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Dear IHPBA Community,

I am writing to express my hearty gratitude to the IHPBA Research Committee for selecting me as the recipient of the Kenneth Warren Fellowship for the 2023/2024 term. Because of the support of Kenneth Warren Fellowship, I am able to receive clinical training and continue my scientific at the National Centre for Global Health and Medicine (NCGM) in Tokyo under the mentorship of Professor Norihiro Kokudo. I am sincerely grateful to the recognition of my past work by the Research Committee, further refinement and specific training in NCGM greatly benefit my future career development.

➤ **Six-month report:**

1. Identified Tertiary lymphoid structures as a potential prognostic biomarker for combined hepatocellular–cholangiocarcinoma.

Combined hepatocellular–cholangiocarcinoma (cHCC–CCA), as a rare primary hepatic tumor, is challenging to accurately assess in terms of the clinical outcomes and prognostic risk factors in patients. The TLSs, with different spatial distributions and densities, of 137 cHCC–CCA were quantified, and their association with prognosis was assessed by Cox regression and Kaplan–Meier analyses. We further validated TLS possible efficacy in predicting immunotherapy responsiveness in two cHCC–CCA case reports. TLS composition and its relationship to CXCL12 expression were analysed by fluorescent multiplex immunohistochemistry (IHC).

We found that a high intratumoural TLS score was correlated with prolonged survival, whereas a high TLS density in adjacent tissue indicated a worse prognosis in cHCC–CCA. Mature TLSs were related to favorable outcomes and showed more CD8 positive T cells infiltrating tumor tissues. We further divided the cHCC–CCA patients into four immune grades by combining the peri-TLS and intra-TLS, and these grades were an independent prognostic factor. In addition, our reported cases suggested a potential value of TLS in predicting immunotherapy response in cHCC–CCA patients. Our findings suggested that CXCL12 expression in cHCC–CCA tissue was significantly correlated with TLS presence.

The spatial distribution and density of TLSs revealing the characteristics of the cHCC–CCA immune microenvironment, significantly correlated with prognosis and provided a potential immunotherapy response biomarker for cHCC–CCA.

2. Multi-omics Characterization of Cholangiocarcinoma Reveals Distinct Prognostic and Therapeutic associated Molecular Subtypes

We performed a large-scale integrative analysis of the multiomics sequencing, clinical and pathological data of 450 cholangiocarcinoma (CCA) samples with various anatomical subtypes from multiple Chinese centers. Notably, integrative clustering defined 4 CCA subgroups (C1-C4) with distinct clinical-pathological features, tumor microenvironment (TME) characteristics, putative therapeutics and prognoses.

Then, a 160-gene classifier was created, and this classifier was able to classify the vast majority of CCA cases into the C1-C4 subtypes and was verified to be valuable for guiding clinical treatment. Patients with C3 characteristics had the best prognosis and exhibited higher responsiveness to immune checkpoint blockade therapy than others (75% vs. 15%). Moreover, we demonstrated that the CpG island methylator phenotype (CIMP) could serve as an indicator of immunocyte infiltration in the TME of CCA. *SOS1* and *ATP2B1* were identified as potential new driver genes and significant prognostic biomarkers of CCA.

This study yield insights into the heterogeneity and clinical-molecular classification of CCA and could aid in the establishment of diagnostic and individualized therapeutic strategies.

3. A predictive radiotranscriptomics model based on DCEMRI for tumor immune landscape and immunotherapy in cholangiocarcinoma

This study aims to determine the predictive role of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) derived radiomic model in tumor immune profiling and immunotherapy for cholangiocarcinoma.

To perform radiomic analysis, immune related subgroup clustering was first performed by single sample gene set enrichment analysis (ssGSEA). Second, a total of 806 radiomic features for each phase of DCE-MRI were extracted by utilizing the Python package Pyradiomics. Then, a predictive radiomic signature model was constructed after a three-step features reduction and selection, and receiver operating characteristic (ROC) curve was employed to evaluate the performance of this model. In the end, an independent testing cohort involving cholangiocarcinoma patients with anti-PD-1 Sintilimab treatment after surgery was used to verify the potential application of the established radiomic model in immunotherapy for cholangiocarcinoma.

Two distinct immune related subgroups were classified using ssGSEA based on transcriptome sequencing. For radiomic analysis, a total of 10 predictive radiomic features were finally identified to establish a radiomic signature model for immune landscape classification. Regarding to the predictive performance, the mean AUC of

ROC curves was 0.80 in the training/validation cohort. For the independent testing cohort, the individual predictive probability by radiomic model and the corresponding immune score derived from ssGSEA was significantly correlated.

In conclusion, radiomic signature model based on DCE-MRI was capable of predicting the immune landscape of cholangiocarcinoma. Consequently, a potentially clinical application of this developed radiomic model to guide immunotherapy for cholangiocarcinoma was suggested.

➤ **Publications and International Conference Presentations:**

1. Wenchen Gong[#], Su Zhang[#], Xiangdong Tian[#], Wenshuai Chen, Yuchao He, Liwei Chen, Tingting Ding, Peiqi Ren, Lin Shi, Qiang Wu*, Yan Sun*, **Lu Chen***, Hua Guo*, Tertiary lymphoid structures as a potential prognostic biomarker for combined hepatocellular-cholangiocarcinoma, *Hepatol Int*, 2024, 18(4):1310-1325. 5-year *IF*:6.6. (*Co-Corresponding author*)
2. **Lu Chen**[#], Guotao Yin[#], Ziyang Wang[#], Zifan Liu, Chunxiao Sui, Kun Chen, Tianqiang Song, Wengui Xu, Lisha Qi*, Xiaofeng Li*, A predictive radiotranscriptomics model based on DCE-MRI for tumor immune landscape and immunotherapy in cholangiocarcinoma, *Biosci Trends*, 2024, 18(3):263-276. 5-year *IF*:3.9. (*First Author*)
3. **Lu Chen**, Wenchen Gong, Xudong Zhang, Peipei Song, Wei Tang, Tianqiang Song, Norihiro Kokudo. Tertiary Lymphoid Structure is a Prognostic Marker and Predict Immunotherapy Response in Combined Hepatocellular-Cholangiocarcinoma, The 14th Asia-Pacific Primary Liver Cancer Expert Meeting, July 2024, Hawaii, USA. (*Poster*)

Yours sincerely,



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