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ALTERING HEMATOPOIETIC STEM CELLS TO POTENTIALLY TREAT FIP

PROJECT STUDY:
Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis
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Feline coronavirus (FCoV) infection is widespread in domestic cats, and while most FCoV-infected cats are healthy, or only display mild enteritis, a small number develop fatal feline infectious peritonitis (FIP). The pathogenesis of FIP is related to mutations in the virus that lead to its ability to infect monocytes/macrophages and the proposed lack of a suitable cell-mediated immune response, which results in systemic virus proliferation.

In a study funded previously, the investigators proved that RNA interference (RNAi) could be used to inhibit FCoV replication *in vitro* (in cell cultures outside the body). Therefore, RNAi could be used therapeutically if the small interfering RNA (siRNA) could be delivered in sufficient quantity to monocytes and macrophages. Therefore, in this study, they assessed the ability to transduce (alter) hematopoietic stem cells with FCoV-specific, siRNA-coding DNA (miRNA) in a laboratory environment using a non-replicating lentivirus as the carrier. The effect is to produce successor monocytes and macrophages that subsequently express these siRNAs, therefore inhibiting coronavirus replication and protecting these target cells from infection.

Four miRNA sequences were designed and cloned for transfer into a lentivirus plasmid. The lentiviruses were then generated to contain the coding sequence for one designed miRNA per virus (4 viruses total). As the lentivirus enters a target cell, the viral RNA is transcribed in reverse, imported into the cell nucleus and integrated into the host genome (DNA).

In the next phase, hematopoietic stem cells were transduced with the lentivirus producing genetically altered cells that would express one miRNA (miRNAL-2) that resulted in the largest reduction of FIPV and FECV genomic RNA. Evaluating the stem cells microscopically and through growth characteristics, the infection did not produce any negative effects on the stem cells.

To assess the effectiveness of miRNAL-2 in inhibiting FCoV replication in stem cells, the altered hematopoietic stem cells were infected with FCoV. Accordingly, miRNA-L2 resulted in a significant reduction in the extracellular virus titers of FIPV and FECV as compared with the negative control.

These results prove that genetic modification of hematopoietic stem cells for subsequent production of this anti-coronavirus siRNA will reduce FCoV replication *in vitro*. According to the investigators, this work will potentially lead to introduction of genetically modified hematopoietic stem cells for the treatment of FIP in cat patients.

Summary prepared by Vicki L. Thayer, DVM, DABVP (Feline) © 2015