Cell Genomics

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Preview

Genetic perturbations go spatial

Katie Teng[1,2] and Hartland Warren Jackson[1,2,3,*]
[1]Centre for Molecular and Systems Biology, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada
[2]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada
[3]Ontario Institute for Cancer Research, Toronto, ON, Canada
*Correspondence: hjackson@lunenfeld.ca
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Tissue-tumor interactivity is the culmination of cell intrinsic features and their extrinsic interactions with the environment. Recently in *Cell*, Dhainaut and Rose et al. established a strategy to track pooled CRISPR-modified cells *in vivo* using protein barcodes (Pro-Codes) and measure their impact on the tumor microenvironment through multiplexed imaging and spatial transcriptomics of intact tissues.[1]

Our understanding of cancer is continually evolving and now aims to include external environments and internal, spatially distinct clones and niches. Half of the established cancer hallmarks involve direct tumor-environment interactions, and these are tightly intertwined with intracellular hallmarks and drivers.[2] The study of extrinsic hallmarks initiated the development of therapeutics targeting angiogenesis and immune exhaustion or avoidance and has generated cell-based immunotherapies. However, the prevalence of therapeutic resistance has highlighted various mechanisms by which tumors alter their environment and how intra-tumor heterogeneity requires the targeting of all subclones and cellular environments in order to move from treatments to cures.[3]

Technological advances are facilitating the comprehensive study of the cellular components of tissues and tumors through transcriptome-wide measurement of cell phenotypes with single-cell RNA sequencing and their organization by spatial transcriptomics.[4] Simultaneously, high-throughput CRISPR screening strategies have enabled unbiased but precise methods to identify genes causally driving specific phenotypes.[5] Pooled CRISPR screens paired with single-cell transcriptomic readouts, such as Perturb-seq, are powerful tools to understand the function of unknown genes or test the impact of cancer-associated mutations.[6] Unfortunately, these approaches can be costly, require enrichment of cellular phenotypes of interest, and are dependent on physically dissociated single cells. Therefore, genetic perturbation studies are limited to cell-intrinsic phenotypes and lack the ability to link single genes to their impact on multi-cellular tissues, internal and external environments, and association with clinically relevant histopathology classifications of cellular organization.

To study both cell-intrinsic and -extrinsic features, Dhainaut and Rose et al. developed Perturb-map, a combination of pooled *in vivo* CRISPR screening, protein barcodes (Pro-Codes) to track CRISPR-modified cells, and spatially resolved transcriptomics and single-cell measurements to quantify the location, organization, and extrinsic alterations arising from genetic perturbations[1] (Figure 1). This strategy was used to investigate how genes that are associated with immunotherapy outcomes modify the tumor microenvironment in a mouse model of lung cancer. This is especially important for tumor immunity as immune cell composition and localization within and around tumors affect immunotherapy response and patient outcome.[7]

From their library of 35 immune cell interaction genes, the authors identified both positive and negative regulators of tumor growth. Image analysis of entire lung lobes from only 11 mice facilitated the quantification of >8 million CRISPR-modified cells, their organization, and immune environments in ~1,750 lesions. The area and number of cells imaged were limiting factors, since combinations of antigen tags (Pro-Codes) are able to provide thousands of potential barcodes. The ability to measure single cells with increased throughput enabled identification of both enriched and depleted genes and phenotyping of variably sized lesions. Measurement of immune cell infiltration or exclusion at lesion boundaries showed that two of the largest and most enriched genes had contrasting immune microenvironments. Loss of *Tgfbr2* resulted in immune-deserted environments containing only myeloid cells, whereas *Socs1*-deficient lesions were immune hot, containing all immune cell types measured. Other gene deletions resulted in immune-cell-type-specific infiltration or exclusion, with various perturbations impacting infiltration distance. This indicates the potential of this approach to interrogate cytokine- and chemokine-specific immune recruitment and inform how diverse immune cell networks arise in spatially distinct regions of human tumors.

