

KERATINS IN AMELOBLASTOMAS, DEVELOPING TOOTH, ORAL EPITHELIUM AND DENTIGEROUS CYSTS

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تم استخدام أجسام مضادة لمادة القرنية بالطيف العريض للأوزان الجزئية على شرائح البرافين وقد شمل البحث ٧ حالات براعم أسنان جنينية بمصاحبة الصفيحة السنية والنسيج البشري الفمي، ١١ حالة ورم مينائي منها ٣ حالات خبيثة و٧ حالات أكياس ناجية.

وقد عمل اختبار إيجابي وآخر سلبي وقد تشابه في توزيع مادة القرنين في الورم المينائي (السليم والخبيث) النسيج البشري الفمي وأكياس حاملة الأسنان وقد كان التصيغ شديداً في حالات أورام الأكياس الناجية الخبيثة، بينما كانت سلبية في حالات صفيحات الميناء النسيج البشري الفمي الجنيني غير المتقرن والصفيحة السنية. مما سبق نستنتج ما يلي:

- ١ - التشابه في توزيع مادة القرنين في النسيج البشري الفمي والأورام والخويصلات وهذا يفسر الدور الهام للنسيج البشري الفمي كمصدر للورم المينائي (سليم خبيث) والأكياس الناجية.
- ٢ - يمكن اعتبار الأورام المينائية ناتجة من الأكياس السنية.
- ٣ - من التفاعل السليبي لمادة القرنين لكل من الصفيحة السنية وبراعم الأسنان الجنينية قد فسر المفهوم من أن هذه الأنسجة بالرغم من كونها بشرية سنية إلا أنها ليست هي المصدر الأساسي للورم المينائي والأكياس الناجية.
- ٤ - احتمال وجود علاقة بين الخلايا الصافية الموجودة في النسيج البشري الفمي الناضج وفي الورم المينائي حيث إن كلاهما صيغاً سلبياً بالقرنين وهذه الخلايا يمكن اعتبارها غير مكونة لمادة القرنين.
- ٥ - الاصبغ السلبي لهذه الخلايا من الدلالات التشخيصية لتحديد الخلايا غير المكونة للقرنين.
- ٦ - نظراً لوجود هذه الخلايا الصافية في النسيج البشري الناضج وعدم تواجدها في النسيج البشري الجنيني يدل على تواجدها في مراحل الأكثر تقدماً.
- ٧ - لأول مرة استخدمت الأجسام المضادة لمادة القرنين لدراسة الأورام المينائية الخبيثة.
- ٨ - نظراً لظهور تفاعل قوي في حالات الأورام المينائية الخبيثة يمكن استنتاج أن هذا التبدل في القرنين قد يكون سبب قوي للتجديد الحلوي السريع.
- ٩ - ويمكن اعتبار الاصبغ السلبي أو الإيجابي لمادة القرنين يعتمد على درجة النمو والنضج كما أنه يعتبر ناتج عن مراحل متميزة مختلفة.
- ١٠ - ولأول مرة تتم دراسة مقارنة لأنواع عديدة من النسيج البشري الفمي تشمل مراحل نمو جنينية مختلفة الأشكال ومختلفة في الأماكن التشريحية ومراحل مرضية لتقديم شرح مبسط لاختلاف تغير مادة القرنين تحت هذه الأحوال.

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- ١١- وقد أظهرت هذه الدراسة الأولية أن الإطار التعبيري لمورثات القرنين ترتبط ارتباطاً وثيقاً بدرجة التميز للنسيج البشري والتي من الممكن أن تعتبر كعلامة لهذا النسيج .
- ١٢- إمكان استخدام بيانات الحركة الخلوية مع نتائج الدراسات المناعية الكيميائية الخلوية لإظهار العلاقة الوظيفية لهذا النسيج .
- ١٣- ولمزيد من الضوء يمكن استخدام دراسة المناعة الكيميائية الخلوية لبروتينات بشرية أخرى من المجهر الإلكتروني لإيجاد أصل الأورام المينائية والأكيس التاجية وذلك لاستنباط أساس متين للتشخيص وعلاج هذه الحالات .

