Mechanism investigation of low-temperature plasma selective killing effect on human breast cancer cell

L. Du[1], C. Miron[2], T. Motooka[3], M. Hori[2], M. Mizuno[3], S. Toyokuni[2,4], H. Kajiyama[3], and H. Tanaka[2]

[1]*Department of Electronics, Graduate School of Engineering, Nagoya University, Nagoya, Japan*
[2]*Center for Low-temperature Plasma Sciences, Nagoya University, Japan*
[3]*Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan*
[4]*Department of Pathology and Biological Responses, Nagoya University, Nagoya, Japan*

Abstract: In this contribution, we report that low-temperature plasma-activated solution induced MCF-7 cell source exosome showed an inhibition effect on MCF-7 cells, which suggests that the expression of exosomes and information in exosome was changed due to plasma-activated solution.

1. Introduction

Plasma-activated solutions selectively kill cancer cells while less harm to the normal cells were reported. [1,2] My former research confirmed that the Plasma-treated L-arginine (PTA) solution has selective killing effects on breast cancer MCF-7 cells.[3] Exosome is a kind of small vesicle generated from cells, size around 30-200 nm, contains protein, RNA, etc., as information, and plays an important role in cell-to-cell communication.[4] After PTA treats MCF-7, the exosomes generated from MCF-7 may change and the research about exosomes during this process is necessary to explain the mechanism of PTA selective killing effect.

2. Methods

MCF-7 cells were cultured in DMEM medium with 10% FBS and 1% P/S, incubated at 37 ℃, 5% CO2. After 80% confluent, remove the medium and use Advanced DMEM with 1% GlutaMax supplementary incubate for 48 h, then collect the supernatant for exosome extraction. Centrifuge the supernatant at the speed of 150,000g for 70 min to gather exosomes. Observe the 1000-fold diluted exosomes by Nanosight to analyze exosome particle number, size and size distribution. The exosome samples were adsorbed onto a 200-mesh copper grid and negatively stained with 2% uranyl acetate, observed using TEM to examine the morphology of exosomes. MTS assay was performed to identify the effects of MCF-7 source exosomes and PTA-treat MCF-7 source exosomes on MCF-10A.

3. Results and Discussion

Nanosight results showed that both the MCF-7 source and PTA-treated MCF-7 source exosomes have similar size and size distribution, but after PTA treatment, the cells generated fewer exosomes than before. TEM showed both types of exosomes have similar morphologies. However, the PTA-treated MCF-7 source exosomes have higher protein concentration was confirmed by Bradford reagent protein assay.

The results of MTS assay showed that MCF-7 cell source exosomes increased MCF-10A cell viability dependent to culture time and exosome dose, but PTA-treated MCF-7 source exosomes lost the promotion effect on MCF-10A at the same dose and time. We believe that during the process of PTA treatment, MCF-7 cells were influenced by PTA and reacted to stimulation, leading to the information, such as protein and RNA contained in the exosome, were changed.

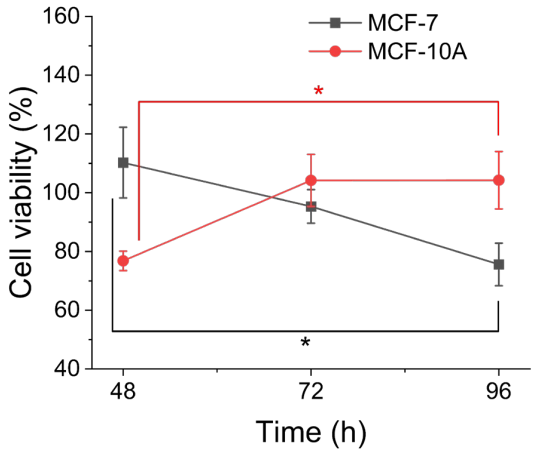

Fig. 1. PTA treated MCF-7 source exosomes decreased MCF-7 cell viability.

4. Conclusion

Investigation of exosomes from PTA-treated MCF-7 and untreated MCF-7 showed there are some changes between the two different types of exosomes, proving that PTA treatment on MCF-7 changed the exosome expression.

Acknowledgment

This material is based upon work supported by Grant-in-Aid for Specially Promoted Research (No. 19H05462) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Miron, C. *et al.* *Free Radical Research* **0**, 1–14 (2023).
[2] Tanaka, H. *et al.* *Sci Rep* **6**, 36282 (2016).
[3] Du, L. *et al.* Plasma Processes and Polymers n/a, e2400192 (2024).
[4] Kalluri, R. & LeBleu, V. S. *Science* **367**, eaau6977 (2020).