SANTA CRUZ BIOTECHNOLOGY, INC.

CYB561D2 siRNA (m): sc-142649

BACKGROUND

CYB561D2 (cytochrome b561 domain-containing protein 2), also known as putative tumor suppressor protein 101F6, is a 222 amino acid highly hydrophobic protein that contains 6 membrane spanning domains. Localized to endosomes and endoplasmic reticulum of the perinuclear region, CYB561D2 contains two heme-binding sites, through which it reduces ferric ions. CYB561D2 is ubiquitously expressed, with highest levels in lung, liver and kidney. Though expression of CYB561D2 is abundant in normal lung bronchial epithileal cells, CYB561D2 is absent in most lung cancers. The gene encoding CYB561D2 maps to human chromosome 3p21.3, a region that is prone to genetic alterations and is associated with frequent and early allele loss in many human cancers. Exogenous expression of CYB561D2 leads to the induction of caspase-independent apoptotic and autophagic pathways, resulting in the synergistic killing of tumor cells. This suggests an important role for CYB561D2 as a therapeutic intervention in cancer treatment.

REFERENCES

1. Lerman, M.I., et al. 2000. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. The international lung cancer chromosome 3p21.3 tumor suppressor gene consortium. Cancer Res. 60: 6116-6133.
2. Ji, L., et al. 2002. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities *in vitro* and *in vivo*. Cancer Res. 62: 2715-2720.
3. Chow, L.S., et al. 2004. RASSF1A is a target tumor suppressor from 3p21.3 in nasopharyngeal carcinoma. Int. J. Cancer 109: 839-847.
4. Angeloni, D. 2007. Molecular analysis of deletions in human chromosome 3p21 and the role of resident cancer genes in disease. Brief Funct. Genomic Proteomic. 6: 19-39.
5. Ohtani, S., et al. 2007. Tumor suppressor 101F6 and ascorbate synergistically and selectively inhibit non-small cell lung cancer growth by caspase-independent apoptosis and autophagy. Cancer Res. 67: 6293-6303.
6. Mizutani, A., et al. 2007. Involvement of 101F6, a homologue of cytochrome b561, in the reduction of ferric ions. J. Biochem. 142: 699-705.

CHROMOSOMAL LOCATION

Genetic locus: Cyb561d2 (mouse) mapping to 9 F1.

PRODUCT

CYB561D2 siRNA (m) is a pool of 2 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 CYB561D2 shRNA Plasmid (m): sc-142649-SH and CYB561D2 shRNA (m) Lentiviral Particles: sc-142649-V as alternate gene silencing products.

For independent verification of CYB561D2 (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-142649A and sc-142649B.

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

CYB561D2 siRNA (m) is recommended for the inhibition of CYB561D2 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

CYB561D2 (C-2): sc-373870 is recommended as a control antibody for monitoring of CYB561D2 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 CYB561D2 gene expression knockdown using RT-PCR Primer: CYB561D2 (m)-PR: sc-142649-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.