Development of Novel Anticancer RNA Therapeutics with Dual-Targeting siRNA that Silences Ubiquitin Family Members

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Abstract

Ubiquitination is a metabolic process in eukaryotic cells that modifies proteins with ubiquitin leading to protein degradation via the ubiquitin-proteosome pathway. Cellular levels of ubiquitin are driven by the expression of RPS27A, UBA52, UBB and UBC. In cancer, the dysregulation of ubiquitination can affect various aspects of tumor progression including cell cycle regulation and gene expression. Ubiquitin B (UBB) gene has been shown to be over-expressed in some tumor types, including NSCLC and cervical cancer, and under-expressed in other tumors, in particularly a subset of ovarian cancers. A role for ubiquitin C (UBC) in cancer has not been well established but UBC has been described as a biomarker in renal cancer and CLL. In recent studies, repression of UBB in ovarian cancer cells was associated with sensitivity to UBC knockdown and demonstrated a synthetic lethal relationship. Since both UBB and UBC show substantial sequence homology, we explored the possibility of developing dual targeting siRNA that would silence both UBB and UBC. We identified unique individual siRNA sequences that efficiently silenced both UBB and UBC and led to rapid cytotoxicity in several cancer cell lines including the colon cancer cell line HCT-116, the breast cancer cell line SK-BR3, and the prostate cancer cell line 22Rv1. Treatment with either UBB-specific or UBC-specific siRNA did not show the same lethal phenotype. Dose response assays using the dual targeting siRNA designated U21 indicated EC_{50} for cytotoxicity between 50–500 pM in several cancer lines. Treatment of cancer cell lines with U21 leads to rapid apoptosis and cell death. Chemical modifications to pharmacologically stabilize the U21 siRNA sequence did not change its activity. The efficient cytotoxic activity of the U21 siRNA sequence has been integrated into our SeekR™ platform to create aptamer-siRNA chimeras for targeted delivery of the lethal U21 payload to specific cancer cell types.

Background

- Ubiquitination is a metabolic process in eukaryotic cells that modifies proteins with ubiquitin leading to protein degradation.
- Cellular levels of ubiquitin are driven by the expression of RPS27A, UBA52, **UBB** and **UBC**.
- **Ubiquitin B (UBB)** has been shown to be over-expressed in some tumor types, including NSCLC and cervical cancer, and under-expressed in other tumors, in particularly a subset of ovarian cancers.
- A role for **ubiquitin C (UBC)** in cancer has not been well established, but UBC has been described as a biomarker in renal cancer and CLL.
- In recent studies, repression of UBB in ovarian cancer cells was associated with sensitivity to UBC knockdown and demonstrated a synthetic lethal relationship. (Tsherniak et al., Cell 2017; Kedves et al., J Clin Invest 2017).
- We have identified an siRNA sequence that induces cellular cytotoxicity through knockdown of both UBB and UBC.
- The UBB/UBC targeting siRNA sequence has been introduced into SeekR™ molecules, an RNA therapeutic platform for multi-targeting of cancer cells currently being developed at Oligon.

