SANTA CRUZ BIOTECHNOLOGY, INC.

Smo siRNA (m): sc-40162

BACKGROUND

Overexpression of either Wnt-1 or the GLI proteins results in cancer; however, the molecular basis for this transformation was poorly understood. The Wnt-1 and GLI proteins have now been placed in a signaling cascade downstream of the mammalian homologs of the *Drosophila* hedgehog and patched proteins. The *Drosophila* segment polarity gene hedgehog (hh) encodes a secreted protein that appears to function in embryonic and imaginal disc patterning. The ptc gene, also identified as a *Drosophila* segment polarity gene, encodes the transmembrane protein patched, the expression of which is precisely regulated during embryonic development. Hedgehog has been shown to enhance the expression of the Wnt family of proteins through a signaling cascade involving the GLI transcription factors, while patched functions as a repessor opposing the effects of the hedgehog. Smoothened (Smo), a seven transmembrane receptor, is complexed with patched in many tissues and is believed to be an essential component in the Hh signaling pathway.

REFERENCES

1. Nusslein-Volhard, C., et al. 1980. Mutations affecting segment number and polarity in *Drosophila*. Nature 287: 795-801.
2. Kinzler, K.W., et al. 1987. Identification of an amplified, highly expressed gene in a human glioma. Science 236: 70-73.
3. Parkin, N.T., et al. 1993. Activity of Wnt-1 as a transmembrane protein. Genes Dev. 7: 2181-2193.
4. Roelink, H., et al. 1995. Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. Cell 81: 445-455.
5. Marti, E., et al. 1995. Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature 375: 322-325.
6. Johnson, R.L., et al. 1996. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272: 1668-1671.
7. Stone, D.M., et al. 1996. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. Nature 384: 129-134.

CHROMOSOMAL LOCATION

Genetic locus: Smo (mouse) mapping to 6 A3.3.

PRODUCT

Smo siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Smo shRNA Plasmid (m): sc-40162-SH and Smo shRNA (m) Lentiviral Particles: sc-40162-V as alternate gene silencing products.

For independent verification of Smo (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40162A, sc-40162B and sc-40162C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Smo siRNA (m) is recommended for the inhibition of Smo expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Smo (E-5): sc-166685 is recommended as a control antibody for monitoring of Smo gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Smo gene expression knockdown using RT-PCR Primer: Smo (m)-PR: sc-40162-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.