BALB/c-hFcgRs/hCD47/hSIRPA

Strain Name: BALB/cJGpt-*Cd47*$^{em1Cin(hCD47)}$*Sirpa*$^{em1Cin(hSIRPA)}$Tg(hFcgR)8/Gpt

Strain Type: Knock-in/BAC-TG

Strain Number: T055268

Background: BALB/cJGpt

Description

CD47, also known as integrin-associated protein (IAP), is widely expressed on the surface of cells. CD47 can interact with inhibitory receptor signaling protein alpha (SIRPA), thrombospondin (TSP1), and integrins mediating a series of reactions such as apoptosis, proliferation, and immunity [1, 2]. Studies have confirmed that CD47 molecules are over-expressed in many malignant tumors, such as acute myeloid leukemia (AML), B-cell and T-cell acute leukemia, and non-Hodgkin's lymphoma, and their expression level is negatively correlated with the prognosis of the disease. Tumor cells can escape the immune surveillance of macrophages through the CD47-SIRPA signaling pathway. Therefore, blocking the binding of CD47 to SIRPA by CD47 antibody can activate the phagocytosis of macrophage and the antigen presentation of DC cells. CD47 can combine with other immunotherapies to inhibit tumor growth [3,4].

Blocking CD47-SIRPA interactions has been shown to promote the destruction of cancer cells by phagocytes, including macrophages and neutrophils. Furthermore, there is growing evidence that targeting the CD47-SIRPA axis may also promote antigen-presenting cell function and thereby stimulate adaptive T cell-mediated anti-cancer immunity [5-8]. These identify the CD47-SIRPA axis as a promising innate immune checkpoint in cancer.

Fcγ receptor (FcgR) belongs to the immunoglobulin superfamily, and FcgR is an IgG Fc segment receptor [9], which is mainly expressed on the immune cell membrane. In mice, FcgR is divided into *Fcgr1(CD64), Fcgr2b(CD32), Fcgr3(CD16) and Fcgr4(CD16-2).* FcgR binds to the Fc region of the antibody attached to the target cell, activating the antibody-dependent cell-mediated cytotoxicity mechanism (ADCC), resulting in the lysis of the target cell [10]. For antagonist antibodies, inhibiting the binding of Fc to FcgR can avoid side effects caused by ADCC, and so on[11]. With the increasing efficacy and safety requirements of monoclonal antibodies, the selection of appropriate IgG subtypes and Fc region transformation is a hot spot in the development of monoclonal antibodies.

GemPharmatech used BAC transgenic technology to create the FcgRs transgenic model carrying the FcgRs encoding genes (*FCGR1, FCGR2A, FCGR2B, FCGR2C, FCGR3A, FCGR3B*) in BALB/c-hCD47/hSIRPA mice. The phenotypically validated mouse model can be applied to assess the Fc-mediated ADCC effect of Fc binding to FcgR of human CD47 or SIRPA antibodies to screen for safer antibody drugs.

Strategy

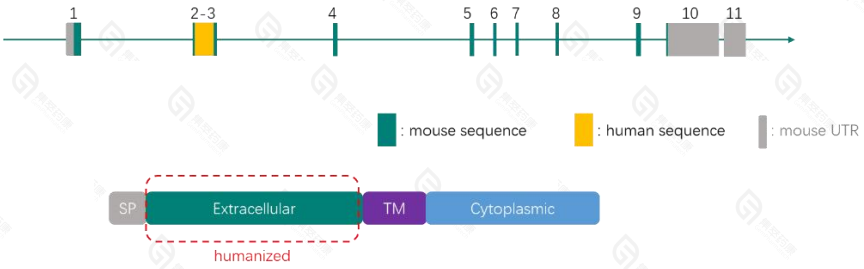

Fig 1. Schematic diagram of CD47 humanization strategy in BALB/c-hFcgRs/hCD47/hSIRPA mice.

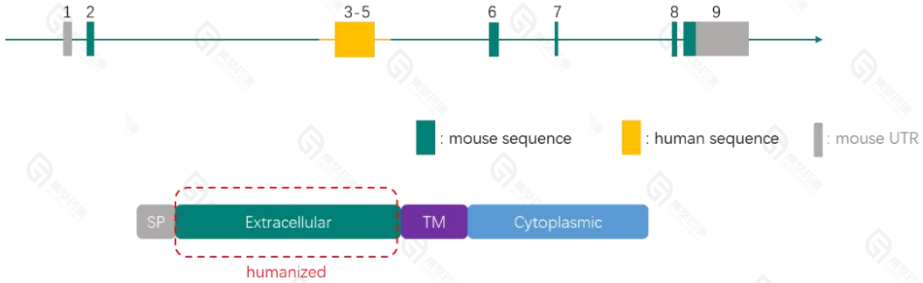

Fig 2. Schematic diagram of SIRPA humanization strategy in BALB/c-hFcgRs/hCD47/hSIRPA mice.

