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Features of microvessel density (MVD) and angiogenesis inhibitors in therapeutic approach of hepatocellular carcinoma (HCC)

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Abstract. – OBJECTIVE: The curative hepatocellular carcinoma (HCC) therapy was traditionally based on surgical or loco-regional ablation approach. However, HCC is a solid tumor characterized by a highest level of vascularization; therefore, angiogenesis inhibitor could play a pivotal role in the pharmacological therapeutic approach. Despite the low number of approved drugs, a wide range of multi-kinase and MET inhibitor is currently being evaluated in phase II and III study. In this review, we described all the drugs that have shown efficacy in recently and ongoing trials.

Moreover, the immunotherapy represents a recent challenge in the HCC treatment. The strategy based on the production of multi-epitope, multi-HLA peptide vaccine naturally processed and presented on primary tumor tissues of HCC patients. A further upgrade of cancer vaccine could be represented by the combination of metronomic chemotherapy and checkpoint inhibitors.

Key Words:
HCC, Angiogenesis inhibitor, Tumor microvessel density, Sorafenib, Lenvatinib, Regorafenib, Cancer vaccine.

Introduction

Primary liver cancer (PLC) is one of the most common cancers worldwide. Hepatocellular carcinoma (HCC) is the main histological type, accounting for 90% of the cases of PLC and is the 3rd leading cause of cancer-related deaths all over the world[1-3]. Cirrhosis of viral etiology is the major HCC risk factor especially in United States and Europe[4]. An increasing association was reported between the non-alcoholic fatty liver disease (NAFLD) and HCC[5,6]. As shown in several studies, the HCC NAFLD related is characterized by appearance in elderly patients, larger tumor, higher risks of tumor recurrence, and shorter survival time[7,8].

The ultrasound represents a low cost and reliable method of screening for HCC and enables the surveillance both in the chronic hepatitis patients and the healthy population[9]. Contrast enhancement computed tomography (CT) and magnetic resonance imaging (MRI) confirm the diagnosis in typical pattern[9,10]. The histological sample allows to characterize the genetic profile of HCC and could provide the valuable information for therapeutic choices[9,10].

Surgical resection or liver transplantation in selected cases could be the therapeutic curative choice. Likewise, based on HCC stage, loco regional approach as mean radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) or trans-arterial chemoembolization (TACE), represents further therapeutic option[11,12].

So far, high efficacy drug therapies have not been available. However, the identification of

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cellular pathways has led to the development of targeted drugs evaluating the mutational profile in HCC[13].

Vascular Features and Microvessel Density

HCC represents one of the solid tumors with the highest levels of vascularization and therefore angiogenesis has an important role in its development, progression and metastatic disease[14].

The main vascular change in HCC is represented by capillarization and arterialization of hepatic sinusoids[15].

These vascular spaces in normal condition have discontinuous endothelium (fenestrated capillary) in order to promote nutrient exchanges. In conditions of chronic hepatic injury sinusoids acquire a muscle-endothelial wall, as well as real capillaries and arterioles. These vascular changes can already be seen in precancerous lesions, such as dysplastic nodules and cirrhosis[16].

In literature there are many reports that demonstrate the evaluation and quantification of neovascularization and tumor microvessel density (MVD) by immunohistochemistry using endothelial markers as CD34, CD31, and von Willenbrand Factor (vWF) (Figure 1)[17,18].

However, there is no standardized and accepted MVD grading system in the literature and the first report of MVD assessment was done by Weidner et al[19] in invasive breast cancer. The system of Weidner et al[19] was subsequently used to evaluate MVD in several solid tumors and it is applicable on HCC too[20,21].

The Weidner system consists, after immunohistochemistry was performed, to identify areas of most intense neovascularization (hotspots areas) at high power (40x). After this, individual microvessels are counted in this hotspot area under medium power (20x) and the average vessel count in 5 hotspots is taken as the MVD.

