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Review

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Application of multi-omics in hepatocellular carcinoma: new prospects for classification and precise diagnosis and treatment

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Abstract

Hepatocellular carcinoma (HCC) represents a significant global health challenge, with a complex etiology and limited treatment options. The integration of multi-omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, has revolutionized our understanding of HCC, offering novel insights into its molecular underpinnings. This comprehensive review synthesizes the current knowledge on the application of multi-omics in HCC, highlighting its role in disease classification, early detection, and the development of targeted therapies. We discuss the identification of key driver mutations and single nucleotide polymorphisms (SNPs) that enhance risk prediction models, with implications for personalized medicine. The multi-omics approach has facilitated the discovery of distinct HCC subtypes, each with unique molecular signatures and tumor microenvironments (TME), which are critical for predicting prognosis and guiding treatment strategies. Furthermore, we explore the implications of these findings for precision medicine, emphasizing the potential of biomarker identification and targeted therapies, including immune checkpoint blockade (ICB). The review concludes by underscoring the transformative impact of multi-omics on HCC research and clinical practice, heralding a new era of personalized medicine with the promise of improved patient outcomes.

Keywords: Hepatocellular carcinoma, multi-omics, precision medicine, molecular classification, targeted therapy, immune checkpoint blockade

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common pathological type of primary liver cancer, accounting for more than 90% of primary liver cancers[1,2]. HCC has the fifth-highest incidence globally and the second-highest mortality rate, indicating its severity and threat to public health[3]. In Asia, especially China, the incidence and mortality of HCC are relatively high[4]. The causes of HCC are diverse, including hepatitis virus infection, cirrhosis, aflatoxin exposure, smoking, obesity, diabetes, and other factors[5]. In terms of treatment, the therapeutic strategies for HCC include local treatment such as surgery, ablation, and interventional therapy. However, less than 30% of patients are suitable for radical treatment at the first diagnosis[6]. For patients with advanced HCC, the systemic therapy regimen of targeted combined immunotherapy has become the new standard of first-line therapy for advanced HCC. Immunocombination therapy, especially the combination of immune checkpoint inhibitors and macromolecular anti-angiogenesis drugs, has achieved remarkable results and significantly improved the survival rate of patients[7]. However, the low early diagnosis rate and high recurrence rate, which affect patient survival, remain treatment challenges of HCC. Current surveillance tools, including abdominal ultrasound, are insufficiently sensitive to detect early HCC, especially in obese and/or non-viral liver disease patients.

Multi-omics techniques, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiome, provide comprehensive information about cell function and state[8]. The application of multi-omics technology in cancer research not only deepens our understanding of disease, but also provides new strategies and methods for cancer diagnosis, treatment, and prevention, showing broad application prospects[9,10]. In recent years, with the vigorous development of multi-omics technologies, HCC molecular typing systems have been discovered and established. By further refining the classification using multiple omics characteristics, subtypes with different molecular characteristics and clinical prognoses can be identified, providing a new approach to precise diagnosis and treatment in HCC.

MULTI-OMICS OF HCC CLASSIFICATION

Genetic variations in HCC classification

The genetic susceptibility to HCC is characterized by genetic heterogeneity[11]. Genomic studies have identified numerous driver mutations in HCC, including alterations in key genes such as *TERT*, *TP53*, *CTNNB1*, *ARID1A*, and *KMT2C* (Chang *et al*., 2023)[12]. These mutations contribute to the development of HCC through various mechanisms, including promoting cell proliferation, inhibiting apoptosis, and disrupting cellular metabolism. The traditional clinical model created by Cadier *et al.*[13] was improved to better predict the probability of HCC development in patients with cirrhosis, by incorporating information on seven single nucleotide polymorphisms (SNPs), including rs738409 (*PNPLA3*), rs58542926 (*TM6SF2*), rs187429064 (*TM6SF2*), rs641738 (*MBOAT7*), rs72613567(*HSD17B13*), rs429358 (*APOE*), and rs708113 (*WNT3AWNT9A*). However, the model is primarily intended for Europeans, and its applicability to East Asians remains uncertain[14]. The research of Chen *et al*. indicates a strong association between four SNPs rs429358 (*APOE*), rs58542926 (*TM6SF2*), rs708113 (*WNT3AWNT9A*), rs738409 (*PNPLA3*) and liver cancer in Europeans[15]; however, only one SNP, rs738409 on the *PNPLA3* gene, is linked to HCC in East Asians, with the other three showing no such correlation. This suggests that current European models may not be directly applicable to the East Asian population. Additionally, the SNP rs429358 located on the *APOE* gene is also not significantly associated with Europeans. A genome-wide systematic comparison of genetic differences between Europeans and East Asians in HCC showed that on chromosome 6, East Asians exhibit more significant sites, with SNP rs200715955 being the most prominent signal, while Europeans lack notable SNPs on this chromosome. Both populations demonstrate strong genetic signals on chromosome 19; however, for East Asians, the most significant SNP is located at the *IFNL4* gene (rs8107030), while for

