J Oral Pathol Med (2006) 35: 560–7

www.blackwellmunksgaard.com/jopm

Expression of complement restriction factors (CD46, CD55 & CD59) in head and neck squamous cell carcinomas

Naren M. H. Ravindranath, Charles Shuler

Center for Craniofacial Molecular Biology, University of Southern California, Health Sciences Campus, Los Angeles, CA, USA

BACKGROUND: Tumor cells can escape complement-dependent cytotoxicity (CDC) by expressing complement restriction factors (CRFs), CD46, CD55 and CD59. CRF-expression in non-neoplastic mucosa of the head and neck was compared with biopsies of the head and neck squamous cell carcinoma (HNSCC) and cell lines derived from oral squamous cell carcinomas (OSCC).

METHODS: Normal mucosa and HNSCC tumor tissue (poor, moderate, or well differentiated) specimens were immunostained with anti-CRF monoclonal antibodies. Immunostaining of the OSCC cell lines (SCC12 and SCC71) was examined under laser scan fluorescence microscopy.

RESULTS: CD46, CD55 and CD59 were highly expressed in HNSCC cells including T1/T2N0M0 stages. The CRF expression was much lower or absent in non-neoplastic squamous epithelia or in the submucosa of both normal and tumor tissues.

CONCLUSIONS: Enhanced staining of tumor tissues at stages T1/T2 indicates that the CRFs are overexpressed by primary tumors before metastasis to either lymph nodes or organs (N0M0 stage) suggesting that CRFs are formed early during tumorigenesis.

J Oral Pathol Med (2006) **35**: 560–7

Keywords: complement restriction factors; complement-dependent cytotoxicity; decay-accelerating factor CD55; head and neck squamous cell carcinoma; membrane co-factor protein CD46; oral cancer cell lines; protectin CD59; SCC12; SCC71; TNM stages

Introduction

Cancer patients often produce antibodies against tumor-antigens (1–6). Cancer vaccines that will generate antibodies against tumor associated antigens and mediate killing of tumor cells have been considered (7, 8). Antibody binding to the tumor cell surface would activate the complement cascade system to promote formation of the membrane attack complex (MAC) on the tumor cell surface (9). The series of events that lead to MAC formation can be arrested by complement restriction factors (CRFs). Decay-accelerating protein (CD55) and membrane co-factor protein (CD46) are two different CRFs that inhibit autologous C3/C5 convertases at the early stages of complement activation (10, 11). Another CRF, protectin (CD59) binds to the C8-binding protein in the complement pathway and inhibits MAC formation (12). Tumor cell enhanced cell surface expression of CRFs would represent a mechanism for tumor cells to escape complement-mediated cell death. Consequently, there would be a need to down-regulate the expression of CRFs on the tumor cell surface to improve the efficacy of antibody-mediated killing of tumor cells.

It has been shown that tumor cells utilize CRFs to restrict the effectiveness of anti-tumor antibodies (13). CRFs are overexpressed in several types of carcinomas [renal, (14); skin, (15, 16); colorectal, (17, 18); gastric, (19); ovary, (20); cervical, (21, 22)]. The expression patterns of CD46, CD55 and CD59 are not identical for all the cancer types. Some tumor cell lines do not express one or more of the CRFs (23, 24). It is not known whether CRF-overexpression in carcinomas is correlated with TNM stages of tumor progression. There is a need to identify the type of CRF prevalent on the primary tumor cells *in vivo*. This is critical to develop a CRF-blocking strategy to generate an effective autologous complement-dependent cytotoxic (CDC) therapy. We hypothesize that neoplastic transformation of squamous epithelial cells of the head and neck may result in overexpression of CRFs to prevent antibody-mediated attack. Expression of CRFs on head and neck squamous cell carcinoma (HNSCC) may define the level of resistance to CDC. The objectives were to identify the CRFs on normal oral mucosa, biopsies of head and neck SCC and HNSCC cell lines and compare their patterns to determine if CRF expression is found during early stages of SCC.

Correspondence: Naren M. H. Ravindranath, Center for Craniofacial Molecular Biology, University of Southern California, 2250 Alcazar Street, CSA 103, Health Sciences Campus. Los Angeles, CA 90033, USA. Tel: 323 442 3170. Fax: 323 442 2981. E-mail: buduwa 2002@yahoo.com.
Accepted for publication May 9, 2006

Table 1 Histological characteristics of squamous cell carcinoma from oral cavity and associated tissues in HNSCC patients with different TNM stages

