SANTA CRUZ BIOTECHNOLOGY, INC.

EDA siRNA (m): sc-39826

BACKGROUND

Affected males of X-linked anhidrotic ectodermal dysplasia show hypotrichosis, abnormal teeth and absent sweat glands. Some of the patients reported by Halperin and Curtis showed mental defect also, but this is not an invariable feature. Ectodysplasin A (EDA) is a trimeric type II membrane protein that co-localizes with cytoskeletal structures at the lateral and apical surfaces of cells. EDA is expressed in hair follicles and in the epidermis of adult skin. The sequence of the longest isoform includes an interrupted collagenous domain of 19 Gly-X-Y repeats and a motif conserved in the tumor necrosis factor (TNF)-related ligand family. EDA is a member of the TNF-related ligand family involved in the early epithelial-mesenchymal interaction that regulates ectodermal appendage formation. Similar to other members of collagenous membrane proteins and members of TNF-related ligands, EDA is a type II membrane protein which forms trimers.

REFERENCES

1. Halperin, S.L., et al. 1942. Anhidrotic ectodermal dysplasia associated with mental deficiency. Am. J. Ment. Defic. 46: 459-463.
2. Buckle, V.J., et al. 1985. Comparative maps of human and mouse X chromosomes. Cytogenet. Cell Genet. 40: 594-595.
3. Kere, J., et al. 1996. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nat. Genet. 13: 409-416.
4. Ezer, S., et al. 1999. Ectodysplasin is a collagenous trimeric type II membrane protein with a tumor necrosis factor-like domain and co-localizes with cytoskeletal structures at lateral and apical surfaces of cells. Hum. Mol. Genet. 8: 2079-2086.
5. Huang, C., et al. 2006. A novel *de novo* frame-shift mutation of the EDA gene in a Chinese Han family with hypohidrotic ectodermal dysplasia. J. Hum. Genet. 51: 1133-1137.
6. Pummila, M., et al. 2007. Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. Development 134: 117-125.
7. Bal, E., et al. 2007. Autosomal dominant anhidrotic ectodermal dysplasias at the EDARADD locus. Hum. Mutat. 28: 703-709.

CHROMOSOMAL LOCATION

Genetic locus: Eda (mouse) mapping to X C3.

PRODUCT

EDA 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 EDA shRNA Plasmid (m): sc-39826-SH and EDA shRNA (m) Lentiviral Particles: sc-39826-V as alternate gene silencing products.

For independent verification of EDA (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-39826A, sc-39826B and sc-39826C.

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

EDA siRNA (m) is recommended for the inhibition of EDA 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

EDA (3E12): sc-517135 is recommended as a control antibody for monitoring of EDA 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 EDA gene expression knockdown using RT-PCR Primer: EDA (m)-PR: sc-39826-PR (20 µl, 571 bp). 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.