We can distinct 2 types of microvessels in HCC: capillary-like, with small capillaries with or without narrow lumen, and sinusoid-like with wide lumen[22]. In a series of 98 patients with histo-pathologically confirmed HCC, 45 patients (45.9%) have capillary-like microvessel, 21 patients (21.4%) have sinusoid-like microvessel, and 32 patients (32.7%) have both types of microvessels. In the latter category, the pattern was determined by dominant microvessel type[23]. Microvessels have been described to be heterogeneously represented in the HCC and the highest density is often observed at the growing edge[24].

Despite the hypervascular characteristic of most HCC, the prognostic significance of MVD still remains controversial.

These discrepancies are conditioned by methodological procedures, such as the use of different endothelial markers, tissue sampling sites, evaluation method of MVD or the characteristics of the patients[25].

Murakami et al[26] show the correlation between MVD and histological grade, tumor size, DFS, and OS. In this work, the authors highlight an unfavorable correlation to histologic grade and the tumor size; therefore, lower MVD is associated with poorly differentiated and large tumors. Moreover, in their study a decreased MDV represents an important poor prognostic factor for Disease Free Survival (DFS) and Overall Survival (OS).

The predictive role of MVD in HCC and in solid tumors in general still remains unclear. However, development of MVD is based on the dysregulation of several angiogenic pathways, suggesting that they could be involved in the pathogenesis of HCC[27].

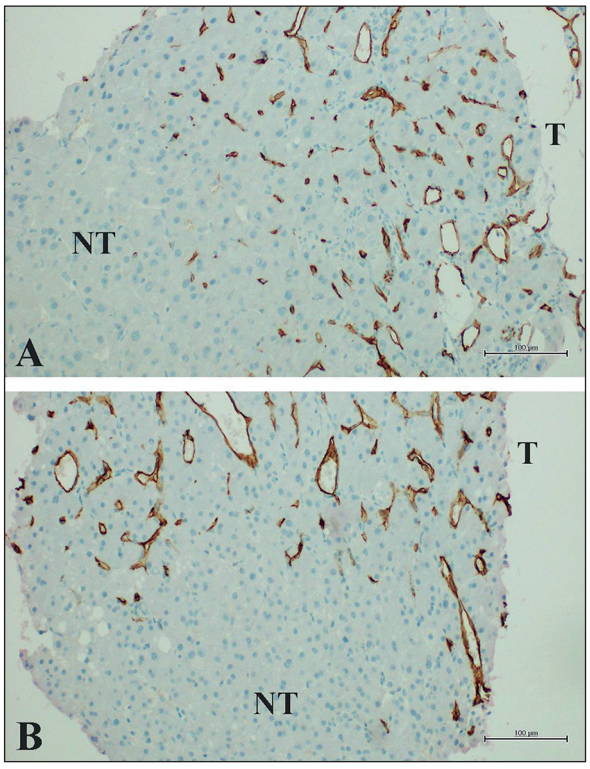

Figure 1. Assessment of microvessel density (MVD) in Hepatocellular Carcinoma (HCC) by CD34 **(A)** and CD31; 10× magnification **(B)** immunostaining. High levels of MVD were detected in tumor tissue (T) than the non-tumor tissue (NT); 10× magnification.

Indeed, most currently approved therapies for advanced HCC, in 1st and 2nd line settings, target angiogenic pathways[11].

The primary focus in antiangiogenic target therapy in HCC is represented by the Vascular Endothelial Growth Factor (VEGF) -VEGFRs pathway[28].

In HCC the serum VEGF levels are high, and they are correlated to tumor angiogenesis and progression[29]. In this scenario VEGF-A has been considered as a potential prognostic and predictive biomarker for VEGF-targeted therapy with Bevacizumab[30].

In SHARP study, plasma concentration of VEGF and Angiopoietin 2 (Ang2) are identified as an independently prognostic factor for survival in patients with advanced HCC, although neither predicted effectiveness or benefit[31].

Horwitz et al[32] highlighted that amplification of VEGF-A in human HCC could predict OS in patients treated with sorafenib and that amplification increases tumor sensitivity to sorafenib.

In a recent phase 3 trial (REACH 2), the levels of alpha-fetoprotein (AFP) were evaluated as a potential biomarker of selection for ramucirumab therapy, based on the hypothesis that inhibition of VEGFR-2 by this monoclonal antibody is more effective in this subtype of patients[33,34].