ID	*Tissue source*	*Sex*	*Age*	*TNM stage*	*CRF tested*	*Nature*	*Histological differentiation*
A3,4	SCC: lip, lower	M	90	T2N0M0	CD46	Papillary	Well to moderately differentiated (with extension to muscle layer)
A5,6	SCC: lip, upper	M	63	T4N3M0	CD46, CD59	Ulcerofungating; invasive depth:1.2 cm	Poorly differentiated invasion Lymph node metastasis (5/53) Salivary gland, free of tumor
A11	Normal lip, lower	F	59	0	CD55	Non-neoplastic,	Lip, lower
B1,2	SCC: oral soft palate	M	75	T2N0M0	CD59	Basaloid	
B5,6	SCC: oral hard palate	M	61	T2N0M0	CD46, CD55, CD59	Invasive depth: 8 mm	Moderate (i) Lymph nodes negative (ii) Submandibular glands: free of tumor
B11	Normal	M	65	0	CD46, CD55, CD59	Non-neoplastic	Oral cavity
C3,4	SCC: oral second/ third molar left	M	59	T2N0M0	CD55, CD59	Invasive	Well differentiated
C5,6	SCC: oral gingiva, left	F	78	T1N0M0	CD46 CD55, CD59	Invasive	Well differentiated
C11	Normal	F	58		CD46, CD55, CD59	Non-neoplastic	Esophagus
D3,4	SCC: oral	F	74	T3N0MO	CD46, CD55	Invasive, extension to subcutaneous fat tissue	Well differentiated (i) Lymph nodes (0/54) (ii) Soft tissue, free of tumor
D5,6	SCC: oral	F	67	T2N0M0	CD46	*In situ* SCC, invasive to tongue musc. depth: 5 mm	(i) Lymph nodes, negative (ii) Tonsil, left: free of tumor (iii) Submandibular glands, bilateral: free of tumor (iv) Soft tissue, free of tumor
D11	Normal				CD46, CD55, CD59	Non-neoplastic	Hypopharynx
E3,4	SCC: oral buccal floor	M	65	T3N0M0	CD46	Invasive, depth: 8 mm	Well differentiated (i) Lymph nodes negative (ii) Soft tissue, free of tumor (iii) Salivary glands, free of tumor
E7,8	SCC: esophagus	M	69	T1N0M0	CD46, CD55	Fungating SCC	Well differentiated, focal necrosis (i) Extension to the submucosa (ii) No lymphovascular permeation (iii) No perineural invasion (iv) Regional L nodes, negative (v) Omentum: free of tumor
E9,10	SCC: esophagus	F	58	T2N0M0	CD55		Moderately differentiated, extension to the proper muscle Lymph node, regional: free of tumor
F7,8	SCC: esophagus	M	67	T2N0M0	CD46, CD55		Moderately differentiated, extension to muscularis propria and occasional lymphatic permeation (i) Resection margin, free of tumor (ii) Regional lymph nodes, free of tumor
F9,10	SCC: larynx	M	63	T4aN0M0	CD46		Well differentiated, extension to thyroid cartilage
G7,8	SCC: larynx	M	79	T4aN0M0	CD46, CD59		Moderately differentiated, extensive necrosis; marked fibrosis, due to radiation (i) Size: $4.5 \times 4.5 \times 4$ cm (ii) Expanding to supraglottis, glottis, subglottis, through the cricoid, thyroid cartilages, other tissue beyond the larynx (soft tissue, skeletal muscle of the neck and perithyroidal soft tissue (T4), extension to epiglottis (iii) no metastasis to regional L nodes

Table 1 (Continued)

ID	*Tissue source*	*Sex*	*Age*	*TNM stage*	*CRF tested*	*Nature*	*Histological differentiation*
H7,8	SCC: hypopharynx	M	64	T3N1M0	CD55	Invasive	Well differentiated, extension to the larynx and surrounding soft tissue (i) Upper pharyngeal: tumor but moderate dysplasia (ii) Lateral haryngeal: tumor free (iii) Lower trachea: tumor free (iv) Posterior: tumor free (v) Regional L nodes (3/50) (vi) Salivary gland: tumor free (vii) Thyroid: tumor free
H9,10	SCC: hypopharynx	M	62	T4N2cM1	CD46		Well differentiated, right pyriform sinus (i) Extension to pharyngeal muscle (ii) Extension to submucosa of vocal cord
I9,10	SCC: hypopharynx	M	64	T3N1M0	CD46, CD55		Moderately differentiated with extension to the surrounding strap muscle (i) Epiglottis: free of tumor (ii) Resection margin, proximal: tumor involvement (iii) Resection margin, distal: free of tumor (iv) Oropharyngeal lateral wall: tumor involvement (v) Regional lymph nodes (1/108): tumor involvement in left level II (1/15)

Materials and methods

Normal and malignant tissues

Normal SSE and primary SCC tissues (TNM stages T1 to T4) were obtained as tissue arrays (AccuMax™ Array from Petagen, an affiliate of ISU Chemical in Korea, distributed by MTR Scientific (9639 Dr Perry Road, Ijamsville, MD, USA). Surgically resected tissue biopsies from patients were processed after obtaining appropriate consent from individual patients. Cancer tissues and non-neoplastic control tissues from 45 different patients were obtained immediately after surgery, both cancer and adjacent normal tissues were fixed in 10% Formalin and embedded in paraffin. The tissue array is coded as A219 Lot no. 122120312031. Additionally two Oral SCC cell lines (SCC12 and SCC71) were obtained from Dr David Crowe, CCMB-USC and Los Angeles.

Immunohistochemistry

The CRFs were identified by using murine monoclonal antibodies (MAb) as follows: CD46 (purified MAb 143–30, IgG2a, Neomarkers); CD59 (purified MAb 1972-B1, IgG2a, Neomarkers); CD55 (purified MAb 193–27, IgG2a, Neomarkers). For immunohistochemistry, paraffin sections (5 μm thick) were rinsed in phosphate buffered solution (PBS), blocked with PBS–human serum albumin (1% HSA) for 1 h at 37°C, rinsed in PBS and incubated with one of the MAbs, diluted 1/100 in PBS-HSA for 2 h at room temperature. For tissue localization, biotinylated secondary antibody (DAKOCytomation, K3954), peroxidase conjugated-Streptavidin (DAKOCytomation, P0397) and DAB chromogen (DAKO, Carpinteria, CA, USA) were used. The sections were counterstained with hematoxylin. All observations were made on two different sections from each specimen, each observation was carried out at a different site in the different tumor.

