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KERATIN PROTEIN PROFILE IN SQUAMOUS CELL CARCINOMA AS INDUCED IN THE HAMSTER CHEEK POUCH WITH DMBA (PRELIMINARY REPORT)

LI-MIN LIN and REZA MOSTOFI*

The purpose of this study is to compare the keratin profile in DMBA-induced squamous cell carcinoma with that in normal hamster cheek pouch epithelium. The pattern of keratin expression in these two groups was studied by the methods of SDS-PAGE and immunoblot analysis on the electrophoretic keratin protein. A study of the specific changes of this protein were revealed as an essential step for analyzing the consequent changes in keratin expression that occurs in the course of development of a fully malignant lesion.

Key words: carcinogen, keratin profile, DMBA-induced carcinoma

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Oral cancer, primarily squamous cell carcinoma (SCC), occurs in approximately 4% of all malignant tumors in the United States. The American Cancer Society estimated 27,710 cases in 1981 with the 10,000 cancer deaths in that same year (2.4% of all cancer caused deaths), despite treatment.[1] The overall five-year survival rate after diagnosis of an intraoral neoplasm has been postulated at 30%. The poor prognosis of lesions in an area that is readily accessible to examination is caused in part by the inadequate early detection of premalignant lesions. Despite numerous studies on oral hyperkeratotic lesions (leukoplakia), there is no reliable test to differentiate benign hyperkeratotic lesions (leukoplakia) from those with the potential for malignant transformation. Silverman *et al*[2] reported 17.5% of their 257 patients with oral leukoplakia subsequently developed squamous cell carcinoma. To this time, the most reliable method of assessing the neoplastic potential has been microscopic observation of changes in cellular morphology consistent with a metaplastic change. The observed metaplasia most likely results from changes in the cytoskeleton due to an unknown cause consistent with a neoplastic process. Recently, several classes of intermediate cytoskeletal filaments (IF) have been identified.[3-5] These IFs are good molecular markers of cellular origin and have been proven to of value in the pathological diagnosis of undifferentiated tumors.[6,7] Keratin IFs specifically are evidenced only in cells of epithelial origin. It has been demonstrated that the keratins of human epidermis consist of several distinct proteins of different molecular weights, each of them translated from a unique mRNA.[8] It is shown that cells in the inner layer of human epidermis contain smaller keratin molecules. Where as cells in outer layers contain keratin molecules with a larger molecular weight. These changes in keratin gene expression occur during terminal differentiation of the keratinocytes.[8] Recently, several investigators have examined human squamous cell carcinoma to determine the presence of keratin polypeptides in the malignant tissues.[9] These analyses have shown that the squamous cell carcinoma demonstrates a keratin profile distinctly different from that of the normal tissue counterpart. Due to the spontaneous nature of the squamous cell carcinoma studied and the unknown etiology, it has been impossible

School of Dentistry, Kaohsiung Medical College Kaohsiung City 80708, Taiwan, Republic of China.
*Zoller Dental Clinic, University of Chicago. U.S.A.

to correlate a specific keratin profile with the tumors. One method to examine keratin in the similar squamous cells is using the chemically induced tumor model. Induction of squamous cell carcinoma in a hamster cheek pouch by DMBA (7, 12-dimethylbenzanthracene) application has been widely used for morphologic studies for several years.[10,11] However, few molecular studies have been done on such tumors and changes in keratins have not been examined.[12-14]

The purpose of this study is to compare the keratin profile in DMBA-induced squamous cell carcinoma with squamous cells found in normal hamster cheek pouch epithelium and to set up a foundation for further investigation of keratin profiles at different stages in tumor development.

MATERIALS AND METHODS

Ten adult male Syrian hamsters were divided into two groups. Each group consisted of five experimental and five control hamsters. The right cheek pouch of animals, in the experimental and control groups, was painted thrice weekly for twelve weeks with either 0.5% DMBA in mineral oil or mineral oil alone, respectively. They were observed, sampled and sacrificed. Each tissue sample was divided in half. One half was snap frozen and used for keratin preparations. The other half was fixed in 10% buffered formalin, processed by conventional techniques embedded in paraffin, sectioned at five microns and stained with hematoxylin and eosin. The tissue for keratin extraction[14] was thoroughly homogenized in a small well fitting grinder in 10mm tris-HCL, 1mm EDTA pH 7.6. The homogenate was centrifuged at 8000Xg for 8 minutes. The pellets were then washed three more times with the same buffer to remove the water soluble products. The water insoluble cytoskeletal extract was subsequently solubilized in 8 M urea and 1% mercaptoethanol, heated at 37°C for 20 minutes and

