Identification of a Cell Surface Protein with a Role in Stimulating Human Keratinocyte Proliferation, Expressed During Development and Carcinogenesis

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In an attempt to define cell surface molecules with an important role in the development of squamous cell carcinomas (SCCs), we generated monoclonal antibodies (MoAbs) to a human keratinocyte cell line (FEP18–11-T1) capable of giving rise to SCCs in nude mice. MoAb 10G7 was selected for further study because it bound to a cell surface component preferentially expressed by this cell line as compared with normal human foreskin keratinocytes. This MoAb recognizes a cell surface protein (10G7 antigen) that is not detectable on normal keratinocytes in the foreskin *in vivo*, but whose expression is induced when the keratinocytes are dissociated from this tissue and placed in culture. Interestingly, the 10G7 antigen is downregulated upon keratinocyte differentiation *in vitro*. Consistent with its expression in hyper-proliferative epithelia *in vitro*, 10G7 antigen exhibited a classic oncofetal pattern of expression *in vivo*. Thus, although no reactivity was obtained with MoAb 10G7 in the epithelia of normal foreskin or cervical tissue, strong reactivity was detected in epithelia from genital lesions ranging from benign warts to invasive SCCs. Epidermis from developing fetal tissue also exhibited strong reactivity with MoAb 10G7. We have been able to demonstrate that this MoAb is capable of stimulating FEP18–11-T1 keratinocyte proliferation *in vitro* in a concentration-dependent manner in the *absence* of growth factors, suggesting that the 10G7 antigen may play an important role in regulating cellular proliferation during development and in carcinogenesis in epithelial tissues. *Key words: skin/squamous cell carcinoma/growth/monoclonal antibody. J Invest Dermatol 109:194–199, 1997*

Carcinogenesis is a complex, multistep process involving a cascade of cellular changes, ultimately leading to metastatic carcinomas. Studies in mouse epidermis have shown that this process can be separated into three distinct stages: initiation, promotion, and progression. During carcinogenesis, epithelial cells progressively become capable of unregulated growth, microinvasion through the basement membrane, and dissemination through the vasculature to form metastases at secondary sites. The process of neoplastic conversion in epithelia reflects changes occurring both at the intracellular level and at the cell surface. A critical role for a variety of cell surface components in tumor growth and spread has been demonstrated in the literature, including key factors such as growth factor receptors (GFRs), proteolytic enzymes, and cell adhesion molecules, which mediate both intercellular and cell–extracellular matrix adhesion (reviewed in Hart and Saini, 1992; Van Roy and Mareel, 1992).

Uncontrolled proliferation can be achieved by the activation of oncogenes and inactivation of tumor suppressor genes (Klein and Klein, 1985), as well as by the overexpression of GFRs at the cell surface (reviewed in Aaronson, 1991). Cell surface molecules involved in the invasion and metastasis of epithelial tumors include proteolytic enzymes for degradation of the extracellular matrix (Liotta *et al*, 1983); intercellular adhesion molecules such as E-cadherin, which restrict invasiveness (Frixen *et al*, 1991); those that permit tumor cell motility, such as the CD44 meta-1 variant (Gunthert *et al*, 1991); and those involved in mediating adhesion of epithelial tumor cells to endothelial cells lining blood vessels at distal sites, such as sialylated forms of the Lewis^a and Lewis^x carbohydrate antigens (Hakomori *et al*, 1984), which bind to endothelial cell adhesion molecules such as P-selectin (Aruffo *et al*, 1992) and E-selectin (Rice and Bevilacqua, 1989). These studies have demonstrated an important functional role for cell surface molecules expressed preferentially by tumor cells in mediating tumor cell growth and spread, and provide important insights into the mechanisms involved in malignant tumorigenesis.

Relatively little is known about the keratinocyte cell surface and the role of such components in the development of squamous cell carcinomas (SCCs). We therefore investigated alterations at the cell surface level occurring during the neoplastic conversion of the keratinocyte to SCC. We reasoned that generating monoclonal antibodies (MoAbs) to cell surface components would be a valuable approach and would provide useful tools (antibodies) for functional

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Abbreviation: GFR, growth factor receptor.

