

provides a suitable strategy for the generation of optimised xenodonor pigs.

TRR 127- *Biology of xenogeneic cell, tissue and organ transplantation – from bench to bedside.*

331.9 (and poster P.231) | Introduction of the TLA sequencing analyses for a better identification of the transgene integrations, to select porcine founder animals for breeding

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Xenotransplantation of modified pig islets could be a possible medical treatment for T1/T2 diabetic patients. Tailored genetic engineering of a genomic safe harbor locus assisted by programmable nucleases (ZFNs, TALENs and CRISPR/Cas9) is making easier and reproducible also the generation of new transgenic large animal models. To validate in vivo our preliminary in vitro data (Mourad et al., 2017), we generated cloned transgenic porcine lines targeting the Insulin-GLP1Ser8M3R transgene (I-GS8M3R) into the genomic safe harbor V2G locus (Chromosome 9-EP2921048), using TALENs. Resulting transgenic pancreatic islets presented an increased glucose-stimulated insulin secretion.

The aim of our work was to detect all the possible transgene integrations occurred in our targeted V2G/I-GS8M3R founder lines, introducing the Targeted Locus Amplification (TLA) technique as a new support to our genotyping procedure.

TALENs-mediated integrations of transgene I-GS8M3R in V2G locus were achieved nucleofecting two different PERV-C negative and low PERV-A/B lines (female and male). Resulting Puromycin-resistant colonies were genotyped with 4 different PCR assays to select ones, having an intact transgene copy and a targeted V2G-allele, as nuclear donors for the Somatic Cell Nuclear Transfer (SCNT) experiments. At birth tissue biopsies were taken from alive piglets for genotyping analyses, identifying the donor colony, and to establish primary fibroblast cell lines for cryopreservation. TLA sequencing analyses of selected founders were outsourced to Cergentis (Netherlands).

We selected by PCR, 6 V2G-targeted colonies (1 female = 1D5; 5 males = 1C2, 1F2, 2C2, 2E3 and 2E7) for SCNT. V2G-targeting of InsGLP1Ser8-M3R transgene resulted monoallelic in colonies 1D5, 2C2, 2E3 and 2E7 and bi-allelic in colonies 1C2 and 1F2. Characterization of alive born piglets revealed that colonies 1D5, 1C2, 1F2 and 2E3 produced healthy animals. TLA sequencing analyses confirmed the V2G-targeting of the I-GSer8M3R expression cassette for the male clones (1C2, 1F2 and 2E3), identifying also different transgenes tandems in lines 1C2 and 1F2. Instead, the TLA

results on the 1D5 female line, revealed that an unexpected rearrangement occurred eliminating the I-GS8M3R transgene from the targeted V2G locus and that an intact copy of the I-GSer8M3R was randomly integrated only into the Chromosome 17.

Conclusion: Mono- or bi-allelic V2G-gene targeting of the I-GSer8M3R construct using TALENs was suitable for the generation of healthy pig clones. At the same time the access to the Next Generation Sequencing technologies benefits, integrating the TLA analyses in our classical molecular PCR screenings, permitted us to define better the characterization of the V2G-targeting events and to detect new random transgene integrations. Following this strategy, we will be able to select the founder animals suited for establishing a breeding colony.

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331.10 (and poster P.226) | Humanization of Von Willebrand factor in pigs reduces platelet sequestration in a xenogeneic lung perfusion model

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Purpose: Von Willebrand factor (vWF) is an important element of hemostasis, mediating platelet adhesion and promoting platelet aggregation under shear stress conditions. Non-physiologic activation of human platelets by porcine vWF (pvWF) leads to immediate platelet aggregation in vitro. We hypothesized that expression of humanized vWF (hvWF) by pig donor lungs prevents the activation of the coagulation cascade and platelet sequestration when pig lungs are perfused with human blood. Here we report for the first time results from lung perfusion experiments, using pigs with humanized vWF.

Methods: GalTKO.hCD46 transgenic lungs, in which specific domains of the porcine vWF was replaced with the human ortholog sequences, were perfused with freshly collected human blood and compared to identical lungs, missing the vWF modification (n = 4 each). The blood in both groups was treated with aGPIIb Fab, histamine receptor blocker and a thromboxane synthase inhibitor. Lungs were perfused for 8 h or until the organs failed. Platelet counts were measured by flowcytometry.

Results: Both groups showed similar survival time with one lung in each group failing at 440 min and all other organs reaching the time of elective termination. Pulmonary vascular resistance remained very low and constant in both cohorts. No significant differences were observed in BTG levels and neutrophil counts. Platelet sequestration was significantly reduced and delayed in lungs with hvWF