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ChM-1 shRNA (h) Lentiviral Particles: sc-43279-V

BACKGROUND

ChM-1 is a cartilage-specific matrix glycoprotein that stimulates the growth of chondrocytes. ChM-1 inhibits angiogenesis by disrupting the tube formation of endothelial cells and thus is responsible for the avascular nature of cartilage. ChM-1 is strongly expressed by the proliferating and hypertrophic zones in the epiphyseal plate of long bones. ChM-1 accumulates in the interterritorial matrix around the lacunae. During development, downregulation of ChM-1 permits angiogenesis and ultimately bone formation on the cartilage template. ChM-1 expression is downregulated in the presence of several growth factors including TGF β 2, FGF2 and PTHLH. ChM-1 expression may also play a role in the hypovascularity and chondroid formation of pleomorphic adenomas. The gene encoding human ChM-1 maps to chromosome 13q14.3.

REFERENCES

1. Hiraki, Y., et al. 1991. Molecular cloning of a new class of cartilage-specific matrix, chondromodulin-I, which stimulates growth of cultured chondrocytes. *Biochem. Biophys. Res. Commun.* 175: 971-977.
2. Hiraki, Y., et al. 1997. Inhibition of DNA synthesis and tube morphogenesis of cultured vascular endothelial cells by chondromodulin-I. *FEBS Lett.* 415: 321-324.
3. Hiraki, Y., et al. 1997. Identification of chondromodulin I as a novel endothelial cell growth inhibitor. Purification and its localization in the avascular zone of epiphyseal cartilage. *J. Biol. Chem.* 272: 32419-32426.
4. Shukunami, C., et al. 1999. Spatiotemporal pattern of the mouse chondromodulin-I gene expression and its regulatory role in vascular invasion into cartilage during endochondral bone formation. *Int. J. Dev. Biol.* 43: 39-49.
5. Shukunami, C., et al. 2002. Differentiation of cartilage stem cells and their clinical applications. *Clin. Calcium* 12: 194-199.

CHROMOSOMAL LOCATION

Genetic locus: LECT1 (human) mapping to 13q14.3.

PRODUCT

ChM-1 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 ChM-1 siRNA (h): sc-43279 and ChM-1 shRNA Plasmid (h): sc-43279-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.

PROTOCOLS

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

APPLICATIONS

ChM-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of ChM-1 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

ChM-1 (H-10): sc-365693 is recommended as a control antibody for monitoring of ChM-1 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 ChM-1 gene expression knockdown using RT-PCR Primer: ChM-1 (h)-PR: sc-43279-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.