Europeans, it is the SNP at the *SUGP1* gene (rs739846)[15]. SNPs on the *IFNL4* and *SUGP1* genes are not currently included in existing predictive models; in the future model construction, it is necessary to consider the genetic effects of multi-population, so as to improve the prediction efficiency and increase the external validity of the model. In addition, the use of genetic risk scores associated with the phenotype of HCC can also effectively improve the efficacy of the model. For example, the causes of HCC mainly include hepatitis virus infection, alcoholic liver disease, non-alcoholic liver disease, *etc*. If the genetic scores of related risk factors are added to the future prediction model, the predictive efficacy of the model will be greatly improved. If these genetic scores can be combined into a single valid score and associated with related pathways, it will greatly simplify the model and detection costs.

Multi-omics improves the gross classification of HCC

In 1987, Moriyama's group classified HCC into five gross subtypes based primarily on tumor shape: single nodular type (type I), single nodular type with extra nodular growth (type II), contiguous multinodular type (type III), poor demonstrated type (also named as infiltrative type, type IV), and early HCC type[16]. Several limited retrospective studies have suggested a correlation between tumor shape and prognosis in patients with HCC; even imaging features reflecting the gross appearance of HCC have been proposed to be predictive of outcomes after Radiofrequency Ablation (RFA), transcatheter arterial chemoembolization (TACE), and even lenvatinib therapy[16,17]. Despite the utility of the classification system for HCC, its clinical implementation has been limited due to diagnostic challenges associated with certain subtypes and a scarcity of cases representing each subtype that would facilitate robust clinical investigation. Thus, selecting interventions for patients with solitary HCC remains a challenge. Despite gross classification being proposed as a potential prognostic predictor, its widespread use has been restricted due to inadequate studies with sufficient patient numbers and the lack of established mechanisms.

Fan *et al.* sought to investigate the prognostic impacts on patients with HCC of different gross subtypes and assess their corresponding molecular landscapes. Multi-omics technology such as transcriptomics, proteomics, copy number variation (CNV analysis), Weighted Gene Co-Expression Network Analy (WGCNA) were performed on tumors and non-tumor tissues from 49 patients to investigate the mechanisms underlying gross classification[18,19]. Inverse probability of treatment weight (IPTW) was used to control for confounding factors. A prospective cohort of 400 patients who underwent hepatic resection for solitary HCC was reviewed and analyzed and gross classification was assessed. The research provides an easy-to-use modified gross classification system (MMC) for HCC based solely on margin morphology. It finds distinct molecular expression patterns, gene mutations, and components of tumor microenvironment (TME) among the four gross subtypes. Infiltrative type HCC exhibits the most similarities to intrahepatic cholangiocarcinoma in terms of gross appearance, prognosis, and downregulated expression profiles. Only infiltrative type HCC shows the response to adjuvant TACE[19], indicating the potential effective treatment methods for different gross subtypes of HCC.

By combining transcriptome and genomic profiling, HCC can be classified into molecular subtypes G1 ~ G6 with different biological characteristics. G1 and G2 are associated with HBV infection, and both AKT pathways are activated. G3 ~ G6 are associated with HCV infection and excessive alcohol consumption. *TP53* and *TCF1* mutations occur in G3 and G4, respectively. G5 and G6 have a high *CTNNB1* mutation rate and activated WNT pathway, indicating a good prognosis. The study also found that 50% of tumor WNT or AKT pathways are activated, suggesting that inhibiting these signaling pathways may benefit relevant patients.

