Dissecting the Contributions of the Extracellular Matrix to Cancer Progression:
a proteomics-based approach

The extracellular matrix (ECM) is a complex meshwork of cross-linked proteins that provides biophysical and biochemical cues that are major regulators of cell migration, differentiation, survival, etc. ECM deposition (desmoplasia) is a hallmark of tumor progression and pathologists have used excessive ECM as a marker of tumors with poor prognosis long before the composition and the complexity of the ECM was even uncovered. However, the biochemical properties of ECM proteins (large size, insolubility) have compromised systematic characterization of ECM composition.

In the first part of my talk I will present the proteomic pipeline we devised to characterize with high throughput the composition of *in vivo* ECMs. The pipeline consists in 1) the sequential extraction of soluble intracellular proteins and the concomitant enrichment of ECM proteins, 2) the digestion of proteins into peptides, 3) the analysis of the peptide composition using liquid chromatography coupled to tandem mass spectrometry and 4) computational data analysis. Proof of concept experiments on normal tissues demonstrated that the ECM of any given tissue is composed of 150+ proteins and that reproducible differences can be detected between tissues [1].

I will then discuss the results of our study aimed at characterizing the composition of poorly and highly metastatic mammary carcinoma. Using human mammary tumor xenografts in mice, we demonstrated that tumors of differing metastatic potential differ in both the tumor- and the stroma-derived ECM. We also demonstrated that a high proportion of the proteins differentially expressed between tumors of differing metastatic potential have causal effects on metastasis [2].

I will finally discuss how the experimental pipeline we developed can be applied to human samples for biomarker discovery studies. Using sets of patient-derived samples (primary metastatic colorectal tumors, paired metastases to liver and normal colon and liver samples), we identified consistent differences in the ECMs of i) colon tumors as compared to normal colon, ii) colon cancer-derived metastases to the liver and normal liver, and iii) primary tumors as compared with metastases derived from them. Based on these changes, we demonstrate that robust signatures of ECM proteins characteristic of each tissue, normal and malignant, can be defined using relatively small samples and from small numbers of patients [3].

Altogether, our results illustrate that the proteomic analysis of the composition of tumor ECMs offers promise for development of diagnostic and prognostic signatures of the metastatic potential of tumors. In addition, the fact that reliable results can be obtained using small tissue samples from limited numbers of patients opens the way to application of these methods to other tumor types or diseased samples.

[1] Naba A, Clauser K.R, Hoersch S, Liu H, Carr S.A and Hynes RO. (2012) The matrisome: *in silico* definition and *in vivo* characterization by proteomics of normal and tumor extracellular matrices. Molecular and Cellular Proteomics, 11(4):M111.014647.

[2] Naba A, Clauser K.R, Lamar J.M, Carr S.A and Hynes RO. (2014a) Extracellular matrix signatures of human mammary carcinoma identify novel metastasis promoters. eLife, 3:e01308.

[3] Naba A, Clauser K.R, Whittaker C.A, Carr S.A, Tanabe K.K and Hynes RO. (2014b) Extracellular matrix signatures of human primary metastatic colon cancers and their metastases to liver. BMC Cancer, 14(1):518.