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SCCA1/2 shRNA (h) Lentiviral Particles: sc-44087-V

BACKGROUND

Metastasis of a primary tumor to a distant site is determined through signaling cascades that break down interactions between the cell and extracellular matrix proteins. Among the proteins mediating metastasis are serine proteases, such as neutrophil elastase. In 1985, Dr. Jim Travis and Dr. R.W. Carrell designated an emerging family of serine protease inhibitors as the serpin family, which share homology in both primary amino acid sequence and tertiary structure. Serpins contain a stretch of peptide that mimics a true substrate for a corresponding serine protease. Serine proteases bind to this substrate mimic in a 1:1 stoichiometric fashion and become catalytically inactive. Aberrant expression of serpin family members can contribute to a number of conditions, including emphysema (α -1 antitrypsin deficiency), fatal bleeding (elastase to thrombin specificity) and thrombosis (antithrombin deficiency), and are indicators of cancer stage phenotypes (circulating levels of squamous cell carcinoma antigen, known as SCCA1, increase in advancing stages of some cervical, lung, esophageal and head and neck cancers). Human chromosome position 18q21.3 contains a cluster of serpins, including a tandem duplication of the SCCA gene, plasminogen activator inhibitor type 2, and maspin. SCCA is transcribed by two nearly identical genes (SCCA1 and SCCA2), and is mainly produced as SCCA1. The human SCCA1 gene encodes a 390 amino acid protein that was originally isolated from a metastatic cervical squamous cell carcinoma.

REFERENCES

1. Kato, H., et al. 1977. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer* 40: 1621-1628.
2. Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 600517. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Hefler, L., et al. 1999. Serum concentrations of squamous cell carcinoma antigen in patients with vulvar intraepithelial neoplasia and vulvar cancer. *Int. J. Cancer* 84: 299-303.

CHROMOSOMAL LOCATION

Genetic locus: SERPINB3/SERPINB4 (human) mapping to 18q21.33.

PRODUCT

SCCA1/2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SCCA1/2 siRNA (h): sc-44087 and SCCA1/2 shRNA Plasmid (h): sc-44087-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

SCCA1/2 shRNA (h) Lentiviral Particles is recommended for the inhibition of SCCA1/2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

SCCA1/2 (B-9): sc-28384 is recommended as a control antibody for monitoring of SCCA1/2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SCCA1/2 gene expression knockdown using RT-PCR Primer: SCCA1/2 (h)-PR: sc-44087-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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