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Concise reviews

Progress of targeted and immunotherapy for hepatocellular carcinoma and the application of next-generation sequencing

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ABSTRACT

Hepatocellular carcinoma (HCC), leading cancer worldwide, has a high degree of genetic heterogeneity; next-generation sequencing (NGS) technology has contributed significantly to the discovery of driving genes as well as high-frequency mutations in HCC. The detection of gene alterations may allow us to predict prognosis and adverse drug reactions for individuals, paving the way for personalized medicine in HCC patients. In this review, we summarized the common systemic therapy regimens for HCC and the predictive efficacy of genetic biomarkers on the prognosis of patients under these treatments. Finally, we put forward a future perspective on the potential of NGS technology for the guidance of targeted therapy and immunotherapy in HCC.

Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer and is mainly caused by hepatitis B or C virus. In recent years, HCC caused by liver disease associated with alcohol and metabolic syndrome has been on the rise [1,2]. Chronic inflammation, viral infection, and liver regeneration can induce genetic and epigenetic damage [3]. Through the accumulation of these genomic changes, most HCC gradually develop from these precancerous stages. With the rapid development of metagenomic sequencing technology, HCC has been proved to have significant heterogeneity, and its high therapeutic resistance and poor prognosis pose a challenge to systemic treatment [4].

Next-Generation Sequencing (NGS) can analyze the comprehensive profile of cancer genome and transcriptome on a large scale, allowing quick identification of potential driving gene events in cancer, especially potential molecular therapy targets [5]. In non−small cell lung cancer (NSCLC), genetic biomarkers have been proved to predict responses to targeted therapy, especially those targeting specific tyrosine kinase receptors and immune checkpoint inhibitors [6]. Gene detection also plays an essential role in guiding the proper treatment of breast cancer, especially patient stratification [7,8]. Targeted therapy, immune checkpoint suppression therapy, and combination therapy of HCC have shown superior efficacy in clinical trials. Biomarkers including gene alterations and pathway activations are of great significance for designing proper treatment regimens, and biomarker-driven therapies have shown gratifying benefits [9]. In the future, gene detection may be included as a routine process in the systemic treatment of HCC.

1. Progress of targeted therapy for HCC

Except for REACH-2 clinical trials, all effective drugs in phase III clinical trials are multi-kinase inhibitors in HCC [10]. Many studies have determined the scope of mutant genes and drug development targets, and about 25% of the mutant genes are considered to have potential drug properties. However, the mutant genes have targets co-expressed by normal liver tissues and cancerous ones, resulting in severe toxic and other side effects and compromised efficacy of targeted drugs [4]. Therefore, it is necessary to understand the therapeutic mechanism of various targeted drugs and use gene detection

Abbreviations: HCC, Hepatocellular carcinoma; uHCC, unresectable Hepatocellular carcinoma; CRLM, Colorectal liver metastases; HBV, Hepatitis B virus; NSCLC, Non-small cell lung cancer; NGS, Next-generation sequencing; PCR, Polymerase chain reaction; RT-PCR, Reverse transcription-polymerase chain reaction; mPCR, Multiplex Polymerase Chain Reaction; IHC, Immunohistochemistry; FISH, Fluorescence in situ hybridization; WGS, Whole-genome sequencing; OS, Overall survival; mOS, Median overall survival; ORR, Objective effective rate; PFS, Progression-free survival time; TTP, Time to progress; DCR, Disease control rates; TCGA, The Cancer Genome Atlas; NCCN, National Comprehensive Cancer Network; TKI, Tyrosine kinase inhibitor; RTK, Receptor tyrosine protein kinase; EGFR, Epidermal growth factor receptor; VEGFR, Vascular endothelial growth factor receptor; PDGFR, Platelet-derived growth factor receptor; FGFR, Fibroblast growth factor receptor; ICI, Immune checkpoint inhibitors; PD-1, Programmed death 1; PD-L1, Programmed death ligand 1; IFN, Interferon; PTK, Protein tyrosine kinases; CCL5, Chemokine ligand 5; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; IPM, Immune prognosis model; Treg, Regulatory T cells; TRM, Resident memory T cells; HRR, Homologous recombination repair

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to identify unique biomarkers that can be utilized as drug targets or prognostic markers. These efforts may finally help clinicians select proper drugs, minimalize drug resistance, and improve the prognosis of patients.