Immunolocalization with laser scan confocal microscopy

The SCC-12 and SCC-75 cells were cultured in sterile eight-well Nunc Lab-Tek II Chamber Slide System (Cat No 154534; Nalge Nunc International, Naperville, IL, USA). For immunostaining, 10 000 cells were suspended in RPMI 1640 (0.5 ml) with glutamine supplemented with 10% fetal calf serum, HEPES buffer, and antibiotics; the cells were incubated at 37°C in a humidified (95% air/5% CO_2) chamber. After 24 h, the medium was removed and the cells were washed in cold D-MEM/F-12 medium (Invitrogen, Carlsbad, CA, USA; Cat No 11039–021) once and incubated in the same cold medium for 30 min. All the experiments were performed on an ice chamber. The cells were blocked with cold PBS (pH 7.2)-human serum albumin (HSA, 2%) for 2 h and rinsed with PBS-HSA (1%). CRFs were identified by using murine MAbs (CD46: MAb 122–2, IgG1, R.D.I; CD59: MAb MEM43, IgG2a, R.D.I; CD55 MAb IA10, IgG2a, Pharmingen, San Diego, CA, USA) diluted in cold PBS-HSA (1%) with the concentration adjusted to 2 ng/ml. The cells were incubated on ice with MAbs for 90 min. After washing [in PBS-HSA (1%) for 5 min × 3], the cells were then incubated for 30 min in fluorescein-isothiocyanate FITC conjugated goat anti-mouse IgG (diluted in cold PBS-1% HSA 1:200; Jackson Immunoresearch, Westgrove, PA, USA; cat no: 115–095–071). After three cold buffer rinses, the slides were mounted with 95% glycerol with 5% phosphate buffer. In some preparations, the cells were washed and fixed for 1 min in 4% paraformaldehyde (cold). The glycerol-mounted slides, under oil immersion were examined under a laser scan confocal fluorescence microscope (LSCFM, LSM

510 Carl Zeiss, Oberkochen, Germany) equipped with a 514 argon and a 543 helium-neon laser.

Results

Tumor biopsies: immunostaining on paraffin sections

The TNM stages and histological characteristics of HNSCC biopsies obtained from different regions including oral cavity, esophagus, hypopharynx and larynx are presented in Table 1. The level of CRF was low in the non-neoplastic squamous epithelial cells when compared with the intensity observed for tumor cells. SCC from the oral cavity and hypopharynx had high levels of CRF on the cell surface even in tumors that were staged T1N0M0 and T2N0M0. A uniform feature of the CRF quantitation was that the levels of all CRFs was strikingly greater (++ or +++) in tumor tissues than in the corresponding normal, non-neoplastic regions (+) or (−) of the same patients. Both non-neoplastic stratified squamous epithelia (SSE) and submucosa (SM) of tumor tissues served as positive and negative controls for the location and intensity of the CRFs normally. No endogenous peroxidase activity was detected in neoplastic tissues. In tumor biopsies, CD59 was consistently overexpressed in both cytoplasm and cell membrane of the malignant cells. Although CD46 and CD55 were overexpressed, their intensity was lower than CD59. The CRFs were present at low levels in non-neoplastic SSE and absent in the connective tissue cells or stroma in the region of SM and lamina propria (LP). The connective tissue and stromal cells were effective internal negative controls, providing contrast to both SCC and non-neoplastic SSE cells. There was no observed difference in the CRFs levels based on the grading of the tumors as either poor, moderate or well differentiated. Overexpression of CRFs was observed in HNSCC at stages T1N0M0 to T4N0M0 at the

