Case Study

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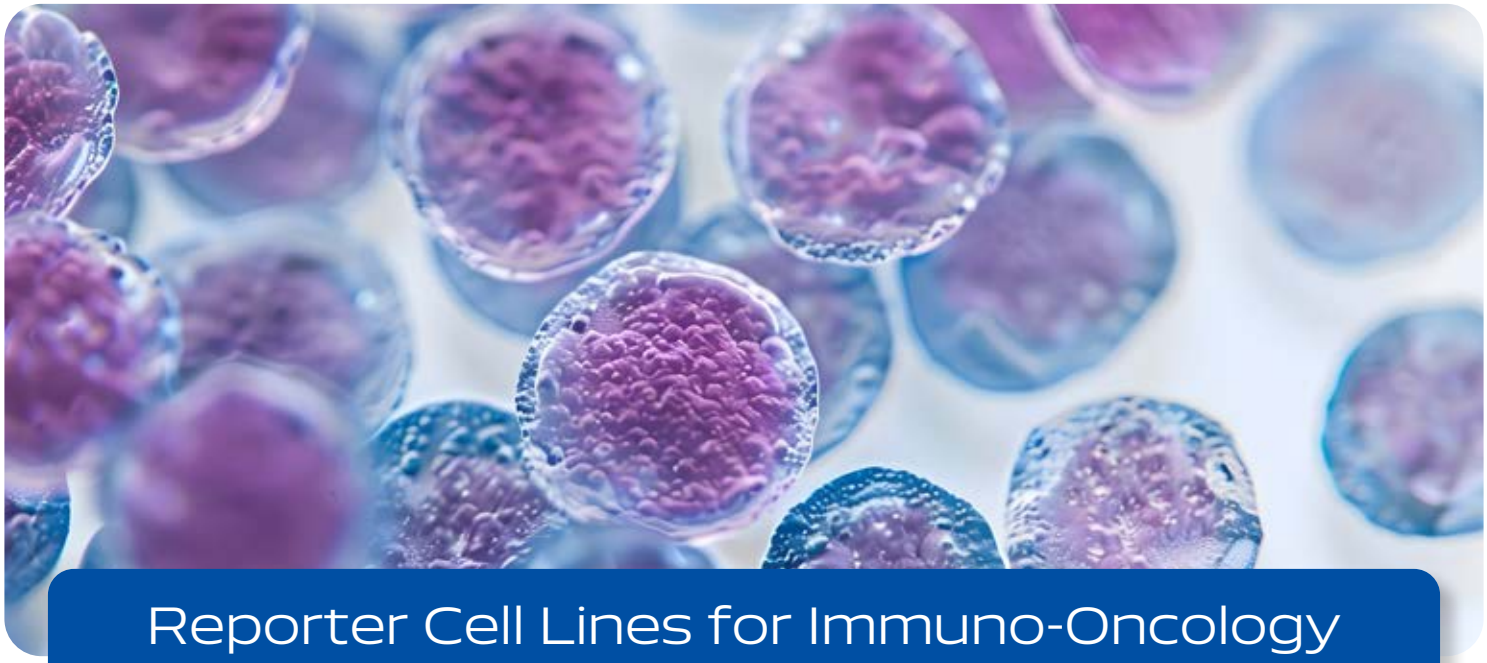

Reporter Cell Lines for Immuno-Oncology Drug Discovery: Targeting T-Cell Activation

Overview

Reporter cell lines are genetically engineered cells that express reporter genes, allowing monitoring of cellular processes through detectable signals like luminescence or fluorescence. In immuno-oncology, these cell lines are useful for monitoring T-cell activation, a critical functional readout for assessing immune responses to therapies. By linking reporter gene expression to pathways like the NFAT (Nuclear Factor of Activated T cells) pathway, these systems enable precise, quantifiable measurements of T-cell activation, critical for evaluating immunotherapies, offering valuable insights into the effectiveness of immunotherapies.

This case study details the development of a Jurkat-NFAT-Luc reporter cell line, optimized for sensitive, reproducible detection of T-cell activation. By incorporating an NFAT-responsive luciferase construct into the Jurkat T-cell background, this system offers a reliable platform for assessing immune-modulating agents, supporting early-stage evaluation and high-throughput screening.

Our Approach

We generated NFAT-Luc reporter Jurkat cells by introducing a plasmid carrying the luciferase gene under the NFAT response element via electroporation. Following antibiotic selection, stable Jurkat-NFAT-Luc cells were established. The functionality of these cells was validated by stimulating them with T-cell activators or chemical stimulants, to confirm stable integration and activation of luciferase expression through both TCR-dependent and independent signaling pathways.

Methodology

To evaluate the responsiveness of the Jurkat-NFAT-Luc reporter cell line to T-cell stimulation, a 96-well plate-based functional assay was performed that involved:

1. TCR-Dependent Stimulation Using Anti-CD3/CD28 Beads

i. Jurkat-NFAT-Luc and Jurkat wild-type (WT) cells (25,000 per well) were seeded in a 96-well culture plate containing complete medium (RPMI with 10% FBS) and incubated for 24 hours.

ii. The next day, anti-CD3/CD28 activation beads were washed with phosphate-buffered saline (PBS) and resuspended in complete medium.

iii. Beads were added to wells at varying cell-to-bead ratios (1:0 to 1:8).

iv. Cells were incubated at 37°C with 5% CO_2 for 6 hours.

v. After incubation, cells were lysed using a luciferase assay reagent.

vi. Luminescence was measured using a multi-mode plate reader.

