

REVIEW

Artificial Intelligence & Precision Oncology

Multi-Omics and Its Clinical Application in Hepatocellular Carcinoma: Current Progress and Future Opportunities

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Abstract Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the fourth leading cause of cancer related death worldwide. China covers over half of cases, leading HCC to be a vital threaten to public health. Despite advances in diagnosis and treatments, high recurrence rate remains a major obstacle in HCC management. Multi-omics currently facilitates surveillance, precise diagnosis, and personalized treatment

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decision making in clinical setting. Non-invasive radiomics utilizes preoperative radiological imaging to reflect subtle pixel-level pattern changes that correlate to specific clinical outcomes. Radiomics has been widely used in histopathological diagnosis prediction, treatment response evaluation, and prognosis prediction. High-throughput sequencing and gene expression profiling enabled genomics and proteomics to identify distinct transcriptomic subclasses and recurrent genetic alterations in HCC, which would reveal the complex multistep process of the pathophysiology. The accumulation of big medical data and the development of artificial intelligence techniques are providing new insights for our better understanding of the mechanism of HCC via multi-omics, and show potential to convert surgical/intervention treatment into an antitumorigenic one, which would greatly advance precision medicine in HCC management.

INTRODUCTION

HEPATOCELLULAR carcinoma (HCC) is the sixth most prevalent cancer and the fourth leading cause of cancer-related death worldwide.^[1] China has the highest incidence and mortality of HCC, while a fast increase of HCC is also observed in part of Europe and the USA.^[2] The estimated number of future affected individuals turns out to be over 1 million annually.^[3] Although surgical interventions are the mainstay of curative treatment, the highly occurred recurrence rendered the 5-year survival considerably low.^[4, 5] It urgently requires a new way to reveal the mechanism of this highly heterogeneous cancer. With the development of medical data accumulation and artificial intelligence techniques, multi-omics that integrate radiological imaging, genetic and proteomic information provides advancement in our understanding of HCC in all stages of malignant progression.

Medical imaging, as the main tool for diagnosis of HCC, evolves from simple qualitative radiological analysis into quantitative radiomics calculation, which harnesses mineable features from encrypted medical images to produce evidence-based clinical decision support system.^[6, 7] Multi-parametric and multi-modality imaging based radiomics has been widely applied in benign and malignant diagnosis, histopathological outcome prediction, and treatment response evaluation in HCC.^[8-10] The artificial intelligence based novel technique could shift forward original pathological gold standard diagnosis, thus provide a new solution in the paradigm of HCC management. From another perspective, high-throughput next generation sequencing and gene expression profiling enabled a new way to reveal the complex multistep process of hepatocarcinogenesis.^[11-13] Cancer driver gene mutations and molecular

classes of HCC are substantially elucidated based on main molecular drivers and pathways involved, or depending on the immune status of the tumor.^[14] Genomics and proteomics may provide new insights for application of systemic therapies including immune checkpoint inhibitors, tyrosine kinase inhibitors and monoclonal antibodies, as well as promotion of antitumorigenic one.

Here, this review is structured in five parts including multi-omics in the clinical workflow of HCC, design and implementation of clinical cohort for HCC omics study, methodology of radiomics, genomics and proteomics in HCC, and final summary of current limitation and future opportunities of multi-omics in HCC studies.

MULTI-OMICS IN THE CLINICAL WORKFLOW OF HCC

Clinical application of radiomics in HCC

By profiling quantitative imaging information behind human eyes and developing highly accurate predictive models based on artificial intelligence techniques, radiomics can play a pivotal role throughout diagnosis, treatment decision-making, therapeutic efficacy evaluation, and prognosis prediction of HCC.

