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Expression of Tumor-associated Glycoantigen, Sialyl Lewis^a, in Human Head and Neck Squamous Cell Carcinoma and Its Application to Tumor Immunotherapy

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The glycoantigen sialyl Lewis^a (sLe^a) is widely expressed on a variety of gastrointestinal tumor cells. Here, we immunohistochemically demonstrated the expression of sLe^a antigen in 54% (7 out of 13) of human head and neck squamous cell carcinoma (H-NSCC) samples. Frequent expression of sLe^a antigen was also demonstrated on a variety of H-NSCC cell lines using flow cytometry. Both CD4$^+$ and CD8$^+$ T cells, which were activated with immobilized OKT3 monoclonal antibody plus interleukin-2, showed augmented cytotoxicity against sLe^a-positive H-NSCC, including autologous tumor cells, on targeting with anti-CD3 × anti-sLe^a bispecific antibody, suggesting that sLe^a antigen is a good target molecule for bispecific antibody-dependent adoptive tumor immunotherapy of human head and neck cancer.

Key words: Sialyl Lewis^a — Head and neck squamous cell carcinoma — Immunotherapy

Squamous cell carcinoma represents the major histological type of neoplasm arising from head and neck, cervix, skin, and lung.[1)] For the diagnosis and therapy of H-NSCC,[4] mAbs recognizing tumor-associated antigens are potentially powerful tools.[1,2)] Such mAbs have been used as targeting reagents for the selective delivery of antitumor drugs or radionuclides.[2–5)] Recently, it was also demonstrated that BSAb prepared with anti-CD3 × anti-tumor mAbs is effective for the targeting of anti-tumor effector cells to a tumor site.[6–9)] During screening for a mAb which can react with H-NSCC, we found that sLe^a antigen detected by KM231 mAb was strongly expressed on H-NSCC. sLe^a antigen is a tumor-associated antigen expressed on a variety of gastrointestinal tumor cells, while its expression on normal cells is restricted.[10–12)] Therefore, a mAb against sLe^a should be suitable for application to tumor immunotherapy. We show herein that H-NSCC express sLe^a antigen in high frequency and present evidence indicating that mAb against sLe^a is a good reagent for the preparation of BSAbs useful for adoptive immunotherapy of H-NSCC.

The expression of sLe^a antigen in 13 H-NSCC was examined by immunohistochemical analysis. As summarized in Table I, 7 out of 13 tumor samples were positive for sLe^a expression. Typical staining patterns in two tumor specimens (tumors no. 4 and 5 in Table I) are shown in Fig. 1. As is clear from the staining profiles (Fig. 1b and d), only the tumor, indicated by "T" in Fig. 1a and c, showed a strong reactivity with KM231 mAb, while normal tissues around the tumor were not stained with KM231 mAb. To confirm the frequent expression of sLe^a antigen on the cell surface of H-NSCC cells, we also investigated sLe^a expression on 6 primary H-NSCC cell lines established in our laboratory. Flow cytometry analysis showed that 5 out of 6 established H-NSCC cell lines expressed sLe^a antigen on their cell surface (Fig. 2).

To determine whether it is possible to target antitumor effector T cells to H-NSCC using anti-sLe^a mAb, we prepared anti-CD3 × anti-sLe^a BSAb by the method shown in Fig. 3. The BSAb reacted with both CD3$^+$ T cells and sLe^a H-NSCC but not with sLe^a-negative Daudi B lymphoma cells (Fig. 3). The effect of BSAb on the induction of cytotoxicity mediated by activated T cells was also determined. CD4$^+$ T cells and CD8$^+$ T cells isolated from the blood of a patient with H-NSCC were activated with immobilized OKT3 plus IL-2 for 14 days. Then, their cytotoxicity against sLe^a-positive H-NSCC Ga cell line was measured in the presence or absence of BSAb. As shown in Fig. 4, both CD4$^+$ and CD8$^+$ activated T cells showed higher cytotoxicity in the presence of BSAb compared with that in the absence of BSAb. Moreover, activated CD4$^+$ T cells and CD8$^+$ T cells showed augmented cytotoxicity against autologous H-NSCC cells in the presence of BSAb. Such augmentation of cytotoxicity by BSAb was not observed when sLe^a-negative IMR32 glioma cells were used as target cells.