There is remarkably little pertinent information available about the correlation between oral epithelium and the possible role in the etiology of dentigerous cysts and ameloblastomas (benign or malignant). Monoclonal antibodies, the wide spectrum screening type was applied on paraffin section, using the peroxidase-antiperoxidase (PAP) procedure. Seven human embryonic cap, bell-stage teeth with the associated dental lamina and oral epithelium, eleven cases of ameloblastoma including three malignant cases, and seven cases of dentigerous cysts were immunocytochemically studied. Keratin profiles were similar in distribution in ameloblastoma (benign and malignant), the mature oral epithelium and dentigerous cysts. The reaction was strong in the malignant ameloblastoma while the dental organs, thin non-keratinized embryonic oral epithelium and dental lamina displayed negative reaction. Interestingly, certain individual cells revealed negative reaction at the stratified squamous adult oral epithelium. These clear negative cells were also present in ameloblastoma. Since the keratin reaction is related to the development and differentiation of tissues and was negative in the thin non-keratinized epithelium, dental lamina, dental organ but positive in adult epithelium and ameloblastoma, it seems that both dental organs and dental lamina are the principal origin of ameloblastoma and dentigerous cysts.

Introduction

Recent progress in understanding the biology of keratin together with the development of monoclonal antibodies to individual keratin proteins provide the foundation for studying keratin expression in normal and pathological oral epithelia.

Keratins are a group of water insoluble proteins characterized by the occurrence of a zonal distribution of different molecular weights keratin that form 10 nm to non-filament in a wide variety of epithelial cells.^{1,2} Compared with other types of intermediate filament protein (vimentin, desmin, neurofilament and glial filament) keratin is very complicated in terms of sub-unit composition.³ The sub-unit composition of keratin filaments varies with cell type,^{4,5} period of embryonic development,⁶ stage of histologic differentiation,^{7,8} cellular growth environment,⁹ and disease state.¹⁰⁻¹⁴

Ameloblastoma are odontogenic tumors of epithelial origin. The resemblance of this tumor's epithelium to the normal enamel organ indicates that ameloblastoma arises from the dental epithelium, or at least very closely connected with

it, but the precise point of origin is unknown.^{15,16}

Spouge¹⁷ reported that this tumor arises from the dental lamina or its derivatives. Additionally, other authors suggested that ameloblastoma could also originate from either surface epithelium, or epithelium of odontogenic cysts, particularly the dentigerous cysts.¹⁸⁻²⁴ The aims of this work are:

1. To study the keratin profiles and the cyto-keratin expression in oral epithelium including:
 - a. Embryonic non-keratinized epithelium:
 - i.) thin up to 2–3 layers
 - ii.) thick, up to 10 layers
 - b. Dental lamina
 - c. Ameloblastic epithelium of ameloblastoma
 - d. Dentigerous cyst epithelium
 - e. Adult oral epithelium:
 - i.) non-keratinized
 - ii.) keratinized
2. To examine whether immuno-histological localization of epithelial proteins could be used to determine the origin of ameloblastoma and dentigerous cysts.

Materials and Methods

Eleven cases of ameloblastoma (including three malignant), 7 cases of dentigerous cysts, 7 human embryonic cap and bell stage teeth with the associated dental laminae, and adult oral epithelium were taken from the files of Oral Biology and Oral Pathology Departments, Faculty of Dentistry, University of Alexandria.

Five of the ameloblastomas were of the follicular type, 3 were plexiform type, whereas the other 3 were malignant. All tumors, cysts, and embryonic dental tissues were fixed in 10% formalin and embedded in paraffin. Sections, 4 microns thick, were cut for the immunohisto-chemical detection of keratin proteins with the use of wide spectrum screening monoclonal antibodies to correlate between the oral epithelium and the role in the etiology of dentigerous cysts and ameloblastoma (benign and malignant).

Immunohistochemical Methods

Deparaffinized sections were treated for 20 minutes with a methanol solution containing 0.3% H_2O_2 to permit inactivation of endogenous peroxidase. The sections were rinsed well and treated with normal rabbit serum for 30 minutes and plotted dry with filter paper. Subsequently, they were reacted with a monoclonal antibody to the wide spectrum keratin (poloconal) for one hour, rinsed three times in phosphate-buffered saline (PBS), and reacted for 30 minutes with horse radish peroxidase (HRP). The sections were labelled with rabbit anti-mouse immunoglobulin (1:20 dilution, Dakopatts, Copenhagen, Denmark) and rinsed well. Finally, the sections were immersed for 5 minutes in 0.005M TRIS buffer solution (pH 7.6) with 0.05% 3.3 diaminobenzidine tetrahydrochloride (DAB) or by using another chromogen, 3-amino-9-ethyl-carbazole (AEC) in dimethylformide.