HCC is currently the only solid tumor that could be diagnosed noninvasively without further requirement for histopathological confirmation, where investigators have explored the potential of radiomics in diagnosing HCC based on different imaging modalities,^[15-18] and most efforts have been oriented on magnetic resonance imaging,^[15-17] in particular with the aid of deep learning techniques.^[16] Furthermore, radiomics allowed accurate profiling of HCC aggressiveness; for example, tumor differentiation,^[19-21] proliferation (annotated by Ki-67 status),^[22] microvascular invasion,^[19,23-25] immune

cell infiltrates,^[26, 27] and molecular landscape.^[28] As most of these markers are beyond the assessment of naked human eyes, the fruit of radiomics is well suited to improve pre-treatment characterization of tumor biology, thereby aiding in personalized treatment decision-making. In addition to pre-treatment assessment, radiomics also showed great potential in stratifying HCC prognosis and predicting antitumoral treatment effect in the post-treatment stages, with encouraging results reported on predicting post-ablation^[29] and postoperative survival,^[8,30,31] treatment responses to transarterial chemoembolization,^[32, 33] transarterial radioembolization,^[34] tyrosine kinase inhibitors,^[35] and immune therapies.^[26]

Identification of molecular alterations during hepatocarcinogenesis

The recent development of multi-omics techniques has enabled the profiling of the molecular landscape of HCC.^[36-38,40-43] The major pathways involved in hepatocarcinogenesis comprise 1) telomere maintenance via TERT promoter mutations, 2) Wnt/ β -catenin signaling activation caused by CTNNB1 mutations or AXIN1/APC inactivations,^[36,41,42] and 3) cell cycle regulation controlled by TP53, RB1, CCNA1, CCNE1, PTEN, etc. Notably, TERT promoter, CCNA1 and CCNE1 mutations are frequently observed as a result of hepatitis B virus (HBV)-mediated insertions,^[44, 45] revealing the possible underlying mechanisms via which chronic HBV infection induces malignant hepatocyte transformation. Additionally, other genetic and epigenetic changes which involved in oxidative stress and activation of various oncogenic signaling pathways (including PI3K/AKT pathway, RAS/MAPK pathway and those of receptor tyrosine kinases^[46]) play additional pivotal roles in the pathogenesis of HCC.

Apart from cancer driver gene mutations, the synergy of risk factors also facilitates hepatocarcinogenesis. In specific, HBV infection has been shown to reinforce the toxic effect of aflatoxin B1.^[47, 48] Furthermore, PNPLA3, TM6SF2 and HSD17B13 polymorphisms were correlated with HCC development in non-alcoholic steatohepatitis (NASH) patients (chronic heavy drinkers in particular).^[49, 50]

Development of HCC molecular classification

Development of molecular classifications of HCC can aid in personalized clinical decision-making and refine tumor prognostication. Prior multi-omics studies on

HCC gene expression signatures,^[38, 39, 51-55] histopathological profiles, metabolic characteristics, and immunological traits^[56, 57] have helped to establish a comprehensive molecular and immune classification system of HCC.^[1,36,52-55]

In general, HCC can be divided into 2 major classes: the proliferation class and the non-proliferation class, each accounting for about 50% of tumors.^[46] The proliferation class is more prevalent in HCC developed in chronic HBV carriers and is associated with a more aggressive phenotype, higher serum alpha-fetoprotein (AFP) levels, and worse clinical outcomes.^[36,37,58] At the histological level, these tumors demonstrate a propensity towards poorer differentiation and are usually classified as macrotrabecular massive HCC, scirrhous HCC, or progenitor HCC.^[46,52,53,55,59] At the molecular level, the proliferation class is characterized by frequent TP53 mutations, substantial chromosomal instability, global DNA hypomethylation, and activation of signaling pathways involved in cell cycle regulation.^[38,52,55] This class can be further divided into two subclasses: the progenitor cell group (accounts for 25% - 30% of HCC),^{[46][52]} and the Wnt-TGF β group (accounts for about 20% of HCC). The progenitor cell group features activation of cell proliferation pathways (e.g., PI3K/AKT pathway, RAS/MAPK pathway, and IGF2 pathway),^{[37][38]} expression of stem cell markers (e.g., CK19, alpha-fetoprotein, and EpCAM), and is often immune-active. Conversely, the Wnt-TGF β group is characterized by upregulation of the Wnt-TGF signaling pathway and represents an immune-exhausted phenotype driven by TGF β -mediated CD8⁺ T cell exhaustion.^[56]