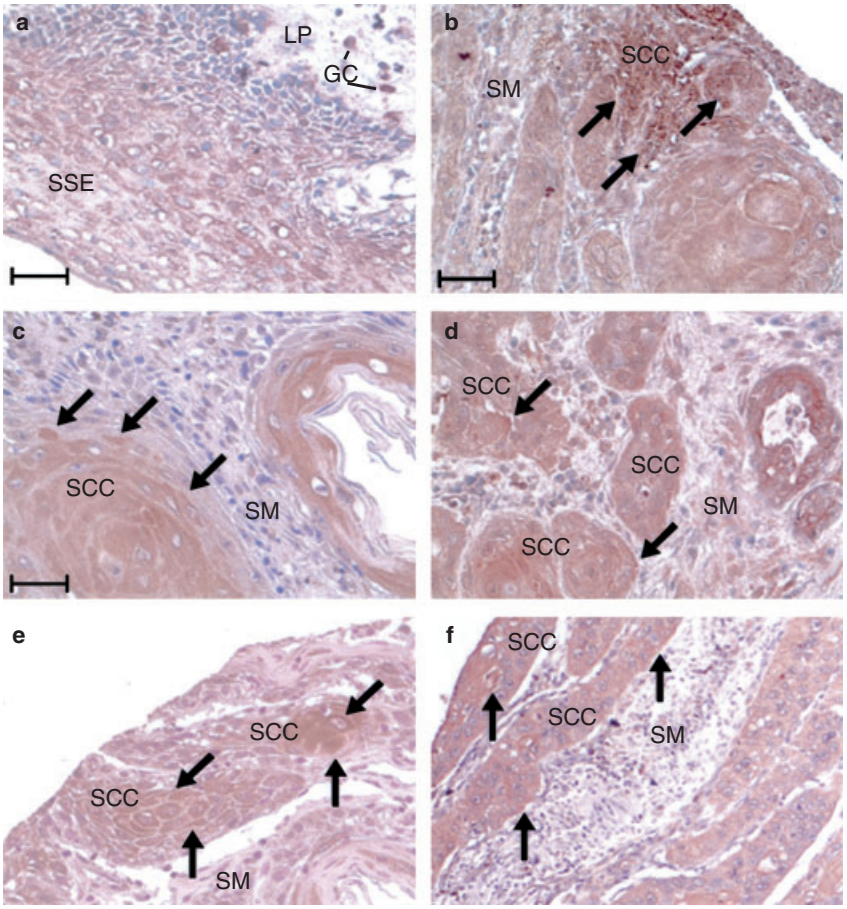

Figure 1 (a) (patient ID: B11, for details see Table 1) Non-neoplastic squamous epithelia from oral cavity stained with anti-CD46 MAb (143–30) and peroxidase conjugated secondary Ab. Stratified squamous epithelium (SSE) stained weakly, No staining was observed in the connective tissues and stroma in sub-mucosa (SM) and lamina propria (LP). In the connective tissues, some cells stained darkly in the region of LP and SM, which are granulocytes (GC) whose endogenous peroxidase activity was not blocked. (b) (patient ID: B6 stage T2N0M0) Invasive OSCC (depth 8 mm) from hard palate, tumor cells are differentiated moderately. Both cytoplasm and the cell membrane of the tumor cells stained intensely with anti-CD46 MAb (arrows). (c) (patient ID: C6 stage T1N0M0) Invasive OSCC in the gingival region; Tumor cells are well-differentiated tumor cells and stained intensely with anti-CD46 MAb (arrows). (d) (patient ID: B5 stage T2N0M0) Invasive OSCC (depth 8 mm) at the hard palate, moderately differentiated. Tumor cell surface (arrows) indicates intense staining. (e) (C5) Invasive OSCC at the left gingival, well-differentiated tumor cells with intense staining of the membrane in contrast to SM. (f) (patient ID: E3 stage T3N0M0) Invasive OSCC (8 mm) at the buccal floor, well-differentiated, both cytoplasm and cell surface are intensely stained. In contrast, connective tissues in the SM did not stain. However, SM is infiltrated with lymphocytes some of which stained intensely.

primary site. No change in CRF expression was observed after nodal (N1) or organ metastasis (M1) of HNSCC.

Distribution of CD46

The CD46 was present at low level in non-neoplastic SSE from oral cavity. It was absent in the connective tissues and stroma in SM and LP (Fig. 1a). In HNSCC cells, both cytoplasm and the cell membrane showed the presence of CD46 (arrows in Fig. 1b–f). In all the neoplastic lesions examined (Fig. 1b–f), CD46 was absent from the connective tissues in the SM. The level of CD46 was markedly elevated in moderately differentiated invasive OSCC (T2N0M0) from hard palate (Fig. 1b,d), well-differentiated confined OSCC (T1N0M0) from gingival region (Fig. 1c,e) and invasive OSCC (T3N0M0; Fig. 1f).

Distribution of CD59

Stratified squamous epithelia obtained from non-neoplastic oral cavity had minimal expression of CD59; whereas CD59 was absent in the connective tissues, the stroma in SM and the LP (Fig. 2a). CD59 was present in abundance in all SCC tissues examined including well-differentiated oral SCC (T1N0M0) from gingival (Fig. 2b,d) and moderately differentiated invasive OSCC (T2N0M0) from the hard palate (Fig. 2c). Consistently, CD59 was absent in the connective tissue stroma of all tumor specimens.

Distribution of CD55

The CD55 was present at low levels in the epithelium of the non-plastic specimens (Fig. 3a,b). CD55 was at high levels (++/+++) in the cytoplasm and cell membrane of a well-differentiated invasive OSCC (T2N0M0; Fig. 3c,d, magnified), and a well-differentiated OSCC of the gingival (T1N0M0; Fig. 1f) and in a more advanced stage OSCC of the gingival (T3N0M0l; Fig. 3d), whereas HNSCC in the hypopharynx the CD55 was present at low levels (data not shown).

