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A STUDY OF INTERCALATED / LIGHT CELLS OF DORSAL SKIN IN SOUTH INDIAN FROG *RANA HEXADACTYLA* WITH LIGHT AND ELECTRON MICROSCOPE

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ABSTRACT

In Amphibians, the dorsal and ventral skin differs from each other in structure and function. Histology of both skins show two major type of cells, Principal cells (P-cells) and Intercalated / Light cells (L-cells). Since the morphology of ventral skin has already been demonstrated in our previous study, the authors undertook the present study to observe the morphology and functions of P-cells and L-cells in the dorsal skin. We conclude from the results that P-cells are the major cell type and are found in all four strata of the epidermis of dorsal skin. These P-cells are tightly couple by gap junctions to form a syncytium. L-cells, the minor cell type, are found interspersed among P-cells. Some L-cells are flask shaped while others are polygonal. L-cells are larger than P-cells. L-cells did not display communicating junctions as between P-cells. The ultrastructural observation of L-cells showed absence of mitochondria density, predicting that they are not involved in active ionic transport. The presence of secretory granules in the L-cells indicates that the dorsal skin plays a protective role through its secretion. The pigmentation at the dermo-epidermal junction is responsible for the colouration of dorsal skin. Since ventral skin, according to our finding in previous study, also showed two types of L-cells viz. mitochondria rich cells and cells without mitochondria density, the presence of L-cells in dorsal skin needs to be further explored to know the role of these cells in the ventral as well as in the dorsal skin.

Key Words: *Rana hexadactyla*, Dorsal skin, Epidermis, Principal cells, Light cells

INTRODUCTION

Amphibian skin has several functions. It serves as a protective mechanical barrier (Farquhar and Palade, 1965) helps to maintain water, electrolyte and pH balance, excretes metabolic wastes (Sullivan et al., 2000), serves as a cutaneous sense organ (Koyama et al., 2001) and thermo regulator (Duellman and Trueb, 1994), which also regulates gas exchange (Talbot and Stiffler, 1992).

The ventral abdominal skin plays a role to maintain homeostasis of the body fluid (Feder et al., 1993). The dorsal skin involve in protective function (Goniakowska and Kubiczek, 1998) that have rich nerve supply and serves as a sensory organ (Watts and French, 1985). Both ventral and dorsal are slippery in nature due to the presence of various glands like mucous and serous (Goniakowska and Kubiczek, 1998). The morphology of ventral skin is discussed in our previous publication (Indirani et al., 2009). Literature review revealed that the available information on the epidermis of the ventral skin in amphibian species were gathered from investigations on *bullfrog* (Talbot, 1992), *Pacific tree frog* (Passch and Talbot, 2001), *Bufo viridis* (Katz, 1978), *Rana ridibunda* (Rosenberg and Warburg, 1978), *Rana catesbeiana* (Robinson and Heintzelman, 1987) and *Rana esculenta* (Masoni and Garcia-Romeu, 1979). There are very few studies on the structure and functions of dorsal skin of Indian frog *Rana hexadactyla* (*euphlyctishexadactyla*). Hence the present study focused on the histology of the epidermis and particularly on the distribution and morphology of Principal cells (P-cells) and Intercalated / Light cells (L-cells) in dorsal skin. The L-cells of dorsal skin was studied due to its structural variation from that of the ventral skin.

Research Article

MATERIALS AND METHODS

The adult frogs used in the present study were South Indian origin, *Rana hexadactyla* - *euphyctishexadactyla*. They were bought from the vendor and reared in fresh water frog tank until used. Frogs were anaesthetized with ether and pithed. The study protocol was approved by the institutional research committee and ethical committee and adhered to the legal requirements of the country.

Dorsal skin was incised at midline and reflected. Care was taken not to mechanically damage the skin with the dissecting instruments. The samples were immediately placed in frog Ringer's solution to wash off blood. 1 mm size skin pieces were cut and fixed in 3% glutaraldehyde in phosphate buffer solution at pH 7.4. This was followed by cryoprotection with 4% sucrose solution in phosphate buffer; the samples were osmicated with 2% osmium tetroxide for the appropriate period. After washing off excess osmium, samples were dehydrated in ascending grades of alcohol, cleared in propylene oxide and resin-propylene oxide mixture and embedded in resin. Semithin sections were cut with Lieca ultracut UCT using DIATOME diamond knife; sections were mounted on clean glass slides precoated with poly-L lysine. They were stained with toluidine blue, cover slipped and air-dried. All sections were examined under Light microscopy (LM) and photographed at different magnifications.

