Association of intrinsic pathways with altered tumor immune infiltration in hepatocellular carcinoma: New targets for combining immune therapy

Dear Editor,

Though cancer immune therapy has achieved benefits in partial patients’ population, the overall responses remain low, and low immune infiltration status has been deemed as a key factor leading to low response. In hepatocellular carcinoma (HCC), we found that tumor intrinsic oncogenic pathways were related to tumor infiltrating immune cells: mutation of catenin beta 1 (CTNNB1) or activation of the canonical b-catenin pathway was related to poor immune infiltration, while high expression of Kirsten rat sarcoma viral oncogene homolog (KRAS) was related to a better immune infiltration status, which provides new thoughts on combining immune therapy by targeting tumor intrinsic pathways and gives new markers for preference of treating methods.

In this study, we studied changed oncogenic pathways, as well as immune infiltration status of tumors, with bioinformatic methods, using publicly available sequencing data and somatic mutation data in TCGA (LIHC) and ICGC (LIRI JP, LICA FR) databases, which covered HCC patients from different ethics and backgrounds (Supplementary Table S1). After calculation of tumor immune infiltration levels of each patient, we found high immune infiltration levels in HCC tissues could predict better survival of HCC patients, and high Th1 cell tumor infiltration was a consistent good indicator for better survival across different datasets (Figure 1). After clustering of patients according to the Th1 cell score, we found in low Th1 cell infiltration patients, genes, related to adaptive immune response signal transduction and T cell stimulation, were suppressed across datasets, and in those patients, signals of the Wnt/b-catenin pathway and KRAS downregulated genes were highly enriched (Supplemental Figure S1). After matching sequencing data with somatic mutation information, we found that the mutation status of CTNNB1 in HCC patients was related to high expression of CTNNB1 mRNA levels in tumor; in CTNNB1-mutated patients, the Wnt/b-catenin pathway was activated with less infiltrated immune cells in comparison to patients with no CTNNB1 mutation (Supplemental Figure S2; Figure 2). Besides, in CTNNB1 mutation patients, genes concerning metabolic changes, such as xenobiotic metabolism, fatty acid metabolism and bile acid metabolism, were also enriched (Supplemental Figure S3).

Further clustering of patients according to 13 T cell populations showed in the low T cell inflammation group of patients, enriched KRAS downregulated genes (including GPRC5C, CPEB3, THRBL, CDKAL1, THNSL2, PDK2, and HNF1A) were highly overlapped between datasets of TCGA LIHC, LIRI JP, and LICA FR, and KRAS was differentially expressed between groups (Supplemental Figure S4). After comparing KRAS, Harvey Rat Sarcoma Viral Oncogene Homolog (HRAS) and Neuroblastoma RAS Viral Oncogene Homolog (NRAS) levels between high- and low-T cell inflammation groups, we found only KRAS was differentially expressed between groups. We correlated PDK2, HNF1A, and KRAS mRNA expression with 28 types of immune cell scores, and results showed KRAS was positively correlated with most immune cell types, while PDK2 and HNF1A were negatively correlated with most immune cells (Figure 3).

Former studies showed that tumor intrinsic pathways can influence immune infiltration by targeting immune regulators or chemokine axis in immune cell recruitment and differentiation. We wondered if KRAS, HNF1A, and PDK2 could also influence immune microenvironment of HCC through those mechanisms, and we further examined correlations between mRNA expression of KRAS, HNF1A, PDK2, and well-known immune regulators, as well as chemokines. It turned out that KRAS was positively correlated with a series of immune stimulators and inhibitors, and across three projects, CXCL16, TNFSF4, CD80, TGFBRI, and CCR4 were significantly correlated to KRAS’s expression, while HNF1A and PDK2 were inversely...
FIGURE 1 Infiltrating levels of immune cells were correlated to patients’ survival. (A)-(H) Survival difference between high- and low-activated B cell, CD56 bright natural killer cell, effector CD8+ T cell, eosinophil, natural killer cell, plasmacytoid dendritic cell, T follicular helper cell, and type 1 T helper cell infiltrating groups of LIRI JP project. (I)–(M) Survival difference between high- and low-eosinophil, immature B cell, macrophage, natural killer T cell, and type 1 T helper cell infiltrating groups of TCGA LIHC project.
FIGURE 2 Immune infiltration scores between CTNNB1 mutation and no mutation groups. (A)–(C) Comparison of scores for 28 immune infiltration cell types between CTNNB1 mutation and no mutation groups in projects of LICA FR, LIRI JP, and TCGA LIHC (*p < .05, **p < .01, ***p < .001) (D) Survival curves for CTNNB1 mutation and no mutation groups were similar in project of LIRI JP. (E) Survival curves for high- and low- CTNNB1 mRNA expression groups showed high CTNNB1 expression group had worse overall survival in project of LIRI JP. (F) Survival curves for CTNNB1 mutation and no mutation groups were similar in project of TCGA LIHC. (G) Survival curves for high- and low-CTNNB1 mRNA expression groups showed high CTNNB1 expression group had worse overall survival in project of TCGA LIHC.
Correlated with a wide range of immune regulators and chemokines (Additional file 1–3).