The Effect of UBB Targeting siRNAs on Cell Growth

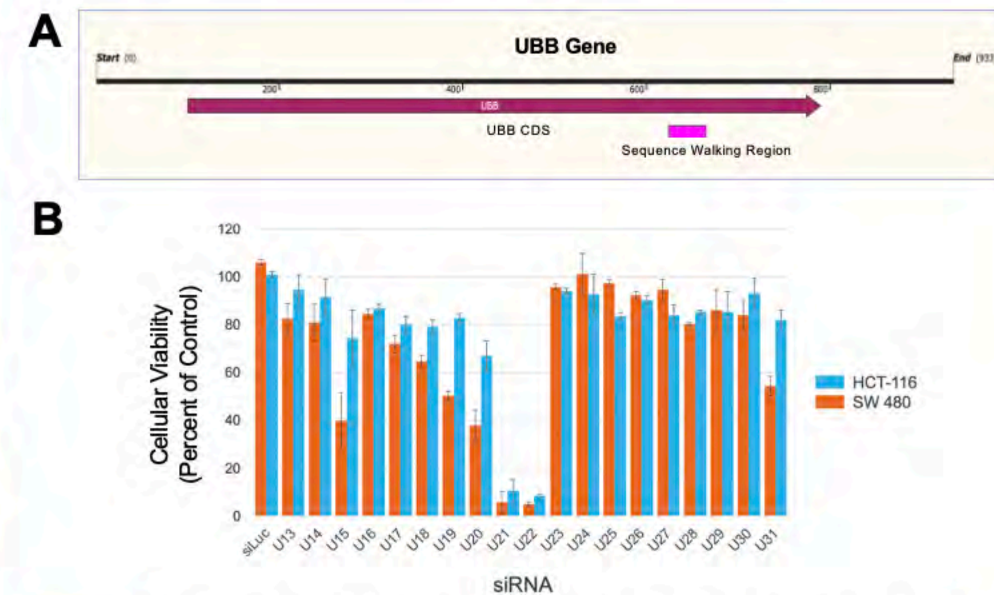

Figure 1. **A)** Map of the UBB gene and region where sequence walking of UBB siRNA was conducted. **B)** Cellular viability assay was performed on HCT-116 and SW480 cells transfected with 19 siRNA to UBB and control siLuc.

Induction of Cytotoxicity by UBB siRNA

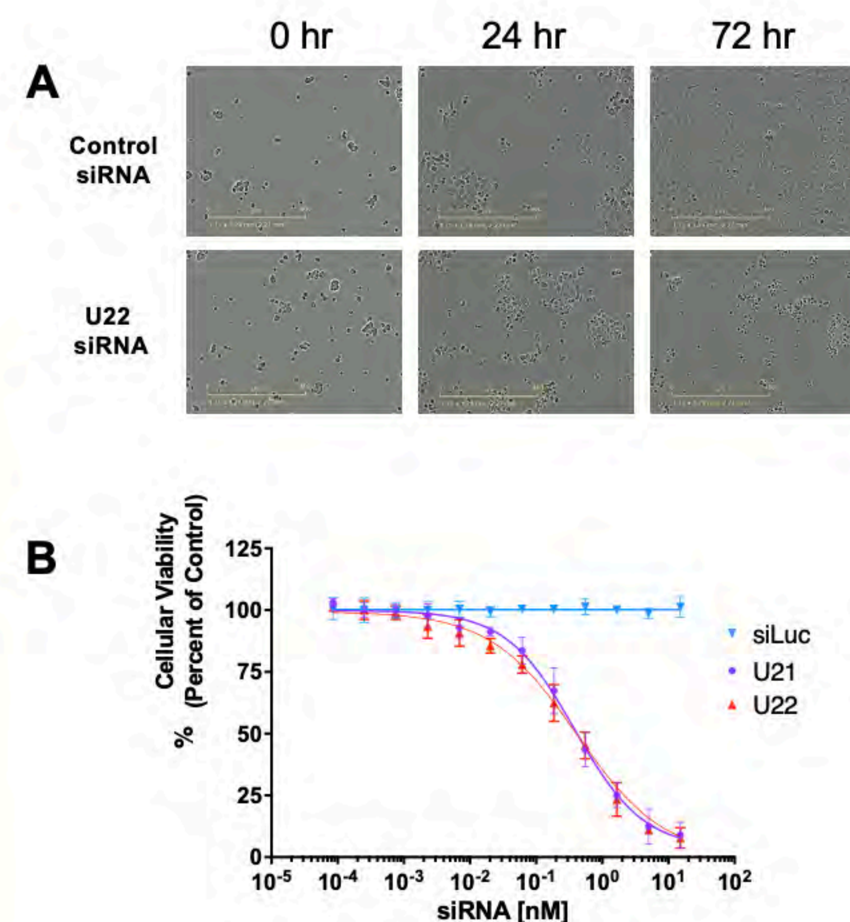

Figure 2. **A)** Induction of rapid cellular cytotoxicity of HCT-116 cells treated with control siRNA siLuc, or U22 siRNA. Cell death was seen as early as 24 hr. **B)** Dose response of HCT-116 cells transfected with various concentrations of UBB siRNAs U21 and U22. EC_{50} values were similar approximately 400 pM.

