International Mini-Seminar:
Application of Extracellular Vesicles "Exosomes" for Future Therapy
大阪府立大学 研究推進機構 第 31 回ケミカルバイオロジー研究所セミナー
大阪府立大学 大学院理学系研究科 第 101 回生物科学フロンティアセミナー

Date: **April 23 (Tue), 2019**
Time: **3:15pm-5:35pm**
Location: **Osaka Prefecture University, Nakamozu Campus**
Bldg. C10, Rm. 512 (5F)
Organizer: **Ikuhiko Nakase** (*Associate Professor, Graduate School of Science, Osaka Prefecture University*) (E-mail: i-nakase@21c.osakafu-u.ac.jp)

3:15pm-3:20pm
Opening Remarks: Ikuo Fujii
(*Professor, Graduate School of Science, Osaka Prefecture University*)

3:20pm-3:35pm:
Biofunctional Peptide-Modified Exosomes for Intracellular Delivery of Therapeutic Molecules
Ikuhiko Nakase
(*Associate Professor, Graduate School of Science, Osaka Prefecture University*)

3:35pm-3:55pm:
Clinical Application of Mesenchymal Stem/Stromal Cell Therapy for Central Nervous System Diseases
Hidetaka Nishida
(*Associate Professor, Department of Veterinary Science, Osaka Prefecture University*)

3:55pm-4:15pm:
Pharmacokinetics of Exosomes Evaluated by Using Exosome Labeling Methods
Yuki Takahashi
(*Associate Professor, Graduate School of Pharmaceutical Sciences, Kyoto University*)

Special Lecture
4:15pm-5:30pm:
Engineered Extracellular Vesicles for Drug Delivery Editing
Niek Dekker
(*Principal Scientist, Associate Director Biomedical Assay Development, AstraZeneca*)

5:30pm-5:35pm:
Closing Remarks: Ikuhiko Nakase
(*Associate Professor, Graduate School of Science, Osaka Prefecture University*)

Special Lecture

Engineered Extracellular Vesicles for Drug Delivery Editing

Niek Dekker

(*Principal Scientist, Associate Director Biomedical Assay Development, AstraZeneca*)

Abstract

Extracellular Vesicles (EVs) represent an exciting opportunity as biological delivery vehicle for therapeutic cargo with excellent safety, low intrinsic immunogenicity, cell specific tropism and biological delivery efficiency. There are multiple approaches for the introduction of protein and RNA cargo into EVs, including physical, chemical and cell engineering. We have engineered Expi293F suspension cells with transient expression of fusion proteins for reversible loading with protein cargo with examples for Cre recombinase and Cas9 for CRISPR gene editing. We have developed a single molecule fluorescence microcopy technique to quantify cargo loading at a single particle level, showing excellent loading for GFP fusions with CD63 with on average 70 copies of the fusion protein per particle. Functional delivery of Cre recombinase, as measured in a reporter cell line, was dependent on addition of small molecule or peptide enhancers of endosomal escape. Using RNA-binding proteins fused to exosomal markers we were able to enrich EVs with RNA cargo with up to 10-fold higher loading of sgRNA compared to loading from passive mass redistribution. Human Expi293F cell-derived EVs did not trigger any significant immune response in vitro in human blood. The *in vivo* assessment following a single intravenous administration of these EVs in BALB/c mice did not reveal marked haematological changes, cytokine induction or histopathological effects. Labeling of EVs using fluorescent mCherry, luminescent NanoLuc or radio-isotope 111Indium marker allowed for on line analysis of bio-distribution *in vivo*. Opportunities of naïve and engineered EVs for drug discovery and their potential for therapeutic applications will be discussed.