Chapter

Research Frontier of Accurate Diagnosis and Treatment Guided by Molecular Typing of Hepatocellular Carcinoma

Haicaho Zhao, Changzhou Chen and Jiefeng He

Abstract

Liver cancer will continue to be a major disease threatening the lives and health of our people in the next few decades. In recent years, with the development of early diagnosis and treatment of liver cancer, precise liver resection, and the development of targeted and immunotherapeutic drugs, the survival rate of liver cancer patients has been improved. Nevertheless, due to the high heterogeneity of liver cancer, patients with liver cancer in the same clinical stage still have great differences in response to treatment and prognosis. New staging and classification indicators are urgently needed to facilitate accurate diagnosis and treatment of liver cancer, so as to further improve the survival rate of patients. The continuous progress and development of multi-omics technology, single-cell technology, tumor molecular visualization technology and medical artificial intelligence, etc., make the molecular classification of liver cancer more and more approaching the true nature of tumor biological characteristics, thus contributing to the accurate diagnosis and treatment of liver cancer.

Keywords: hepatocellular carcinoma, tumor heterogeneity, molecular typing, diagnosis, treatment

1. Introduction

Liver cancer is a major disease that seriously threatens the lives and health of our people. In recent years, the clinical diagnosis and treatment of liver cancer and innovative research have made remarkable progress. Nevertheless, due to the high heterogeneity of liver cancer, patients with liver cancer of the same clinical stage still have great differences in response to treatment and prognosis. There is an urgent need for new staging and classification indicators to facilitate accurate diagnosis and treatment of liver cancer, so as to further improve the survival rate of patients.

Liver cancer is considered to be one of the most heterogeneous tumors [1]. Due to the high heterogeneity of liver cancer, no "cancer-dependent genes" related to liver cancer have been found so far, which makes the therapeutic effect of molecular targeted therapy of liver cancer very small and lacks theoretical basis [2]. The heterogeneity of liver cancer includes inter-tumor heterogeneity and intra-tumor heterogeneity, both of which are distinguished from each other and closely related.

Among them, there are both genetic heterogeneity and microenvironment heterogeneity. Tumor heterogeneity indicates the insufficiency of "genetic characteristics and microenvironmental information obtained from a single biopsy", which has important theoretical value and clinical significance for studying the development history of individual liver cancer, overcoming drug resistance, and achieving individual precise treatment. The continuous progress and development of multi-omics technology, single-cell technology, tumor molecular visualization technology, and medical artificial intelligence have brought the molecular classification of liver cancer closer to the true nature of tumor biological characteristics, thereby helping the implementation and health of accurate diagnosis and treatment of liver cancer China's strategic planning.

2. Molecular typing based on transcriptome

With the progress of gene chips and second-generation sequencing technology, it is possible to analyze tumor gene expression changes without bias at the whole genome level, and the molecular typing of liver cancer first started from the exploration of transcriptomics. The gene microarray analysis of primary and metastatic HCC showed that the gene expression signature of primary HCCs with accompanying metastasis was very similar to that of their corresponding metastases, implying that genes favoring metastasis progression were initiated in the primary tumors. The constructed 153 gene expression markers could divide HCC into metastatic and non-metastatic types with a prediction accuracy of 78% [3]. At present, a number of studies have divided liver cancer into proliferative and non-proliferative types through transcriptomics methods, with the two molecular types each accounting for 50% [4–10]. The proliferative type is characterized by activation of PI3K-Akt–mTOR, Ras-MAPK, MET, and other cell proliferation-related signaling pathways, which are usually associated with HBV infection, and are driven by TP53 inactivation, FGF19, and/or CCND1 amplification, and has a poor prognosis. The non-proliferative type is more heterogeneous and is usually associated with alcoholic liver disease and HCV infection, with a relatively good prognosis. The proliferative type can be further divided into Hoshida S1 and S2 subtypes [4]. Strong enrichment of the WNT signature in subclass S1 compared with S2 or S3, suggesting preferential WNT activation in S1 tumors. Hoshida S2 tumors were strongly enriched in signatures of EpCAM、AFP and IGF2 positivity. The non-proliferative Hoshida S3 subtype is still heterogeneous, including the classical Wnt pathway activation subtype mediated by CTNNB1 mutation [5]. The Cancer Genome Atlas (TCGA) analyzed 363 hepatocellular carcinoma cases by whole-exome sequencing and DNA copy number analyses, DNA methylation, RNA, miRNA, and proteomic expression also. Integrative molecular HCC subtyping incorporating unsupervised clustering of five data platforms identified three subtypes:iClust1 ~ 3. Then iClust1 consisted predominantly of Hoshida S2 patients whereas iClust 2 subtype corresponds to Hoshida S3 subtype (CTNNB1 mutant subtype), and iClust 3 subtype corresponds to TP53 mutation and Hoshida S1 subtype [7]. These transcriptome-based molecular typing revealed the intrinsic molecular characteristics of liver cancer and had potential clinical significance.