Spatial screening confirmed tumor-promoting roles of both innate and adaptive immune checkpoints (PD-L1 and CD47) and the need for major histocompatibility complex class I presentation for adaptive immune control. Furthermore, Perturb-map clarified the role of IFNγ signaling and *Tgfbr2*. Through upregulation of PD-L1, the IFNγ pathway was shown to promote tumor growth even in the presence of extensive immune influx. Similarly, *Tgfbr2* functionally regulated tumor growth, histopathology, and the tumor immune microenvironment. *Tgfbr2* mutations are present in numerous human cancers and are associated with immune checkpoint inhibitor resistance in lung cancer,[8] but the functional role of this gene mutation is unknown. *Tgfbr2* knockout generated large lung tumors with fibro-mucinous histopathology and T cell exclusion and unexpectedly resulted in increased TGFβ signaling. Utilizing spatially resolved measurements, the

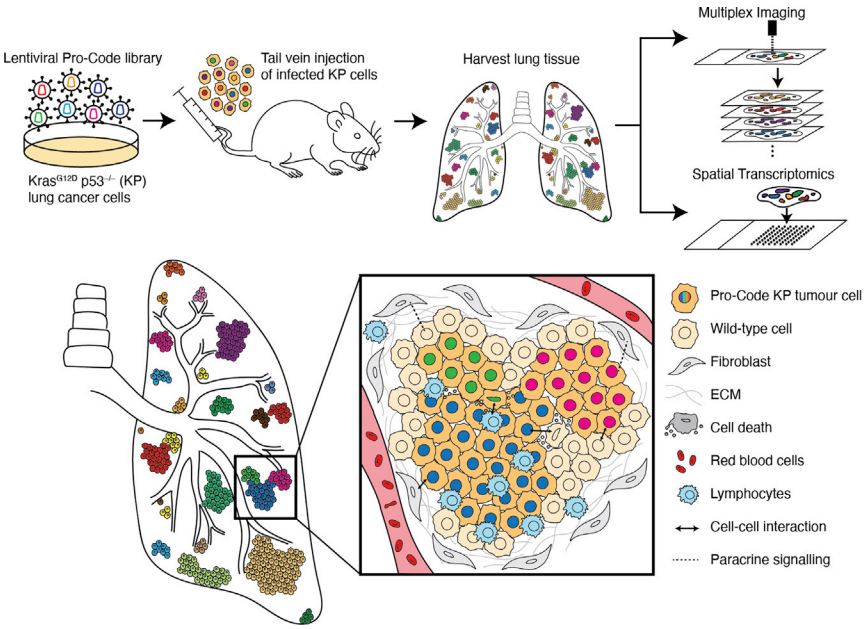

Figure 1. Probing novel cancer biology through spatial methods

Kras^{G12D} p53$^{-/-}$ (KP) lung cancer cells were infected with a protein-barcoded (Pro-Code) lentiviral library targeting genes regulating cytokine signaling and injected by tail vein into mice. Lung tissue was harvested for multiplexed imaging, histopathological analysis, and spatial transcriptomics. With this innovative Perturb-map approach, Dhainaut and Rose et al. were able to track modified cells and investigate their interactions with the surrounding tumor microenvironment.[1] This study emphasizes the need to study cancer within intact tissues to acquire a more comprehensive view of tumor biology.

authors determined that this activated TGFβ signature likely originated from surrounding fibroblasts and suggest that in the absence of *Tgfbr2*, increased TGFβ availability created a pro-tumorigenic microenvironment. This highlights that *in situ* measurements from models that recapitulate tissue structure and cellular interactions reveal counterintuitive feedback loops and intercompartment signaling, which drives distinct phenotypes and histopathologies.

Socs1- and *Tgfbr2*-deficient clones maintained distinct immune-hot or -cold microenvironments even in close proximity to each other. Such clonal- or subclonal-specific activity may be responsible for distinct immune environments observed in human metastases and spatially distinct regions of primary tumors. Subclonal cooperation can alter the immune environment to promote tumor progression and metastasis,[9] and even early human lesions contain multiple interacting clones.[10] Although this could not be investigated in the predominantly clonal model of lung cancer used here, the authors detailed the ability of Perturb-map to quantify interacting tumor clones and their patterns of organization and dispersion. This feature could be utilized for lineage tracing, tracking clones spatially within a tumor or between sites during metastasis, and investigating clonal interactions.

