

# MR Microscopy of ECM Alterations in a Mouse Model of Pancreatic Cancer

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**Introduction** Early changes in pancreatic cancer (PC) go undetected because of the lack of reliable detection methods. The hallmarks of PC include marked stromal fibroblast proliferation and deposition of extracellular matrix (ECM) components, resulting in extensive fibrosis. It has been previously shown that MR microimaging coupled with volume rendering enables visualization of the pancreatic ECM as well as its exocrine and endocrine components, based only on endogenous tissue contrast [1]. In this study, we have used MR microscopy to examine the stromal changes in a transgenic mouse model of PC that expresses mutant Kras, namely, the Pdx1-Cre/LSL-Kras (PCK) mouse [2]. PCK transgenic mice develop ductal lesions similar to human PanINs that infrequently progress to invasive cancer in adult mice. From high spatial resolution MR images, we have calculated the volume fraction, thickness, and compactness of the ECM as a method to quantify stromal changes associated with pancreatic fibrosis in the PCK mouse.

**Experimental** Formalin-fixed pancreata of Pdx1-Cre/LSL-Kras transgenic and normal control mice were examined using MR microscopy. High resolution MR microimages were acquired on a 600MHz imaging spectrometer using a 3D RARE8 pulse sequence with fat suppression. The imaging parameters were: TR 2500ms, effective TE 40ms, FOV 20mm x 16mm x 3.55mm, matrix size 320x256x100 for pixel size of 62 $\mu$ m x 62 $\mu$ m x 35 $\mu$ m. For fat suppression, a frequency-selective Gaussian 90-degree pulse was applied at a frequency offset of 3.5ppm from water. 3D MR images were visualized using VolView (Clifton Park, NY) volume rendering software that allows the manipulation of the voxel opacity as a function of MR signal intensity. The ECM thickness distribution was computed using a two-step semi-automated approach. First, the ECM was manually segmented in a representative 3D region of contiguous slices using ITKSnap [3]. Then the ECM thickness distribution was computed by using 2D mathematical morphology (erosions, dilations, and topological skeletonization). For the manually segmented ECM, we also computed the ECM compactness, a rotation invariant shape descriptor, defined as  $\sqrt{\{(4\pi A)/P^2\}}$ , where P = perimeter of ECM and A = its area.

**Results and Discussion** High spatial resolution T<sub>2</sub>-weighted spin-echo MR images of the mouse pancreas demonstrate excellent contrast between the structures within the pancreas. In the normal mouse pancreas, acinar cells have the lowest intensity and constitute >80% of the gland (Figure 1, left). Islets have intermediate signal intensity and are easily identified from their circular or elliptical appearance. ECM has the highest signal intensity: in 2D displays, it appears as a network of curves with varying thickness; in 3D volume images, ECM appears as sheets (not shown). Images of the pancreas from a PCK mouse appear markedly different. PCK mouse ECM has slightly lower signal intensity relative to normal mouse ECM; however, it is more extensive, indicating an increase in the amounts of ECM material as a result of fibrosis (Figure 1, right). Increased fibrosis in the PCK mouse pancreas was verified histologically (Figure 2).

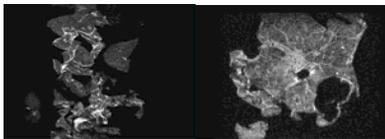


Figure 1. Stromal architecture in MR images of control and PCK mouse pancreas. Left: Pancreas from an 11 month old control mouse. ECM appears as a network of hyperintense curves. Right: Pancreas from a 17-month old PCK mouse. ECM appears with slightly reduced signal intensity, but is clearly more extensive in the PCK pancreas.

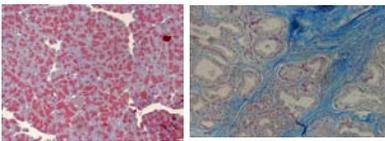


Figure 2. Trichrome staining of control and PCK mouse pancreas. Left: Pancreas from a control mouse at 10 months showing no fibrosis. Right: Pancreas from a PCK mouse at 9 months shows extensive staining of the connective tissue (blue) as a result of fibrosis. Fibrosis increases with age in the PCK mouse.

Several tissue changes that are associated with lesion development in the PCK mouse pancreas are captured elegantly by MR microimaging as shown in Figure 3. Briefly, islets are deformed, acinar volume is reduced, lesions appear and stroma proliferates in the PCK mouse.

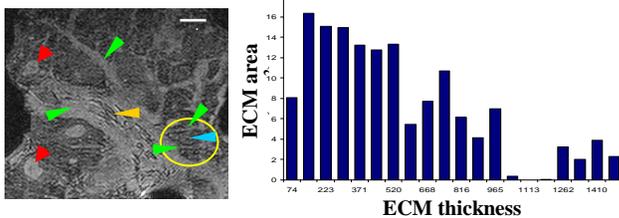


Figure 3. Magnified view of a representative slice from an MR microimage of a 17-month old PCK mouse pancreas. Regions of fibrosis (defined as increased ECM deposition and loss of acinar cells) have higher signal intensity (green arrow heads). Extensively branching ECM surrounds lesions (gold arrowheads) and groups of acinar cells (blue arrow). Islets (red arrows) often appear deformed. Note the overall loss of contrast in the image due to stromal infiltration into acinar lobules. The gold circle includes examples of interlobular fibrosis (green arrows) and intralobular fibrosis (blue arrow). Scale bar = 1mm. The histogram of the distribution of ECM thicknesses ( $\mu$ m) measured in the PCK transgenic mouse pancreas quantifies the extensive fibrosis. (ECM area is given in  $\text{mm}^2$ )

Volume fraction of the ECM in the PCK mouse pancreas (36.9%) is markedly higher than that in the normal pancreas (12.3%), illustrating the presence of increased ECM material and loss of acinar cells in fibrosis. The thickness of normal ECM was below 0.25mm, with 80% of the ECM area  $\leq$ 0.10mm. In contrast, fibrosis has led to much thicker ECM in the transgenic mouse pancreas as shown by the histogram in Figure 3. The ECM in the PCK mouse pancreas is also denser as shown by increased compactness (0.15 compared to 0.09 in the normal pancreas).

Our data demonstrate that the PC-associated ECM remodeling can be detected and quantified using high spatial resolution MR imaging. The ability to image the spatial and temporal progression of ECM alterations might be helpful in evaluating therapies that target stroma in PC in preclinical studies.

## References

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