3. Molecular typing based on tumor microenvironment

The immunoinflammatory microenvironment is the seventh characteristic of tumors [11]. Hepatocellular carcinoma is a typical immunoinflammatory and

microenvironment-related tumor. Imbalance of immune-inflammatory response in the microenvironment is one of the key mechanisms for the occurrence and development of liver cancer [12, 13]. It has been discovered that the prognosis model of HCC constructed by integrating microenvironmental immune response, angiogenesis, and interstitial reaction can accurately predict the recurrence and metastasis of patients after surgery, highlighting the importance of stromal biology in HCC progression [14]. Based on immune-related gene expression level in the tumor microenvironment, HCC can be divided into the type of immune activation, the depletion of immune and immune exemption, various accounts for 10% ~ 25%, including immune activation type high expression of adaptive immune-related genes, immune depletion type high expression of TGF-β mediated immune suppression and T cell depletion related genes, immune exemption type is characterized by lack of T cells and CTNNB1 mutations [8]. According to the situation of immune cell infiltration, it can be divided into three subtypes: Immune-high, Immune-mid, and Immune-low. The Immune-high subtype was characterized by increased B−/plasma-cell and T cell infiltration, and the Immune-high subtype and B-cell infiltration were identified as independent positive prognostic factors. Low immune subtypes with a high Treg/CD4 ratio had the worst prognosis [15]. Further research found that: Comprehensive liver cancer immune microenvironment score (CD3, CD27, CD68, CD103, PD1) and tumor size, degree of differentiation, the prognosis model constructed by GGT is significantly better than the traditional clinical staging, and patients can be divided into high, medium, and Low-risk 3 groups [16]. Immune microenvironment typing has a certain clinical guiding value. For example, patients with Immune exemption type characterized by CTNNB1 mutations do not respond to programmed death-receptor-1(PD-1) / programmed death-ligand 1(PD-L1) inhibitors due to the lack of T cell infiltration. In addition, β-catenin activation conferred resistance to anti–PD-1 therapy in murine models [17]. There is no doubt that microenvironmental immune cells are highly plastic and heterogeneous. The results of single-cell sequencing showed that there were 11 T cell subsets with different functional phenotypes in the HCC microenvironment. It is necessary to further elucidate the microenvironmental characteristics and regulatory mechanisms of each subtype [18]. In addition, the liver itself is the most common metastatic organ for liver cancer, and the interaction between liver cells and immune cells creates a "metastasis-promoting microenvironment." The results of a number of studies have shown that the microenvironment of the adjacent liver tissue or tumor junction area plays an important role in the invasion and metastasis of liver cancer [19, 20]. Studies have shown that 17 inflammatory cytokine gene expression markers such as CSF1 can divide the adjacent tissues into metastasis-promoting microenvironment type and anti-metastatic microenvironment type, among which metastasis-promoting microenvironment type has high expression of Th2 cytokines and low expression of Th1 Cytokine as a feature [21]. Hoshida *et al.* [22] analyzed the expression profile of adjacent tissues of liver cancer and found that gene expression markers composed of 186 genes related to liver function and inflammation can divide liver cancer into good prognosis and poor prognosis. The poor prognosis is characterized by late recurrence, suggesting that the gene markers in the adjacent tissues may be related to the new liver cancer.

The presence of multifocal tumors, developed either from intrahepatic metastasis (IM) or multicentric occurrence (MO), is a distinct feature of hepatocellular carcinoma (HCC). The results of the study show that there are significant differences between IM and MO tumors, and their immune microenvironment also shows temporal and spatial heterogeneity: IM has fewer T lymphocytes and abundant M2 macrophage infiltration, while MO has higher Suppressive immune checkpoints, which also resulted in immune editing mainly occurring in MO rather than IM.

Similar to the mutation profile, the neoantigens and TCR components shared in tumors are higher in IM patients, but very few in MO. In addition, the loss of HLA heterozygosity occurs in 17% of multifocal liver cancers, which prevents a large number of predicted neoantigens from being effectively presented to the immune system and reduces the actual mutation load, especially in IM patients [23].

Immune inflammatory cells in the tumor microenvironment are not only an important prognostic factor but also determine their response to specific treatment methods, especially tumor immunotherapy. With the advancement of flow cytometry, immunostaining, and biological information technology, we can identify and classify the microenvironmental immune cell population with unprecedented precision. Immune inflammatory cells in the liver cancer microenvironment have significant inter-tumor heterogeneity and intra-tumor tissue heterogeneity in terms of density, location distribution, phenotype, and functional status; while the migration and differentiation of immune cells in tissues have temporal and spatial differences Qualitatively, liver cancer cells use this characteristic of immune cells to dynamically domesticate and edit them, leading to local immunosuppression, suggesting the plasticity of the liver cancer microenvironment [24–26].

