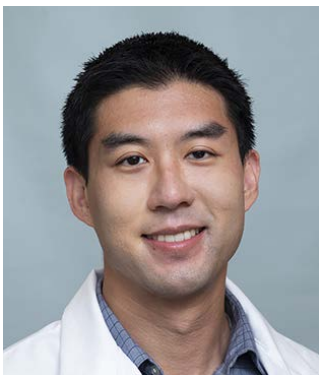
William L. Hwang, MD, PhD



Hwang Laboratory

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The Hwang laboratory focuses on the immense phenotypic, temporal and spatial heterogeneity of tumor ecosystems and the many insights that can only be gleaned by studying these systems at the level of their individual components — single molecules or cells. We study tumor-stroma interactions at unprecedented resolution through the development and application of techniques in spatial and systems oncology, advanced microscopy, genetic engineering and computational biology to patient-derived specimens, stromal tumoroids and mouse models. Our goals are to elucidate mechanisms of (1) therapeutic resistance mediated by genetic, epigenetic, and phenotypic factors including cell state plasticity; (2) treatment-mediated remodeling of the spatial microarchitecture of tumors and underlying cancer cell-stromal interactions; and (3) tumor-nerve-immune crosstalk, which plays a critical role in the pathophysiology and morbidity of many malignancies but remains understudied.

Single-cell dynamics

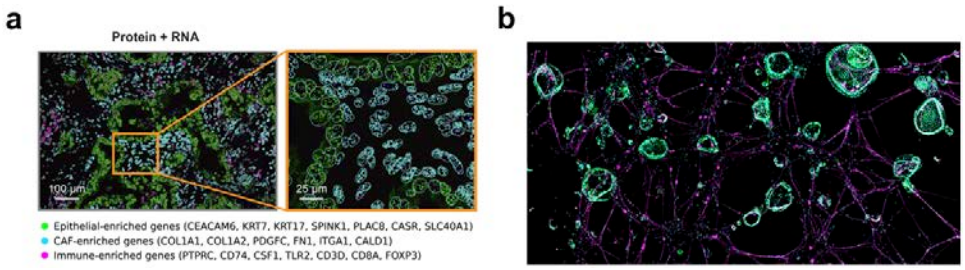
Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal and treatment refractory disease. Molecular subtyping of PDAC is rudimentary and does not currently inform clinical management or therapeutic development. We optimized single-nucleus RNA-seq to discover treatment-associated changes in cellular composition and state, including enrichment of a novel neural-like malignant program in residual tumors after chemoradiation. Our high-resolution molecular framework elucidates the inter- and intra-tumoral diversity of PDAC, treatment-associated remodeling and clinically relevant prognostication to enable precision oncology in PDAC.

Ongoing projects:

1. Identifying key regulators, context dependence and therapeutic vulnerabilities of resistant cell states
2. Elucidating (epi)genetic contributions to cell state plasticity in therapeutic resistance
3. Investigating mechanisms of tumorigenesis using single-cell multiomics to enable chemoprevention and early detection
4. Studying developmental lineages and mechanisms of metastasis in pancreatic neuroendocrine tumors

Spatial oncology

Dissociative single-cell approaches enable detailed characterization of the different cell types and states that compose a heterogeneous tumor but sacrifice in situ spatial relationships among cells. Leveraging recent advances in spatial proteo-transcriptomics enabling single-cell resolution and high molecular plex, we performed spatial molecular profiling (SMI) on a cohort of patient-derived PDAC tumors and developed a novel method for inferring multicellular interactions. Spatially Constrained Optimal Transport Interaction Analysis (SCOTIA) that considers both spatial distance and ligand-receptor (LR) expression (collaborator: Martin Hemberg). We used SCOTIA to dissect the remodeled pancreatic tumor microenvironment in response to neoadjuvant chemoradiation and uncovered marked changes in LR interactions between cancer-associated fibroblasts and malignant cells, which was supported by orthogonal experiments using a murine tumoroid co-culture system (*https://tinyurl.com/2xtdytxt*).



(a) Spatial coordinates of RNA transcripts for canonical epithelial (green), CAF (cyan) and immune (magenta) marker genes, overlaid on an immunofluorescence image from spatial molecular imaging (SMI). Inset depicts a magnified region with cell segmentation boundaries (cyan). (b) 3D co-culture of genetically-engineered cancer organoids (green) with sensory neurons (magenta) in a Matrigel dome.