By contrast, the non-proliferation class is more common in alcohol-/NASH/hepatitis C virus-related HCC and is associated with a less aggressive phenotype, lower serum AFP levels, and better clinical outcomes.^[36,37,58] At histology, these tumors are usually well-differentiated and display the steatohepatitic or CTNNB1 mutated phenotypes.^[37, 60] At the molecular level, the genetic and epigenetic profile of these tumors mirrors that of mature hepatocyte,^[37] and is characterized by frequent mutations in TERT promoter and CTNNB1 and chromosomal stability. The non-proliferation class is also further divided into two subgroups: the Wnt/ β -catenin subgroup and the G4 subgroup.^[46, 61] The Wnt/ β -catenin subgroup constitute a homogenous subtype with mutations in CTNNB1,^[61] upregulation of the Wnt/ β -catenin signaling pathway, and histological traits featured by well differentiation,

microtrabecular and psudoglandular architecture, and intratumoral cholestasis. Of note, the Wnt/ β -catenin subgroup represents a group of immune-excluded tumors with a paucity of CD8+ T cell infiltrates and increased regulatory T cells,^[46,55,56] thus primarily resistant to the immune-checkpoint inhibitors.^[62] In contrast, the G4 subgroup displays the steatohepatic phenotype with activation of the IL6/JAK/STAT pathway, and is generally immune-active.^[60]

DESIGN AND IMPLEMENTATION OF CLINICAL COHORT FOR HCC OMICS STUDY

Clinical cohort design

Similar to the population- or community-based cohort design, clinical cohort is a type of nonexperimental or observational study design, but focuses on patients other than general population. In a clinical cohort study, the participants (*e.g.*, HCC patients) do not have the outcome of interest to begin with. They are selected based on the exposure status of the individual. They are then followed over time to evaluate the occurrence of the outcome of interest. A cohort study is the best way to identify incidence and natural history of a disease (*e.g.*, HCC progression), and can be used to examine multiple outcomes after multiple exposures (**Figure 1**).

Types of cohort design

Prospective cohort

In prospective cohort, the investigators conceive and design the study, recruit participants, and collect baseline exposure data on all participants, prior to any of the participants have developed any of the outcomes

of interest (**Figure 2**). The weakness of a prospective cohort design is relative long follow-up time and high economic cost, which makes it inefficient for studying rare clinical outcome and difficult to replicate in future studies. However, designers can intensively define the incidence and causes of a condition, measure a variety of variables including multiple exposures, confounding factors, and clinical outcomes.

For example, a nationwide prospective cohort study of 8,510 HCC patients in Japan was conducted to investigate the overall survival rate of unresectable HCC after transcatheter arterial lipiodol chemoembolization, and reveal the factors affecting the survivals. After 8 years of follow-up, 3,605 patients died. The 5-year survival rate was 26%, and the degree of liver damage, TNM stage, and AFP value were determined as risk factors for HCC survival.^[63]

Retrospective cohort

In retrospective cohort, both the exposure and outcome are ascertained retrospectively, and pre-existing records are used to reconstruct the sequence of events (*i.e.*, exposure and outcome are past events at the study baseline). Retrospective cohort is seen as the inexpensive and efficient design for studying rare events and is an attractive alternative in hospital settings, where it is feasible to find good-quality medical records. However, unlike prospective cohort design, researchers have limited control over the construction of the sample and the quality of the monitoring. For example,^[64] Kim S *et al.* designed a retrospective cohort to evaluate the prognostic value of a preoperative radiomics model in predicting recurrence after curative resection in HCC, and pointed out that the radiomics model showed a prognostic performance.^[65]