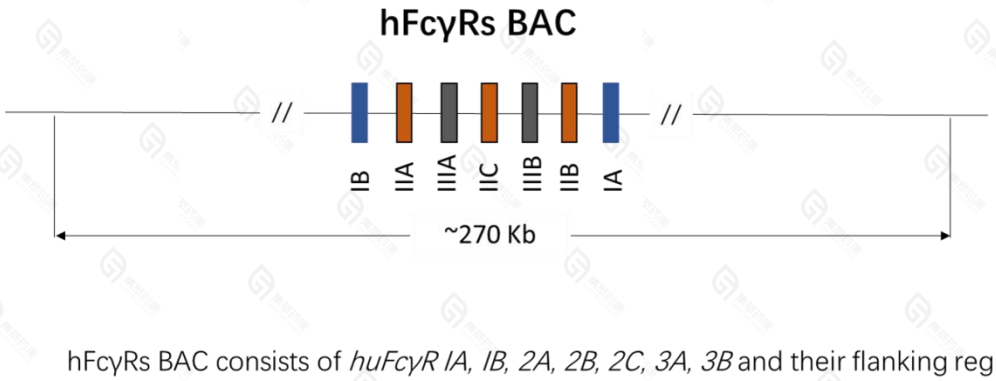

Fig 3. Schematic diagram of the hFcgRs BAC.

Applications

1. Screening for human CD47 or human SIRPA related drugs
2. Efficacy and safety evaluation of human CD47 or human SIRPA related drugs
3. Immune system related research

Data support

1. Detection of CD47, SIRPA, and FCGR expression

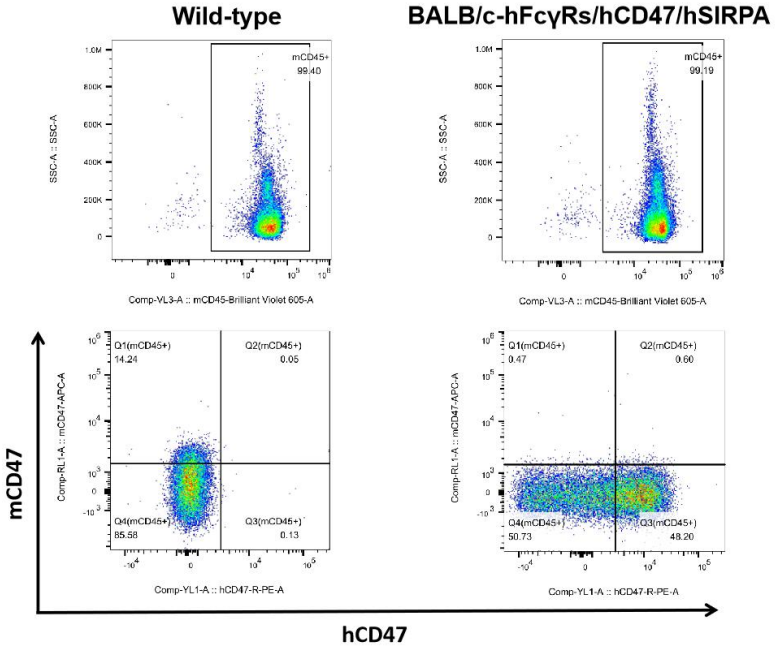

Fig 4. Detection of CD47 expression in peripheral blood lymphocytes on BALB/c-hFcgRs/hCD47/hSIRPA mice.

Peripheral blood lymphocytes were collected from wild-type (6-7 weeks) and BALB/c-hFcgRs/hCD47/hSIRPA mice (6-7 weeks) and analyzed for CD47 expression with flow cytometry. The results indicated human CD47 expressing was detected on mCD45+ cells in BALB/c-hFcgRs/hCD47/hSIRPA mice(hFcgRs-TG, hCD47 homo, hSIRPA homo).