Another potential biomarker related with angiogenesis is represented by Platelet-derived growth factor (PDGF) and its receptor[35]. In HCC, the overexpression of PDGFR-α is related to MVD and worse prognosis, and its pathway may be inhibited by sorafenib and other tyrosine kinase inhibitors. Nevertheless, the clinical importance of targeting PDGF pathway for inhibition of angiogenesis in HCC still remains to be clarified[36].

Scholars[37,38] have demonstrated a synergism between Fibroblast Growth Factor (FGF) and VEGF pathways in stimulating angiogenesis and tumor growth and this may contribute to the drug resistance of HCC to the sorafenib.

Other biomarkers that have been observed with pro-angiogenic effects in HCC are Angiopoietin 1 (Ang1) and 2 (Ang2) and their receptor Tie 2[39] and Endoglin (CD105)[40], however their clinical relevance in antiangiogenetic therapies has yet to be proven.

Antiangiogenesis Systemic Chemotherapy: What We Learned

Considering that HCC is a highly vascularized cancer, the use of antiangiogenic drugs seemed logical in all patients. However, the HCC patients are fragile and the overall benefits of chemotherapy results from a fine balance between efficacy and safety.

Until 2007, there was no efficient systemic treatment of HCC, an unparalleled situation in oncology, when sorafenib, a multityrosine kinase inhibitor targeting particularly VEGFR, PDGFR, and RAF family kinases, significantly improved overall survival *vs.* placebo in advanced HCC[41,42].

Since its approval, in 2007, sorafenib is considered as first-line treatment agents in advanced HCC. Nonetheless, the median survival time in patients treated with sorafenib is less than 12 months and less than 5% of tumors respond.

From that, a lot of efforts were dedicated to drugs with less anti-tumoral activity or high toxicity, therefore a strong need of newer and more effective drugs for advanced HCC is needed (Table I).

Table I. Phase III trials with results in advanced HCC.

SHARP	Sorafenib	10.7	0.69	< 0.001
	Placebo	7.9		
Asian-Pacific	Sorafenib	6.5	0.68	0.01
	Placebo	4.2		
REFLECT	Lenvatinib	13.6	0.92	< 0.05
	Placebo	12.3		
RESORCE	Regorafenib	10.6	0.63	< 0.001
	Placebo	7.8		
CELESTIAL	Cabozantinib	10.2	0.76	< 0.05
	Placebo	8.0		
REACH	Ramucirumab	9.2	0.86	0.13
	Placebo	7.6		
REACH2	Ramucirumab	8.5	0.71	0.019
	Placebo	7.3		
METIV	Tinvatinib	8.4	0.76	0.0049
	Placebo	9.1		
CHECK-MATE459	Nivolumab	8.5	0.71	0.019
	Sorafenib	7.3		

Other antiangiogenic drugs were tested in Phase III clinical trials, either alone or in combination *vs.* sorafenib, but until now, none of the agents examined offer increased survival benefit over sorafenib or showed non-inferiority, compared to lenvatinib[43].

Also, lenvatinib is a multiple kinase inhibitor, targeting the VEGFR1-3, FGFR1-4, PDGFR alpha, c-Kit, and the RET proto-oncogene.

To compare lenvatinib *vs.* sorafenib as 1st line treatment, unresectable HCC patients that did not receive any systemic therapy, were enrolled in the REFLECT study. The primary endpoint of the study was the OS and lenvatinib showed non-inferiority to sorafenib (median OS was 13.6 *vs.* 12.3 months). Although both drugs showed comparable results, lenvatinib does still not replace sorafenib for practical reasons: some oncologists used to manage the adverse events of sorafenib, others for the real cost-effectiveness. However, the efficacy and safety of lenvatinib was not assessed in patients with major portal vein thrombosis, bile duct infiltration, and when the disease involves more than 50% of the liver. In addition, new agents have been identified to offer benefits if the disease progressed after sorafenib, whereas no agent has been yet identified in case of failure of lenvatinib.

The approved second-line treatments for advanced HCC patients progressed during sorafenib treatment is regorafenib.

