

UNUSUAL CELL TYPES IN THE DORSAL LINGUAL EPITHELIUM OF THE FROG, RANA RIDIBUNDA

Mukaddes EŞREFOĞLU*
Aysel TEMELLİ**
Muammer EŞREFOĞLU***

*İnönü Üniversitesi Tıp Fakültesi,
Histoloji ve Embriyoloji Anabilim
Dalı
MALATYA
**Atatürk Üniversitesi Kazım
Karabekir Eğitim Fakültesi Biyoloji
Bölümü
ERZURUM
*** İnönü Üniversitesi Tıp
Fakültesi Dermatoloji Anabilim Dalı
MALATYA

Yazışma Adresi:
Doç. Dr. Mukaddes Eşrefoğlu
İnönü Üniversitesi Tıp Fakültesi,
Histoloji ve Embriyoloji Anabilim
Dalı,
MALATYA

GSM: 0532 3465239
E-mail: drmukaddes@hotmail.com

Objectives: We investigated ultrastructural features of electron-dense granular cells, mucus and ciliated cells located in the dorsal lingual epithelium of *Rana ridibunda* recently. Within this epithelium, in addition to these cells, there were several kinds of cells. In the present study we investigated electron microscopic features of these cells.

Materials and methods: Six frogs were used in this study. Anterior parts of their tongues were excised and cut into small pieces. These were fixed in 3% glutaraldehyde and 0.1% osmium tetroxide. Specimens were dehydrated in acetone and embedded in Araldide CY212. Ultrathin sections stained with uranyl acetate and lead citrate were examined in an JEOL-100SX electron microscope.

Results: Mitochondria rich-cells, electron-lucent clear cells and plasma cells were observed within the dorsal lingual epithelium of *Rana ridibunda*. Mitochondria-rich cells contained many mitochondria and glycogen granules in their cytoplasm and long microvilli on their free surface. We concluded that cuboid cells that contained a large nucleus, well developed granular endoplasmic reticulum and many lysosomes in their cytoplasm were macrophages. In addition to these cells, many plasma cells which originated from connective tissue were located within the epithelium.

Conclusion: All the cells which we observed within the dorsal lingual epithelium of *Rana ridibunda* are unusual cell types for the dorsal lingual epithelium of mammals.

Key words: Frog, dorsal lingual epithelium, electron microscopy

Rana ridibunda'nın dorsal lingual epitelinde olağan dışı hücre tipleri

Amaç: Daha önceki bir çalışmamızda *Rana ridibunda*'nın dorsal lingual epitelinde yer alan elektron yoğun granüllü hücrelerin, mukus hücrelerinin ve silli hücrelerin ultrastrüktürel özelliklerini incelemiştik. Epitelde bu hücrelerden başka bir kaç hücre türü daha bulunmaktaydı. Çalışmamızda bu hücrelerin elektron mikroskopik özellikleri incelendi.

Materyal ve metod: Çalışmada altı adet kurbağa kullanıldı. Dillerinin ön bölümleri çıkarılarak küçük parçalara ayrıldı. Parçalar %3'lük glutaraldehit ve %1'lik osmium tetroksitle fikse edildi. Asetonla dehidrate edildikten sonra Araldid CY212'ye gömüldü. Uranil asetat ve kurşun sitratla boyanan ince kesitler JEOL-100SX elektron mikroskopta incelendi.

Bulgular: *Rana ridibunda*'nın dorsal lingual epitelinde mitokondriden zengin hücreler, elektron-lüsent şeffaf hücreler ve plazma hücreleri izlendi. Serbest yüzeylerinde uzun mikrovilluslar bulunan mitokondriden zengin hücrelerin sitoplazmalarında bol mitokondri ve glikojen bulunmaktaydı. Geniş nükleusu, iyi gelişmiş granüler endoplazma retikulumu ve lizozomları olan küboid şekilli hücrelerin makrofaj olduğu sonucuna vardı. Bu hücrelere ilaveten epitelde bağ dokusundan kaynaklanan pek çok plazma hücresi yer almaktaydı.

Sonuç: *Rana ridibunda*'nın dorsal lingual epitelinde gözlemlediğimiz bütün bu hücreler memelilerin lingual epitelinde bulunmayan hücre tipleridir.

