Proceedings of ICMMGH 2026 Symposium: Cell and Molecular Biology: Understanding Immune Cell Functions
DOI: 10.54254/2753-8818/2026.PJ31758

Research Progress on Molecular Subtypes and Transdifferentiation Mechanisms of Hepatocellular Carcinoma

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Abstract. Hepatocellular carcinoma (HCC) occurred with high occurrence rates and fatal incidence, and both clinical and basic research have challenges because HCC is highly heterogeneous and resistant mechanisms exist. Recently, assisted by further advances in molecular biotechnology, the HCC cells were categorized into different molecular subtypes, and the difference of gene expression profiles for various HCC subtypes was defined. These subtypes have clear differences in gene mutation signatures and the statuses of different signalling pathways, which not only determine the clinical behaviours of the tumours, but are also closely associated with both patient prognosis and drug responses. During molecular subtype evolving to cell phenotype, transdifferentiation also plays a role in promoting hepatocytes to return to biological lineage plasticity to respond to a certain microenvironment or gene mutation, developing into a stem or progenitor cell-like characteristic, and improving the plasticity and invasion capacity of tumors. Epithelial-mesenchymal transition (EMT) is an irreplaceable part of this process, giving HCC cells greater plasticity and drug resistance, particularly in high-risk subtypes. With the detailed molecular subtyping of HCC, the researchers can now not only more accurately and precisely recognise different clinical subtypes of disease but also provide a basis for identifying subtype-specific biomarkers and subtype-specific targeted therapies to optimise treatment outcomes and tackle the complexity of tumour heterogeneity. Not only is analyzing the mechanism of transdifferentiation helping to understand how HCC may develop but also helping to understand how some subtypes of HCC will have different outcomes as a result of different mechanisms, leading to better prognosis and more effective treatment in certain highly resistant subtypes.

Keywords: Hepatocellular carcinoma, Transdifferentiation, Epithelial-mesenchymal transition

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide [1]. The tumour heterogeneity and poor clinical outcome of HCC are well known; it is notoriously difficult to diagnose and treat and metastasises aggressively. Cholangiocarcinoma (CCA) is another common

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hepatobiliary cancer, which is even more challenging in terms of diagnosis, treatment, and prognosis [2]. Early transcriptome studies showed HCC molecular subtypes that predict prognosis; subsequent studies have described better evidence for the existence of HCC subtypes, involving both cholangiocytes and stem cells with specific gene characteristics, and these have been more invasive and have a worse prognosis than classic HCC [3].

Classical diagnosis for HCC is based on histopathology. Classical HCC diagnosis has the problem that tumours that resemble HCC biologically tend to have very different courses and outcomes and may suggest different molecular drivers exist. Heterogeneity of HCC is manifested in many aspects, for example, histological aspects, genomic alterations, clinical course, outcomes, responses to treatments, etc. This heterogeneity poses significant challenges to clinical management. Traditional pathological classification only depends on the overall morphology of lesions and requires even more detailed molecular subtypes to stratify "diseases" on the basis of the driving molecular mechanisms to identify subtype-specific biomarkers and effective targeted therapies for resolving the challenges due to HCC heterogeneity and the optimal personalized treatment.

Transdifferentiation refers to the transmutation of one fully differentiated mature cell type into another, at a condition not involving a multipotent state of this cell type. It is seen in hepatic cells, where hepatic stem cells are bipotent and can differentiate into hepatocytes or cholangiocyte progenitor cells. This developmental homogeneity provides the potential for their interconversion [4]. This more complex and dynamic model of tumorigenesis explains, in part, the fact that clinical and molecular evidence for intermediate (tumours that have both HCC and CCA characteristics) tumours exists. Transdifferentiation can both facilitate tumorigenesis and also contribute to drug resistance. Understanding this mechanism is important to understand HCC pathogenesis, prognosis, and overcoming resistance, in particular, high-risk HCC subtypes.

2. Genomic characteristics of HCC subtypes

With increased high-throughput sequencing technologies such as RNA-seq, HT-microarrays, and single-cell sequencing, we are now able to analyse the pattern of HCC tumor activities in much more accuracy. This way, the research scientists can also differentiate specific signatures, connected to each carcinogenic process, existing in the tumors. Classification systems that encompass each of these different features are important because they emanate from new sequence technologies and new technologies that allow investigators to assess functional genomics data on a large scale in a way that is not possible to examine in its entirety by more traditional methods (such as the tumour pathogenesis).

HCC can be traditionally separated into two groups: proliferation and inflammation. Inflamed subtypes usually feature the high expression levels of immune genes, with a relatively stable genome, and exhibit a slower clinical progression and better prognosis. On the contrary, proliferation subtypes normally express at a high level cell cycle-related genes or cancer-related genes, which are relatively complicated and heterogeneous. The Cancer Genome Atlas (TCGA) further divides HCC into multiple subtypes, including S1, S2, S3, and S4 subtypes [5]. Each of these subtypes is identified by a distinct gene expression profile, which harbours different molecular pathways and clinical behaviour.