Immunochemical localization of CRFs on live SCC cells with laser scan confocal microcopy

Immunohistochemical observations show immunostaining of tumor cells but fail to clearly identify whether CRFs are found in the cell surface. In order document cell surface expression of CRFs unequivocally, we performed immunochemical localization of CRFs on cultured cells from cell lines SCC12 and SCC71 (Fig. 4). Presence of CRFs on the cell surface was distinct and appeared as bead-like aggregations (arrows). Uniform distribution of CD46 was observed in both SCC12 and SCC71 (Fig. 4a,b). A similar distribution was also observed with CD55 in both cell lines (Fig. 4e,f). The

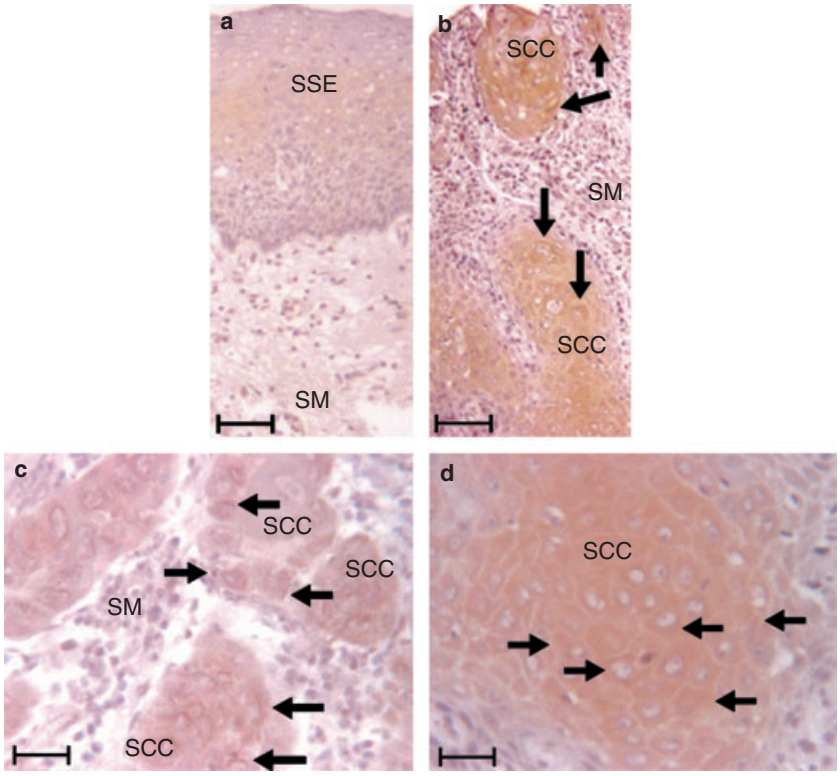

Figure 2 (a) (patient ID: B11) CD59 immunostaining with anti-CD59 MAb (1972-B1) on non-neoplastic oral cavity. SSE showed weak staining, whereas connective tissues and stroma in SM did not stain. Darkly stained cells could be GC or lymphocytes whose endogenous peroxidase activity was not blocked. (b) (patient ID: C6 stage T1N0M0) Invasive SCC at the left gingival, the tumor is well-differentiated and intensely stained for CD59. Arrows indicate intense staining of cytoplasm and cell membrane. (c) (patient ID: B6 stage T2N0M0) Invasive OSCC in the region of the hard palate, moderately differentiated with arrows indicate staining of both cytoplasm and/or the cell membrane with anti-CD59 MAb. (d) (patient ID: C5 stage T1N0M0) Invasive OSCC in the gingival region, that is well-differentiated and intensely stained at the cytoplasm and cell membrane.