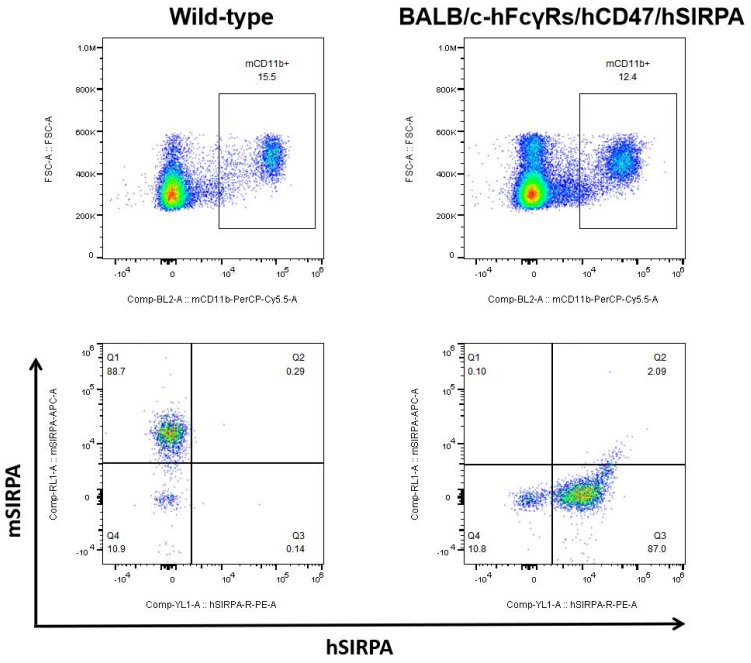

Fig 5. Detection of SIRPA expression in peripheral blood lymphocytes on BALB/c-hFcgRs/hCD47/hSIRPA mice.

Peripheral blood lymphocytes were collected from wild-type (6-7 weeks) and BALB/c-hFcgRs/hCD47/hSIRPA mice (6-7 weeks) and analyzed for SIRPA expression with flow cytometry. The human SIRPA expression of mCD11b+ cells in BALB/c-hFcgRs/hCD47/hSIRPA (hFcgRs-TG, hCD47 homo, hSIRPA homo) is comparable to the mouse SIRPA counterpart in wild-type BALB/c mice.

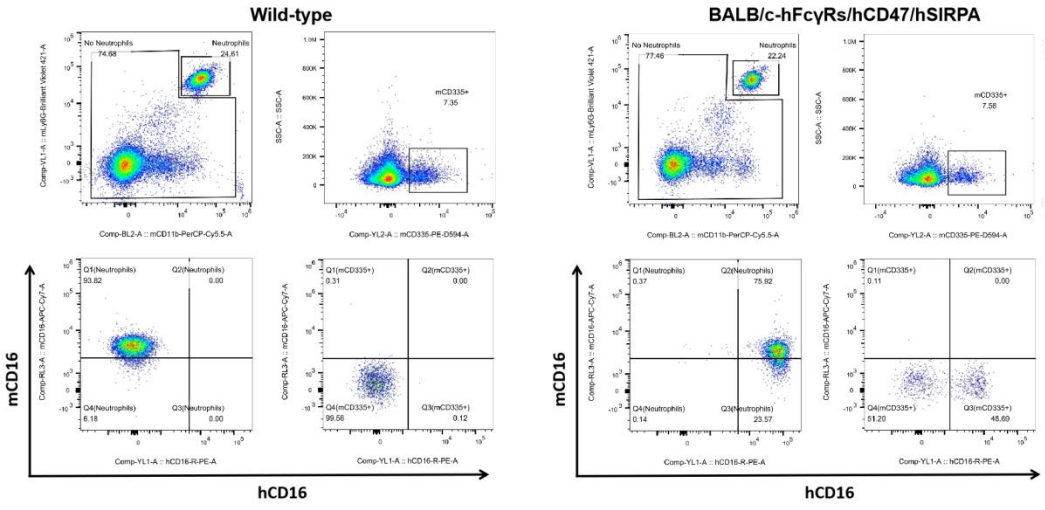

Fig 6. Detection of CD16(FCGR3) expression in peripheral blood lymphocytes on BALB/c-hFcgRs/hCD47/hSIRPA mice.

Peripheral blood lymphocytes were collected from wild-type (6-7 weeks) and BALB/c-hFcgRs/hCD47/hSIRPA mice (6-7 weeks) and analyzed for CD16(FCGR3) expression with flow cytometry. The results indicated human CD16 expressing was detected on both neutrophils(mCD11b+, mLy6G+) and NK cells(mCD335+) in BALB/c-hFcgRs/hCD47/hSIRPA mice(hFcgRs-TG, hCD47 homo, hSIRPA homo), whereas mouse CD16 expressing was detected on neutrophils but not NK cells.