From the toluidine blue stained sections, relevant areas of skin were selected and processed for electron microscopic (EM) study. Ultra-thin sections were cut, collected on copper grids and stained with uranyl acetate and lead citrate, air-dried and visualized under Philips EM 201 C Electron microscope at 40 KV. Fields of interest were analyzed at different magnifications and photographed to demonstrate the Principal cells (P-cells) and Intercalated / Light cells (L-cells) in the dorsal skin samples.

RESULTS

Light Microscopic (LM) Study of Dorsal Skin

Four samples of dorsal skin were studied under Light microscopy (LM). The epidermis of dorsal skin comprised of four layers viz. stratum corneum, stratum granulosum, stratum spinosum, and stratum

A

B

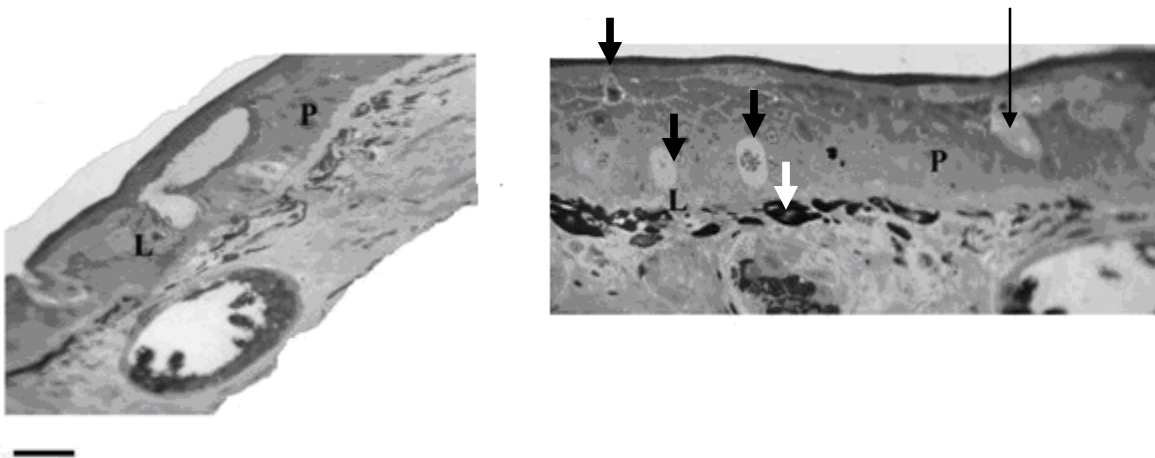


Figure 1A and B. Dorsal skin - Toluidine Blue Stained Light Microscopic (LM) Picture

A. P - Principal cell (P-cell); L - Light cell (L-cell) / Intercalated cell.

P cells are the predominant type; the L-cells are seen dispersed among the P-cells.

B. Some of the L-cells are flask shaped (short arrows) while others are polygonal in shape (long arrow).

The L-cells in dorsal epidermis are larger than P-cells. Note the pigmentation at the dermo-epidermal junction in the dorsal skin (white arrow).

Scale bar: 125 & 165 microns.

Research Article

basale. Two types of cells called Principal cells (P-cells) and Intercalated / Light cells (L-cells) were identified. The P-cells were the predominant type. The L-cells were seen dispersed among the P-cells. L-cells were seen in all layers of epidermis excluding the superficial stratum corneum. Some of the L-cells were flask shaped while others were polygonal in shape. The L-cells in dorsal epidermis were larger than P-cells. Glands were seen in the dermis. The dermo-epidermal junction was marked by pigments. The pigmentation is shown with white arrow (Figure 1A and B).

Electron Microscopic (EM) Study of Dorsal Skin

Four samples of dorsal skin were studied under Electron microscopy (EM). EM picture shows pigmented dorsal skin. L-cells were clearly demonstrated in the stratum basale or stratum germinativum. The P-cells were connected by desmosomes that were absent in the L-cells. These L-cells lacked mitochondrial density hence were not 'Mitochondrial rich cells' (MRCs), but contain dark granules. The dark pigmented layer is clearly seen at the junction of epidermis and dermis (Figure 2A and B).