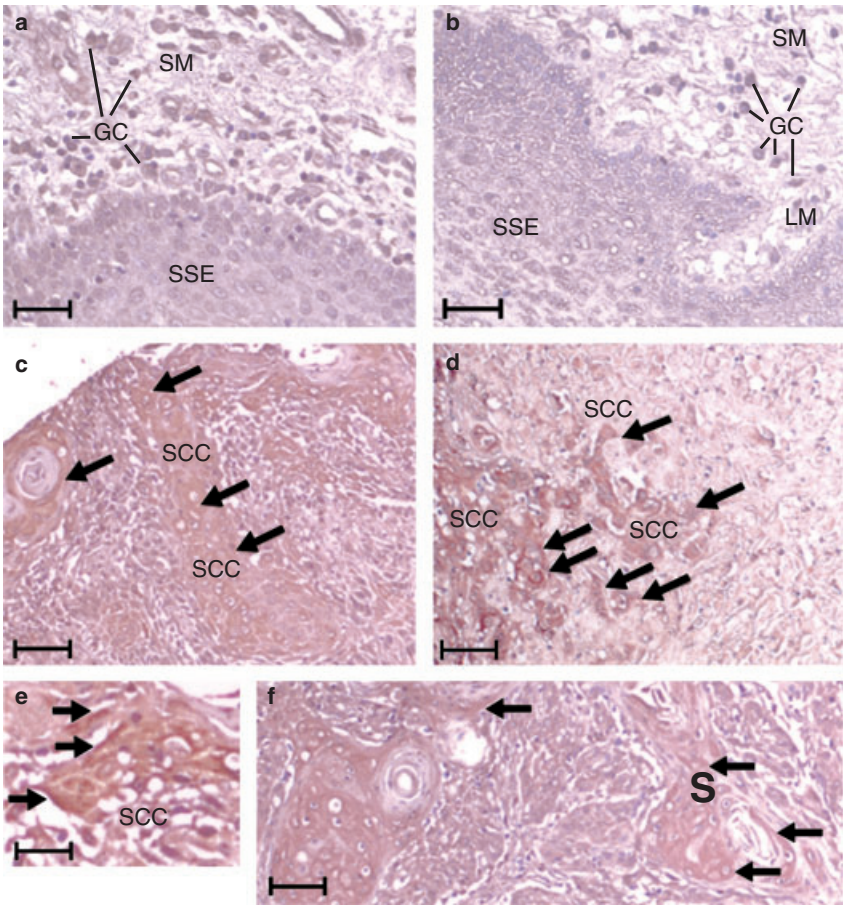

Figure 3 (a) (patient ID: A11) Non-neoplastic lower lip region, SSE and SM are distinct. SSE stained feebly with anti-CD55 Mob (193–27) and the connective tissues in SM failed to stain. Darkly stained cells are GC whose endoperoxidase activity was not blocked. (b) (patient ID: B11) Non-neoplastic SSE of oral cavity weakly stained. The darkly stained cells are GC whose endoperoxidase activity was not blocked. (c) (patient ID: C4 stage T2N0M0) Invasive OSCC in the region of second and third molar (left), with well-differentiated tumor cells. Arrows indicate intense staining of the cytoplasm and membrane. (d) (patient ID: D4 stage T3N0M0) Invasive OSCC extending to subcutaneous fat tissue, well differentiated cells, both cytoplasm and cell membrane stained intensely (arrows). (e) (patient ID: C4 stage T2N0M0) Invasive OSCC in the region of second and third molar, magnified to show distinct CD55 positive staining of the cell membrane. (f) (patient ID: C6 stage T1N0M0) Invasive SCC at the gingival region, with well-differentiated tumor cells, anti-CD55 MAb showed intensive staining at the cytoplasm and the cell membrane, whereas the connective tissues in SM showed poor staining.

distribution of CD59 on the cell surface of SCC12 was somewhat sparse and found aggregated on the cell surface as spheres. Such aggregation reaches larger size in SCC71.

Discussion

Assessment of the expression of CD46, CD55 and CD59 using a tissue array of neoplastic and non-neoplastic human tissues specimens provides a means to compare CRFs in normal vs. neoplastic tissues. CRFs, CD46, CD55 and CD59 are present in high levels in tumor biopsies of HNSCC (Figs 1–3). The normal non-neoplastic tissues expressed CRFs at low levels whereas they were absent in submucosal and stromal tissues of tumor biopsies. All three CRFs were found at very high levels in tumor biopsies of HNSCC obtained from stage T1N0M0 and T2N0M0 indicating that CRFs are present at high level before nodal (N stage) or organ metastasis (M stage). The confocal microscopic observations on SCC12 and SCC71 cells confirmed the presence of these CRFs on the cell surface of HNSCC (Fig. 4).

The significance of the expression of all the three different kinds of CRFs in HNSCC is far from clear. Presence of high levels of CRFs could be of adaptive value and survival benefit for tumor cells. The first step to understand the role of the tumor microenvironment on the overexpression of CRFs is to evaluate quantitative differences, if any, in the cell surface expression of CD46, CD55 and CD59. The environment surrounding a tumor has many features that would enhance the antibody-mediated cell killing and the CRFs would represent a means to avoid the cell killing. The targets for antibody mediated killing may include proteins and glycoconjugates of tumor or of viral etiology. Particularly, the neoplastic transformation of oral SCC involves highly immunogenic viral pathogens, such as human papilloma virus (HPV) (25). One mechanism for the malignant transformation of oral mucosa is attrib-