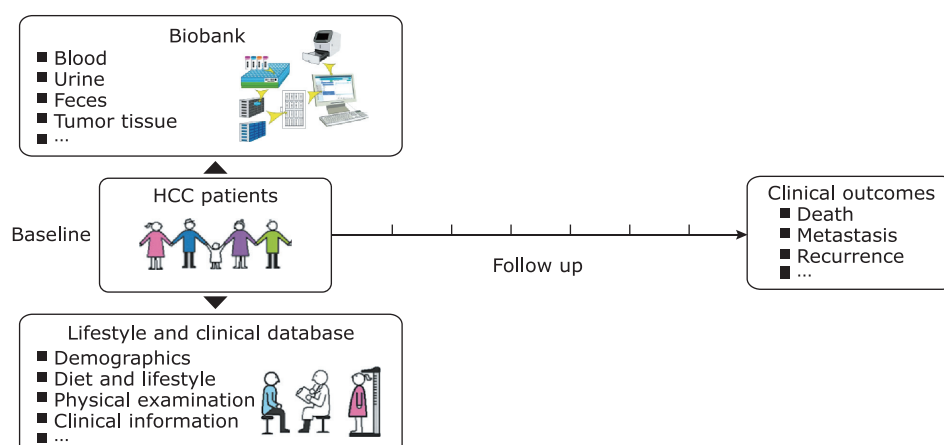


Figure 1. Features of a cohort study

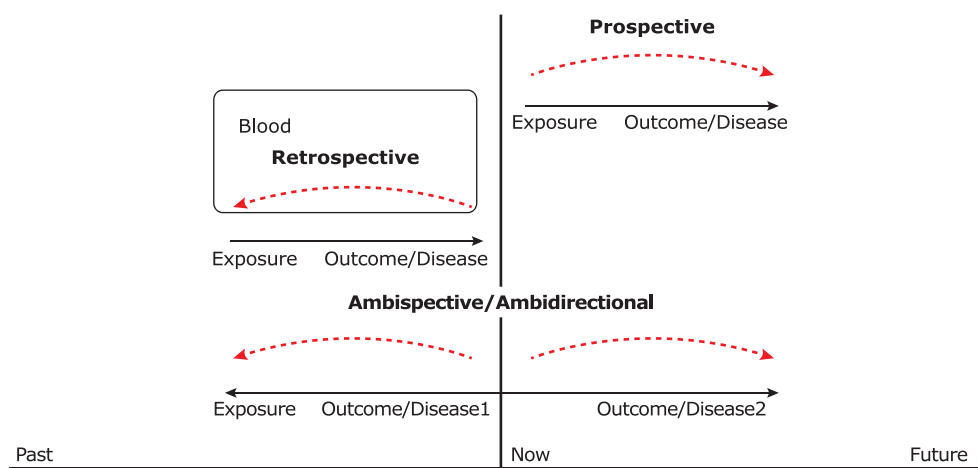


Figure 2. Types of cohort design

Dashed red lines with arrows denote when and how data collection occurs.

Ambispective or ambidirectional cohort

There are both retrospective and prospective phases of the study in ambispective or ambidirectional cohort. This design uses pre-existing exposure data, documents, clinical outcomes, and allows for prospective/future monitoring and follow-up. It shares elements of the advantages and disadvantages of retrospective and prospective cohort, and is more suitable for investigating exposures that may have both short and long effects, or more than one outcome.^[66] For example, an ambispective cohort study was conducted to identify predictors of short-term mortality, major bleeding, and venous thromboembolism recurrence among cancer patients.^[67]

Challenges in the establishment of clinical cohort

Selection bias

Because only a finite number of liver cancer cases are recruited in the cohort, selection bias is an unavoidable issue. The selection of cancer cases from one or a few hospitals may cause bias considering patients have chosen the one or few hospitals based on referral or their own preference. To reduce bias due to differential hospital selection by patients, one may consider using multi-center cohort design.

