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dsg3 siRNA (h): sc-43115

BACKGROUND

Pemphigus is an autoimmune disease of skin adhesion associated with auto-antibodies against a number of keratinocyte antigens, such as the adhesion molecules desmoglein (dsg) 1 and 3 and acetylcholine receptors. Desmogleins, Type I membrane proteins, are important for cell adhesion and are expressed in great abundance at the desmosomes, which are adhesive cell junctions. Desmogleins belong to the cadherin family and consist of dsg1, dsg2 and dsg3. Calcium binds to the putative calcium binding sites at the extracellular N-terminal domain, which has cadherin-like repeats. Unlike normal human keratinocytes, the squamous cell carcinoma cells exhibit diminished or unusual expression of dsg3 and dsg1, which bear pemphigus vulgaris and pemphigus foliaceus antigens, respectively. Several carcinoma cell lines constantly express dsg2 and dsg3 mRNA, whereas cultured normal human keratinocytes always express dsg1 and dsg3 mRNA, with or without dsg2 mRNA. This expression pattern indicates that desmoglein isoforms exhibit abnormal expression and may be related to tumor cell kinetics, such as cell invasion and metastasis. dsg2 is the fundamental dsg common to all desmosome-possessing tissues and is the largest desmoglein in the family.

REFERENCES

1. Amagai, M., et al. 1991. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell* 67: 869-877.
2. Niles, L.A., et al. 1991. Structural analysis and expression of human desmoglein: a cadherin-like component of the desmosome. *J. Cell Sci.* 99: 809-821.
3. Wheeler, G.N., et al. 1991. Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules. *Proc. Natl. Acad. Sci. USA* 88: 4796-4800.
4. Schafer, S., et al. 1994. Identification of the ubiquitous human desmoglein, dsg2 and the expression catalogue of the desmoglein subfamily of desmosomal cadherins. *Exp. Cell Res.* 211: 391-399.
5. Iwatsuki, K., et al. 1995. Differences in the expression of pemphigus antigens during epidermal differentiation. *Br. J. Dermatol.* 133: 209-216.
6. Harada, H., et al. 1996. Abnormal desmoglein expression by squamous cell carcinoma cells. *Acta Derm. Venereol.* 76: 417-420.

CHROMOSOMAL LOCATION

Genetic locus: DSG3 (human) mapping to 18q12.1.

PRODUCT

dsg3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see dsg3 shRNA Plasmid (h): sc-43115-SH and dsg3 shRNA (h) Lentiviral Particles: sc-43115-V as alternate gene silencing products.

For independent verification of dsg3 (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-43115A, sc-43115B and sc-43115C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

dsg3 siRNA (h) is recommended for the inhibition of dsg3 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 μ M in 66 μ l. 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

dsg3 (5H10): sc-23912 is recommended as a control antibody for monitoring of dsg3 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 dsg3 gene expression knockdown using RT-PCR Primer: dsg3 (h)-PR: sc-43115-PR (20 μ l). 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.