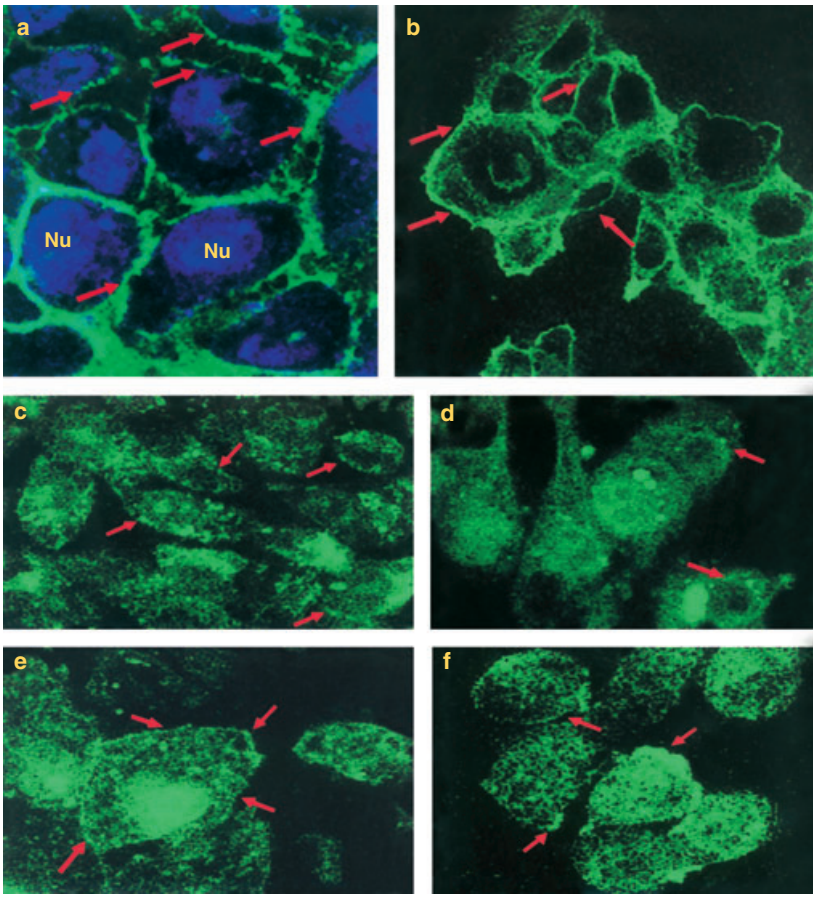

Figure 4 Confocal fluorescence microscopic assessment of expression of complement restriction factors, CD46, CD55 and CD59 in oral squamous carcinoma cell lines SCC12 (a,c,e) and SCC71 (b,d,f). (a,b) Anti-CD46 MAb 122–2 IgG2 (R.D.I) stained the cell surface of both the cell lines distinctly. The beaded nature on the cell surface revealed the nature of distribution of CD46 in specific and uniform clusters (arrows). (c,d) Anti-CD59 MAb MEM43 (IgG2a, R.D.I.) shows the distribution of CD59 on the cell surface of both SCC12 and SCC71. Compared with CD46, the distribution of CD59 is sparse and aggregate on the cell surface. (e,f) Anti-CD55 MAb IA10 (IgG2a, Pharmingen) stained the cell membranes of both cell lines distinctly. Uniform bead-like cluster indicates the nature of distribution of CD55 on the cell surface. The expression of CD55 appears more intense than CD59. Confocal comparison of the three CRFs reveals that SCC12 and SCC75 are similar.

uted to HPV infection correlated with p53 mutation (26–30). Woods et al. (31) noted that HPV infection and p53 mutation contribute to production of IL-6 by human OSCC. Indeed, OSCC cell lines secreting the IL-6 had mutant rather than wild-type p53. The primary function of human IL-6 is to stimulate B cell proliferation and production of potential complement fixing IgM antibodies (32). IL-6 mRNA expression is highly prevalent at early stage of tumorigenesis (33). Nakano et al. (34) found IL-6 production in T1and T2 stages of tumor growth, suggesting that IL-6 production is a continuous process since T1 stage. We speculate that the cell surface expression of the CRFs in HNSCC could be an escape strategy adopted by tumor cells to circumvent antibody mediated CDC.

References

1. Irie RF, Irie K, Morton DL. Natural antibody in human serum to a neoantigen in human cultured cells grown in fetal bovine serum. *J Natl Cancer Inst* 1974; **52**: 1051–8.
2. Carey TE, Takahashi T, Resnick LA, Oettgen HF, Old LJ. Cell surface antigens of human malignant melanoma: mixed hemadsorption assays for humoral immunity to cultured autologous melanoma cells. *Proc Natl Acad Sci U S A* 1976; **73**: 3278–82.
3. Shiku H, Takahashi T, Oettgen HF. Cell surface antigens of human malignant melanoma. II. Serological typing with immune adherence assays and definition of two new surface antigens. *J Exp Med* 1976; **144**: 873–81.
4. Old LJ. Cancer immunology: the search for specificity – G. H. A. Clowes Memorial lecture. *Cancer Res* 1981; **41**: 361–75.
5. Ueda R, Ogata S, Morrissey DM et al. Cell surface antigens of human renal cancer defined by mouse monoclonal antibodies: identification of tissue-specific kidney glycoproteins. *Proc Natl Acad Sci U S A* 1981; **78**: 5122–6.
6. Vlock DR, Scalise D, Schwartz DR et al. Incidence of serum antibody reactivity to autologous head and neck cancer cell lines and augmentation of antibody reactivity following acid dissociation and ultrafiltration. *Cancer Res* 1989; **49**: 1361–5.
7. Bystryn JC, Ferrone S, Livingston P. Specific immunotherapy of cancer with vaccines. New York Academy of Sciences. *Ann N Y Acad Sci* 1993; **690**: 1–411.

