Deleting the Gal Epitope from the Donor Pig

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The galactose-α1,3-galactose (Gal) epitope is abundantly expressed on pig endothelium and is now well recognized as the major xenoantigen in the pig-to-human combination.[1] Because of the pivotal role of Gal in hyperacute rejection and its likely involvement in acute vascular rejection, its deletion from the donor organ would be a major advance in xenotransplantation. One approach to achieving this is to use gene targeting technology to 'knock-out'(KO) the α1,3-galactosyltransferase gene (α1,3-GT) responsible for producing Gal. This has not yet been realized in the pig, but the recent achievement of cloning pigs by nuclear transfer has paved the way for this development in the near future[2] provided the Gal epitope is dispensable in this species.

Gene Targeting Technology

Gene targeting in embryonic stem (ES) cells has been used to generate α1,3-GT knockout (Gal KO) mice.[3,4] This process involves manipulation of undifferentiated, pluripotent ES cells in vitro to insert a disrupting sequence into the α1,3-GT gene, thus rendering it non-functional. ES cells carrying the Gal KO mutation were selected and injected into blastocysts to produce chimeric mice. Offspring from those chimera exhibiting germline transmission of the mutation were then interbred to generate Gal KO mice. This process has not been possible in pigs because despite intensive efforts, ES cells have not been isolated. Primordial germ cells, another cell type which can be used for targeted gene disruption in mice, have been used to produce chimeric pigs,[5] but germline transmission could not be achieved. Even if this were possible the logistics of breeding pigs makes this process extremely resource intensive. Rate-limiting steps include the lag time before germline transmission is demonstrated, and breeding the mutation onto a desired background could take several years in itself.

Cloning Technology

Recent advances in cloning technology has offered the possibility of bypassing the need for pluripotent cells, chimera production, and screening for the genetic modification. Wilmut and co-workers first demonstrated that cloning by nuclear transfer was possible in mammals using a somatic cell from a fetus[6] and subsequently from an adult animal.[7] Transfer of the somatic cell nucleus to an unfertilized egg was followed by activation with an electrical pulse, and the resultant developing embryo was implanted in a surrogate mother resulting in the birth of a 'clone' of the original animal. The name given to this sheep, 'Dolly', has become synonymous with the cloning of mammals. These investigators took the technology a step further by producing a transgenic lamb by nuclear transfer.[8] In this case, the donor cell was a fetal fibroblast transfected with the human blood clotting factor IX gene.

The pig is a more difficult prospect because of the need for sows to have multiple viable embryos in a litter to complete a pregnancy, and the earlier timing of embryonic genome activation has been hypothesized to greatly reduce cloning efficiency by mechanisms that remain unknown. PPL Therapeutics have modified the normal nuclear transfer process to generate a litter of five cloned piglets[2] by using serial nuclear transfer, whereby the donor nucleus from a granulosa cell was first transferred to a zygote before a second transfer to an enucleated

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oocyte. This process is thought to facilitate nuclear reprogramming and hence blastocyst formation. Overall cloning efficiency was low (less than 1% of nuclear transfers performed), but in practical terms only a single animal cloned using the nucleus from a manipulated cell is needed to establish a herd of Gal KO pigs.

Strategy to Produce a Gal KO Pig

It is in fact the characteristics of the nuclear donor cell that currently prevent the generation of a Gal KO pig by nuclear transfer. Gene targeting to disrupt the α1,3-GT gene in a pig cell suitable for nuclear transfer has been hindered by several factors. Some pig somatic cells like the granulosa cells used to successfully clone pigs either cannot clonally propagate indefinitely in culture or have a very low frequency of homologous recombination compared to the mouse ES cells on which the technique was perfected.[9] Additional methods of enhancing the targeting process, culture and the selection of cells with the desired modification will require a robust cell line such as fetal fibroblasts.[10] Gene targeting in ovine fetal fibroblasts to place a transgene at the procollagen locus and production of live sheep by nuclear transfer was recently described, raising hopes that the process would be possible in pigs.[11]

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The Dispensability of Porcine Gal Expression

A critical issue in deleting Gal expression in pig is the possibility that this cell surface molecule is not dispensable. Galilli has found that Gal expression in membranes from pig organs is a hundred to a thousand-fold higher than in corresponding mouse organs.[12] He speculated that this higher expression in pigs may be associated with a essential role for Gal in cell function or survival, raising the possibility that the generation of a Gal KO pig will not be possible. Evidence against this notion is the evolutionary knockout of α1,3-GT in higher primates and the successful generation of Gal KO mice. However, the question can only be answered when gene targeting technology is perfected in the pig.

Conclusions

The appeal of donor organ modification over current ineffective and often toxic modalities of immunosuppression can only be entertained in cross-species organ transplantation. The discovery of Gal as the major xenoantigen has established the deletion of Gal from the pig as the primary goal in xenotransplantation research. Technology has thus far failed to deliver, but given recent breakthroughs and the resources being invested by both scientific and commercial interests, it is likely that a conclusion will be reached in the near future. The scientific advances made along the way will provide powerful tools with which to overcome subsequent barriers to successful xenotransplantation.

Note added in proof: A further two groups have reported successful cloning of pigs. Onishi A, Iwamoto M, Akita T et al. Pig cloning by microinjection of fetal fibroblast nuclei. Science 2000; 289:1188-1190. Betthauser J, Forsberg E, Augenstein M et al. Production of cloned pigs from in vitro systems. Nature Biotechnol 2000; 18:1055-1059.

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