Immune signaling reporter cell lines enable quantitative monitoring of crosstalk among cancer, innate, and adaptive immune cells in tumor microenvironment model

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Abstract

Background: T cell-targeted immunotherapies have led to major clinical gains, yet many patients fail to respond or develop resistance due to the immunosuppressive tumor microenvironment (TME). Increasing evidence shows that B cells and myeloid cells also influence antitumor immunity, but accessible models capable of capturing interactions among cancer cells and multiple immune cell types remain limited. To address this gap, we developed immune signaling reporter cell lines that allow real-time, quantitative monitoring of NFAT- and NF-κB-driven activation pathways. These models enable capturing dynamic interactions among multiple immune cell lineages and cancer cells relevant to immunotherapy response.
Methods: Six luciferase-based reporter lines derived from T cells, B cells, or myeloid cells were engineered with NFAT or NF-κB response elements driving luciferase expression. The models retain high endogenous expression of checkpoint receptors, including PD-1, TIGIT, and GITR in T cell reporters and SIRPα, Siglec-10, LILRB1, and B7-1 in myeloid reporters. Reporter activation was assessed following pathway-specific stimulation: PMA/ionomycin for NFAT and TNF-α or T cell-conditioned media for NF-κB. The B cell NF-κB reporter with elevated basal activity was further tested with an NF-κB inhibitor. In addition, all reporters were evaluated in co-culture with primary immune and cancer cells.
Results: Stimuli activating NFAT or NF-κB signaling produced strong, dose-dependent increases in luciferase activity, while pathway inhibition reduced signal as expected. Co-culture with primary immune and cancer cells generated diverse activation patterns, reflecting context-dependent signaling shaped by interactions within the TME.
Conclusions: These reporter cell lines provide a scalable platform for monitoring NFAT- and NF-κB-driven immune activation across T, B, and myeloid lineages. They support sensitive, reproducible evaluation of immune responses, enable mechanistic studies of dynamic immune cross-talk, and evaluation of combinatorial immunotherapy strategies within the TME.

Multi-lineage immune reporter platform for modeling tumor-immune signaling

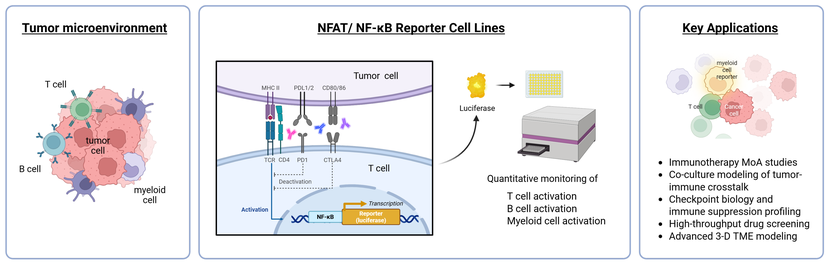

Figure 1: Schematic of immune signaling reporter cell lines enabling quantitative monitoring of crosstalk among cancer, innate, and adaptive immune cells in tumor microenvironment models. Tumor cells dynamically interact with T, B, and myeloid immune cells in tumor microenvironment. Engineered NFAT-Luc2 (T cell) and NFkB-Luc2 (B and myeloid cells) reporter cell lines generate luciferase signals in response to pathway-specific stimulation and co-culture interactions, allowing real-time quantitative assessment of immune activation. Luminescence-based readouts capture dynamic, context-dependent interactions in co-culture systems, supporting mechanistic studies and evaluation of immunotherapy responses. Image created with BioRender.com

Results

1. Reporter cells robustly detect NFAT and NF-κB pathway activation

NFAT reporter activation by TCR stimulation

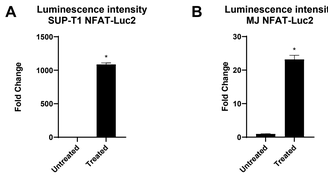

Figure 2: NFAT-Luc2 T-cell reporters display strong activation following TCR-mimetic stimulation. (A) SUP-T1 NFAT-Luc2 (ATCC® CRL-1942-NFAT-LUC2™) and (B) MJ NFAT-Luc2 (ATCC® CRL-8294-NFAT-LUC2™) cells were stimulated with PMA (50 ng/ mL) and ionomycin (10 ug/ mL) for 6 h. NFAT activation was measured by luciferase assay. Luciferase activity was quantified using the Bright-Glo Luciferase Assay System (Promega) and measured on SpectraMax i3x (Molecular Devices). Data represent mean ± SD (n=3). *P<0.05.