Multi-omics of HCC and precise diagnosis and treatment

Liquid biopsy is an innovative diagnostic technique that detects and monitors diseases by analyzing molecular components such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, microRNAs (miRNAs), and tumor education platelets in blood and other biological fluids such as urine, saliva, *etc*. Compared with traditional tissue biopsy, liquid biopsy has the characteristics of noninvasiveness, high reproducibility, easy operation, and real-time monitoring of disease progression. The main application areas of liquid biopsy include: early cancer detection, tumor staging and monitoring, and treatment response monitoring. Liquid biopsy and multi-omics improve the precise diagnosis and treatment of HCC both in research and clinical settings. Currently, major advances and breakthroughs in precision medicine primarily rely on genomic analysis. Advancements in multi-omics techniques have provided a theoretical foundation for personalized and targeted therapies. Based on the multi-omics technology, the biomarkers discovered in the blood of HCC patients play an important role in disease diagnosis, early detection, prognosis evaluation, and treatment response monitoring. In primary HCC, as protein biomarkers, AKR1B10 is overexpressed in early stages of highly and moderately differentiated liver cancer, and downregulated in late-stage tumors with lower differentiation, indicating potential for early diagnosis of HCC. AKR1B10 is an effective serological marker for detecting liver cancer, with a sensitivity and specificity of 72.7% and 95.7%, respectively. Especially in AFP-negative HCC patients, AKR1B10 has a sensitivity of 71.2% and a specificity of 92.6% for diagnosing HCC. miRNAs such as miRNA-21, miRNA-221, and miRNA-224 are unregulated in liver cancer and can serve as potential novel serum biomarkers. Additionally, miRNAs such as miRNA-18a, miRNA-221, miRNA-222, and miRNA-224 also play a role in diagnosis and prognosis. The downregulation of miRNA-101, miRNA-106b, miRNA-122, miRNA-195, and miRNA-125b expression is associated with the inhibition of HCC progression. Extracellular vesicles (EVs) contain various biochemical signals, such as genetic material, proteins, *etc*., and are considered biomarkers for early detection of HCC. In a small study, the sensitivity of EV detection reached 94.4% and the specificity reached 88.5%. CTCs are malignant cells derived from primary tumors or metastasized to the systemic circulation, which are measured through liquid biopsy and, to some extent, represent samples of tumor lesion cells in patients. Detecting the quantitative abundance, biological characteristics, and genomic heterogeneity between CTCs can predict the disease prognosis and treatment response of liver cancer patients. Though these biomarkers typically require a combination of multiple biomarkers and other diagnostic tools for comprehensive evaluation, the multi-omics technology is reshaping biomedical research, integrating multidimensional data such as genomics, transcriptomics, proteomics, and spatial profiling, opening up new paths for disease mechanisms, diagnosis, treatment, and drug development[20-26]. The development of multi-omics has promoted the molecular state monitoring of HCC, and may pave the way for new methods to overcome tumor progression and treatment resistance. The development of multicenter, multi-omics, and large-scale liver cancer data analysis and high-throughput, multi-platform validation technologies is expected to provide important support for precise drug target selection and optimization for patients. At the same time, conducting multi-omics analysis on tumor biopsy samples - pre- and post-treatment, as well as in cases of primary recurrence - can help monitor tumor subclonal evolution, further deepening the understanding of genotype-phenotype correlations, as well as the mechanisms of treatment sensitivity and resistance. The application of multi-omics technology in the precise diagnosis and treatment of HCC is shown in Figure 1.

Approved therapy for HCC

The target therapy for HCC

Systemic therapy is the main treatment method for advanced HCC. Targeted therapy is a treatment approach designed at the cellular molecular level that specifically targets known oncogenic sites. The drugs used in this therapy enter the body and selectively bind to these oncogenic sites to exert their effects, leading to the specific death of tumor cells without affecting the surrounding normal tissue cells. Molecular targeted

