

New gene modification may help prevent corneal graft rejection

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in London**

GENE therapy modification of donor corneas prior to transplantation could increase graft survival, the Moorfields Bicentenary Meeting heard. Lab and animal work demonstrates that therapies could address several of the underlying rejection mechanisms either individually or in combination.

Frank Larkin MD FRCOphth, consultant ophthalmologist at Moorfields Eye Hospital, London, UK, outlined three strategies that could improve the chances of graft survival. Two aim at maintaining or improving corneal endothelial cell density, while the third aims at mitigating, or ideally neutralising, the immune response in the grafted eye.

Earlier work found that an adenovirus vector, with no immunomodulatory effect but simply expressing a histochemical marker gene, was expressed mainly in the endothelium even though the researchers exposed the whole cornea.

"This finding was fortuitous in the context of corneal transplantation because we know that the endothelial monolayer is critical to transplant survival. This type of experimental approach indicated the potential for using recombinant adenovirus vectors for transferring genes into corneal endothelial cells in eye bank type conditions that have been standard for many years," said Dr Larkin.

What's more, these genetically modified corneas survived transplantation in rabbits. In those tests the adenovirus antigens did not increase the immunogenicity of the donor cornea. The findings have led to new research to find the best gene therapy strategies and the best candidate genes to

reduce graft rejection.

Preventing apoptosis and inducing replication

The strategies to maintain cell-density involve two different approaches, one is to prevent or reduce cell death, the other is to induce cell replication in the endothelial cells.

To test the anti-apoptotic approach, Dr Larkin modified the donor cornea by incubation with an adenoviral vector expressing the gene for the enzyme, catalase. The enzyme limits the amount of endothelial cell death caused by exposure to hydrogen peroxide and oxidative cell stress in stored donor corneas. His research showed that most of the modified cells survived hydrogen peroxide exposure, he noted, adding: "This is just one example of an application of genetic modification of donor cornea in eye bank storage which would be useful in preventing cell death."

For the second strategy, Dr Larkin sought to push the endothelial cell cycle to replication. Dr Larkin and his colleagues used gene therapy to induce over-expression of the transcription factor E2F2. Increased expression of this factor induces cell cycle progress from the G phase to the S phase and then on to mitosis. The team exposed human corneas to adenoviruses expressing either E2F2 or a marker gene.

"What we have found is that you do indeed see division and an increase in cell density of the corneas that are exposed to E2F2. However, the adenovirus antigens which are expressed with E2F2 might increase donor corneal immunogenicity. Therefore this type of strategy has to be tested in vivo in an animal

model before it can be proposed for corneal storage."

If this strategy proves viable, however, it could be of great benefit to corneal transplants. Currently, grafts suffer an enormous loss of endothelial cell density during recovery, storage, and transplantation. Post-graft surgical interventions, such as cataract extraction, and natural cell loss further reduce endothelial cell density and deturgescent function over the life of the graft.

Neutralising the immune response

Dr Larkin's third strategy for maximising the potential of corneal graft success focuses on modified donor corneas attempting to actively neutralise the immune response in the graft eye.

"If you look within the aqueous humour for cytokines, for example tumour necrosis factor (TNF), one finds biologically active cytokines within the aqueous and in the anterior chamber long before observed rejection onset," said Dr Larkin.

With colleague Dr Andrew George, Dr Larkin hypothesised that if endothelial cells could be induced via adenovirus vector to express a cytokine receptor, in this case TNF-receptor, the receptor might absorb the cytokine before rejection onset, preventing or mitigating graft rejection.

Results were disappointing, however. Though the receptor was expressed, it had an insignificant effect on graft survival. Another research group did show better graft survival in donor corneas expressing cDNA for interleukin (IL)-10, but Dr Larkin believes that these adenoviral vectors produce

immunogenic and cytopathic effects.

Research on modifying the immune response in the grafted eye continues, but he now uses lentiviruses for this type of corneal modification. Early work using modified lentivirus vectors and a modified regime are promising though Dr Larkin is not ready to publish details.

In any case, Dr Larkin believes adenovirus vectors are not the way forward. "As it is likely that adenovirus

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vectors increase the immunogenicity of donor corneas, I am very doubtful that these types of virus will be used in clinical practice and it is more likely that we will move either to non-viral vectors or to recombinant lentiviruses. Nonetheless, gene-based approaches have major potential in corneal transplantation and one must consider possible applications of the types I have discussed in combination," said Dr Larkin.

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