GENE SILENCING TECHNOLOGY AS A TOOL TO INDUCE PERMANENT STERILITY

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Mass sterilization of feral cats and dogs via surgery is expensive and inefficient, and chemical/immunological sterilization techniques mostly result in transient infertility. We are performing studies to lay out the groundwork for implementation of a novel approach intended to cause permanent sterility. Our strategy calls for the combined use of a "gene silencing" technology with a "gene therapy" delivery system that can target silencing molecules to specific regions of the brain critical for fertility, and cause long-term suppression of gene expression. Because the vector to be employed remains active for years, permanent sterility is expected to occur following a single systemic administration of our delivery vehicle. We are currently using rats to test the general feasibility of this approach, which is based on the combined use of two complementary methodologies: One employs a process known as RNA interference (RNAi) that can be used to silence genes involved in the control of reproduction; the other is intended to deliver RNAi selectively to the hypothalamus (where these genes are expressed) via systemic injection of a vehicle engineered to target this brain region. We selected the hypothalamus because it contains neurons expressing *Kiss1 and Tac2*, two genes essential for reproduction and fertility. We first used a phage display library and a technique termed bio-panning to identify peptide sequences able to target the hypothalamus. These studies resulted in the isolation of a peptide epitope that homes selectively to the hypothalamus – and within this region – to the arcuate nucleus (ARC), where *Kiss1* neurons are located. We next modified the tropism of a non-pathogenic adeno-associated virus (AAV2) by inserting this peptide into the viral capsid, and observed increased tropism of the modified virus (mV) for the hypothalamus after intravenous injection. When mV was further engineered to carry *Kiss1* silencing molecules estrous cyclicity was disrupted, suggesting that ovulation had been compromised. Because fertility was obliterated in only 20% of the rats injected with this construct, we are currently attempting to increase the viability and titer of the virus by modifying the purification procedure, and are preparing a new construct carrying both *Kiss1* and *Tac2* silencing molecules. These results suggest that gene silencing delivered to the brain via modified viral vectors may represent a viable method for the eventual sterilization of cats and dogs.