Using E-cadherin dynamics to predict cancer spread and response to anti-invasive therapies: Insights from intravital imaging

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E-cadherin-mediated cell-cell junctions play a prominent role in maintaining epithelial architecture. Their dysregulation in cancer can lead to the collapse of tumour epithelia and subsequent invasion and metastasis. Recent evidence suggests that, apart from modulating E-cadherin expression, cells are able to mobilise E-cadherin within their cell-cell junctions upon migration and invasion, which can be impaired using Src kinase inhibitors. Here, we have developed a new tool to assess the spatiotemporal dynamics of epithelial tumour cell-cell junctions (endogenous E-cadherin biosensor mouse).

Utilizing a next-generation E-cadherin-GFP knock-in mous[5], we have established Fluorescence Anisotropy Imaging Microscopy (FAIM) to quantify the dynamics of E-cadherin clustering. This enables real-time, *in-vivo* assessment of E-cadherin-based cell-cell junction strength and integrity. By crossing our endogenous E-cadherin-GFP mouse to the highly invasive and metastatic MMTV-PyMT mouse model of breast cancer we can watch E-cadherin clustering dynamics during cancer development and metastasis.

Using subcellular imaging we show that:
(1) E-cadherin clustering is dynamically regulated over the course of mammary gestation with clustering increasing during lactation and decreasing during involution, when tissue remodelling occurs.
(2) E-cadherin clustering becomes de-regulated in invasive and metastatic cancer compared to healthy tissue. Additionally, a decrease in E-cadherin clustering at tumour borders (where cancer cells can invade into adjacent tissue) is seen compared to the tumour centre.
(3) Cancer cells isolated from these mice can be used in a variety of *in-vitro* assays, that mimic key stages of the metastatic cascade, to assess E-cadherin clustering in response to anti-invasive therapies

We suggest that our new biosensor mouse models can be used as:
(1) **novel tools** to fundamentally expand our understanding of cell-cell junction dynamics and cancer invasiveness *in vivo* in native microenvironments.
(2) **novel pre-clinical drug-screening platforms** to predict cancer spread and to estimate the efficacy of anti-invasive treatment *in vivo*.