Anahtar kelimeler: Kurbağa, dorsal lingual epitel, elektron mikroskopi

Stratified squamous epithelium covers the surface of the tongue in mammals.^{1,2} Many features of this epithelium which are closely related to the environmental conditions, varies in the members of different genus and species. In our previous study, we reported undulant,

irregular, branching papillae in the dorsal lingual surface of the frog, *Rana ridibunda* which were not similar to any of the lingual papillae in mammalian tongue. These papillae were lined by pseudostratified columnar epithelium instead of stratified squamous one.

We investigated electron microscopic features of the granular, mucus and ciliated cells located in this epithelium(unpublished data). In addition to these cells, we observed mitochondria-rich cells, electron-lucent clear cells and plasma cells within this epithelium. In the present study, we investigated electron microscopic features of these unusual cell types.

MATERIALS AND METHODS

Six frogs (three male and three female) were used in the present study. The animals were killed by decapitation and the anterior parts of their tongues were excised and cut into pieces. These were fixed in 3% gluteraldehyde buffered with 0.2 M $\text{NaH}_2\text{PO}_4 + \text{NaHPO}_4$ (pH=7.2-7.3), and postfixed in 0.1% osmium tetroxide buffered with 0.2 M $\text{NaH}_2\text{PO}_4 + \text{NaHPO}_4$ (pH=7.2-7.3). Specimens were dehydrated in acetone and embedded in Araldite CY 212. Semithin sections were studied with toluidin blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in an JEOL-100SX electron microscope.

RESULTS

The pseudostratified columnar epithelium covering the lingual papillae was composed of different cell types. By light microscope, some granular and nongranular cells were distinguishable(Figure 1).

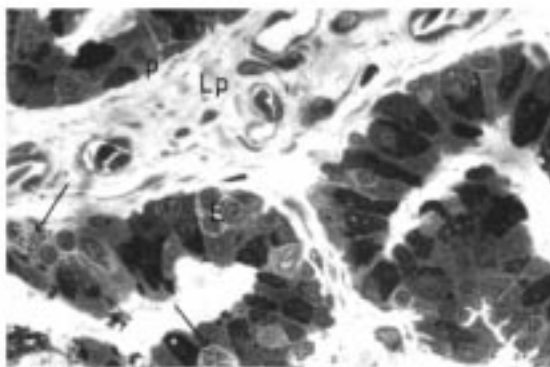


Figure 1. The pseudostratified columnar epithelium(E) composed of different cell types covers the lingual papillae. Some granular and nongranular cells are distinguishable. Lamina propria containing many blood vessels is observed beneath the epithelium. Cuboidal cells with clear cytoplasm are clearly visible(arrows). A plasma cell can be distinguishable(p). Toluidin blue x40.

The large portion of the epithelium was composed of granular, mucus and ciliated cells. In addition to these cells, on rare occasion, columnar cells which lacked electron-dense granules, mucus granules, vacuoles and cilia were observed within the epithelium. In the cytoplasm of these cells, a large number of mitochondria, free ribosomes and glycogen particles were scattered. Nuclei were generally irregular in shape. There were some electron-dense small bodies in the cytoplasm(Figure 2). Microvilli on the free surfaces of the cells were well developed.

Cuboidal cells with clear cytoplasm were visible by light microscope. These cells were located just above the basal membrane, thus their apical side did not reach the free surface of the epithelium. Some of these cells contained electron-dense granules (Figures 1,2). Rough endoplasmic reticulum was generally well-developed and was not usually organized as parallel tubules. The elements of endoplasmic reticulum contained a fine granular material (Figures 3-6). Golgi apparatus was well-developed (Figures 3-5). Mitochondria, ribosomes and many lysosomes were distributed in the cytoplasm (Figures 3-5). The nucleus membrane surrounding the irregular nucleus was indented and perinuclear space was distended (Figures 5,6). The contents of the lysosomes were heterogeneous (Figure 3-5).

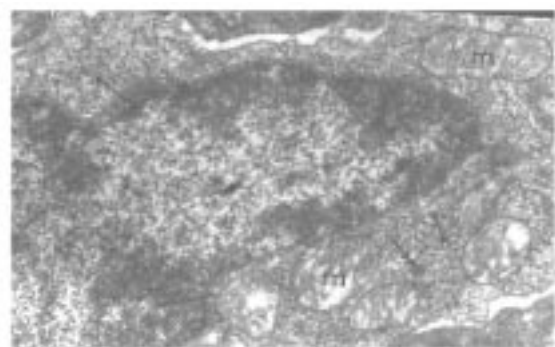


Figure 2. Mitochondria-rich cell which lacks electron-dense granules, mucus granules and vacuoles is observed. In the cytoplasm of these cells a large number of mitochondria(m) and glycogen particles(arrows) are scattered. Nucleus is irregular in shape. There are some electron-dense small bodies(double arrowhead) in the cytoplasm. Uranyl acetate and lead citrate x15.000.