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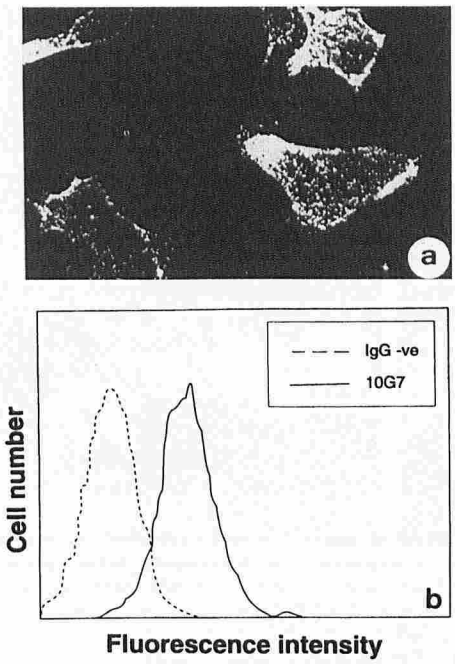

Figure 1. MoAb 10G7 detects a cell surface component. *(a)* Immunofluorescent staining of nonpermeabilized FEP18–11-T1 cells with MoAb 10G7 and *(b)* flow cytometric analysis of trypsinized FEP18–11-T1 cells. IgG negative (−ve) antibody is 1D4.5.

studies as well as for expression cloning. We therefore generated MoAbs to cell surface components that are preferentially expressed on the FEP18–11-T1 human keratinocyte cell line as compared with normal human foreskin keratinocytes. The FEP18–11-T1 cell line was derived from an invasive SCC induced by subcutaneous injection of a human papillomavirus type-18 immortalized foreskin keratinocyte cell line (FEP18–11) into nude mice, as described previously (Kaur and McDougall, 1989; Hurlin *et al*, 1991). We have raised several MoAbs that identify interesting keratinocyte cell surface components. Here we describe the cell surface antigen recognized by MoAb 10G7, which appears to have an important role in tumorigenesis.

MATERIALS AND METHODS

Keratinocyte Culture All keratinocytes (normal foreskin keratinocytes and FEP18–11-T1s) were cultured in keratinocyte growth medium (Clonetics Corporation, San Diego, CA).

Generation of MoAb 10G7 Balb/c mice were immunized intraperitoneally with 3×10^6 trypsinized tumorigenic FEP18–11-T1 cells together with 50 μg of the adjuvant muramyl dipeptide (Sigma Chemical Co., St. Louis, MO), three times at 4-wk intervals. After a final intravenous boost, the immune splenocytes were fused with the murine myeloma cell line SP2 using polyethylene glycol according to standard protocols (Kohler and Milstein, 1975). Supernatants from HAT-resistant hybridomas were screened for binding to monolayers of live, adherent FEP18–11-T1 cells in 96-well trays using a sensitive enzyme-linked immunosorbent assay method developed for cell surface antigens. Briefly, cells were incubated with 50 μl of hybridoma supernatant for 1 h at 4°C, washed in wash medium (RPMI 1640, 10 mM HEPES, 10% serum), and incubated sequentially with rabbit anti-mouse antibody, diluted 1:600 in wash medium for 30 min, and peroxidase-conjugated goat anti-rabbit antibody (1:200) for 30 min, all at 4°C. After washing, the cells were incubated for 5–10 min with peroxidase substrate consisting of 1 mg O-phenylene-diamine per ml in 100 mM citrate buffer, pH 6.5, plus 0.03% H_2O_2. The enzyme reaction was quenched with 1 M H_2SO_4, and optical density at 490 nm was measured on a Biorad 3550 microplate reader. All supernatants exhibiting cell surface binding to FEP18–11-T1 cells were then subjected to a second screen against monolayers of normal foreskin keratinocytes. Those supernatants showing increased or exclusive binding to FEP18–11-T1 cells were then selected for further analysis. These hybridomas were cloned by limiting dilution. MoAb 10G7 was selected because it exhibited increased reactivity to the tumor FEP18–11-T1 keratinocytes compared with normal foreskin keratinocytes. The isotype of MoAb 10G7 was determined as IgG_{2a} using a commercially available isotyping kit (Southern Biotechnology, Burlingame, CA).

Immunofluorescent Staining For *in situ* staining, cells were plated in eight-chamber slides and cultured overnight. The cells were fixed briefly in 2% formaldehyde for 2 min and stained by first incubating with neat hybridoma supernatant for 1 h, washing with phosphate-buffered saline (PBS), and incubating with a 1:80 dilution of anti-mouse IgG–fluorescein isothiocyanate-conjugated secondary antibody, all at room temperature. For flow cytometric analysis, cells were harvested by trypsinization and incubated in suspension with undiluted hybridoma supernatant at 4°C for 1 h. Cells were then washed and incubated with the appropriate fluorescein isothiocyanate-conjugated secondary antibody for 1 h at 4°C. The cells were washed and post-fixed in 2% formaldehyde and then analyzed on a Coulter Epics Profile II flow cytometer. Isotype-matched negative control MoAb 1D4.5 (IgG_{2a}), kindly provided by Dr. Leonie Ashman, Hanson Center, was used in all flow cytometry experiments.

