The effects of released exosomes from stimulated NK-Cells with IL15 on the apoptosis of HL-60 cell line

(Experimental Hematology / Oncology Section)

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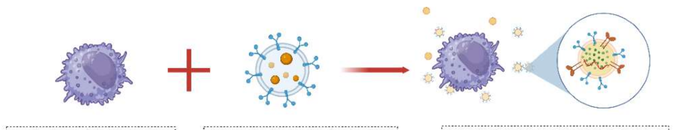
INTRODUCTION

Acute myeloid leukemia (AML) is a prevalent hematologic malignancy in adults over 60, treated primarily with chemotherapy, which can cause significant side effects like fatigue, nausea, and severe hair loss. Consequently, researchers are exploring alternative treatments like immunotherapy to enhance recovery and minimize complications. Immunotherapy modifies immune cells to boost the body's defense against tumors, but it faces challenges such as cancer cells' evasion tactics and hostile tumor environments. Among the immune cells, Natural Killer (NK) cells are crucial early responders that destroy cancer cells with cytotoxic proteins and receptors, enhanced by cytokines like IL-2 and IL-15. However, their efficacy is hindered by tumor-derived suppressive factors. Research on NK cell-derived exosomes, which carry NK cell components like perforin and NKG2D, shows promise. These nanoparticles, adapted to acidic environments, can deliver lethal doses to cancer cells more effectively than their source cells, presenting a novel approach in immunotherapy.



NK cell features:
- Perforin, Granzyme and Fas-L
- NK markers like: NKG2D, CD56, NCRs and etc.

Exosome features:
- Low immunogenicity
- Biostability
- source cell cargo
- Exosome markers: CD81, CD63 and etc.

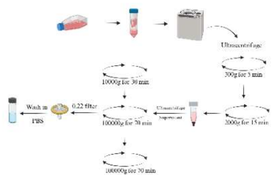
NK- derived exosome features:
- Anti-tumor ability did not diminish in the present of TGF-b and IL-10
- More perforin and Fas-L in comparison with NK cell.
- More tendency to low-PH environment

OBJECTIVE

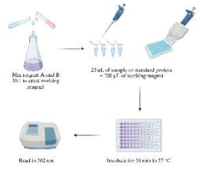
- To determine whether IL-15 could change the cytotoxicity of NK-92-derived exosome against AML-M3 leukemia.
- Compare the final yield of exosome between stimulated NK-92 with IL-15 and control group.

METHODS

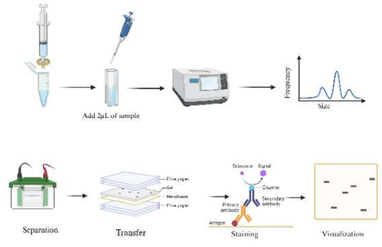
1) Ultracentrifuge
Purpose: Exosome isolation



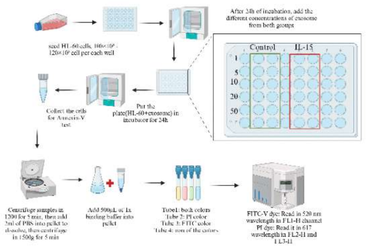
2) BCA test
Purpose: Exosome concentration determining



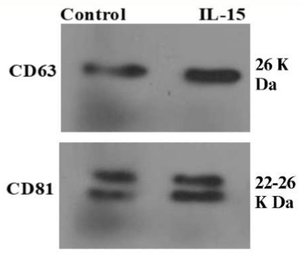
3) DTL and Western blotting test
Purpose: For exosome size and distribution and CD63 & CD81 determining



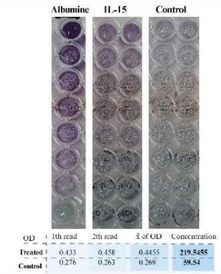
4) Annexin-V test
Purpose: Cell apoptosis detecting



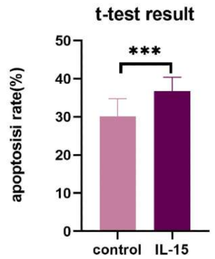
IL-15, EXOSOME YIELD & CYTOTOXICITY INCREASEMENT



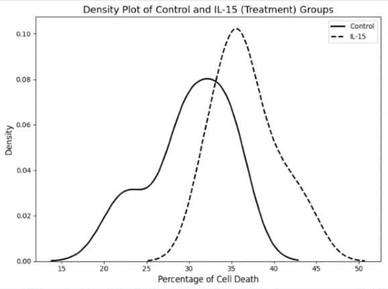
- Thicker bands of CD63 and CD81 in the exosomes derived from NK-92 cells stimulated with IL-15 western blotting test.



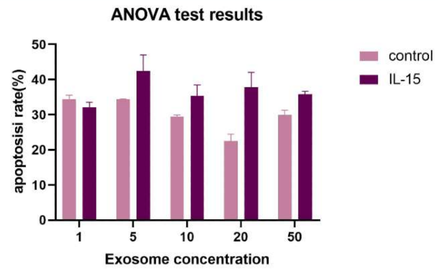
- The higher optical density and concentration observed in the exosomes derived from NK-92 cells stimulated with IL-15 in the BCA test.



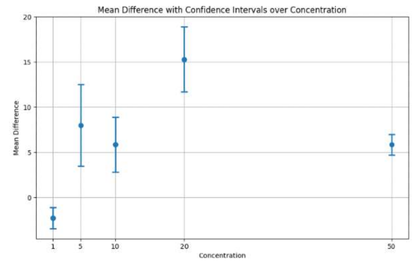
- The results of the t-test derived from the analysis of the data demonstrate that the results are significant.



- Density plot: This could imply that IL-15 has a consistent and pronounced effect on increasing cell death among treated cells, making it a potentially effective treatment in promoting cell apoptosis.



- ANOVA test, shows the difference between the control and treatment group in each concentration



- Welch's *t*-test: P-values associated with IL-15 concentrations are notably low. This suggests that a significant proportion of the variability in apoptosis rate can be explained by the model, which likely accounts for the effect of IL-15 at different concentrations.

CONCLUSION

- The addition on IL-15 to the NK-92 medium leads to a higher exosome yield.
- The exosomes from the IL-15 pre-treated group enhance the apoptosis rate compared to the control group.
- In future researches, treatment group and control group's cargo could be assessed.

REFERENCES

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- Using natural killer cell-derived exosomes as a cell-free therapy for leukemia. *Aladin Samara. Hematological Oncology. 2022*
- NK cell-derived exosomes enhance the anti-tumor effects against ovarian cancer by delivering cisplatin and reactivating NK cell functions. *Heyong Luo. Frontiers in Immunology. 2023*

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