

# David T. Ting, MD



## Ting Laboratory

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Gastrointestinal cancers are highly lethal cancers where the vast majority of patients are diagnosed too late and conventional therapies have largely been ineffective, making early detection and novel drug targets greatly needed.

**The Ting laboratory** has been utilizing innovative technologies to characterize RNA expression patterns in cancer. Using single molecule sequencing, we have discovered a significant amount of “non-coding” repeat RNAs to be produced in high amounts at the earliest stages of cancer development, but not in normal tissues. These repeat RNAs can serve as a novel early detection cancer biomarker and they can be targeted as a new therapeutic avenue. In parallel, we have used single cell, microfluidic chip technologies, and spatial transcriptomics to understand the factors involved in cellular plasticity in cancer. Our studies in circulating tumor cells (CTCs) have revealed the importance of tumor-stromal crosstalk in pancreatic cancer. We are using these studies to generate new therapies to stop the metastatic spread of cancer.

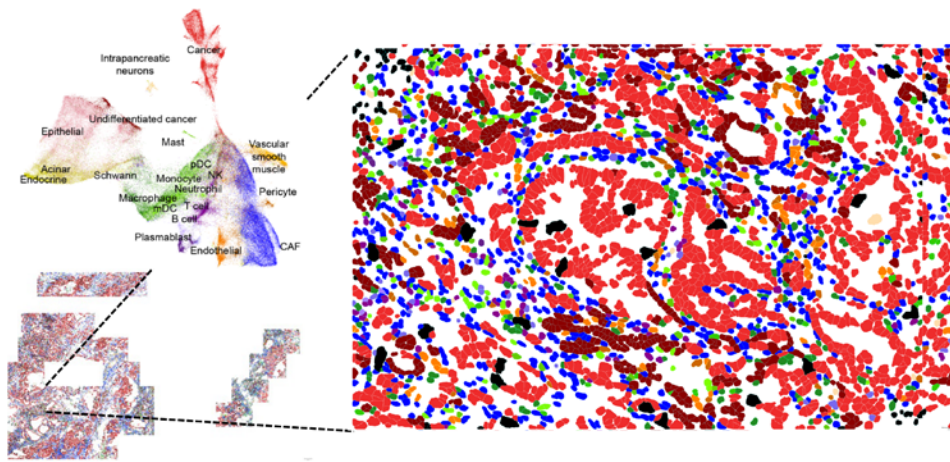
The Ting laboratory has utilized RNA-sequencing, RNA in situ hybridization, and spatial transcriptomic technologies to understand the complex transcriptional landscape of cancers. We have used these technologies to characterize non-coding repeat RNA expression across cancer and normal tissues. This has provided novel insight into the role of the repeatome in cancer development and offers a method to identify novel biomarkers and therapeutic targets. In addition, we have used single cell, spatial transcriptomic, and microfluidic technologies to understand cancer cell heterogeneity and plasticity. We have now found the aberrant expression of repeat RNAs are linked with cancer cell plasticity.

### Repeat non-coding RNAs

RNA sequencing of a broad spectrum of carcinomas demonstrated a highly aberrant expression of non-coding repeat RNAs emanating from regions of the genome previously thought to be inactive due to epigenetic silencing. Analysis of all human repeats identified the HSATII satellite as being exquisitely specific for epithelial cancers, including carcinomas of the

pancreas, colon, liver, breast, and lung. HSATII expression was confirmed by RNA in situ hybridization (RNA-ISH), and was present in preneoplastic lesions in mouse models and human specimens of the pancreas and colon suggesting satellite expression occurs early in tumorigenesis, which provides for a potential biomarker for early detection and a novel therapeutic avenue. Recently, we have discovered that HSATII is reverse transcribed in cancer cells and can integrate back into the genome and expand these pericentromeric regions. These expansions were found to be a poor prognostic marker in cancer. Moreover, our work has found that these satellite repeats can affect the local tumor microenvironment with implications for immunotherapies.

This has led to a Phase II clinical trial of a nucleoside reverse transcriptase inhibitor (NRTI) 3TC in metastatic colorectal cancer, which is demonstrating promising single agent activity in 25% of patients. We are now trying to identify the HSATII reverse transcriptase and better understand the biological role of satellites in cancer progression and tumor immune response.