Dual Silencing of UBB and UBC, and Cytotoxicity Induced by UBB siRNA

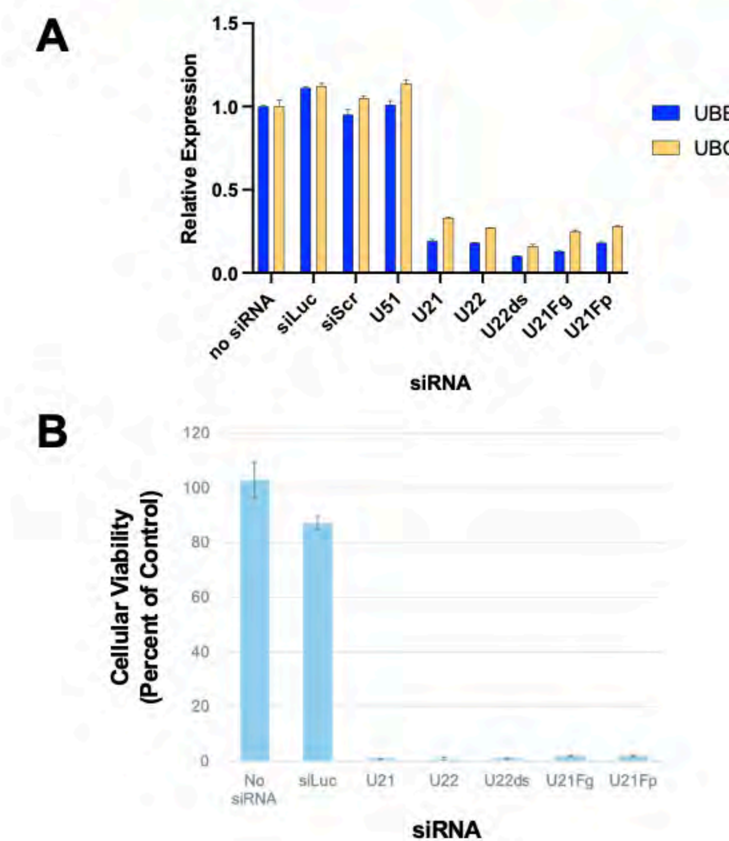

Figure 3. **A)** The effect of control and UBB siRNAs on UBB and UBC expression. HCT-116 cells were transfected with control siRNAs siLuc, siScr (scrambled), U51 (siGFP) and UBB targeting siRNA U21, U22, U22ds (dicer substrate), U21Fg (2'F-pyrimidines on guide strand), and U21Fp (2'F-pyrimidines on passenger strand). Expression analysis was performed after 24 hr siRNA treatment. **B)** Cellular viability assay was performed on HCT-116 transfected with control siRNA siLuc or UBB siRNA as before. Cell viability was assessed at 96 hr.

Selection of Aptamers to EPCAM and HER3 for the SeekR™ Platform

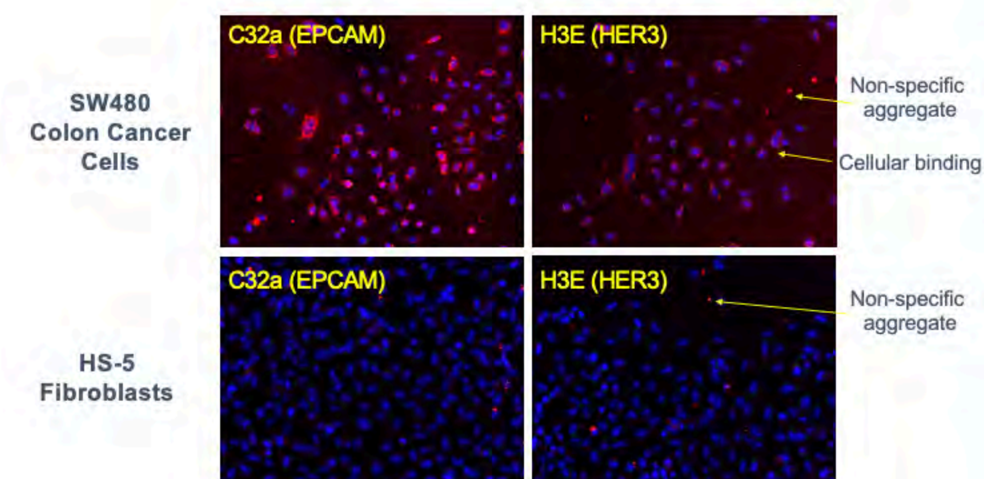

Figure 4. Selective binding of aptamers to EPCAM and HER3 expressed on SW480 colon cancer cells but not HS-5 fibroblasts. Both cell types were treated with Cy3-labeled aptamer-siRNA chimeras C32a, which binds EPCAM, and H3E, which binds HER3, and imaged for binding.

