Intravital optical window imaging of RhoA-, Rac1- and Akt-FRET biosensor mice monitoring drug treatment response in cancer.

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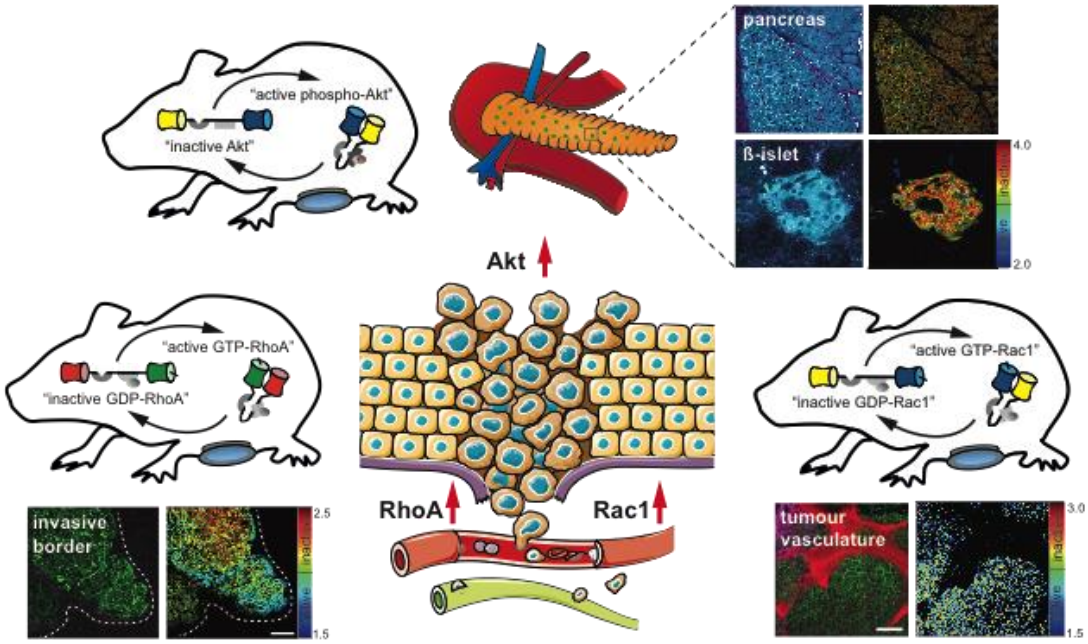
Small GTPases such as Rac1 and RhoA enable cells to migrate during development as well as metastasize during cancer progression by actively remodelling the cytoskeleton of cells. Co-option of this activity has been demonstrated both in mammary and pancreatic cancer. Furthermore, in pancreatic cancer the PI3K pathway is aberrantly regulated in ~21% of cases. More specific, time-resolved monitoring of these key drivers ranging from an *in vitro* to *in vivo* settings can be achieved by the use of FRET-biosensors and genetically engineered mice expressing these biosensors to track protein activity and the effect of therapeutic intervention.

Here, we describe the generation and characterization of these FRET-biosensor mice to examine RhoA[1], Rac1[2] and Akt kinase activity in an *in vivo* setting in a variety of cell types in homeostasis as well as in mouse models of cancer. Time-correlated single photon counting (TCSPC) fluorescent lifetime imaging (FLIM) on a multiphoton system of these signalling biosensors in live mice was achieved by the application of optical windows[3]. Elevated levels of Rac1 and RhoA activity were observed in models of invasive mammary and pancreatic cancer such as the polyoma-middle-T-antigen (PyMT) model and the KPC ($KRas^{G12D/+}$ and $p53^{R172H/+}$ driven) model. There, spatially defined to the invasive borders, high small GTPase activity was observed, absent in non-invasive mouse models such as the KC ($KRas^{G12D/+}$ alone) and KPflC ($KRas^{G12D/+}$ and p53 KO). Finally, spatiotemporally resolved imaging of the inhibition of RhoA, Rac1 and Akt activity live *in vivo* was achieved by employing optical windows implanted on top of developed tumours. Treatment was monitored for a period of up to 24h and the therapeutic response further correlated live to the local extra-cellular matrix and to the local vasculature. This allowed for unprecedented insight into treatment dynamics and the strong potential for further tailoring of targeted therapeutics in *in vivo* settings.

In conclusion, the development and use of the FRET biosensor mice represents a strong resource in understanding tissue context specific signalling events during cancer progression and drug target validation *in vivo*.



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