Trypsin Pretreatment

Deparaffinized sections were treated for 30 minutes at 37°C with 100 ml of phosphate-buffered saline (PBS), which contained 0.1 g each of trypsin and $CaCl_2$, prior to immuno-histochemical staining.

Results

Negative keratin reaction was found in epithelium of all the human embryonic cap and bell

stages of enamel organs and dental laminae. The embryonic thin 2-4 layers of cell, on-keratinized oral epithelium, were also keratin negative [Fig. 1]. The thick 10-15 rows of embryonic cells of non-keratinized oral epithelium revealed very weak staining for keratin in the basal and parabasal layers. The spinous cells developed keratin protein expressions and stained moderately orange red color when using AEC chromogen [Figs. 2,3].

The parakeratinized adult oral epithelium expressed keratin in a sequential order of reaction from the weak stain in the basal and parabasal cell layers to a moderate stain in the superficial spinous cell layer. The parakeratinized layer revealed strong antigen reaction.

The same distribution of keratin proteins were expressed in the ortho-keratinized oral adult

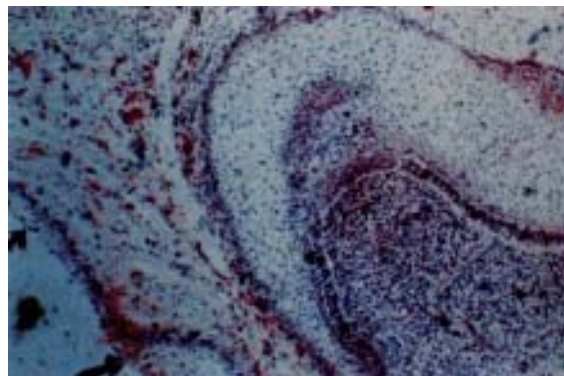


Figure 1. Bell stage of dental organ revealing negative brown reaction for keratin. The embryonic thin non-keratinized oral epithelium shows complete negative reaction. PAP-DAB (x 120)



Figure 2. Stratified squamous epithelium, the thick non-keratinized embryonic epithelium demonstrate positive orange-red reaction labelling the keratin distribution at the spinous (arrow) cell layers. Note the weak reaction at the basal cell layer. PAP-AEC(X 250)

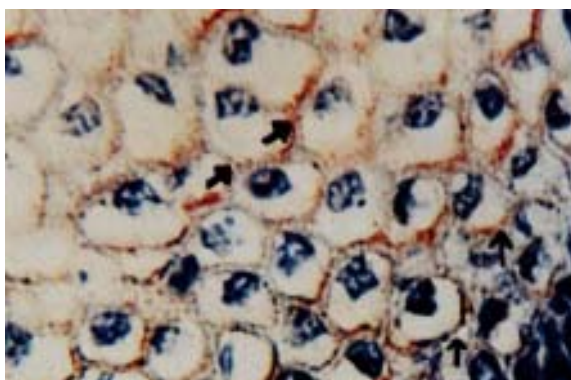


Figure 3. Higher magnification of Figure 2 revealing the orange-red reaction at the spinous cell layers (arrow), while the basal and parabasal cell layers (arrow) are very weak in reaction to keratin. (X 450)

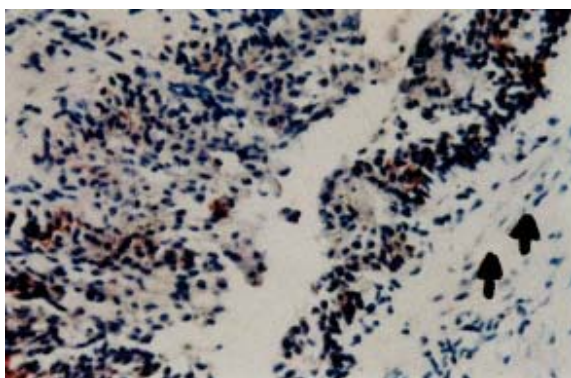


Figure 5. The epithelium lining of dentigerous cyst demonstrates red stain indicating the presence of keratin proteins. Notice the negative reaction at the fibrous capsule (arrow). PAP-AEC (x 120)

epithelium (weak staining reaction of the basal and parabasal cell layers and moderate staining in the spinous layers). The superficial spinous cell layers and the granular cell layers exhibited strong reaction assuming a deep brown color when using DAB chromogen and deep red color with AEC [Fig. 4].