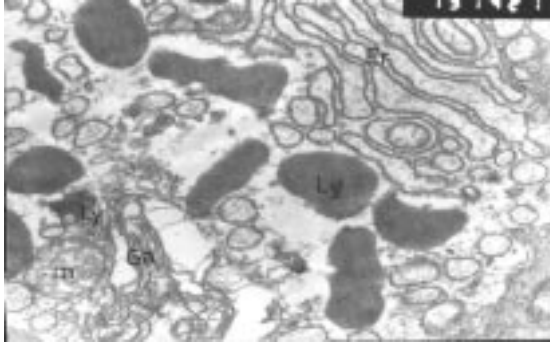


Figure 3. Cytoplasm of the electron-lucent, clear cell. A well-developed rough endoplasmic reticulum(Er) and Golgi apparatus(Ga) are seen. The elements of endoplasmic reticulum contains a fine granular material. Mitochondria(m), ribosomes and many lysosomes(Ly) are distributed in the cytoplasm. The contents of the lysosomes are heterogeneous. Uranyl acetate and lead citratex15.000.

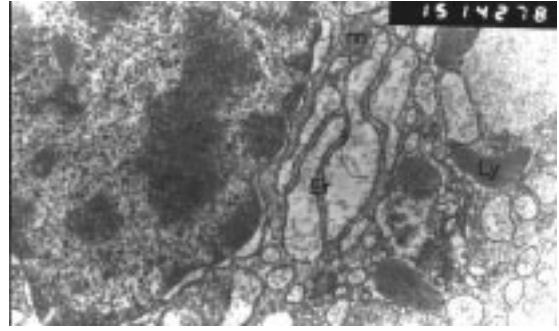


Figure 4. Cytoplasm of the electron-lucent, clear cell. Rough endoplasmic reticulum(Er), Golgi apparatus(Ga), mitochondria(m) and lysosomes(Ly) are observed near the nucleus. The contents of the lysosomes are heterogeneous. Uranyl acetate and lead citratex15.000.

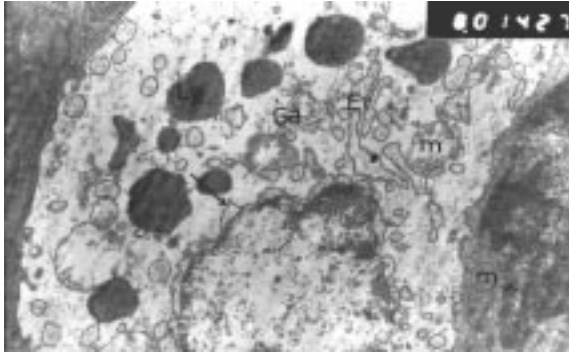


Figure 5. Cytoplasm of the electron-lucent, clear cell. The nucleus membrane surrounding the irregular nucleus is indented and perinuclear space is distended(arrow). There are elements of rough endoplasmic reticulum(Er), a Golgi apparatus(Ga), mitochondria(m) and lysosomes(Ly) in the cytoplasm. The contents of the lysosomes are heterogeneous. Uranyl acetate and lead citratex8.000.

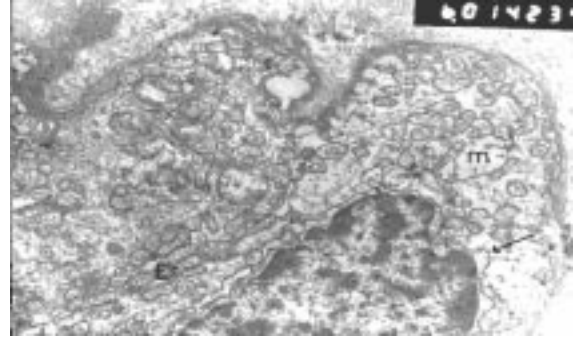


Figure 6. Cytoplasm of the electron-lucent, clear cell. The nucleus membrane surrounding the irregular nucleus is indented and perinuclear space is distended(arrow). There are elements of rough endoplasmic reticulum(Er), mitochondria(m) and ribosomes in the cytoplasm. Uranyl acetate and lead citratex6.000.

