UNIVERSITI TEKNOLOGI MARA

TUMORIGENIC ACTIVITY OF EOSINOPHILS IN BREAST CANCER MOUSE MODEL POST-IRRADIATION THERAPY

NURHASLINA BINTI HASAN

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ABSTRACT

COVID-19 pandemic forcing everyone to adapt to a new norm, including cancer management. Due to the minimal risk of cross-infection, hypofractionation radiotherapy was suggested, although its efficacy and toxicity are still unknown. The potential and mechanism of action of radiotherapy on the tumour microenvironment (TME) has become a matter of concern as it either has the capability to induce immunogenic cell death that might attract the systemic immune response known as abscopal effect, or it just an unintentional or bystander effect. One unsolved finding related to radiotherapy and the TME was an increased gene expression of Eosinophil-associated ribonuclease family 11 (EAR11) following 7.5 Gy irradiation that potentially enables eosinophils to modulate immune response. However, up to date, the role and the recruitment of eosinophils remain unclear. Thus, this present study was divided into three main objectives; 1) to develop the custom-made lead shield for targeted irradiation of breast cancer mouse model based on calorimetric changes of Gafchromic film; 2) to investigate the effect of single targeted 2 Gy and 8 Gy absorbed doses of gamma irradiation on the circulating immune cells particularly eosinophils and those infiltrating the TME's and 3) to investigate the effect of IL-33 sensitisation alone or with the combination of radiotherapy on the immune cell recruitment and the tumour progression. Objectives 2 and 3 also measured the cytokine concentration correlated to either pro- or anti-tumorigenic activity. As a result, 28 Gy and 75 Gy delivery doses were required to achieve the respective 2 Gy and 8 Gy absorbed doses, using the 5 cm thickness of the lead shield. In Study 2, the untreated mice tumour model presented with an unaffected systemic immune population ant significant splenomegaly. The early response of single targeted irradiation was reduced systemic total white blood cells (TWBC) with a significant decrease in eosinophils and basophils' absolute count and percentage. Subsequently, the exposure with 8 Gy gamma irradiation in the tumour model significantly increased the percentage of systemic neutrophils and monocyte. Overall, the immune suppression was dose-dependent, with 8 Gy irradiation shown more significant suppression compared to 2 Gy irradiation with both doses presented with a short-term immunosuppression effect. In contrast, irradiation-treated groups showed a significant decrease in neutrophils but an increased lymphocyte percentage within the TME. In line with these findings was a declined in protumorigenic cytokines that are IL-6, GM-CSF, and VEGF. In Study 3, instead of inducing the eosinophils, the tumour model sensitised with IL-33 presented with systemic neutrophilia and lymphopenia. The sensitised and the non-sensitised tumour model exposed to 8 Gy gamma irradiation showed a similar immune suppressive effect except for significant temporary neutropenia. There was a significant increase in $CD45^{+}$ cells and neutrophils in acute phase compared to early phase across the sensitised tumour models with or without irradiation treatment. A significantly slower tumour growth was observed in synergistic effect between IL-33 sensitisation and single targeted 8 Gy irradiation. As conclusion, the single targeted 8 Gy gamma irradiation produced using the customised lead shield, caused an increased eosinophil percentage within the TME with the concentration of cytokines possess the anti-tumorigenic activity. Subsequently, IL-33 triggered the neutrophils infiltration instead of eosinophil that supressed the lymphocyte population within the TME. However, further investigation with a more extended experimental period is needed to understand the detailed mechanism.

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