Since the hybridoma technique was developed, many investigators have considered the application of mAbs to tumor immunotherapy.[2–5, 13)] Indeed, it has been demon-

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[4] Abbreviations: H-NSCC, head and neck squamous cell carcinoma; sLe^a, sialyl Lewis^a; mAb, monoclonal antibody; BSAb, bispecific antibody; IL, interleukin.

Table I. Summary of sLe^a Expression in Human Head and Neck Squamous Cell Carcinoma

Tumor sample	Sex/Age	TNM classification	sLe^a expression[a]
1. Laryngeal carcinoma	M/67	Stage IV (T3, N2, M0)	+
2. Laryngeal carcinoma	M/77	Stage III (T3, N0, M0)	+
3. Laryngeal carcinoma	M/78	Stage III (T3, N1, M0)	−
4. Maxillary carcinoma	M/56	Stage III (T3, N0, M0)	+
5. Maxillary carcinoma	M/42	Stage IV (T4, N0, M0)	+
6. Maxillary carcinoma	F/43	Stage IV (T4, N1, M0)	−
7. Maxillary carcinoma	M/47	Stage III (T3, N0, M0)	−
8. Maxillary carcinoma	M/78	Stage IV (T4, N0, M0)	−
9. Carcinoma of tongue	F/49	Stage IV (T4, N0, M0)	−
10. Carcinoma of tongue	F/42	Stage III (T3, N0, M0)	−
11. Carcinoma of mouth floor	M/59	Stage IV (T3, N2, M1)	+
12. Hypopharyngeal carcinoma	M/53	Stage IV (T3, N2, M0)	+
13. Hypopharyngeal carcinoma	M/78	Stage II (T2, N0, M0)	+

a) The expression of sLe^a antigen was determined by immunohistochemical analysis. Briefly, a paraffin-embedded section of tumor tissues were deparaffinized, blocked with normal goat serum, and treated with mAb against sLe^a antigen (KM231 mAb, kindly donated by Dr. N. Hanai, Kyowa Hakko Co., Ltd., Tokyo) for 1 h. After washing with phosphate-buffered saline, the sections were further treated with peroxidase-conjugated goat anti-mouse Ig for 1 h. The tissues were developed with 0.2 mg/ml of diaminobenzidine tetrahydrochloride. +, positive; −, negative.

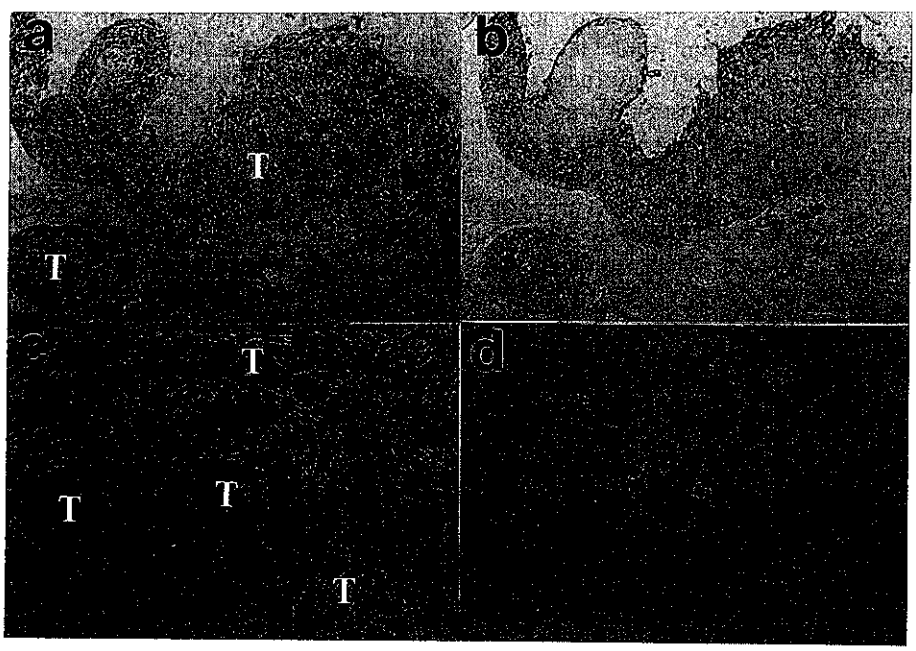

Fig. 1. Immunohistochemical staining of sLe^a antigen in H-NSCC. The staining profile of two tumor tissues is shown. (a, b), tumor sample no. 4 in Table I; (c, d), tumor sample no. 5 in Table I; (a, c), hematoxylin and eosin staining; (b, d), sLe^a expression detected by KM231 mAb indirect peroxidase staining; (T), tumor area.

