Intended transcriptional gene silencing with siRNA to the human Vascular Endothelial Growth Factor (VEGF) promoter results in VEGF repression through sequence-specific off-targeting

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Background & Aims
RNA interference (RNAi) mediates post-transcriptional gene silencing in the cytoplasmic fraction of the cell by targeting mRNA for either sequence-specific degradation or inhibition of translation1, 2. In addition, it has been proposed that short interfering RNA (siRNA) may act in the nucleus as a modulator of gene activity at the transcription level with both suppressive and inductive effects being reported in human cells3-5. In these studies, the promoter region of genes implicated in human disease were targeted by siRNA and initial assessments determined that these offered a high level of suppression and exquisite sequence-specificity, similar to those seen for cytoplasmic RNAi, as single-nucleotide mismatches dramatically altered silencing activity. This study aims to critically evaluate the potential for transcriptional regulation of the Vascular Endothelial Growth Factor (VEGF) gene using siRNA designed to target specific sites within the VEGF gene promoter.

Methods & Results

SiRNA Transfection Assay: A series of 21 nt siRNA designed to target the sense-strand of genomic DNA at sites within the human VEGF promoter were transfected into human cell lines, HeLa and ARPE-19. SiVFp(-992) demonstrated ≥ 50% inhibition of VEGF expression in both cell lines (Figure 1A). Sequence specificity for siVFp(-992) was established using mismatch variants and siVFp(-992)m1, which has several mismatches, had no activity. Interestingly, suppression was observed with siVFp(-992)m2 which carried mismatches in the seed region of the molecule, suggesting that siVFp(-992) may have miRNA-like properties (Figure 1B).

Dual Luciferase Assay: Reporter plasmids carrying the VEGF promoter with either the wildtype, mutated or deleted siVFp(-992) target sequences (Figure 2A), were cloned to express the firefly luciferase gene. When each reporter construct was co-transfected into HeLa cells, siVFp(-992) and its mismatch variants revealed a similar pattern of activity against luciferase expression regardless of whether these carried wildtype, mutated or deleted VEGF promoter target motifs (Figure 2B). Furthermore, this pattern was similar to that demonstrated when targeting endogenous VEGF production (compare with Figure 1B).

These data suggest that siVFp(-992) mediated inhibition of VEGF expression was not occurring through specific targeting of the VEGF promoter, but was more likely due to sequence-specific off-targeting of an unintended gene involved with VEGF transcription.

Conclusions

• SiRNA designed to target the VEGF gene promoter revealed a putative candidate for transcriptional gene silencing. However, mutation or deletion of the target site demonstrated that the observed VEGF knockdown was the result of a sequence-specific off-target effect. This is consistent with recent findings with a HIV-LTR model8 in which proposed transcriptional activation was in fact due to off-target sequence-specific repression of mRNA akin to previous reports on siRNA7, 8.

• A systematic approach involving combined bioinformatic and microarray analyses of genes implicated in VEGF transcriptional control followed by knockdown and Western blotting experiments presented one candidate, GRB2, as an unintended target of the VEGF promoter-specific siRNA.

• These data support the need for evidence of target-specificity in studies of transcriptional regulation of genes by siRNA.

References