1.1. Tyrosine kinase inhibitor (TKI)

Sorafenib is a multi-target TKI with anti-angiogenic and anti-proliferative effects. Between 2007 and 2016, Sorafenib was the only systemic drug licensed for the treatment of HCC [11]. This drug can inhibit up to 40 kinds of kinases, including angiogenesis induced by receptor tyrosine protein kinases (RTKs; including VEGFRs and PDGFR β) and drivers of cell proliferation (such as RAF1, BRAF, and KIT) [12].

Lenvatinib is an oral small molecule multi-receptor TKI targeting the VEGF receptor, FGFR1-FGFR4 axis, RET, KIT, and PDGFR-α, etc. Its efficacy has been tested in Phase II and Phase III clinical trials of patients with advanced HCC [13,14]. Compared with Sorafenib, Lenvatinib is comparable in overall survival rate (OS), but with a higher objective effective rate (ORR), longer progression-free survival time (PFS), and time to progress (TTP) [15].

Donafenib is a novel oral small molecule multi-kinase inhibitor and derivative of Sorafenib deuterated. Recently, Qin et al. [16] conducted a multicenter Phase II-III trial in systemic-treatment-naive patients with unresectable liver cancer or liver metastasis cancer, with a Child-Pugh score < 7. The results showed that the median overall survival time (mOS) of Donafenib was significantly higher than that of Sorafenib (12.1 vs. 10.3 months, $P = 0.0245$). The progression-free survival (PFS), objective response rate (ORR), and disease control rates (DCR) were not significantly different. From the perspective of pharmacokinetic features, compared to Sorafenib, Donafenib has a more stable antioxidant capacity, enhancing the anti-tumor activity *in vivo*. In addition, Donafenib has a higher original drug plasma concentration and lower metabolite concentration, which means that Donafenib monotherapy may have a better curative effect [17]. Donafenib is expected to be the first-line treatment of advanced HCC in the future.

Regorafenib is a new multi-kinase inhibitor for advanced hepatocellular carcinoma resistant to Sorafenib. It has recently been approved for the treatment of HCC patients previously treated with Sorafenib, based on the significant improvement in the time to progression (TTP) and OS in 573 patients according to the Phase III trial [18]. It suppresses tumor proliferation by inhibiting several critical targets, including vascular endothelial growth factor receptor (VEGFR 1, 2, and 3), platelet-derived growth factor receptor beta (PDGFR-β), Raf, Ret, and Kit kinases [19]. In addition, a recent study has shown that Regorafenib provided clinical benefit to patients regardless of the drug dose and disease progression status in prior Sorafenib treatment [20].

At present, there are no targeted drugs to specifically block the pathways and inhibit tumor growth in HCC due to the tumor heterogeneity and multi-target inhibitive nature of these drugs. There are no standard guidelines for selecting the first-line drugs, and most decisions are empirical. Therefore, it is crucial to detect the gene alterations and guide the personalized administration of targeted drugs.

1.2. Non-tyrosine kinase inhibitors

ARQ197 (Tivantinib) is a small oral molecule MET inhibitor, inhibiting the growth and inducing apoptosis of tumor cells [21]. A study in HCC cell lines confirmed the sensitivity of Tivantinib to HCC and showed anti-tumor activity in various mouse xenograft tumor models [21]. Twelve tumor biopsies treated with Tivantinib also showed that MET expression decreased following treatment, and expression of genes related to the downstream pathway was related to Tivantinib [22]. Furthermore, preclinical studies showed that the combination of Tivantinib and Sorafenib had a synergistic effect, enhancing the inhibitory effect of Tivantinib [23]. Whether MET inhibitors can be a potential treatment for some patients with advanced HCC and whether they can solve the drug resistance problem of Sorafenib still need follow-up mechanism research to decide [23]. In addition, Tivantinib is thought to have a mechanism independent from inhibiting MET, which may imitate cytotoxic agents and attack a wide range of targets [11]. At present, new selective oral compounds, such as Tepotinib [24] and Capmatinib [25], have been developed in HCC. Currently, the focus is on reducing their toxicity and determining the effectiveness of MET-positive patients in Phase II clinical trials[26]. The mechanism of Tivantinib, Tepotinib and other MET inhibitors against MET-positive HCC may be highly complex, and more evidence from follow-up studies is needed.