Immunoperoxidase Staining of Tissue Sections Eight-micrometer cryostat sections of unfixed human tissue including neonatal foreskin, fetal tissue, and biopsies of genital epithelial lesions were subjected to immunoperoxidase staining using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Briefly, tissue sections were blocked in 3% normal goat serum and incubated sequentially with hybridoma supernatant as primary antibody for 1–2 h, followed by biotinylated goat anti-mouse IgG secondary antibody diluted to 10 μg per ml in 3% normal goat serum for 1 h. After thorough washing in PBS, the sections were incubated with ABC reagent (avidin-biotinylated horseradish peroxidase complex) for 1 h, and finally with the AEC (3-amino-9-ethyl carbazole) substrate. Fetal tissue was kindly provided by Dr. William Carter (Fred Hutchinson Cancer Research Center, Seattle, WA). Positive control MoAb AE3 to cytokeratin was kindly provided by Dr. Henry Sun (New York University Medical School, New York, NY). Isotype-matched control MoAb was as described for flow cytometry experiments.

Western Blotting Cell lysates were prepared with 0.5% Nonidet P-40, as described later, and were separated on an 8% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel. Lysate from approximately 5×10^6 cells was loaded per lane. The gel was blotted to nitrocellulose and blocked in "blotto" (5% nonfat milk in PBS) for 4 h at room temperature. The blots

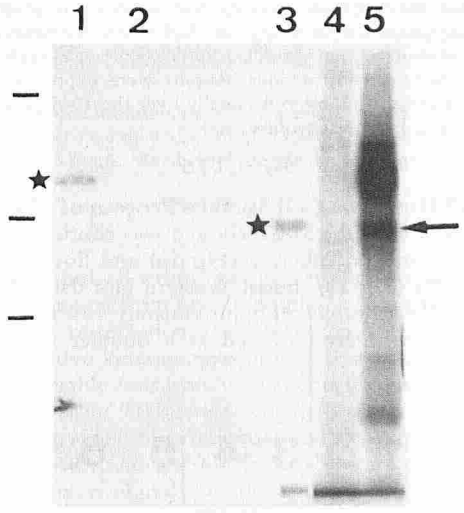

Figure 2. MoAb 10G7 recognizes a cell surface protein of about 130 kDa. *Lanes 1,2,* Western blot analysis with MoAb 10G7: FEP18–11-T1 lysate *(lane 1)* and negative control neutrophil lysate *(lane 2)* under reducing conditions. The positions of molecular weight markers of 200, 116.5, and 89.5 kDa are indicated *(bars)*. *Lanes 3–5,* immunoprecipitation from surface biotinylated FEP18–11-T1 cells under reducing conditions. Immunoprecipitations were performed sequentially with MoAb 10G7 *(lane 3)*, SP2 control hybridoma supernatant *(lane 4)*, and MoAb P4C10 to β_1 integrin *(lane 5)*. This integrin subunit migrates at 120 kDa (→), and several associated α subunits are co-immunoprecipitated. 10G7 protein is indicated (★) and is estimated to migrate at approximately 130 kDa. These data are typical of several separate immunoprecipitation experiments (n = 3).

were then incubated in hybridoma supernatant overnight at 4°C, washed with PBS-Tween (0.1%), and incubated in horseradish peroxidase-conjugated goat anti-mouse IgG at 1:1000 in blotto for 1 h. Blots were washed as before and finally incubated with enhanced chemiluminescence reagents (Amersham, Littlechalfont, UK) before being exposed to film.

Immunoprecipitation of Cell Surface Antigen Cells were harvested by trypsinization and labeled at the surface by biotinylation according to published procedures (Cole *et al*, 1987). Briefly, the cells were washed extensively with cold PBS and resuspended in PBS at 10^8 per ml. N-Hydroxysulfosuccinimide-biotin (Pierce, Rockford, IL) was then added at 2.2 mg per ml and incubated at 4°C for 30 min, with occasional mixing. Cells were washed and resuspended in PBS at 10^8 per ml and subsequently lysed by adding an equal volume of 1% Nonidet P-40 in TSE (50 mM Tris-HCl, 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, pH 8.0), containing protease inhibitors, at 4°C for 30 min. The cell lysates were centrifuged in an Eppendorf centrifuge for 15 min to pellet the nuclei, and the supernatants were used for immunoprecipitation. In other experiments, unlabeled cells were lysed as described above for immunoprecipitation.