Regorafenib is a multikinase inhibitor targeting VEGFR2, TIE2, c-KIT, B-Raf, PDGFR, and FGFR. In preclinical studies, regorafenib showed higher antitumoral activity compared to sorafenib, thereby it was approved for refractory metastatic colorectal cancer and gastrointestinal stromal tumors treatment.

The phase I/II studies showed a good tolerability together with antineoplastic activity in advanced HCC, moving forward a randomized phase III trial (RESORCE) in HCC patients whose disease progressed during sorafenib treatment. Regorafenib showed a median OS of 10.6 months *vs.* 7.8 months with placebo, with a significant improvement of the secondary endpoints (PFS, TTP, ORR, and DCR)[44]. Thus, regorafenib is the only systemic second-line treatment with an overall survival benefit in HCC patients progressed during sorafenib therapy. Although the sequential treatment (sorafenib and regorafenib) improves the outcomes of HCC patients, many patients leave the sequential therapy, due to deterioration of hepatic function. Thus, it is crucial to stratify which subjects can be treated with regorafenib.

The deterioration of hepatic function is therefore a good prognostic marker to follow during the systemic therapy, and the albumin-bilirubin score is an adequate biochemical marker.

Yukimoto et al[45] followed 267 HCC patients progressed with sorafenib to identify the candidates that could obtain a benefit with regorafenib. They chose the albumin-bilirubin score (ALBI) as primary endpoint. Using univariate analysis, they demonstrated that the ALBI score <-2.53 was the only significant predictor to identify regorafenib-responder HCC patients. According to these results, the median OS was 15.6 months in patients that were treated with regorafenib *vs.* 6.8 months in the regorafenib non-candidate group ($p < 0.01$).

More recently, a good efficacy in the OS was obtained with cabozantinib, a multi-kinase inhibitor, such as VEGFRs 1, 2, and 3, MET, and AXL (NCT01908426).

Patients who received previous treatment with sorafenib, had disease progression after at least 1 systemic treatment, and may have received up to 2 previous systemic treatments for advanced HCC were enrolled in cabozantinib trial[46]. The study showed significantly longer overall survival (OS: 10.2 months *vs.* 8.0 months) with cabozantinib *vs.* placebo and the progression free was more than twice (PFS: 5.2 months with cabozantinib and 1.9 months with placebo) with only grade 3 or 4 adverse events. The most frequent high-grade events were palmar-plantar erythrodysesthesia (17% with cabozantinib *vs.* 0% with placebo), hypertension (16% *vs.* 2%), increased AST level (12% *vs.* 7%), fatigue (10% *vs.* 4%), and diarrhea (10% *vs.* 2%)[47].

Ramucirumab, a human IgG1 monoclonal antibody that inhibits ligand activation of VEGFR2, also showed initial anti-tumor activity. The REACH study was set up to test the efficacy and safety of ramucirumab monotherapy in 565 patients with advanced HCC who progressed after sorafenib[33]. ramucirumab did not significantly improve the overall survival compared to the placebo. However, the only clinically meaningful and significant improvement in survival was noted in those patients with baseline AFP concentrations of 400 ng/mL or greater. Starting from this point, the REACH2 trial investigated the efficacy and safety of ramucirumab monotherapy in HCC patients with baseline AFP concentration of 400 ng/mL or greater after intolerance to, or progression, during sorafenib treatment. Recently published

data showed that 292 subjects were randomly assigned to the ramucirumab group and to the placebo group. The REACH-2 study met its primary endpoint, showing improved OS for ramucirumab in patients with HCC and AFP concentrations of at least 400 ng/mL, after sorafenib progression. Ramucirumab was well tolerated, with an acceptable safety profile, although the reported grade 3 or worse adverse events were reported in 35% of patients treated with ramucirumab.

To date, REACH-2 is the only phase III trial done in a biomarker-selected patient population with HCC[34].

Another promising drug seemed Tivantinib (ARQ 197), a selective, oral MET inhibitor. The encouraging results obtained in the phase II study were not confirmed in the phase III study (NCT01755767), where 340 subjects were randomly assigned to receive Tivantinib (n=226) or placebo (n=114)[48]. Tivantinib did not improve the overall survival compared with placebo group in HCC patients with high MET expression previously treated with sorafenib and therefore whether MET inhibition could be a potential therapy for subset of HCC patients with advanced disease is still an issue[49,50].

