学位論文内容の要旨

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学位論文題名

Adiponectin is partially associated with exosomes in mouse serum

（マウスの血清においてアディポネクチンの一部はエキソソームに存在する）

Exosomes are membrane vesicles with a size of 30–120 nm that are released by many different cell types. They have been found in many biological fluids including blood, bronchoalveolar lavage fluid, urine, bile, and breast milk. Exosomes harbor a wide variety of proteins, lipids, mRNA, and microRNA, which can be transferred to another cell, and are implicated in cell-to-cell communication by transferring molecules. Thus, exosomes play an important role in physiological and pathophysiological processes and also are applicable as a source of disease biomarkers.

Previous studies suggest that adipocyte-derived exosomes play a role in cell-to-cell communication during the development of metabolic diseases. However, the characteristics and function of exosomes released from adipocytes *in vivo* remain to be elucidated. Clearly, exosomes released from adipocytes could exist in the circulation. In addition, because the composition of exosomes is heterogenic, depending on the cellular origin of the exosome, adipocyte-derived exosomes could be accompanied by molecules produced specifically in adipocytes. In this context, this study postulated that such molecules associated with exosomes in the serum could be markers for adipocyte-derived exosomes *in vivo*. This study particularly focused on secretory proteins produced specifically in adipocytes, namely adipocytokines including adiponectin, leptin, and resistin.

1. Serum adiponectin is partially associated with exosomes

Based on western blotting, CD63, a well-known protein marker of exosomes, was concentrated in the pellet of mouse serum after ultracentrifugation, suggesting successful isolation of exosomes. Western blotting detected adiponectin but no leptin

and only trace amounts of resistin in the exosome fraction. After ultracentrifugation on a discontinuous gradient, both adiponectin and CD63 were detected in a fraction at a density of 1.17 g/mL, consistent with the density of exosomes. The adiponectin signal in the exosome fraction was decreased by proteinase K treatment and completely quenched by a combination of proteinase K and Triton X-100. These results suggest that a portion of adiponectin exists as a transmembrane protein in the exosomes in mouse serum.

2. Exosome-associated adiponectin may be a physiologically relevant form

Adiponectin exists as low-molecular-weight (LMW), middle-molecular-weight (MMW) and high-molecular-weight (HMW) forms in the circulation, and the latter has more relevant roles in its physiological functions (e.g., protecting metabolic diseases). Western blotting following SDS-PAGE under nonreducing and unheated conditions indicated that, although MMW forms are the predominant form in the serum, HMW forms are present principally in the exosome fraction. These findings suggest that exosome-associated adiponectin and exosome-free adiponectin may have different physiological and pathological functions *in vivo*. Indeed, quantitative ELISA showed that the concentration of adiponectin in the serum and the ratio of adiponectin to total protein in the exosome fraction were lower in obese mice than in lean mice.

In conclusion, this study showed that serum adiponectin is partially associated with exosomes in mice. Considering that adiponectin is produced exclusively by adipocytes, adiponectin-associated exosomes in serum could be derived from adipocytes. This study proposes that adiponectin could be a marker for exosomes released from adipocytes *in vivo*.