Cell lysates were precleared by incubation with Sepharose-4B beads for 4–6 h, and subsequently were incubated with primary antibody overnight. Rabbit anti-mouse IgG-coated Sepharose 4B beads were then added for 1–2 h, and the antigen-antibody-bead complex was spun down. The beads were washed alternately with low-salt buffer (25 mM Tris, 0.15 M NaCl, 1% Triton X100, 0.5% deoxycholate, 0.05% SDS) and high-salt buffer (25 mM Tris, 0.5 M NaCl, 1% Triton X100, 0.5% DOC). Laemmli's reducing sample buffer was added, and the samples were boiled and then subjected to SDS-PAGE on an 8% gel. The gel was blotted onto nitrocellulose, and the immunoprecipitates were visualized by incubating the blot sequentially with Streptavidin-horseradish peroxidase and enhanced chemiluminescence reagents and then exposing the blot to film. Antibody to the β_1 integrin (P4C10) was kindly provided by Dr. William Carter (Fred Hutchinson Cancer Research Center). Immunoprecipitation experiments were repeated several times.

Cell Proliferation Assays Keratinocytes were plated at 2.5×10^3 cells per well in 96-well trays in keratinocyte growth medium and were allowed to attach overnight in the incubator. The medium was aspirated and replaced with keratinocyte basal medium (Clonetics Corporation) lacking all growth factor supplements (i.e., epidermal growth factor, hydrocortisone, insulin, and whole bovine pituitary extract, present in keratinocyte growth medium), to which the appropriate purified MoAb (10G7 or 1D4.5) had been added to the desired concentration. Three replicate wells were tested for each experimental condition. Cells were maintained in culture as usual for a period of 10 d, during which they were refed with keratinocyte basal medium containing fresh antibody twice a week. The assays were then processed for quantification using an 3-[4,5 dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide proliferation kit (Promega, Madison, WI), as per the manufacturer's instructions. Results were expressed as mean ± SEM. Assays were also monitored visually over the 10-d period to distinguish cell survival from cell proliferation.

RESULTS

MoAb 10G7 Detects a Cell Surface Protein of 130 kDa Cell surface expression of the 10G7 antigen was confirmed by both *in situ* immunofluorescent staining **(Fig 1*a*)** and flow cytometry on trypsinized cells **(Fig 1*b*)**. Initial Western blot data identified the 10G7 antigen as a protein of approximately 130 kDa in FEP18–11-T1 cells, sometimes observed as a doublet under reducing conditions **(Fig 2, *lane 1*)**. Subsequent immunoprecipitation studies using cells labeled at the cell surface with biotin were able to confirm that 10G7 was indeed a 130-kDa cell surface protein in FEP18–11-T1 cells **(Fig 2, *lane 3*)**. The size of this protein was estimated by comparison with immunoprecipitation of the β_1 integrin subunit, which migrates at 120 kDa under reducing conditions **(Fig 2, *lane 5*)**.

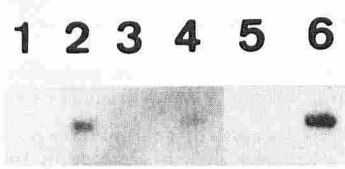

Figure 3. Downregulation of 10G7 protein in differentiating foreskin keratinocytes. Immunoprecipitation blot of 10G7 antigen from proliferating *versus* differentiating foreskin keratinocytes and FEP18–11-T1 cells. Equivalent amounts of protein (300 μg) from cell lysates of proliferating foreskin keratinocytes *(lanes 1,2)*, differentiating foreskin keratinocytes *(lanes 3,4)*, and FEP18–11-T1 cells *(lanes 5,6)* were sequentially subjected to immunoprecipitation with control MoAb 1D4.5 *(lanes 1,3,5)* and MoAb 10G7 *(lanes 2,4,6)*. The immunoprecipitates were separated on an SDS-PAGE gel and blotted. The filter was then probed with MoAb 10G7.

