Cytokine-Induced Cancer Cell-Derived Migrasomes Proteomic Analysis

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Migrasomes are extracellular vesicles released from migrating cells, playing critical roles in intercellular communication[1]. Cytokines, such as tumor necrosis factor α (TNF-α) and IL-6, play an important role in the body's response to infection, inflammation, or injury[2]. IL-6 amplifier is a phenomenon of excessive secretion of IL-6 by immune cells and non-immune cells. IL-6 amplifier starts when the immune cells receive massive inflammatory signals[3]. Our results showed that the peptide interface increased migrasomes formation and retained the migrasomes on the peptide substrates after cell detachment[4]. Applying this method, migrasomes derived from cancer cells that stimulated with IL-6 increased the IL-6 expression in the cells cultured afterward.

However, cytokine stimulation's impact on the IL-6 amplifier condition in cancer-derived migrasomes remains underexplored. This study aims to investigate the proteomic changes in migrasomes derived from MDA-MB-231 cells, a triple-negative cancer cell line under an IL-6 amplifier condition. Cells were cultured under standard conditions with serum-containing medium, starvation conditions by replacing to serum-free medium, and subjected to IL-6 stimulation. Migrasomes released by cells were isolated using differential centrifugation. Gene expression was analyzed via quantitative PCR, and proteomic profiling was conducted using mass spectrometry.

As a result, 1,339 proteins commonly expressed across conditions, with Ferroptosis and Proteasome related protein groups, were upregulated after starvation and cytokines stimulation based on the KEGG Pathway analysis results. The JAK-STAT pathway's role in inflammation, cell survival, and immune regulation directly intersects with ferroptosis and proteasome. Therefore, we tried to identify the expression of genes involved in the JAK-STAT pathway in migrasomes after stimulation by IL-6, namely JAK1, STAT1, and STAT3, respectively, also the suppressors of cytokine signaling (SOCS) protein group, namely SOCS1 and SOCS3. Following starvation, the expression of IL-6, JAK-1, SOCS-1, and SOCS-3 in migrasomes was upregulated, but subsequent cytokine stimulation led to downregulation. Conversely, STAT-1 (pro-inflammatory) was increased during starvation and reduced after stimulation, while STAT-3 (anti-inflammatory) exhibited the opposite results. This suggests that migrasomes are involved in signaling modulation under stress conditions under starvation and IL-6 stimulation.

1) Ma, L., et al., Cell Res. **2015**, 25 (1), 24–37. 2) Yuan, S., et al., Front. Immunol. **2021**, 12, 659419. 3) Murakami, Y. et al. Sci. Rep. **2017,** 7, 1, 44042 4) Saito, S., et al., Mater. Sci. Eng., C **2021**, 124, 112495.