strated that immunotoxin is effective to inhibit tumor growth in an *in vivo* animal model.[14, 15] Recent studies have also demonstrated that use of BSAb prepared with anti-tumor and anti-effector cell mAbs is an efficient method for the targeting of antitumor effector cells to tumor cells.[6–9] This specific targeting therapy should be an effective strategy for tumor immunotherapy if a good target molecule expressed on tumor cells can be found. As previously demonstrated, sLe^a is expressed on a variety of tumor cells, while normal tissues generally express

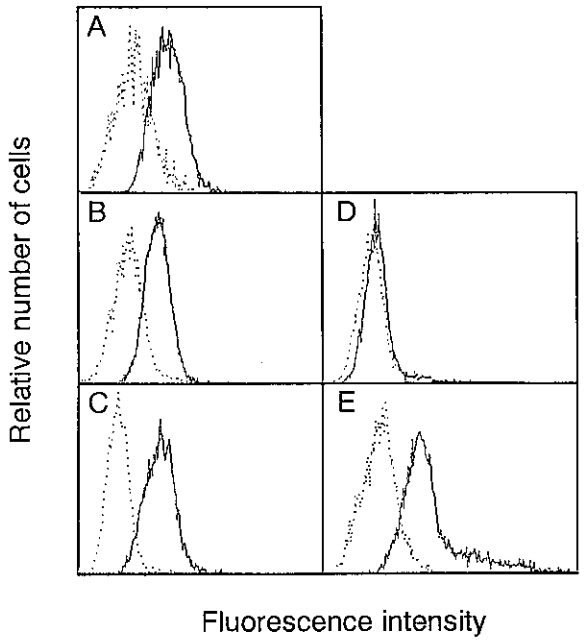

Fig. 2. The expression of sLe^a antigen on established H-NSCC lines. The reactivity of KM231 mAb with various H-NSCC cell lines was examined by FACScan as described previously.[19] A, Ga cells; B, KH cells; C, KM-2 cells; D, Q2 cells; E, TY cells. The solid lines show the staining profiles and the dotted lines indicate control curves.

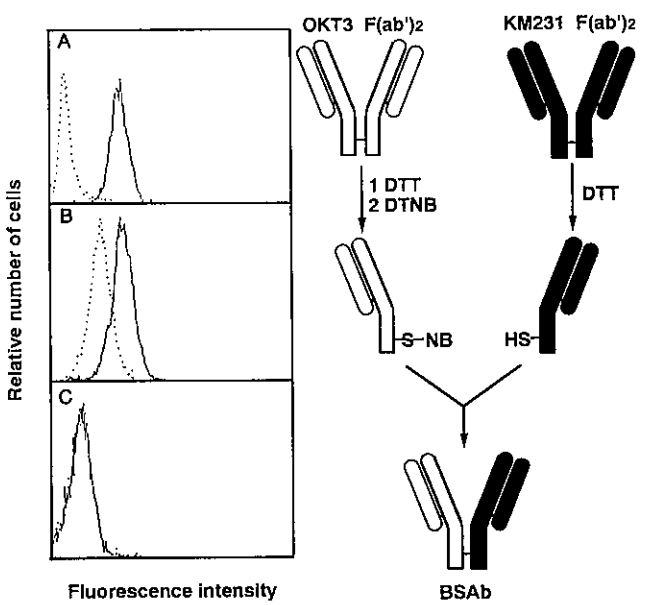

Fig. 3. Scheme for preparation of BSAb and its reactivity with T cells and H-NSCC. The procedure for preparation of BSAb was described in detail in a previous paper.[20] The reactivity of anti-CD3×anti-sLe^a BSAb was determined using CD3$^+$CD8$^+$ T cells (A), sLe^a-positive Ga H-NSCC cells (B) or sLe^a-negative Daudi B lymphoma cells (C).

