

ABSTRACT

Purpose: To evaluate the antitumor efficacy of monoclonal antibodies (mAbs) raised against desmoglein 3 (DSG3), a transmembrane cell adhesion molecule found in the desmosomes in stratified squamous epithelia, for therapy of lung squamous cell carcinoma (SCC).

Experimental design: Expression of DSG3 in human lung SCC was investigated by gene expression profiling and immunohistochemical analysis. Antitumor activity of mAbs against human (hDSG3) or mouse DSG3 (mDSG3) was evaluated in vitro and in vivo.

Results: DSG3 mRNA expression was found in 9 of the 14 lung SCC. Immunohistochemical analysis detected DSG3 protein in all 5 lung SCC samples tested. mAbs generated against hDSG3 or mDSG3 did not induce dissociation of primary cultured keratinocytes. Compared to its fucosylated counterpart, defucosylated mouse IgG2a (mIgG2a) mAb against hDSG3 induced superior antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro and tumor growth inhibition in a SCID mouse xenograft model. Furthermore, defucosylated mAb against mDSG3 exhibited profound antitumor efficacy in a syngeneic mouse lung SCC model with little toxicity.

Conclusions: DSG3 was identified as a target molecule for antibody-based therapy of lung SCC.

Generation of antibodies

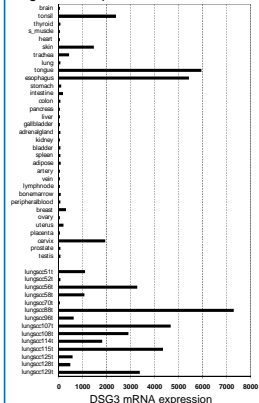
Autoantibodies against DSG3 are known to cause pemphigus vulgaris, an autoimmune disease characterized by suprabasal blisters on the skin and mucous membrane. Inhibition of homophilic DSG3 adhesion by the antibodies may cause disruption of keratinocyte adhesion leading to the disease. It has been reported that pathogenic antibodies are primarily directed to the N-terminus of DSG3. In this study, mAbs with no cell dissociation activity were generated against hDSG3 or mDSG3.

Anti-hDSG3 mAbs std-DF366m and df-DF366m
mAb DF366 (mIgG1κ) was generated by immunizing MRL/lpr mice with purified protein comprising hDSG3 ectodomain fused to the mIgG2a Fc domain. DF366 bound to GST-fusion protein containing the membrane proximal ectodomain of hDSG3 (Asn₉₀₁-Arg₉₁₅) by ELISA. Fucosylated (std-DF366m) or defucosylated (df-DF366m) chimeric mIgG2a mAbs were generated in a CHO DG44 cell line or a GDP-fucose transporter^{-/-} CHO cell line, respectively. The binding affinity (K_d) of std-DF366m to hDSG3 was 526 pmol/L. std-DF366m did not bind to human DSG1 or 4.

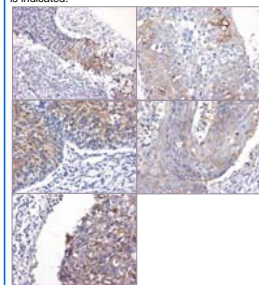
Anti-mDSG3 mAbs std-18-1m and df-18-1m
mAb 18-1 (mIgG1κ) was generated by immunizing DSG3^{-/-} mice with cDNA for mDSG3 using Helios Gene Gun (Bio-Rad). mDSG3 binding was Ca²⁺-independent, indicating it does not recognize the conformational adhesive interface of mDSG3. Fucosylated (std-18-1m) or defucosylated (df-18-1m) mIgG2a mAbs were generated similar to DF366. The K_d of std-18-1m to mDSG3 was 90.1 pmol/L.

Expression of DSG3 in human lung SCC

■ Nine out of 14 lung SCC samples expressed DSG3 mRNA. Expression in normal tissue was limited to those with stratified squamous epithelial cells.
■ DSG3 protein was detected in 5/5 lung SCC samples.



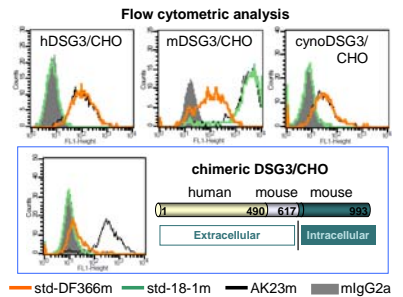
DSG3 mRNA expression was analyzed by Human Exon 1.0 ST Array. Average number of core probes is indicated.



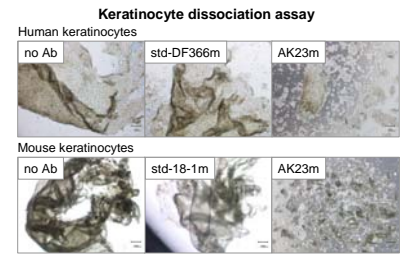
Human lung SCC tissues were stained with anti-DSG3 mAb (clone 5G11) and counterstained with hematoxylin. Magnification, ×200.