This image represents a spatial transcriptomic “map” of a pancreatic cancer with individual molecules of repeat and coding RNAs quantified with precise spatial coordinates in a human primary tumor sample. Individual cell types can be determined based on transcriptional profiles with mapping to understand cell-cell interactions within tissue.

### Pancreatic cancer cellular heterogeneity

The high lethality of pancreatic cancer results from an intrinsic ability to resist chemotherapy and the propensity to metastasize. The etiology of this behavior is multifactorial, but our group has identified cancer cell heterogeneity and plasticity as key elements of aggressive pancreatic cancer. Our initial work using a microfluidic device to isolate rare circulating tumor cells (CTCs) offered a window into understanding the metastatic cascade. These studies demonstrated the inherent heterogeneity of pancreatic CTCs and their ability to seed metastases through a partial epithelial mesenchymal transition (EMT) program. We have recently uncovered the importance of stromal cancer associated fibroblasts (CAFs) in inducing EMT single cell heterogeneity consistent with phenotypes observed in CTCs and the plasticity of EMT phenotypes in the setting of chemoresistance and metastasis. Moreover, we defined pancreatic cancer intratumoral heterogeneity in discrete tumor glands using RNA-ISH and high content digital image analysis. We are now using spatial transcriptomic methods to fully characterize the relationship of tumor

cell plasticity and CAF heterogeneity. In addition, this platform provides a strategy to understand the spatial relationship of these cell types important for pancreatic cancer pathogenesis. More recently, we have now identified repeat RNA intercellular delivery via extracellular vesicles as a mechanism to drive cellular heterogeneity in pancreatic cancer. The understanding of cellular crosstalk of pancreatic cancer cells with CAFs and other microenvironmental cells will provide new mechanistic insight in the drivers of cancer cell heterogeneity and CTC generation, identify biomarkers in predicting patient outcomes, and reveal novel therapeutic avenues targeting tumor cell microenvironment interactions.

### Selected Publications:

Porter RL\*, Sun S\*, Flores MN, Berzolla E, You E, Phillips IE, Kc N, Desai N, [...] Stott SL, Deshpande V, Liu JF, Solovyov A, Matulonis UA, Greenbaum BD†, and **Ting DT**†. Satellite repeat RNA expression in epithelial ovarian cancer associates with a tumor-immunosuppressive phenotype. *J Clin Invest*, 2022 Aug 15;132(16):e155931.

Rajurkar M\*, Parikh AR\*, Solovyov A\*, You E, Kulkarni AS, Chu C, Xu KH, Jaicks C, Taylor MS, Wu C, Alexander KA, Good CR, Szabolcs A, Gerstberger S, [...] Deshpande V, Rivera MN, Aryee MJ, Hong TS, Berger SL, Walt DR, Burns KH, Park PJ, Greenbaum BD†, and **Ting DT**†. Reverse Transcriptase Inhibition Disrupts Repeat Element Life Cycle in Colorectal Cancer. *Cancer Discovery*, (2022).

Parikh AR\*, Szabolcs A\*, Allen JN, Clark JW, Wo JY, Raabe M, Thel H, Hoyos D, Mehta A, [...] Greenbaum BD, **Ting DT**†, and Hong TS†. Radiation therapy enhances immunotherapy response in microsatellite stable colorectal and pancreatic adenocarcinoma in a phase II trial. *Nat Cancer*, (2021); 2(11): 1124-1135.

Franses JW\*, Philipp J\*, Missios P, Bhan I, Liu A, Yashaswini C, Tai E, [...] Ryan DP, Maheswaran S, Haber DA, Daley GQ, and **Ting DT**. Pancreatic circulating tumor cell profiling identifies LIN28B as a metastasis driver and drug target. *Nature Communications* (2020); 11(1): 3303.

Porter RL, Magnus NKC, Thapar V, Morris R, Szabolcs A, Neyaz A, Kulkarni AS, Tai E, [...] Ferrone CR, Haber DA, and **Ting DT**. Epithelial to mesenchymal plasticity and differential response to therapies in pancreatic ductal adenocarcinoma. *PNAS* (2019); 116(52): 26835-26845.

Ligorio M\*, Sil S\*, Malagnon-Lopez J, Nieman LT, [...] Fernandez-Del Castillo C, Ferrone CR, Haas W, Aryee M†, **Ting DT**†. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. *Cell*. (2019 Jun 27); 178(1):160-175.e27.

\*Equal contribution

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