

Inflammatory Pseudotumors Induced by AdCre in Transgenic Oncopigs After *In Situ* Tumor Induction

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Background

Comparable anatomy and physiology of pigs make them an ideal model for many human diseases, and few large animal tumor models exist for use in interventional radiology. Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide. HCC, reportedly may be induced in transgenic oncopigs with hepatic injection of adenovirus encoding Cre-recombinase (AdCre). AdCre activates mutant KRAS^{G12D} and TP53^{R167H} genes and induces tumors local to the administration site. This study evaluated cellular composition of AdCre-induced oncopig liver lesions at three time points. The original goal was to produce a hepatocellular carcinoma model within 4 weeks post-injection. The objective of this study was to characterize these lesions using multiplex immunofluorescence assays.

Methods

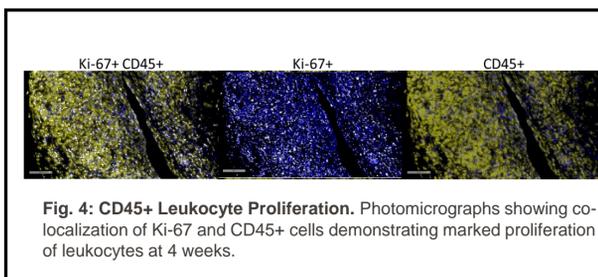
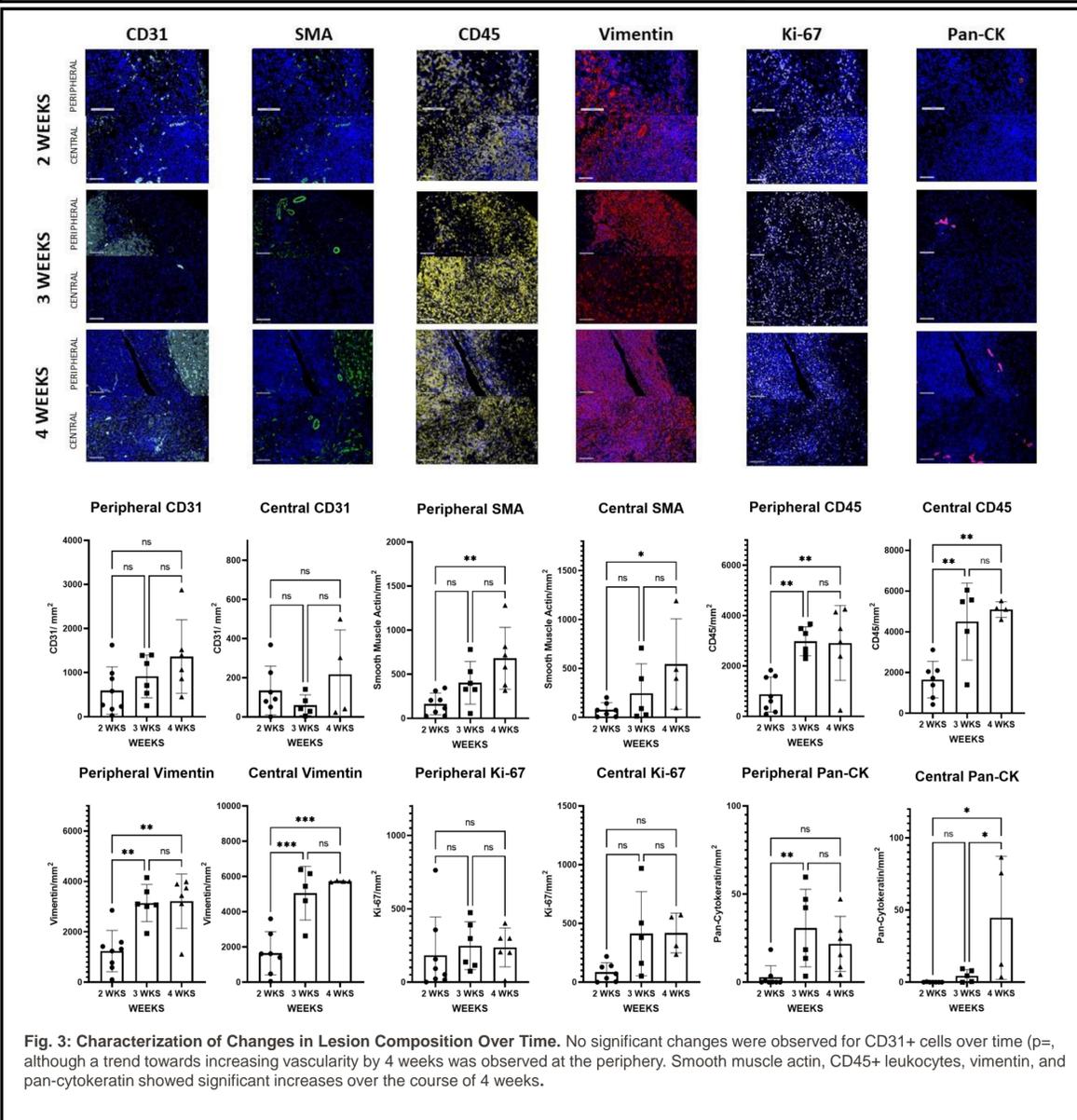
