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WORD COUNT	7842	TIME SUBMITTED	19-MAY-2021 10:49AM
		PAPER ID	72303555

Name of Journal: *World Journal of Clinical Cases*
Manuscript NO: 66006
Manuscript Type: REVIEW

Encouraging specific biomarkers-based therapeutic strategies for hepatocellular carcinoma

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Abstract

The prevention, early diagnosis, effectively treatment, and poor prognosis of patients with hepatocellular carcinoma (HCC) remain a global medical challenge. Surgery operation is still mainly treated for HCC with auxiliary vascular embolization, radio frequency, radiotherapy, chemotherapy, and biological therapy. Applications of multikinase inhibitor sorafenib, chimeric antigen receptor T cells, or PD-1/PD-L1 inhibitors can prolong the median survival of HCC patients. However, the treatment efficacy is still unsatisfactory because of HCC metastasis and postoperative recurrence. Liver tissues in hepatocarcinogenesis or hepatocyte malignant trans- formation can express and secrete a variety of molecules with specific biomarkers or oncogenic antigens into blood, for example, alpha-fetoprotein, glypican-3, Wnt3a (one of the key signaling molecules in the Wnt/β-catenin pathway), insulin-like growth factor (IGF)-II or IGF-I receptor, vascular endothelial growth factor, secretory clusterin, and so on. In addition, combinatorial immunotherapy with non-coding RNAs might improve anticancer efficacy. These biomarkers not only contribute to the diagnosis and prognosis of HCC, they but also might become the molecular-targets for HCC treatment under developing or clinical trials. This article reviews the recent progress of some emerging biomarkers in basic researches or clinical trials for HCC immunotherapy.

Key Words: Hepatocellular carcinoma; Immunotherapy; Carcinoembryonic proteins; Specific biomarkers; Wnt/β-catenin pathway; Signal molecules

Yao M, Yang JL, Wang DF, Wang L, Chen Y, Yao DF. Encouraging specific biomarkers-based therapeutic strategies for hepatocellular carcinoma. *World J Clin Cases* 2021; In press

Core Tip: Tissues in hepatocellular carcinoma (HCC) or hepatocyte malignant transformation can express and secrete a variety of molecules with specific biomarkers or oncogenic antigens into blood. These biomarkers not only contribute to the diagnosis and prognosis of HCC, they but also might become the molecular-targets for HCC treatment under developing or clinical trials. This article reviews the recent progress of some emerging biomarkers in basic researches or clinical trials for HCC immunotherapy.

INTRODUCTION

The prevention, early monitoring or diagnosis and accurate or effectively treatment of hepatocellular carcinoma (HCC) are still urgent medical problems[1,2]. Occurrence of HCC is mainly associated with chronic persistent infection of hepatitis B virus (HBV) or hepatitis C virus (HCV), chemical carcinogens intake, and nonalcoholic fatty liver disease (NAFLD)[3]. In the past decade, NAFLD has become a leading cause of chronic hepatitis and liver cirrhosis, as well as an important risk factor for HCC[4]. Innate and adaptive immunity play a pivotal role in determining tumor control *vs* progression. Genomic instability and abnormal signaling in the setting of chronic liver inflammation that promotes fibrogenesis and angiogenesis lead to tumorigenesis, along with how they may be exploited in the development of novel therapeutics[5]. The activation of oncogenes or HCC-related genes, inactivation of anti-oncogenes or reactivations of some oncogenes during the embryonic period can induce malignant transformation of

hepatocytes[5], many kinds of specific markers can be expressed, and then secreted into blood in the process of initiation, promotion and evolution[1]. Notably, HCC oncoimmunology depends on diverse genetic and environmental factors that together shape cancer-promoting inflammation and immune dysfunction- critical processes that control HCC malignant progression and response to therapy[6,7].

Nowdays, surgery operation is still mainly treated for HCC with auxiliary vascular embolization, radio frequency, radiotherapy, chemotherapy, and biological therapy[8,9]. Application of multikinase inhibitor sorafenib can prolong the median survival of HCC patients. However, the treatment efficacy is still unsatisfactory because of HCC metastasis and postoperative occurrence[10,11]. Undoubtedly, the integration of data obtained from both preclinical models and human studies can help to accelerate the identification of robust predictive biomarkers of response to targeted or immune-therapy[12,13]. HCC tissues express the specific antigens such as the key molecules of HCC-related signal pathways, growth factors and receptors, vascular endothelial growth factor (VEGF), and the products of oncogenes that some mediated tumor progression and could be potential molecular-targeted for anti-cancer therapy with highly specificity and application prospects[14,15]. This review presents new advances of few promising carcino-embryonic biomarkers for HCC immunotherapy on basic studies or clinical trials.

