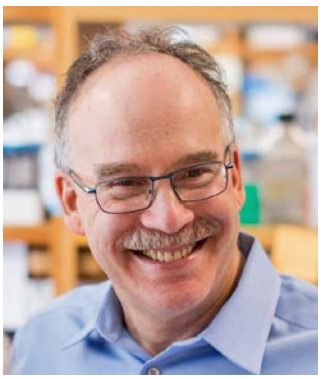
Daniel A. Haber, MD, PhD



Haber Laboratory*

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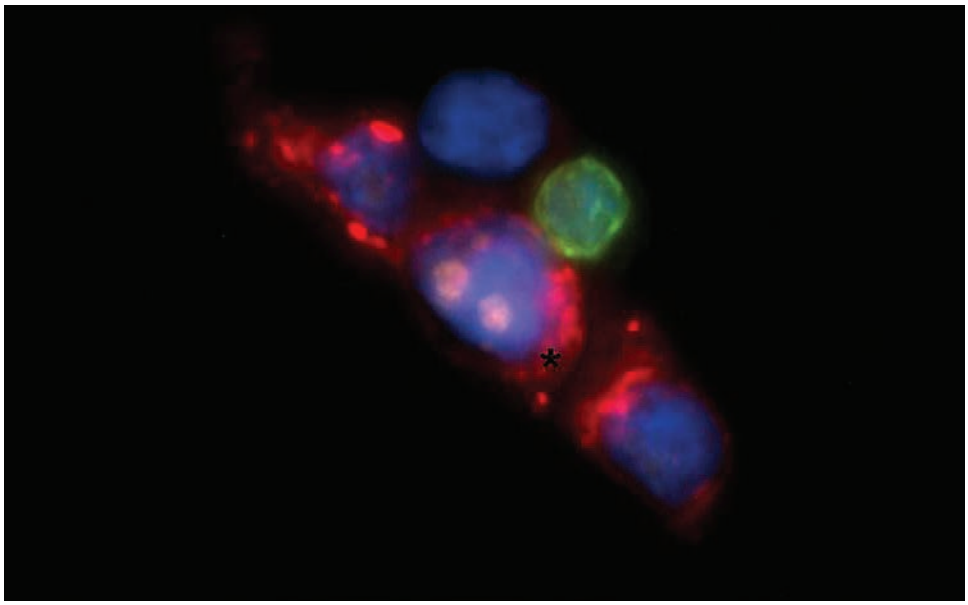
The Haber laboratory focuses on understanding mutations that are acquired by tumors and render them susceptible to specific targeted drug therapies. In 2004, we identified mutations in the EGFR gene in lung cancers which confer dramatic sensitivity to drugs that specifically inhibit that pathway. This finding triggered the application of targeted therapies in lung cancer, and more generally pointed to the critical importance of mutational analysis for treatment selection in common epithelial cancers. Since then, we have collaborated with the bioengineering team led by Dr. Mehmet Toner, the molecular biology group led by Dr. Shyamala Maheswaran, and the MGH Cancer Center clinical disease centers to develop, characterize and apply microfluidic devices to isolate rare circulating tumor cells (CTCs) in the blood of patients with cancer. Using these technologies, our lab seeks to explore 1) blood-based early detection of cancer, 2) noninvasive monitoring of cancer for the emergence of drug resistance, and 3) understanding mechanisms of tumor cell dissemination and metastasis, with the ultimate goal of suppressing blood-borne spread of cancer.

Our laboratory is interested in the genetics of human cancer. Current projects include the use of a microfluidic device to capture circulating tumor cells (CTCs) and its application in early detection of invasive cancer, molecular-directed therapy, and in the study of human cancer metastasis.

Circulating tumor cells and molecular genetics underlying targeted cancer therapeutics

Activating mutations in the epidermal growth factor receptor (*EGFR*) were identified in our laboratory in the subset of non-small cell lung cancer (NSCLC) with dramatic responses to the tyrosine kinase inhibitor gefitinib. We have studied mechanisms underlying such oncogene addiction, as well as the pathways that lead to the acquisition of resistance to targeted therapies, including the application of irreversible kinase inhibitors to circumvent mutations that alter drug binding affinity. Following these efforts to monitor the emergence of drug resistance mutations, we established collaborations with the Toner and Maheswaran laboratories to characterize novel microfluidic devices capable of isolating CTCs from the blood of cancer patients. Our most advanced version of these CTC-Chips relies upon blood flow through a specialized chamber, which allows the high efficiency depletion of antibody-tagged leukocytes, thereby enriching for intact CTCs without selection bias. We have shown that the number of captured CTCs correlates with clinical evidence of tumor response, and that the cells can be used to define molecular markers characteristic of the underlying malignancy, including *EGFR* mutations in lung cancer and measurements of androgen receptor (AR) activity in prostate cancer. We have applied next generation single-molecule RNA sequencing and RNA-in-situ hybridization to characterize the heterogeneous expression profiles of individual CTCs in breast, prostate and pancreatic cancers, as well as melanoma and glioblastoma. To facilitate CTC quantitation and provide the sensitivity and specificity required for early cancer



