Immunological characterization of the Oncopig model and detection of cell-mediated immune responses to cancer

Poster 1659

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BACKGROUND

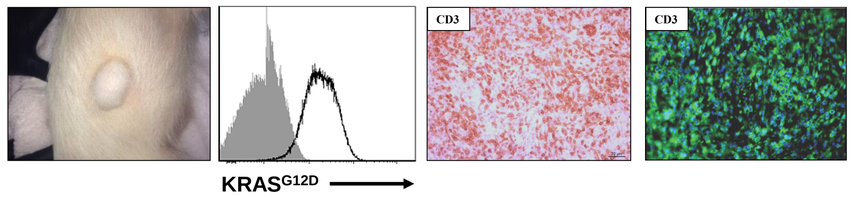
Immunotherapy has recently shown great breakthroughs; but the majority of preclinical studies has been based solely on rodent models, and it is now well established that mice "lie" and often poorly mimic many human diseases[1]. Therefore, the translation of therapies to human patients can be troublesome. Due to the high degree of similarities in both the immunome, metabolism, and size between humans and pigs[2], there is a great potential in using pigs as a large animal model for translational cancer research[3]. The Immunoscore is a new approach for staging cancer patients, which underlines the importance of the immune status within the tumor microenvironment and how it affects both patient's outcome and response to therapies[4]. For this reason, it is crucial to know the immunological landscape of the tumors in order to push the therapeutic developments forward.

THE ONCOPIG MODEL

The Oncopig is a novel transgene porcine model which, following an injection with the adenovirus encoding a Cre recombinase (AdCre), will start expressing $KRAS^{G12D}$ and $TP53^{R167H}$; two mutations commonly found in human patients. This will subsequently result in tumor formation at any desired place[5].

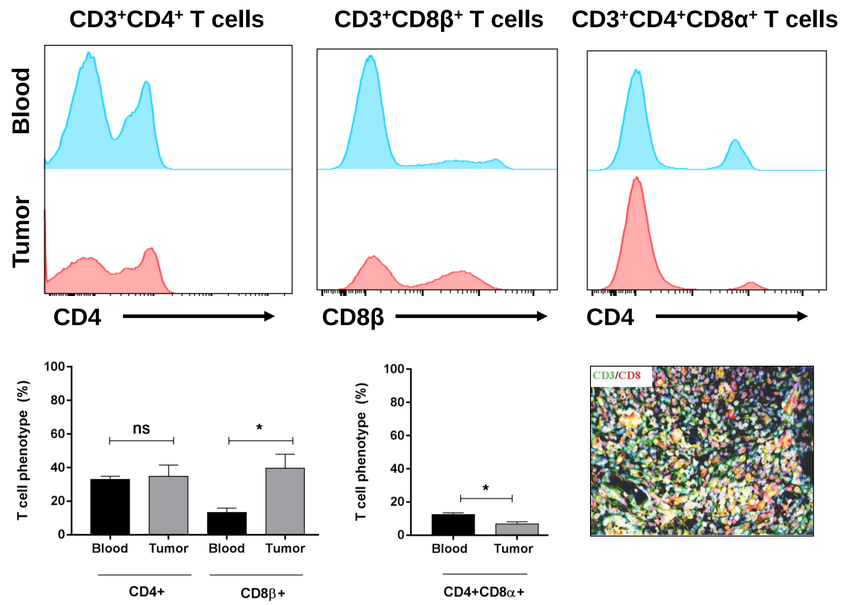
RATIONALE: A large animal model enables a more efficient translation of preclinical immunotherapies to human patients.