ALPHA-FETOPROTEIN

A glycoprotein of alpha-fetoprotein (AFP) synthesized from human fetal liver or HCC tissues[16], consisting of 609 single-chain amino acid polypeptides and containing 24 Leading signal points (9-10 amino acid) residues located in three N-terminal domains, the major histocompatibility complex (MHC) class I or II molecules recognize these precursor signals and present them to $CD4^+$ T cells and $CD8^+$ T cells, the activated T cells recognize the body's immunodominant or sub-immunodominant epitopes[17]. Amino acid peptide sequences and immunogenicity of human AFP epitopes are shown in Table 1. These immunogenic or sub-immunogenic AFP peptide chains could play an

immune-modulatory role in human, having the function and ability of polypeptide vaccine, and could induce and stimulate human anti-AFP specific immune responses.

AFP peptide chains have several fragments showing immunodominant or sub-immunodominant epitopes, which could be recognized by the MHC-I molecules, specifically induce T cells to activate and recognize AFP antigen. AFP positive peripheral blood mononuclear cells (PBMC) containing five human leukocyte antigen (HLA)- A*24:02 restricted T cell epitopes, AFP-derived peptide induces cytotoxic T lymphocyte (CTL) to produce interferon-γ (INF-γ), which can kill AFP positive HCC cells. Although it has been in clinical trials, the function of dendritic cells (DC), specific CTL, $CD8^+$ T cells response and targeting therapy for AFP positive HCC cells remains to be studied. Now, T cell receptor (TCR) has been prepared by induction and screening *in vitro*, which can specifically recognize and bind AFP/HLA-A*02 antigen that is restricted to AFP158-166 peptide (FMNKFIYEI) to lay a foundation for HCC immunotherapy[18]. A novel HLA-A*24:02 antigen was found to be more common than the HLA-A*02:01 in Asian HCC patients. Its restrictive peptide (KWVESIFLIF, AFP2-11signal) was found to be soluble in healthy human monocyte AFP 2-11-HLA- A*24:02-specific TCR (KWV3.1). T cells could be activated specifically and kill AFP positive T2-A24 HCC cells that contained AFP 2-11 and HLA-$A^*24:02^+$ antigen, indicated that AFP^+HLA-$A^*24:02^+$ antigen might be a new immunotherapeutic target for HCC[19].

Combination of anti-CTL-A-4 therapy (Tremelimumab) together with ablation in advanced HCC cases has shown that the killing tumors by direct methods can result in immune system being activated or switched on. There are new drugs available known as immune checkpoint inhibitors (ICIs) which could enhance anti-HCC effect. After the patients with Tremelimumab treatment, the blood $CD4^+$- HLA-DR^+, $CD4^+$PD-1+, $CD8^+$HLA-DR^+, $CD8^+$PD-1^+, $CD4^+$$ICOS^+$, and $CD8^+$$ICOS^+$ T cells increased, the patients with higher CD^+PD-1^+ cells responded well to the treatment, with increasing specific $CD8^+$PD-1 T cells for AFP & survivin, and higher $CD3^+$T cells for tumor infiltrating, suggesting that Tremelimumab with ablation is a novel potential method with

increasing $CD8^+$ T cells and decreasing circulating HCV amount for advanced HCC effective therapy[20].

