Studies on ganglion cell-like (GL) cells in the skin of Djungarian hamsters (*Phodopus sungorus*)

(ジャンガリアンハムスター (*Phodopus sungorus*) の
皮膚神経節細胞様 (GL) 細胞に関する研究)

Summary

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There are specific cells in the dermis of the abdominal and thoracic skin of Djungarian hamsters (*Phodopus sungorus*), which have been called ganglion cell-like (GL) cells. Microscopically GL cells have one or two ovoid nuclei and abundant basophilic, foamy cytoplasm and mimic ganglion cells. GL cells more often appear in males than in females and the foci increase in size and number after sexual maturation. Immunohistochemically the nuclei of GL cells are consistently positive for androgen receptor (AR), and the cytoplasms are always positive for vimentin. In addition, the stroma of the foci includes fibers positive for type I and II collagen. These results suggest that the cells may have an androgen-dependent biological behavior and an ability of collagen fiber production. However, the detailed nature and function of GL cells remain unclear.

In this study, the author tried to confirm the *in vivo* reactivity of GL cells to androgen, paying attention to the fact that GL cells express androgen receptor (AR) and find out a lectin specific to GL cells by lectin histochemistry and thereafter identify the core protein modified with its lectin-binding glycan by MALDI-TOF MS analysis and western blotting.

1. Androgen–dependent biological behavior on ganglion cell-like cells in the skin of Djungarian hamsters (*Phodopus sungorus*) following gonadectomy (Chapter II)

To confirm whether GL cells have androgen-dependent biological behavior, the changes of GL cells in the skin of Djungarian hamsters following gonadectomy were evaluated histologically. Both sexes were gonadectomized at the age of 4 weeks, and then necropsied at the age of 18 weeks. The growth grade, distribution, and proliferative activity of GL cells in the thoracoabdominal and dorsal skins of gonadectomized and

intact animals were evaluated. The castrated males showed more lowered growth grade and proliferative activity, and smaller distribution than the intact males. Regarding these 3 parameters similar trends were seen between ovariectomized and intact females, and between intact males and intact females.

These results suggest that GL cells of Djungarian hamster have sex difference in its distribution and proliferative activity, and that androgen is involved in the development of GL cells.

2. Morphologic changes of GL cells in the skin due to the *in vivo* long-term testosterone stimulation in gonadectomized Djungarian hamsters (Chapter III)

To access the effect of androgen on GL cell proliferation, after being gonadectomized, both sexes were given low-dose (5mg/kg) or high-dose (20mg/kg) of testosterone propionate (TP) subcutaneously once every week for short-term (12wks) or long-term (24wks) period and were evaluated similarly in Chapter II. In both sexes of short-term and long-term TP treated groups, an increase in growth rate and proliferative activity of GL cells was found in a dose-dependent manner. Moreover, in the low-dose groups the thickness of foci and proliferative activity of GL cells were higher in long-term treated animals than in short-term treated ones. On the other hand, there was no apparent term-related difference in both sexes in the high-dose groups.

The growth inhibition of GL cells occurred following gonadectomy similarly in the Chapter II, but dose-dependent proliferation of GL cells was induced by TP administration. From these results, GL cells have high sensitivity for androgen such as testosterone and androgen is strongly involved in proliferation of GL cells.

3. Lectin binding of GL cells in the skin of Djungarian hamsters (Chapter IV)

To detect a new marker of GL cells besides AR and vimentin, lectin histochemistry using 8 lectins: Con A, DBA, PNA, RCA_{120}, SBA, UEA-I, WGA and succinylated WGA (sWGA) was performed. WGA and sWGA specifically reacted to GL cells; Con A reacted to GL cells as well as adjacent stratified muscle fibers. GL cells were consistently negative for remaining 4 lectins.

This indicates that GL cells contain certain proteins glycosylated with *N*-acetylglucosamine (GlcNAc) and/or sialic acid. Thus, WGA lectin is thought to be available as a marker of GL cell.

4. Identification of WGA lectin-conjugated protein in GL cells in the skin of Djungarian hamsters (Chapter V)

Since WGA lectin specifically reacted to the cytoplasm of GL cells in the skin as described in Chapter IV, the author speculated that the protein modified with WGA lectin-binding sugar chain may be associated with the function of GL cells. Therefore, the glycosylated proteins eluted from the abdominal skin tissue of Djungarian hamsters were purified using WGA lectin affinity column and subjected to MALDI-TOF MS analysis and western blotting. The results suggested the presence of MGAT2 (mannosyl (alpha-1,6-)-glycoprotein beta-1,2-*N*-acetylglucosaminyltransferase) and β-actin.

Recent studies have revealed that β-actin functions not only as cytoskeleton for the maintenance of cell shape, but also as signal transduction molecules associated with the transcriptional regulation. Although the significance of glycosylated β-actin remains obscure at present, the possibility exists that this specific glycosylation may take a

functional role of GL cells.

In conclusions, it became evident that androgen had a significant influence on the growth of GL cells of Djungarian hamsters. Furthermore, it became clear that glycosylation with GlcNAc by MGAT2 and/or silalic acid took place in the cytoplasm of GL cells and that β-actin was one of those glycosylated proteins. In the future, the specific reactivity with WGA lectin may be available as a new marker of GL cells to elucidate the functional role of the cells. Although the author speculated about their secretion of a pheromone-like material, it was impossible to be demonstrated.