Figure 1. Oncopig tumors are heavily infiltrated by T cells



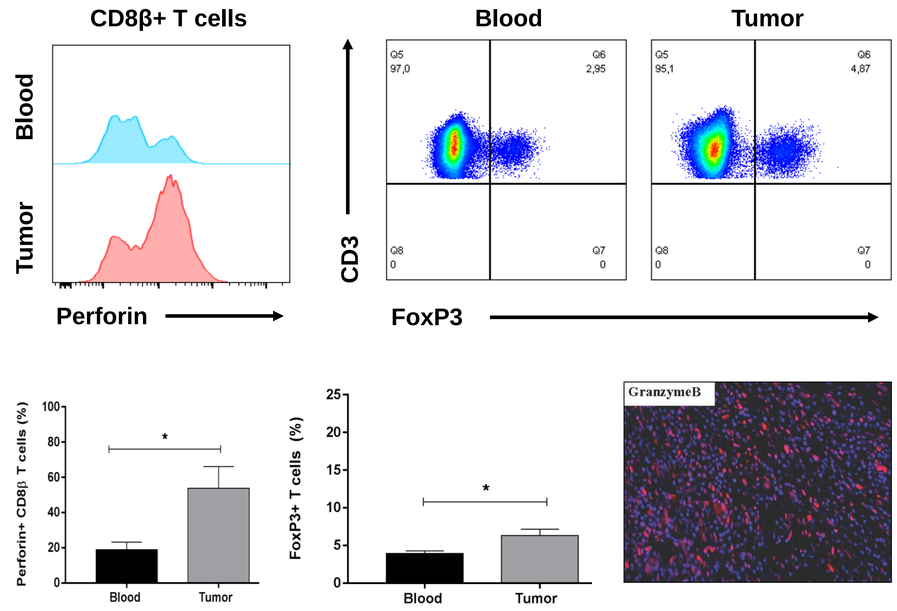
AdCre injection results in tumor formation. $KRAS^{G12D}$ expression in tumor biopsies was detected by flow cytometry and tumor-infiltrating $CD3^+$ cells were detected by both immunohistochemistry and immunofluorescence.

Figure 2. $CD8\beta^+$ T cells specifically infiltrate the tumors



Flow cytometric detection of T cell subsets in blood samples and tumor biopsies obtained from Oncopigs 21 days post AdCre injection. Immunofluorescence staining on tumor sections was used to detect both $CD3^+$ and $CD8\alpha^+$ T cells.

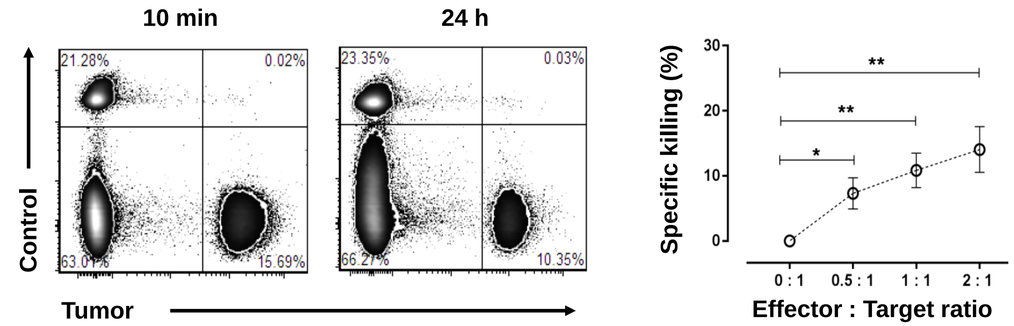
Figure 3. The tumor microenvironment contains cytotoxic and regulatory T cells



Intracellular flow cytometry was used to detect both $perforin^+$ and $FoxP3^+$ T cells in circulation and in tumor biopsies.

Detection of Granzyme B^+ cells in the tumor was done using immuno-fluorescence.

Figure 4. Autologous tumor cells are specifically killed by the immune cells



In vitro fluorescence-based cytotoxicity assay with tumor cells ($eFluor450^+$) and control cells ($eFluor670^+$) being co-cultured with effector cells. Samples were harvested at 10 min (baseline) and 24 h post co-culturing and flow cytometry was used to reveal the percentage specific killing of tumor cells.

CONCLUSIONS

Taken together, our results show that the established Oncopig tumors are infiltrated by T cells exhibiting an either cytotoxic or regulatory phenotype, thus indicative of a tumor microenvironment mimicking the complexity seen in human patients. Additionally, we were able to measure cell-mediated immune responses to cancer in this novel, large animal model, and both the cytokine production and tumor-specific killing hence underline the potential in using the Oncopig for future testing of immune therapies against human cancer.

REFERENCES

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