In multi-omics cohort study, a biospecimen (e.g., blood, tumor tissue, and urine) as well as imaging (for radiomics analysis) retrieval rate is almost inevitably less than 100%. In this case, if specimen or imaging availability is correlated with patient outcome, the selection bias may occur. For example, tumor tissue availability could be related to tumor size and thereby

be associated with liver cancer prognosis. Several analytical methods such as inverse probability-of-censoring weight (IPW) method can be carried out to correct such bias.^[68]

Other selection bias include loss to follow-up bias. It normally occurs when the dropout rates are different between study groups (exposure status), or the patients who drop out are different from those who do not drop out.^[69] Some suggested that <5% loss leads to little bias, while >20% poses serious threats to validity. Training interviewers and developing mechanisms to retain and contact participants are ways to reduce such bias.^[70]

Other bias

In clinical cohort design, errors in measurement can lead to misclassifications of both the exposure status of the participant and the clinical outcomes of interest. This should be considered and handled during the design process, with all criteria clearly defined from both clinical and operational perspectives (e.g., threshold values, scales to be used, and laboratory methods). In addition, the questionnaires that were used to collect post-diagnostic factors (e.g., alcohol use, smoking, diet, and physical activity) need to be validated, and the interviewers should be trained.

Confounding bias often occurs in cohort studies. Confounding variable is associated with both exposure and clinical outcome of interest, but not a result of exposure. Beside randomization and matching in the study design phase, stratification (e.g., Mantel-Haenszel methods), or adjustment for known confounding variables in the multivariable-adjusted model

could avoid or decrease such bias.

Sample size

Smaller sample size can cause lower statistical power with a large variation of an effect size estimate and wide confidence limits; and may also lead to selection bias and publication bias,^[71] which may preclude further intensive analysis and hamper the robustness of the conclusion. Sample size is particularly important in HCC omics cohort study, because multiple exclusions were conducted in the omics study based on availability of specimen and valid assay results, even if the parent HCC cohort is large-scale. In addition, HCC subtyping according to omics data that guide precision medicine is increasing in future study, which requires larger sample size. Nonetheless, single-center omics study generally has limited sample size due to the low incidence of HCC worldwide, particularly in Europe and North America. Therefore, a large-scale, multi-center HCC cohort is essential for future omics study.

Biospecimen issues

Biospecimen collection and processing for multi-omics study are major concerns in the liver cancer cohort establishment. Although individual genome datum does not change across lifespan, profiles of other omics targets, such as metabolites and proteins, could be affected by many factors, such as treatments, cancer stage, fasting status, genetic variations, diet, and other lifestyles. Therefore, detailed information on these factors should be collected to evaluate their influence on omics data, ideally in a pilot study. Based on the evaluation, stringent protocols for the collection, transfer, processing, and storage of biological samples suitable for omics analyses need to be developed during the design process. Regarding influence of factors that were not feasible to be addressed or were not sufficiently addressed in the design, calibration or further adjustment for these factors in the data analysis stage can be considered.

Data integration

The integration of multi-omics data, or the integration of omics data and non-omics data such as demographics (e.g., age, sex, and social economic status), clinical features (e.g., HBV and HCV infection status, AFP, tumor size), and post-diagnostic lifestyles (e.g., alcohol, smoking, diet, and physical activity after treatment or cancer diagnosis) can help revealing novel risk factors of liver cancer prognosis, illuminate mechanisms

for hepatocarcinogenesis and disease heterogeneity, increase the predictive ability of prediction model, distinguish patients with positive response to treatment, and thereby assist precision medicine. Nonetheless, such data integrative efforts are still in their infancy, partly due to the lack of analytical strategies towards the data integration.^[72, 73] Another possible reason is the lack of multi-disciplinary research team and collaborations.

Multi-disciplinary collaboration and cross-training

Using cohort design to conduct omics study for liver cancer requires expertise of diverse fields, including but not limited to epidemiology, biostatistics, hepatology, radiology, and clinical oncology. Hence, a collaborative research team is essential, and cross-training and education are extremely important to advance this interdisciplinary area of science.

