pro-contractile transforming growth factor-β/bone morphogenetic protein (TGF-β/BMP) signal (inducing a quiescent 'contractile' phenotype) and the pro-synthetic platelet-derived growth factor (PDGF) signal (causing a proliferative 'synthetic' phenotype) [112, 113]. This suggests that microRNA-24/microRNA-429 may play an important regulative role between DRR capacity and angiogenesis. Taken together, this evidence proves that low DRR-induced MVD augmentation is regulated by the amount of DNA damage.

Evidence from in vitro and in vivo studies further shows that dysregulation of DRRs and signaling to cell cycle checkpoints (CCCs) may modify hepatocarcinoma angiogenesis. CCCs involving in DRRs mainly encompass G1/S and G2/M checkpoint [114]. During G1/S checkpoint, both ATR and ATM act as central activators for DRR via inducing the phosphorylation of p53 protein which can activate p21 (a Cdk inhibitor). ATM/TP53/P21 pathway also plays an important function controlling G2/M procession [114]. The dysregulation of these factors and signal pathways can change the status of angiogenesis [115–119]. For example, Qin et al. [115] found that E2F1, an important cell cycle regulator, can modify angiogenesis via controlling VEGF expression by p53-dependent way. In this control model, deficient phenotype of E2F1 will result in VEGF overexpression, while its positive phenotype decreases VEGF expression [115]. Factors controlling cell shape and cytosol can regulate the cycle of vessel endothelial cells and angiogenesis [116, 117]. In mice model with the deficiency of BCL-2 (an important regulatory factor in DDRs), cells featured increasing DNA damage [118]; the inhibition of BCL-2 will result in the arrest of cells in S phrase and suppression of tumor angiogenesis [119]. In an integrated genomic study (including 5 hepatocarcinoma patients with hepatitis D visus [HDV] and 7 HDV-positive cirrhosis cases), Diaz et al. [120] investigated the association between HDV-related hepatocarcinoma and potential signal pathways involved in DNA damage and repair and cell cycle and found significant interactions of DDR/cell cycle-related genes, such as BRCA1, BARD1, CDK1, CDKN2C, CCNA2, CCNB1, CCNE2, GSK3B, H2AFX, MSH2, NPM1, PRKDC, and TOP2A. Results from the t-SNP (*t*-distributed stochastic neighbor embedding analyses) further exhibited that HUS1, BRCA1, BARD1, GADD45, DNA-damage-induced 14-3-3σ, and MSH2 gene involving in DRRs valuably scored with regulatory genes (such as ATM, TP53, NO, and epidermal growth factor), which involve in G2/M checkpoint and angiogenesis [120]. The dysregulation of HUS1 and corresponding genotoxin-activated checkpoint complex (also termed as Rad9-Rad1-Hus1complex) will cause abnormal DRR capacity and cell cycle in response to DNA damage and promote the alteration of hematogenous metastatic phenotype for hepatocarcinoma [121, 122]. The genetic alterations and abnormal expression of BRCA1 and GADD45 (two important regulatory factors in DRR and apoptosis pathways) in hepatocytes can also change TP53-dependent CCCs and VEGF expression [123, 124]. Altogether, these studies have proved that the dysregulation of DDRs can cause the abnormal regulation of CCCs and change the status of hepatocarcinoma angiogenesis.

Detailed molecular mechanisms of DRR dysregulation promoting hepatocarcinoma angiogenesis have still not been fully understood. Several possible pathways may play some important roles. First, DNA damage agents induce NO synthase and increase the expression of VEGF and HGF [125, 126]. Second, DNA damage agents like AFB1 cause the mutations of such genes as TP53, ras, and DNA repair genes. Activation of oncogenes and inactivation of tumor suppression genes and

DNA repair genes lead to uncontrolled expression of genes involving in angiogenesis such as VEGF and Ang-1/2 [5, 6]. Third, genetic alterations in DRR pathways may alter the microenvironment of tumor and promote angiogenesis [127–129]. Fourth, the abnormal DRRs may accelerate the accumulation of DNA damages and trigger the dysregulation of angiogenesis-related genes and the progression of hepatocarcinoma. Finally, some metabolic products (such as AFBO) or nucleotide sequences (HBx) of DNA agents can bind to genomic DNA of hepatocytes and may increase the activation of VEGF HREs [22, 40, 41, 45]. Taken together, under the conditions of low DRR capacity and/or chronic risk factors, DNA damages will accumulate in hepatocytes and ultimately induce hepatocarcinogenesis and tumor angiogenesis.