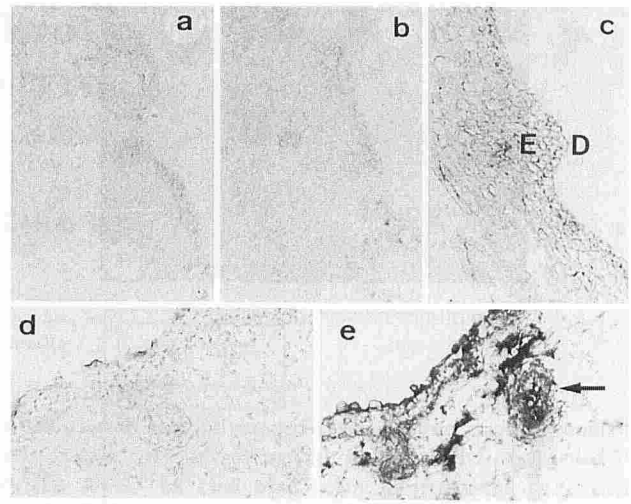

Figure 4. The 10G7 protein is not expressed in human neonatal foreskin but is expressed in developing fetal epithelial tissue. Immunoperoxidase staining of human foreskin tissue with *(a)* IgG negative control MoAb, *(b)* MoAb 10G7, and *(c)* positive control MoAb AE3 to keratins. The position of the epidermis (E) and the dermis (D) is indicated for orientation. Pigmentation is evident in all these sections in the basal layer. Immunoperoxidase staining of 92-d human fetal scalp epithelium was done with *(d)* IgG negative control MoAb and *(e)* MoAb 10G7. These two sections *(d,e)* were counterstained with methyl green. Note reactivity of hair follicles with MoAb 10G7 (→).

10G7 Antigen Expression Is Correlated With Hyper-Proliferative Keratinocytes *In Vitro* During routine flow cytometry experiments with different cultures of foreskin keratinocytes, we observed variable expression of 10G7 antigen as judged by the mean peak of fluorescence intensity. Upon closer examination, it became evident that the level of 10G7 antigen expression was correlated with the density of cells at harvest, before staining. Because foreskin keratinocytes are proliferative when subconfluent and differentiate rapidly at confluence, we compared the expression of 10G7 antigen in subconfluent mitotic foreskin keratinocytes *versus* confluent, postmitotic, differentiated cultures. In a typical experiment, the mean peak of fluorescence intensity obtained with proliferating foreskin keratinocytes was 6.07, whereas that of differentiated foreskin keratinocytes was 2.63. These observations were confirmed by experiments in which equivalent amounts of unlabeled protein (300 μg) from proliferative and differentiated foreskin keratinocyte cell lysates were used to immunoprecipitate 10G7 antigen. The immunoprecipitates were subjected to SDS-PAGE separation, and 10G7 antigen was detected by Western blot analysis. The results shown in **Fig 3** demonstrate that the 10G7 antigen is downregulated in differentiating keratinocytes **(Fig 3, *lane 4*,** differentiated keratinocytes *versus lane 2*, proliferating keratinocytes). This experiment also confirmed the initial results of enzyme-linked immunosorbent assay screening, which indicated that 10G7 antigen is overexpressed in FEP18–11-T1 cells as compared with foreskin keratinocytes **(Fig 3, *lane 6*)**.

10G7 Antigen Expression Is Also Correlated With Hyper-Proliferative Keratinocytes *In Vivo*, During Development, and in Carcinomas Having demonstrated that 10G7 antigen was expressed under hyper-proliferative conditions *in vitro*, we investigated the possibility that this might also be reflected *in vivo*.