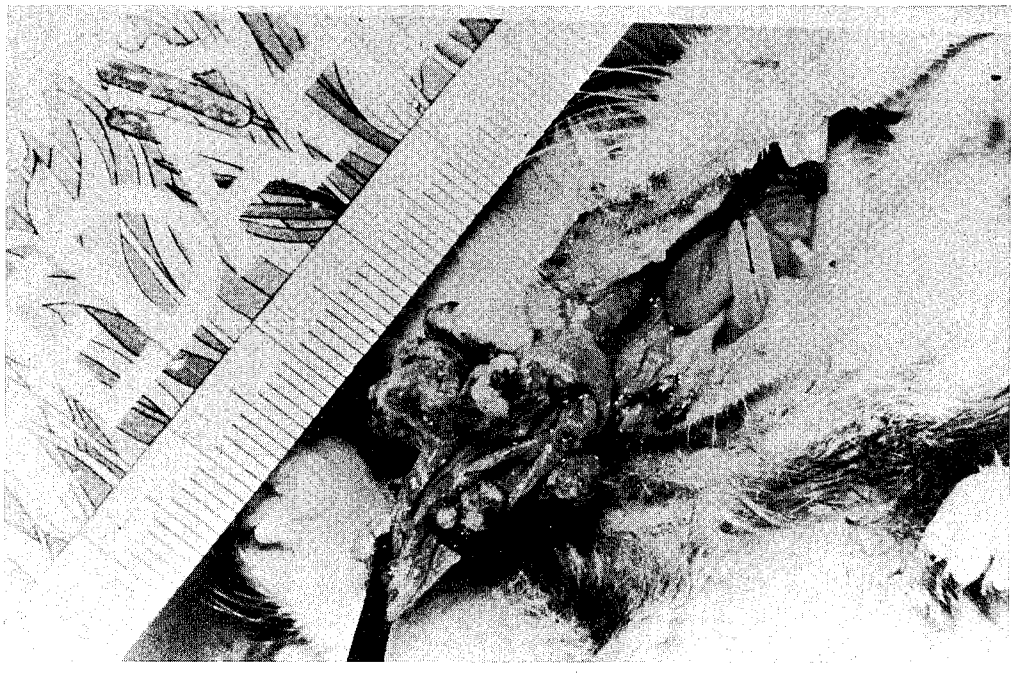

Fig. 1. DMBA induced squamous cell carcinoma of the hamster buccal pouch. This hamster was painted thrice weekly with 0.5% DMBA for 12 weeks. Note multiple exophytic and sessile tumors. These tumors were excised and divided in half. One half was processed for routine histology, as described, and the keratin was extracted from the remaining half.

sonicated (4 x 15 sec.). The methods for SDS-PAGE of keratin proteins and immunoblot analysis of electrophorectically separated keratins were previously described.[15-17]

RESULTS AND DISCUSSION

It is now well documented that the thrice weekly application of 0.5% solution of DMBA to the buccal pouch mucosa produces areas of histologically hyperkeratosis and dysplasia at six to eight weeks, early squamous cell carcinoma at eight to ten weeks, and finally, invasive squamous cell carcinoma at ten to twelve weeks.[13] During this experiment, the tissue was observed and areas with suspected malignancy were easily sampled. In this study, the keratins were examined by SDS-PAGE in squamous cell cacinoma induced by DMBA following the protocol described[14] (Figs. 1–4). It was noted that the keratin profile of the induced lesions was obviously different from that of the normal pouch epithelium.

The SDS-PAGE and Antikeratin immunoblot patterns of keratin protein samples from the investigated epithelia are shown in Fig. 3 and 4. The individual composition of the major keratin polypeptides from the hamster foot pad, palate and buccal pouch epithelium samples revealed no qualitative differences. The keratinized normal epithelium consistently showed polypeptides varying in molecular weight from 43 to 68 K daltons, whereas the squamous cell carcinoma in the hamster buccal pouch lacked the large polypeptides and showed an almost missing upper half of the polypeptides with molecular weights from only 43 to 58 K daltons. The exceptions to this were found in group E of Figs. 3 and 4 which demonstrated few polypeptides and group B of Fig. 4 showed similar polypeptides as normal buccal pouch.

