Observation of Cultured Adipogenic Stem Cell Differentiation by Transmission Electron Microscopy

Kahyung Han[1,2], Eun-Kyung Park[1,2], Chang-Hyun Park[3], and Chang-Sub Uhm[1,2]

[1]Department of Anatomy, [2]Brain Korea 21 Biomedical Sciences, [3]Electron Microscope Facility, Korea University College of Medicine, Seoul, 136-705 Korea

Adipose tissue is an important energy-storaging tissue. Recently, stem cells in the adipose tissue draw attentions. These stem cells are able to self-renew and capable of differentiation along multiple lineages. Last year, we reported the adipogenesis of the stem cells isolated from adipose tissue by scanning electron microscopy. In this study, to understand the adipocyte differentiation, cultured stem cells were observed by transmission electron microscopy.

Preadipocytes are adipogenic stem cells directed to differentiate into adipocytes. Preadipocytes were isolated from perirenal fat tissue of male Sprague-Dawley rats and cultured using DMEM (Dubelcco's modified Eagle's medium) with 10% FBS in 5% CO_2 atmosphere at 37°C. Differentiation was classified into 4 stages based on the degree of the lipid accumulation. Cells of each differentiation stage were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) overnight and post-fixed with 1% osmium tetroxide in the same buffer for 1 hr. Cells were dehydrated using ascending series of ethyl alcohol and were embedded in Epon 812. Thick sections were stained with toluidine blue. Thin sections of the selected area were cut with a Ultracut E (Leica) ultramicrotome using a diamond knife. Sections were double-stained with uranyl acetate and lead citrate. And, stained sections were observed with a Hitachi H-7500 TEM.

At stage I, preadipocytes were characterized by well-differentiated rough endoplasm reticulum (rER) with extremely expanded lumen filled with furry material. Many lysomes are scattered throughout the cytoplasm. Cytoskeletons were also charactristic structures. Some area contained a bundle of intermediate filaments. At stage II, most characteristic findings were the appearance of small lipid droplets, and many small mitochondria in the cytosol. In some cells, numerous small lipid droplets filled the cytosol. In most cases, lysosomes and dilated rER were not

present. Few scattered rER were present. Golgi apparatus was present, but the cisternae were shrinked. These lipid droplets grew to multiple large lipid droplets. At stage III, nucleus was displaced to the cell periphery or was bean-shaped due to the accumulated lipid droplets. Also, several large lipid droplets came to contact each other. Mitochondria had granular matrix. At stage IV, cytoplasm was present only at the periphery of the cell. Lipid droplets coalesced to form one single large droplet. At the periphery of lipid droplet, small irregular lipid droplets, which had not fused, were present.

Throughout the observation, developing adipocytes were characterized by large vesicular nuclei with prominent nucleoli, rather active protein synthetic activity, evidenced by many mitochondria, rER, and Golgi apparatus. Lipid appears in the cytoplasm as small droplets, which eventually fused to form a large droplet.

This study shows that during the early stages of adipocyte differentiation, protein synthesis was more prominent than energy storage. The organelles related to the energy storage and metabolism such as lipid droplet, mitochondria, and sER tend to increase following cellular differentiation. Further study on the nature of protein synthesized during the early period of adipocyte differentiation, and their relation to the energy metabolism is needed.

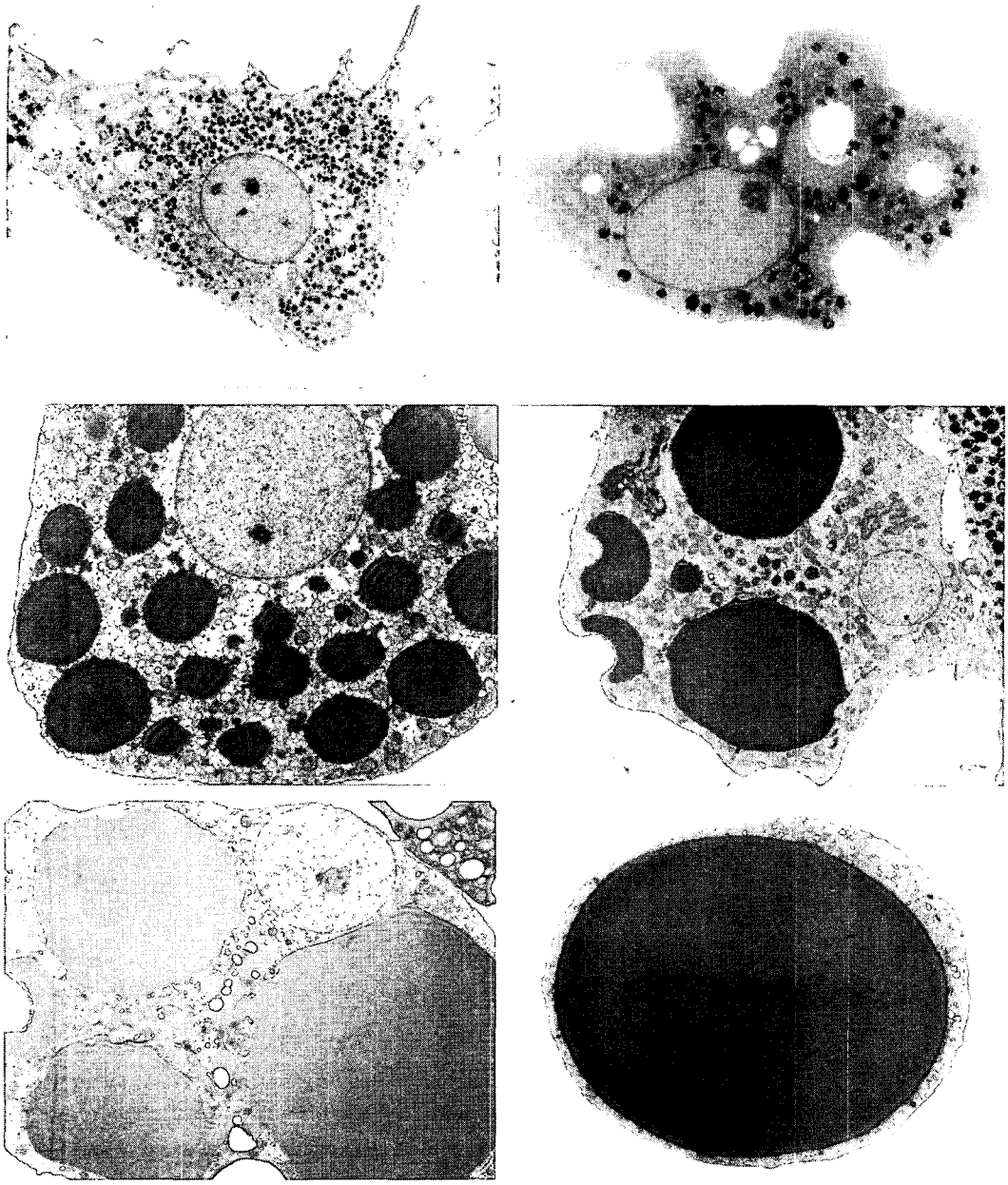

Figure. Differentiating adipocytes at various stages. As cell differentiates, small lipid droplets appeared in the cytoplasm, which eventually fused to form large lipid droplets and to displace the nucleus to the periphery.