Signaling reporter cell lines for monitoring immune activation enable the study of dynamic crosstalk among cancer cells, innate immune cells, and adaptive immune cells in tumor microenvironment

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Abstract 13

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Abstract

Background: Although T cell–targeted immunotherapies have achieved significant clinical success, a considerable proportion of patients either do not respond or eventually relapse, largely due to the immunosuppressive characteristics of the tumor microenvironment. Growing evidence indicates that additional immune cell types, including B cells and myeloid cells, play vital roles in regulating the effectiveness of cancer immunotherapies. Despite this, widely accessible immunological models that accurately recapitulate the complex, multidirectional interactions between cancer cells and both adaptive and innate immune cells remain limited.
Methods: To address this need, we developed six luciferase-based immune activation reporter cell lines derived from T cells, B cells, or myeloid cells. These lines were engineered to express luciferase under the control of either the nuclear factor of activated T cells (NFAT) or nuclear factor kappa B (NF-κB) signaling pathways. In addition, the cell lines endogenously express high levels of relevant immune checkpoint receptors, including PD-1, TIGIT, and/or GITR for T cells, and SIRPα, Siglec-10, LILRB1, and/or B7-1 for myeloid cells, facilitating their use in immune checkpoint research.
Results: To validate the reporter functionality, NFAT-luciferase T cell lines were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, while NF-κB-luciferase myeloid lines were activated using tumor necrosis factor-alpha (TNF-α) or T cell–conditioned media. A B cell–derived NF-κB reporter line (BDCM-NFκB-Luc2), characterized by high basal luciferase activity, was treated with an NF-κB pathway inhibitor. These reporter lines were further evaluated in co-culture with primary immune and/or cancer cells to investigate immune cell interactions. Luciferase assays were used for rapid and quantitative assessment of reporter activity under all experimental conditions. Results demonstrated that stimuli activating NFAT or NF-κB pathways led to dose-dependent increases in luciferase expression, while pathway inhibition caused corresponding decreases. Notably, co-culture with various other immune and cancer cell types elevated luminescence signals in different levels indicating that interactions with other cell types in tumor microenvironment led to varying degrees of signaling activation.
Conclusions: These newly developed immune reporter cell lines provide a robust and scalable ex vivo platform for the evaluation of cancer immunotherapies. They enable sensitive, reproducible monitoring of the dynamic interplay between cancer cells and components of the innate and adaptive immune systems, offering a powerful tool for assessing combinatorial immune responses within the tumor microenvironment.

Background

Protein and RNAseq profiling of immune checkpoint expression in T cell lines and myeloid cell lines

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Cell Lines	ATCC® catalog #	HLA Class I	HLA Class II	PD-1	CTLA4	LAG-3	TIM-3	BTLA	VISTA	TIGIT
Jurkat E6-1	TIB-152™	+	-	45	0	71	83	300	2406	17
TALL-104	CRL-11386™	+	-	75	2	159	1090	301	1051	0
MOLT-3	CRL-1552™	+	+	230	75	107	42	193	3771	32
HH	CRL-2105™	+	+	243	24	3046	749	606	3878	1995
Hut 78	TIB-161™	+	+	231	20	416	267	1154	2886	88
SUP-T1	CRL-1942™	+	-	2076	218	81	29	487	1339	18
HMJ	HB-8587™	+	-	361	46	120	0	464	4095	221
MJ (611)	CRL-8294™	+	-	272	91	348	281	2780	1607	4727
CCRF-CEM	CCL-119™	+	-	108	13	91	111	222	118	53
Primary CD8+ T cells	PCS-800-017™	+	-	812	98	274	33765	623	1378	88
Primary CD4+ T cells	PCS-800-016™	+	-	321	106	78	181	756	1009	32