Refametinib is an effective, non-ATP competitive and highly selective inhibitor of MEK1 and MEK2. Its anti-tumor activity as a monotherapy or in combination with Sorafenib has been confirmed in both in vitro and *in vivo* preclinical studies [27]. Refametinib monotherapy was well tolerated and showed therapeutic advantages for patients with advanced solid tumors, including HCC, according to a phase I research (NCT00785226). The efficacy of Refametinib and Sorafenib in combination in preclinical HCC models relies on one or two underlying mechanisms. The first is to block the MAP signaling pathway (RAF combined with Sorafenib and MEK combined with Refametinib); the second is to inhibit the parallel signaling pathways (MAPK combined with Refametinib and VEGF receptor-mediated signaling pathway combined with Sorafenib). The inhibition of these pathways shows enhanced anti-tumor activity in HCC [28]. Under a low concentration of Sorafenib, the phosphorylation level of MEK and ERK in HCC cells increased due to abnormal activation of RAF signal, so the dual inhibition of Sorafenib combined with Refametinib may be an effective way to treat HCC. Moreover, MEK inhibitors have increased inhibitory activity on cancer cells containing RAS mutations. Therefore, compared to wild-type RAS, HCC patients with RAS mutation have a superior clinical response to Refametinib combined with Sorafenib [29], indicating that detection of RAS genotype may help drug selection.

The amplification of FGF19 occurs in 5-10% of HCC, which has been proved to be a carcinogenic driver of Sorafenib resistance in some studies and a potential prognostic marker of FGFR kinase inhibitors [30]. Clinical trials of specific FGFR4 kinase inhibitors are underway, including BLU554 (NCT02508467) [31], H3B-6527 (NCT02834780) [32] and FGF401 (NCT02325739). These drugs are currently under evaluation using biomarker-based methods, mainly based on immunohistochemistry of FGF19 and FGFR4, and β-klotho, a transmembrane protein that enhances FGF19-FGFR4 interaction and signal transmission. Moreover, BLU-554 has made the fastest progress in clinical research. Preliminary data show that the effective rate of FGFR4 high expression group (FGF19 expression ≥1% in IHC) is 16%, while the effective rate of FGFR4 negative group is 0% [31]. Regardless of the amplification status of FGF19 negative group, there will be some toxic reactions, which are generally mild, including diarrhea, nausea, vomiting and elevated AST and/or ALT levels (transaminase tends to rise to grade 3-4) [31].

2. Advances in immunotherapy of liver cancer

A great challenge facing the development of immune checkpoint inhibitors (ICI) is identifying the biomarkers predicting response. So far, the treatment of HCC with Nivolumab and Pembrolizumab has not shown the correlation between PD-L1 expression or the etiology of cirrhosis and clinical benefits [33]. The FDA has approved Pembrolizumab to treat advanced cancer with high microsatellite instability or mismatch and subsequent lack of DNA repairment. However, the

low incidence of these defects in HCC makes the indication of Pembrolizumab usage unclear[34].

According to the immunohistochemical analysis of immunoregulatory molecules, the immune microenvironment of HCC was divided into three different subtypes: high, medium, and low immunity. Cases with tumors of high immunity subtype that are usually rich with progenitor/proliferative gene expression patterns may be ideal candidates for ICI treatment, as this type usually shows a high degree of immune infiltration and PD-1 expression in the tumor microenvironment[35]. Various biomarkers have predictive value for immunotherapy combined with targeted therapy in HCC, but so far, there has been no specific biomarker to predict prognosis in these treatments [36].

2.1. PD- 1/PD- L1 inhibitor

PD-1 is a receptor expressed by T cells and mainly provides negative regulatory signals during the effective period of T cell response. In tumor pathogenesis, PD-1 on T cells can combine with PD-L1 and PD-L2 ligands in the tumor microenvironment, contributing to the immunosuppressive microenvironment. Monoclonal antibodies against PD1 (Nivolumab and Pembrolizumab) or PD-L1 (Atezolizumab, Avelumab and Durvalumab) have been approved for the treatment of various malignant tumors[11].