ANGIOGENIC FACTORS

Most patients with HCC are diagnosed at an advanced stage of disease. Until recently, systemic treatment options that showed survival benefits in HCC have been limited to tyrosine kinase inhibitors, antibodies targeting oncogenic signaling pathways or VEGF receptors[21]. Angiogenesis plays an important role in HCC progression, and VEGF and angiopoietin (Ang) are key drivers of HCC angiogenesis. A better understanding of the relation between VEGF and HCC angiogenesis or progression may reveal their potential as biomarker for HCC diagnosis and therapy. VEGF- targeting strategies already represent an important component of today's systemic treatment landscape of HCC, whereas targeting the Ang/Tie2 signaling pathway may harbor future potential in this context due to reported beneficial anticancer effects when targeting this pathway[22,23]. Following a decade of negative Phase III trials since the approval of sorafenib, more recently several drugs have proven efficacy both in first line *vs* sorafenib (lenvatinib) or in second line *vs* placebo (regorafenib, cabozantinib, ramucirumab/Cyramza®). A fully human anti-VEGFR-2 recombinant IgG1 monoclonal antibody (Ramucirumab) has been approved as monotherapy for HCC patients with AFP levels ≥ 400 ng/mL who have been treated with sorafenib, with significantly prolonged overall survival (OS) and progression-free survival. Its safety profile was consistent with that expected for agents targeting the VEGF/ VEGFR axis. The potential clinical development of systemic treatments in HCC, focusing on combination therapies with immunotherapy and treatment sequences as a way to maximize survival benefit[24,25].

HCC microenvironment is characterized by a dysfunction of the immune system through multiple mechanisms, including accumulation of various immune- suppressive factors, recruitment of regulatory T cells and myeloid-derived suppressor cells, and induction of T cell exhaustion accompanied with the interaction between immune

checkpoint ligands and receptors. ICIs have been interfered this interaction and have altered therapeutic landscape of multiple cancer types including HCC. Intermediate-stage HCC with different levels of liver function, tumor size, and number of lesions may all have intermediate-stage disease according to the BCLC staging system. Their treatments includes conventional or drug-eluting bead transarterial chemoembolization, yttrium-90 radioembolization, thermal ablation, bland embolization, and combination therapy with VEGF inhibitors or ICIs. The clinical evidence supported available locoregional treatment options for intermediate-stage HCC[26]. Although optimal sequencing is an area of ongoing investigation, multiple targeted therapies have improved OS in intermediate or advanced HCC[27]. Several targeted agents including multi-tyrosine kinase inhibitors and immunotherapy agents have been approved for use beyond the frontline setting in patients with advanced HCC, combinatorial therapeutic strategies is an evolving approach showing early promising signal[23,28]. Success of PD-1 monotherapy, combinatorial regimens with PD-1/PD-L1 inhibitors plus VEGF targeted agents shown positive results in various malignancies including HCC. These innovative approaches enhance the intensity of cancer-directed immune responses and will potentially impact the outlook of this aggressive disease[29].

GLYPICAN-3

Regarding HCC, one promising antigen appears to be glypican-3 (GPC3) that is over-expressed by HCC tissues and has been associated with worse disease-free survival and overall survival. GPC3 is involved in many signaling cascades that promote cell growth and invasion, including the Wnt pathway that is well- known for its role in embryogenesis. GPC3 as an oncofetal proteoglycan anchored to the cell membrane of HCC, is normally detected in the fetal liver but not in the healthy adult liver[30,31]. However, the abnormal levels of GPC3 expression in tissues or sera of HCC patients are expressed at GPC3 mRNA gene transcription or protein levels, and predicts a poor prognosis of HCC. Mechanistic studies have revealed that GPC3 functions by binding

to molecules such as the Wnt/β-catenin signaling or growth factors during HCC occurrence and progression. Moreover, specific serological GPC3 has been used as a diagnostic or prognostic serological marker, and a molecular-targeted for molecular imaging or therapeutic intervention in HCC[32-34]. GPC3 as a molecular target for HCC immunotherapy is shown in Table 2. Up to date, GPC3-targeted magnetic resonance imaging, positron emission tomography, and near-infrared imaging have been investigated at early stage of HCC, and immunotherapeutic protocols targeting GPC3 have been developed, including the use of humanized anti-GPC3 cytotoxic antibodies, peptide/DNA vaccines, immuno- toxin therapies, and genetic therapies.