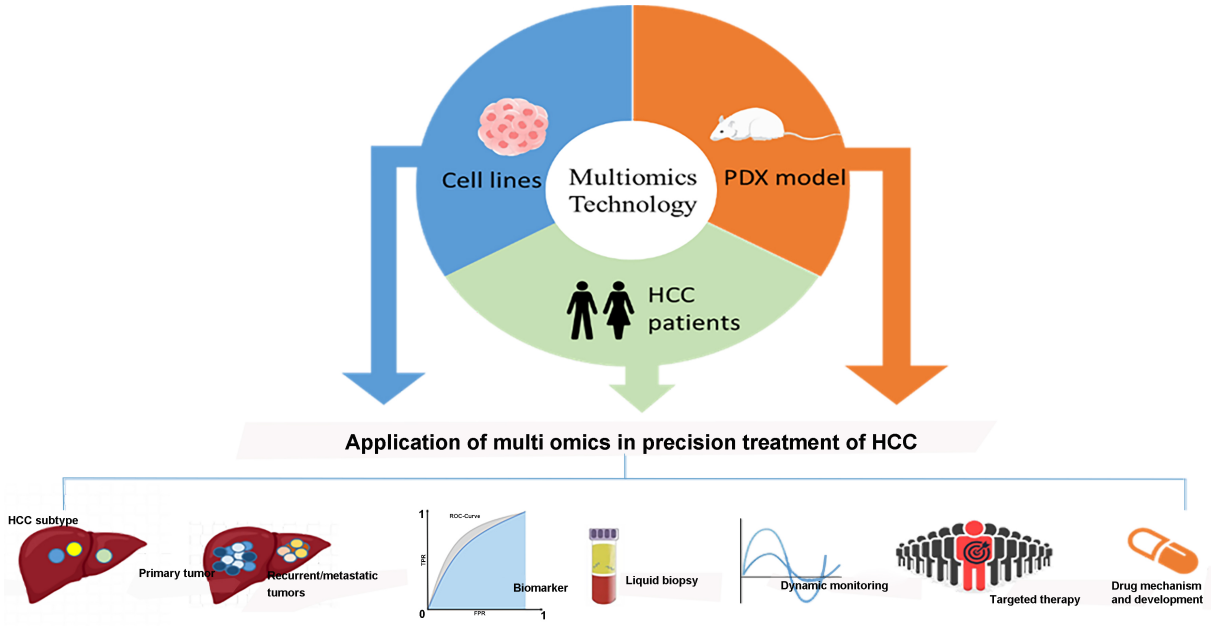

Figure 1. Application of multi-omics in precision treatment of HCC.

drugs primarily intervene in the treatment of malignant tumors by targeting key points in their pathophysiological development. Currently, there is no unified classification method for molecular targeted drugs. At therapeutic doses, drugs that act on only one target point are called single-target inhibitors; in contrast, drugs that act on multiple targets at therapeutic doses are called multi-target inhibitors. The multi-target inhibitors such as sorafenib, lenvatinib, donafenib, regorafenib, cabozantinib, ramucirumab, and apatinib have become the backbone of systemic therapy for advanced HCC. Sorafenib is the first approved multi-target receptor tyrosine kinase (RTK) inhibitor, mainly for its effects on proliferation and angiogenesis, and has been used as a first-line treatment for HCC since 2007. However, only about 5% of patients have a partial response to sorafenib. Lenvatinib is another multi-target RTK inhibitor that shares certain common targets with sorafenib and uniquely inhibits fibroblast growth factor receptor (FGFR). Serum biomarkers, including VEGF, ANG2, and FGF19, have been shown to be closely associated with the efficacy of lenvatinib. The approved Targeted therapy for HCC is shown in Table 1. Despite significant treatment progress, most advanced HCC patients still exhibit treatment resistance and disease progression. Therefore, screening patients who are most likely to benefit from specific therapies is extremely important, as it can maximize potential benefits, reduce avoidable toxicity, and save medical resources[27-34].

The immunotherapy for HCC

Besides the multi-target drug mentioned above, the cell signal of HCC also showed the potential for the target therapies [shown in Table 2]. Current immunotherapy drugs for HCC primarily target PD-1/PD-L1 and CTLA-4. Research is also exploring new targets and combination therapies[27-46].

Given the complexity of HCC, the current therapeutic effect is still very limited, and how to more accurately identify patients suitable for certain drugs or treatment modalities has become the focus of clinical attention. Multi-omics technology provides key technologies for these studies and has uncovered potentially effective precision treatments.