Immunohistochemistry study of the seven dentigerous cysts demonstrated keratin reactivity in all layers of the epithelial lining (a regular layer of para-keratinized stratified epithelium). The antigen was localized exclusively in the cytoplasm of the cells. The spinous cell layers and the superficial parakeratinized layers were more strongly labelled [Fig. 5].

The 11 cases of ameloblastoma were immunocytochemically reacted with the antikeratin antiserum. The tumor epithelium in all cases of

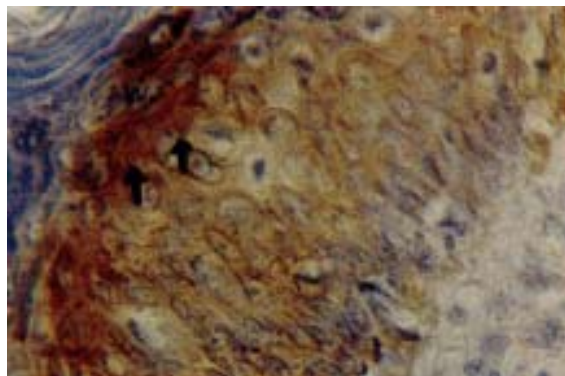


Figure 4. Keratinized stratified squamous epithelium, showing the intensity of the brown reaction in a sequential order from the basal and parabasal cell layers to the strongest reaction at the granular cell layers (arrow). Notice the negative reaction at the clear cells. PAP-DAB (x 250)

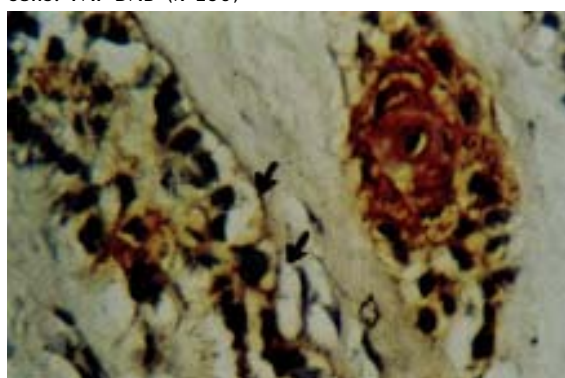


Figure 6. Acanthomatous ameloblastoma. The squamous metaplastic area was strongly reactive for keratin antibodies. Note the clear cells that showed negative brown reaction (arrow). PAP-AEC (x 450)

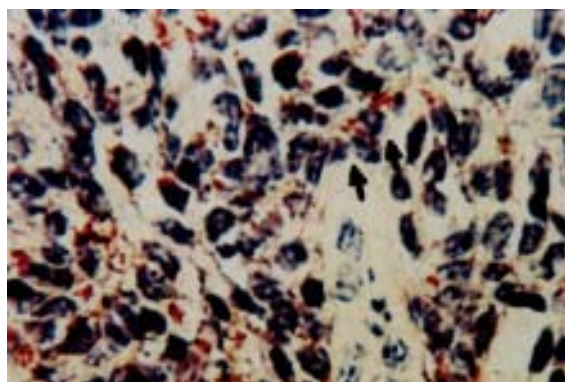


Figure 7. Well-differentiated malignant ameloblastoma revealing cellular pleomorphism and hyperchromatism. The cells display strong red color denoting the presence of keratin antigens (arrow). PAP-AEC (x 450)

ameloblastoma showed strong red or brown stain when reacted with antibodies against keratin. The pre-ameloblast, like peripheral cells, and the stellate reticulum, like central cells, in the follicles were keratin positive. However, cells undergoing degeneration appeared as vacuolated clear cells. The plexiform ameloblastoma revealed anastomosing or continuous inter-connected strands of epithelium that were keratin positive. Also, in the case of acanthomatous ameloblastoma, the tumor epithelium were strongly positive in keratin reaction [Fig. 6].

In malignant ameloblastoma, two of the cases were undifferentiated (malignant transformation) in certain areas. The malignant criteria were hyperchromatism, pleomorphism, and increase in mitotic figures. The third tumor was a poorly differentiated type (ameloblastic carcinoma) that appeared similar to a squamous cell carcinoma. The tumor exhibited keratin pearl formation, individual cell keratinization, hyperchromatism, pleomorphism and increased mitotic figures. All these three cases revealed strong keratin antigen expression that was variable in intensity at different locations. Clear cells were detected in ameloblastoma, which do not express any keratin protein reaction [Fig. 7].

