AN NFAT PROMOTER BASED FLUORESCENT JURKAT CELL PLATFORM FOR HIGH-THROUGHPUT SCREENING OF CHIMERIC ANTIGEN RECEPTOR (CAR) CONSTRUCTS

Brikena Gjerci*, Sadik Kassim, Julian Scherer. *Vor Biopharma, Cambridge, MA, USA*

Background CAR T-cells have exhibited efficacious treatment of hematological malignancies, such as Acute Myeloid Leukemia (AML). Upon antigen binding, CARs initiate Ca^{2+} -dependent signaling pathways, leading to an increased intracellular concentration of nuclear factor of activated T cells (NFAT), which stimulates downstream T cell effector functions.^{1 2} However, an objective high-throughput platform to compare CAR-induced T cell activity has been lacking. Here, we report on a CAR screening platform that utilizes an NFAT-sensitive promoter driving fluorescent protein expression in Jurkat T cells. This approach, termed 'IL-2 Reporter System' (IRS), is employed for the identification of functional CD33CARs to improve AML CAR T-cell therapies.

Methods Lentiviral transduction of two fluorescent proteins (mOrange2 and mTurquoise2) under either the constitutively active EF1alpha (FP1) or the NFAT-sensitive minimal IL-2 promoter (FP2) into Jurkat cells resulted in two IRS cell lines. IRS cell function was confirmed by treatment with phorbol myristate acetate (PMA) and ionomycin to induce activation and thus the expression of FP2. To screen CARs with previously described potential in AML therapy, IRS cells were transduced with eight distinct CD33CAR constructs and cocultured with CD33+ cells (MOLM13-WT) and CD33 knock-out (MOLM13-CD33KO) cells. IRS cell analysis was performed using flow cytometry, fluorescent microscopy, and IncuCyte live cell imaging.

Results As expected, live cell fluorescence imaging revealed that PMA/ionomycin treatment induced expression of FP2. Peak expression occurred at 12–15 hours post-treatment and consistent, high FP2 signal was detected for 24h in both cell lines. Flow cytometry analysis 24hr post treatment determined that 60–80% of transduced IRS cells expressed FP2. Normalized FP2 expression was comparable between both IRS cell lines but varied across the eight CD33CARs. Six of the eight CD33CAR constructs resulted in significant increases (up to 8-fold) in FP2 signal upon exposure to CD33+ cells compared to control CD33KO cells.

Conclusions Our results indicate that IRS cells can be used as an objective, fast, and reliable reporter system for CD33CAR activity. The constitutively expressed FP1 eliminates false negative outcomes and verifies successful transduction of the reporter construct. FP2 expression, driven only in activated cells, represents antigen recognition by and activity of the CAR. Importantly, the use of IRS cells is not restricted to just CD33CARs but provides a platform for CAR and recombinant TCR screenings against any antigen. Additionally, efforts are underway to adapt this platform entirely to microplates and a liquid handling system, allowing for high-throughput screens of several AML-targeting CAR constructs.

REFERENCES

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