

## **Three-dimensional morphology of the novel desmin-immunopositive perivascular (DIP) cell in rat anterior pituitary gland by using FIB/SEM tomography**

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Besides the hormone-producing cells, there were other cell types which did not produce hormones in the anterior pituitary gland, i.e., folliculo-stellate cell, capsular fibroblast, macrophage, endothelial cell and pericyte. Recently, a new cell type of perivascular cell - novel desmin-immunopositive perivascular (DIP) cell - was identified in the anterior pituitary of rat by immunoelectron and conventional transmission electron microscopy (Jindatip et al. 2012). This cell type totally differed from pericyte which also occupied in the perivascular space in the gland (Figure 1). The novel DIP cell was stained by desmin and had a unique rough endoplasmic reticulum (rER) characteristic. However, the actual morphology of this novel DIP cell has not been well clarified. In this study, therefore, we introduced the technique of focused ion beam scanning electron microscopy (FIB/SEM) with three-dimensional (3D) reconstruction to reveal the external morphology and rER feature of the novel DIP cell including the pericyte.

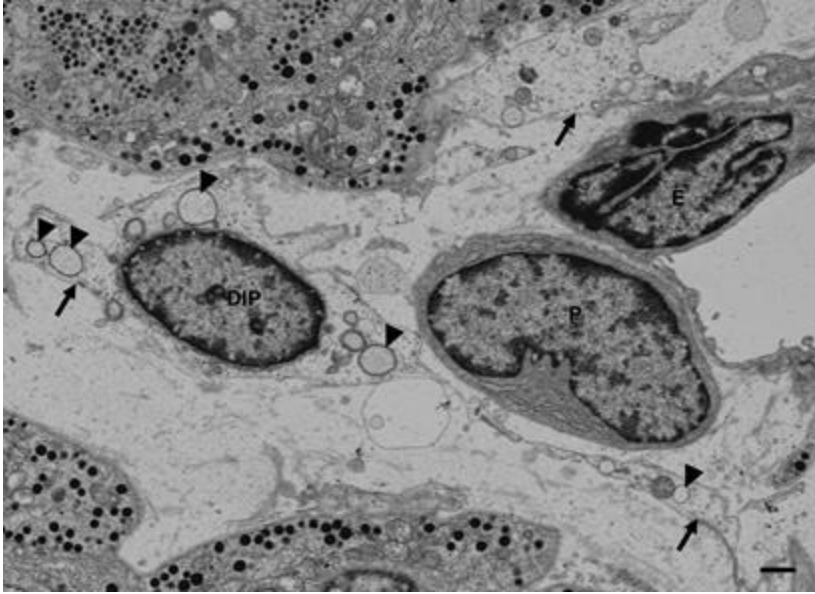
Pituitary epoxy resin blocks were cut for semithin and ultrathin sections to observe the capillary areas under light and conventional transmission electron microscopy. All specimens were coated by an ion sputter coater, and then placed into the vacuum chamber of the FIB/SEM. Selected area on the resin block was milled away 60 nm by using the gallium ion beam and imaged by scanning electron microscopic system with back-scattered electron detector. This process was repeated until finished the entire cell.

FIB/SEM tomography and 3D analysis clearly showed that the novel DIP cells had large cytoplasm and multidirectional expanded processes, whereas pericytes exhibited small cytoplasmic area and extended their fine processes on the capillary wall. It is noted that some processes of the novel cells also reached to the vessel. However, these processes were loosely wrapped the capillary when compared to those of pericytes. Additionally, we succeeded in clarifying the 3D characteristic of dilated rER. Almost rERs were glomus shape and dispersed throughout the cytoplasm (Figure 2). Moreover, we found a single primary cilium which protruded from the cytoplasm of the novel DIP cell during the observation of FIB/SEM tomographs. This cilium extended from mother centriole of centrosome and orthogonally arranged with the daughter centriole. The results of the present study clearly show that FIB/SEM tomography and 3D reconstruction are necessary for getting a better insight into the real characteristics of the novel DIP cell.

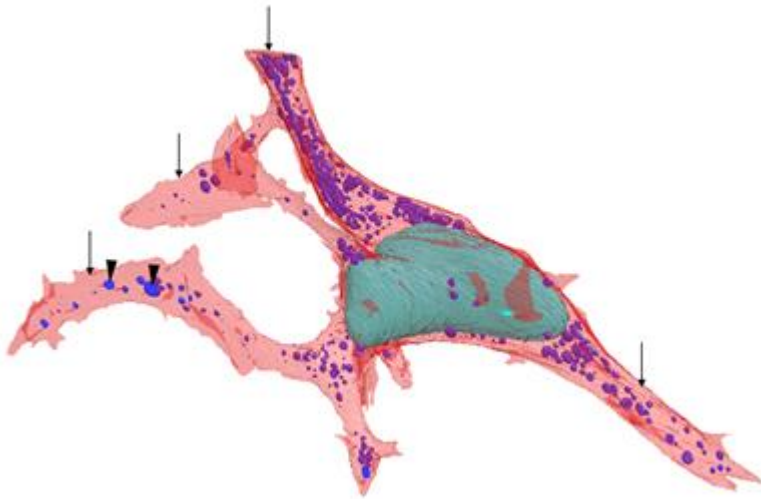
### Reference

Jindatip D, Fujiwara K, Kouki T, Yashiro T (2012) Transmission and scanning electron microscopy study of the characteristics and morphology of pericytes and novel desmin-immunopositive perivascular cells before and after castration in rat anterior pituitary gland. *Anat Sci Int* 87:165 - 173

The authors acknowledge funding from the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (CU-56-638-HR).



**Figure 1.** Conventional transmission electron microscopy of anterior pituitary gland of adult normal rats. Pericyte (*P*) and novel desmin-immunopositive perivascular cell (*DIP*) occupy in the perivascular space. Shown are cytoplasmic processes (*arrows*) and rER of DIP cell (*arrowheads*). *E*, Endothelial cell. Bar 1  $\mu\text{m}$



**Figure 2.** Three-dimensional reconstruction of FIB/SEM serial micrographs of the novel desmin-immunopositive perivascular cell. Note multidirectional processes (*arrows*) containing a number of glomus rER (*arrowheads*). *green*, nucleus, *pale-red*, cytoplasm