Novel Systemic Antiblastic Chemotherapy to Treat HCC: Ongoing Trials

Although no other antiangiogenetic drugs had produced any positive results in terms of efficacy up to now, different clinical trials are still ongoing, testing also molecules with different targets.

Donafenib

Donafenib is the deuterium derivative of sorafenib, an orally available drug.

Upon oral administration, donafenib (also known as CM4307) binds and blocks the activity of Raf kinase and inhibits Raf-mediated signal transduction pathways. This inhibits cell proliferation in Raf-expressing tumor cells. In addition, this agent may inhibit unidentified RTKs, and thus may further block tumor cell proliferation in susceptible tumor cells.

The ongoing phase III trial (NCT02645981) is evaluating the efficacy of donafenib, in a randomized, controlled, multicentre study. Donafenib is administered at the dose of 200 mg BID, compared to the controlled drug sorafenib, at 400 mg BID. The primary endpoint is OS. The first results will be available at the end of 2019.

Icaritin

Icaritin is a newly discovered small active prenyl-flavonoid derived from Epimedium genus, which is high selective ERalpha modulators. Preclinical studies conducted on prostate cancer PC-3 cells[51] and breast cancer cell growth through activation of ERK signaling[52] showed the antitumor activity of icaritin. When used with HCC cancer cells, it can inhibit the growth having pro-apoptotic function with HepG2, KYN-2, and Huh-7 lines and primary human HCC cells, inhibiting sphingosine kinase 1 (SphK1) activity, leading to pro-apoptotic ceramide production and JNK1 activation[53].

The phase III trial (NCT03236636) enrolled 28 HCC patients of which 12 received 600 mg of icaritin once, twice per day, after meal 30 minutes, 6 received 800 mg of icaritin once, twice per day, after meal 30 minutes. Preliminary results showed that in the 600 mg arm it was observed one case of partial response (10%), 5 cases of stable disease (50%), and 4 cases of Progressive Disease (40%) with no grade 3 or above adverse effects. No published data are available yet, but although the small number of treated patients, Icaritin has been approved in few registered trials, due to the disease stabilization and less unfavorable adverse effects.

Varlitinib

Varlitinib (ASLAN001) is a small molecule tyrosine kinase inhibitor against HER1 (EGFR), HER2, and HER4. EGFR overexpression in HCC and matched non-tumor tissues were found in (32.5%) and (28.6%), respectively. Moreover, missense and silent mutations were detected in (39.4%) and (33.3%) of HCC tissues, respectively. *In vivo* studies on HER2/3 expression in patient-derived xenograft (PDX)-HCC humanized mice models suggested the inhibition of pERB B2/3, pERK1, and pERK2 with treatment with ASLAN001 (varlitinib) and vessel normalization[54].

After the recommended dose has been determined, the phase Ib portion of the trial will evaluate the efficacy of ASLAN001 in HCC patients who have progressed on 1st line sorafenib or lenvatinib. This is a single-arm, open label study (NCT03499626) with the primary objective to evaluate the maximal tolerable dose (MTD) of ASLAN001 (varlitinib) in the study population. The results will be available on 2020.

Immune Checkpoints Inhibitors to Treat HCC Cancer

All approved systemic therapies remain unsatisfactory, with limited ORRs and poor OS. Immune checkpoint inhibitors (CPIs) offer a great promise in the treatment of solid cancers. The rationale of this strategy is based on the ability of the immune system to recognize tumoral cells as "foreign" in the body. This lets the immune system attack the foreign cells, leaving the normal cells alive. To do this, it uses "checkpoints" – molecules on certain immune cells that need to be activated (or inactivated) to start an immune response, also in liver[55].

