A Potential Role for Peripheral Natural Killer (NK) Cell Activity Induced by Preoperative Chemotherapy in Breast Cancer Patients

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INTRODUCTION

• Tumor-infiltrating lymphocytes, including cytotoxic T lymphocytes and natural killer (NK) cells, play a key role in achieving a pathologic complete response to neoadjuvant chemotherapy (NAC) in patients with breast cancer.
• The prognostic value is predictive of good outcomes in patients with human epidermal growth factor receptor-2 (HER-2)-positive or triple negative (TN) breast cancer, but not in patients with luminal type (L) cancer.
• NK cells are critical for innate immunity-mediated eradication of tumor cells in a way that involves a cooperative relationship with T-cell amplified adaptive immunity.
• The precise role of NK cells in human breast cancer remains unclear, and the behavior of NK cells following NAC remains to be clarified.

AIMS

• To investigate the functional role of peripheral NK (pNK) cells in terms of systemic immune response to NAC in patients with breast cancer.
• To assess pNK cell activity and analyze whether it was associated with pathologic therapeutic efficacy of NAC and tumor subtype.
• To assess the relationship between systemic and local immune responses to NAC, tumor microenvironmental factors (TMEFs) were evaluated.

METHODS

• Patients: Thirty-nine patients with stage II-IV breast cancer received NAC between July 2012 and August 2017 at our clinic.
• Measurement of pNK cell activity: Peripheral NK cell activity was measured in blood samples collected immediately before and three weeks after NAC.
• Assessment of TMEFs: TMEFs were assessed by next-generation sequencing in a subset of 26 patients, and disappearance of axillary lymph node metastases (Ax+as) was analyzed in a subset of 23 patients, for CD8, CD45, NK, FOXP3, CTLA-4, PD-1, PD-L1, IL-2, IL-6, IL-12, IFN-γ, IL-10, TGF-β, and VEGF in formalin-fixed paraffin embedded sections collected from preoperative vacuum-associated biopsy samples and surgical specimens.
• Statistical analysis: Continuous variables were analyzed with Mann-Whitney tests and categorical variables were analyzed with chi-square tests. Independent variables were analyzed with Mann-Whitney tests. Univariate and multivariate analyses were performed to evaluate associations between clinicopathologic factors, pNK cell activity, and TMEF levels. Odds ratios (ORs) are reported with 95% confidence intervals (CIs). A p-value less than 0.05 was considered statistically significant.

RESULTS

• Pathologic therapeutic effect: G1a (n = 8), G1b (n = 13), G2a (n = 7), G2b (n = 4), G3 (n = 7). The PCR (y = 0.36 + 1.24x) rate was 17.9%.
• Changes in pNK cell activity and their association with clinicopathologic factors: Increased pNK cell activity was associated with the disappearance of Ax+ (Table 1). In multivariate analyses, increased pNK cell activity following NAC was associated with disappearance of Ax+ (OR = 1.41, 95% CI: 1.19-24.52; p = 0.023). Increased pNK cell activity did not associate significantly with any tumor subtype.
• Involvement of TMEFs in therapeutic effect following preoperative chemotherapy: Increased pNK cell activity tended to be associated with decreased PD-L1 after chemotherapy. A G2 or better therapeutic effect was associated significantly with high post-NAC levels of CTLA-4, and nonsignificant trends was observed with respect to pre- and PD-L1 and TGF-β levels potentially being related to better therapeutic effect (Table 1). In multivariate analyses, nonsignificant trends suggestive of a G2 or better effect potentially being associated with higher NK after NAC (OR = 2.61, 95% CI: 0.995-7.09; p = 0.058), and increased TGF-β before chemotherapy (OR = 0.995, 95% CI: 0.990-1.000; p = 0.379).
• Factors related to disappearance of Ax+: A significant relationship between Ax+ disappearance and tumor subtype, with HER-2 positive and TN tumors was observed. Disappearance of Ax+ was significantly associated with high pre-NAC CTLA-4 levels and elevated post-NAC CD4 levels (Table 2). The disappearance of Ax+ tended to show increases in CD4 and decreases in CTLA-4 from pre-NAC to post-NAC assessments.

HYPOTHESIS

A hypothesis of systemic and local immune activation interactions in the therapeutic effect induced by NAC in patients with breast cancer. According to this model, in NAC responders, elevated pNK cell activity works cooperatively with not only a release from local suppression of antitumor immunity (imposed by tumor cell expression of PD-L1) and decreased CTLA-4 expression in Treg cells, but also with local-immunity activation of CD4-expressing and NK cells in the presence of IL-12 in the tumor microenvironment. Increased TGF-β may contribute to activation of tumor immunity, contrasting with its otherwise inhibitory functions.

CONCLUSIONS

• The presence of CD4+ and NK cells within tumor-infiltrating lymphocyte population profiles may favor NAC-induced antitumor immunity in breast cancer patients.
• PD-L1 and CTLA-4 in formation of the immunosuppressive tumor microenvironment suggest that immune checkpoint inhibitors targeting PD-L1 and CTLA-4 may improve NAC efficacy for breast cancer.
• Good NAC treatment responders were found to exhibit post-NAC TGF-β increases, though it is unclear whether increase is related to a functional activation of antitumor immunity or an overwhelmed attempt by tumor cells to further suppress immunity.
• Systemic activation of pNK cell activity may improve elimination of metastatic tumors in breast cancer patients owing to local release from immunosuppression and immune activation in the tumor microenvironment.

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