ImmunoTools *special* Award 2024

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Restoring of NK Cell Function of Cancer Patients by NLRP3 Inflammasome Inhibition

Introduction

Natural killer (NK) cells are immune cell population crucial for anti-tumoral response and can be easily accessed from peripheral blood. However, many cancer patients struggle with defects in NK cells' cytotoxic functions.

NLRP3 inflammasome is a multimeric protein complex present in the endoplasmic reticulum and cytosol of immune cells. Its activation plays a crucial role in the recognition and immune response to pathogens.

However, dysregulation of NLRP3 plays a crucial role in driving several pathologies associated with chronic inflammation, including cancer. The high expression of NLRP3 in tumors is overall associated with overall poor prognosis and worse survival rates.

The NLRP3 inflammasome can play distinct roles within cancer progression and the tumor microenvironment (TME) and is still not well understood. Recent study of Blanquart et al. (2024, Blood) has characterised a NK cell population with reduced LFA-1, CD16 and CD226 markers, which is characterised by adhesion defects and reduced effector functions. Moreover, increase in this LFA-1^{low}CD16^{low}CD226^{low} population of NK cells is associated with worse prognosis in melanoma patients and correlates with patients' clinical outcomes. Based on RNAseq data presented in this

study, increasement of this NK cell population highly correlated with NLRP3 upregulation in cancer cells. However, the study did not further focus on the NLRP3 inflammasome.

Hypothesis

I suggest identifying LFA-1^{low}CD16^{low} population of NK cells might be not only great prognostic marker in cancer, but it might also be a valuable predictive biomarker for NLRP3-targeted treatment. My hypothesis is that interaction of immune cells with cancer cells bearing the NLRP3 upregulation leads to enhancement of this NK cell subpopulation and contribute to poor prognosis. The inhibition of NLRP3 may have positive effect on reduction of LFA-1^{low}CD16^{low} NK cell population and restoring the NK cell anti-tumoral cytotoxic capacity.

Project aims

1. Build **FlowISiAM panel for NK cells' phenotyping**.
2. Analyse **NK cells' subpopulations changes** after the co-cultivation with cancer cells with or without NLRP3 amplification.
3. **Restoring anti-tumoral cytotoxic capacity of NK cells** by analysing NK cells' subpopulations changes after the co-cultivation with cancer cells with cancer cells with NLRP3 amplification and NLRP3 inhibition by small molecular inhibitor MCC950.
4. Analyse the overall **potential of LFA-1^{low}CD16^{low} NK cell population as prognostic biomarker** in cancer.

Methodology

In this study, we will isolate immune cells from peripheral blood of healthy donors and prostate cancer patients. In addition, cancer cell lines PC3 (NLRP3 amplification) and LNCap (no NLRP3 amplification) will be involved. For addressing all of project's aims, ImmunoTools NK cells' phenotyping panel is required. For this panel CD56-FITC was chose as a robust marker of NK cells. To distinct T cells from NK cells CD3-PerCP (marker of T cells) will be used. To analyse the NK cell population of our interest, the next markers are: CD16-PE (marker of NK cells, differs in NK cell subpopulations) and CD11a-APC (subunit of LFA-1 adhesive molecule on NK cells). Subsequently, ELISA assay for IL-10 will be performed to analyse the regulatory properties of NK cells. CD226 in not father considered as it is associated especially

with melanoma. In addition, induction of NK cell apoptosis will be evaluated by Annexin V-APC analysis. Since IL-1beta might be involved in inducing the NK cell phenotype, we will also test NK cells changes in its presence.

Conclusion

In this project, I would like to focus on NK cells phenotyping and especially on the LFA-1^{low}CD16^{low} NK cell population. This award would help me to build a panel for analysis of NK cells phenotype within these settings. It might not only contribute to the understanding the impact of NLRP3 on TME, but LFA-1^{low}CD16^{low} population of NK cells can also become a great prognostic and target in malignant diseases.

ImmunoTools *special* AWARD for **Katerina Kalkusova** includes 10 reagents

FITC - conjugated anti-human CD56

PE - conjugated anti-human CD16

PerCP - conjugated anti-human CD3

APC - conjugated anti-human CD11a, Annexin V

recombinant human cytokines: rh IL-1beta

human IL-10 ELISA set (counted as 4 reagents)

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