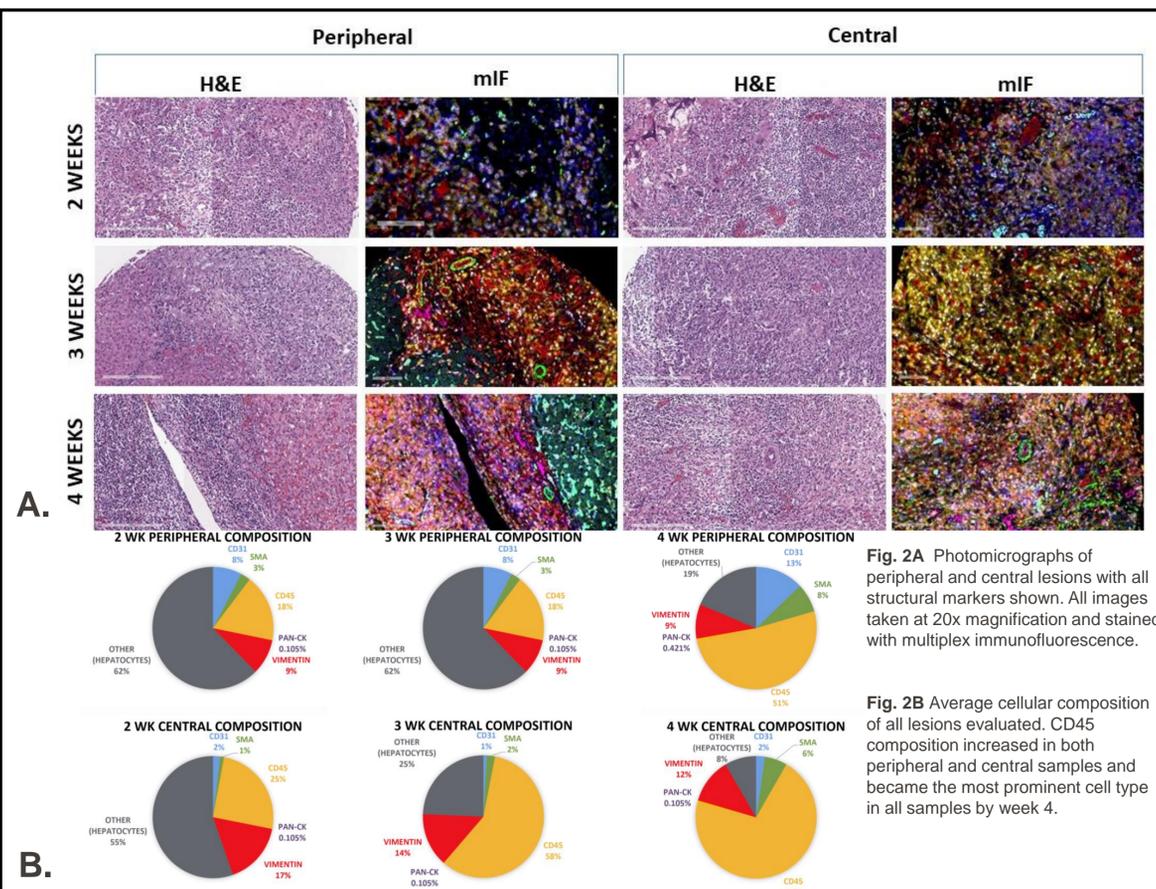
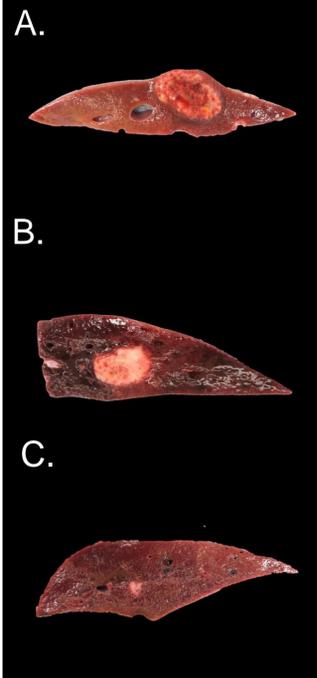
Oncopigs (n=9) received AdCre injections in the liver. Pigs were sacrificed at 14 days (n=3), 21 days (n=3), and 28 days (n=3). Liver tissue was collected in 10% neutral buffered formalin, processed, and paraffin embedded. Tissue microarray (TMA) was created containing representative central and peripheral areas of liver lesions. 4µm thick sections were stained using a Leica Bond Rx autostainer and Akoya Biosciences Opal 7-color kit. Slides were imaged using a Leica Versa 8 whole slide fluorescent scanner. Samples were evaluated using the nuclear stain DAPI in conjunction with CD31 (endothelium), smooth muscle actin, CD45 (leukocytes), vimentin (mesenchymal cells), Ki-67 (proliferation), and pan-cytokeratin (epithelium) to determine total cell counts and relative abundances of each marker in the samples. Cell composition was evaluated using Halo and analyzed with GraphPad Prism for statistical significance.