Effect of SeekRs™ Containing UBB siRNA on Colon Cancer Cells

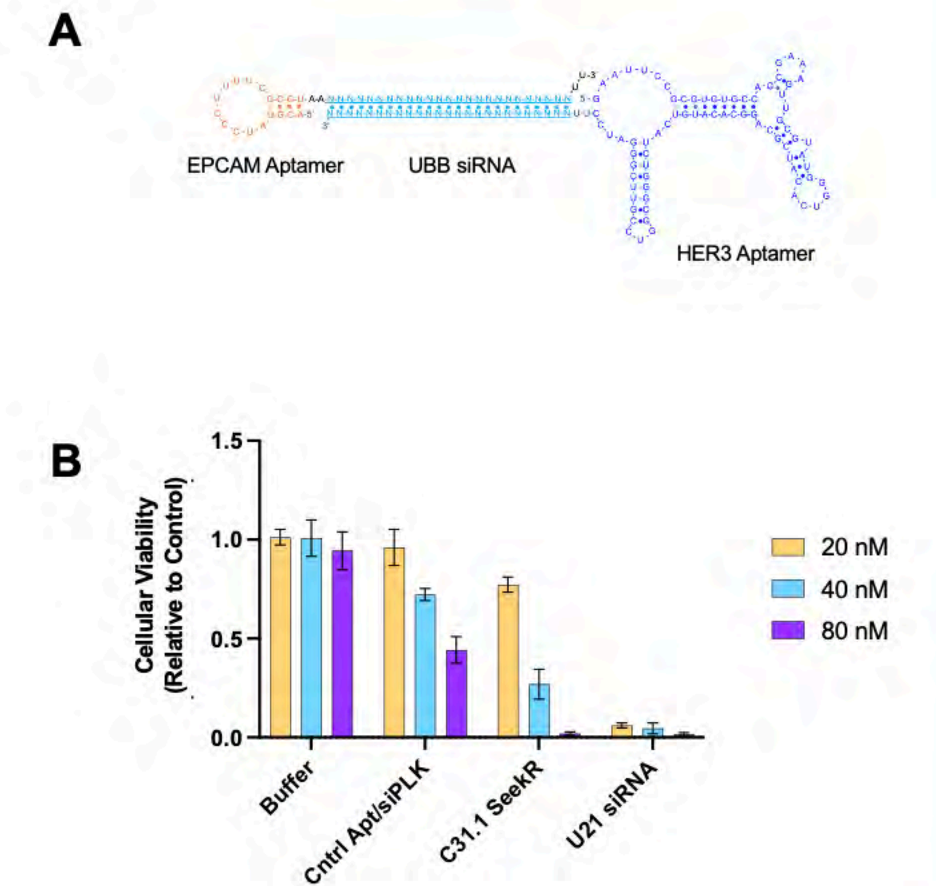

Figure 5. **A)** Schematic of the dual aptamer, single siRNA C31.1 SeekR targeting EPCAM, HER3 and UBB. **B)** Cellular viability assay was performed on HCT-116 transfected with control aptamer-PLK siRNA chimera, C31.1 SeekR™ and U21 siRNA at three concentrations. Cellular viability was assessed at 72 hr and plotted relative to control treatment.

Conclusions

- We have identified siRNA sequences to UBB that silences both UBB and UBC and induced rapid cellular cytotoxicity.
- Modifications using 2'F-pyrimidines in the UBB siRNA do not affect activity.
- SeekR™ constructs using EPCAM and HER3 aptamers and the UBB siRNA can induce cellular cytotoxicity.
- siRNA sequences in the SeekR™ platform can lead to targeted silencing of specific genes and produce a desired phenotype.

Future Directions

- We are developing SeekR™ molecules that utilize the UBB/UBC dual-targeted siRNA with novel high-performing aptamers to other cell membrane proteins for improved targeted delivery in other cancer indications.
- We are utilizing the SeekR™ platform to develop efficient multi-targeting molecules to not only tumor cells, but also dysfunctional immunomodulatory cells involved in the tumor response.