Editorial

Single-Cell Landscape of Liver Cancer in Response to Immunotherapy

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Primary liver cancer is the sixth most commonly diagnosed cancer in the world, with 905,677 new cases of liver cancer in 2020, and the third leading cause of cancer death globally.[1] The liver cancer burden in China is substantial, with more than 40% of new liver cancer cases occurred in China.[2,3] Hepatocellular carcinoma and intrahepatic cholangiocarcinoma comprise the majority of liver cancers. Both of them are characterized by extensive intra- and inter-tumor heterogeneity.[4,5] This may be caused by genomic complexity and the presence of multiple etiological factors.[6]

The initiation and progression of cancers, including liver cancer, is a complex evolutionary process, in which natural selection and genetic/epigenetic drift play a key role. A small proportion of tumor cells can survive by acquiring genetic/epigenetic alterations when they are exposed to a microenvironment that is not conducive to tumor growth. This "survival of the fittest" feature, which is common in cancers, leads to intra-tumor heterogeneity.[7] Just as biodiversity promotes the survival and development of organisms in the ecosystem, intra-tumor heterogeneity plays a similar role in maintaining tumor survival and promoting tumor metastasis. Additionally, intra-tumor heterogeneity is the main cause of drug resistance in targeted therapies and severely affects the prognosis of cancer patients.[8,9] An in-depth understanding of the biodiversity and clonality of tumors can be of great help at the diagnosis and treatment of tumors. However, the underlying mechanisms that regulate tumor cell evolution are still unknown. Although the existing single-cell analysis techniques can observe intra-tumor heterogeneity in solid tumors, these techniques cannot detect interpreting tumor clonality and tumor evolution.

Previous studies traced the tumor cell clonality by analyzing somatic mutations in tumor cells. However, most of the somatic mutations observed in these studies are passenger mutations. They are not the drivers of tumor evolution and do not participate in positive selection during the evolution. Therefore, tumor mutational data acquired from single-cell sequencing cannot be used to reconstruct tumor clonality due to a high false positivity. In a recent study, Marjanovic *et al.*[10] analyzed the cell status of lung cancer and found that the high-plasticity cell state drives cell heterogeneity. It is worth noting that the genetic variation in lung cancer evolution did not affect the cell state heterogeneity. These findings suggest that it may be possible to better define the functional clonality of tumor cells and the heterogeneity of their cell states by identifying tumor cell lineages and evolutionary trajectories, as well as tumor cell clusters with transcriptomic similarities at single-cell level.

Previous clinical trials showed that only a small subset of liver cancer patients have a durable response to immune checkpoint inhibitors alone or in combination with anti-vascular endothelial growth factor therapy.[11] A single-cell transcriptome analysis conducted by Ma *et al.*[12] revealed that inter-tumor heterogeneity was correlated with liver cancer prognosis. It is one of the reasons why liver cancer patients have different reactions to antitumor

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therapies. However, the molecular mechanism that drives and regulates inter-tumor heterogeneity in liver cancer is still unclear. In a study recently published in the *Journal of Hepatology*, titled "Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma," Ma *et al.*[13] extended their previous study. They used single-cell transcriptome analysis to delineate the evolution of tumor cells in hepatocellular carcinoma or intrahepatic cholangiocarcinoma tissue samples to determine the driving mechanism of liver cancer intra-tumor heterogeneity.

A total of 46 hepatocellular carcinoma and intrahepatic cholangiocarcinoma tissues analyzed in this study were collected from 37 patients who were enrolled by the National Institutes of Health Clinical Center. The authors tracked cell status of malignant and nonmalignant cells (n = 57,000) in tumor mass by newly developed machine learning-mediated consensus clustering method based on functional clonality analysis of tumor cell transcriptomes. Next, they used RNA velocity and reverse graph embedding to determine the tumor cell relationships. Furthermore, the cellular state and evolution of tumor were determined by analyzing four sets of longitudinal samples collected from liver cancer patients. Finally, the findings of the experiments mentioned above were validated in 488 hepatocellular carcinoma and 277 intrahepatic cholangiocarcinoma patients through bulk transcriptome analysis. The authors found that the increased heterogeneity of tumor cell state was closely related to the prognosis of liver cancer patients. The increase of functional clonality was accompanied by an increase in preexhausted T-cells and other polarized immune cell landscapes. Additionally, they demonstrated that secreted phosphoprotein 1 (SPP1) plays an important role in tumor microenvironmental reprogramming and tumor cell evolution. Furthermore, the authors used these data to develop scAtlasLC, a knowledge base of liver cancer single-cell atlas (https://scatlaslc-ccr-cancer-gov.eproxy.lib.hku.hk).

In fact, we can observe intra-tumor heterogeneity by while-genome sequencing of bulk tumors, but the resolution of whole-genome sequencing is not sufficient enough to accurately define the functional clonality of tumor cells and their distinct evolutionary trajectories. In addition, the current approaches to define driver mutations have certain limitations, because they cannot define somatic copy number and epigenetic alterations, missense mutations, and structural variants. Therefore, it is necessary to study tumor evolution at single-cell level through new detection technologies and collaborative effort. Unfortunately, the error rate of mutational information at the genomic level detected by single-cell sequencing is very high. This study used single-cell transcriptomics to determine the clonality of liver cancer. Unlike single-cell sequencing at the genomic level, single-cell transcriptomic analysis has been widely used to define different cell types. This approach is based on functional clonality to distinguish cell types, reflecting the status and phenotypic characteristics of different cell types in various organs. Since most of the genetic/epigenetic alterations may not be linked to the phenotype of tumor cells, using functional clonality to directly detect tumor evolution will yield more accurate results. Using this strategy, the authors found that the cluster number was negatively correlated with patient survival in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Phylogenetic analysis of subclusters showed that tumor evolution in hepatocellular carcinoma and intrahepatic cholangiocarcinoma was consistent with the Darwinian evolution model proposed by Nowell.[14] Copy number variation analysis revealed that the clonality of the subclones defined by copy number variation was similar to that defined by transcriptome. These findings support the hypothesis that functional diversity is the consequence of genetic heterogeneity.

The tumor cell evolution can promote the increase in tumor cell diversity, and thereby produce drug-resistant cells. Therefore, identifying participants in tumor cell evolution can facilitate the development of better cancer intervention approaches. This study used a new strategy to identify SPP1 as a driver for tumor cell evolution in liver cancer. The authors reported that the expression pattern of SPP1 followed the hierarchical relationship of liver cancer cell branching evolution. Osteopontin (OPN) encoded by SPP1 has been reported to play an important role in HCC progression. For example, OPN can induce HCC invasion and metastasis and regulate the function of tumor-infiltrating immune cells.[15] These abilities may be linked to the initiation of tumor cell evolution that can adapt the tumor microenvironment for tumor cell survival.

Overall, this study revealed that intra-tumor heterogeneity is a key feature of liver cancer and may link to treatment failure. Additionally, it presented a single-cell atlas of liver cancer and described the cell states and hierarchical relationship of liver cancer cells. This work creates an enormous pool of resources and opportunities to understand the complex liver cancer ecosystem.

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Conflicts of interest

There are no conflicts of interest.

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