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Specific Keratins as Molecular Markers for Neoplasms with a Stratified Epithelial Origin[1]

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ABSTRACT

The expression of specific keratin polypeptides in human neoplasms was investigated by the immunoblot technique using monoclonal anti-keratin antibodies. M_r 50,000 and 58,000 keratins, recognized by AE1 and AE3 antibodies, respectively, were detected only in carcinomas of stratified epithelial origin, but not in carcinomas derived from simple epithelia. No keratin was detected in nonepithelial tumors including melanoma, lymphoma, neurofibroma, and sarcoma. The results suggest that the M_r 50,000 and 58,000 keratins provide useful molecular markers for identifying neoplasms of stratified squamous epithelial origin.

INTRODUCTION

Intermediate-sized or 10-nm filaments are present in almost all mammalian cells. So far, 5 major types of intermediate filaments have been identified, each with a restricted tissue distribution: keratin filaments are found mainly in epithelial cells; desmin filaments in muscle cells; neurofilaments in neurons; glial filaments in astrocytes; and vimentin filaments mainly in mesenchymal cells (11). This tissue distribution pattern appears to be faithfully preserved in neoplasms (1–3, 15, 17, 19). Thus, immunohistochemical staining of tissue sections with antibodies to different types of intermediate filaments can provide useful information for identifying and typing various tumors (for a recent review, see Ref. 15).

Recent data from several groups have demonstrated that all known human epithelial keratin polypeptides can be divided into 2 subfamilies [Subfamily A (acidic keratins that are recognized by AE1 monoclonal antibody and are related to "type I" wool keratins) and Subfamily B (basic keratins that are recognized by AE3 monoclonal antibody and are related to "type II" wool keratins)] according to their charge properties, immunoreactivities, and complementary DNA sequence (4, 6, 9, 10, 21, 27; for a recent review, see Ref. 23). Within these 2 subfamilies, the expression of specific keratin molecules appears to correlate with different types of epithelial differentiation. In particular, M_r 50,000 and 58,000 keratins (of Subfamilies A and B, respectively) are found to be present in various quantities in all stratified epithelia, both *in vivo* (12, 27) and in culture (12, 14), but not in any simple epithelia. To determine whether these 2 keratins are also retained in neoplasms, we have analyzed keratins of 40 well-characterized human neoplasms by the immunoblot technique (26, 29), using AE1 and AE3 monoclonal antibodies. We found that a great majority of neoplasms derived from stratified squamous epithelia expressed these 2 keratins, whereas neoplasms from simple epithelia did not. These results suggest that the M_r 50,000 and 58,000 keratins may be regarded as molecular markers for stratified squamous epithelial cells not only in normal tissues (12, 27) and cultured cells (14), but also in neoplasms.

MATERIALS AND METHODS

Tumor Selection. Forty cases of human neoplasms including 20 cases of stratified origin (13 squamous cell carcinomas, 2 basal cell carcinomas, one verrucous carcinoma, one benign thymoma, and 3 bladder transitional carcinomas), 15 cases of simple epithelial origin (12 cases of adenocarcinomas, one infiltrating ductal carcinoma from breast, one medullary carcinoma of the breast, and one adenosquamous carcinoma of endometrium), and 5 cases of nonepithelial tumors (one giant cell tumor of the bone, one neurofibroma, one malignant melanoma metastatic to lymph node, one meningioma, and one lymphoma) which were diagnosed by routine histological methods were selected for the present study. Cases with controversial diagnoses were not included.

Keratin Analysis. After the surrounding tissues were carefully removed under a dissecting microscope, the tumors were snap frozen in liquid nitrogen and stored at −70° until use. The tumor mass was minced and subsequently homogenized at 4° in approximately 100 volumes of a buffer containing 25 mM Tris HCl (pH 7.4), 1% Triton X-100, 1 mM EDTA, 1 mM ethyleneglycol-bis-(β-aminoethylether) *N,N'*-tetraacetic acid, antipain (Sigma; 5 μg/ml), Pepstatin A (Sigma; 5 μg/ml), and 1 mM phenylmethylsulfonyl fluoride (27, 29). After the homogenate was centrifugated at 10,000 × *g* for 10 min at 4°, the "water-insoluble cytoskeletal" proteins were dissolved by heating at 95° for 10 min in 1% sodium dodecyl sulfate containing 25 mM Tris HCl (pH 7.4) and 10 mM dithiothreitol. For immunoblot analysis, 30 to 50 μg of such cytoskeletal proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5% acrylamide), transferred electrophoretically to nitrocellulose paper (Millipore type HA with a pore size of 0.45 μm) using an E-C blotting apparatus (2.5 hr at 4° with a power supply setting of 65%) (26, 29), and then stained with mouse monoclonal antibodies AE1 and AE3 by the peroxidase-antiperoxidase technique (29).

