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Monitoring of pancreatic ductal adenocarcinoma in mouse models via multiparametric magnetic resonance imaging

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease due to the late diagnosis and the lack of effective chemo/radiotherapy. The development of strategies for early tumor detection and evaluation of new treatments in models with high predictive value is of outmost importance. To characterize the spontaneous course of tumor progression, we monitored tumor growth kinetics and physiology in genetically engineered mouse models (GEMMs) of PDAC by multiparametric magnetic resonance imaging (MRI).

For generation of conditional endogenous PDAC models, *Ptf1a*$^{+/Cre}$ mice were crossed to *Kras*$^{+/LSL-G12D}$*(K)Tgfa(T), K;p53(P)*$^{+/fl}$, *K;P*$^{fl/fl}$, *K;P*$^{+/R172H}$*;T* and *K;P*$^{+/fl}$*;T* GEMMs and subjected to serial T_2-weighted (T_2w) MRI from 4−6 weeks of age onwards. Upon detection of solid tumor, diffusion-weighted imaging and dynamic contrast enhanced-MRI protocols were implemented. Survival analysis and histopathological characterization of PDAC were performed and correlated with MRI data.

Tumor growth kinetics as determined by semi-automated segmentation of T_2w data showed good correlation with the animal genotype. In *K;P*$^{+/fl}$ animals, tumors were first detected at 12−15 weeks of age while significant tumor burden was observed around week 20. Tumor appearance was highly heterogeneous, with cystic and solid tumor parts clearly distinguishable. Additional TGFa overexpression led to earlier onset, faster tumor growth and significantly shorter lifespan. Large premalignant IPMN lesions, seen in TGFa expressing models, appeared hypointense compared to PDAC. Deletion of both p53 alleles led to development of PDAC already at 4 weeks of age. These tumors appeared more homogeneous and were histologically confirmed as highly fibrotic well differentiated PDAC. ADC values correlated well with the histological grading of tumor. Tumor perfusion revealed differences among the tumors and allowed identification of spontaneous necrosis.

Conclusions: Using non-invasive multiparametric MRI we characterized PDAC development and progression in mouse models. This information is valuable for correct interpretation of spontaneous and drug-induced changes in tumor physiology when conducting preclinical therapy studies. Strong heterogeneity in tumor morphology emphasizes the necessity for an individualized tumor assessment and response monitoring.

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Early detection of pro-apoptotic effects of chemotherapy using a caspase-3 fluorescent probe in human breast tumorgraft models

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Background: Apoptosis is a programmed cell-death process believed to play an important role in tumor response to chemotherapy. As an early effector in the apoptotic cascade, caspase-3 (CASP3) activation is an attractive target for *in vivo* molecular imaging. We used a noninvasive molecular imaging technology to study early detection of CASP3 activity *in vivo,* as a potential marker of therapeutic efficacy in a panel of human breast tumorgraft models.

Methods: Human breast tumor xenografts were established directly onto *Nude* mice from patient tumor material (Marangoni et al. Clin Cancer Res 2007; 13: 3989–3998). Ten breast tumor models were characterized for their response to Adriamycin/Cyclophosphamide (A/C) treatment, defining two groups of 5 responders and 5 non-responder tumors. *In vivo* measurement of tumor CASP3 proteolytic activity was carried out 6−72 h after A/C treatment in groups of three mice bearing subcutaneous tumors. QCASP3.2 probe, a FRET probe emitting in the near-infrared wavelength (QUIDD, France) was injected intravenously (7 nmol) and fluorescence imaging was performed 6−24 h later with an optical imaging system (IVIS-Lumina II, Caliper, CA). For *in vitro* assays, tumor protein extracts were prepared in lysis buffer containing protease inhibitor cocktail, and incubated during 1 h at 4ºC. Total proteins were quantified by the microBCA protein assay kit (Pierce, Rockford, IL). Caspase activity was measured on synthetic substrate Ac-DEVD-AFC for caspases 3/7 (AnaSpec, San Jose, CA). Enzymatic reactions were allowed to proceed after 30 mn at room temperature. Fluorescence intensity was measured at different time points on a PerkinElmer LS 50B spectrofluorometer (PerkinElmer Life Sciences, Norwalk, CT). Specificity of the fluorometric signal was confirmed by adding specific caspase inhibitor to the reaction mixture (casp3 inhibitor, z-DEVD-fmk). Levels of cleaved CASP3 and PARP were assessed in parallel by western blotting.

