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Nano-elasticity - novel diagnostic biomarker for breast cancer

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Marko Loparic[1], Marija Plodinec[1], Philipp Oertle[1], Roderick Y.H. Lim[1]

[1]Biozentrum and the Swiss Nanoscience Institute, University of Basel

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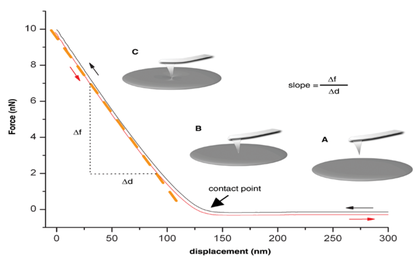
The Center for Molecular Life Sciences

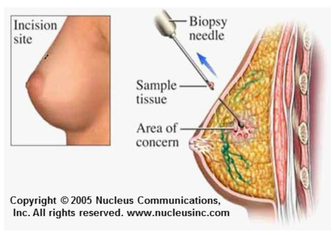
Primary carcinomas (epithelial-derived tumors) are rarely fatal, however cancer cells that invade and metastasize to other parts of the body are responsible for > 90 % cancer -related deaths. Emerging evidence shows that a key to the problem lies in the physicality of the tumor tissue environment and highlight the potential role of the physical properties of cancer cells in detachment from the primary tumor, invasion into the surrounding stromal tissue and finally distant metastases. To adress this issue, we have recently developed an atomic force microscope (AFM) - based apparatus known as "ARTIDIS" (Automated and Reliable Tissue Diagnostics) to identify distinct tumor stages of native human breast biopsies. Our data show that nanomechanical profiles obtained on normal and benign tissues are characterized by a single distinctive stiffness peak. In contrast, malignant tissues exhibit a broad stiffness distribution due to tissue heterogeneity, with a prominent low - stiffness peak representing cancer cells. Because the molecular and environmental etiology of patients is extremly variable, we further validated our findings in MMTV- PyMT (mouse mammary tumor virus polyoma middle T antigen) mice, which confirmed human biopsy measurements. Further investigation reveals that compliant regions in primary malignant tissues are largely hypoxic indicating an increased migration potential in late cancer. Similarities in stiffness profiles between distant metastatic lesions in murine lungs with the primary tumors provide compelling evidence of this. Overall, these findings indicate direct correlation between hypoxia-related tissue softening and cancer progression to metastasis.

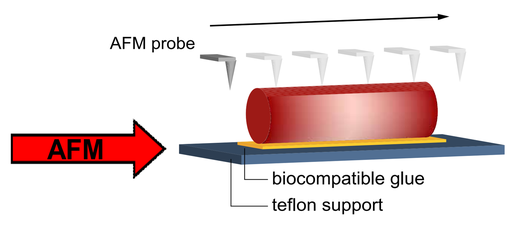
Principle of AFM

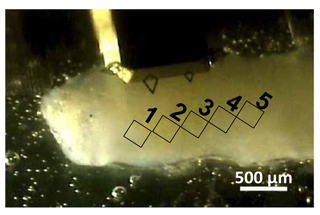


The Atomic Force Microscope (AFM) measures the nanomechanical properties of a sample under physiological conditions. Force measurements are aquired by cyclically pressing the AFM probe against the sample and recording the probe deflection. The sample stiffness is then computed from the slope of the unloading curve. Here we show stiffness maps that were generated by making 32 x 32 force measuremens within a given area.

Experimental design for IT-AFM testing of human breast biopsies





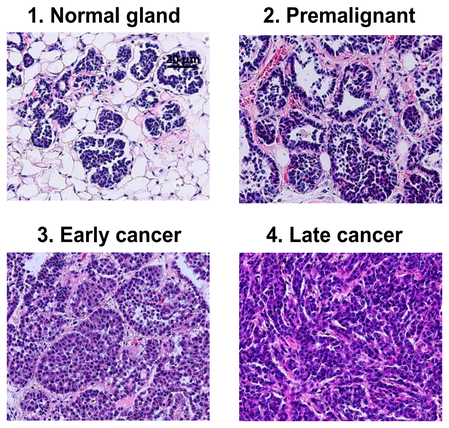


Measurements are performed on native tissues under physiological conditions.

Following tumor progression and hypoxia in MMTV - PyMT mice

MMTV - PyMT transgenic mice provide a reproducible and well - controlled model to study the development and progression of human breast cancer.

