A Novel Marker for Basal (Stem) Cells of Mammalian Stratified Squamous Epithelia and Squamous Cell Carcinomas

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ABSTRACT

We have developed a monoclonal antibody (174H.64) which selectively recognizes antigens shared by the basal cell layers of human, bovine, canine, feline, and murine squamous cell carcinomas (SCC). Histopathological and immunological studies of the frozen tissue sections demonstrated selective binding of this antibody to SCCs of a variety of organs. The antibody reacts with SCCs of human, bovine, canine, feline, and murine origin. Tumors of other histological types did not show reactivity with the antibody. In well-differentiated SCCs the peripheral layer of the tumor showed preferential binding of the antibody, suggesting that the antigens are associated with the proliferative compartment of the tumor. Studies on normal human tissues showed selective binding of the antibody to the basal layers of stratified epithelia, simple epithelia, or tissues of nonepithelial origin. A similar pattern of antibody binding was also observed for bovine and murine skin with staining of the basal layer. The antigens detected by monoclonal antibody 174H.64 were characterized from cytoskeletal protein extracts of normal human keratinocytes as well as human and bovine SCC tissues by using an immunoblotting technique. The antigens detected in normal human keratinocytes consisted of two major protein bands of approximate molecular weights of 48,000-50,000 and 57,000. In bovine SCC tumor the antigen detected was the M, 48,000-50,000 band and in the human SCC tumor it was the M, 57,000 band. A murine lung SCC model was developed with a murine SCC cell line KLN-205. The lung tumor obtained was reactive against the antibody and showed selective staining of the peripheral layer of the tumor containing the stem cell population. The antigens described by monoclonal antibody 174H.64 appear to be molecules associated with the stem cell populations of normal stratified epithelium and squamous cell carcinoma.

INTRODUCTION

Squamous cell carcinoma is a common histological type of cancer of the head and neck, lung, oral cavity, and cervix. Identification and characterization of molecular markers associated with SCC is an important approach to earlier diagnosis and treatment of SCCs. Functional studies on SCC markers would aid in better understanding of the biology of this type of cancer. Monoclonal antibodies are useful tools as molecular and functional probes for studying tumor-associated antigens. The majority of the Mabs raised against SCCs were produced by using SCC cell lines as the immunogen (1-8). Antigenic molecules defined by such Mabs include cell surface proteins (1-6), and cytoskeletal components (7). Most of these antibodies either show cross-reactivity with other types of cancers or their reactivity is limited to SCCs of certain organs only. A recently reported IgM antibody (Mab 17.13) is one of the few SCC-specific antibodies which react with SCCs of a variety of organs (8). The antigen recognized by Mab 17.13 has as yet not been reported. Another approach has been to develop Mabs against keratinocytes and evaluate their reactivity with SCCs (9). A Mab produced against human keratinocytes, VM-2, has been reported to bind selectively to the basal layer of the epidermis, as well as squamous and basal cell carcinomas (9, 10).

Monoclonal antibodies developed against cytokeratins have been used as probes for studying the keratin expression in normal and neoplastic stratified epithelium (11-16). Cytokeratins exhibit a differentiation-specific pattern of distribution in different layers of stratified epithelium (16). Specific keratin polypeptides have also been reported as molecular markers for SCC (17). Antiketatin Mabs have been proposed as tools for diagnosis of tumors, including SCCs (13, 18). We report here the characterization of a novel IgG1 Mab (174H.64) with high degree of selectivity toward SCCs of various origins. We describe evidence suggesting that this antibody recognizes a unique epitope on cytoskeletal proteins which may serve as a marker for the stem cell population in normal stratified epithelia and squamous cell carcinomas.

MATERIALS AND METHODS

Immunization and Hybridoma Production. RBF/Dn mice were immunized by two consecutive i.p. injections, 1 week apart, of a mixture of human choriogonadotrophin (50 µg) and a synthetic carbohydrate antigen Tn-HSA (50 µg) (19) in complete Freund’s adjuvant. On the 12th day the animals were given another i.p. injection of a mixture of HCG (400 µg) and Tn-HSA (400 µg) in PBS. On the 13th day the mice were further immunized by an i.v. and an i.p. injection, each consisting of a mixture of HCG and Tn-HSA (200 µg each). The latter procedure was repeated on the 14th day. On the 15th day, the spleen cells from mice having high antiserum titers against HCG and Tn-HSA were fused with FOX-NY cells (HyClone Laboratories, Logan, UT). Hybrids with positive reactivity against Tn-HSA and negative reactivity with HSA were selected and recloned. The supernatants from the reclones were tested for reactivity against tumor tissues by immunohistological staining. A hybridoma designated 174H.64 was chosen for further studies, based on its selective reactivity with SCCs.
were embedded in OCT compound (Tissue-Tek; Miles Scientific, Naperville, IL) and coated with 50 µl of 0.2 M sodium bicarbonate solution containing 0.15 M potassium chloride, pH 8.1. To this a solution of modified poly-L-lysine (BDH) was added and incubated at room temperature for 20 min. The reaction was stopped by addition of 1 M ammonium chloride solution, pH 6.0 (50 µl), and the solution was extensively dialyzed against PBS.

