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Actual and Predicted Off-target Effects Induced By siRNAs Targeting the HPV16 E7 Oncogene Demonstrate No Correlation in Human Papillomavirus-negative Keratinocytes *in vitro*

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Short-interfering RNAs (siRNAs) are short, non-coding RNAs designed to cause sequence-specific downregulation of target genes. Consequently, siRNAs may be used to target specific genes known to be dysregulated in different disease states. When designing siRNAs for in vitro or clinical use, the most common mechanism employed to assess off-target effects (OTEs) is bioinformatic prediction of the number of sequence-dependent OTEs by identifying transcripts that either demonstrate complete complementarity to the siRNA sequence or contain matches to the siRNA 'seed' sequence (5'-nucleotide positions 2-8) in their 3' untranslated regions. It is assumed that the higher the number of predicted OTEs, the greater the size of the OTE signature and the more likely a detrimental phenotypic effect will be induced. In this study, we sought to answer two critical questions. Firstly, do predicted OTEs correlate with actual OTEs? Secondly, does the cell type studied have an effect on the actual OTEs observed? In initial work, we bioinformatically predicted numbers of OTEs for siRNAs designed to target the human papillomavirus 16 (HPV16) oncogene E7. Next, we undertook comprehensive OTE analysis using microarrays in two different HPV-negative keratinoctye cell lines, using four of these E7-targeting siRNAs. We found there was no correlation between the predicted and actual number of OTEs induced, nor any significant overlap between OTEs induced in the two different keratinocyte cell lines. Critically, the siRNA with the lowest number of predicted OTEs was the only siRNA to induce both sequence-specific and immune-stimulatory OTEs. Our data highlight limitations of the current strategy employed for rational siRNA design; further development of current algorithms is required for more accurate prediction of OTEs.