Results

Fig. 1A Liver transection from an oncopig in the 2-week cohort. The lesion was focal, pale tan to yellow, firm, well-demarcated, firmly adhered microlobulated mass. Surrounding tissue architecture appeared to be affected by the mass.

Fig. 1B Liver transection from an oncopig in the 3-week cohort. The lesion was focal, pale tan, somewhat firm, less demarcated, firmly adhered mass. Surrounding tissue had fewer defined hepatic lobules than observable in the 2-week sample.

Fig. 1C Liver transection from an oncopig in the 4-week cohort. The lesion was focal, pale tan, firm, poorly demarcated, firmly adhered mass considerably smaller than prior masses. Overall, week 4 masses were smaller than prior weeks.



Discussion

Liver lesions were induced in transgenic oncopigs after *in situ* tumor induction. Lesions appeared to variably regress by 4 weeks. We optimized a 7-color multiplex immunofluorescence panel to characterize the lesions. We found a trend towards increasing CD31+ endothelial cells at the periphery of lesions possibly suggesting early neoangiogenesis. The most remarkable findings were significant increases in vimentin, alpha smooth muscle actin, CD45+ leukocytes, and pan-cytokeratin+ bile ducts over time. By 4 weeks, lesions consisted predominantly of CD45+ leukocytes and vimentin. Ki-67+ appeared to predominantly co-localize with CD45+ cells consistent with immune cell proliferation. Vimentin and smooth muscle actin both increased with statistical significance throughout the experiment, with co-expression in some areas consistent with myofibroblasts. An increase in myofibroblasts could suggest wound healing after localized injection trauma caused by the AdCre. A statistically significant uptick in CD45-expressing cells is strongly suggestive of a leukocyte-dominant inflammatory response. Additionally, Ki-67 expression did not significantly increase over time. Ki-67 is expressed by proliferating cells and increases in cancerous cells which divide without cessation. Finally, Pan-cytokeratin inconsistently increased throughout the lesions in areas of reactive biliary hyperplasia. Characteristics of increasing proliferating leukocytes, fibroblasts, and myofibroblasts share some features of a group of benign entities referred to as “inflammatory pseudotumors” (IPT) that resemble neoplasia clinically and on diagnostic imaging. IPT frequently presents as a single mass and consists of variable inflammatory, granulomatous, and myofibroblastic reactions. Additional characterization of immune infiltration may aid in elucidating the immunopathogenesis of inflammatory pseudotumors induced in transgenic pigs after direct injection with AdCre.

Conclusion

Hepatic AdCre injection induces inflammatory pseudotumors in transgenic oncopigs resulting in targetable lesions for interventional radiology studies, although the biological significance of such models is currently unclear.

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