Xu et al. conducted a phase II study on the efficacy and safety of Camrelizumab (anti-PD-1 monoclonal antibody) combined with Apatinib (VEGFR-2 tyrosine kinase inhibitor) in the treatment of advanced HCC. Their research shows that this combination has good efficacy and manageable safety in patients with advanced HCC. This conclusion may provide a new option for patients with advanced HCC in the future[37]. Finn et al. reported the results of Lenvatinib combined with Pembrolizumab in unresectable HCC (uHCC) in a phase I b study. The results showed that Lenvatinib combined with Pembrolizumab had good anti-tumor efficacy against uHCC, and manageable toxic and side effects[38]. These researches further proved the benefits of combining tumor immune microenvironment manipulation with targeted therapy.

TCGA researchers observed that 22% of HCC had lymphocyte infiltration, consistent with the previous study of HCC immune category. About 27% of patients had high infiltration of immune cells, high PD-1/PD-L1 expression, and active IFN-γ signal. The immune rejection phenotype appeared in 25% of HCC patients, with CTNNB1 mutation, low immune infiltration (based on immune specific gene markers) and overexpression of PTK2, a carcinogenic pathway related to poor infiltration of T cells into malignant tissues[39]. These findings are in line with melanoma studies, demonstrating that activation of the Wnt/-catenin (CTNNB1) pathway is linked to T cell rejection and immunotherapy resistance[40]. De Galarreta et al. proved that β-catenin-activated tumors were resistant to PD-1 treatment in mice models, and the expression of chemokine ligand 5 (CCL5) resumed immune surveillance[41].

To sum up, the activation mutation of β-catenin may be a negative predictor of ICI treatment for HCC patients. However, it is worth noting that the response to Nivolumab of HCC patients seems irrelevant to PD-L1 expression. New biomarkers are needed to anticipate the reaction degree of HCC patients to anti-PD-1 therapy[11].

2.2. CTLA-4 monoclonal antibody

CTLA-4 is expressed by regulatory T cells on a constant basis, but it is also up-regulated in activated cytotoxic T cells. CTLA-4 is a dominant negative signal molecule, monoclonal antibodies against CTLA-4 such as Ipilimumab and Tremelimumab block the negative feedback reaction and lead to deep and lasting reactions in cancer patients.

The combination of Ipilimumab and Nivolumab ("O+Y") has been approved for second-line treatment. The adverse drug reactions of Ipilimumab are known at present, the combination of the two drugs has successfully reduced the side effects. Moreover, the combination's OS and ORR are superior to monotherapy of both in melanoma patients, and median PFS is superior to Ipilimumab[42,43].

Recently, Robin et al. evaluated the safety and efficacy of different doses of Duvariuzumab + Tremelimumab ("D+T") (T300+D: Tremelimumab 300 mg + Durvalumab 1,500 mg; T75+D: Tremelimumab 75 mg + Durvalumab 1,500 mg), T monotherapy and D monotherapy in the first- and second-line treatment of patients with advanced uHCC. The results showed that T300+D presented an encouraging benefit-risk profile compared to both monotherapies and T75+D, and both mOS (18.7 months) and ORR (24%) of the T300+D further support its application in advanced uHCC. Furthermore, its toxicity is favorable compared with other CTLA-4/PD-1(L1) combination therapies and is consistent with the toxicity of monotherapy. A comparative study between the D+T and Sorafenib regimen is now underway, and it is likely to become a novel therapeutic regimen for liver cancer in the future[44].

Long et al. established an immune prognosis model (IPM) by using differentially expressed immune-related genes in TP53 mutant liver cancer samples. Studies have shown that the high expression of TREM1 and EXO1 is related to the high expression of CTLA-4, PD-1 and TIM-3, and may be related to the better outcome after using immunosuppressants[45]. Some miRNA and lncRNA may participate in the "cancer immune cycle" regulated by CTLA-4 and PD-L1/PD-1, which may become the subject of liver cancer research in the future [46]. As the clinical trials and studies on CTLA-4 inhibitors have not yet reached a clear conclusion, further efforts should be made to determine biomarkers to guide the selection of HCC patients suitable for CTLA-4 inhibitors.