5. Summary and further direction

Abnormal angiogenesis and DNA damages/DRRs are two important pathophysiological events in the process of hepatocarcinogenesis. Recently, it has become a growing evidence of DNA damage and repair and angiogenesis in hepatocarcinogenesis. Low DRR capacity resulting genetic or obtained alterations may lead to the accumulation of DNA damages and induce angiogenesis and ultimately promote hepatocarcinoma development. The main challenge for this field is the explanations of molecular basis and regulative signal pathways of DNA damages/DRRs interacting with angiogenesis during hepatocarcinogenesis. A better understanding of hypervascular feature and corresponding mechanisms of hepatocarcinoma on the basis of DNA damage/DRR pathway may be helpful for the medical researchers and clinic doctors exploring and validating hepatocarcinogenesis but also for them designing safe and efficient antiangiogenic drugs.

Acknowledgements

We thank Dr. Yuan-Feng Zhou for literature collection.

Conflicts of interest and source of funding

The authors declare no competing financial interests. This study was supported in part by the National Natural Science Foundation of China (Nos. 81860489, 81760502, 81572353, 81372639, 81472243, 81660495, and 81460423), the Innovation Program of Guangxi Municipal Education Department (Nos. 201204LX674 and 201204LX324), Innovation Program of Guangxi Health Department (No. Z2013781), the Natural Science Foundation of Guangxi (Nos. 2017GXNSFAA198002, 2017GXNSFGA198002, 2016GXNSFDA380003, 2015GXNSFAA139223, 2013GXNSFAA019251, 2014GXNSFDA118021, and 2014GXNSFAA118144), Research Program of Guangxi "Zhouyue Scholar" (No. 2017-38), Research Program of Guangxi Specially-invited Expert (No. 2017-6th), the "12th Five" Planning Program of Guangxi Education Science (No. 2015C397), the Innovative Program of Guangxi Graduate Education (No. JGY2015139), Research Program of Guangxi Clinic Research Center of Hepatobiliary Diseases (No.AD17129025), and Open Research Program from Molecular Immunity Study Room Involving in Acute & Severe Diseases in Guangxi Colleges and Universities (Nos. kfkt20160062 and kfkt20160063).

Abbreviations

AFB1	aflatoxin B1
Ang-2	angiopoietin-2
ATM	ataxia telangiectasia mutated kinase
BER	base excision repair
bFGF	basic fibroblast growth factor
DNA-PKcs	DNA-activated protein kinase catalytic subunit
DSBR	double-strand break repair
DRR	DNA damage repair response
G-CSF	granulocyte colony-stimulating factor
HBV	hepatitis B virus
HCV	hepatitis C virus
MVD	microvessel density
NER	nucleotide excision repair
HGF	hepatocyte growth factor
hOGG1	human oxoguanine glycosylase 1
IL-8	interleukin-8
PARP-1	poly(ADP-ribose) polymerase 1
SSBR	single-strand break repair
TEMs	TIE2-expressing monocytes/macrophages
TP53M	hot-spot mutation at codon 249 of TP53 gene
VEGF	vascular endothelial growth factor
XRCC1	X-ray repair cross complementing 1
XRCC3	X-ray repair cross complementing 3
XRCC4	X-ray repair cross complementing 4
XRCC5	X-ray repair cross complementing 5
XRCC6	X-ray repair cross complementing 6
XRCC7	X-ray repair cross complementing 7
XPC	xeroderma pigmentosum, complementation group C
XPD	xeroderma pigmentosum, complementation group D

Author details

Xi-Dai Long[1,2,3*†], Zhou-Ximao Long[4†], Xiao-Ying Huang[2], Jin-Guang Yao[2], Qiang Xia[1] and Yan Deng[5]

1 Department of Liver Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

2 Department of Pathology, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China

3 Guangxi Clinic Research Center of Hepatobiliary Diseases, Baise, China

4 Zhoupu Middle School, East China Normal University, Shanghai, China

5 Department of Epidemiology, Youjiang Medical University for Nationalities, Baise, China

*Address all correspondence to: sjtulongxd@263.net

† These authors contributed equally to this work

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