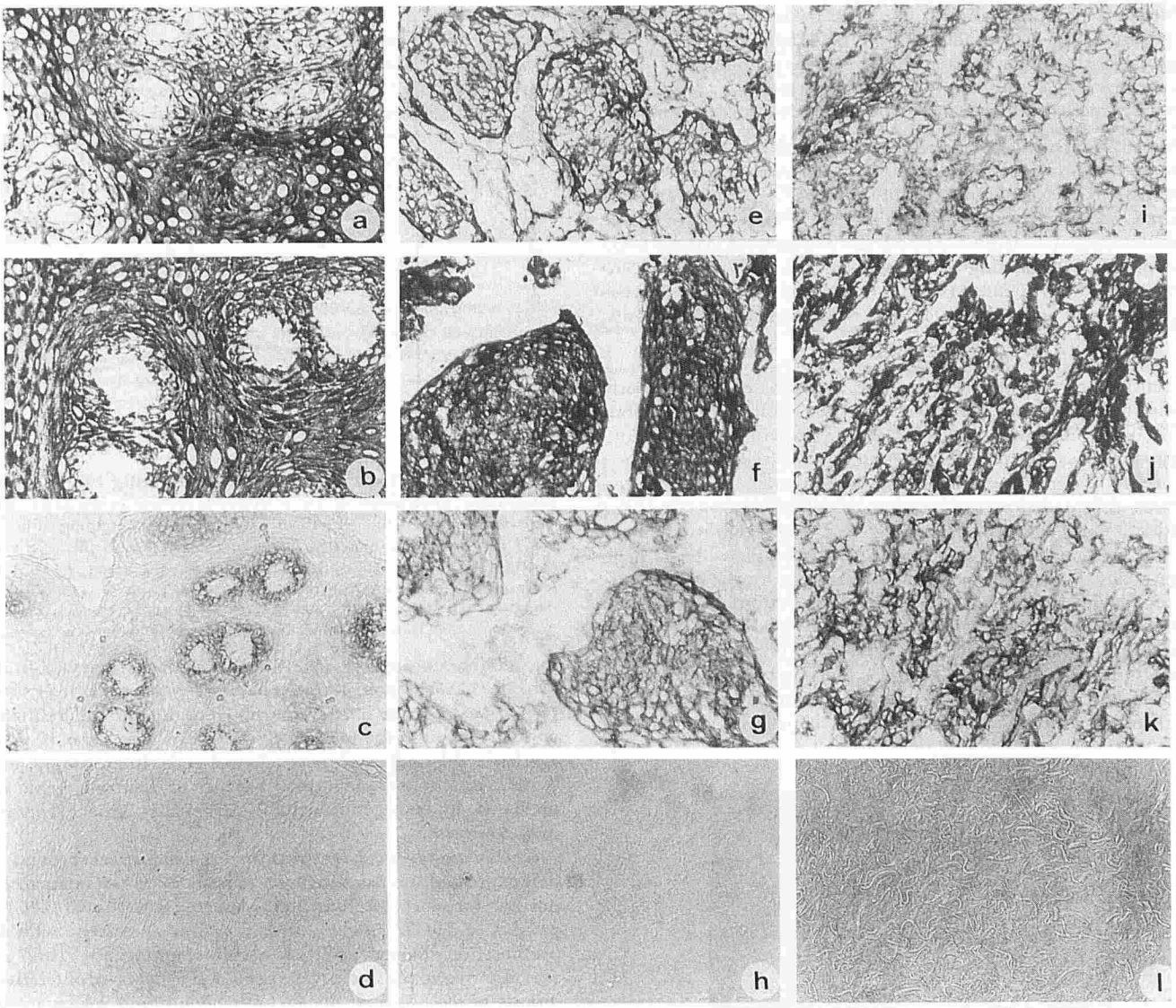

Figure 5. The 10G7 protein is expressed in both benign and malignant genital carcinomas. Immunoperoxidase staining of genital epithelial lesions for 10G7 antigen. *(a-d)* Human papillomavirus (HPV)-6– positive vulval wart; *(e-h)* HPV-16–positive cervical SCC Ib; *(i-l)* HPV-18– positive vulval invasive SCC II. Cryostat sections were stained with hematoxylin and eosin *(a,e,i)*, anti-keratin MoAb AE3 *(b,f,j)*, MoAb 10G7 *(c,g,k)*, and isotype-matched negative control MoAb 1D4.5 *(d,h,l)*.

Our initial characterization of the 10G7 antigen showed that it was sensitive to fixation. Flow cytometry data indicated that the already low levels of expression of the 10G7 antigen **(Fig 1*b*)** could be lost even with brief fixation in 2% formalin. Thus, we were restricted to analyzing frozen tissue. Several samples of normal human foreskin (from which all normal foreskin keratinocytes and FEP18–11-T1s were derived in our studies) were analyzed for 10G7 antigen expression by immunoperoxidase staining. As shown in **Fig 4*b***, no reactivity was obtained with MoAb 10G7, although positive staining with the anti-keratin control was observed **(Fig 4*c*)**. We have consistently been able to demonstrate that freshly isolated keratinocytes exhibit expression of 10G7 antigen (Li A, Simmons PJ, Haylock D, Kaur P. Enrichment for human keratinocyte stem cells. Manuscript submitted), which suggests that the epitope identified by MoAb 10G7 is somehow masked in normal foreskin epithelium.

