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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 microfluidic chip technologies to capture circulating tumor cells (CTCs), the cells that disseminate to distant organs. Using single cell RNA-seq we have gained unprecedented insight into the programs that drive metastatic spread. We are using these studies to develop blood based "liquid biopsy" biomarkers and generate new therapies to stop the spread of cancer.

The Ting laboratory has utilized RNA-sequencing and RNA in situ hybridization technology 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 been able to capture circulating tumor cells (CTCs) with an innovative microfluidic chip technology and successfully applied RNA-sequencing to these cells to understand their role in the metastatic cascade and to develop novel early detection biomarkers.

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, work with others has found that these satellite repeats can affect the local tumor microenvironment with implications for immunotherapies. We are now trying to identify the HSATII reverse transcriptase and better understand the biological role of satellites in cancer progression.

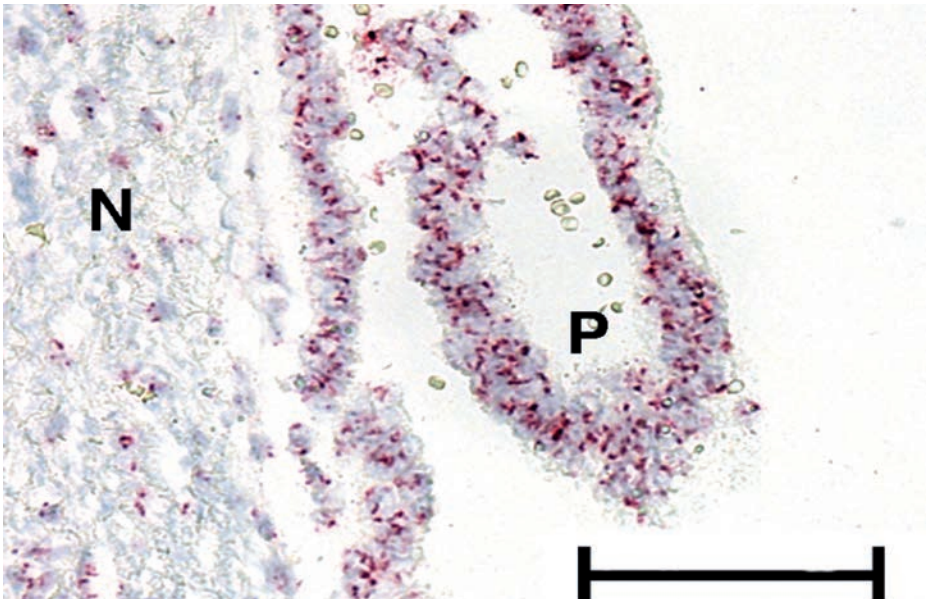

Image of a preneoplastic pancreatic intraepithelial neoplasm (P) positive for the HSATII ncRNA (Red dots).Normal adjacent reactive stroma (N) with minimal expression. Counterstain hematoxylin (blue). Scale bar = 100 μm.

Circulating Tumor Cells: The Liquid Biopsy

The temporal development of circulating tumor cells (CTCs) in tumorigenesis is not well understood, but evidence for CTC shedding in early localized cancers suggests that these cells are heterogeneous and that only a small subset of CTCs have the biological potential to metastasize. Using a novel microfluidic device developed at MGH, we have isolated pancreatic and liver CTCs and perform RNA sequencing on these rare cells. This has revealed the opportunity to develop a novel early detection blood based biomarker and study the metastatic cascade. Using single cell RNA-sequencing, we have characterized the heterogeneity of pancreatic CTCs into three major subclasses, and note that over half of the CTCs are not viable. This illustrates that not all CTCs have the full capacity to metastasize, and that there are likely multiple paths for cancer cell dissemination. In addition, single cell RNA-seq has provided unprecedented transcriptional resolution of CTCs that has revealed significant enrichment for stem cell and epithelial mesenchymal transition markers of these metastatic precursors. Notably, we have also found that CTCs express a significant amount of extracellular matrix proteins normally found in the stroma of primary tumors. This suggests that the seeds of metastasis are in fact producing their own soil during the metastatic cascade. We have recently identified the stromal microenvironment is responsible for generating a significant amount of heterogeneity in pancreatic cancer and drive the development of these CTC phenotypes in both mouse models and patients. The early emergence of CTCs and the opportunity to understand the biology of metastasis in transit offers the potential for developing non-invasive, early detection tools and new strategies to target metastasis.

Selected Publications:

Ligorio M*, Sil S*, Malagnon-Lopez J, Nieman LT, Misale S, Di Pilato M, Ebright RY, Karabacak M, Kulkarni A, Liu A, Jordan NV, Franses JW, Philipp J, Kreuzer J, Desai N, Arora KS, Rajurkar M, Horwitz E, Neyaz A, Tai E, Magnus NKC, Vo KD, Yashaswini CN, Marangoni F, Boukhali M, Fatherree JP, Damon LJ, Xega K, Desai R, Choz M, Bersani F, Langenbucher A, Thapar V, Morris, R, Wellner UF, Schilling O, Lawrence MS, Liss AS, Rivera MN, Deshpande V, Benes CH, Maheswaran S, Haber DA, Fernandez-Del Castillo C, Ferrone CR, Haas W, Aryee M†, **Ting DT**†. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. *Cell* (2019); 178(1):160-175.e27.

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