

Mel-CAM siRNA (m): sc-35919

BACKGROUND

The tumorigenic and metastatic phenotype of melanoma cells correlates well with an increased expression of cell-cell and cell-matrix adhesion receptors. The human Mel-CAM gene encodes a transmembrane glycoprotein, also designated MCAM, MUC18 or CD146, that belongs to the immunoglobulin superfamily and functions as a Ca^{2+} -independent cell adhesion molecule. The deduced human sequence of 603 amino acids consists of a signal peptide, 5 immunoglobulin-like domains, a transmembrane region and a short cytoplasmic tail. Mel-CAM expression is restricted to advanced primary and metastatic melanomas and to cell lines of the neuroectodermal lineage, but not normal melanocytes. Mel-CAM is found on 80% of advanced primary human melanomas and correlates well with development of metastatic disease. Mel-CAM activation initiates an outside-in signaling pathway that involves the protein tyrosine kinases Fyn, FAK and paxillin. Mel-CAM influences the dynamics of Actin cytoskeleton rearrangement and is essential for the maintenance of thymic architecture and function.

REFERENCES

1. Lehmann, J.M., et al. 1989. MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. *Proc. Natl. Acad. Sci. USA* 86: 9891-9895.
2. Sers, C., et al. 1993. Genomic organization of the melanoma-associated glycoprotein MUC18: implications for the evolution of the immunoglobulin domains. *Proc. Natl. Acad. Sci. USA* 90: 8514-8518.
3. Shih, I.M. 1999. The role of CD146 (Mel-CAM) in biology and pathology. *J. Pathol.* 189: 4-11.
4. Seftalioglu, A. and Karakoc, L. 2000. Expression of CD146 adhesion molecules (MUC18 or MCAM) in the thymic microenvironment. *Acta Histochem.* 102: 69-83.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 155735. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Satyamoorthy, K., et al. 2001. Mel-CAM-specific genetic suppressor elements inhibit melanoma growth and invasion through loss of gap junctional communication. *Oncogene* 20: 4676-4684.

CHROMOSOMAL LOCATION

Genetic locus: Mcam (mouse) mapping to 9 A5.1.

PRODUCT

Mel-CAM 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 Mel-CAM shRNA Plasmid (m): sc-35919-SH and Mel-CAM shRNA (m) Lentiviral Particles: sc-35919-V as alternate gene silencing products.

For independent verification of Mel-CAM (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-35919A, sc-35919B and sc-35919C.

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

Mel-CAM siRNA (m) is recommended for the inhibition of Mel-CAM 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

Mel-CAM (P1H12): sc-18837 is recommended as a control antibody for monitoring of Mel-CAM 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Mel-CAM gene expression knockdown using RT-PCR Primer: Mel-CAM (m)-PR: sc-35919-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.