

**302 ALLOGENIC ADOPTIVE NK CELLS AND IRRADIATION
JOIN FORCE TO CONTROL TRIPLE NEGATIVE BREAST
CANCER STEM CELLS**

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Background Previously, our group has shown the long-term synergistic effects of allogeneic expanded natural Killer (NK) cells and Irradiation in a human triple-negative breast cancer (TNBC) model. Primary tumor irradiation enhanced the migration and infiltration of adoptive NK cells into tumor microenvironment (TME), hence increasing the NK cell's ability to lyse tumor cells. Although RT alone successfully controlled primary tumor, however, the primary tumor bioluminescence intensity was significantly reduced only in the RT+NK group compared to the RT alone. To clarify this phenomenon, we investigated cancer stem cells (CSC) population correlated with radioresistance and metastatic propensity.

Methods We analyzed CSC phenotype in human TNBC after irradiation in a time- and dose-dependent manner by using multiparameter flow cytometry. Ex vivo expansion of NK cells from human peripheral blood mononuclear cells was performed by co-culture with conventional K562 cells. NK cell cytotoxicity against CSCs were evaluated under irradiated TME. Mammosphere was generated to investigate the efficacy of combination therapy against CSC. Allogenic NK cells were i.v injected twice after local tumor irradiation in human TNBC xenograft model. Single cells suspension of tumors was prepared on day 14 after treatment and analyzed with flow cytometry

Results We identified ALDH+CD44+CD24- CSC phenotype showing increased absolute number of MDA-MB-231 CSCs population after fractionated RT compared to single-dose RT. Interestingly, NK cells effectively killed CD44+CD24- CSC population in response to an increased effector to target ratio. Combined NK cells with RT significantly decreased CD44+CD24- population of tumors both single-dose and fractionated RT ($p=0.0104$ and $p=0.0188$). We observed a significantly increase NK cells' lysis ability against TNBC mammosphere both single-dose RT ($p=0.0011$) and fractionated RT ($p=0.0145$) compared to non-irradiated group. However, there was no synergistic effect of NK cells and irradiation against parental TNBC cells. Moreover, allogenic NK cells prefer to lyse mammosphere but not parental cells when co-cultured sphere and parental cells together. NK cells combined with single-dose irradiation more effectively killed TNBC mammosphere than fractionated irradiation ($p=0.0212$).

Conclusions Allogenic NK cells effectively killed breast-stem like cells. Combination therapy enhances NK cells antitumor response against breast cancer stem-like cells, suggesting that elimination of stem like cells by NK cells is closely correlated to long-term primary tumor control under irradiated TME. For RT dose scheme when combined with NK therapy, single-dose irradiation improved antitumor response against breast cancer stem-like cells.

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