PD-1/PD-L1 modulation by nivolumab and gamma-ray irradiation in a radioresistant EMT6 mouse mammary carcinoma model

Mohammad Johari Ibrahim[1], Nur Fatihah Ronny Sham[1], Narimah Abdul Hamid Hasani[1], Harissa Husainy Hasbullah[2], Wan Nor I'Zzah Wan Mohammad Zain[1], Suraya Othman[3], Nora Julianna Osman[3], Muhammad Khalis Abdul Karim[4], Syed Baharom Syed Ahmad Fuad[5], Nurhaslina Hasan[6]

[1]Department of Biochemistry & Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Malaysia, [2]Oncology Unit, Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Selangor, Malaysia, [3]Department of Surgery, Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Selangor, Malaysia, [4]Faculty of Science, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Selangor, Malaysia, [5]Department of Anatomy, Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Selangor, Malaysia, [6]Faculty of Dentistry, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Selangor, Malaysia

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ABSTRACT

Radiotherapy resistance poses a significant challenge in breast cancer management, often leading to tumour recurrence and decreased patient survival. Contributing factors include epithelial-mesenchymal transition (EMT) and immune evasion, mechanisms by which tumours evade immune recognition. This study explores the involvement of immune evasion—specifically the PD-1/PD-L1 axis—in gamma-ray-induced radioresistance using a novel EMT6-derived radioresistant model (EMT6RR_MJI) in comparison to the parental EMT6 breast cancer cell line. In this study, mice were divided into two groups, each inoculated with either parental EMT6 cells or EMT6RR_MJI cells. Each group was further subdivided into four subgroups: 1 (control), 2 (gamma-ray irradiation), 3 (gamma-ray + nivolumab), and 4 (nivolumab alone). Subgroups 2 and 3 were treated with gamma rays at a dose of 2 Gy per cycle for eight cycles. All mice were sacrificed 5 days post-irradiation, and tumour sections were collected for analysis. The expression of PD-1 and PD-L1 was determined using qPCR, while PD-1 protein levels were analysed by Western blot and ELISA. In the parental EMT6 tumour model, tumour growth in subgroups 2 ($p<0.01$), 3 ($p<0.001$), and 4 ($p<0.01$) was significantly reduced compared to the control group. Additionally, PD-1 expression was higher in subgroup 2 ($p<0.05$) than in the control group. Notably, PD-1 protein expression increased in subgroup 4 ($p<0.05$) but decreased in subgroup 2 ($p<0.05$) compared to the control. In the EMT6RR_MJI tumour model, tumour growth in subgroup 3 was lower than in the control group ($p<0.001$). Furthermore, PD-L1 gene expression decreased in subgroups 2 and 4 compared to the control group ($p<0.05$). PD-1 protein expression was significantly reduced in subgroups 2 and 3 relative to the control ($p<0.05$). These findings suggest that PD-1 and PD-L1 contribute to the acquired radioresistance of EMT6RR_MJI tumours, highlighting their potential as therapeutic targets to improve radiotherapy outcomes in resistant breast cancer.

Keywords: Breast cancer, Radioresistance, Epithelial-mesenchymal transition (EMT), Nivolumab