Regarding transdifferentiation in liver cancer, there exists a CC-like HCC subtype. Although this subtype is histologically diagnosed as typical HCC, its overall gene expression profile closely resembles that of CCA and is associated with embryonic stem cell characteristics, suggesting that it may have undergone lineage transformation. Patients with this subtype generally exhibit stronger invasiveness and a worse prognosis. Recent studies of more typical HCC tumours have identified 2

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molecular types that are not seen with typical HCC tumours: CCA-like subtype and the Blast-like subtype. The CCA-like subtype maps to the CC-like HCC subtype, and the Blast-like subtype has a transcriptomic likeness to hepatoblastoma [6]. The key driving events, characteristics of signalling pathways of the CCA-like subtype generally endow it with its strong tendency to give rise to cholangiocyte differentiation. At the level of driver genes, it is enriched for genes with classical cholangiocarcinoma hotspot mutations, such as IDH1/2 and BAP1 (the two key driving events for its "cholangiocyte-like" phenotype). Additionally, the mutation rate of conventional HCC driver genes, such as CTNNB1, is very low in this subtype. As for the signalling pathways, pathways closely related to cell lineage reprogramming and epithelial–mesenchymal transition (EMT) are activated predominantly in CCA-like tumours, especially in the NOTCH, TGF-β, and WNT signalling pathways. This is consistent with previous mouse studies where synergistic activation of NOTCH and AKT signalling was shown to be the main element driving transdifferentiation of hepatocytes into cholangiocytes [7]. In contrast, the Blast-like subtype's molecular features indicate a dedifferentiated and highly proliferative cell state. Its principal driving genetic events are the occurrence of mutations in TP53 and the aflatoxin point mutation R249S. This subtype also has strong activation of the pathways affecting cell cycle progression and is, therefore, also fast proliferating.

3. Mechanisms of transdifferentiation

During cellular differentiation and reorganization, EMT is an inevitable middle-point step, and the high plasticity of cells after undergoing EMT serves as the functional basis for cellular transdifferentiation. EMT is an important event of hepatocyte dedifferentiation in HCC and equips tumor cells with the function of cell migration, invasion, and resistance to treatment. Under certain pathologies, hepatocytes undergo dedifferentiation and lose their mature epithelial characteristics to obtain mesenchymal characteristics, while also being activated with stem cell characteristic markers, indicating dedifferentiation has occurred. It is reported that hepatocytes and cholangiocytes can both transdifferentiate into each other, despite being different types of cells that execute different final functions at terminal differentiation [8]. Such plasticity of cells plays a key role in the onset and progression of HCC, particularly in high-risk molecular subtypes. Notably, the epithelial-mesenchymal transition process can be coupled with gaining 'stemness.' Hepatocellular carcinoma cells going through EMT will often express several stem-cell surface markers [9]. And those cell features strongly enhance tumour cell survival and stimulate the self-renewal and differentiation ability of tumour cells, inducing intratumour heterogeneity and increasing sensitivity to chemotherapies (including conventional chemotherapy and targeted drugs).

During HCC transdifferentiation, a few different transcription factors cooperate in controlling the cell-fate switch that drives hepatocytes away from a canonical pathway of cell differentiation to acquire cholangiocyte- or stem-cell-like cell traits. One of the major cholangiocyte/progenitor cell markers and cancer stem cell markers is cytokeratin 19 (CK19). The regulatory network associated with CK19 expression has been summarized: extracellular stimuli are transmitted through signaling pathways (TGF-β, MAPK/JNK, and MEK-ERK1/2) to the cytoplasm, which further induces the activation of important nuclear transcription factors (SALL4, AP1, and SP1) that activate the CK19 promoter [10]. Another few transcription factors, such as SOX9, HNF1β, and those involved in EMT, such as SNAIL and TWIST, have also been reported to have a role in this process. SOX9, a cholangiocyte differentiation-related transcription factor, is mainly expressed in cholangiocytes in normal liver. In a few high-risk HCC subtypes, tumour cells from hepatocytes abnormally overexpress SOX9. Aberrant expression of these markers is often associated with activation of

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NOTCH signalling, which drives the expression of cholangiocyte-specific genes, such as CK19, thus promoting transdifferentiation of hepatocytes into a cholangiocyte-like cell phenotype.

