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論　文　内　容　要　旨

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学位論文題目	Potential solutions for certain hurdles of the implementation of exosomes in drug delivery （ドラッグデリバリーにおけるエキソソームの臨床応用に向けた基礎的検討）

Exosomes (Exos) are nano-sized extracellular vesicles (EVs) which are secreted by various cell types including tumor cells. The potential usage of Exos as delivery vehicles particularly to tumor is promising and increasingly expanding due to their credentials, such as in vivo stability due to their endogenous origin, their innate ability to carry macromolecules to target cells, the presence of a set of uptake-related surface proteins on their surface which can also be engineered for targeting specific cell types. Although these characteristics reveal the preponderance of cancer cell-derived Exos as drug delivery system to tumor, many challenges for that emerging field still exist. For instance, the low yield of secreted Exos, uncertain uptake mechanism, unpredictable Exo biodistribution and poor specific cell-targeting of systemically administered Exos hinders their implementation in tumor targeting delivery. Hence, the aim of this research was to try to overcome such hurdles.

To increase Exo yield, I attempted to stimulate tumor cells via the addition of liposomes in vitro. Neutral-, cationic-bare or PEGylated liposomes were incubated with four different tumor cell lines. The stimulatory effect of liposomes on Exo secretion and cellular uptake propensity of the collected Exos by autologous cells or allogeneic cells was evaluated. Both neutral- and cationic-bare liposomes enhanced Exo secretion in a dose-dependent manner. Fluid cationic liposomes provided the strongest stimulation. Surprisingly, the PEGylation of bare liposomes diminished Exo secretion.

Then, to see if the incubation of cells with liposomes would change the biological properties of these Exos, I investigated the surface proteins and the uptake mechanism of the harvested Exos. Interestingly, Exos induced by solid cationic liposomes lacked some major exosome marker proteins such as CD9, flotillin-1, annexin-A2 and EGF, and subsequently showed lower levels of cellular uptake upon re-incubation with donor cancer cells. However, Exos induced under normal condition and by fluid cationic liposomes, displayed the entire spectrum of proteins, and exhibited higher uptake by the donor cancer cells. Although endocytosis was the major uptake pathway of Exos by tumor cells, endocytosis could occur via more than one mechanism. Higher Exo uptake was observed in donor cells than in allogenic cells, indicating that donor cells might interact specifically with their Exos and avidly internalize them.

Later on, to enhance Exos circulation time and hence their tumor accumulation, PEGylation of Exos was conducted. Then, the biodistribution of intravenously injected PEGylated Exos, namely autologous C26-Exos and allogeneic B16BL6-Exos, were evaluated in C26 tumor bearing mice. Both Exos were remarkably accumulated in the tumor tissue. In addition, the tumor accumulation of autologous Exos was more predominant than that of allogeneic Exos. Overall, the obtained findings indicate that the physicochemical properties of liposomes determine whether they will act as a stimulant or as a depressant on Exo secretion from tumor cells. In addition, liposomes of varying physicochemical properties might control the characteristics of secreted Exos such as the expression of Exos proteins and Exos uptake. Finally, PEGylated autologous cancer cell-derived Exos can be a promising delivery platform to target tumor cells and that may be fruitfully exploited in cancer therapeutics and diagnostics.