Table 1. Approved therapy for HCC

Target drug	Target gene/signaling pathway
sorafenib[35]	RAF kinase, EGFR-2, VEGFR-3, PDGFR-β, KIT, FLT-3/RAF/MEK/ERK
lenvatinib[36]	VEGFR-11, VEGFR-2, VEGFR-3, FGFR1, FGFR2, FGFR3, FGFR4, PDGFR, CKIT, RET
donafenib[37]	VEGFR, PDGFR, RAF
regorafenib[37]	VEGFR-1,2,3, TIE-2, BRAF, KIT, RET, PDGFR, FGFR
cabozantinib[38]	MET, VEGFR1/2/3, ROS1, RET, AXL, NTRK, KIT
ramucirumab[39]	EGFR, ALK

HCC: Hepatocellular carcinoma.

Table 2. Signaling pathways in HCC[46]

Signaling pathway	Drug
Wnt/β-catenin	Celecoxib, PKF118-310, PKF115-584, CGP049090
RAF/MEK/ERK(MAPK)	Sorafenib, Regorafenib, Danoprevir/PD 0325901/PD 0325901
Hippo	Bortezomib
HRR	Inhibitors of ARP
FGF19- FGFR4	BLU-554
HGF/c-MET	Cabozantinib/Tivantinib

HCC: Hepatocellular carcinoma.

The panoramic map of gene mutation and molecular expression of metastatic liver cancer is still weak, and the evolution law of clonal selection in the metastasis process of liver cancer is still unclear, resulting in the lack of sufficient theoretical guidance for the clinical diagnosis and treatment of metastatic liver cancer. Sun *et al.* generated Multi-omics analytical methods, including genomics, transcriptomics, single-cell RNA sequencing (scRNA-seq), spatial transcriptomics, and immunohistochemical techniques, which were used to detect 257 primary and 176 metastatic regions from 182 HCC patients to obtain comprehensive information on primary and metastatic HCC, such as the spatial distribution and interactions of cancer cells, as well as their interactions with the surrounding microenvironment[47]. The research found primary tumors rich in hypoxia signatures facilitated polyclonal dissemination. Genomic divergence between primary and metastatic HCC is high, and early dissemination is prevalent. The remarkable neoantigen intratumor heterogeneity observed in metastases is associated with decreased T cell reactivity, resulting from disruptions to neoantigen presentation. The somatic copy number alterations are highly selected events driving metastasis. Mutations in the Wnt pathway affect *CTNNB1*, *AXIN1*, or, more rarely, *APC*. Subclones without Wnt mutations show a stronger selective advantage for metastasis than those with Wnt mutations and are characterized by a microenvironment rich in activated fibroblasts favoring a prometastatic phenotype. Finally, metastases without Wnt mutations exhibit higher enrichment of immunosuppressive B cells that mediate terminal exhaustion of CD8+ T cells via HLA-E: CD94-NKG2A checkpoint axis. The study indicated that metastatic HCC has a high degree of intratumoral heterogeneity and a complex evolutionary trajectory, suggesting that each patient population may have unique biological characteristics and developmental pathways. Subclonal selection is an important factor in the progression of metastatic liver cancer, and the TME [especially cancer-associated fibroblasts (CAFs) and B cells] plays a crucial role in promoting or inhibiting metastasis. These findings not only advance our understanding of metastatic HCC, but also provide valuable insights for the development of new diagnostic methods and treatment strategies. It is the first time to systematically depict the spatio-temporal evolution panorama of liver cancer metastasis, reveal the complex evolutionary trajectory and clonal selection mechanism of liver cancer metastasis, and provide valuable data and theoretical basis for the development of new markers for

the prediction of liver cancer metastasis and new therapeutic targets.

A major challenge for proteomics-driven precision medicine is how to use the comprehensive proteome to identify subtypes of patients with shared biological factors that can be targeted for treatment[48,49]. Patients with HCC at the same clinical stage can have extremely different prognoses, and molecular subtyping provides an opportunity for individualized precision treatment.

