Modifying the Glycome in Pigs for Xenotransplantation

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Inactivation of glycosyltransferase genes during human evolution is a fundamental cause of differential glycosylation in humans and pigs, resulting in the presence of carbohydrate structures on pig cells that are recognized by natural (preexisting) human antibodies. αGal, the first of these epitopes to be identified, is synthesized by α1,3-galactosyltransferase, encoded by the *GGTA1* gene. The crucial importance of αGal in solid organ xenotransplantation was demonstrated by the finding that knockout of *GGTA1* prevented hyperacute rejection in the pig-to-nonhuman primate (NHP) preclinical model. However, there is evidence to suggest that knockout of additional glycosyltransferase genes will further improve the "compatibility" of porcine xenografts. One such gene is *CMAH*, which is responsible for the synthesis of N-glycolylneuraminic acid (Neu5Gc).

The development of efficient genome editing techniques (ZFNs, TALENs, and CRISPR/Cas9) has revolutionized the genetic engineering of donor pigs, allowing precise and rapid targeted mutagenesis in cultured primary cells, which can then be cloned by somatic cell nuclear transfer. The Tector group was the first to adopt this technology to knock out multiple glycosyltransferase genes, namely, *GGTA1* and *CMAH*.[1] When tested against 121 human serum samples, peripheral blood mononuclear cells from double-knockout (DKO) pigs generally bound less antibody than *GGTA1* single-KO cells.[2]

In this issue of *Transplantation*, the group presents preliminary evidence that the elimination of Neu5Gc may have benefits in pig liver xenotransplantation beyond reducing the binding of natural antibodies.[3] In vivo and ex vivo models have shown that pig livers rapidly sequester human and NHP platelets, resulting in the development of life-threatening thrombocytopenia in the pig-to-baboon model (reviewed by Ekser et al[4]). Inhibition and knockout studies indicate that the asialoglycoprotein receptor-1 (ASGR-1) plays an important role in the binding of human and NHP platelets to porcine endothelium,[5,6] whereas deletion of αGal and/or expression of human CD46 have no effect.[7] In the current study, Butler et al[3] perfused pig livers with human platelets and found that DKO livers sequestered significantly fewer platelets than either *GGTA1* KO or WT livers, suggesting for the first time the involvement of Neu5Gc in xenogeneic platelet uptake. The authors go so far as to propose that the DKO modification is more effective than ASGR-1 KO, although some fine tuning of the model was needed to demonstrate this quite subtle difference. Nevertheless, the potential importance of this finding is that eliminating Neu5Gc may kill two birds with one stone: reducing the targets for non-Gal antibodies, and solving a key problem in liver xenotransplantation.

The study by Butler et al[3] is a pure in vitro and ex vivo investigation and focuses on binding of human platelets to porcine liver endothelial cells in the absence of innate immune mechanisms such as the complement and coagulation cascades. It therefore remains to be seen whether the reduced platelet binding to DKO livers can be confirmed, for example, in perfusion experiments with whole human blood. In addition, the study does not address the mechanism linking Neu5Gc to the binding and phagocytosis of human platelets by porcine liver endothelial cells. It will be very important to understand what deleting Neu5Gc does to the endothelial glycocalyx and its anticoagulant and anti-inflammatory function. Which sugars are expressed on the endothelium when both αGal and Neu5Gc are missing? How does this influence the binding of regulatory proteins and growth factors from the plasma, which are known to be important for endothelial function? Last but not least, could it be that the ASGR-1 expression or function is altered in the DKO pig livers?

A companion paper in the current issue[8] looks at the effect of deleting a third porcine glycosyltransferase, β1,4 N-acetylgalactosaminyl transferase 2, which is encoded by *B4GALNT2* and is thought to synthesize a xenoantigen similar to the SD^a blood group antigen in humans.[9] The first important finding by Wang et al[8] is that renal microvascular endothelial cells (RMEC) from DKO pigs show less binding of natural antibodies in pooled human serum than *GGTA1* KO cells; this confirms the potential benefit of the *CMAH* KO in a cell type that is arguably more relevant in xenograft rejection than previously studied cells. They then immortalized the RMEC and deleted *B4GALNT2* using CRISPR/Cas9 and demonstrated a small but significant further reduction in binding of human natural antibodies to the triple-KO (TKO) cells. The fact that the binding of preformed, naturally occurring human antibodies to the DKO and TKO RMEC is reduced suggests that the genetically modified pig cells may express some carbohydrate structures on their surface, which are similar to the ones on human cells. Whether this is indeed the case remains to be studied. It is also as yet unclear whether the manipulation of the pig glycome as used here will have other, completely unforeseen consequences as

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shown in mouse models,[10] and it remains to be seen whether the TKO pigs will be viable.

Together, these papers highlight the power of genome editing and show, at least in principle, the scope and relative ease of progressive "carbohydrate remodeling" to reduce the antigenicity of pig xenografts.

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