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学位論文要旨
Dissertation Abstract

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論文題名 Title of Doctoral Dissertation: Exosome-Based Molecular Transfer Activity of Macrophage-Like Cells Involves Viability of Oral Carcinoma Cells: Size Exclusion Chromatography and Concentration Filter Method (マクロファージ様細胞のエクソソーム分子送達能は口腔癌細胞の活性に関与する：サイズ排除クロマトグラフィー濃縮フィルター法の考案)

論文内容の要旨（2000字程度） Dissertation Abstract (approx. 800 words)

Extracellular vesicles (EV) heterogeneity is a crucial issue in biology and medicine. In addition, tumor-associated macrophages are key components in cancer microenvironment and immunology. However, macrophage-derived EVs heterogeneity and their effects on receiver cancer cells have not been well understood.

In the present study, our aims were as follows: (i) to separate different sized EVs (such as small exosomes, large exosomes, and large EVs) from macrophage-like cells, (ii) to investigate the EV-based molecular transfer of palmGFP (approximately 30kD) to receiver oral carcinoma cells, and (iii) to examine whether the macrophage-EVs altered the cell viability of receiver oral carcinoma cells.

A human monocytic leukemia cell line THP-1 was differentiated to CD14-positive macrophage-like cells by stimulation with PMA (phorbol 12-myristate 13-acetate) but not M1 or M2 types.

We developed a combination method of size exclusion chromatography and concentration filters (SEC-CF) and aimed to characterize different EV types by their size, cargo types, and functions. Using the SEC-CF method, we first fractionated the culture supernatant into 20 fractions (Fractions 1, 2, 3…20). To simplify the EV analysis, we concentrated these fractions into the following three groups using concentration filters: Fr. 1–6, Fr. 7–9, and Fr. 10–20. In Fr. 1–6 of the particle size was between 100–500 nm and peaked at 208.9 nm, suggesting that Fr. 1–6 contained large EVs (larger than exosomes). In Fr. 7–9, the particle size ranged between 50–300 nm with a peaked size of ap-proximately 150 nm, suggesting large exosomes (EXO-L) from the size. In Fr. 10–20, the size of particles was smaller than 100 nm and peaked at approximately 40 nm, which could contain small exosomes (EXO-S).

To characterize the small and large exosomes or larger EVs using protein markers, we next performed Western blotting of tetraspanins (CD9 and CD63 are established EV markers), HSP90α, HSP90β (often found in EVs), and β-actin. CD9 was markedly detected in the Fr. 7–9. On the other hand, CD63, another tetraspanin family member often found in EV appeared in Fr. 10–20 (EXO-S).

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For a molecular transfer assay, we developed a THP-1-based stable cell line producing a GFP-fused palmitoylation signal (palmGFP) associated with the membrane. The receiver human oral carcinoma cells were fused with tdTomato (palmT). The THP1/palmGFP cells were differentiated into macrophages producing palmGFP-contained EVs. After adding conditioned medium collected from THP1/palmGFP and macrophage/palmGFP to the HSC-3/palmT cells in a 3D culture system, we noticed that macrophage-derived palmGFP was highly transferrable to receiver oral carcinoma cells compared to monocyte-derived palmGFP, suggesting that EVs enclosing palmGFP could alter molecular transfer efficiencies. Then, the macrophage/palmGFP EVs were prepared by SEC-CF method and applied to HSC-3/palmT. The molecular transfer of macrophage/palmGFP EVs can be visualized and monitored. We found EXO-S and EXO-L efficiently transferred the palmGFP to HSC-3/palmT, as compared to other EV types.

We fractioned the macrophage/palmGFP and concentrated them into seven groups: Fr. 1–3, Fr. 4–7, Fr. 7–9, Fr. 10–12, Fr. 13–15, Fr. 16–18, and Fr. 19–21. To molecualrly characterize these fractions, we carried out western blotting of GFP, CD63, and HSP90α. GFP was detected in the Fr. 7–9 (EXO-L), Fr. 13–15, Fr. 16–18, and Fr. 19–21 (EXO-S), suggesting that the EXO-L and EXO-S cotained palmGFP. Indeed, CD63 was detected in Fr. 13–15, Fr. 16–18, and Fr. 19–21, suggesting that these fractions contained EXO-S. HSP90α was also detected in the same fractions (Fr. 13–21).

We also established intercellular communication experiments using a conditioned medium (CM) and a transwell-based co-culture system. To confirm whether macrophage-secreted factors alter the recipient cell's viability, we examined the ATP content of the HSC-3 cells after receiving macrophage-secreted factors in the CM or in the co-culture system, and found macrophage-secreted factors decreased the viability of oral carcinoma cells. In addition, we prepared macrophage-derived EVs by SEC-CF method and confirmed that the macrophage-secreted EXO-S and EXO-L significantly reduced the cell viability (ATP content) in oral carcinoma cells.

Taken together, using SEC-CF method, we could separate different subtypes of EVs from macrophages: (i) rare large EVs (500–3000 nm) reminiscent of apoptosomes, (ii) EVs (100–500 nm) reminiscent of microvesicles (or microparticles), (iii) EVs (80–300 nm) containing CD9-positive large exosomes (EXO-L), (iv) EVs (20–200 nm) containing unidentified vesicles/particles, and (v) EVs (10–70 nm) containing CD63/HSP90-positive small exosomes (EXO-S) and particles. The SEC-CF method is useful for the purification of large and small exosomes with higher molecular transfer activities, potentially enabling efficient molecular delivery to target cells. In addition, the molecular transfer activities of exosomes from macrophage-like cells can reduce viability in oral carcinoma cells.