METHODOLOGY OF RADIOMICS IN HCC

Main development process of radiomics concluded four steps: encrypt imaging quantification, radiomics signature construction, relevant clinical factor selection, and final integrated radiomics model construction. Enrolled imaging dataset should be partitioned into training and validation datasets using random split, time-independent split, or center-independent split. Preprocessing of the imaging dataset is required via resampling and intensity normalization before radiomics analysis.^[74, 75]

Encrypt imaging quantification

To quantitatively extract radiomic features from imaging data, regions of interests (ROIs) should be initially delineated. It exists two ways for segmentation of ROIs, which are manual segmentation and semi-automatic/automatic segmentation. Considering the vague boundary of tumor lesion, most radiomics studies on HCC apply manual segmentation. Feature extraction comes after the step of ROI segmentation. The radiomic features could be classified as four types: shape and size, first-order statistics, textural, and wavelet features. The geometric features describe three-dimensional shape and size of the tumor lesion. The first-order statistics features describe the histogram distribution of the voxel intensities and are contained within the ROI. The textural features describe the heterogeneous texture patterns of the tumor lesion

and are based on the texture matrixes of Gray Level Co-Occurrence (GLCM), Gray Level Run-Length (GLRLM), Gray Level Size-Zone (GLSZM), Gray Level Distance-Zone (GLDZM), Neighborhood Gray Tone Difference (NGTDM), and Neighboring Gray Level Dependence (NGLDM). Beside the original image, 8 filtered images are usually acquired by decomposing the original image in low- and high-frequencies using a three-dimensional wavelet transformation. For feature robustness analysis, the feature stability and reproducibility are examined by concordance correlation coefficients (CCCs) and intra-class correlation coefficients (ICCs), respectively. It randomly chooses 10% - 20% of enrolled patients to accept multi-segmentations, including multiple clinician segmentation, multiple time-point segmentation, and perturbation segmentation for robustness analysis. Semantic features that are originated from empirical experience of radiologists may also be quantified, such as tumor border clearness, tumor circularity, number of nodule heave on the border, and difference between center and cavity. To ensure the feature extraction transparency, numerical values of the radiomic features computed on a set of open-access digital phantoms should be provided. Another type of quantitative features is the deep learning (DL) based features. DL features are extracted from trained network that encode radiological imaging into shape or textural data via different level of layer filters. Certain studies proved DL features could outperform manual engineered features when predicting malignancy of HCC.^[76]

Radiomics signature construction

Based on the extracted radiomic features, radiomics signature construction is carried out, which consists of two processes: feature selection and prediction modeling. Feature selection is designed to select features that correlated with targeted clinical outcome to the maximum extend. Redundant and irrelevant features are supposed to be removed. Prediction modeling is conducted to choose the befitting machine learning classifier based on selected features for prediction of the targeted clinical outcome status.

There are 16 frequently applied feature selection algorithms and 3 most commonly used classifiers in radiomics researches. A brief introduction of the algorithms and classifiers is provided online as supplementary (**Appendix 1**). DL network appears to be another important tool for radiomics signature con-

struction. Being different from manual engineered features, DL method provides an end-to-end self-learning strategy that extract high-dimension deep features automatically via convolution layers and generate an output as the signature. However, DL method requires a larger sample size to train the network compared with traditional classifiers.

Predictive clinical factors selection

Uni-variable analysis is initially conducted to pick up clinical factors correlated with targeted clinical outcome with p -value less than 0.1. Further, multi-variable analysis with forward selection likelihood ratio should be performed to select clinical factors with p -value less than 0.05. All qualified clinical factors would be defined as candidates for integrated model construction.

Integrated model construction

To comprehensively construct the gathered radiomics system for prediction of targeted clinical outcome of HCC, the clinical candidate parameters are combined with the established radiomics signatures by logistic regression or simple classifiers such as random forest (RF) or support vector machine (SVM). Heatmap are often provided to represent the correlation between the clinical factors and the radiomics signature.

GENOMICS, PROTEOMICS AND RADIOMICS IN HCC

Genomics

Since the beginning of the era of high-throughput sequencing (Next Generation Sequencing, NGS), genomics has entered a stage of rapid development. In the field of human disease research, in-depth understanding of the pathogenesis of disease has been realized. The conventional genomics workflow consists of three parts: genome library construction, gene sequencing and sequence assembly, and bioinformatics analysis. As the fundamental source of controlling various mechanisms and phenotypes of human body, genome has been highly concerned by the medical community. After scientific and rigorous sample inclusion screening, standard DNA library extraction procedures are required for tissue or blood samples. At present, NGS technology is commonly used to achieve genome sequencing at three levels: whole genome sequencing (WGS), whole exome sequencing (WES), and targeted

sequencing, which can achieve different sequencing depths, coverage and research purposes. The assembly and bioinformatics analysis of post-sequencing data is a top priority in the genomics workflow, and the detection of gene mutation sites can be the key to prevention, early detection, diagnosis, treatment, and prognosis of cancers like HCC.