In one study, genomic, transcriptomic, proteomic, and phosphoproteomic profiling of primary tumor tissues and paired para-tumor tissues from HCC patients (N = 160) are integrated. Proteomic profiling identifies three HCC subtypes (SI-SIII) with different clinical prognoses[50]. These subtypes differ significantly in overall survival (OS) and relapse-free survival (RFS) and are associated with clinical stage, tumor markers, and other pathological features, which are validated in three publicly available external validation sets. A simplified panel of nine proteins associated with metabolic reprogramming is further identified as a potential subtype-specific biomarker for clinical application. Multi-omics analysis further reveals that three proteomic subtypes have significant differences in genetic alterations, microenvironment dysregulation, kinase-substrate regulatory networks, and therapeutic responses. There were significant differences in the response of the three subtypes to sorafenib. Specifically, SII and SIII tumors showed high sensitivity to sorafenib, while SI was primarily resistant to it. After 4 days of treatment, the response of each patient-derived cell (PDC) to sorafenib was measured by calculating the AUC of the dose-response curve, and the differences in response to sorafenib were compared among the three protein subtypes. The results showed that sorafenib effectively inhibited the proliferation of PDC with broad drug sensitivity, indicating that HCC PDC is highly heterogeneous. Notably, growth inhibition of PDC was significantly stronger in SII and SIII compared to SI, especially at concentrations of 32 mM or higher of sorafenib. In addition, the percentage of tumors that reached the semi-maximum inhibitory concentration (IC50) was significantly higher in SIII than in SI, suggesting that SIII tumors may benefit more from sorafenib. These findings demonstrate that proteomics holds great promise in identifying HCC-subtype patients with different prognoses who might benefit from further clinical treatment.

The revolutionary progress of immune checkpoint blockade (ICB) therapy, such as anti-PD-1/L1 and anti-CTLA4 antibodies, has extended the survival of various cancer patients. Although many efforts have been made to enhance the clinical benefits of HCC immunotherapy, particularly with T cells possessing different cell lytic activities, the response of these therapies in HCC patients is still very limited. Most previous studies on scRNA seq datasets in the HCC TME have only focused on the heterogeneity of TME at the cellular level, neglecting its spatial structure and potential impact on treatment response[51-53]. In another study, the authors integrated single-cell and spatial multi-omics data to identify and characterize the tumor immune barrier (TIB) structures associated with immune therapy efficacy in the HCC TME. They found that secreted phosphoprotein 1 (SPP1) macrophages and CAFs interact to promote the formation of TIB structures, which limit tumor immune invasion, indicate the structure present in the HCC microenvironment was identified, $SPP1^{+}$macrophages are a potential clinical therapeutic target for HCC[54,55]. These findings represent an important step toward discovering more effective therapies for HCC and offer new insights for precision medicine in HCC treatment.

Chronic inflammatory stimulation of HCC patients may cause their immune microenvironment to develop into a state resistant to immunotherapy. In fact, most HCC patients exhibit resistance or relapse after ICB, which is related to the transformation of the tumor immune microenvironment into an immunosuppressive one[55-58]. This transformation is related to the dysfunction of innate immunity and adaptive immunity. Therefore, reshaping the immune microenvironment has become a promising therapeutic strategy for

advanced HCC patients. However, the specific mechanisms by which different cells regulate the tumor immune microenvironment to induce resistance to immunotherapy remain unclear[59-61]. Using single-cell sequencing technology (scRNA-seq), comparative analysis, and experimental verification, researchers found that neutrophils are associated with immunotherapy resistance in HCC. Specifically, a subpopulation of $CD10^+ALPL^+$ neutrophils in HCC patients is associated with resistance to PD-1 therapy[62]. They further found that tumor cells reprogram $CD10^+ALPL^+$ neutrophils via the NAMPT-NTRK1 signaling pathway. These findings reveal the mechanism through which $CD10^+ALPL^+$ neutrophils contribute to resistance to anti-PD-1 therapy. Tumor cells reprogram CD10 + ALPL + neutrophils to maintain an immature state, preventing their maturation and activation, while these neutrophils drive T cells into an irreversible state of depletion, ultimately leading to drug resistance in patients. These results also provide valuable insights into potential new immunotherapy targets and possible synergistic treatment approaches.

