PROJECT

Supervisor: ____Donato Inverso_____

Title: ____ VASCULAR CONTROL NASH PROGRESSION _____

Curriculum: _ ___Basic and Applied Immunology and Oncology _____

Link to the personal page of the University or relevant hospital site website:

https://inversolab.org/

https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/units/vascular-pathobiology.html?ts=202407051214

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

The liver parenchyma is organized in a complex architecture with heterogeneous gene expression alongside its lobule axis, a process referred to as "lobular zonation". Specifically, 50% of the genes expressed by the hepatocytes have a significant spatial distribution, primarily regulated by the WNT ligands derived from the liver endothelial cells. This architecture becomes progressively dysregulated during prolonged liver injuries, such as non-alcoholic steatohepatitis (NASH), leading to cirrhosis and hepatocellular carcinoma (HCC). The combination of anti-PD-L1 and anti-VEGF demonstrated a dramatic improvement in overall survival in HCC patients, compared to the standard treatment with sorafenib6. Notably, anti-PD-L1 as single therapy does not provide any survival benefit compared to sorafenib7, indicating that the anti-angiogenic therapy is instrumental in effective immune therapy. These observations indicate the crucial role of the liver vasculature in regulating hepatic zonation as well as disease progression. However, how zonation disruption impacts the NASH-to-HCC transition and how VEGFR therapy synergizes with immune-checkpoint inhibition still needs to be clarified.

Rationale and hypothesis

To study these questions, one major restriction is our limited capacity to integrate gene expression data into a complex morphological context. This is further exacerbated at the proteomics and post-translational level, where obtaining highly resolved insights into the heterogeneity of complex tissues remains challenging. Recently, we established a spatially-resolved multiomics approach that correlates thephosphoproteomics of endothelial cells with their anatomical position. In doing so, we identified tyrosine phosphorylation as the most zonated signalling event of the liver lobule8. In particular, the major targets of current anti-angiogenic therapies (including VEGFRs) are receptor-tyrosine-kinases (RTK), further suggesting a mechanistic link between RTK phosphorylation and disease outcomes.

Objectives and specific aims

this project will focus on two major points:

i) a phosphorylation signature of NASH-to-HCC transition and of HCC heterogeneity, to identify signalling pathways that cannot be studied by conventional sequencing approaches.

ii) Dissect molecular mechanisms downstream anti-VEGF therapy and the impact on immune-checkpoint inhibitors.

Expected outcomes

In this project we will provide a spatially-resolved analysis of the proteins activation during NASH-to-HCC transition and during tumor progression in order to identify novel signaling events that cannot be identified with the traditional sequencing approach and that could have a significant prognostic potential. Moreover, by defining the phosphorylation pattern consequent to the anti-angiogenic treatment, we will clarify the mechanism supporting its enhanced effectiveness when combined with the immune-checkpoint inhibition therapy.

Skills that the student should acquire (max. 600 characters including spaces):

The student will acquire different skills including: murine model of dietary-induced NASH and HCC, isolation of liver parenchymal and non-parenchymal cells, basic and advanced imaging techniques and a strong background in multiomics and relative data analysis. Moreover, as part of the PhD training, the student will implement crucial skills such as long-term experimental planning, scientific writing and presentation, biostatistics, and strategic planning for independent funding.

References (max. 15)

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2. Huang, D. Q., El-Serag, H. B. & Loomba, R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 18, 223-238 (2021).
3. Yang, J. D. *et al.* A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* **16**, 589-604 (2019).
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6. Brescia, P. & Rescigno, M. The gut vascular barrier: a new player in the gut-liver-brain axis. *Trends Mol Med* **27**, 844-855 (2021).
7. Wolf, M. J. *et al.* Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* **26**, 549-564 (2014).
8. Moor, A. E. *et al.* Spatial Reconstruction of Single Enterocytes Uncovers Broad Zonation along the Intestinal Villus Axis. *Cell* **175**, 1156–1167 e1115 (2018).
9. Giesen, C. *et al.* Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods* **11**, 417-422 (2014).
10. Villani, T. S., Koroch, A. R. & Simon, J. E. An improved clearing and mounting solution to replace chloral hydrate in microscopic applications. *Appl Plant Sci* **1** (2013).