

The upregulation of anti-neonatal Nav1.5 antibody in the serum of 4T1 orthotopic breast cancer mice model (*in vivo*): An immunosurveillance marker for metastatic breast cancer

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Introduction: Traditionally, breast cancer was deemed as immunologically 'silent'. However, with the aid of research advancements, the prospect of merging the role of immune system in treating breast cancer is gaining global attention. In this study, we would like to introduce a novel marker known as anti-neonatal Nav1.5 antibodies (anti-nNav1.5-Ab). These antibodies are intended to target neonatal Nav1.5 (nNav1.5) antigen which promotes the metastasis of breast cancer. **Methodology:** A total of 40 mice were utilised in this study. Seventeen mice were used for the development of 4T1 orthotopic mice model by injecting the cultured 4T1 murine mammary cells at the 3rd mammary pad of female BALB/c mice (day 0). The other 20 mice were kept as controls. Three mice were used for the development of positive control by injecting nNav1.5 peptide, subcutaneously. After a period of 42 days (week 6) of tumour development, the mice were sacrificed. The serum samples were collected from the three groups of mice. An in-house enzyme-linked immunoassay (ELISA) assay was developed to detect the presence of anti-nNav1.5-Ab in the serum samples. Lung metastasis clonogenic assay was performed by retrieving fresh lungs from both 4T1 orthotopic and control groups. In addition, commercial sandwich ELISA was performed to quantitate the expression of Interleukin-6 (IL-6) in the serum samples of both groups ($n=11$ each). Statistical analyses were performed, and the significance was set at $P<0.05$. **Results:** By week 5 (day 29-35) of tumour development, there was a drop in the volume of 4T1 tumour. The serum samples obtained from 4T1 orthotopic mice models exhibited significantly higher expression of anti-nNav1.5-Ab compared to control group ($P<0.0001^{****}$). Based on the clonogenic assay, 6-thioguanine resistant 4T1 cells were only present from lungs retrieved from 4T1 orthotopic mice and absent from the lungs of control mice. The expression of IL-6 in the serum of 4T1 orthotopic mice was higher than those of control mice but the difference was not significant ($P=0.1650$). In addition, there was a significant positive correlation between the expression of anti-nNav1.5-Ab and the concentration of IL-6 in the serum of 4T1 orthotopic mice model ($P=0.0366^*$, $r=0.6430$).

Discussion and Conclusion: The upregulated expression of IL-6 and presence of lung metastasis originating from the primary 4T1 tumour, signifies the presence of metastasis in the 4T1 orthotopic mice model. The presence of anti-nNav1.5-Ab in the serum reflects the immunogenicity and robust expression of nNav1.5 in 4T1 tumour. The positive correlation between anti-nNav1.5-Ab and IL-6 may elucidate the potential of anti-nNav1.5-Ab as an immunosurveillance marker for the screening of metastatic breast cancer.



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