Figuring out the mutant gene is the key point in the research of liver cancer. Zhang P *et al.* used a microarray dataset in the high-throughput Gene Expression Omnibus (GEO) database, combined with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) for gene annotation and pathway enrichment analysis. Corresponding protein-protein interaction network was constructed. They also confirmed 10 candidate genes and pathways affecting the low overall survival rate of HCC patients.^[81] Zhou X *et al.* identified S100A10 as a new gene in HCC through comprehensive analysis of prospective data sets and The Cancer Genome Atlas (TCGA) data, and proposed a new treatment strategy for HCC.^[82] Chen DB *et al.* demonstrated that FFX5 gene can promote HCC by activating KDM4A pathway.^[83] Huo J *et al.* integrated gene expression profiles and clinical related information in TCGA database and International Cancer Genome Consortium (ICGC) database, used Gene Set Enrichment Analysis (GSEA) to determine the difference of gene expression patterns between TP53 wild-type and mutant in TCGA cohort. Through regression analysis and survival analysis, six genes were reported and included in the construction of a prognosis model. In addition, this study found three possible causes of poor prognosis of HCC: high proliferative activity, low metabolic activity, and immunosuppression.^[84] Sun Y *et al.* obtained nearly 17,000 cells from tumor tissues and tumor-adjacent tissues of 12 patients with primary HCC and 6 patients with early recurrent HCC, and sequenced the full-length transcripts. Compared with primary tumors, the level of regulatory T cells in early recurrent tumors decreased, dendritic cells (DC) increased and infiltrated CD8+ T cells increased. For CD8+ T cells in recurrent tumors, KLRB1 overexpressed and showed a congenital low cytotoxicity state with low clonal expansion. The enrichment of these cells is related to poor prognosis. Differential gene expression and interaction analysis revealed the potential immune escape mechanism in recurrent tumor cells, which can inhibit DC antigen presentation and recruit innate-like CD8+ T cells.^[85]

Proteomics

Proteomics is an important area of research that has received a significant amount of attention in recent years with the great promise of developing biomarkers for the early detection of HCC. A common proteomics workflow is comprised of four main steps: protein extraction, digestion, data acquisition, and analysis. Proteins are part of a complex network of interacting biomolecules that regulate their function and localization within the cell. Extraction and isolation of proteins from chemical and physical interactions with other biomolecules from specific cellular subcompartments have become a critical step for their global analysis in a biological context. Then, trypsin cleaves proteins to generate peptides with a lysine or arginine residue at the carboxy terminus. High performance liquid chromatography-Mass Spectrometry (HPLC-MS) is performed to acquire the MS data for the prepared samples. Analyzing the MS data can get the peptides and proteins sequences and intensities, which are formatted into a “panel” or “matrix”. Bioinformatic methods are involved to detect the biomarkers, which serve as molecular indicators for the severities of HCC or its early response to treatment.

Identifying HCC biomarkers is an important application of proteomics methods. Li N *et al.* identified the differentially expressed protein profiles between HCC and tumor-adjacent tissues by using proteomic methods, revealed six new proteins differentially expressed in HBV related HCC, and clarified that there are common characteristics between chronic hepatitis B (CHB)-developed HCC and liver cirrhosis (LC)-developed HCC, which are potential diagnostic markers or therapeutic targets for HBV related HCC.^[86] Wei D *et al.* revealed the differentially expressed proteins between HCC induced by HBV and HCV, and provided basic profiles for further researches.^[87] Du Z *et al.* identified a combination of three proteins as the potential biomarker of HCC diagnosis.^[88]