Circulating prostate tumor cell cluster stained for PSA (green) along with Ki67 (orange) and CD45 (red).

detection, we have we have applied high throughput CTC isolation from blood with molecular genetic and epigenetic markers.

Understanding metastasis through CTC biology

In addition to noninvasive detecting and monitoring of cancer, CTCs provide a window to study the process of blood-borne metastasis. We demonstrated treatment-associated epithelial-to-mesenchymal transitions (EMT) within CTCs from women with breast cancer. Using a combination of mouse models and patient-derived studies, we observed that tumor-derived fragments generate CTC-Clusters, which have greatly enhanced metastatic propensity compared with single CTCs. CTC-Clusters are held together by plakoglobin, whose knockdown dramatically suppresses CTC-Cluster formation and metastatic spread of breast cancer cells. We successfully established long-term *in vitro* cultures of CTCs from patients with estrogen-receptor (ER)-positive breast cancer, identifying treatment-associated mutations in the estrogen receptor (ESR1), as well as acquired mutations in druggable therapeutic targets, such as *PIK3CA* and *FGFR*. In a recent study of prostate tumorigenesis, from the earliest Gleason stages through to metastatic CTCs, we tracked, at single cell level, core DNA hypomethylation domains that arise early in tumorigenesis, thereby silencing genes that are colocalized within a chromosomal locus. Early hypomethylation-induced silencing targets immune-related genes, notably the lipid antigen presentation pathway involved in native immunity, while sparing proliferation-associated genes. Ongoing studies are directed at using patient-derived CTCs and mouse models to understand key steps in cancer metastasis, including the shift from cell quiescence to proliferation, viability during blood-borne transit, and resistance to targeted and immune therapies.

Selected Publications:

Guo H, Vuille JA, Wittner BS, Lachtara EM, Hou Y, Lin M, Zhao T, Raman AT, Russell HC, Reeves BA, Peskow HM, Wu CL, Meissner GA, Efstathiou JA, Lee RJ, Toner M, Aryee MJ, Lawrence MS, Miyamoto DT*, Maheswaran S*, **Haber DA***. DNA hypomethylation silences anti-tumor immune genes in early prostate cancer and CTCs. *Cell*, 186:2765-2782, 2023 PMID 37327786.

Micalizzi DS, Che D, Nicholson BT, Edd JF, Desai N, Lang ER, Toner M, Maheswaran S, Ting DT, **Haber DA** Targeting breast and pancreatic cancer metastasis using a dual-cadherin antibody. *Proc Natl Acad Sci USA* 119: e2209563119, 2022, PMID 36256815.

Guo H, Golczer G, Wittner BS, Langenbucher A,...Vasudevan S, Zou L, Mostoslavsky R, Maheswaran S*, Lawrence MS*, **Haber DA***. NR4A1 regulates expression of immediate early genes, suppressing replication stress in cancer. *Mol Cell*. 81(19): 4041-4058, 2021 PMID: 34624217.

Hong X, Roh W, Sullivan RJ, Wong KHK, Wittner BS,...Toner M, Stott SL, Getz G, Maheswaran S*, **Haber DA***. The lipogenic regulator SREBP2 induces transferrin in circulating melanoma cells and suppresses ferroptosis. *Cancer Discovery*. 11:678-95, 2021 PMID 33203734.

Ebright RY, Lee S, Wittner BS, Niederhoffer KL,...Ting DT, Toner M, Vasudevan S, **Haber DA***, Maheswaran* S, Micalizzi DS. Deregulation of ribosomal protein expression and translation promotes breast cancer metastasis. *Science*. 367(6485):1468-1473, 2020. PMID 32029688.

Miyamoto DT, Lee RJ, Kalinich M, LiCausi JA, Zheng Y,...Toner M, Maheswaran S, **Haber DA**. An RNA-based digital circulating tumor cell signature is predictive of drug response and early dissemination in prostate cancer. *Cancer Discovery*. 8: 288-303, 2018. PMID: 29301747.

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