

Cell Culture

[0196] Human hepatocyte Hep3B, human hepatocyte HepG2 cells, mouse hepatoma Hepa1-6 cells, and human renal proximal tubule epithelial cells (RPTEC) were cultured using conditions known in the art (see, e.g. Current Protocols in Cell Biology). Details of the cell lines used in the experiments described herein are provided in Table 5.

TABLE 5

| Cell lines | | | | | | | |
|------------|-------------|---------|--------|------------------|-----------------|--------------|--------------------|
| Cell Line | Source | Species | Gender | Type | Tissue | Status | Culture Conditions |
| RD RMS | ATCC | human | F | rhabdomyosarcoma | skeletal muscle | immortalized | DMEM + 10% FBS |
| HSKM | Gibco | human | M | muscle | skeletal muscle | normal | DMEM + 10% FBS |
| SK-N-AS | ATCC | human | F | neuroblast | brain | immortalized | MEM + 10% FBS |
| am002fDMD | DVBiologics | human | M | muscle | skeletal muscle | normal | M-gro + supplement |

Oligonucleotide Design

[0197] Oligonucleotides were designed within PRC2-interacting regions in order to upregulate UTRN. The sequence and structure of each oligonucleotide is shown in Table 2.

[0198] The following table provides a description of the nucleotide analogs, modifications and intranucleotide linkages used for certain oligonucleotides tested and described in Table 2.

TABLE 3

| Oligonucleotide Modifications | |
|-------------------------------|---------------------------------------|
| Symbol | Feature Description |
| bio | 5' biotin |
| dAs | DNA w/3' thiophosphate |
| dCs | DNA w/3' thiophosphate |
| dGs | DNA w/3' thiophosphate |
| dTs | DNA w/3' thiophosphate |
| dG | DNA w/3' phosphate |
| dU | deoxyuridine w/3' phosphate |
| enaAs | ENA w/3' thiophosphate |
| enaCs | ENA w/3' thiophosphate |
| enaGs | ENA w/3' thiophosphate |
| enaTs | ENA w/3' thiophosphate |
| fluAs | 2'-fluoro w/3' thiophosphate |
| fluCs | 2'-fluoro w/3' thiophosphate |
| fluGs | 2'-fluoro w/3' thiophosphate |
| fluUs | 2'-fluoro w/3' thiophosphate |
| InaAs | LNA w/3' thiophosphate |
| InaCs | LNA w/3' thiophosphate |
| InaGs | LNA w/3' thiophosphate |
| InaTs | LNA w/3' thiophosphate |
| omeAs | 2'-OMe w/3' thiophosphate |
| omeCs | 2'-OMe w/3' thiophosphate |
| omeGs | 2'-OMe w/3' thiophosphate |
| omeTs | 2'-OMe w/3' thiophosphate |
| InaAs-Sup | LNA w/3' thiophosphate at 3' terminus |
| InaCs-Sup | LNA w/3' thiophosphate at 3' terminus |
| InaGs-Sup | LNA w/3' thiophosphate at 3' terminus |
| InaTs-Sup | LNA w/3' thiophosphate at 3' terminus |
| InaA-Sup | LNA w/3' OH at 3' terminus |
| InaC-Sup | LNA w/3' OH at 3' terminus |
| InaG-Sup | LNA w/3' OH at 3' terminus |
| InaT-Sup | LNA w/3' OH at 3' terminus |
| omeA-Sup | 2'-OMe w/3' OH at 3' terminus |
| omeC-Sup | 2'-OMe w/3' OH at 3' terminus |
| omeG-Sup | 2'-OMe w/3' OH at 3' terminus |

TABLE 3-continued

| Oligonucleotide Modifications | |
|-------------------------------|---------------------------------------|
| Symbol | Feature Description |
| omeU-Sup | 2'-OMe w/3' OH at 3' terminus |
| dAs-Sup | DNA w/3' thiophosphate at 3' terminus |
| dCs-Sup | DNA w/3' thiophosphate at 3' terminus |
| dGs-Sup | DNA w/3' thiophosphate at 3' terminus |
| dTs-Sup | DNA w/3' thiophosphate at 3' terminus |
| dA-Sup | DNA w/3' OH at 3' terminus |
| dC-Sup | DNA w/3' OH at 3' terminus |
| dG-Sup | DNA w/3' OH at 3' terminus |

In Vitro Transfection of Cells with Oligonucleotides

[0199] Cells were seeded into each well of 24-well plates at a density of 25,000 cells per 500 μ L and transfections were performed with Lipofectamine and the single stranded oligonucleotides. Control wells contained Lipofectamine alone. At 48 hours post-transfection, approximately 200 μ L of cell culture supernatants were stored at -80° C for ELISA. At 48 hours post-transfection, RNA was harvested from the cells and quantitative PCR was carried out as outlined above. The percent induction of target mRNA expression by each oligonucleotide was determined by normalizing mRNA levels in the presence of the oligonucleotide to the mRNA levels in the presence of control (Lipofectamine alone). This was compared side-by-side with the increase in mRNA expression of the "control" housekeeping gene.

Results:

In Vitro Delivery of Single Stranded Oligonucleotides Upregulated UTRN Expression

[0200] Oligonucleotides were designed as candidates for upregulating UTRN expression. A total of 65 single stranded oligonucleotides were designed to be complementary to a PRC2-interacting region within a sequence as set forth in SEQ ID NO: 1 or 2. Each of the oligonucleotides was tested in at least duplicate. The sequence and structural features of the oligonucleotides are set forth in Table 2. Briefly, cells were transfected in vitro with each of the oligonucleotides as described above. UTRN expression in cells following treatment was evaluated by qRT-PCR. Oligonucleotides that upregulated UTRN expression were identified. Further details are outlined in Table 2.