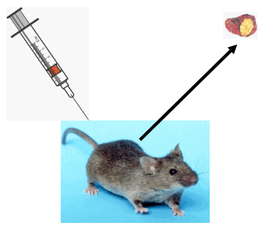
Tumor progression in MMTV - PyMT



Correlating hypoxia with nanomechnical properties

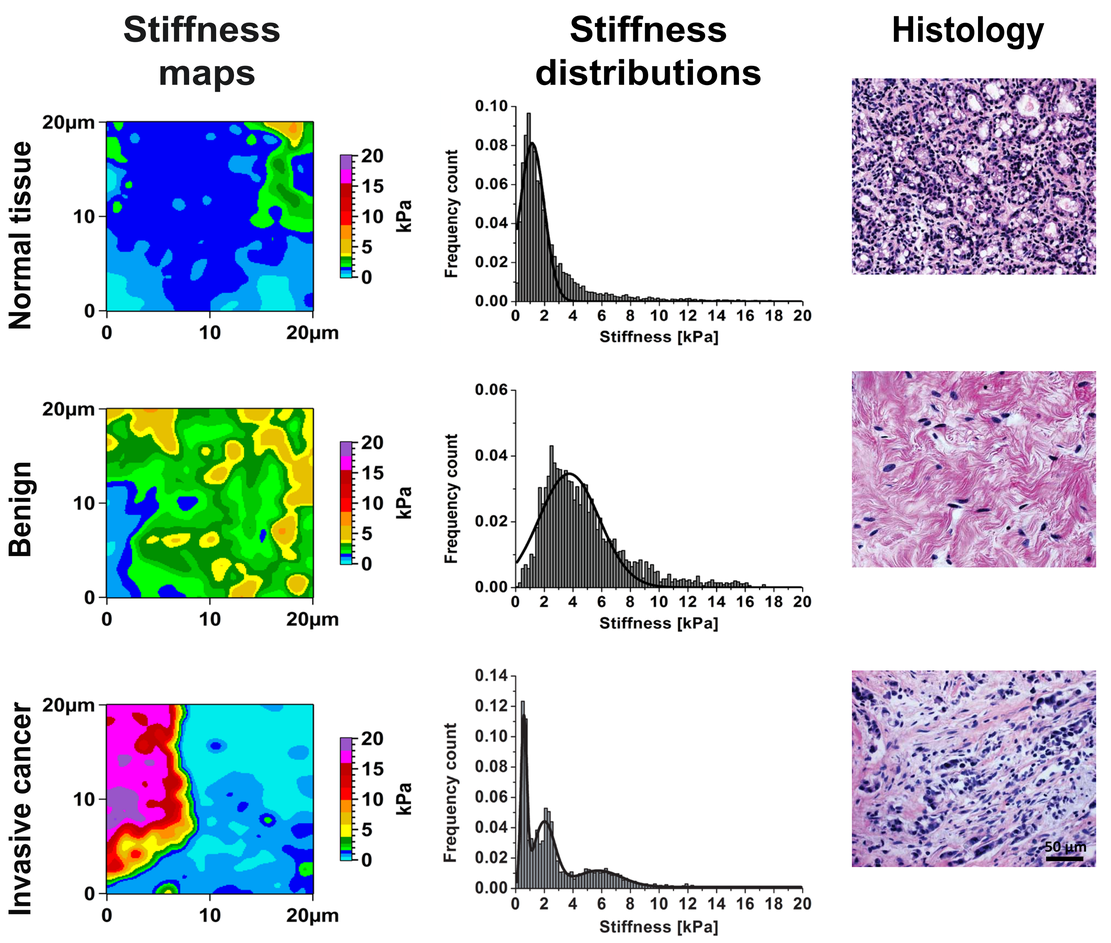
Hypoxyprobe-1 (pimonidazole)

excised tissue for AFM testing



Hypoxia is measured *in situ* by injecting a hypoxia marker **Hypoxyprobe-1 (pimonidazole)** in a living mouse. After 1.5 hours, the murine mammary tissue is excised and probed by AFM followed by immunohistochemistry analysis.

