

**GENETIC ANALYSIS OF TUMOR CELLS DERIVED FROM THE STRESS OF IMMUNE RESPONSE FOR UNDERSTANDING OF IMMUNOTHERAPY**

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**Background** Cancer immunotherapy with immune checkpoint inhibitors (ICIs), unlike conventional anticancer agents and molecular targeted drugs, shows Kaplan-Meier curves characteristic of long-term survival in patients with advanced stage IV cancer, which is so-called the ‘kangaroo tail phenomenon’. However, the response rate of ICI is limited at only 20–30%, and improvement of therapeutic efficacy needs to be developed. We believed that a thorough elucidation of the mechanism of the kangaroo tail phenomenon would be one of the most important aspects to improve the therapeutic efficacy of ICIs.

**Methods** Using a mouse model of breast cancer 4T1 cells subcutaneously transplanted into wild type (natural immunological response) and immunocompromised mice with CTL transferred (tumor specific immunological response) (RAG-/- + ACT), we isolated tumor cells that had shrunk once and then re-expanded in two systems and performed whole exon RNA sequencing using tumor cells immediately after transplantation as controls. We analyzed the genetic changes induced by anti-tumor immune stress that tumors undergo from natural immune response.

**Results** In all samples, Tumor Mutational Burden and MSI scores increased compared to the control which is under no immunological stress, and many new genetic mutations were observed. In addition, the immunogenicity was maintained, with high affinity between neoantigens and MHC, which was inferred from the mutation information with amino acid changes. This suggests that the kangaroo tail phenomenon is caused by the repeated recognition and cytotoxic activity of neoantigens by CTLs with  $10^{18}$  repertoire T cell receptors.

On the other hand, the genetic mutations common to each sample appeared the genes related to immune response, and mutations were also found in genes related to repair mechanisms such as DNA repair. For elective splicing, intron retention was found to have more genes related to DNA repair, while exon skipping had more genes related to DNA repair and the cell cycle. Copy number analysis also showed loss of heterozygosity in chromosome 17, which is located at MHC. These results were suggested that immune evasion by immunological pressure may be derived from the mutations such as abnormal immune response and loss of antigen-presenting ability.

**Conclusions** This study provides the first molecular biological insights into the mechanisms by which ICI produces long-term survivors. Furthermore, this study also provides clues to further improve the response rate of ICI through the understanding of the mechanism of action of ICIs in effective cases, at the same time understanding the immune evasive mechanism that occurs in ineffective cases.

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