4. Molecular typing based on proteome

Proteins are the direct executors of life activities and proteomics is one of the effective methods to search for molecular markers. The molecular characteristics of 110 cases of early hepatocellular carcinoma were analyzed and compared by proteomics [27]. The heterogeneity of early hepatocellular carcinoma was divided into the subtypes S-I, S-II, and S-III, each of which has a different clinical outcome. TGF-β and other tumor proliferation-related proteins were highly expressed in the S-III subtype, which was consistent with the Hoshida S1 subtype, and the prognosis was poor. S-II and S-I subtypes were characterized by high expression of Wnt and CTNNB1, consistent with Hoshida S2 and S3 subtypes. Proteomics is also an effective way to find drug targets. At present, the direct targets of liver cancer-targeted drugs with multi-kinase inhibitors and immunotherapy with immune checkpoint inhibitors are all proteins. S-III, which is characterized by disrupted cholesterol homeostasis, is associated with the lowest overall rate of survival and the greatest risk of a poor prognosis after first-line surgery. The knockdown of sterol O-acyltransferase 1 (SOAT1)-high expression of which is a signature specific to the S-III subtype-alters the distribution of cellular cholesterol, and effectively suppresses the proliferation and migration of hepatocellular carcinoma. Finally, on the basis of a patient-derived tumor xenograft mouse model of hepatocellular carcinoma, that treatment with avasimibe, an inhibitor of SOAT1, markedly reduced the size of tumors that had high levels of SOAT1 expression, which indicates that SOATI may become a new target of S-III subtype, namely Hoshida S1 subtype liver cancer [27]. Gene mutation induced by aristolochic acid is a characteristic pathogenic factor in China and even in Asia except for viral hepatitis B [28]. The mutation "fingerprint" of aristolochic acid is significantly positively correlated with tumor mutation burden, tumor neoantigen burden, CD8+ T cell infiltration, and immune microenvironment tolerance, suggesting these patients may benefit from immunotherapy [18]. On the other hand, the microenvironment of CTNNB1 mutation patients is immuno-privileged and may not benefit from immunotherapy. Further multi-omics analysis of liver cancer found that CTNNB1 mutation is related to the phosphorylation of serine 36 in ALDOA (fructose-1,6-bisphosphate aldolase) [29]. ALDOA phosphorylation promotes tumor cell proliferation by promoting anaerobic glycolysis and knocking

down ALDOA significantly inhibits tumor proliferation. Therefore, ALDOA may be an important potential therapeutic target for CTNNB1 mutant liver cancer.

5. Molecular typing based on metabolic characteristics

Cell metabolism is downstream of gene regulation and protein action network, reflecting the terminal information of life activities. The liver is the largest metabolic organ of the human body, and metabolic reprogramming undoubtedly plays an important role in the occurrence and development of liver cancer [30]. Multi-omics research results show that glycolysis and fatty acid metabolism are up-regulated in liver cancer tissues, while liver-specific metabolic pathways are down-regulated in liver cancer tissues, such as gluconeogenesis, detoxification, bile acid metabolism, and urea-ammonia metabolism [23]. The combined markers of glycine cholic acid and phenylpropionate tryptophan identified based on metabonomics technology can accurately diagnose liver cancer 1 year in advance [31]. The high heterogeneity of the liver cancer mutation spectrum and expression spectrum will inevitably lead to the heterogeneity of its metabolome level. By constructing a genome-scale metabolic network model, liver cancer can be divided into iHCC type 1 to 3. iHCC1 showed the highest fluxes in the metabolism of amino acids, cofactors and coenzymes, pyruvate, fatty acid oxidation, carnitine shuttle, steroids, TCA, and oxidative phosphorylation. iHCC2 exhibited specific features including lower fatty acid biosynthesis and high glutamine metabolism, and β-catenin–associated up-regulated fatty acid oxidation. Finally, iHCC3 tumors were associated with multiple features of malignant tumors, including hypoxic behavior, epithelial-to-mesenchymal transition, higher fluxes in fatty acid biosynthesis, and a strong Warburg effect [32]. Whether tumor metabolic reprogramming is the initiating factor of cancer or the accompanying result of cancer, there is still much controversy. Preliminary research results show that amino acid metabolism-related genes such as proline synthase PYCR1 play an important role in the occurrence and development of liver cancer [33].

6. Conclusion

In recent years, many breakthroughs have been made in the treatment of liver cancer. Following sorafenib, lenvatinib, regorafenib, cabozantinib and combination therapies centered on immune checkpoints have come out to promote the progress of liver cancer drug treatment. However, due to the high heterogeneity of liver cancer, the overall effectiveness of the above drugs is still limited. Accurate molecular classification of liver cancer not only contributes to the decision-making of individualized diagnosis and treatment of liver cancer, and personalized drug treatment, but also greatly deepens clinicians' understanding of the complexity and heterogeneity of liver cancer, so as to formulate a more accurate and effective treatment strategy. The new molecular typing system should be closely integrated with clinical-pathological information, which can not only reflect changes at the molecular level but also have guiding significance for clinical diagnosis and personalized treatment or predicting prognosis. The author believes that with the progress and development of multi-omics technology, single-cell technology, tumor molecular visualization technology, and medical artificial intelligence, the molecular classification of liver cancer will become closer and closer to the essence of tumor biological characteristics, and ultimately achieve disease precision treatment.

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