BALB/c-hPD1-hPDL1-hCD73-hCD39

Strain Name： BALB/cJGpt-*Pdcd1*$^{em1Cin(hPDCD1)}$*Cd274*$^{em1Cin(hCD274)}$*Nt5e*$^{em1Cin(hNT5E)}$ *Entpd1*$^{em1Cin(hENTPD1)}$/Gpt

Strain Type： Knock-in

Strain Number： T058749

Background： BALB/cJGpt

Description

PD1 (programmed death 1) is a programmed death receptor 1, a type I transmembrane glycoprotein of the immunoglobulin superfamily, an important immunosuppressive molecule and a member of the CD28 superfamily. PDL1 is a ligand for PD1, called programmed cell death 1 ligand 1 (PDL1). Under normal circumstances, the immune system responds to foreign antigens that accumulate in lymph nodes or the spleen, promoting the proliferation of antigen-specific T cells. PD1, in combination with PDL1, transmits inhibitory signals and reduces the proliferation of antigen-specific T cells. Immunomodulation targeting PD1/PDL1 has important implications for antitumour, anti-infection, autoimmune diseases and organ transplant rejection.

Numerous studies have confirmed that increased PDL1 expression on the surface of tumour cells in the tumour microenvironment simultaneously binds to PD1 on activated T cells, delivering negative regulatory signals. This leads to apoptosis or immune deactivation of tumour antigen-specific T cells, thereby suppressing the immune response and thus contributing to the escape of tumour cells. Blocking the PD1/PDL1 signalling pathway with antibodies has become a classical approach to tumour immunotherapy[1-3].

CD73 is a glycoprotein anchored to the plasma membrane by glycosylphosphatidylinositol (GPI), which is widely distributed on the surface of human tissue cells and has extracellular nucleotidase activity, hydrolysing AMP to produce adenosine. Stagg J et al. found that CD73 activity was significantly increased in bladder cancer cells with high malignancy and promoted bladder tumor formation through the purinergic signaling pathway[4]. This suggests that CD73 promotes the growth of tumour cells in vitro and in vivo[5]. It has also been shown that CD73 can promote invasion and migration of breast cancer cells through adenosine[6].

Leukaemic T lymphocytes with upregulated CD73 expression specifically inhibit TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis and induce multidrug resistance, thereby promoting tumour cell survival. Studies with CD73 antibodies in mice have shown that inhibition of CD73 activity has a significant inhibitory effect on tumour growth, apoptosis and invasion[7].

CD39 is an extracellular nucleotidase that is localized on the cell surface to catalyse the production of AMP from ATP/ADP, also known as ENTPDase1 (NTPDase1). CD39 in combination with CD73 catalyzes the production of adenosine from extracellular ATP. In the tumour microenvironment, adenosine binding to adenosine receptors inhibits the activity of T cells and NK cells, thereby suppressing the immune system. In some human tumours, upregulation of Treg cells expressing CD39 and upregulation of Treg cells promotes tumour growth and metastasis. The use of antibodies to CD39 and CD73 blocks the hydrolysis of ATP to adenosine, which transmits inhibitory signals, and the combination of the two restores the activity of isolated T-cells in tumour patients in vitro by activating macrophages and dendritic cells. The use of CD39 antibodies in mice expressing human CD39 increased the antitumour activity of oxaliplatin, a chemotherapeutic agent induced by ATP[8]. These results validate that CD39 and CD73 monoclonal antibodies have good potential for development in the field of oncology therapy.

BALB/c mice are inbred mice widely used in immunology and oncology research. There are many homologous tumour cell lines derived from the BALB/c background, such as CT26 colon cancer cells, 4T1 breast cancer cells, H22 liver cancer cells and A20 B lymphoma cells.

Using gene editing technology, we replaced the extracellular regions of the murine Pd1, Pdl1, Cd73 and Cd39 genes with the homologous human PD1, PDL1, CD73 and CD39 fragments, while retaining the intracellular signal transduction regions of the corresponding murine genes to ensure that the correct cell signaling was not affected. Two humanized mouse models, BALB/c-hPD1-hPDL1-hCD73 and BALB/c-hPD1-hPDL1-hCD39, were constructed respectively. And the BALB/c-hPD1-hPDL1-hCD73-hCD39 mice were obtained by breeding these two models. BALB/c-hPD1-hPDL1-hCD73-hCD39 mice would be an ideal animal model for screening human PD1, PDL1, CD73 and CD39 inhibitor drugs.

Strategy

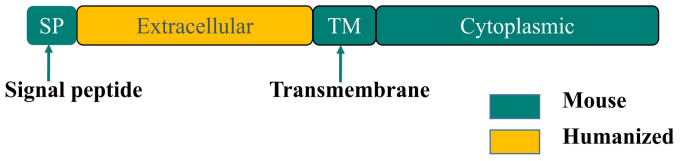

Fig.1 Schematic diagram of humanized PD1 strategy in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice.

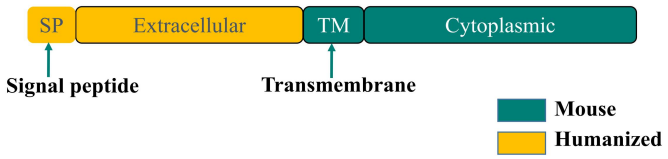

Fig.2 Schematic diagram of humanized PDL1 strategy in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice.

Fig.3 Schematic diagram of humanized CD73 strategy in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice.