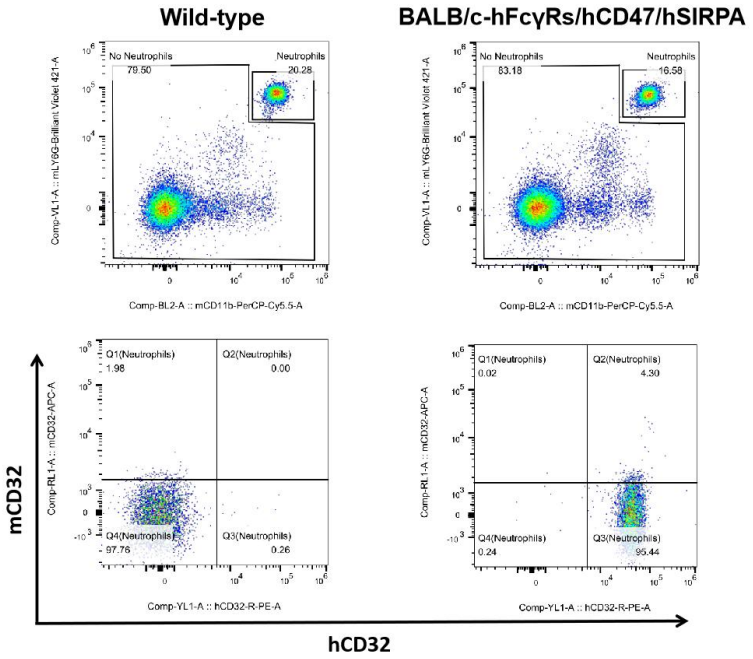

Fig 7. Detection of CD32(FCGR2) expression in peripheral blood lymphocytes on BALB/c-hFcgRs/hCD47/hSIRPA mice.

Peripheral blood lymphocytes were collected from wild-type (6-7 weeks) and BALB/c-hFcgRs/hCD47/hSIRPA mice (6-7 weeks) and analyzed for CD32(FCGR2) expression with flow cytometry. The results indicated human CD32 expressing was detected on neutrophils(mCD11b+, mLy6G+) in BALB/c-hFcgRs/hCD47/hSIRPA mice(hFcgRs-TG, hCD47 homo, hSIRPA homo).

2. Leukocyte subpopulation analysis

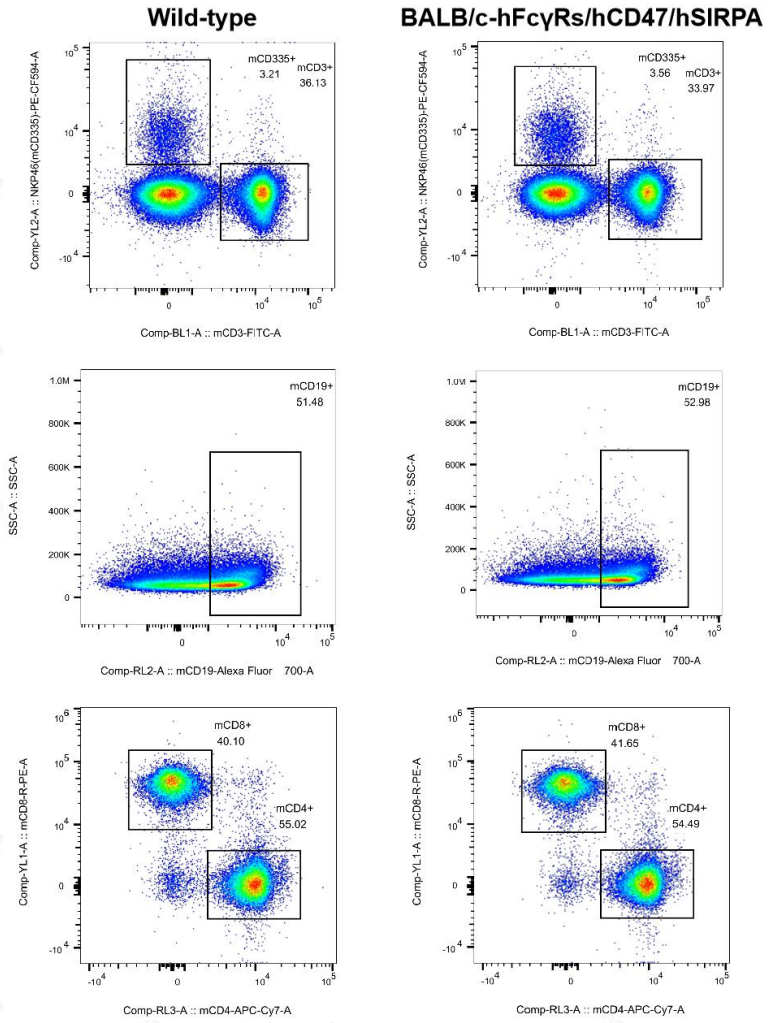

Fig 8. Analysis of leukocyte subpopulation in splenocytes on BALB/c-hFcgRs/hCD47/hSIRPA mice.

Splenocytes were collected from wild-type (6-7 weeks) and BALB/c-hFcgRs/hCD47/hSIRPA mice (6-7 weeks) and analyzed for leukocyte subpopulation with flow cytometry. The ratio of T cells (mCD3+, mCD4+, and mCD8+), B cells (mCD19+), and NK cells (mCD335+) in the spleen of BALB/c-hFcgRs/hCD47/hSIRPA (hFcgRs-TG, hCD47 homo, hSIRPA homo) mice were similar to that of wild-type BALB/c mice.

References

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