436e Immune Activation in Response to Short Interfering Rnas (Sirnas) and Implications for RNA Interference (Rnai) in Mammalian Cells

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RNA interference (RNAi) is a natural phenomenon observed in many eukaryotic species that can be used to facilitate disease diagnosis, treatment, and an overall understanding of biological processes. It provides a means for potent and primarily specific gene silencing that is initiated by the presence of short interfering RNAs (siRNAs). siRNA is the double-stranded effector molecule (~21-23 base pairs long) in RNAi that provides the enzyme complex responsible for mRNA cleavage its sequence-specific recognition capability.

While this mRNA cleavage was originally thought to be highly specific, it is in fact possible for these siRNAs to elicit a non-specific immune response in the form of interferons, a family of cytokines that mediate antiviral and antigrowth responses. The two known pathways responsible for this immune response are through Protein Kinase R (PKR) and Toll-like Receptor 3 (TLR3). Both proteins recognize dsRNA and signal interferon production as well as stimulation of several transcription factors, such as NF-kB. Use of siRNAs in a therapeutic setting will require complete understanding of these phenomena.

Here we have inhibited, in a dose-dependant manner, the green fluorescent protein (GFP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes using siRNA in various human cell lines. In addition, we have measured the immune responses in these cell lines. The important siRNA sequence and structural characteristics are discussed, as are the implications for application of siRNA in the therapeutic context.