Interestingly, the expression of 10G7 antigen could be detected readily in hyper-proliferative epithelia *in vivo*, confirming our *in vitro* findings. Fetal tissue was stained by immunoperoxidase methods, and epithelium from several sites exhibited abundant staining with MoAb 10G7. Staining in the scalp epidermis is shown in **Fig 4*e***. Notably, hair follicles within this epidermis, which also contain keratinocytes, showed strong staining. Analysis of several gynecologic tumors ranging from benign dysplasias to invasive carcinomas confirmed the observation that 10G7 antigen is expressed by hyper-proliferative keratinocytes *in vivo*. Specifically, frozen tissue sections of genital lesions from ten patients used in a previous study (Mackenzie *et al*, 1994) were selected for early or late stages of neoplastic progression to carcinomas and used for immunoperoxidase staining. All tumors tested (n = 15) were positive for 10G7 antigen. Notably, benign dysplastic lesions (warts) showed staining restricted to the basal layer **(Fig 5*c*)**, whereas malignant lesions such as SCC I and SCC II exhibited staining throughout the epithelium **(Fig 5*g,k*)**. In addition, three samples of macroscopically and histologically normal ectocervical and endocervical epithelium obtained from patients having cone biopsy or hysterectomy for noncervical tumors were analyzed for 10G7 expression. All three samples stained in parallel with one of the positive lesions (shown in **Fig 5*g***) showed no detectable 10G7 antigen (data not shown). Interestingly, in tissue sections obtained from a patient with SCC III, we were able to obtain normal endocervical epithelium adjacent to the neoplastic epithelium **(Fig 6*a*)**. Staining for 10G7 antigen on these sections showed clearly that the normal epithelium

was negative **(Fig 6*d*)**, whereas the adjacent neoplastic epithelium was positive **(Fig 6*c*)**. In addition, we have been able to demonstrate that both benign and malignant breast carcinomas (n = 10) also express 10G7 antigen, whereas their normal counterparts (mammary reduction tissue, n = 3) do not (data not shown).

MoAb 10G7 Stimulates Keratinocyte Proliferation To determine a functional role for the cell surface component recognized by MoAb 10G7, we tested its ability to affect keratinocyte proliferation. Under conditions in which FEP18–11-T1 cells were unable to proliferate (basal medium without growth factors), MoAb 10G7 was capable of stimulating the growth of these cells in a concentration-dependent manner **(Fig 7)**, compared with an isotype-matched control antibody (1D4.5), in three replicate experiments.

DISCUSSION

The cell surface of human keratinocytes remains relatively poorly characterized, and little is known about the molecular alterations that occur at the cell surface level during growth, differentiation, and carcinogenesis. Molecules expressed at the cell surface have a critical role in regulating numerous processes such as growth, cell adhesion, differentiation, and invasion. In addition, the cell surface provides a more accessible site for therapeutic approaches via bioactive peptides or MoAbs. Important advances have been made in identifying some of the cell adhesion molecules used by keratinocytes in attaching to extracellular matrix components of the basement membrane and in intercellular adhesion, such as integrins (Carter *et al*, 1990a, 1990b) and cadherins (Frixen *et al*, 1991). Several GFRs with tyrosine kinase activity expressed by human keratinocytes have also been identified, including epidermal growth factor, insulin-like growth factor, hepatocyte growth factor, and keratinocyte growth factor receptors (reviewed in Aaronson, 1991; Sachs *et al*, 1996). These receptors have been shown to play a major role in the morphogenesis and differentiation of epithelia through the binding of their respective ligands, generally synthesized by adjacent mesenchymal cells. Interestingly, many of these receptors were identified because of their oncogenic activity mediated by mitogenic signals, particularly when amplified or overexpressed.

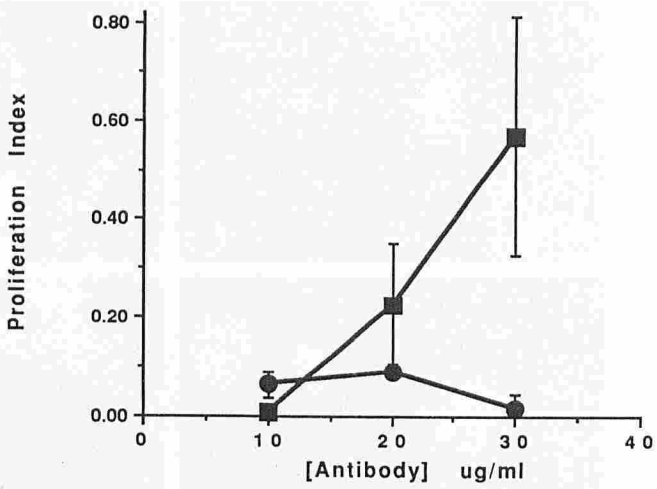

Figure 7. MoAb 10G7 is capable of stimulating FEP18–11-T1 keratinocyte proliferation in a concentration-dependent manner. Cells were assayed for growth in basal medium containing either MoAb 10G7 (■) or isotype-matched negative control MoAb (●) at the various concentrations after 10 d. Data points represent mean ± SEM from a representative experiment.

