Supplemental material and methods

Immunohistochemistry and Immunofluorescence

Immunohistochemistry was performed on PFA fixed and paraffin embedded samples, cut on microtome (3-5μm thickness) and stained according to the standard protocols. Antibodies used in murine studies were: rabbit monoclonal to β3 integrin (ab75872, Abcam), goat polyclonal to αv integrin (sc-6618, Santa Cruz), rat monoclonal to CK19 (TromaIII, Developmental Hybdridoma Bank Iowa) and rabbit polyclonal to CD31 (ab28364, Abcam). For immunohistochemistry of human samples, mouse monoclonal to β3 integrin antibody (mab2008, Millipore) was used. Human samples were investigated by using tissue microarrays (each sample in triplicate) isolated from the tumor core and prepared from PFA fixed surgical samples after written informed consent was obtained from all patients. Sampling was approved by the local Institutional Review Board and ethics committee.

For microscopic fluorescence analysis, Integrisense680 was injected into wildtype and *CKP* animals and allowed to circulate for 24h. OCT-embedded PDAC were then cryo-sectioned at thickness of 10-20μm and stained with an antibody against β3 integrin.

Western Blotting

Western blot was performed according to standard laboratory protocols. Briefly, snap-frozen murine pancreatic tissue samples were snap frozen and preserved in –80C until use. Samples were then homogenated with a static rotor homogenizer in appropriate lysis buffer, protein concentration was measured and proteins were separated on SDS-PAGE.

Primary cancer cell lines were isolated from *CKP* animals as previously described (*1*) and their lysates were prepared for Western blot according to standard protocols. Western blots were performed with the following antibodies: rabbit monoclonal to β3 integrin (ab75872,

Abcam), goat polyclonal to αv integrin (sc-6618, Santa Cruz), rabbit polyclonal to HSP90 (Sigma). HSP90 was used as loading control.

^{68}Gallium labelling of NODAGA-RGD

NODAGA-cyclo(RGDyK) (abbr. NODAGA-RGD) was purchased from ABX GmbH (Radeberg, Germany). The cold reference complex ^{nat}Ga-NODAGA-cyclo(RGDyK) was prepared by mixing equimolar solutions of $Ga(NO_3)_3$ and NODAGA-RGD. The formation of ^{nat}Ga-NODAGA-RGD was verified by MS analysis (LC-MS analysis was performed with an Ion-trap 500 Varian system with ESI (Varian Deutschland GmbH, Darmstadt, Germany). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained from MERCK.

Radio-TLC of ^{68}Ga-NODAGA-c(RGDyK) was performed on VARIAN glass microfiber chromatography papers impregnated with silicic acid. Radio-TLCs were evaluated using a BIOSCAN TLC scanner consisting of B-MS-1000 scanner, B-EC-1000 detector with a B-FC-3600 GM tube. Radio-HPLC was performed on a SYKAM system using a Chromolith column (MERCK, 100×4.6 mm), flow rate 1.0 ml/min with radioactivity and UV detection (220 nm). Eluents were water (A) and acetonitrile (B), both containing 0.1% TFA. HPLC was performed using an isocratic elution with 3% B for 2 min, followed by a gradient to 60% B in 6 min and isocratic elution with 95% B for 3 min.