New immune-modulatory agents are introduced for oncological therapy, targeting programmed death-1 (PD-1), programmed death-ligand 1 (PD-1), or cytotoxic T lymphocyte antigen-4 (CTLA-4). In 2015, Food and Drug Administration (FDA) and European Union approved drugs targeting PD-1 for their antitumor activity in several cancers, such as: pembrolizumab, nivolumab, and cemiplimab. The approved anti-CTLA-4 is Ipilimumab (Table II). PD-L1 activation together with DNA methyltransferases (DNMTs) overexpression provided therapy resistance. In sorafenib-resistant HCC cell lines, highly upregulated DNMT1 positively correlated with PD-L1 overexpression[56]. On the other hand, Chen et al[57] revealed that HCC PD-1-positive patients have poorer OS than PD-1-negative patients, therefore these patients might take advantage from combination treatment with anti-PD-1 antibody and sorafenib.

In 2017, the Checkmate 040 study of nivolumab was approved to assess its safety and clinical benefit in different HCC patients, including those progressed from HCV or HBV infection[58]. Treatment with nivolumab resulted in significant tumor reductions regardless if the patients received sorafenib and the disease progressed after this therapy or showed intolerance to this therapy, suggesting that nivolumab might offer the ultimate therapeutic option and has handy adverse effects[59,60].

Based on the obtained results in Checkmate 040 study, the FDA approved nivolumab for the treatment of patients with HCC that had failed treatment with sorafenib, opening the possibility to use nivolumab in the 1st line setting. This trial, Checkmate 459, is currently ongoing, using Response Evaluation Criteria in Solid Tumors (RECIST) as response criterion.

Studies with an anti-CTLA-4, tremelimumab or pembrolizumab, gave controversial results. Tremelimumab gave more adverse events than those caused by anti-PD-1 antibodies[61]. In 2016, in contrast to the previous study, Truong et al[62] reported a case of advanced HCC that responded dramatically to pembrolizumab after the progression during sorafenib. Different phase III trials were initiated, with pembrolizumab

Table II. A part of promising trials for advanced HCC.

Drugs	Typology	Targets	HCC therapeutic indication	Primary endpoint	Ongoing study
Donafenib	Small multikinase inhibitor	RAF VEGFR	Donafenib *vs.* sorafenib	Overall survival	NCT02645981
Icaritin	Small multikinase inhibitor	sphK1, JNK1	Inoperable HCC	Tumour stabilization rate	NCT03236636
Varlitinib	Small multikinase inhibitor	EGFRs	Varlinitib *vs*. sorafenib	Overall survival	NCT03499626
NIvolumab	Anti-PD-1	PD-1	Nivolumab *vs.* sorafenib	Overall survival	NCT02576509
Pembrolizumab	Anti-PD-1	PD-1	Pembrolizumab	Overall survival *vs.* placebo	NCT002702401
Pembrolizumab + Regorafenib	Anti-PD-1 + multikinase inhibitor	PD-1 VEGFR EGF		Overall survival	NCT03347292
Nivolumab + Lenvatinib	Anti-PD-1 + multikinase inhibitor	PD-1 VEGFR EGF		Overall survival	NCT03841201
Nivolumab + BMS-986205	Anti-PD-1 + IDO-1	PD-1 IDO-1 inhibitor		Overall survival	NCT03695250

in HCC subjects with different etiologies that progressed or not after other systemic therapies (KEYNOTE-224, one arm, phase III trial KEYNOTE-240), showing that the anti-CTLA-4 was effective and tolerable in patients with advanced HCC who had previously been treated with sorafenib[63].

The combination therapies are a new option, and several clinical trials are ongoing and will show if the combined attack to HCC cells is successful (Table II). A phase Ib study of regorafenib in combination with pembrolizumab is on the way and also the combination of nivolumab with lenvatinib for Advanced Stage HCC (IMMUNIB) started.

Novel molecules have been identified as immune system modulators; also, for these reasons the oncologist paid attention to see whether could be effective in inhibiting tumor growth. An example derives from the identification of an IDO1 inhibitor, the BMS-986205 drug, which inhibits indoleamine 2,3-dioxygenase 1 (IDO1), with potential immunomodulating and antineoplastic activities[64]. Upon administration, BMS-986205 specifically targets and binds to IDO1, a cytosolic enzyme responsible for the oxidation of the amino acid tryptophan into the immunosuppressive metabolite kynurenine. By decreasing kynurenine in tumor cells, BMS-986205 restores and promotes the proliferation and activation of various immune cells, including dendritic cells (DCs), natural killer (NK) cells, and T lymphocytes, and causes a reduction in tumor-associated regulatory T cells (Tregs). The activation of the immune system arrested the growth of IDO1-expressing tumor cells (NCT03695250).