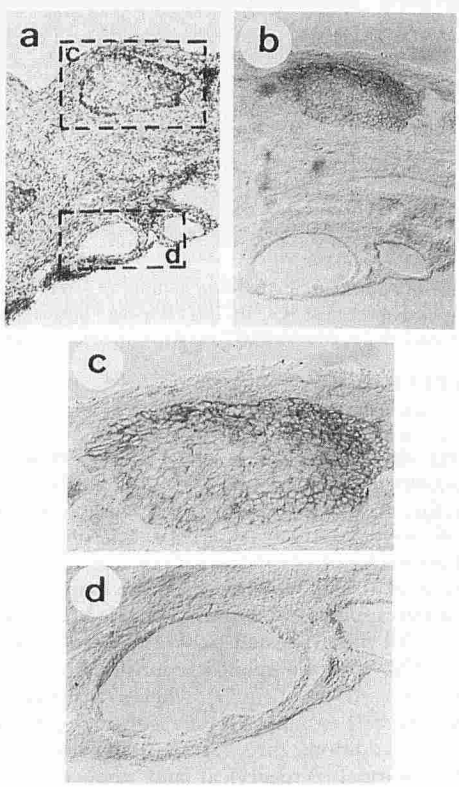

Figure 6. The 10G7 protein is preferentially expressed in neoplastic but not in normal cervical epithelium. Immunoperoxidase staining with MoAb 10G7 of a section containing normal and neoplastic cervical epithelium from a patient with SCC III. Cryostat sections were stained with hematoxylin and eosin *(a)* and MoAb 10G7 *(b-d)*. *(c)* Magnification of the positively stained neoplastic epithelium indicated by the box marked "c" in the hematoxylin and eosin–stained section; *(d)* negatively stained normal epithelium indicated by box "d."

In this study, we have identified a cell surface protein that is overexpressed on tumorigenic keratinocytes as compared with normal foreskin keratinocytes, detected by MoAb 10G7. The pattern of 10G7 antigen expression was consistent with hyperproliferation of keratinocytes in culture. Significantly, 10G7 antigen was also overexpressed by epithelia under hyper-proliferative conditions *in vivo*, specifically in both benign and malignant genital carcinomas and in developing fetal tissue, whereas no expression was detected in normal genital epithelium *in vivo*. The ability of MoAb 10G7 to stimulate the proliferation of FEP18–11-T1 keratinocytes in the absence of growth factors provides strong evidence that the 10G7 protein has an important role in regulating cell growth. It is possible that MoAb 10G7 could elicit this response by acting as a surrogate ligand, capable of transducing a signal resulting in cellular proliferation, and this theory merits further investigation. These data, taken together with the overexpression of 10G7 antigen observed in carcinomas, as reported for known GFRs such as the epidermal growth factor receptor and her-2/*neu* (Xu *et al*, 1984; Slamon *et al*, 1987), allow us to speculate that the 10G7 antigen may also be a GFR or form part of a GFR complex. We have compared the characteristics of the 10G7 antigen in terms of its molecular size, its tissue distribution, and its functional role in stimulating the proliferation of keratinocytes with known GFRs or indeed any other cell surface components already described in the literature. We have thus far been unable to ascertain its identity in this manner and conclude that either the 10G7 protein is a novel cell surface component or it is a known molecule to which such characteristics have not been attributed previously. Hence, our current goals are to clone the gene encoding this antigen and to determine how widespread its expression is in carcinomas from different tissues, as well as in development and wound healing. The precise role of the 10G7 antigen in carcinogenesis needs to be determined and will form the basis of future studies. Interestingly, we have been able to use MoAb 10G7 to enrich for putative human epidermal stem cells on the basis of low levels of 10G7 antigen

expression in conjunction with high levels of α_6 integrin expression. Basal epidermal cells with high levels of 10G7 antigen expression represented the transit amplifying population of epidermal cells (Li A, Simmons PJ, Haylock D, Kaur P. Enrichment for human keratinocyte stem cells. Manuscript submitted). Given the ability of MoAb 10G7 to detect early benign changes in genital epithelial cells as well as malignant cells, and the absence of binding to normal epithelia, this MoAb may prove to be a useful diagnostic tool.

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