The emergence of scRNA-seq technology has demonstrated its powerful ability to explore cell diversity and tumor heterogeneity[63]. The study revealed intertumor and intratumoral transcriptome heterogeneity in HCC and identified molecular signatures associated with TME reprogramming. Rna-seq can classify malignant and non-malignant cells based on aneuploid copy number profiles and identify clonal substructures in different subclusters[64]. It enables the analysis of tissue heterogeneity at the single-cell level and provides insight into the contribution of different cell subclusters to biological function and pathogenesis. In one study, the TME landscape of HCC was described using RNA-SEQ data and six prognostic-related subclusters were identified[65]. The authors then developed five transcriptome subtypes by unsupervised clustering of subcluster-specific markers (SSMs) in the training cohort. These molecular subtypes exhibit different clinical outcomes, including variations in genomic variation, and immune-infiltrating microenvironments. They investigated the therapeutic responses of HCC patients with different molecular subtypes to ICB, TACE, and targeted therapy using a publicly available clinical treatment cohort and a Xiangya HCC cohort. The characteristics of TME were further validated by RNA-SEQ, single-cell T cell receptor /B cell receptor sequencing, mass spectrometry flow cytometry (CyTOF), and multiple immunofluorescence techniques. The integration of the scRNA-seq data with the overall RNA-seq cohort enabled the authors to generate molecular subtypes with clinical efficacy, providing a theoretical basis for the development of personalized treatment options for HCC patients.

DISCUSSION

The integration of multi-omics technologies into HCC research and clinical practice has opened new avenues for understanding the disease's complexity and developing targeted therapies. However, the journey toward precision medicine is paved with both known and emerging challenges.

Challenges in multi-omics applications in HCC

The technical challenges in multi-omics data analysis are manifold. First, the data generated are often high-dimensional, with many more features than samples, leading to the "curse of dimensionality". This can result in overfitting of models and reduced generalizability. Second, the data are noisy and may contain outliers, which can distort the analysis. Third, the integration of data from different platforms (e.g., genomics, proteomics) requires harmonization of the data, which can be technically challenging due to differences in measurement scales and data types. Lastly, the validation of multi-omics findings in independent cohorts is crucial but often limited by the availability of well-annotated samples.

The theoretical gaps in our understanding of HCC are substantial. For instance, while driver mutations in HCC have been identified, the functional consequences of these mutations and how they interact with each other and with the TME are not well understood. Additionally, the role of non-coding RNAs, epigenetic

modifications, and the microbiome in HCC is still being elucidated. Understanding these complex interactions is essential for developing effective therapies[66].

The heterogeneity of HCC is a significant challenge for precision medicine. This heterogeneity is not only molecular but also extends to the clinical presentation of the disease. For example, patients with the same stage of HCC can have vastly different outcomes, suggesting that current staging systems may not fully capture the complexity of the disease[67]. Moreover, the TME, which includes immune cells, fibroblasts, and vascular cells, can also vary significantly between patients and influence treatment response.

AI in multi-omics of HCC

Artificial intelligence (AI) has the potential to transform the analysis and application of multiomics data in HCC. AI is being utilized to analyze complex datasets generated from multiomics platforms.

AI in biomarker discovery for HCC

Atezolizumab is an immunotherapy that works by blocking a protein called PD-L1, which helps tumors hide from the immune system; bevacizumab is an antibody that inhibits a protein necessary for the growth of blood vessels that feed tumors (antiangiogenic therapy). This study used a retrospective multicenter approach to develop a predictive model, called ABRS Prediction (ABRS-P), for estimating the atezolizumab-bevacizumab response signature (ABRS) in patients with HCC[68]. ABRS is a biomarker associated with progression-free survival (PFS) in patients treated with atezolizumab and bevacizumab[66]. The ABRS-P model was developed using a previously established machine learning pipeline called clustering-constrained attention multiple instance learning (CLAM). The pipeline may be designed to address the complexity of histological sections and identify patterns that predict ABRS expression. The model was trained on a multicenter dataset of the Cancer Genome Atlas (TCGA), which included samples of patients who underwent surgical resection (n = 336). The study shows that the application of AI to analyze digital slices of HCC can be used as a biomarker for PFS in patients treated with the combination of atezolizumab and bevacizumab. This is a new application of AI in precision medicine, where computational models can help predict a patient's individual response to treatment. Compared to traditional biomarker discovery methods, AI-based biomarkers can be developed quickly and at a relatively low cost. This is particularly important in the field of oncology, where timely and cost-effective diagnostics can have a significant impact on treatment decisions and patient outcomes. By using AI to analyze histological slides and spatial transcriptomic data, the study improves the understanding of how different treatments work at the molecular level. This deeper insight could lead to better treatment strategies, the identification of new drug targets, and the development of adjunctive diagnostics.

