556 ESTABLISHMENT OF A NOVEL PRECLINICAL MODEL FOR EVALUATING IMMUNE CHECKPOINT BLOCKADE-INDUCED ANTITUMOR IMMUNOLOGICAL MEMORY IN LUNG CANCER

Takuro Noguchi*, Hitoki Arisato, Akihiko Shiiya, Ichiro Kinoshita, Hirotoshi Akita. *Hokkaido University School of Medicine, Sapporo, Hokkaido, Japan*

Background Immune checkpoint blockade (ICB) has been shown to elicit long-term, durable responses in patients with metastatic or unresectable lung cancer. Similar to immunity against pathogens, memory-like antitumor T cell response may be pivotal in these patients. Nonetheless, due to the absence of suitable clinic-to-bench preclinical animal models, the mechanisms behind the generation of beneficial T cells following ICB in lung cancer remain largely unexplored.

Methods Subclones were obtained from a syngeneic murine lung cancer cell line. Alongside confirming their genomic oncogenicity through whole exome sequencing, MHC class I and class II neoantigens within the subclones were computationally identified. The subclones were subsequently tested in vivo for their sensitivity to ICB, including anti-PD-1 monotherapy and a combination of anti-PD-1 and anti-CTLA-4, both of which are the standard of care for lung cancer. Additionally, we assessed the role of memory-like antitumor T cell response in wild-type and *Batf3* knockout (KO) mice that had previously rejected the lung tumor cells post-ICB treatment.

Results Three subclones, huL1, huL2, and huL3, were established. These retained oncogenic driver mutations, such as *Kras*, *Nras*, and *Trp53*, present in the parental cells, and also shared immunogenic MHC class I and class II neoantigens. Wild-type mice were subcutaneously challenged with $1x10^6$ tumor cells, followed by ICB treatment on days 3, 6, and 9. Upon anti-PD-1 monotherapy, tumor rejection rates in mice bearing huL1, huL2, and huL3 were 30%, 0%, and 50%, respectively. Treatment with a combination of anti-PD-1 and anti-CTLA-4 improved rejection rates to 20% for huL1, 36% for huL2, and 100% for huL3. Following a minimum interval of three weeks after the rejection of huL3 tumors, mice were re-challenged with $5x10^6$ huL3 tumor cells on the opposite flank. Despite remaining untreated, these mice spontaneously rejected the secondary-challenged tumor cells. The same approach was replicated with *Batf3* KO mice, which also rejected huL3 tumor cells when treated with the ICB combination, albeit at a rate of 50%. Interestingly, these tumor-free *Batf3* KO mice could not spontaneously reject re-challenged huL3 tumor cells, unlike their wild-type counterparts.

Conclusions We have developed a novel preclinical lung cancer model that assesses antitumor immunological memory through tumor rejection. Our research findings suggest that *Batf3* is crucial for the development of memory-like antitumor T cells following ICB.

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