Here, and in any transplantation-based tumor model, bottlenecks in cancer cell seeding could impact tumor outgrowths, and though no immune response was detected against Pro-Code antigens, central tolerance against all tags may be necessary in some cases as unintended immunogenicity against tags has been shown to influence metastatic progression.[11]

As tissue architecture is key to our understanding of complex tissues, there is a growing requirement for methods that functionally test modifiers of cellular recruitment and organization. Perturb-map moves spatial profiling tools from only identifying associations to functionally testing multiple hypotheses using a targeted systems biology approach. Its compatibility with many multiplexed imaging technologies for single-cell proteomics and Visium spatial transcriptomics was shown, and we expect that other spatial methods will work with this system.[4] Here, imaging methods had single-cell resolution but were limited to <50 antibodies, and Visium sequencing of arrayed spots measured viral integrations within whole-transcriptome measurements, but not specific Pro-Codes and without single-cell resolution. With improvements in spatial sequencing technologies and single-cell methods, future work will likely extend this approach to enable spatial readouts of whole-genome CRISPR screens by measuring single guide RNAs within multimodal 'omics' readouts. We predict that functional spatial genomics will contribute key insights in the study of secreted proteins, extracellular signaling, and cell interaction networks that control tumor microenvironments, immune avoidance, and immunotherapy response.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Dhainaut, M., Rose, S.A., Akturk, G., Wroblewska, A., Nielsen, S.R., Park, E.S., Buckup, M., Roudko, V., Pia, L., Sweeney, R., et al. (2022). Spatial CRISPR genomics identifies regulators of the tumor microenvironment. Cell *185*, 1223–1239.e20. https://doi.org/10.1016/j.cell.2022.02.015.
2. Hanahan, D. (2022). Hallmarks of cancer: New dimensions. Cancer Discov. *12*, 31–46. https://doi.org/10.1158/2159-8290.cd-21-1059.
3. Dagogo-Jack, I., and Shaw, A.T. (2017). Tumour heterogeneity and resistance to cancer therapies. Nat. Rev. Clin. Oncol. *15*, 81–94. https://doi.org/10.1038/nrclinonc.2017.166.
4. Lewis, S.M., Asselin-Labat, M.-L., Nguyen, Q., Berthelet, J., Tan, X., Wimmer, V.C., Merino, D., Rogers, K.L., and Naik, S.H. (2021). Spatial omics and multiplexed imaging to explore cancer biology. Nat. Methods *18*, 997–1012. https://doi.org/10.1038/s41592-021-01203-6.
5. Chow, R.D., and Chen, S. (2018). Cancer CRISPR screens in vivo. Trends Cancer *4*, 349–358. https://doi.org/10.1016/j.trecan.2018.03.002.
6. Dixit, A., Parnas, O., Li, B., Chen, J., Fulco, C.P., Jerby-Arnon, L., Marjanovic, N.D., Dionne, D., Burks, T., Raychowdhury, R., et al. (2016). Perturb-seq: Dissecting Molecular

Circuits with Scalable single-cell RNA profiling of pooled genetic screens. Cell *167*, 1853–1866.e17. https://doi.org/10.1016/j.cell.2016.11.038.e17.

7. Binnewies, M., Roberts, E.W., Kersten, K., Chan, V., Fearon, D.F., Merad, M., Coussens, L.M., Gabrilovich, D.I., Ostrand-Rosenberg, S., Hedrick, C.C., et al. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat. Med. *24*, 541–550. https://doi.org/10.1038/s41591-018s4150014-x.
8. Li, T., Wang, H., Xu, J., Li, C., Zhang, Y., Wang, G., Liu, Y., Cai, S., Fang, W., Li, J., and Wang, Z. (2021). TGFBR2 mutation predicts resistance to immune checkpoint inhibitors in patients with non-small cell lung cancer. Ther. Adv. Med. Oncol. *13*. 175883592110384. https://doi.org/10.1177/17588359211038477.
9. Janiszewska, M., Tabassum, D.P., Castaño, Z., Cristea, S., Yamamoto, K.N., Kingston, N.L., Murphy, K.C., Shu, S., Harper, N.W., Del Alcazar, C.G., et al. (2019). Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments. Nat. Cell Biol. *21*, 879–888. https://doi.org/10.1038/s41556-019-0346-x.
10. Casasent, A.K., Schalck, A., Gao, R., Sei, E., Long, A., Pangburn, W., Casasent, T., Meric-Bernstam, F., Edgerton, M.E., and Navin, N.E. (2018). Multiclonal Invasion in Breast tumors identified by Topographic single cell sequencing. Cell *172*, 205–217.e12. https://doi.org/10.1016/j.cell.2017.12.007.e12.
11. Grzelak, C.A., Goddard, E.T., Lederer, E.E., Rajaram, K., Dai, J., Shor, R.E., Lim, A.R., Kim, J., Beronja, S., Funnell, A.P.W., and Ghajar, C.M. (2022). Elimination of fluorescent protein immunogenicity permits modeling of metastasis in immune-competent settings. Cancer Cell *40*, 1–2. https://doi.org/10.1016/j.ccell.2021.11.004.