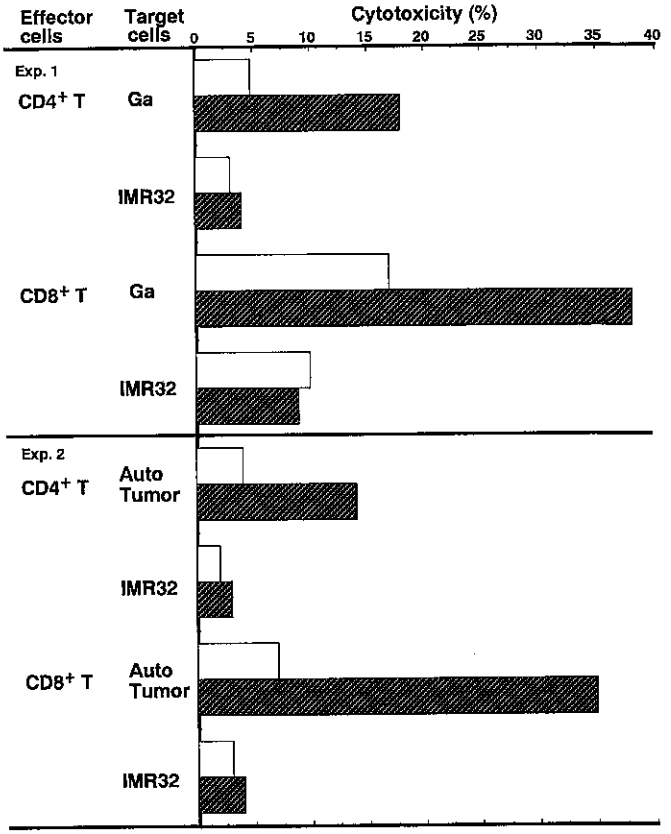

Fig. 4. BSAb-directed cytotoxicity mediated by CD4$^+$ or CD8$^+$ T cells against H-NSCC. The cytotoxic activity of CD4$^+$ T cells or CD8$^+$ T cells, which were activated with immobilized OKT3 mAb plus IL-2, was determined by 4-h ^{51}Cr-release assay in the presence (hatched bars) or absence (open bars) of BSAb (1 μg/ml). Exp. 1, both CD4$^+$ T cells and CD8$^+$ T cells were obtained from an H-NSCC patient and their cytotoxicity against Ga cell lines was determined. Exp. 2, both CD4$^+$ T cells and CD8$^+$ T cells were induced from an H-NSCC patient and their cytotoxicity against autologous tumor cells (Auto tumor) was examined. As a negative control, sLe^a-negative IMR32 glioma cells were used for target cells. The method for preparation of effector T cells was described in detail in a previous paper.[21]

little or no sLe^a antigen.[10-12] It has been demonstrated that pancreatic cancer, gall bladder cancer and hepatoma express sLe^a in high frequency.[14] However, the expression of sLe^a antigen on H-NSCC has not previously been determined. As summarized in Table I, we initially demonstrated that H-NSCC express sLe^a antigen in high frequency (Fig. 1). The flow cytometric analysis of established cell lines confirmed the frequent expression of sLe^a on H-NSCC (Fig. 2). Using CA19-9, which is a well known mAb against sLe^a antigen, it has been demonstrated that sLe^a is a good target molecule for diagnosis and therapy of various tumors.[16,17] As previously re-

ported by Hanai *et al.*,[18] KM231 mAb reacts with sLe^a antigen with higher affinity compared with CA-19-9 mAb. Moreover, it was demonstrated that immunotoxin containing KM231 mAb and ricin A was specifically delivered to the tumor site and inhibited the growth of a tumor implanted in nude mice.[14] Therefore, we thought KM231 mAb might be advantageous for the preparation of a BSAb which can bind both tumor cells and anti-tumor effector cells.

In previous papers,[9,21] we reported that anti-CD3× anti-c-*erbB*-2 BSAb could triger both cytotoxicity and helper function of $CD4^+$ helper/killer T cells. Moreover, the growth of LS174T colon cancer implanted in nude mice was completely inhibited by treatment with BSAb plus $CD4^+$ helper/killer cells. Thus, targeting of $CD4^+$ T cells with killer cells appeared to be effective for the augmentation of local help at tumor sites. As shown in Fig. 4, our anti-sLe^a×anti-CD3 BSAb is effective for triggering both $CD8^+$ T cells and $CD4^+$ helper/killer cells. From the findings that (1) anti-CD3×anti-sLe^a BSAb can react with H-NSCC in high frequency and that (2) the BSAb can stimulate effector cell functions of both $CD4^+$ and $CD8^+$ T cells, it appears that sLe^a antigen is a good target molecule for BSAb-directed adoptive tumor immunotherapy of human H-NSCC.

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