Overall, we demonstrated the immense potential of a translational spatial biology paradigm for deriving novel biological insights and identifying actionable therapeutic targets — one that can be broadly applied to other malignancies and treatment contexts.

Ongoing projects:

1. Discovering gene regulatory networks that modulate tumor-stroma interactions through perturbative spatial screens
2. Developing computational models to infer cell state from integrating intrinsic and extrinsic influences
3. Creating a platform for correlating morphological changes to transcriptional changes through combining live-cell imaging with spatial transcriptomics
4. Integrating matched liquid and spatial biomarkers to assess response to therapy

Cancer neuroscience

Active recruitment of nerve fibers into tumors plays an important role in cancer development, treatment resistance, metastasis and mortality for many malignancies, but the diverse molecular mechanisms underlying tumor-nerve crosstalk remain largely unknown. To address this gap in knowledge, we performed a comprehensive, cell-type specific, spatially resolved whole transcriptome analysis of human PDAC using custom tissue microarrays derived from intratumorally matched malignant areas with (N+) and without (N-) nerve involvement. Whole-transcriptome digital spatial profiling revealed that classical malignant cells were depleted near nerves while basal/mesenchymal and neural-like cancer cells were enriched near nerves. Differential gene expression analysis comparing malignant cells in N+ versus N- regions enabled selection of subtype-specific candidate genes for functional investigation. This research will provide a detailed understanding of the mechanisms by which pancreatic cancer cells and the peripheral nervous system collaborate to confer numerous pro-tumorigenic effects, and guide prioritization for therapeutic intervention in the burgeoning cancer neuroscience field.

Ongoing projects:

1. Identifying cell-type specific mediators of nerve outgrowth, invasion and colonization using patient-derived tumors, tumoroids and GEMMs
2. Determining influence of neuronal subtype and activity on the immune response to cancer in primary tumors and draining lymph nodes
3. Dissecting molecular mechanisms of dynamic physical interactions between cancer cells and nerves
4. Discovering the mechanistic basis for differential central nervous system versus peripheral nervous system tropism across the spectrum of cancer

Selected Publications:

Hwang WL*, Jagadeesh KA*, Guo JA*, Hoffman HI*, Yadollahpour P, Reeves J, … Fernandez-del Castillo C, Liss AS, Ting DT, Jacks T†, Regev A‡. Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. *Nature Genetics* 2022 Aug;54(8):1178-1191.

Shi DD, Guo JA, Hoffman HI, Su J, Mino-Kenudson M, Barth JL, Schenkel JM, Loeffler JS, Shih HA, Hong TS, Wo JY, Aguirre AJ, Jacks TJ, Zheng L, Wen PY, Wang TC, **Hwang WL†**. Therapeutic avenues for cancer neuroscience: translational frontiers and clinical opportunities. *Lancet Oncology*. 2022;23(2):e62-74.

Gu, JA, Hoffman HI, Weekes CD, Zheng LZ, Ting DT, **Hwang WL†**. Refining the molecular framework for pancreatic cancer with single-cell and spatial technologies. *Clinical Cancer Research*. 2021;27(14):3825-3833.

Guo JA, Hoffman HI, Shroff S, Chen P, Hwang PG, Kim DY, Kim DW, Cheng SW, Zhao D, Mahal BA, Alshalafa M, Niemierko A, Wo JY, Loeffler JS, Fernandez-del Castillo C, Jacks T, Aguirre AJ, Hong TS, Mino-Kenudson M, **Hwang WL†**. Pan-cancer transcriptomic predictors of perineural invasion improve occult histopathological detection. *Clinical Cancer Research*. 2021;27(10):2807-2815.

Hwang WL*, Pike LRG*, Royce TJ, Mahal BA, Loeffler JS†. Safety of combining radiotherapy with immune checkpoint inhibitors. *Nature Reviews Clinical Oncology.* 2018;15(8):477-94.

Hwang WL*, Deindl S*, Harada BT, Zhuang X†. Histone H4 tail mediates allosteric regulation of nucleosome remodeling by linker DNA. *Nature*. 2014;512(7513):213-7.

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