Different synergisms have been postulated based on the potential interplay between anti-angiogenic drugs and immunotherapy, with several clinical trials currently testing. Since the most extensively tested combination regimens for advanced HCC comprise anti-PD-1/anti-PD-L1 agents plus anti-angiogenic agents, oncogenic GPC3 is becoming an ideal promising candidate for HCC immunotherapy because of highly expressed in cancerous tissues but limited in normal liver tissues. Recently, the adoptive transfer of hGPC3-specific chimeric antigen receptor T (CAR-T) cells for HCC treatment has been conducted in clinical trials. Due to the rigid construction, conventional CAR-T cells have some intrinsic limitations, like uncontrollable over- activation and inducing severe cytokine release syndrome. By using co-culturing assays and a xenograft mouse model, the *in vitro* and *in vivo* cytotoxicity and cytokine release of the split anti-hGPC3 CAR-T cells were evaluated against various HCC cell lines and compared with conventional CAR-T cells. *In vitro* data demonstrated that split anti-hGPC3 CAR-T cells could recognize and lyse hGPC3 positive HepG2 and Huh7 cells in a dose-dependent manner. Impressively, the split anti-hGPC3 CAR-T cells produced and released a significantly lower amount of pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-6, and GM-CSF, than conventional CAR-T cells. When injected into immune-deficient mice inoculated subcutaneously with HepG2 cells, our split anti-hGPC3 CAR-T cells could suppress HCC tumor growth, but released significantly lower levels of cytokines than conventional CAR-T cells. The split anti-hGPC3 CAR-T cells could suppress tumor

growth and reduce cytokine release, and represent a more versatile and safer alternative to conventional CAR-T cells for HCC treatment[35,36]. The most recent data on novel combination strategies and targets, as well as looking, ahead to the future role of molecular therapies in the treatment of advanced HCC. Current barriers of CAR-T therapy include its high production cost and need to identify validated extracellular HCC-specific antigens[33,37].

WNT3a

Several signaling pathways involved in HCC have been studied, including STAT3-NFκB, JAK-STAT, RAS MAPK, PI3K-AKT-mTOR, and Wnt-β-catenin. Of these, cascades involving mitogen-activated protein kinase (MAPK) emerge as key regulators of HCC. Bath of HBV and HCV infection can induce the activation of Wnt/β-catenin signal pathway and participate in HCC progression[38,39]. Oncogenic HBx of HBV can activate Src kinase to inhibit GSK3β activity and make intracellular β-catenin accumulation, promote the expression of DNA methyl-transferase I and Wnt3a to bind and silence secreted frizzled related protein 1 and 5[40]. HBx can reduce the inhibiting role for deacetylase 1 to β-catenin, and activating Wnt pathway promotes HCC development[41]. Also, the core protein of HCV can promote Wnt3a expression, induce TCF dependent transcription, inhibit GSK3β, increase and stabilize intracellular β-catenin to nucleus transport, and up-regulate the expressions of cyclinDl, c-myc, WISP2, Wnt3a, Wnt1 and CTGF to promote the HCC growth, DNA synthesis for HCC progression[42]. Wnt3a is a critical signal molecule among the 19 mammalian Wnt proteins. The higher level of Wnt3a expression was only found in sera or tissues of HCC patients from a cohort cases with chronic liver diseases[43,44], and it is the first time to report as a novel specific marker for HCC diagnosis and prognosis[45,46].

Abnormal Wnt3a expression is involved in the development and metastasis of HCC[47], and might be a novel strategy for HBV or HCV-related HCC therapy. Hepatic higher LINC00662 correlated with poor survival of HCC patients[48,49], and might up-regulate Wnt3a expression by competitively binding miR-15a, miR-16 and miR-107,

with tumor-associated macrophages as a major component of tumor microenvironment in HCC progression, and they have been revealed the associations between Wnt3a signaling and cancer initiation, tumor growth, metastasis, dormancy, immunity and tumor stem cell maintenance[40]. Wnt3a is one of HCC-related Wnt signals exhibited numerous genetic abnormalities as well as epigenetic alterations including modulation of DNA methylation. Targeted *Wnt3a* gene transcription might be an effective molecule-targeted therapy for HCC. Novel Crispr/Cas9-gsRNA lentiviral vector system with the advantages of higher targeting accuracy has been successfully used to affect the Wnt3a gene transcription in human HCC cell lines at mRNA level *in vitro* and confirmed at protein level *in vivo* with transplanted tumor studies[44,50].