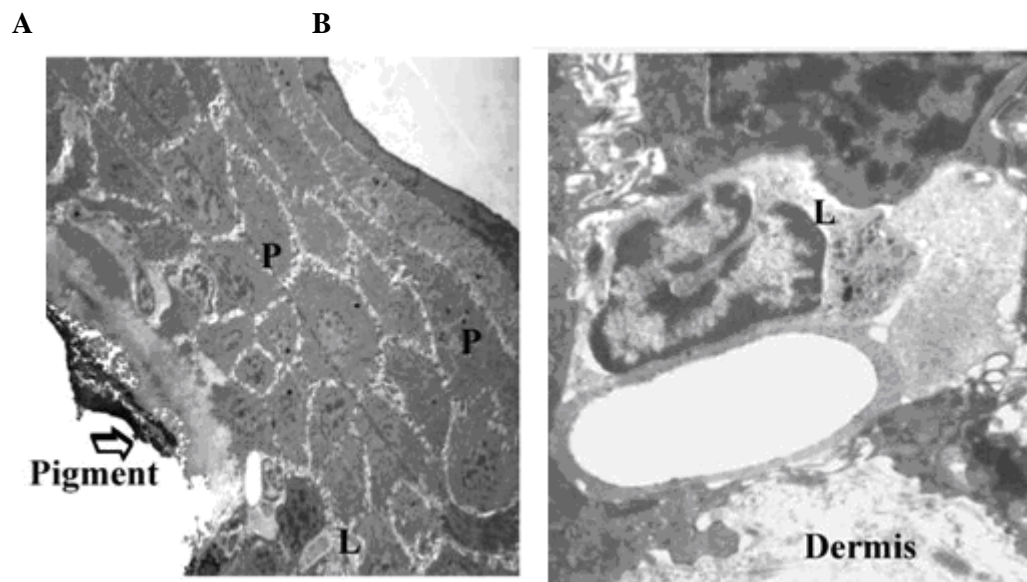


Figure 2A and 2B. Dorsal Skin - Electron Microscopic (EM) Picture

A. P- Principal cell; the desmosomes are clearly seen between the P- cells. The L-cells are shown in the stratum germinativum (the basal layer of the epidermis). Scale bar: 6.2 microns.

B. L- Light cell. A large Light cell lacking mitochondria density, but contain dark granules. Scale bar: 0.8 micron.

DISCUSSION

In human kidney, the second half of the distal convoluted tubules (DCT) and collecting duct (CD) epithelium contain two types of cells namely, Principal cells (P-cells) and Intercalated cells. The P-cells are responsible for the fine-tuning of ionic concentration for body fluid homeostasis. The intercalated cells are involved in acid-base regulation of body fluid and are aligned in-between the P-cells. These intercalated cells are less in number (Fejes-Toth and Naray-Fejes-Toth, 1992). The frog skin also consists of two types of cells similar to human kidney, namely Principal cells (P-cells) and Light cells (L-cells). These cells are also involved in regulation of ionic concentration and pH of body fluid. L-cells of frog skin epithelium are considered similar to the intercalated cells in the renal epithelium (Indirani et al.,

Research Article

2009). L-cells are rich in mitochondria hence also named as Mitochondria Rich Cells (MRCs). The presence of rich mitochondria in cell is an indication of active transport of ions between cell cytoplasm and environment.

In our previous study (Indirani et al., 2009), we showed that the ventral skin epidermis was divided into four strata. The outer mucosal side is formed by a layer of dead cells – the stratum corneum, followed (towards serosal side) by stratum granulosum, stratum spinosum and stratum basale or germinativum. Stratum corneum is formed by a layer of dead cells. The other strata have three types of cells. They are 1) Principal cells or epithelial cells (P-cells), 2) Intercalated cells or Mitochondria rich cells (MR-cells) or Light cells (L-cells) with rich mitochondria and 3) Light cells with less mitochondrial density.

The P-cells are the major cell type in the ventral skin. They are found in all strata of the epidermis; these cells are tightly coupled by gap junctions to form a syncytium (Indirani et al., 2009). These P-cells are mainly involved in ionic transport across the epithelium, especially sodium transport. Even though that the epidermis is multi-layered, it functions as a single compartment with mainly two types of membranes i.e. the mucosal and the serosal membranes. Sodium passively enters the cells through the epithelial sodium channels (ENaCs) and raises the intracellular sodium concentration. To maintain the intracellular negativity these sodium ion would be transported out via the sodium-potassium pump on the serosal membrane (Jared and Rao 2011). Secretion and absorption of potassium is through the P-cell and it determines body fluid homeostasis (Field et al., 1984). Most of the ionic transport would be regulated by the effect of hormones like ADH (Jared et al., 2009), aldosterone (Urbach et al., 1996a), insulin (Blazer-Yost et al., 1998), oxytocin (Schoen et al., 1985), prolactin (Takada, 2005) etc.