AI in HCC classification and early detection

Many other studies show that machine learning algorithms can identify patterns and correlations in large datasets that might be missed by traditional analysis methods[68]. For instance, AI has been employed to classify HCC subtypes based on gene expression profiles, predict patient outcomes, and identify potential therapeutic targets. As AI algorithms become more sophisticated and data sets grow larger and more diverse, the potential for AI in HCC research and treatment is vast. Future applications may include real-time analysis of patient data to guide personalized treatment strategies and the development of dynamic models that can predict disease progression and response to therapy. AI could also play a role in the early detection of HCC by analyzing multi-omics data to identify individuals at high risk of developing the disease. A liquid biopsy technique called DELFI used whole-genome cell-free DNA fragment analysis to evaluate 724 individuals, including HCC patients or individuals at average or high risk for liver cancer, from the United States, the European Union, and Hong Kong, China[69]. Among these 724 plasma samples, 501

were collected in the United States and the European Union, including samples from 75 HCC patients, to train and validate machine learning models, which are powered by AI and use data and algorithms to improve accuracy. To further verify the results, 223 plasma samples from individuals in Hong Kong were analyzed, including 90 HCC patients, 66 patients with hepatitis B virus (HBV), 35 patients with HBV-related cirrhosis, and 32 samples from individuals without potential risk factors. The DELFI technology demonstrated the ability to detect liver cancer at its early stages with an overall sensitivity or accuracy of 88% and a specificity of 98%, indicating a very low likelihood of false-positive results in average-risk populations. For high-risk individuals, the test achieved a sensitivity of 85% and a specificity of 80%, further emphasizing its potential as a reliable tool for early detection in these groups.

Future trends in HCC precision medicine

Integrative approaches

The future of HCC precision medicine lies in the integration of multi-omics data with clinical information to create a comprehensive view of each patient's disease. This will enable the development of personalized treatment plans that take into account the unique molecular characteristics of each patient's tumor. For example, integrating genomic data with clinical outcomes can help identify patients who are likely to respond to specific targeted therapies.

Therapeutic personalization

As our understanding of the molecular basis of HCC improves, so too will our ability to develop targeted therapies. This will lead to a shift from a one-size-fits-all approach to treatment to one that is tailored to the specific molecular profile of each patient's tumor. Personalized medicine will also involve the use of pharmacogenomics to predict how individual patients will respond to specific drugs, allowing for the optimization of treatment regimens.

Early detection and prevention

Multi-omics technologies have the potential to improve early detection of HCC, which is critical for improving patient outcomes. By identifying individuals at high risk of developing HCC, we can implement preventive measures and early interventions. This could involve the use of liquid biopsies to detect ctDNA or tumor-educated platelets, which can serve as early indicators of HCC development.

Immunotherapy and combination therapies

The success of immunotherapy in other cancers has sparked interest in its potential for HCC. Multi-omics analysis can help identify patients who are likely to respond to immunotherapy and may also reveal synergistic effects when combined with other treatments. For example, the identification of specific immune cell populations within the TME could inform combination therapies that enhance immune responses.

CONCLUSION

HCC is a globally significant health burden, with multi-omics technologies playing a pivotal role in advancing our understanding and treatment of the disease. Our review synthesizes the current landscape, highlighting the impact of genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiome studies on HCC. Multi-omics has been transformative for HCC, providing a rich dataset for diagnosis, prognosis, and therapy. As we move toward personalized medicine, the challenge lies in harnessing this wealth of information to develop more effective treatments and improve patient outcomes. The future is poised to see a reduction in HCC's global impact through the continued integration of multi-omics data into clinical practice.

DECLARATIONS

Authors' contributions

Contributed to the conception and design of the study: He J, Hu X, Chen L, Jiang Y

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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