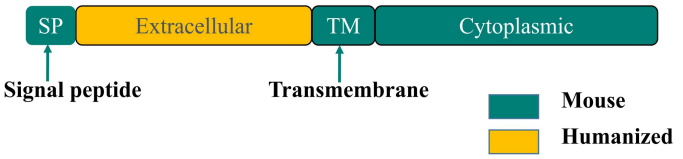

Fig.4 Schematic diagram of humanized CD39 strategy in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice.

Applications

1. Human CD73 or CD39 inhibitors and evaluation of performance, e.g. screening for oncology therapeutics or neutralizing antibodies
2. Evaluation of the efficacy of human CD73 or CD39 inhibitors in combination with human PD1 or PDL1 inhibitors
3. Anti-tumour studies
4. Tumour immunology and autoimmune disease research

Data support

1. Detection of CD73 and CD39 expression in spleen T cells

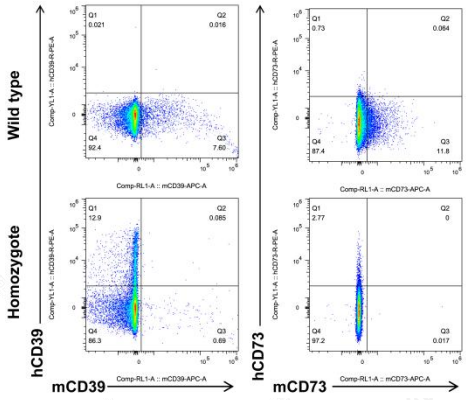

Fig 5. Detection of hCD73 and hCD39 expression in spleen cells of BALB/c-hPD1-hPDL1-hCD73-hCD39 mice.

Spleen cells were collected and the expression of CD39 and CD73 in mice was detected separately by flow cytometry using specific antibodies. In BALB/c mice, only mouse CD39 and CD73 were detected. In BALB/c-hPD1-hPDL1-hCD73-hCD39 homozygous mice, only human CD73 and CD39 was detected, mouse CD39 and CD73 were not detected.

2. Analysis of spleen leukocyte subpopulations in mice

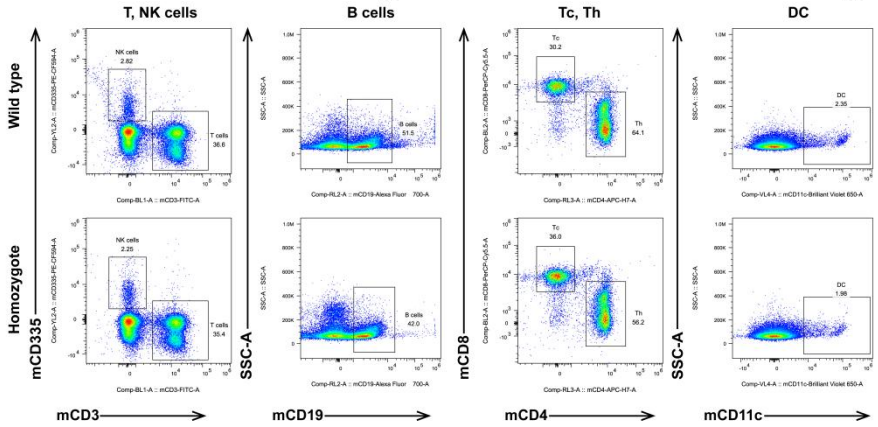

Fig 6. Detection of splenic leukocyte subpopulations in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice by FACS.

Splenocytes from 6-week-old BALB/c mice and BALB/c-hPD1-hPDL1-hCD73-hCD39 homozygous mice were taken for flow cytometry to determine the ratio of their immune cell fractions. The results showed that BALB/c-hPD1-hPDL1-hCD73-hCD39 mice had almost identical T, B, NK and DC cell fractions compared to BALB/c mice.

3. Analysis of spleen leukocyte subpopulations in mice

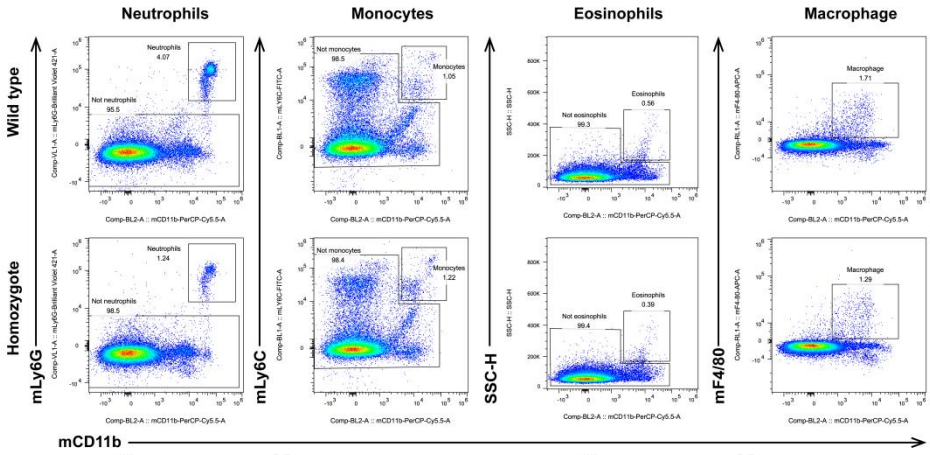

Fig 7. Detection of splenic leukocyte subpopulations in BALB/c-hPD1-hPDL1-hCD73-hCD39 mice by FACS.

Splenocytes from 6-week-old BALB/c mice and BALB/c-hPD1-hPDL1-hCD73-hCD39 homozygous mice were taken for flow cytometry to determine the ratio of their immune cell fractions. The results showed that BALB/c-hPD1-hPDL1-hCD73-hCD39 mice had almost identical Neutrophils , Monocytes , Eosinophils and Macrophage compared to BALB/c mice.

References

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