Furthermore, multi-omics methods are interacted to illustrate the deep molecular mechanisms of HCC. Gao Q *et al.* used proteogenomics methods for paired tumor tissues and tumor-adjacent tissues from 159 patients to characterize HBV related HCC, revealed activation status of key signal pathways and liver specific metabolic reprogramming in HBV related HCC, and divided HBV related HCC into three subgroups.^[89] This study is conducive to clinical practice and disease prognosis. Zhang F *et al.* performed a combined proteomic

and metabolomic analysis of paired tumor tissues and tumor-adjacent tissues from 200 patients, showing that liver specific metabolic reprogramming and metabolic changes occur with the increase of tumor size, revealing metabolomic changes related to advanced disease and adverse clinical outcomes. Several prognostic metabolic markers involved in metabolic reprogramming of HCC were also identified, which is expected to provide effective treatment for HCC.^[90] Based on TCGA, ICGC and GEO databases, Lu S *et al.* revealed that ATP1B3 may be an oncogene and an independent prognostic factor which is related to the immune infiltration of HCC, proved the prognostic role of atp1b3 in the progression of HCC, and provided new insights into potential immune regulation and treatment strategy for HCC.^[91] Wang L *et al.* used RNA sequencing and proteomic data and corresponding clinical information from TCGA database and Clinical Proteomic Tumor Analysis Consortium (CPTAC) database respectively, identified 406 differentially expressed RNA binding proteins (RBP) between HCC tumors and tumor-adjacent tissues at transcriptional and protein levels. Among the proteins, 11 proteins were raised as the biomarker to establish the risk assessment model for grouping HCC patients. The reliability of the model was proved through the life cycle, indicating that these proteins may be used as potential drug targets.^[92]

Radiogenomics and Radioproteomics

Non-invasive radiographic images can visually display changes in the site of the disease, so as to realize the diagnosis of the disease, and experienced clinicians can also judge the patient's disease by some obvious pathological features in radiographic images. The imaging features of these lesions are highly related to the changes of the genome and proteome in human body, and it would be destructive, expensive and time-consuming to study pathology in clinical diagnosis solely by genomics and proteomics. Radiogenomics and radioproteomics are the emerging disciplines that combine radiomics with genomics and proteomics, mainly including image acquisition, tumor area artificial labeling, image features extracting, expression modules of genome and proteome setting up, disease prediction model training and model validation. Segal E *et al.* combined gene expression microarray data of HCC samples with their imaging features in three-phase contrast-enhanced CT scans, found that combinations of 28 imaging features can reconstruct 78% of

the global gene expression profiles, which revealed the related cell proliferation, liver synthetic function, and it can also represent patient prognosis.^[93] Therefore, differentially expressed genome in HCC patients can be highly characterized by non-invasive imaging features. Radiogenomics and radioproteomics are also advancing rapidly in the studies of ovarian cancer,^[94] colorectal cancer and some other cancers,^[95, 96] which is promising to open up a new era of precision medicine through the combination of multiple-omics and computer technologies such as deep learning.

CURRENT LIMITATION AND FUTURE OPPORTUNITIES

Although multi-omics has gained great progress on precise diagnosis, personalized treatment, decision making, as well as hepatocarcinogenesis mechanism revealing in HCC, it still exists several obstacles for its clinical application. Firstly, reciprocal correlations among radiomic features, histopathological outcomes, and proteomic/genomic data are unrevealed, thus cannot comprehensively and synergistically depict or substantiate tumoral heterogeneity in HCC. Secondly, high-quality cohort is yet to be built with prospective and multi-center data. Multi-institutional data sharing and intensive collaborations on data cleansing and labelling should be strengthened to solve the problem of "isolated islands of information". Last but not least, multi-omics results are very sensitive to different technical acquisition parameters, especially among different vendors. If standard-of-care acquisition could be conducted among multi-centers, it would further drive genuine knowledge via multi-omics analysis in HCC researches.

Supplementary materials

available online at <http://cmsj.cams.cn/EN/10.24920/003984>.

Appendix 1: Commonly used feature selection algorithms and classifiers in radiomics studies.

Conflict of interests

None.

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