NF-κB reporter activation by inflammatory stimuli

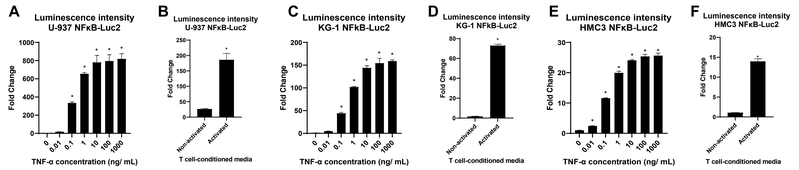

Figure 3: Myeloid NFkB-Luc2 reporters show robust activation in response to inflammatory stimuli and T-cell-derived signals. (A-B) U-937 NFkB-Luc2 (ATCC® CRL-1593.2-NFkB-LUC2™), (C-D) KG-1 NFκB-Luc2 (ATCC® CCL-246-NFkB-LUC2™), and (E-F) HMC3 NFκB-Luc2 (ATCC® CRL-3304-NFkB-LUC2™) cells were stimulated for 6 h with (A, C, E) TNF-α or (B, D, F) conditioned media from non-activated or activated human primary CD4+T cells. Activated conditioned media were collected three days after stimulation with anti-CD2/CD3/CD28 beads. NF-κB activation was quantified by luciferase assay. Data represent mean ± SD (n=3). *, P < 0.05.

2. Reporter cells capture immune cell crosstalk and pathway modulation

CD4+ T cell co-culture activation

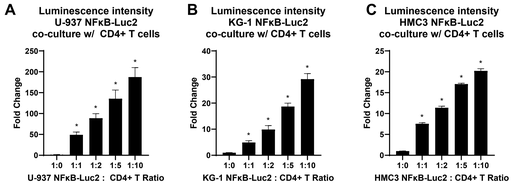

Figure 4: Primary CD4+ T cells induce NF-κB activation in myeloid reporter cells. (A) U-937 NFκB-Luc2, (B) KG-1 NFkB-Luc2, and (C) HMC3 NFκB-Luc2 cells were co-cultured with human primary CD4+ T cells at increasing ratios for 6 h. NF-κB activation was quantified by luciferase assay. Data represent mean ± SD (n=3). *P<0.05.

B cell NF-κB inhibition with Selinexor

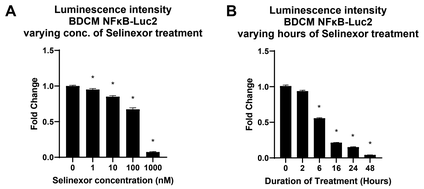

Figure 5: Pharmacologic inhibition of NF-κB signaling suppresses reporter activity in B-cell NFkB-Luc2 cells. BDCM NFκB-Luc2 (ATCC® CRL-2740-NFkB-LUC2™) cells were treated with (A) increasing concentrations of Selinexor for 24 h or (B) 1 μM Selinexor over time. NF-κB activity was quantified by luciferase assay. Data represent mean ± SD (n=3). *P<0.05.

3. Reporter platform models complex tumor-immune interactions

CAR-T/ tumor/ myeloid triple co-culture

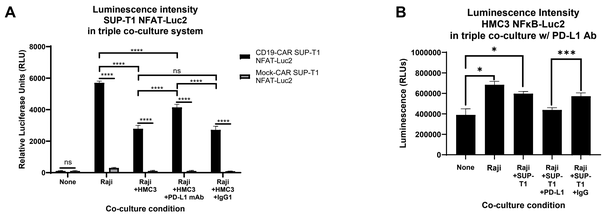

Figure 6: Myeloid cells suppress CAR-T activation through checkpoint-dependent signaling in triple co-culture. (A) CD19-CAR or mock-CAR SUP-T1 NFAT-Luc2 reporter cells were co-cultured with Raji tumor cells ± HMC3 parental myeloid cells for 6 hours in the presence of PD-L1 mAb or IgG control (1,000 ng/ mL). (B) HMC3 NFkB-Luc2 myeloid reporter cells were co-cultured with CAR-T cells and Raji cells for 6 hours in the presence of PD-L1 mAb or IgG control (1,000 ng/ mL). Cell ratios were 1:1:0.5 (CAR-T : Raji : HMC3). NFAT or NF-κB activity was measured by luciferase assay. Data represent mean ± SD (n=3). *P<0.05; ***P<0.001; ****P < 0.0001.

Glioblastoma microenvironment model

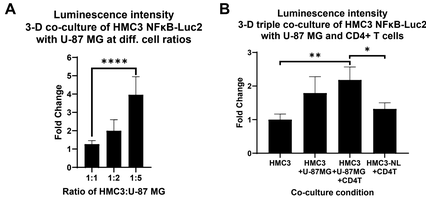

Figure 7: 3-D glioblastoma co-culture models microglia-T cell inflammatory crosstalk captured by NF-κB reporters. U-87MG spheroids were co-cultured with HMC3 NFkB-Luc2 microglia reporters for 48 h (A) at increasing cell ratios or (B) ± primary CD4+ T cells at cell ratios of 1:1:1. NF-κB activation was quantified by luciferase assay. Data represent mean ± SD (n=3). *P<0.05; **P<0.01; ****P < 0.0001.

Conclusion

- Immune reporter cell lines enable quantitative monitoring of NFAT and NF-κB signaling across immune lineages.
- The platform captures immune-immune crosstalk and checkpoint signaling.
- Complex co-culture models reveal tumor microenvironment mechanisms relevant to immunotherapy.

Key References

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