Epitope and cell dissociation activity of anti-DSG3 mAbs

■ std-DF366m recognized hDSG3 or cynomolgus monkey DSG3 but bound weakly to mDSG3.
■ std-DF366m had little affinity to chimeric DSG3 in which membrane proximal ectodomain was converted to mDSG3 sequence.
■ std-18-1m bound to mDSG3 but not to chimeric DSG3.
■ Neither std-DF366m nor std-18-1m induced dissociation of keratinocytes.
■ AK23m, a recombinant mAb constructed from VH and VL sequences of pathogenic anti-DSG3 mAb AK23, served as a positive control (J Immunol (2003) 170:2170).



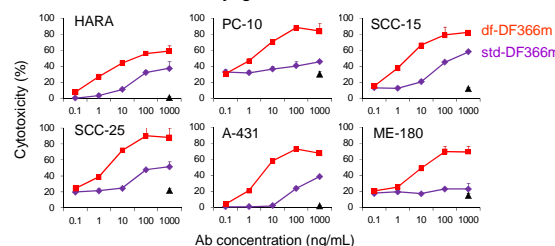
CHO cells transfected with hDSG3, mDSG3, cynomolgus monkey DSG3, or chimeric DSG3 were stained with 10 μg/mL of indicated mAbs and analyzed by flow cytometry. FITC-labeled goat anti-mouse IgG was used as a 2nd antibody.



Primary human or mouse keratinocytes were cultured overnight with 10 μg/mL of indicated mAbs. Cells were further incubated 2 h with exfoliating toxin A, released with dispase, and exposed to mechanical stress.

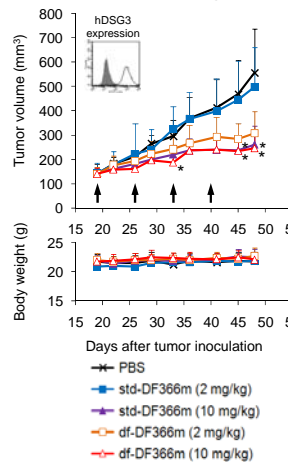
In vitro ADCC and in vivo antitumor efficacy of anti-DSG3 mAbs

ADCC activity against human SCC cell lines

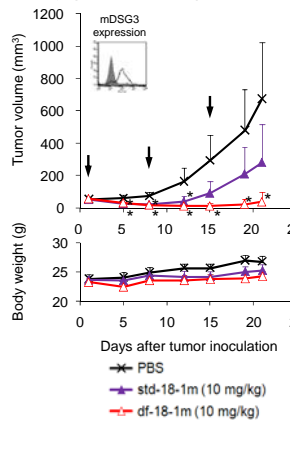


Human lung SCC cell lines HARA and PC-10, human tongue SCC cell lines SCC-15 and SCC-25, human skin SCC cell line A-431, and human cervix SCC cell line ME-180 were used as target cells. DSG3 expression was 1.8×10⁴, 4.8×10⁴, 9.3×10⁴, 3.3×10⁴, 8.3×10⁴, or 4.0×10⁴, respectively (Qifkit, Dako). ⁵¹Cr-labeled target cells were incubated 4h with mouse FcγR4-transfected NK92 cells at an E:T ratio of 5. mIgG2a was used as a negative control (▲).

(A) hDSG3-SK-HEP-1 xenograft model



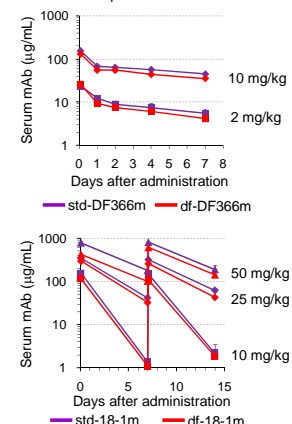
(B) Syngeneic mouse lung SCC model



(A) Female SCID mice were inoculated sc with human hepatocellular carcinoma cell line SK-HEP-1 transfected with hDSG3. DSG3 expression was 1.9×10⁴/cell (Qifkit, Dako). mAbs were administered iv qw×4 starting from day19 (n=5). (B) Male Balb/c mice were inoculated sc with a tumor block of in vivo passaged mouse lung SCC cell line LC-12. mAbs were administered iv qw×3 starting from day1 (n=10). *, significant difference from the vehicle-treated group (p<0.05).

PK profile in mice

■ Fucosylated and defucosylated mIgG2a mAbs showed similar pharmacokinetic profiles.
■ std- or df-18-1m was rapidly cleared from the blood. A dosage of 25 mg/kg was required to saturate endogenous mDSG3 expressed in mice.



SCID mice were administered iv with indicated mAbs (n=3). Serum concentration of the mAbs was analyzed by flow cytometry.

CONCLUSIONS

■ DSG3 was highly expressed in human lung SCC.
■ Anti-human DSG3 mAb DF366m is not likely to cause pemphigus vulgaris because it bound to the membrane proximal ectodomain of human DSG3 and did not induce keratinocyte dissociation.
■ Defucosylated (df-)DF366m elicited stronger antitumor efficacy in SCID mice xenograft model compared to its fucosylated counterpart (std-DF366m).
■ Defucosylated anti-mouse DSG3 mAb (df-18-1m) demonstrated significant antitumor efficacy in a syngeneic mouse lung SCC model despite its rapid clearance from blood.
■ std-18-1m, as well as df-18-1m, indicated little toxicity. Body weight loss was minimal and no signs of pemphigus vulgaris were observed in treated mice.
■ DSG3 was identified as a target molecule for antibody-based therapy of lung SCC.