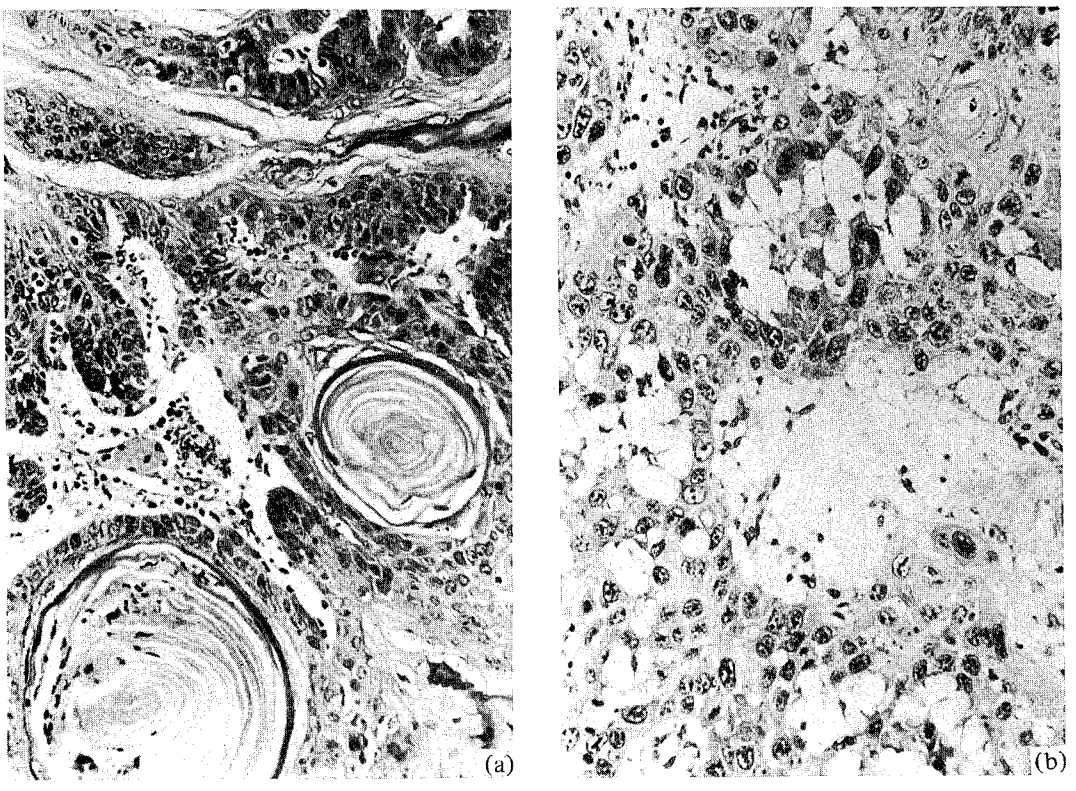

Fig. 2. (a),(b) (H & E original magnification x200)
High power view of the DMBA induced carcinoma of hamster buccal pouch.

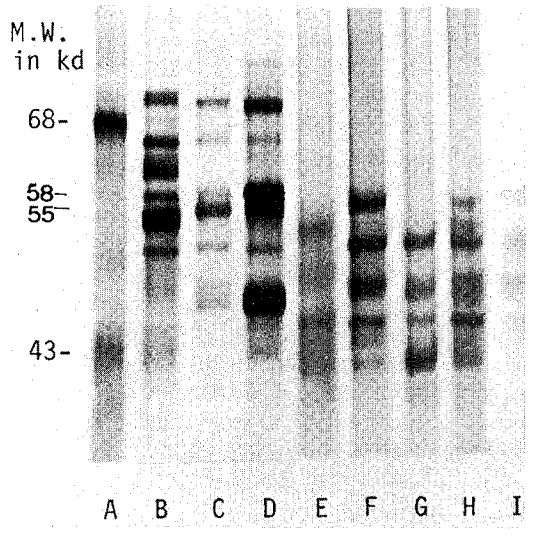

Fig. 3. SDS-PAGE of keratin proteins extracted from normal hamster stratified squamous epithelium and DMBA induced SCC of the hamaster buccal pouch. Normal tissue and tumors were removed from sacrificed hamsters. The proteins were electrophoretically separated on 10% polyacrylamide gels and visualized by silver staining (A) molecular weight markers (BRL), (B) Hamster foot pad epithelium, (C) Hamster palate epithelium, (D) Hamster buccal pouch epithelium, (E, F, G, H, I) SCC in hamster buccal pouch.