Discussion

It has been shown that several types of epithelial cells have the potential to differentiate into odontogenic epithelium when exposed to proper inductive signals.²⁵ Thesleff reported that oral mucosa was readily differentiated into ameloblasts when experimentally combined with dental mesenchyme.²⁶ However, there is little pertinent information available about the pathway and life span of the dental lamina in the permanent molar region.^{27,29} It was stated that the morphology of dental lamina epithelium shows a striking resemblance to the epithelial lining of a keratocysts.^{14,21} Also, the palisading of the basal cells, the hyperchromatic nuclei along with the slight parakeratosis, the apical polarization of the nuclei, were common features in the epithelial lining of cysts in which ameloblastoma arise.³⁰

In the present study, wide spectrum screening monoclonal keratin antibodies were used to correlate between various types of epithelium and their possible role in the etiology of dentigerous cysts and ameloblastoma (benign and malignant).

The reactivity was identified in all epithelia except in the primitive oral epithelium (thin non-keratinized embryonic stratified squamous epithelium), the dental laminae and the enamel organs (cap and bell stage).

The findings of negative reaction on thin non-keratinized embryonic epithelium, dental laminae, enamel organs strong in adult epithelium, ameloblastoma and cysts suggest that the keratin expression for epithelial cells depends on the stage of development and maturation. It also indicate that these epithelia could be the primary tissue of origin of the ameloblastoma and dentigerous cysts.

In accordance with Steolinga,²⁴ dental lamina and developing tooth epithelium are unlikely to be the principal origin of ameloblastoma and dentigerous cysts. Keratin profiles observed in normal squamous epithelia were similar to dentigerous cysts, ameloblastoma, and malignant ameloblastoma. Accordingly, very few ameloblastoma are likely to arise from dentigerous cysts, which is in agreement with the findings of Kuusela *et al.*¹¹

Using the monoclonal antibodies, it was shown that the keratin pattern of the epidermis varies during normal differentiation.^{31,32} Also, in oral mucosa the turn-over of non-keratinized epithelia is generally faster than that of keratinized mucosa.^{27,29,32}

One of the rather striking immunohistochemical results of this study was the presence of clear negative cells to keratin proteins in ameloblastoma. It seems likely that keratin genes expression have similar profiles in oral epithelium and ameloblastoma and is closely linked to the differentiation of epithelium, hence it could be used as epithelial marker.

Accordingly, it was possible to provide localization and distribution of the monoclonal keratin antibodies profile in the malignant ameloblastoma. On the basis of this finding, we suggest that such strong reaction might be due to various levels of differentiation, or it could be considered as a marker of epithelial maturation.

Antibodies, against other epithelial proteins, may be used in the future to solve the problem of whether there are specific cell types in the enamel organ or the oral mucosa, which are progenitors of ameloblastoma. It would be necessary to combine cell kinetic data with immunocytochemical results

in exploring a possible functional relationship.

Nevertheless, the keratins, which is tightly linked to differentiation, constitute important biological markers. The proteins are stable, relatively resistant to degradation, show great fidelity of expression, and are very antigenic. Therefore, it is probable that the monospecific antikeratin antibodies will prove to have fruitful applications, not only in oral biology but also in many areas of oral diagnosis.

Further immunocytochemical studies against other epithelial proteins, utilizing electron microscopy, may throw light on the origin of ameloblastoma and dentigerous cysts. Such a finding may establish a basic foundation in accurate diagnosis and treatment.