2. TCR-Independent Stimulation Using PMA and Ionomycin

i. Jurkat-NFAT-Luc and Jurkat-WT cells (50,000 per well) were treated with a mixture of PMA (0.081 µM) and ionomycin (1.34 µM) in complete medium.

ii. Cells were incubated for 6 hours at 37°C in 5% CO_2.

iii. Following stimulation, cells were lysed using a luciferase assay reagent.

iv. Luminescence was recorded using a multi-mode plate reader.

Outcomes

- **TCR-Dependent Stimulation with Anti-CD3/CD28 Beads:** Jurkat-NFAT-Luc and Jurkat wild-type (WT) cells were stimulated with anti-CD3/CD28 beads at varying cell-to-bead ratios for 6 hours. This stimulation activates the TCR/CD3 complex and provides a costimulatory signal through CD28, mimicking T-cell activation via antigen-presenting cells. Activation of the TCR/CD3 pathway triggers the translocation of the NFAT transcription factor to the nucleus, inducing the expression of cytokines such as IFN-γ and TNF-α. The luciferase activity measured in the assays reflects T-cell activation via the NFAT pathway, demonstrating high specificity, precision, and linearity (Figure 1), and confirming the ability of the Jurkat-NFAT-Luc cell line to accurately monitor T-cell activation in response to immune-modulating agents.

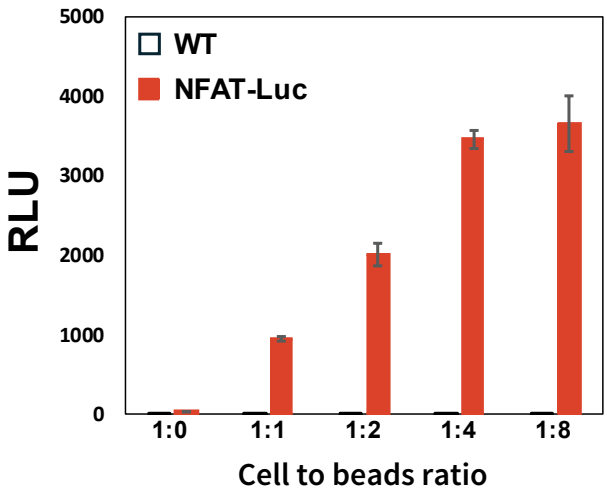

Figure 1: Response of Jurkat-NFAT-Luc and Jurkat wild-type (WT) cells to anti- CD3/CD28 beads. Error bars represent ±SE.

- **TCR-Independent Stimulation with PMA and Ionomycin:** Jurkat-NFAT-Luc and Jurkat-WT cells were treated with a combination of PMA and ionomycin for 6 hours. This treatment bypasses the TCR/CD3 complex and stimulates the NFAT pathway through protein kinase C (PKC) activation and increased calcium influx. Consequently, NFAT translocates to the nucleus, leading to luciferase expression. A significant increase in the luciferase activity (Figure 2) confirms T-cell activation through a TCR-independent mechanism, demonstrating the Jurkat-NFAT-Luc cell line's responsiveness to various signaling pathways and its consistency in activation assays.

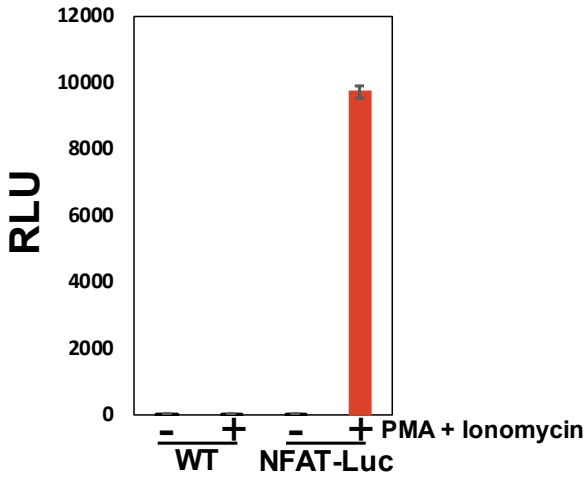

Figure 2: Response of Jurkat-NFAT-Luc cell line upon treatment with a combination of PMA and ionomycin. Error bars represent ±SE.

These findings confirm the Jurkat-NFAT-Luc cell line as a reliable tool for tracking T-cell activation, both through TCR-dependent and TCR-independent pathways, supporting its use in evaluating immuno-oncology therapeutics.

Why Aragen?

At Aragen, we specialize in custom cell line development for drug discovery, including immuno-oncology applications. We offer:

- **Comprehensive Solutions:** A complete range of cell lines (CHO, HEK293, cancer cells) and reporter genes (GFP, luciferase).
- **Rigorous Quality Control:** Extensive testing for expression levels, mycoplasma, sterility, and stability, ensuring reliable results.
- **Flexible Platforms:** Choose from different cell backgrounds and expression systems (intracellular, secretory, membrane) designed for high-yield production and functional assays.
- **Immuno-Oncology Expertise:** Specialized reporter cell lines like Jurkat-NFAT-Luc track T-cell activation, ideal for evaluating checkpoint inhibitors, CAR-T cells, and TCR modulators.
- **Efficient Timelines:** Tool cell lines in 4-6 weeks, monoclones in 10-12 weeks, and production cell lines in 16-18 weeks, accelerating your research.

Accelerate your research with our reliable, high-quality cell line development.
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