Immunohistochemical and Ultrastructural Study on Pilomatrixoma

by

Norio HORIE, Masahito INOUE, Atsushi SAITO, Kaori FUNATSU, Masanori SATO, Kazuhiro KIUCHI and Shinichiro UMEMURA

(Received 29 March 1985)

Introduction

Pilomatrixoma, or calcifying epithelioma of Malherbe, is a benign dermal tumor with differentiation toward hair and hair follicles. The head and neck portions of children and young adults are the most common sites of this tumor\(^{[1,2]}\). Tumor cells are mainly composed of shadow and basophilic cells corresponding to the cortex cells of normal hair. In addition to these cells, the presence of other cell types such as hair cuticular, follicular or sebaceous cells have also been observed in this tumor\(^{[3]}\).

It has been described that whole keratin antibody recognizes keratin protein in squamous epithelium and in normal hair follicles\(^{[4]}\). Precise localization patterns of keratin protein in normal hair follicles and pilomatrixoma have not yet been extensively examined.

In this study, hair follicular elements of pilomatrixoma compared to normal hair and hair follicles were examined with Rhodamine B and whole keratin antibody.

Materials and Methods

Ten cases of biopsied pilomatrixoma and 5 samples of human scalp from autopsy were used in this study. The specimens were fixed in 10\% buffered formalin and embedded in paraﬃn. The sections were stained with hematoxylin and eosin.

For electron microscopic examination 1 specimen was used. Parts of the tumor tissue were fixed in 2.5\% glutaraldehyde in 0.1 M cacodylate buffer for 2 hrs., refixed in 1.0\% osmium tetroxide and dehydrated and embedded in Quetol 812. Thin sections were stained with uranyl acetate and lead citrate.

For histochemical staining with Rhodamine B, formalin fixed sections were used. Deparaffinized sections were stained with toluidine blue solution for 10 min. and stained with Rhodamin B for 10 min. as described by PINCUS et al.\(^{[5]}\).

For examination of keratin protein, the peroxidase-antiperoxidase (PAP) method was applied to the formalin-fixed sections. Deparaffinized sections were washed with phosphate buffered saline (PBS) and were incubated with normal swine serum for 20 min. Washed sections were incubated with rabbit anti-human whole keratin antibody (DAKO-patts) for 60 min., then with swine anti-rabbit IgG for 20 min. and with PAP complex for 20 min. Diaminobenzidine reaction was performed for 5 min.
Before primary antibody incubation, sections were immersed in 0.1% pronase for 15 min. at room temperature to increase antigenicity.

**Results and Discussion**

Light microscopic findings: The majority of tumor cells in the pilomatrixoma were mainly composed of two types of cells, shadow and basophilic cells. Shadow cells were seen in 10 cases and basophilic cells in 2 cases. The transition of basophilic cells into shadow cells with a gradual loss of their nuclei was also seen. In some areas, parallel or concentric layers of keratinized cells, so-called hair-like structures,[3] were found in all cases. Basophilic granules were occasionally present in keratinized cells (Fig. 1).

Electron microscopic findings: Basophilic cells contained rare localization of keratin filaments in the cytoplasm. Shadow cells showed a gradual increase in the number of keratin filaments and were finally filled with large amounts of keratin masses. However, no granular elements were found in these cells. Parallel or concentric layers of keratinized cells were composed of several layers of imbricated cells which were interlocked. Characteristically dense materials with spherical or irregular shapes probably corresponding to trichohyalin granules were present in the cytoplasm. Various amounts of ribosomes and scattered keratin filaments were also seen. Distinct cell membranes were observed and desmosomes were frequently seen (Fig. 2).

Ultrastructurally, parallel or concentric layers of keratinized cells were considered

![Fig. 1 Tumor cells are mainly composed of shadow and basophilic cells (pilomatrixoma, H-E stain)](image1)

![Fig. 2 Shadow cells [S] are filled with large amounts of keratin masses. Adjacent to shadow cells, parallel layers of keratinized cells [K] are seen. They contain dense materials and keratin filaments and desmosomes are frequently seen. (pilomatrixoma, ×2600)](image2)
to be the cells corresponding to hair cuticular or hair follicular cells. Similar findings have been described by Hashimoto et al.\textsuperscript{[3]} and McGavran\textsuperscript{[8]}.

Histochemical findings: Rhodamine B stain has been described as a staining method for cornified materials in various tissues\textsuperscript{[7]}. Intense staining for Rhodamine B was observed in the inner root sheath in the normal hair follicles and medulla, but no intense staining was present in the outer root sheath in the normal hair follicles (Fig. 3).

Pincus et al.\textsuperscript{[5]} reported that inner root sheath trichohyalin granules in normal hair follicles and medulla stain intensely.

In pilomatrixoma intense staining was observed in some of the parallel or concentric layers of the keratinized cells, while faint staining was present in the shadow cells. No staining was found in the basophilic cells (Fig. 4).

Taking ultrastructural findings into consideration, some of the keratinized cells were regarded as the cells of the inner root sheath in normal hair follicles.

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{fig3.png}
\caption{Intense staining of trichohyalin granules of inner root sheath and medulla. (normal hair and hair follicle, Rhodamine B stain, toluidine blue counterstain)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{fig4.png}
\caption{Intense staining in keratinized cells and faint staining in shadow cells. (pilomatrixoma, Rhodamine B stain, toluidine blue counterstain)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{fig5.png}
\caption{Only the cells of the outer root sheath react positively with whole keratin antibody. (normal hair and hair follicle, PAP method)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{fig6.png}
\caption{The keratinized cells react positively with whole keratin antibody, but no reaction product is present in the shadow cells. (pilomatrixoma, PAP method)}
\end{figure}
Immunohistochemical findings: Positive reaction products for whole keratin antibody were found only in the cells of the outer root sheath in normal hair follicles (Fig. 5). No reaction product was present in the shadow and basophilic cells of pilomatrixoma, while positive reaction products were localized in some of the keratinized cells (Fig. 6).

It has been reported that the keratinization process of hair and hair follicles is different from that of epidermis and only the outer root sheath has a similar process to epidermis\(^8\). It has also been described that keratin antibody recognizes keratin proteins in all layers of squamous epithelium and normal hair follicles\(^4\). However, precise localization patterns in normal hair follicles and pilomatrixoma have not been examined extensively. Our results showed that keratin proteins in some of the keratinized cells have a similar nature to those of the outer root sheath in normal hair follicles.

Conclusions

Histochemical, immunohistochemical and electron microscopic studies were performed on hair follicular elements of pilomatrixoma. It was suggested that the keratinized cells of pilomatrixoma correspond to the cells of the inner and outer root sheath in normal hair follicles.

References