Culture Lines. All the SCC cell lines were obtained from American Type Culture Collection, Rockville, MD, and were grown in RPMI 1640 medium (Gibco, Canada, Inc., Burlington, Ontario, Canada) containing 10% fetal calf serum and gentamicin (50 units/ml). Normal human epidermal keratinocytes were obtained from Clonetics Corp., San Diego, CA, and were cultured in serum-free keratinocyte growth medium (Clonetics Corp.).

Isolation of Mouse Epidermal Keratinocytes. Epidermal keratinocytes were isolated from neonatal (1-day-old) ICR mice according to a reported procedure (23). Briefly, mice were decapitated, skin was removed and washed in Hanks' balanced salt solution. The skin was then placed dermal side down in Hanks' balanced salt solution containing trypsin (0.025%-EDTA (0.02%) and incubated at 37°C for 1 h. The trypsin-EDTA solution was aspirated and replaced with RPMI 1640 medium containing 10% fetal calf serum and gentamicin (50 units/ml). Normal human epidermal keratinocytes were obtained from Clonetics Corp., San Diego, CA, and were cultured in serum-free keratinocyte growth medium (Clonetics Corp.).
The reactivity of the antibody to the peripheral layers of the tumor. The normal tissues adjacent to the tumor showed no binding with the antibody. Several spontaneously arising tumors from other mammals were also studied for their reactivity against Mab 174H.64 (Table 1). Canine squamous and basal cell carcinoma tissues showed selective binding with the antibody, while other carcinomas and tumors of mesenchymal origin did not show any reactivity. The reactivity of this antibody was also observed for bovine and feline SCCs. Fig. 1B shows the staining of a well-differentiated metastatic bovine lung SCC with increased antibody binding to the peripheral tumor cells. Among the benign epithelial tumors of dogs, basal cell tumors showed homogeneous staining, whereas in papillomas and perianal adenomas the staining was limited to basal cells only (Fig. 1C). A normal human keratinocyte suspension enriched in basal cells was prepared by trypsin dissociation of the neonatal mouse skin. A cell smear of this suspension on immunoperoxidase staining showed 30–50% of the cells to be positive for their reactivity with the antibody (Fig. 1H). A normal human keratinocyte culture having basal cell characteristics demonstrated all cells to be positive for their reactivity with the antibody (Fig. 1H). The reactivity of this antibody with thymic epithelium could also be demonstrated in mice, cows, and dogs.

Reactivity of Mab 174H.64 with Cell Lines. Several human SCC cell lines (SCL-1, SIHA, ME 180, C-33A) were tested for their reactivity with the antibody by cell smear immunoperoxidase staining and were found to be negative. In contrast, a murine metastatic lung SCC cell line (KLN-205) was reactive with the antibody.

Characterization of Antigens Detected by Mab 174H.64. The antigens detected by Mab 174H.64 were characterized from bovine and human SCC tissues as well as normal human keratinocytes by using an immunoblotting technique. A human adenocarcinoma tumor was used as a negative control sample. The immunoblots of the SDS extracts of bovine SCC, human SCC, and human keratinocytes showed selective binding of 125I-labeled Mab 174H.64 with specific protein bands (Lanes 5–7), while the immunoblots of the corresponding NP-40 extracts (Lanes 1–3) did not show any binding with the antibody (Fig. 2). The NP-40 and SDS extracts of adenocarcinoma (Lanes 4 and 8) showed no binding with 125I-labeled Mab 174H.64. The immunoreactive protein bands in the keratinocyte SDS extract were of approximate molecular weight of 48,000–50,000 (possibly a doublet) and Mr, 57,000. The bovine and human SCC SDS extracts showed only one prominent band each with the approximate molecular weight of 48,000–50,000 for the former and Mr, 57,000 for the latter. A negative control antibody (155H.7) failed to bind with any of the above tumor extracts.

DISCUSSION

During our effort to develop Mabs against HCG and Tn antigen, we have serendipitously isolated a hybridoma specific for SCC. The origin of the hybridoma 174H.64 is not clearly understood. Since this antibody fails to react with HCG and Tn-HSA, it is unlikely to be related to the antigens used for immunization. The reactivity of this murine Mab against the basal cell population of the mouse skin suggests that its origin may be related to an autoreactive B-cell clone present in the mouse used for the immunization. Such autoantibodies against cytokerin or other cytoskeletal components have been observed in laboratory rats and mice (29). The characterization of the antigens detected by this antibody as cytoskeletal components is consistent with this hypothesis.