The inhibitory effect of Wnt3a on the proliferation of HCC cells or the growth of HCC xenograft models has been demonstrated that interfering with *Wnt3a* gene transcription could significantly inhibit the expressions of down-stream β-catenin and related-signal molecules[51]. The xenograft model of knockout of Wnt3a gene in HepG2 cells resulted in a slower growth, significant reduction of tumor size or loss of weight. The molecular mechanism is Wnt3a cascade reaction involving multiple targets, can block upstream GPC-3 signal and downstream β-catenin to nucleus transport[52,53], and inhibiting or delaying cancer progression by using specific antibodies (OMP-54F28, OTSA101)[54] and small size peptide SAH-BCL-9[55]. As the abnormality of liver and circulating Wnt3a expression in HCC has provided initial evidence, the tumor volume after intervening Wnt3a mRNA transcription with specific shRNA was 355.0 ± 99.9 mm^3 in the intervention group with significantly lower than that (869.4 ± 222.5 mm^3) in the negative group, and the tumor formation days of the intervention group were longer than that of the negative group; the tumor weight (0.35 ± 0.11 g) of the intervention group was markedly lower than that (0.88 ± 0.20 g) of the negative group. Immunohistochemistry showed that Wnt3a was strongly inhibited in the intervention group[56], and indicated that targeted-Wnt3a signaling could be a promising target or an effective target for HCC therapy.

CLUSTERIN

Secretory clusterin (sCLU) is a stress-induced heterodimer sulfated glycoprotein, located on chromosome 8q21-q12, which is highly conserved between species and has cytoprotective effect. Its biological function as a small molecule partner is almost similar to that of heat shock protein[57]. Basic and clinical studies showed that sCLU expression was low in normal liver tissues and its activation during the malignant transformation of hepatocytes was progressive over-expression[58,59], which was closely associated with HCC progression by contributing to angiogenesis, chemo- resistance, cell survival, and metastasis[60]. The positive rate of hepatic sCLU expression was up to 73.3% at stage I of HCC by immunohistochemsical analysis. Its expression at mRNA or protein level were increased with the clinical staging of HCC, indicated that sCLU could be a biomarker for differentiating benign from malignant liver diseases[61].

Recurrence and metastasis after hepatectomy are the main causes of poor prognosis of HCC[62]. Hepatic sCLU plays an important role in the proliferation, multidrug resistance, invasion and metastasis of HCC cells[63,64]. The sCLU mediated the expression of MMP-2, p-AKT and E-cadherin in human HCC BEL-7402 and SMMC-7721 cell lines, and down-regulating sCLU expression can significantly reduce the invasive ability of HCC cells by selective COX-2 inhibitor meloxicam combined with specific sCLU-shRNA plasmids[65,66]. These data indicated that sCLU should be a new effective target for the occurrence, invasion and metastasis of HCC, and should have s bright future in HCC immunotherapy.

INSULIN-LIKE GROWTH FACTOR AXIS

Hepatic insulin-like growth factor (IGF) axis contains ligands, receptors, substrates, and ligand binding proteins. Accumulating data demonstrated that aberrant IGFs signaling molecules may lead to malignant transformation of hepatocytes and HCC progression, especially in IGF-II or IGF-I receptor (IGF-IR) as key molecules in hepatocarcinogenesis[67] or rat xenograft models[68], affects the molecular pathogenesis of HCC, thus providing the rationale for targeting IGF axis in HCC[69]. The biological

activities of IGF-II or IGF-IR not only promote HCC cell proliferation or xenograft growth, but also confer resistance to standard treatments[70]. Several strategies targeting this system including monoclonal antibodies against IGF-1R and small molecule inhibitors of the tyrosine kinase function of IGF-1R are under active investigation. For example, DX-2647, a recombinant human antibody, potently neutralizes the action of IGF-II, which is overexpressed in primary HCC[71] and impairs the xenograft growth of Hep3B but not HepG2 cell line with high p-STAT3 Levels, suggesting that STAT3 activation as one pathway that might mediate resistance to IGF-II-targeted therapy in HCC[72].

The over-expression of hepatic IGF-IR in human HCC promotes HCC cell proliferation, and attaching importance to IGF-IR might improve the prognostic or therapy of HCC[73]. Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) as a regulator of promoting IGF-IR induced sorafenib resistance of HCC *in vitro* by directly transcriptionally repressing a set of microRNAs including miR-101, miR-122, miR-125b, and miR-139[74-76]. A model for an EZH2-miRNAs-IGF-IR regulatory axis might provide insights into how to reversal sorafenib resistance in HCC. Silencing IGF-IR gene by specific shRNA on the inhibition of cell proliferation *in vitro* or rat xenograft growth *in vivo* to elucidate it as a novel molecular-targeted therapy for HCC. Several strategies targeting this system including monoclonal antibodies against IGF-IR and inhibitors of the tyrosine kinase function of IGF-IR are under active investigation. Gene-specific shRNA against IGF-signaling molecules as well as IGF-IR selective receptor tyrosine kinase (RTK)-inhibitors (tyrphostins) may therefore offer new therapeutic options[77,78]. However, since specific shRNA is currently not applicable in HCC therapy, selective RTK-inhibitors represent the most promising approach for future therapeutic strategies.