Monoclonal Anti-Keratin Antibodies. The preparation and characterization of mouse monoclonal anti-keratin antibodies have been described previously (29). The AE1 and AE3 antibodies recognize acidic and basic keratins, respectively, and are therefore subfamily specific (6, 23, 27).

RESULTS

Water-insoluble cytoskeletal proteins were prepared from various tumor specimens and analyzed with AE1 and AE3 monoclonal anti-keratin antibodies by immunoblotting (29). Using this technique, we have shown previously that AE1 and AE3 react with 2 mutually exclusive subfamilies of keratins. In combination, these 2 antibodies recognize a great majority of known human epithelial keratins (6, 21, 27, 29).

As reported earlier, AE1 antibody recognizes in various epithe-

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lia an M_r 56,500 keratin which may be regarded as a marker for keratinization (as defined morphologically by the formation of a granular layer and an anucleated stratum corneum) (22, 27, 29), an M_r 50,000 keratin present in various quantities in all stratified epithelia, an M_r 48,000 keratin characteristic of hyperproliferative keratinocytes (28), and an M_r 40,000 keratin present in a wide variety of epithelial cells (22, 27). Immunoblot analysis of human neoplasms demonstrated that the M_r 50,000 keratin (Fig. 1, *upper dot*) was present in most carcinomas originating from stratified epithelia (16 of 20 cases examined so far), including a metastatic squamous cell carcinoma (Fig. 1, *Lane 2*), a verrucous carcinoma from mouth (Fig. 1, *Lane 3*), a squamous cell carcinoma from vulva (Fig. 1, *Lane 4*), a squamous cell carcinoma from esophagus (Fig. 1, *Lane 4*), a benign (thymic epithelium derived) thymoma (Fig. 1, *Lane 6*; Ref. 3), and a transitional cell carcinoma from bladder (Fig. 1, *Lane 7*). In contrast, this M_r 50,000 keratin was not detected in carcinomas with a simple epithelial origin (15 cases) including a colon adenocarcinoma (Fig. 1, *Lane 8*), a breast adenocarcinoma (Fig. 1, *Lane 9*), a pancreatic adenocarcinoma (Fig. 1, *Lane 10*), and an adenocarcinoma metastatic to the liver (Fig. 1, *Lane 11*). Consistent with the current understanding that keratins are epithelium specific (7, 8, 24, 25), no AE1-positive keratin proteins were detected in nonepithelial neoplasms (8 cases) including a lymphoma (Fig. 1, *Lane 12*), a giant cell tumor of the bone (Fig. 1, *Lane 13*), and a melanoma metastatic to a lymph node (Fig. 1, *Lane 14*).

Our earlier results indicated that AE3 antibody recognizes in various epithelia an M_r 65,000 to 67,000 keratin (a marker for keratinization) (22, 27, 29), an M_r 58,000 keratin (a keratinocyte or stratified epithelial marker) (14, 27), an M_r 56,000 keratin (a marker for hyperproliferative keratinocytes) (28), and several other lower-molecular-weight keratin species (M_r 54,000, 52,000, etc.). A survey of various neoplasms showed that, like the M_r 50,000 keratin, the M_r 58,000 keratin was detected only in neoplasms originating from stratified epithelia (19 of 20 cases; *upper dot* in Fig. 2, *Lanes 2* to *7*), but not in 15 neoplasms originating from simple epithelia (Fig. 2, *Lanes 8* to *11*). Nonepithelial tumors (Fig. 2, *Lanes 12* to *14*) did not contain any significant amount of AE3-reactive keratin species.

DISCUSSION

The results from this study have demonstrated that the M_r 50,000 and 58,000 keratin proteins, which are readily detected by the immunoblot technique using AE1 and AE3 monoclonal antibodies, respectively, may be regarded as molecular markers for stratified squamous epithelial cells not only in normal tissues (12, 27) and in culture (14), but also in neoplasms. Moll *et al.* (13) have shown recently by 2-dimensional polyacrylamide gel electrophoresis that complex keratin patterns exist in various human carcinomas. Their results indicated that the M_r 50,000 (No. 14 according to their nomenclature) and 58,000 (No. 5) keratins are present in several carcinomas of the skin, tongue, and noncornified stratified epithelia (12, 13). These results are in excellent agreement with ours and provide independent support for the concept that the M_r 50,000 and 58,000 keratins represent useful markers for tumors with a stratified epithelial origin.