3. Gene detection and precision medicine

Precision medicine consists of two aspects: precise diagnosis and precise treatment. Gene detection is regarded as the core of precision medicine. From diagnosis to treatment of malignant tumors, the etiology is first dissected from the histological, cellular, and molecular level, and then personalized measures are taken. Gene detection is widely used in breast cancer, colorectal cancer, leukemia, lymphoma, head and neck tumor, ovarian cancer, lung cancer, liver cancer, and so on.

For example, 50% of non-small cell lung cancers have driving gene mutations, indicating that even the same type of lung cancer potentially has different treatment methods. Researchers are looking for drugs targeting the corresponding driving genes to treat different types of lung cancer. In May 2003, FDA approved the first-on-the-world targeted drug Gefitinib to treat patients with advanced non-small-cell lung cancer (NSCLC) after chemotherapy failed.

The molecular-genetics difference of individuals is the decisive factor for the variance of response to drugs. When Gefitinib and Erlotinib were first put on the market, the effect was not significant[47]. After it was found that they had a significant effect on patients with EGFR mutation, the two drugs became the first-line drugs for some lung cancer subtypes. DNA sequencing (including first- and second-generation sequencing), polymerase chain reaction (PCR), and other technologies are now used to detect gene alterations in NSCLC. Further development of gene detection technologies has dramatically improved the sequencing depth and throughput.

In liver disease, gene mutation may lead to related genetic diseases, such as hereditary hemochromatosis, Gilbert syndrome, α-1 antitrypsin deficiency, Wilson's disease, etc. Harding et al. [48] aimed to determine whether the application of NGS in modern clinical practice provides predictive and/or prognostic information for HCC patients receiving systematic treatment. Tumor/normal DNA from HCC patients (N = 127) was compared and analyzed using NGS analysis based on hybridization capture. The WNT/β-catenin pathway (45%) and TP53 (33%) were associated with highly malignant and more aggressive

molecular alterations subsets. Oncogenic changes in the PI3K-MTOR pathway in Sorafenib-treated patients were associated with lower disease control rates (DCR), shorter median progression-free survival (PFS), and shorter median overall survival (OS). Experiments on the regulation of proliferation and apoptosis of liver cancer cells by miR-19a targeting the homologous deletion phosphatase-tensin (PTEN) on chromosome 10 showed that the increased expression of miR-19a was related to the pathology and prognosis of liver cancer. Overexpression of miR-19a significantly inhibited PTEN. After overexpression of PTEN plasmid and miR-19a mimic were co-transfected into hepatocellular carcinoma cells, the effects of promoting cell proliferation and inhibiting apoptosis were reversed [49]. In the future, targeted inhibitory drugs designed for miR-19a can block its role in promoting tumor proliferation and inhibiting tumor suppressor genes.

At present, gene detection includes DNA and RNA detection. DNA detection can find the genes with point mutation to determine whether target sites, such as KRAS mutates, are used as sites for drug development. RNA detection can observe the expression of DNA by transcription to predict the situation after blocking the target.

Despite the progress in treatment, colorectal liver metastases (CRLM) have always been a complex problem in surgery/oncology field. Postoperative survival remains highly varied due to the lack of reliable prognostic biomarkers and evidence from clinical trials. At present, the expression of KRAS, BRAF, TP53, PIK3CA, APC and Ki-67 and the existence of microsatellite instability seem to have decisive impact on the prognosis and treatment response of CRLM patients. At the same time, there is a correlation between tumor pathological results and the genomic alterations found in gene detection. If specific biomarkers are found in treatment, the prediction of prognosis and the selection of targeted drugs will be significantly increased [50].