Nanomechanical signature of human breast biopsies

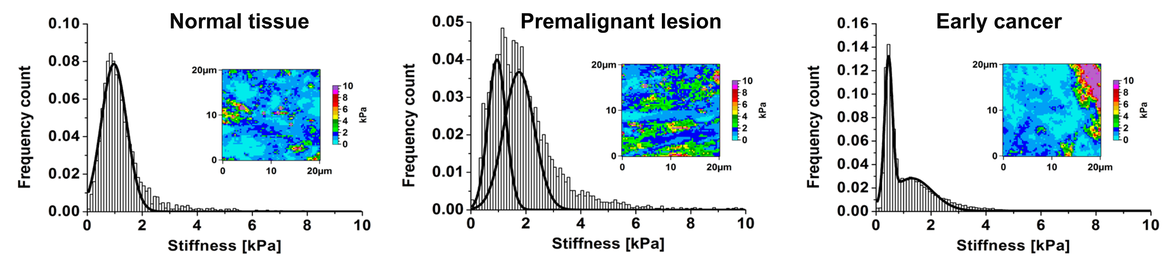


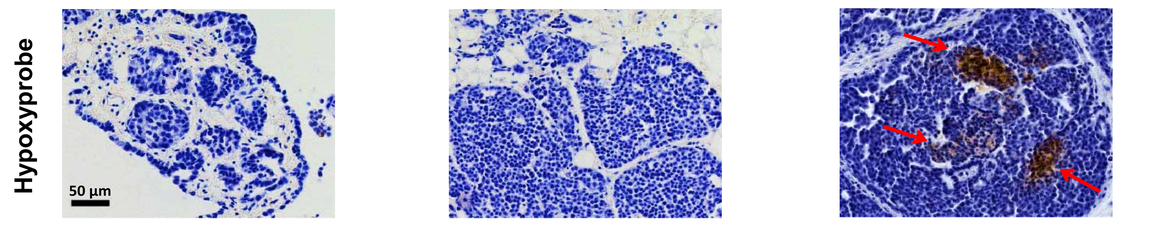
Human breast cancer exhibits heterogeneous nanomechanical properties. Invasive cancer cells exhibit 2-fold softening surrounded by the stiffer peripherial stroma.

What is the origin of heterogeneous nanomechanical behavior of human breast cancer?

Change in nanomechanical properties
Hypoxia ⟷ Tumor progression

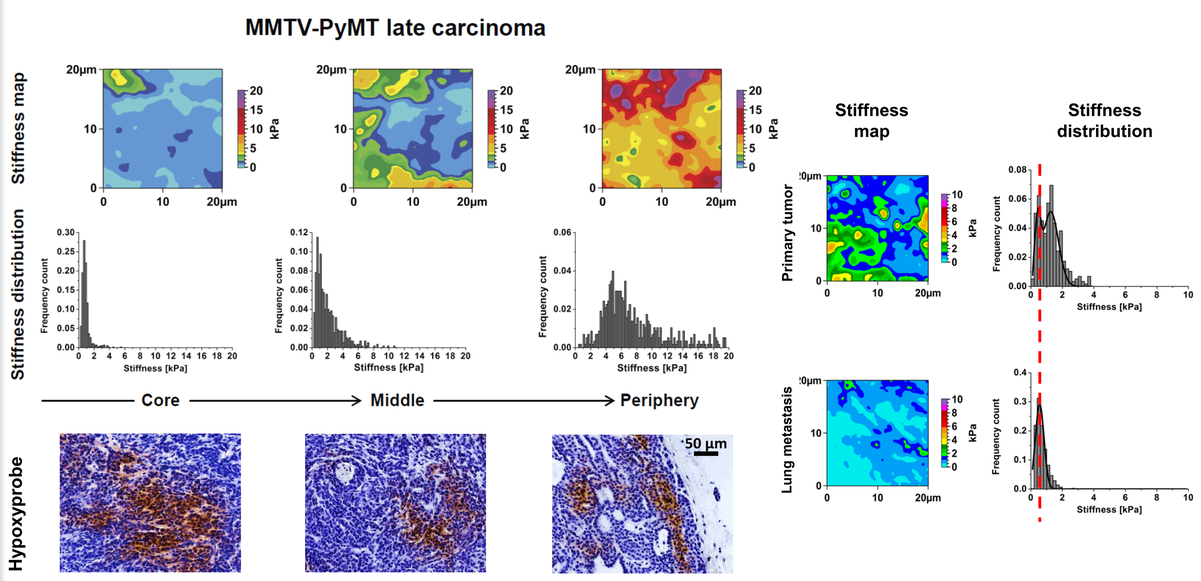
Nanomechanical profiles of murine mammary tissues





Hypoxia in the early cancer correlates with the central softening.

Nanomechanical properties of late cancer and lung metastasis



We find that an overall broader stiffness distribution results from increased tumor heterogeneity in invasive cancer. However, when comparing across regions, tumor core correlates with a predominance of hypoxic cells whereas tumor periphery shows mixture of soft (hypoxic) and stiff features (collagen I). The presence of soft cancer cells in secondary lung tumors supports the notion that cell softening promotes metastases.

Soft and hypoxic cell phenotype is conducive to lung metastasisis.

(1) AFM measurements reveal distinct distinct nanomechanical changes in breast tumor tissues that correlate with each stage of tumor progression. (2) This study suggests how stiffness heterogeneity (i.e., high ratio of soft versus stiff areas may be a phenotypic indicator of cancer progression. (3) Nanomechanical tissue diagnostics might prove a valuable prognostic marker for cancer progression with significant implications for treatment.