Considering the limitation of bulk data yielded by sequencing technology, we corroborated results in the Human Protein Atlas database, and we found CTNNB1 expression levels were relatively high in normal liver tissues, though carcinogenesis may additionally increase CTNNB1 protein expression. On the contrary, protein expression of KRAS was not detectable in normal liver and could be found in HCC tissues. Expression of HNF1A...
and PDK2 was increased in HCC in comparison to normal liver cells (Figure 3). In addition, we used HCC tissue microarray of 90 patients, stained by antibodies of CD4, CD8, CTNNB1, and KRAS to examine the correlation between protein expression of CTNNB1 and KRAS and immune cells of CD4+ and CD8+ T cells. It demonstrated correlations between CD4+ T cells in paratumor tissues, and CD8+ T cells in tumors and KRAS protein expression were close to significance; high expression of KRAS could indicate better tumor infiltration of CD8+ T cells in HCC, though only 33 out of 90 (37%) patients demonstrated high expression of KRAS. Although correlations between CTNNB1, CD4, and CD8 protein stains were not significant, we still observed patients with high CTNNB1 expression showed less infiltration of CD4+ and CD8+ T cells in the tissue microarray, which counted for 71% of whole patient cohort.

Former studies have shown that the β-catenin pathway in the melanoma model could suppress recruitment of CD103+ macrophages, which play important roles in tumor-specific CD8+ T cells’ development.3 Also, some studies testified KRAS was related to low B cell infiltration in lung adenocarcinoma, and in colorectal carcinoma, mutation of KRAS was related to low immune infiltration in tumor microenvironment.4,5 The knowledge of HCC intrinsic oncogenic pathways’ influence on immune microenvironment is limited, and CTNNB1 is deemed as a specific mutation in HCC with a high portion of patients bearing CTNNB1 mutation classified as a worse subtype, in which patients may suffer from poor prognosis.6,7 Our results showed that CTNNB1 and KRAS were related to infiltrated CD4+ and CD8+ T cells in HCC, providing new targets for combining immune therapy and indicators for immune status evaluation. The protein expression of KRAS in HCC tissues was seen in only 37% of patients, while CTNNB1 was highly expressed, which may explain the immune suppressive microenvironment in liver tumor. On the other hand, high CTNNB1 expression could indicate high metabolic status of cancer cells, which could also depress the signals of adaptive immune responses. The explicit mechanisms concerning KRAS’s and CTNNB1’s influences on HCC immune infiltration still need further investigations to clarify.

CONCLUSION

Tumor intrinsic pathways of CTNNB1 and KRAS in HCC were related to immune infiltration levels, in which CTNNB1 was related to low immune infiltration, while KRAS indicated high infiltration levels. Also, KRAS, HNF1A, and PDK2 were highly correlated with immune regulators and the chemokine axis in HCC microenvironment, which provides new targets for combining immune therapy.

Zheng Chen1, Mincheng Yu1, Jiuliang Yan1, Binghai Zhou1,2, Wentao Zhang1, Lei Guo1, Bo Zhang1, Shuang Liu1,3, Lei Jin1, Jian Zhou1, Jia Fan1, Qinghai Ye1, Hui Li1, Yongsheng Xiao1, Yongfeng Xu1

1 Liver Cancer Institute, Zhongshan Hospital, Fudan University and Key Laboratory of Carcinogenesis and Cancer Invasion, Ministry of Education, Shanghai, People's Republic of China
2 Department of Hepatobiliary and Pancreatic Surgery, the Second Affiliated Hospital of Nanchang University, Nanchang, People’s Republic of China
3 Neurosurgery Department of Zhongshan Hospital, Fudan University, Shanghai, People’s Republic of China

Correspondence
Qinghai Ye, Hui Li, Yongsheng Xiao, Yongfeng Xu, Liver Cancer Institute, Zhongshan Hospital of Fudan University, Xuhui District, Shanghai, People’s Republic of China, 200032.
Email: ye.qinghai@zs-hospital.sh.cn (Q.Y.); li.hui1@zs-hospital.sh.cn (H.L.); xiao.yongsheng@zs-hospital.sh.cn (Y.X.); xu.yongfeng@zs-hospital.sh.cn (Y.X.)

Funding Information: National Natural Science Foundation of China (Grant/Award Number): 81502487, 81572301, 81572844, 81802893, 81871924.
Zheng Chen, Mincheng Yu, and Jiuliang Yan contributed equally to the study.

ORCID
Zheng Chen https://orcid.org/0000-0002-5791-1740
Mincheng Yu https://orcid.org/0000-0001-9998-5708

REFERENCES
1. Jindal A. Tqq sxxhadi A and Shailubhai K. Hepatocellular carcinoma: etiology and current and future drugs. J Clin Exp Hepatol. 2019;9:221-232.
2. Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. WNT/β-catenin pathway activation correlates with immune exclusion across human cancers. *Clin Cancer Res*. 2019;25:3074-3083.

3. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. *Cancer Cell*. 2017;31:711-723.e714.

4. Pinto R, Petriella D, Lacalamita R, et al. KRAS-driven lung adenocarcinoma and B cell infiltration: novel insights for immunotherapy. *Cancers*. 2019;11:8.

5. Lal N, White BS, Goussous G, et al. KRAS mutation and consensus molecular subtypes 2 and 3 are independently associated with reduced immune infiltration and reactivity in colorectal cancer. *Clin Cancer Res*. 2018;24:224-233.

6. Hoshida Y, Nijman SM, Kobayashi M, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res*. 2009;69:7385-7392.

7. Shimada S, Mogushi K, Akiyama Y, et al. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. *EBioMedicine*. 2019;40:457-470.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.