Meanwhile, TGF-β signalling is also the driver of EMT induction. The activated SMADs upregulate a cascade of EMT-related transcription factors (e.g., SNAIL, SLUG, and ZEB1), which directly repress epithelial marker genes such as E-cadherin and facilitate the mesenchymal markers' expression. Besides, the TGF-β pathway interacts with not only NOTCH but also with the WNT pathway extensively and retains the dedifferentiation and transdifferentiation states. WNT/β-catenin and NOTCH abnormal activations are closely related to the EMT process and interaction, which in turn increases the dedifferentiation phenotype. These transcription factors, however, are not working alone, but as a complex, multi-layered regulatory network. In addition, epigenetic regulatory mechanisms such as DNA methylation and histone modification have extremely important roles in tuning up the activity of these key transcription factors, establishing the conditions for turning on and proceeding with transdifferentiation at the deep level.

4. Clinical significance and future perspectives

Traditional indicators of pathology, such as the size, and grade of a tumor do sometimes predict a certain level of heterogeneity, but they do not do so directly for a tumour. Unlike traditional indicators, transdifferentiation-related biomarkers reflect whether tumour cells are going towards a phenotypically more aggressive state; it approximates the biological behaviours and potential of a particular patient, rather than inherently reflecting the pathological situation of a certain type of tumour. Detecting such biomarkers may further subdivide HCC with similar morphology and identify subgroups that are inherently more invasive, such as CC-like HCC subtypes. In predicting HCC, transdifferentiation features with multiple important proteins may be more effective than single protein labeling. The tumor cells involved in transdifferentiation will usually present particular molecular characteristics, such as upregulation of cholangiocyte fate-related transcription factors like SOX9 and HNF1β, accompanied by downregulation of epithelial markers (e.g., E-cadherin) and upregulation of mesenchymal markers (e.g., vimentin). This comprehensive molecular map can more fully capture the degree of cell differentiation. It is possible to make a transdifferentiation scoring system using high-throughput methods like RNA sequencing: a score can be generated using the expression levels of numerous genes. Tumours with high scores may always have very unstable cellular identities and strong plasticity, which are attributes of rampant disease progression and treatment resistance. Against HCC's heterogeneity, the establishment of various molecular subtyping systems offers a theoretical basis and practical guidance. And scholars understand early that one size does not fit all for HCC, which makes sense for different kinds of HCC with different biology. At the same time, one-size-fits-all treatment cannot be the same for each HCC subgroup. However, using our molecularly defined classification, many patients will derive more benefit from certain specific treatments.

Molecular subtypes have extensive application prospects to guide targeted drug selections. For example, HCC proliferation subtypes with c-Met expression would be highly sensitive to c-Met inhibitors. HCC subtypes with active mTOR signalling pathways will also be highly sensitive to mTOR pathway inhibitors. Molecular subtypes also provide information to predict the possibility of treatment resistance. For example, subtypes of HCC that express bile duct cell markers, such as CK19, or underlie EMT are typically invasive and much more likely to develop resistance to the multi-kinase inhibitors, such as sorafenib. Identifying these high-risk patients on the basis of molecular subtypes prior to treatment can motivate clinicians to proceed with combination therapy or other therapies as early as possible and avoid futile treatment to improve the prognosis. But

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translation of molecular subtypes to clinical application still remains challenging. The current classification system mainly comes from retrospective studies, and its clinical practicality needs to be validated in large-scale prospective clinical trials. The heterogeneity of tumors within the same patient's body is another issue that cannot be ignored. A biopsy of a single site may not fully reflect the molecular structure of the entire tumor, which may lead to misclassification or omission of key information.

In the future, with the development of more reliable detection technologies for circulating tumor DNA (ctDNA), these methods can be used in a less invasive manner, making non-invasive dynamic molecular subtype monitoring possible. Meanwhile, multi-omics integration involving genomics, transcriptomics, proteomics, and clinical information addition may lead to more accurate and robust predictive models. Lastly, generation of these models may help the personalized, better management of treatment plans for each HCC patient.

5. Conclusion

This review describes briefly the situation of HCC molecular subtypes and the key role of cell transdifferentiation. Deep molecular analysis invariably classifies the HCC into different subtypes, which are correlated with disease states and patients' survival. Among them, the definition of some of the high-risk subtypes is that they have lineage plasticity and acquire properties of other liver cell types.

Understanding the process involved is crucial to unraveling this phenotypic heterogeneity. That process is transdifferentiation itself: profound reprogramming of cellular identity driven by inhibition of maintenance of liver cell-specific factors coupled to abnormal activation of regulatory transcriptional networks implicated for other lines. This reprogramming is coordinated by key developmental and stress response signaling pathways that promote the loss of epithelial features and the acquisition of progenitor-like or alternative differentiation states. It is crucial that this transdifferentiation phenotype is closely related to invasive clinical behavior. Tumors exhibiting transdifferentiation characteristics show stronger invasion ability, higher tumor heterogeneity, and greater resistance to traditional therapies. Therefore, the presence and degree of transdifferentiation are important biomarkers for identifying high-risk HCC patients, emphasizing their importance in moving beyond traditional histology towards mechanism-based disease understanding.

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