8. Dalgleish AG, Browning M. *Tumor Immunology*. Cambridge, UK: Cambridge University Press, 1996; 1–353.
9. Terachi T, Stanescu G, Pontes JE, Medof ME, Caulfield MJ. Coexistence of autologous antibodies and decay-accelerating factor, an inhibitor of complement, on human renal tumor cells. *Cancer Res* 1991; **51**: 2521–23.
10. Medof ME. Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity in vitro. *J Clin Invest* 1988; **81**: 1122–28.
11. Seya T, Hara T, Matsumoto M, Akedo H. Quantitative analysis of membrane cofactor protein (MCP) of complement. High expression of MCP on human leukemia cell lines, which is down-regulated during cell differentiation. *J Immunol* 1990; **145**: 238–45.
12. Schonermark V, Rauterberg EW, Shin ML, Loke S, Roelcke D, Hansch GM. Homologous species restriction in lysis of human erythrocytes: a membrane-derived protein with C8-binding capacity functions as an inhibitor. *J. Immunol* 1986; **136**: 1772–76.
13. Koretz K, Bruderlein S, Henne C, Moller P. Decay-accelerating factor (DAF, CD55) in normal colorectal mucosa, adenomas and carcinomas. *Br J Cancer* 1992; **66**: 810–4.
14. Magyarlaki T, Mosolits S, Baranyay F, Buzogany I. Immunohisto-chemistry of complement response on human renal cell carcinoma biopsies. *Tumori* 1996; **82**: 471–9.
15. Sayama K, Shiraishi S, Miki Y. Distribution of complement regulators (CD46, CD55 and CD59) in skin appendages, and in benign and malignant skin neoplasms. *Br J Dermatol* 1992; **127**: 1–4.
16. Whitlow MB, Klein LM. Response of SCC-12F, a human squamous cell carcinoma cell line, to complement attack. *J Invest Dermatol* 1997; **109**: 9–45.
17. Bjorge L, Vedeler CA, Ulvestad E, Matre R. Expression and function of CD59 on colonic adenocarcinoma cells. *Eur J Immunol* 1994; **24**: 1597–603.
18. Juhl H, Helmig F, Baltzer K, Kalthoff H, Henne-Bruns D, Kremer B. Frequent expression of complement resistance factors CD46, CD55, and CD59 on gastrointestinal cancer cells limits the therapeutic potential of monoclonal antibody 17–1A. *J Surg Oncol* 1997; **64**: 222–30.
19. Inoue T, Yamakawa M, Takahashi T. Expression of complement regulating factors in gastric cancer cells. *Mol Pathol* 2002; **55**: 193–9.
20. Bjorge L, Hakulinen J, Wahlstrom T, Matre R, Meri S. Complement-regulatory proteins in ovarian malignancies. *Int J Cancer* 1997; **70**: 14–25.
21. Simpson KL, Jones A, Norman S, Holmes CH. Expression of the complement regulatory proteins decay accelerating factor(DAF, CD55), membrane cofactor protein (MCP, CD46) and CD59 in the normal human uterine cervix and in premalignant and malignant cervical disease. *Am J Pathol* 1997; **151**: 1455–67.
22. Gelderman KA, Blok VT, Fleuren GJ, Gorter A. The inhibitory effect of CD46, CD55, and CD59 on complement activation after immunotherapeutic treatment of cervical carcinoma cells with monoclonal antibodies or bispecific monoclonal antibodies. *Lab Invest* 2002; **82**: 483–93.
23. Shinoura N, Heffelfinger SC, Miller M et al. RNA expression of complement regulatory proteins in human brain tumors. *Cancer Lett* 1994; **86**: 143–9.
24. Goslings WR, Blom DJ, de Waard-Siebinga I et al. Membrane-bound regulators of complement activation in uveal melanomas. CD46, CD55, and CD59 in uveal melanomas. *Invest Ophthalmol Vis Sci* 1996; **37**: 1884–91.
25. Flaitz CM, Hicks MJ. Molecular piracy: the viral link to carcinogenesis. *Oral Oncol* 1998; **34**: 448–53.
26. Kojima A, Maeda H, Sugita Y, Tanaka S, Kameyama Y. Human papillomavirus type 38 infection in oral squamous cell carcinomas. *Oral Oncol* 2002; **38**: 591–6.
27. Minawaer L, Ahmatjan A, Suzuk L. Detection of HPV type 16, 18 infection and p53 protein overexpression in oral squamous cell carcinoma. *Zhonghua Kou Qiang Yi Xue Za Zhi* 2001; **36**: 451–3.
28. Cao J, Zhang ZY, Patima, Zhang YX, Chen WT. Human papillomavirus infection and p53 alteration in oral squamous cell carcinoma. *Chin J Dent Res* 2000; **3**: 44–9.
29. Shima K, Kobayashi I, Saito I et al. Incidence of human papillomavirus 16 and 18 infection and p53 mutation in patients with oral squamous cell carcinoma in Japan. *Br J Oral Maxillofac Surg*. 2000; **38**: 445–50.
30. Mao EJ, Schwartz SM, Daling JR, Oda D, Tickman L, Beckmann AM. Human papilloma viruses and p53 mutations in normal pre-malignant and malignant oral epithelia. *Int J Cancer* 1996; **69**: 152–8.
31. Woods KV, Adler-Storthz K, Clayman GL, Francis GM, Grimm EA. Interleukin-1 regulates interleukin-6 secretion in human oral squamous cell carcinoma in vitro: possible influence of p53 but not human papillomavirus E6/E7. *Cancer Res* 1998; **58**: 3142–9.
32. Kanda N, Watanabe S. Ganglioside GD1a enhances immunoglobulin production by human peripheral blood mononuclear cells. *Exp Hematol* 2000; **28**: 672–9.
33. Wang YF, Chang SY, Tai SK, Li WY, Wang LS. Clinical significance of interleukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. *Head Neck* 2002; **24**: 850–8.
34. Nakano Y, Kobayashi W, Sugai S, Kimura H, Yagihashi S. Expression of tumor necrosis factor-alpha and interleukin-6 in oral squamous cell carcinoma. *Jpn J Cancer Res* 1999; **90**: 858–66.

Acknowledgements

Thanks are due to Dr Donald L Morton, for permitting to carry out a part of this study at John Wayne Cancer Institute, Santa Monica, Mr Pablo Bringas (CCMB-USC) for assistance in immunohistochemisty, Dr David Crowe (CCMB-USC), for providing OSCC cell lines, Dr Xaio Mei Cui (CCMB-USC), for training in cell culturing techniques and Dr S.R. Selvan, Hoag Cancer Center, NewPort Beach, for critically editing the manuscript.