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GP78 shRNA (h) Lentiviral Particles: sc-43809-V

BACKGROUND

GP78 is the autocrine motility factor (AMF) receptor. AMF (also known as neuroleukin or NLK) is a tumor-secreted cytokine that induces *in vivo* invasion and metastasis. AMF induces tumor cell motility *in vitro* through interaction with GP78. GP78 is distributed evenly across the membranes of normal cells but localizes to the leading and trailing edges of carcinoma cells. In gastric cancer, GP78 surface expression correlates to the pathologic stage and grade of tumor penetration. AMF and GP78 interactions may be involved in a synaptic mechanism for learning and memory formation. GP78 and AMF expression increases in the hippocampi of rats after maze learning. Specifically, GP78 is a RING finger-dependent ubiquitin protein ligase (E3) of the endoplasmic reticulum (ER). GP78 recruits UBC7, an ubiquitin-conjugating enzyme (E2). The E3 activity of GP78 suggests a possible link between metastasis and ubiquitin-mediated protein degradation. In humans, alternative splicing of the GP78 mRNA gives rise to two distinct isoforms, 1 and 2, a type I membrane protein and an integral membrane protein, respectively.

REFERENCES

1. Watanabe, H., et al. 1991. Purification of human tumor cell autocrine motility factor and molecular cloning of its receptor. *J. Biol. Chem.* 266: 13442-13448.
2. Silletti, S., et al. 1993. Autocrine motility factor receptor in human bladder carcinoma: gene expression, loss of cell-contact regulation and chromosomal mapping. *Int. J. Oncol.* 3: 801-807.
3. Hirono, Y., et al. 1996. Expression of autocrine motility factor receptor correlates with disease progression in human gastric cancer. *Br. J. Cancer* 74: 2004-2007.
4. Luo, Y., et al. 2002. A link between maze learning and hippocampal expression of neuroleukin and its receptor gp78. *J. Neurochem.* 80: 354-361.

CHROMOSOMAL LOCATION

Genetic locus: AMFR (human) mapping to 16q13.

PRODUCT

GP78 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 GP78 siRNA (h): sc-43809 and GP78 shRNA Plasmid (h): sc-43809-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

GP78 shRNA (h) Lentiviral Particles is recommended for the inhibition of GP78 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

GP78 (F-3): sc-166358 is recommended as a control antibody for monitoring of GP78 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 GP78 gene expression knockdown using RT-PCR Primer: GP78 (h)-PR: sc-43809-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.