

## Progress in siRNA Targeting in the Central Nervous System: Where Are We on Proof of Principle? – *Dissen*

The phenomenon of RNA interference (RNAi) was initially discovered in *Caenorhabditis elegans*, wherein it was found that gene expression could be silenced by the introduction of double-stranded RNAs containing the sequence specific for the target. In the last few years, RNAi has also been shown to regulate gene expression in mammals, playing a critical role during development, throughout adulthood and in various disease states. There are many naturally occurring small RNAs that are capable of RNAi, one class of which are known as small interfering RNAs — siRNAs, a powerful research tool to examine physiologic processes and perform mechanistic-based experiments. For example, siRNAs offer an inexpensive method to knock down the expression of virtually any gene and have allowed for the production of siRNA libraries that can target genes on a genome-wide scale.

As with all oligonucleotide transcriptional/translational regulators, delivery to the interior of the cell is critical to function. Methods of delivering siRNAs can be classified as either viral or non-viral vector-mediated approaches. In our lab, we use lentiviral vectors and adeno-associated virus (AAV) vectors to deliver siRNAs to multiple cell regions of interest throughout the brain and in peripheral tissues. Lentiviruses offer the possibility of circumventing the difficulty of inserting siRNAs into cells because they can infect and transduce either dividing or nondividing cells. In addition, lentiviruses can permanently integrate into the genome of host cells, and are able to maintain long-term expression. Our laboratory has shown that suppression of the gene Enhanced at Puberty 1 (EAP1) within cells of the hypothalamus blocks fertility. EAP1 is a transcriptional regulator expressed within the hypothalamus of all species examined thus far, and, as its name suggests, EAP1 expression is significantly increased at puberty. We have found that suppression of EAP1 expression results in infertility in both rats and non-human primates. In rats, the intrahypothalamic administration of a lentivirus expressing the EAP1-siRNA causes a delay in puberty and disruption of estrous cyclicity. In non-human primates, decreasing expression of EAP1 in the hypothalamus blocks menstrual cyclicity.

Recently, we have expanded our molecular repertoire to utilize AAVs to deliver siRNAs to the brain and peripheral tissues. Variants of AAV, known as serotypes, determine the tropism (what cells the AAV will infect) of the virus. We, and others, have recently shown that intravenous administration of AAV serotype 9 (AAV9) leads to widespread transduction of neurons and astrocytes throughout the CNS, indicating that AAV9 has the potential to cross the blood brain barrier.

We are also using another approach, referred to as bio-panning, to identify epitopes within a peptide-phage library that will bind to cells of the hypothalamus. The bio-panning procedure consists of injecting the phage library systemically followed by collection of the tissue of interest and isolation of the adherent phage population. Multiple rounds of panning and isolation of the adherent phage are performed until convergence to two or three different receptor epitopes is observed. When we reach this point, we plan to individually insert the sequences of the candidate epitopes into the region of the AAV capsid that determines the tropism of the virus. For example, in AAV2 the sequence would be inserted into loop IV of the capsid site 587.

Once the DNA sequences encoding the selected epitopes are incorporated into the sequence encoding an AAV virus capsid, the chimeric AAV will be unable to bind to its native receptor, but will be endowed with the capability of binding to unique cell-surface recognition molecules present on, and hopefully restricted to, our cells of interest. This modified AAV virus will then be used to deliver RNAi sequences via the vascular system to the hypothalamus with the purpose of silencing genes that, expressed in these tissues, are required for rat fertility. Once the sequence of the epitope is known, work in our laboratories will focus on identifying the cellular receptor responsible for epitope binding using standard biochemical approaches. The reasoning for this is that, if the cellular receptor is identified, we could learn quickly if this receptor was also present in cat and dog hypothalamus, which would streamline work subsequent to proof-of-principle testing in the rodent.

Because the release of gonadotropin releasing hormone from the hypothalamus is the first step in the hypothalamic-pituitary-gonadal axis, there is a strong possibility that partial suppression of a signal in the hypothalamus alone, as opposed to complete ablation, will be sufficient to disrupt fertility. This would be in contrast to a target in the gonad, wherein not destroying all the germ cells could result in recovery of fertility.

In summary, our laboratories are currently using several different strategies to deliver genes of interest to the CNS and peripheral tissues. RNAi is an extremely powerful tool to target and suppress genes of interest. Viral vector delivery of siRNAs to target and suppress genes that control fertility holds great promise in causing permanent infertility in dogs and cats.