The M_r 50,000 and 58,000 markers were undetectable in a few cases of carcinomas derived from stratified or related epithelia (see "Results"). Thus, although the presence of the M_r 50,000 and 58,000 keratins establishes the stratified epithelial origin of a tumor, their absence does not prove the contrary. The significance of the finding that certain stratified tumors (*e.g.*, 3 cases of transitional cell carcinoma and one case of squamous cell carcinoma of the tongue) lack the M_r 50,000 and/or 58,000 keratin markers is unclear. One possibility is that these neoplasms may have arisen from tissues (*e.g.*, esophageal epithelium) which normally express only a small quantity of the M_r 50,000 and 58,000 keratins. However, our data indicated that the tumors as well as cultured cells derived from such epithelia express large quantities of the M_r 50,000 and 58,000 species. An alternative explanation would be that these 2 keratin markers may be lost as a result of certain forms of tumorigenesis. This latter possibility is supported by our recent finding that some SV40-transformed human epidermal keratinocytes cease to express the M_r 50,000 and 58,000 keratins.[3]

We have demonstrated recently that the M_r 48,000 and 56,000 keratins of Subfamilies A and B (Figs. 1 and 2, *lower dots*), respectively, are present in various hyperproliferative epidermal diseases and cultured keratinocytes, but they are absent in normal epidermis or ichthyosis vulgaris (a nonhyperproliferative epidermal disease) (28). In the present study, we have detected these 2 keratins in almost all M_r 50,000 and 58,000 keratin-positive carcinomas originating from stratified squamous epithelia. The only exception so far is a case of thymoma which is a thymic epithelium-derived, slow-growing (nonhyperproliferative), benign tumor (3). These results support our previous suggestion that the M_r 48,000 and 56,000 keratins represent markers for hyperproliferative keratinocytes (28).

Immunolocalization (22, 29) and cell fractionation data (20) have shown that, in normal epidermis, the M_r 50,000 and 58,000 keratins are made in the basal layer, and we have postulated that they may copolymerize to form a heteropolymer early during epidermal differentiation (29). Additional experiments will be needed to elucidate the exact role of the M_r 50,000 and 58,000 keratins during normal epithelial differentiation and in neoplasia. In the meantime, immunoblot assay for the presence of these specific keratins in surgical pathology specimens may be useful as a supplement to routine histological methods for tumor diagnosis, particularly for the poorly differentiated, metastatic cases. Furthermore, the demonstration of keratins specific for a subclass of carcinomas points to the possibility of using monoclonal antibodies highly specific for the M_r 50,000 and 58,000 keratins as a tool for the positive immunohistochemical identification of tumors of stratified epithelia (*cf.* Ref. 5).

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Fig. 1. Immunoblot analysis of keratins from various human neoplasms using AE1 monoclonal antibody. *Lane 1*, keratins of normal human abdominal epidermis showing an M_r 56,500 keratin (a keratinization marker) and an M_r 50,000 keratin (a keratinocyte marker) (21, 27, 29); *Lane 2*, metastatic squamous cell carcinoma; *Lane 3*, verrucous carcinoma of oral cavity; *Lane 4*, squamous cell carcinoma from vulva; *Lane 5*, squamous cell carcinoma from esophagus; *Lane 6*, benign thymoma; *Lane 7*, transitional cell carcinoma of bladder; *Lane 8*, colon adenocarcinoma; *Lane 9*, breast adenocarcinoma; *Lane 10*, pancreatic adenocarcinoma; *Lane 11*, adenocarcinoma metastatic to liver; *Lane 12*, lymphoma; *Lane 13*, neurofibroma; and *Lane 14*, melanoma. Note the presence of an M_r 50,000 keratin (*upper dot*) in tumors of stratified epithelia and its absence in those of simple epithelial or nonepithelial (*NE*) origins. Thymoma, a benign epithelial-derived tumor (3), was unique in that it lacked the M_r 48,000 component (*lower dot*; *cf.* Ref. 28). The M_r 40,000 keratin was detected in almost all nonepidermal neoplasms. The M_r 37,000, AE1-positive band (◀) and several other minor AE1-positive bands were variable in intensity and might represent degradative products of the higher-molecular-weight keratins (27). *M. W.*, M_r.

Fig. 2. Immunoblot analysis of keratins from various human neoplasms using AE3 monoclonal antibody. Samples are the same as those shown in Fig. 1. Note the detection of an M_r 58,000 keratin (*upper dot*) in tumors with a stratified squamous origin but not in significant amounts in simple epithelial or nonepithelial (*NE*) tumors (thymoma lacks the M_r 56,000 keratin; *lower dot*). ◀, an AE3-reactive, M_r 66,000 component which is present in almost all samples, perhaps as a stratum corneum keratin contaminant (14, 16, 27). *M. W.*, M_r.