SANTA CRUZ BIOTECHNOLOGY, INC.

CTMP siRNA (h): 60469

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The Power to Question

BACKGROUND

The Akt family of protein kinases (also designated PKB) play a role in Insulin signaling, cellular survival and transformation. Genetic alterations resulting in an aberrant activation of the phosphoinositol-3-kinase (PI3K)/Akt signaling pathway frequently occur in glioblastomas. Such factors include phosphatase and tensin homolog (PTEN) mutation, epidermal growth factor receptor (EGFR) amplification and rearrangement, and carboxy-terminal modulator protein (CTMP) hypermethylation. CTMP binds to the C-terminal regulatory domain of Akt and acts as a negative regulator. CTMP inhibits phosphorylation of Akt on Serine 473 and Threonine 308, thereby reducing its activity. Decreased expression of CTMP by hypermethylation of its promoter has been linked to the pathogenesis of glioblastomas.

REFERENCES

1. Maira, S.M., et al. 2001. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. Science 294: 374-380.
2. Knobbe, C.B., et al. 2004. Hypermethylation and transcriptional downregulation of the carboxyl-terminal modulator protein gene in glioblastomas. J. Natl. Cancer Inst. 96: 483-486.
3. Knobbe, C.B., et al. 2005. Genetic alteration and expression of the phosphoinositol-3-kinase/Akt pathway genes PIK3CA and PIKE in human glioblastomas. Neuropathol. Appl. Neurobiol. 314: 486-490.
4. Chae, K.S., et al. 2005. Akt activation is necessary for growth factor-induced trafficking of functional K_{Ca} channels in developing parasympathetic neurons. J. Neurophysiol. 93: 1174-1182.
5. Williams, D.L., et al. 2006. Modulation of the phosphoinositide 3-kinase signaling pathway alters host response to sepsis, inflammation, and ischemia/reperfusion injury. Shock 25: 432-439.
6. Martelli, A.M., et al. 2006. Intranuclear 3'-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? Cell. Signal. 18: 1101-1107.

CHROMOSOMAL LOCATION

Genetic locus: THEM4 (human) mapping to 1q21.3.

PRODUCT

CTMP 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 CTMP shRNA Plasmid (h): sc-60469-SH and CTMP shRNA (h) Lentiviral Particles: sc-60469-V as alternate gene silencing products.

For independent verification of CTMP (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-60469A, sc-60469B and sc-60469C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

CTMP siRNA (h) is recommended for the inhibition of CTMP 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

CTMP (H-10): sc-390353 is recommended as a control antibody for monitoring of CTMP 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 CTMP gene expression knockdown using RT-PCR Primer: CTMP (h)-PR: sc-60469-PR (20 µl, 544 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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