Lim et al. [51] identified unique immune subsets in HBV-related HCC. Next-generation sequencing (NGS) and in vitro T cells were used to further investigate phenotypes and functional proliferation assays. Regulatory T cells (Treg) and $CD8^+$ resident memory T cells (TRM) were enriched in HBV-associated HCCs. In contrast, $TIM\text{-}3^+CD8^+$ T cells and $CD244^+$ natural killer cells were enriched in non-virus-associated HCCs. Compared with non-virus-associated HCC, Tregs and TRM in HBV-associated HCC expressed more PD-1, and functionally had stronger inhibitory and depletion effects. Such results showed that NGS could help us find the characteristics of HBV-related HCC microenvironment, which in this case, was a more immunosuppressive and exhausted microenvironment than the non-virus-related HCC.

The treatment based on gene detection provides management of patients at the molecular level. There are still some patients without mature genomic biomarkers; however, with growing knowledge of multi-omic landscapes of cancers and increasing evidence from clinical trials, the future of precision medicine is promising.

4. Future perspective

So far, the etiology of HCC has been well understood, but the knowledge about molecular mechanisms is still negligible. The development of NGS has completely changed the method of gene variation detection in liver cancers. The detection covers point mutation, insertion/deletion, copy number variation, and gene fusion/rearrangement, enabling the estimation of individual response to targeted/immunotherapy. In addition, genotypes of chemotherapy-associated polymorphic loci were also examined to predict the efficacy and side effects of chemotherapy [1,52].

4.1. Application of NGS technology in gene mutations of hepatoma

Cell origins, molecular categorization, and carcinogenesis, as well as tumor heterogeneity, metastasis, and treatment resistance, are all subjects of current research [53]. In fact, the rapid progress and effectiveness of next-generation sequencing technology (NGS) in identifying cancer-driving genomic alterations (GAs) [54].

Basic, translational and clinical studies aim to complete an inventory of cancer drivers and mutations. In this direction, rapidly growing NGS studies from various institutions seek to identify and assess the effectiveness of genetic and genomic changes, assess their clinical utility as biomarkers, and develop new drugs that target specific drug mutations [55].RAS gene is a proto-oncogene of intracellular signal transduction proteins [56], an important oncogene in the EGFR signal pathway and can be used as a molecular switch to participate in signal transduction such as cell growth and proliferation and differentiation. The mutation of KRAS gene will lead to the continuous activation of downstream pathways due to its inability to dephosphorylate, leading to the occurrence and progression of tumors and thus compromising the efficacy of anti-EGFR drugs. Similarly, BRAF V600 mutations are also likely to curb the response to anti-EGFR therapy. A study found that KRAS gene mutation was significantly higher in HCC patients with extrahepatic metastasis than in HCC patients without extrahepatic metastasis[57]. The National Comprehensive Cancer Network (NCCN) identifies KRAS gene status as a predictor of EGFR-targeted monoclonal antibodies' efficacy[58]. BRAF gene testing is also recommended for KRAS wild-type patients [59].

Few studies on PLC WES have been published, with only four reporting on more than 100 patients[60,61]. However, studies have shown that specific mutated genes (CTNNB1, TP53 and AXINI) are associated with environmental risk factors such as alcohol and viral hepatitis. If large-scale clinical trials confirm these findings, a screening program that includes genetic testing and CT could be used for the early detection of HCC[4].

4.2. Research and application of NGS technology in signal transduction pathways of liver cancer

NGS can dissect essential pathways such as homologous recombination repair (HRR), P13K/mTOR signaling pathway, Wnt-β-catenin pathway. The genes involved in HRR pathway include BRCA1/2, PALB2, ATM, ATR, CHEK1/2, BARD1, BRIP1, MRE11A, RAD51 gene family, and FANC gene family. P13K/mTOR signaling pathway-related genes include PIK3CA, PTEN, STKI1, TSCI, TSC2, mTOR, etc. In HCC, PIK3CA mutation frequency is 4%, and PTEN deletion mutation frequency is about 7%. The frequency of PIK3CA gene variation in biliary tumors is 4%∼5%. Clinical studies suggest that PI3K, Akt and mTOR inhibitors can be used as potential therapeutic targets in tumor patients with PIK3CA mutations (NCT00962611, NCT02449538, etc.) [57]. Kan et al. [62] sequenced the genomes of 88 HCC patients, highlighted the critical differences between HCC and other solid tumors. Changes in EGFR, PI3K and MAPK pathways are also common in other cancer types, while Wnt/β-catenin and JAK/STAT are the two main oncogenic pathways of HCC. This finding may explain why drugs against targets like EGFR do not function well in HCC.