Results: Basal levels of CASP3 activity were variable between tumors. Increased level of CASP3 activity was detected *in vivo* in tumors of mice treated with chemotherapy 48h post-treatment. This increase was confirmed *in vitro* by enzymatic and western blotting analyses. Increased CASP3 activity in tumors was correlated with their sensitivity to A/C treatment as assessed by the extent of tumor growth inhibition 10−20 days later.

Conclusion: The QCASP3.2 probe is efficient in early detection of tumor apoptosis *in vivo.* The data confirm that drug-induced tumor apoptosis can be an early marker of antitumor efficacy. This approach will allow molecular imaging assessment of tumor apoptotic response to therapy in preclinical models. Quantitative imaging of apoptosis *in vivo* with the QCASP3.2 probe will facilitate dynamic screening of compound's activity and the assessment of tumor CASP3 activity as an early predictor of therapeutic efficacy in various tumor models.

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An in vivo-like tumor stem cell-related glioblastoma in vitro model for drug discovery

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The discovery of tumor stem cells being highly resistant against therapy makes new demands to model systems suitable for evaluation of the effects of new drugs on tumor stem cells. The aim of the present study was therefore to develop an in vivo-like in vitro glioblastoma model for testing the effects of new drugs on tumor cells including tumor stem cells. Implantation of glioblastoma cells into organotypic brain slice cultures has previously been published as a model system, but not using a stem cell favourable environment.

Organotypic corticostriatal rat brain slice cultures were prepared and cultured in a serum containing medium replaced after three days with a serum-free stem cell medium. Thereafter fluorescent DiI labelled glioblastoma spheroids from the cell line U87 and the tumor stem cell line SJ-1 established in our laboratory were implanted into the brain slices between cortex and striatum. The growth and invasion of the tumor cells were monitored for six days by confocal microscopy followed by processing for histology and immunohistochemical staining. In addition, in vivo xenografts were performed in BALB/c nude mice with U87 cells and SJ-1 cells.

The results showed an expansive growth of the U87 implants, but no invasion of cells into the brain tissue, neither in vitro nor in vivo. In contrast, SJ-1 was clearly invasive both in vitro and in vivo, but not very expansive. The co-cultures and brains with xenografts were immunohistochemically stained with anti-human vimentin confirming the results obtained with hematoxylin-eosin staining and confocal microscopy. Both in vitro and in vivo, U87 implants had a very high proliferation index, whereas the invasive phenotype of SJ-1 only had a low index as shown by Ki-67 immunohistochemistry. Immunohistochemistry for the stem cell marker CD133 revealed only a few positive cells in contrast to staining for the proposed tumor stem cell marker podoplanin showing a more pronounced staining.

In conclusion, we have established an in vivo-like in vitro co-culture model system that enables us to monitor tumor growth and invasion using a stem cell favourable environment. We propose that this model system could play a role in testing of new drugs targeting tumor stem cells.

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Monitoring radiation therapy in orthotopic mouse models by bioluminescence imaging

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Introduction: Radiation therapy is a standard of care for many cancer indications including brain and prostate. Preclinical methods for using localized radiation (+/−chemotherapy) against tumors in deep tissues may increase clinical relevance and predictive power of models. The ability to non-invasively detect tumor burden and radiation response in deep tissue tumors would be an additional advantage. We describe application of localized radiation therapy in two orthotopic tumor models with parallel luciferase driven imaging of tumor burden and response.