The L-cells (MR-cells) have a large number of mitochondria which substantiate the important role of active ionic transport across the ventral skin. L-cells, which do not contain abundant mitochondria, are, probably the precursor cells of MRCs or reserve of MRCs (Indirani et al., 2009). The L-cells, which are minor cell type, interspersed among P-cells. Unlike P-cells, L-cells are not connected to the neighboring cells with gap junctions. They are spread throughout the thickness of the epidermis as seen in other amphibian species.

In the present study, the LM and EM examination of dorsal skin sample elaborated some facts.

Firstly, dorsal epithelium has four strata similar to ventral skin (Indirani et al., 2009). This study demonstrated two types of cells in the dorsal skin of the amphibian viz. the P-cells and the L-cells. The P-cells are the major cell type in all four strata of dorsal skin and are tightly coupled to each other by gap junctions to form a syncytium.

Secondly, the dorsal skin showed pigmentation which is responsible for the dark colouration. The P-cells have more pigments which absorb sunlight and are responsible for skin colouration and also responsible for formation of Vit-D (Goniakowska and Kubiczek, 1998).

Thirdly, L-cells of dorsal epidermis were different from the ventral skin. The L-cells of dorsal epidermis as seen in EM do not belong to Mitochondria Rich cells (MR-cell) type. These cells may not have role in ionic regulation. In *Rana hexadactyla* ventral skin is more important in acid / base balance and ion exchange as it touches the water surface more than dorsal skin. This could be the reason why dorsal skin lacks MR-cells in this species. Further study is required to understand the role of these L-cells in the dorsal skin.

Fourthly, the presence of secretory granules in the L-cells proved that dorsal skin is involved in protective function through its secretion.

Fifthly, the L-cells are not exclusively flask shaped in all layers (Whitaker, 1975). It is a mixture of flask shaped as well as polygonal shape cells.

Sixthly, this study also proved the presence of L-cells both in superficial as well in the deep layers of the epidermis. The remarkable finding of this shape and position of L-cells are inter-related to each other. Previous histochemical studies have shown that mature L-cells are present in the superficial layer of epidermis (Brown et al., 1981). However, Denefle et al., (1987), demonstrated L-cells in the deeper layer of epidermis also. According to many authors, mature L-cells are positioned at the superficial layer of the

Research Article

epidermis in the adult and indistinguishable L-cells permanently exist in stratum germinativum (Zaccone et al., 1986). Because the L-cell in the superficial layer is flask shape and cell in the deep layer is polygonal in its shape. This proves that flask shaped L-cells are mature cells positioned in the superficial layer whereas polygonal cells are immature cells located in the deeper layer of the epidermis.

Seventhly, as expected, our ultrastructural study has demonstrated a relatively clear space surrounding all demonstrable L-cells, indicating the absence of gap junction between P-cells and L-cells (Blankemeyer and Shahin, 1993).

Finally we found that, irrespective of the shape, all L-cells are larger than the P-cells.

We conclude from our results that there are two classes of cells viz. P-cells and L-cells (non-mitochondria rich) in the dorsal skin of *Rana hexadactyla*. The P-cells are the major cell type and found in all four strata of the epidermis of dorsal skin. These P-cells are tightly coupled by gap junctions to form a syncytium. The pigmentation showed at the dermo-epidermal junction, is responsible for the dark colouration of dorsal skin. The L-cells are the minor cell type, interspersed among the P-cells. The ultrastructural observation of L-cells showed low mitochondria density, which suggests revealed that these cells are not involved in active ionic transport. The presence of secretory granules in these cells suggests their role in protective function through its secretion. The L-cells in the superficial layer are flask shaped mature cells while cells in the deeper layer are polygonal immature cells. The presence of L-cells in the germinal layer of the epidermis is a novel observation. The L-cells do not form a structural syncytium suggesting an independent role of each cell. The L-cells are larger than P-cells.

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Research Article

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