Hauke[63] found that changes in Ras-Raf pathway and SMAD family had the highest prognostic significance for the outcome after resection of colorectal cancer liver metastasis (CRLM), and potential prognostic biomarkers were identified by NGS analysis of cancer-related genes. According to Chun et al. [64,65], mutations in BRAF, KRAS, TP53, PIK3CA and SMAD family members were found to be significant predictors of overall survival after liver surgery for CRLM, deleterious changes in SMAD family members, including copy number variations and mutations are most correlated with the prognosis of CRLM[64]. Loss of SMAD signaling is associated with poor prognosis after primary colorectal cancer and CRLM resection. Although there are no targeted therapy options, SMAD analysis appears to be a potent prognostic factor for cancer.

4.3. Defects of NGS technology

NGS-based personalized medicine could help the management of HCC in the way mentioned above or in another. When there are no evidenced prognostic markers detected in a liver cancer patient, if the markers of other malignancies such as lung cancer, breast cancer and colon cancer are highly expressed, corresponding targeted drugs are also recommended.

The NGS technology does offer many advantages over prior methods such as fluorescence in situ hybridization (FISH), reverse transcription-polymerase chain reaction (RT-PCR), and immunohistochemistry (IHC). However, it has its obstacles and dilemmas. Firstly, NGS requires a certain number of materials to complete, which may not be possible with only a small number of samples [66]. Next, NGS may lead to a higher false-positive rate while improving the depth and breadth of sequencing. It takes professional equipment and personnel to sift through and validate the results[67]. In addition, various detection methods based on NGS such as WGS, RNAseq, Hybridization Capture Sequencing, mPCR could be utilized according to the situation to alleviate the cost and inconvenience of NGS.

Despite these challenges and the fact that several companies are currently trying to develop "third-generation" long-read sequencing platforms, NGS has a vast advantage in clinical applications. NGS may become more convenient and accurate with continuous breakthroughs in biotechnology, providing a solid basis for selecting targeted and immunotherapy and achieving more personalized treatment in the context of liver cancers.

Intratumoral heterogeneity of HCC is the main obstacle against proposing standard treatment strategies for HCC. Therefore, the future of HCC treatment will be personalized management. It is crucial to identify the driving alterations in the genome, pathways, and tumor microenvironments of each HCC patient. NGS is an ideal tool assisting this personalized decision-making.

5. Conclusion

The drugs used to treat liver cancer currently include the above-mentioned tyrosine kinase inhibitors, non-tyrosine kinase inhibitors, immune checkpoint inhibitors, etc. Due to the characteristics of hepatocellular carcinoma tumor microenvironment, there is no effective single-target inhibitor at present, and the combination of targeted and immune drugs is the mainstream. NGS has played an essential role in multiple tumor types by identifying driving pathways prominent in recurrent somatic mutations, copy number changes, tumorigenesis, and metastasis. NGS can improve the reliability of clinical diagnosis and provide a basis for a personalized selection of systemic treatment regimens, facilitating optimized decision-making for patients. However, further preclinical studies and clinical trials are still needed to explore the therapeutic effects of these targeted pathways. Given the complicated malignant subtypes and great intra- and inter-tumor heterogeneity of hepatocellular carcinoma, repeatable and reliable results are hard to get. Therefore, it is essential to conduct more global multicenter WGS studies with larger sample sizes better to understand the NGS therapeutic and prognostic potential.

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Ethical disclosure

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Data sharing statement

N/A

Conflicts of interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Fan Yang: Visualization, Writing – review & editing, Investigation, Writing – original draft. **Kaige Deng:** Visualization, Writing – original draft, Writing – review & editing. **Haoran Zheng:** Writing – review & editing, Investigation, Writing – original draft. **Zhenting Liu:** Writing – review & editing, Investigation, Writing – original draft. **Yongchang Zheng:** Visualization, Supervision, Writing – original draft, Writing – review & editing.

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