The specifics and non-specifics of using small interfering RNAs for targeting of viral genes in a fish model

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ABSTRACT

RNA interference (RNAi) is an intracellular mechanism used by the cell to specifically inhibit gene expression through direct interaction with the gene or genes (methylation) or with mRNA copies (cleavage or repression of translation). The cell accomplishes this by the aid of a large protein complex called the RNA-induced silencing complex (RISC). This complex can be programmed with several types of small double stranded RNAs – the type of which defines the destiny of the target. One such class of regulatory RNAs called small interfering RNAs (siRNAs) have received attention due to their high degree of target specificity. These can guide RISC to bind specific mRNAs, defined by sequence complementarity to one of the strands in the siRNA, followed by cleavage of the mRNA. For this reason the use of chemically synthesized siRNAs hold promise in the treatment against cellular diseases, using siRNAs designed to target such genes as oncogenes or viral genes. So far, the usage of siRNAs for such purposes has mainly been exploited in cell culture studies. The low number of published studies on in vivo delivery and activity of siRNAs in vertebrates highlights the need for good vertebrate models.

This work was initiated in an attempt to develop such a model for testing aspects of using siRNAs targeting viral genes for the prophylactic treatment of viral disease in vertebrates. For this purpose a fish model composed of juvenile rainbow trout and a fish pathogenic rhabdovirus was used. Due to the small size of juvenile fish and the use of correspondingly small amounts of siRNA a fish model would be less expensive compared to other vertebrate models such as mice or monkeys. Intraperitoneally injected siRNAs targeting the viral envelope protein and formulated in polycationic liposomes primarily entered free intraperitoneal cells including macrophage-like cells. Uptake correlated with antiviral activity seen as reduced mortality of fish challenged with the fish virus. Protection at the disease level was not dependent upon which one of three tested siRNAs was used and protection correlated with up-regulation of an interferon-related gene in the liver indicating a systemic interferon response.

Initial cell culture studies had further shown that the often-used control siRNAs containing four mismatches compared to the target were not adequate controls in such studies. Inclusion of a heterologous virus as target control was essential for verification of the specificity of siRNA-induced interference with virus multiplication.

Overall the results emphasize the use of controls and the compromise in using transfection reagents for improved uptake of siRNAs, where these reagents also increase the risk of the siRNAs ending up in a cellular compartment in which stimulation of non-specific antiviral defence mechanisms will be initiated.