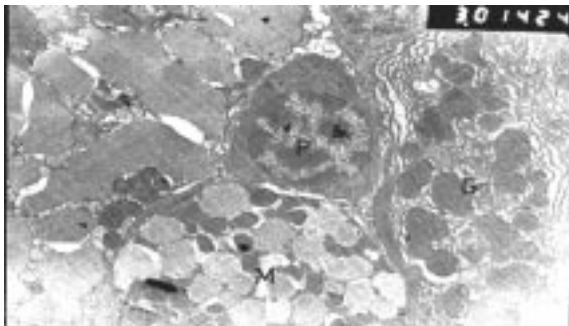


Figure 7. A plasma cell(P) between the granular(G) and mucus cells(M) is observed. Uranyl acetate and lead citratex3.000.

A small number of plasma cells which originated from the connective tissue were seen within the epithelia (Figure 7).

There were no histological differences between males and females with regard to the present results.

DISCUSSION

The cells that lack electron-dense granules, mucus granules and cilia were reported to be located in the dorsal lingual epithelium in *Rana catesbeiana*, *Rana cancrivora* and *Bufo japonicus*.³⁻⁵ These mitochondria-rich cells may very likely be the same kind of cells found in the epidermis of amphibian. The mitochondria-rich cells of amphibian skin have been implicated in the mechanism for passive chloride conductance of chloride ions across the skin.^{3,5} In fish, the active secretion of chloride is carried out by the chloride secreting cells of the opercular epithelium.³ Thus, we suggest that, mucosal epithelium of *Rana*

ridibunda tongue plays a similar role to that of the skin in the passive conductance of chloride ions. The numerous mitochondria in the cytoplasm may function in supplying energy for active ionic exchange. Mitochondria-rich cells have also been recognized in the skin of *Xenopus* that live under conditions of high salinity. These cells may be involved in the elimination of bicarbonate and in osmoregulation because of their capacity to cells are variable in number in different genus and species of the frog.³⁻⁵

In addition to these cells, we observed electron-lucent, clear cells in the epithelium. These cells contained a well-developed rough endoplasmic reticulum, a Golgi apparatus and many lysosomes in their cytoplasm. The nuclear membrane was indented and perinuclear space was distended. We suggested that these cells were macrophages. Macrophages are normally absent in the epithelium in mammals. They are observed in the dorsal lingual epithelium in *Rana cancrivora*.³

Plasma cells located just above the basal membrane have been observed in the dorsal lingual epithelium of *Rana catesbeiana*.⁵ These cells are not present in mammalian epithelium.

transport ions.⁴ The crab-eating frog, *Rana cancrivora* lives in mangrove swamps of high salinity, an environment that is significantly different from the freshwater environments that are typical of habitats of other species of *Rana* (*Rana ridibunda*, *rugosa*, *catesbeiana*). These differences might be expected to affect the fine structure of the lingual epithelium. Mitochondria-rich cells and the other types of

In conclusion, we observed mitochondria-rich cells, macrophages and plasma cells in the dorsal lingual epithelium of *Rana ridibunda*. All of these cells are unusual cell types for the dorsal lingual epithelium of the mammals. To the best of our knowledge, this is the first ultrastructural study about the dorsal lingual epithelium of *Rana ridibunda*.

REFERENCES

1. Fawcett DW. Bloom and Fawcett: Concise Histology; 1st ed. New York: Chapman and Hall. 1997: 182
2. Leeson TS, Leeson CR, Paparo AA. Text/Atlas of Histology; 1st ed. Philadelphia: WB Saunders. 1988: 392-400.
3. Iwasaki S, Wanichanon C. An ultrastructural study of the dorsal lingual epithelium of the Crab-eating frog, *Rana cancrivora*. *Journal Morphol* 1993; 215:89-100.
4. Iwasaki S, Miyata K, Kobayashi K. Fine structure of the dorsal lingual epithelium of the Japanese Toad, *Bufo japonicus*(Anura:Bufonidea). *Zool. Science* 1989; 6:681-689.
5. Iwasaki SI, Kobayashi K. Fine structure of the dorsal lingual epithelium in the Bullfrog, *Rana catesbeiana*. *Zool. Science* 1989; 6:259-267.