SYNERGY OF NON-CODING RNAS

While immunotherapy holds great promise for combating cancer, the limited efficacy due to an immunosuppressive tumor microenvironment and systemic toxicity hinder

the broader application of immunotherapy[79,80]. Combinatorial immunotherapy approach that uses a highly efficient and tumor-selective gene carrier to improve anticancer efficacy and circumvent the systemic toxicity. HCC is one of the multi-genetic diseases, and multiple studies have highlighted the key roles of noncoding RNAs (ncRNAs) in chemoresistance of HCC such as biomarkers and functional modulation of cellular response to sorafenib[81-83]. Targeted chemotherapeutic agent, sorafenib, is known to show a statistically significant but limited overall survival advantage in advanced HCC, linked with the modulation of several intracellular signaling pathways through diverse operating biomolecules including ncRNAs[84-86]. Accumulated evidences have demonstrated that ncRNAs (miRNAs, long ncRNAs or lncRNAs, and circular RNA or circRNA) could serve as biomarkers in diagnosis, prognosis, and treatment of HCC[87,88] that have been well-documented to participate in HCC progression with promoting or inhibiting roles[89,90].

Interestingly, miRNAs varied responses have been linked with the modulation of several intracellular signaling pathways[91]. The abnormality of miR-218 expression was investigated in human HCC tissues or HCC cell lines for evaluating its function and underlying mechanisms of HCC. Gain-of-function and loss-of- function assays indicated that forced expression of miR-218 by inhibited HCC cell migration/invasion and reversed epithelial-mesenchymal transition to mesenchymal- epithelial transition. Serpine mRNA binding protein 1 (SERBP1) was a target gene of miR-218, and targeting the miR-218/SERBP1 signal pathway that inhibit the malignant phenotype formation might be a potential novel way for HCC therapeutics, because of miR-218 functions as a HCC suppressor involves in many biological processes such as tumor initiation, development, and metastasis[92]. Nanotechnology- enabled dual delivery of siRNA and plasmid DNA that selectively targets and reprograms the immune-suppressive tumor microenvironment to improve HCC immunotherapy[93-95].

HCC-associated circRNAs are abundant, and their over/Low expression might promote/inhibit HCC cell proliferation or tumor growth[96-98]. Abnormality of circ-homer1 in HCC cells or tissues was related to tumor size, lymph node metastasis, high

clinical staging and poor prognosis. The molecular mechanism of circ-homer1 over-expression promoted the growth and invasiveness of HCC cell lines *via* mir-1322/cxc16 axis[99]; conversely, interfering the circ-homer1 activation inhibited the proliferation, migration and invasion of HCC cell lines by increasing cell apoptosis. The circ-0051443 from circulating exosomes or HCC tissues could regulated BAK1 expression by combination of mir-331-3p to promote the cell apoptosis or cell cycle arrest of HCC, inhibit the biological behavior of HCC cells *in vivo* or nude mice HCC xenografts[100]. Another interesting study also showed that has_circ_0008450 expression in HCC tissues or cells might inhibit HCC progression by regulating mir-214-3p/ezh2 axis[101,102]. These data suggested that specific ncRNAs were useful molecular targets for HCC therapy.

CONCLUSION

In conclusion, HCC is a multi-gene variant malignant tumor with DNA methylation, microRNA, lncRNA expression and immune response[103]. Immunotherapy for HCC has begun to produce better results, and HCC-specific molecules may be combined with comprehensive intervention such as surgery, interventional therapy, radiotherapy, and chemotherapy to improve the efficacy and prolong the survival time of HCC patients[104]. In spite of the rapid development of genomics and proteomics, advances in molecular pathology, pharmacology and genetic engineering, DNA splicing, gene silencing, interfering transcription, and monoclonal antibody for more specific and less side effects immune therapy techniques[105] that can directly block the signaling molecules involved in HCC growth related signaling pathways (Figure 1) or serve as molecular targets such as radionuclide, drug carriers, and immunotherapy play a unique role in specific or comprehensive treatment of HCC.

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