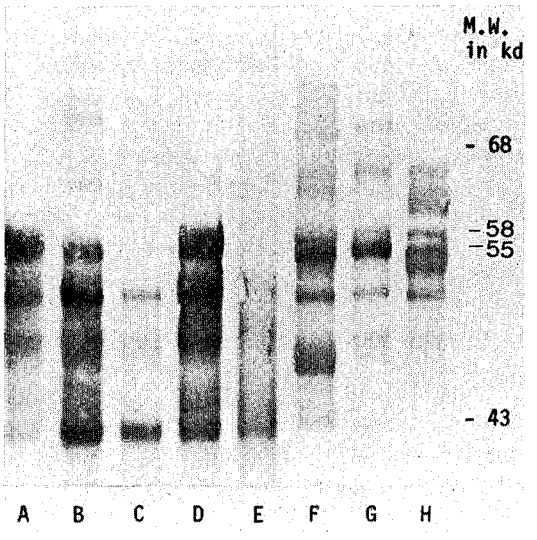

Fig. 4. Antikeratin immunoblot of keratin proteins extracted from hamster stratified squamous epithelium and DMBA induced SCC's of the hamster buccal pouch. Normal tissue and carcinomas were removed from sacrificed hamsters. The proteins were electrophoretically separated on 10% polyacrylamide gels and subsequently electrophoretically transferred and immobilized on nitrocellulose membranes. The immobilized proteins were reacted with a rabbit antikeratin antiserum and the complex recognized by a goat anti-rabbit Ig G horseradish peroxidose conjugated antiserum. The complexes were visualized with diaminobenzidine and hydrogen peroxide. (A, B, C, D, E) squamous cell carcinoma in hamster buccal pouch, (F) untreated hamster buccal pouch epithelium, (G) hamster palate epithelium, (H) hamster foot pad epithelium. Molecular weight determined from co-migrated molecular weight standards.

Despite the keratin pearls are generally thought to be consistent with a high degree of keratinocyte differentiation, the keratin IFs were much more consistent with the degree of differentiation in the basal layer of epithelium. As of this time, it is impossible to determine whether the temporal keratin changes seen in both spontaneous and induced squamous cell carcinoma, occured prior to cellular metaplasia or during the neoplastic process or prior to frank malignancy. The cheek pouch system offers an excellent model to analyze all stages of hyperkeratosis, premalignancy and carcinoma. The morphologic changes that accompany the development of cancer are well known and the cellular morphologic changes themselves are in fact one of the chief distinguishing signs used in the diagnosis of a lesion as malignant.

The keratins as intermediate filaments

are one of the family of proteins that compose the cytoskeleton. These different groups of proteins are deeply involved in the generation of the final shape of a cell. It would be expected therefore that some derangement in the cytoskeleton maybe observed coincidently with the characteristic morphologic changes seen in malignant cells. Since the keratins are the only known cytoskeletal elements specific for epithelial cells, it is postulated that observation of changes in the keratins would yield important information on the metaplastic process in cancer.

The difficulty in examining the changes of keratins in human tumors is that human tumors are spontaneous in nature. The comparisons between individual tumors that may have been induced by extremely different agents are certainly difficult. Therefore, an experimentally induced squamous cell carcinoma appears to be a logical choice as many of the variables can be controlled. However, the choice of location is critical since squamous cell carcinoma in different locations has different characteristic keratin patterns.

In this regard the predominance of studies on skin are inherently complicated by the fact that the pattern of keratins produced by the keratinized squamous cell carcinoma of skin would be suspected to overlap with the keratins of a well differentiated squamous cell carcinoma. The well defined cheek pouch system avoids this problem by the nature of the normal epithelial pattern and yields several other advantages already presented. In this study we showed for the first time the keratin patterns in an experimentally induced cheek pouch tumor. Additionally we also showed that the changes in keratins from the normal condition is distinct and closely linked to the histology of the tumor. Moreover, the changes are not obscured by the background pattern of keratinization.

This study and the specific changes noted revealed an essential step for analyzing the sequential changes in keratin expression that occurs in the course of development of a fully malignant lesion.

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誘癌劑DMBA引發倉鼠頰囊袋之鱗狀上皮樣癌與正常囊袋表皮中之角質蛋白之比較研究（初報）

林立民　Reza Mostofi*

本篇是誘癌劑DMBA所引發倉鼠頰囊袋之鱗狀上皮樣癌與正常囊袋表皮中之角質蛋白之比較研究。實驗比較之方法是運用SDS-PAGE及免疫轉附法，結果發現此兩組中之角質蛋白有明顯的差異。這發現可奠定將來深入研究口腔癌癌變過程時一項有力的比較佳證。

高雄醫學院　牙醫學系
*Zoller Dental Clinic, University of Chicago, U.S.A.