SANTA CRUZ BIOTECHNOLOGY, INC.

BRAG2 siRNA (h): sc-78384

BACKGROUND

The ADP-ribosylation factor (ARF) protein family are structurally and functionally conserved members of the Ras superfamily of regulatory GTP-binding proteins. ARFs influence vesicle trafficking and signal transduction in eukaryotic cells. ARF6 plays a role in protein trafficking near the plasma membrane, including receptor recycling, cell adhesion and cell migration. ARF6 colocalizes with the ARF guanine nucleotide-exchange protein (GEP) BRAG2, also designated GEP100. BRAG2 is ubiquitously expressed as two isoforms, BRAG2a and BRAG2b, which can cycle between the cytoplasm and the nucleus. BRAG2, via its interaction with ARF6, is involved in the regulation of cell adhesion by controlling Integrin β1 endocytosis and E-cadherin redistribution. BRAG2 has also been shown to bind directly to Tyr1068/1086-phosphorylated EGFR to activate ARF6, which induces tumor invasion in MCF7 cells. Therefore, BRAG2 may contribute to the metastasis and malignancy of some breast cancer cells.

REFERENCES

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2. Amor, J.C., et al. 1994. Structure of the human ADP-ribosylation factor 1 complexed with GDP. Nature 372: 704-708.
3. Erickson, J.W., et al. 1996. Mammalian Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus. J. Biol. Chem. 271: 26850-26854.
4. Dunphy, J.L., et al. 2006. The Arf6 GEF GEP100/BRAG2 regulates cell adhesion by controlling endocytosis of β1 integrins. Curr. Biol. 16: 315-320.
5. Hiroi, T., et al. 2006. GEP100/BRAG2: activator of ADP-ribosylation factor 6 for regulation of cell adhesion and actin cytoskeleton via E-cadherin and α-catenin. Proc. Natl. Acad. Sci. USA 103: 10672-10677.
6. Dunphy, J.L., et al. 2007. Nuclear functions of the Arf guanine nucleotide exchange factor BRAG2. Traffic 8: 661-672.
7. Pajcini, K.V., et al. 2008. Myoblasts and macrophages share molecular components that contribute to cell-cell fusion. J. Cell Biol. 180: 1005-1019.
8. Morishige, M., et al. 2008. GEP100 links epidermal growth factor receptor signalling to Arf6 activation to induce breast cancer invasion. Nat. Cell Biol. 10: 85-92.

CHROMOSOMAL LOCATION

Genetic locus: IQSEC1 (human) mapping to 3p25.2.

PRODUCT

BRAG2 siRNA (h) 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 BRAG2 shRNA Plasmid (h): sc-78384-SH and BRAG2 shRNA (h) Lentiviral Particles: sc-78384-V as alternate gene silencing products.

For independent verification of BRAG2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-78384A, sc-78384B and sc-78384C.

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

BRAG2 siRNA (h) is recommended for the inhibition of BRAG2 expression in human 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

BRAG2 (D-10): sc-515803 is recommended as a control antibody for monitoring of BRAG2 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 BRAG2 gene expression knockdown using RT-PCR Primer: BRAG2 (h)-PR: sc-78384-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.