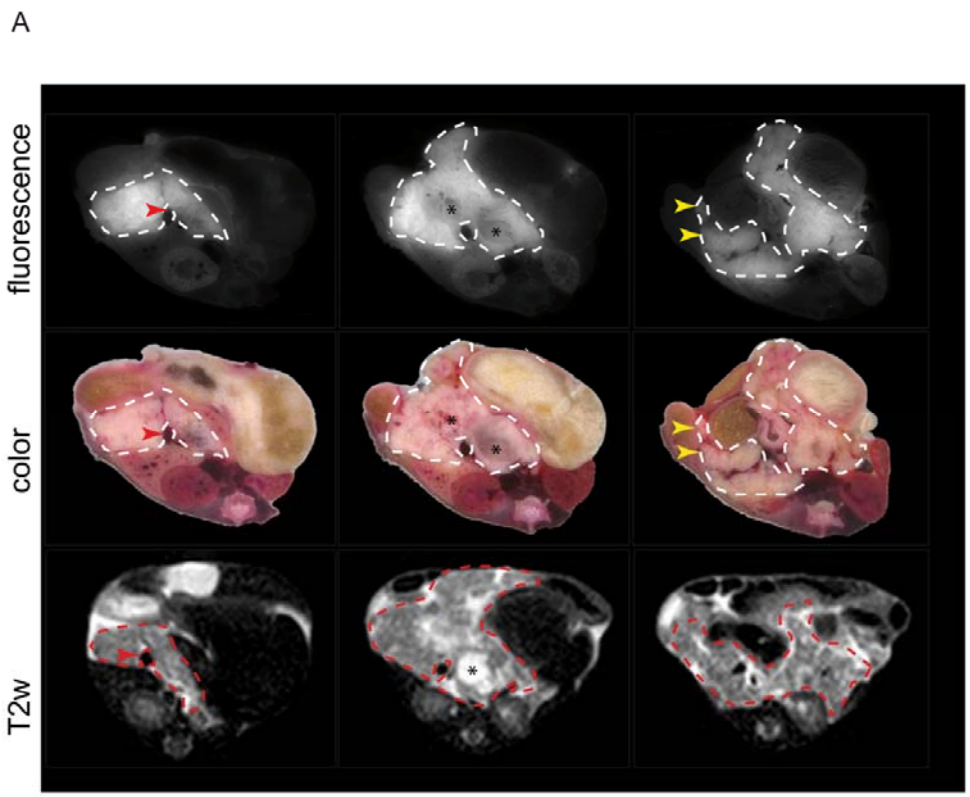
^{68}Ga labeling was performed on a fully automated system (GallElut^{+} from SCINTOMICS GmbH, Germany). A ^{68}Ge/^{68}Ga-generator with SnO_2 matrix (obtained from iTHEMBA LABS, South Africa) was eluted with 1.0 M aq. HCl and a fraction of 1.25 mL containing approx. 80 % of the entire activity (ca. 1.0 GBq) was transferred into a standard reactor vial (ALLTECH, 5 ml) containing the precursor NODAGA-c(RGDyK) (2 nmol) and an aq. solution of HEPES (600 mg HEPES in 500 µö water; resulting pH 3.3). The vial was heated to 95 °C for 5 min while air was slowly bubbled though the solution for agitation. Mixture was passed

through an SPE cartridge (WATERS SepPak® C18 classic) that was previously conditioned by purging with ethanol (5 ml, absolute, Ph. Eur.) and water (10 ml). The cartridge was then purged with water (10 ml) and air (10 ml). The labeled product was eluted from the cartridge into a 10 ml flask with 1 ml ethanol, followed by purging with PBS buffer (1 ml, pH 7.4, for injection) and water (1 ml). The product was concentrated in vacuo to 1 ml, thus leaving no ethanol in the mixture and exhibiting the appropriate pH and osmolality for injection.

Quality control: Radio-TLC of ^{68}Ga-NODAGA-c(RGDyK) was performed using two different eluents. A: 0.1 M aq. sodium citrate as mobile phase, where free $^{68}Ga^{3+}$ is eluted with the solvent front (R_F ca. 0.8–1) and the product stays at the origin (R_F=0.1) and B: 1.0 M NH_4OAc/MeOH (1:1) as mobile phase, $Ga(OH)_4$ stays at the origin (R_F = 0) and the radiolabeled product is eluted (R_F 0.6–0.7). The purity of ^{68}Ga-NODAGA-c(RGDyK) was > 98.0% according to radio-TLC. Radio-HPLC: retention time of ^{68}Ga-NODAGA-c(RGDyK) was t_R = 5.5 min. Purity of the radiolabeled product was > 99.0%.

REFERENCES:

1. Ardito CM, Gruner BM, Takeuchi KK, et al. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer cell.* Sep 11 2012;22(3):304-317.



Supplemental Figure 1:

A) Direct comparison of three T2w and corresponding fluorescence images made at three different positions in the body of the *CKP* animal. Tumor is delineated with a white or red dotted line. Strong intensity contrast of the tumor to surrounding tissue is visible on all fluorescent images. Specific structures that were identifiable on T2w images were also distinguishable on fluorescence images: portal vein (red arrowhead), smaller tumor lobes (yellow arrowhead), large and small cysts (asterisk).

Supplemental Video 1:

Three-dimensional rotational representation of the FMT-XCT reconstruction around animal axis. The animal head is at top of the video and tail at the bottom. The fluorescence signal (orange representing high signal intensity) due to specific accumulation of IntegriSense 680 can be seen in the PDAC.