Ongoing In Vitro Studies: Future Drugs Under Development

Drug resistance is a particular challenging issue in oncology; therefore, strong efforts have been dedicated to the identification of resistance mechanisms and new molecules that could revert the resistance.

The stress-inducible protein Sestrin2 (SESN2) was significantly expressed in both HCC cell lines and tumor tissues compared to normal human hepatocytes, respectively[65]. In addition, SESN2 expression was significantly upregulated after sorafenib treatment and the knockdown of SESN2 exacerbated sorafenib-induced cell arrest and apoptosis[66]. Further study uncovered that SESN2 deficiency impaired both AKT and AMPK phosphorylation and activation after sorafenib therapy. Taken together, this research demonstrates that both phosphor-AKT and phosphor-AMPK activation and SESN2 expression could participate to the mechanism of sorafenib-induced resistance in HCC[65].

The key metabolic sensor AMP-activated kinase (AMPK) was downregulated in HCC sorafenib-resistant cell. Interestingly, AMPK knockdown in cells increased the expression of stem cell markers that blocked sorafenib-induced cell death, suggesting that AMPK might be a novel target to tackle chemoresistance[67,68].

Future Challenges: Cancer Vaccine and HCC

Immunotherapy represents a recent challenge in the cancer treatment and gives raise expectations about future results. It is based on immune response toward the associated-tumor antigens[69-71]. Recently, several HCC associated antigens have been identified, but only a limited number was tested in human clinical trials. In fact, the true neo-antigens are only those showed no homology to self-wild type antigens, able to induce an antitumor T cell response not mitigated by central tolerance[72,73].

The first trial was based on HLA class-I restricted epitopes derived from AFP. This investigation showed an induction of an AFP specific T cell response[74].

Glypican 3 was used in two phases, phase I, and phase II clinical trial, by exploiting specific CD8 and telomerase peptide without significant response[72-75]. Moreover, a phase II clinical trial – GPC3 based as adjuvant after RFA or surgery HCC treatment, is ongoing[76].

Currently, the innovative "HEPAVAC" project has the purpose of identifying shared "off-the-shelf" HCC-specific antigens[70]. The strategy based on the production of multi-epitope, multi-HLA peptide vaccine naturally processed and presented on primary tumor tissues of HCC patients (HLA peptidome). Moreover, the protocol includes a personalized vaccine (APVAC) based on patient-specific HCC-specific neo-antigens[76].

A further implementing rule of cancer vaccine is represented by the combination of metronomic AC and CPI. In this case, cancer vaccine makes use of different biological effects, such as activated T cells and NK functions, or control of the activity of T-regulatory cells[77-80]. Greten et al[81] demonstrated the efficacy of the metronomic AC in decreasing the frequency and suppressor function of circulating CD4+CD25 Foxp3cw2fg4

+ regulatory T cells, without any significant hematologic side effects. HCC is notable for a strong immunosuppressive microenvironment for the presence of immunosuppressive cells, such as CD4+CD25+ regulatory T cells, increased in HCC patients[82]. Likewise, the combination between cancer vaccine and CPI could increase infiltration of effector CD8+ T cell as reported in PLC clinical trial[83,84]. So far, this synergic approach has been evaluated only on aggressive transgenic HCC mouse model[85] and showed a decrease of immunosuppressive cells in tumor microenvironment by activated T cells.

Conclusions

The angiogenesis is a dynamic essential process playing a critical role in tumor growth and metastatic diffusion. The development of new prognostic factors, tumor markers, imaging techniques, and therapeutic modalities is the consequence to understand the basic principles of the biology of angiogenesis. Prognostic angiogenesis biomarker (i.e., VEGF level) plays a key role in the circumstance of several cancer diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

All authors contributed equally to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and approval of the final version.

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