References

- Mori M et al. Immunolocalization of keratins in salivary gland pleomorphic adenoma using monoclonal antibodies. *Oral Surg Oral Med Oral Pathol* 1986; 61:611-6.
- Takai Y, Murase M. Immunohistochemical localization of keratin in experimental carcinoma of the mouse submandibular gland. *J Cell Biol* 1976;65:73.
- Lazarides E. Intermediate filaments: a chemically heterogeneous developmentally regulated class of proteins. *Ann Rev Biochem* 1982;51:219.
- Doran TI, Vidrich A, Sun TT. Intrinsic and extrinsic regulation of the differentiation of skin, corneal, and esophageal epithelial cells. *Cell* 1980;22:17-25.
- Fuchs E, Green H. Changes in keratin gene expression during terminal differentiation of keratinocytes. *Cell* 1980;19:1033.
- Dale BA, Stern IB, Huang LY. The identification of fibrous proteins in fetal rat epidermis by electrophoretic and immunologic techniques. *J Invest Dermatol* 1976; 66:230-5.
- Scraff MA, Johnson NW. Epithelial cell kinetics. A review of methods of study and their application to oral mucosa in health and disease. Part B. *J Oral Pathol* 1982;11:102.
- Vidrich A, Sun TT. The expression of keratin antigens in stratified squamous epithelium. *J Cell Biol* 1980;87:25.
- Paulin D, Jakob F, Weber K, Osborn M. In vitro differentiation of mouse teratocarcinoma cells monitored by intermediate filament expression. *Differentiation* 1982;22:90-9.
- Hill MW. The structure and function of oral mucosa. Oxford: Pergamon Co, 1984:53.
- Kuusela P, Ylipaavalneimi P, Thesleff I. The relationship between the keratocyst antigen (KCA) and keratin. *J Oral Pathol* 1986;15:287.
- Morgan PR, Johnson NW, Leigh JM, Lane FB. Structure of gingival epithelium as revealed by monoclonal antibodies to keratin. *Geneva Med Hyg* 1986:47.
- Stagnet MJ, Viac J, Thivolt J. Keratin polypeptide modifications induced by human papilloma viruses. *Arch Dermatol Res* 1981 ;271:83-90.
- Sun TT, Eichner R, Nelson WC et al. Keratin classes: molecular markers for different types of epithelial differentiation. *J Invest Dermatol* 1983;81:109.
- Lucas RB. Pathology of tumors of the oral tissues. 45th ed. Edinburgh, London, Melbourne, New York; Churchill Livingstone, 1984:32.
- Shafeer WG, Hie MK, Levy BM. Oral pathology. 4th ed. Philadelphia, London, Toronto: WB Saunders Co, 1983:19.
- Spouge JD. Embryonal significance of epithelial odontogenic tumors. *J Can Dent Assoc* 1976;33:200.
- Gardner DC. Peripheral ameloblastoma. *Cancer* 1977; 39:1625.
- Gould AR, Farman AG, Dejean EK, Va Arsdail LR. Peripheral ameloblastoma: an ultrastructural analysis. *J Oral Pathol* 1982; 11:90.
- Hodgkinson DJ, Woods JE, Dahlin DC, Tolman DE. Keratocytes of the jaw. Clinico-pathologic study of 79 patients. *Cancer* 1978;41:803.
- Pindborg JJ. Pathology of the dental tissues. Copenhagen :Munksgaard, 1970:374-7.
- Sicher H, Bhaskar SN. Oral anatomy. 7th ed. St. Louis: CV Mosby, 1980:17-37.
- Stankey HR, Diehl DL. Ameloblastoma potential of follicular cysts. *Oral Surg* 1965;20:260.
- Stoelting PJW. Studies on the dental lamina as related to its role in the etiology of cysts and tumors. *J Oral Pathol* 1976;5:65-73.
- Kollar EJ. The development of the integument: spatial, temporal and phylogenetic factors. *Am Zool* 1972; 12:125.
- Thesleff I. Tissue interactions in tooth development in vitro. In: cell interactions in differentiation. Karkien-Jaaskelainen M, Saxen L and Weizz L ed. London: Academic Press, 1977:191-207.
- Bhaskar SN. Orban's histology and embryology. 8th ed. St. Louis: CV Mosby Co, 1976:18.
- Provenza DV. Oral histology inheritance and development. Toronto: JP Lippincott Co, 1972:312-42.
- Shear M. Cysts of the oral regions. 2nd ed. London, Boston: Wright Press, 1974:252-65.
- Vickers RA, Gorlin RJ. Ameloblastoma: delineation of early histopathologic features of neoplasms. *Cancer* 1970;26:699-710.
- Banks-Schlegel SP. Keratin alterations during embryonic epidermal differentiation: a presage of adult epidermal maturation. *J Cell Biol* 1982;93:551-9.
- Schewizer J, Winter H. Keratin polypeptide analysis in fetal and terminally differentiated newborn mouse epidermis. *Differentiation* 1982;22:19-24.