The results presented in Table 1 show the highly specific reaction of the Mab toward one histological type of tumor, and its reactivity with other classes of tumors is described in Table 1. The antibody to the peripheral layers of the tumor. The normal tissues adjacent to the tumor showed no binding with the antibody. Several spontaneously arising tumors from other mammals were also studied for their reactivity against Mab 174H.64 (Table 1). Canine squamous and basal cell carcinoma tissues showed selective binding with the antibody, while other carcinomas and tumors of mesenchymal origin did not show any reactivity. The reactivity of this antibody was also observed for bovine and feline SCCs. Fig. 1B shows the staining of a well-differentiated metastatic bovine lung SCC with increased antibody binding to the peripheral tumor cells. Among the benign epithelial tumors of dogs, basal cell tumors showed homogeneous staining, whereas in papillomas and perianal adenomas the staining was limited to basal cells only (Fig. 1C). A normal human keratinocyte suspension enriched in basal cells was prepared by trypsin dissociation of the neonatal mouse skin. A cell smear of this suspension on immunoperoxidase staining showed 30–50% of the cells to be positive for their reactivity with the antibody (Fig. 1H). A normal human keratinocyte culture having basal cell characteristics demonstrated all cells to be positive for their reactivity with the antibody (Fig. 1H). The reactivity of this antibody with thymic epithelium could also be demonstrated in mice, cows, and dogs.

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Fig. 1. Immunoperoxidase staining of various normal and tumor tissues of mammalian origin by using Mab 174H.64: (A) human anorectal SCC; (B) bovine SCC; (C) canine papilloma; (D) canine perianal adenoma; (E) stratified squamous epithelium of normal human cervix; (F) normal human thymus; (G) normal human mammary duct; (H) normal mouse keratinocytes; (I) normal human keratinocytes; and (J) murine lung SCC (KLN-205 cells).
Mab REACTIVE AGAINST SCC

The only normal tissues showing reactivity with this antibody are the basal layer of the stratified squamous epithelium, thymic epithelial cells (36), and myoepithelial cells of breast ducts (37). Cytokeratin 14 and 5 have also been reported to be markers for neoplasms originating from stratified epithelium and are not detected in tumors of simple epithelium (17). However, the expression of cytokeratins 14 and 5 in epidermis extends from the basal layer to the well-differentiated squamous cells (34, 35) and therefore does not correlate with the immunoperoxidase staining pattern observed with Mab 174H.64. Similarly, these cytokeratins are believed to be present in all SCC cells irrespective of the state of differentiation. However, Mab 174H.64 fails to stain well-differentiated cells of SCCs. This may be due to the conformational masking of the unique epitope detected by this Mab in the suprabasal layers as reported for other Mabs (38, 39). Alternatively, Mab 174H.64 may be detecting novel cytoskeletal proteins expressed only in the stem cell populations of normal and neoplastic stratified squamous epithelium.

The high specificity of Mab 174H.64 toward SCCs of various origins suggests that it could be used as a reagent for unambiguous diagnosis of squamous tumors by immunohistochemical staining. This could be of special value in cases where histological typing of tumors by conventional staining methods are not satisfactory, as in some cases of adenosquamous carcinomas where it may be difficult to determine whether undifferentiated areas are of squamous or glandular origin. We are currently evaluating the potential of radiolabeled Mab 174H.64 as a reagent for noninvasive diagnosis of SCCs by using the murine lung SCC model described in this paper. Our preliminary results on the biodistribution of this antibody showed greater uptake of 125I-labeled Mab 174H.64 in the tumor-bearing lung as compared to the normal lung, whereas the uptake of a control radiolabeled antibody (125I-labeled MOPC-21) in the tumor-bearing lung was not significantly different from that in the normal lung. These preliminary results suggest that the epitope detected by Mab 174H.64 is expressed also on the cell surface of the KLN-205 cells. Such cell surface domains have been reported for some cytoskeletal proteins (40, 41). Mab 174H.64 is also being evaluated for its potential for antigen-specific targeting of cytotoxic drugs into SCC cells. A daunomycin conjugate of this antibody showed selective killing of KLN-205 cells in vitro (42). Further evaluation of this antibody for radioimmunoimaging and drug targeting are in progress.

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5J. Samuel, T. R. Sykes, A. A. Noujaim, D. M. Haines, and B. M. Longe-
necker, unpublished results.
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