Introduction

Experimental approaches using short interfering RNA (siRNA) molecules to specifically silence gene expression have become more widely used in recent years. As for all techniques in molecular and cellular biology, the importance of protocol optimization and the use of appropriate controls cannot be overstated.

While the induction of interferon-pathway-related stimulatory processes during RNAi is usually attributed to long, double-stranded nucleic acids, it can also be a concern during transfection of short siRNA molecules or in other RNAi-related experiments. A number of parameters, including siRNA chemistry, transfection reagents, cell culture conditions, and siRNA concentration have the potential to cause interferon-pathway-related responses. These responses could interfere with the specific biological effect caused by the siRNA-mediated knockdown of the gene of interest, causing misleading results.

Here we describe assays for quantification of siRNAs which are derived from induced transcription of well characterized interferon-pathway-related genes, using quantitative, real-time RT-PCR. The assays are specific for STAT1, β2-M, IRF5, IFIT1, IFITM1, OAS1, and OAS2. We performed the assays after cells were transfected with functional amounts of synthetic siRNAs or with polyinosinic-polycytidylic acid (poly(I)·poly(C)), which is a long, double-stranded RNA that induces the interferon pathway. Although poly(I)·poly(C) transfection caused dramatic induction of several interferon pathway genes in these cells, as indicated by the assays, such induction was not seen when siRNAs from QIAGEN were transfected.

We conclude that these assays are valuable tools for optimization of experimental conditions and for use as controls, ensuring successful RNAi experiments.

A set of Quant iT® Primer Assays for analysis of the interferon response

- Quant iT® Primer Assays are bioinformatics validated gene-specific primer sets for quantitative, real-time RT-PCR using SYBR® Green based detection
- Quant iT® Primer Assays were used for analysis of the interferon-pathway genes STAT1, β2-M, IFIT1, IFITM1, OAS1, and OAS2

Measurement of induction of interferon genes

Quant iT® Primer Assays were developed to measure the expression of STAT1, β2-M, IFIT1, IFITM1, OAS1, and OAS2 after transfection of poly(I)·poly(C) or a transducing control siRNA into HeLa or MCF-7 cells.

Interferon-pathway-related genes were upregulated after transfection of poly(I)·poly(C). This demonstrates that interferon-pathway–related responses do not cause nonspecific effects via induction of interferon-like responses (Figure 3).

Strong induction of the interferon response by poly(I)·poly(C) but not by siRNA was also shown at the protein level (Figure 4). siRNA transfection did not induce STAT1 protein expression, even using high siRNA concentrations. In contrast, poly(I)·poly(C) strongly induced STAT1 expression, even at low concentrations.

Conclusions

Various reports have shown that interferon-related pathways can be induced by siRNA transfection, leading to nonspecific effects and misleading results.

We have developed Quant iT® Primer Assays for quantitative, real-time RT-PCR of the interferon-pathway–related genes STAT1, β2-M, IFIT1, IFITM1, OAS1, and OAS2. These assays can be used as controls to determine the interferon response in RNAi experiments.

Transfection with equivalent amounts of siRNA from QIAGEN or poly(I)·poly(C) using HiPerfect Transfection Reagent showed that the interferon response was not induced significantly by QIAGEN siRNA and HiPerfect Reagent.

siRNA and HiPerfect Reagent are part of the range of RNAi solutions from QIAGEN that allow efficient knockdown without nonspecific effects.

Measurement of the interferon response in primary cells

HUVEC were transfected with poly(I)·poly(C) or HP (HiPerformance) siRNA and the expression of IFNα was determined after 72 hours, a typical timepoint for phenotypic knockdown evaluation. Knockdown was analyzed by quantitative, real-time RT-PCR.

Expression of Interferon Genes in Transfected Primary Cells

- Poly(I)·poly(C) transfection induced IFNα expression. siRNA transfection did not induce IFNα expression, even after 72 hours in sensitive primary cells.
- Comparison with untransfected cells showed that the ability of siRNAtreated cells was impaired.