Cell Lines	ATCC® catalog #	Exp	Disease	Cell Type	CD274 (PD-L1)	PDCD1 (PD1)	CD80 (B7-1)	HAVCR2 (TIM3)	SIRPA	LILRB1	SIGLEC10
ATCC bulkRNAseq by Psomagen* Log2(FPKM+1)	THP1 TIB-202™	R1	Acute monocytic leukemia	monocyte	0.08	0	0	0.35	4.49	1.32	1.47
		R2			0.27	0	0	0.25	4.47	1.22	1.52
	KG1 CCL-246™	R1	Acute myelogenous	macrophage	0.27	0	0	1.94	2.72	2.31	4.1
		R2			0.34	0	0	2.2	2.57	2.35	3.92
	U937 CRL-1593.2™	R1	Histiocytic Lymphoma	monocyte	0.1	0	0	0.44	6.02	1.97	2.81
		R2			0.07	0	0	0.5	6.11	1.96	2.98
	HMC3 CRL-3304™	R1	Brain microglial cell	microglial cell	3.56	0	0	0	4.05	0	0
		R2			3.63	0	0	0	4.03	0	0

Cell Lines	ATCC® catalog #	Disease	Cell Type	CD274 (PD-L1)	PDCD1 (PD1)	CD80 (B7-1)	HAVCR2 (TIM3)	SIRPA	LILRB1	SIGLEC10
CCLE Expression 22Q2 Public Log2(TPM+1)	THP1 TIB-202™	Acute monocytic leukemia	monocyte	0.33	0	0.06	0.45	4.72	1.68	1.99
	KG1 CCL-246™	Acute myelogenous	macrophage	0.83	0.04	0.06	2.41	2.44	3.5	4.43
	U937 CRL-1593.2™	Histiocytic Lymphoma	monocyte	0.15	0.03	0.01	1.88	6.07	2.11	2.81
	HMC3 CRL-3304™	Brain microglial cell	microglial cell	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B

C

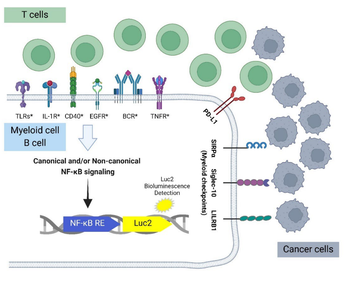

Figure 1: Protein profiling data of selected T cell lines and RNA-seq profiling data of selected myeloid cell lines for immune checkpoint molecule expression. (A) Immune checkpoint expression levels in T cell lines were profiled by flow cytometry. Table values represent median fluorescence intensity (MFI) of sample subtracted by isotype control MFI. Each column was color-coded separately to avoid cross comparison. (B) Immune checkpoint gene expression levels in myeloid cell lines were profiled by RNA sequencing and the results were compared with Cancer Cell Line Encyclopedia (CCLE, Broad Institute) RNA-seq expression data. (C) Mechanism of action of the NFκB-Luc2 myeloid cell lines for myeloid checkpoint studies. Created with BioRender.com.

Results

Myeloid reporter cells demonstrate NF-κB signaling pathway activation via luciferase expression

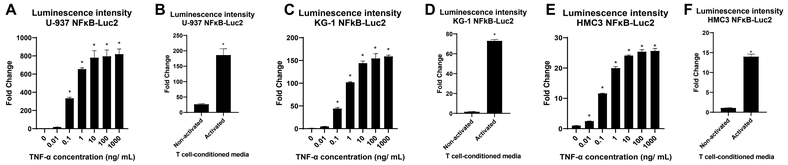

Figure 2: Activation of NF-κB signaling induces luciferase expression in myeloid reporter cell lines. (A-B) U-937 NFκB-Luc2 cell line (ATCC® CRL-1593.2-NFκB-LUC2™) with high endogenous SIRPα expression was stimulated for 6 hours with (A) TNF-α or (B) T cell–conditioned media. (C-D) KG-1 NFκB-Luc2 cell line (ATCC® CCL-246-NFκB-LUC2™) with high endogenous siglec-10 expression was stimulated for 6 hours with (C) TNF-α or (D) T cell–conditioned media. (E-F) HMC3 NFκB-Luc2 cell line (ATCC® CRL-3304-NFκB-LUC2™) with high endogenous PD-L1 and SIRPα expression was stimulated for 6 hours with (E) TNF-α or (F) T cell–conditioned media. For the conditioned media stimulation, the cells were administered with the conditioned media collected from non-activated or activated human primary CD4+ helper T cells. The activated conditioned media were harvested 3 days post-activation with anti-CD2/CD3/CD28 beads. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

B reporter cells demonstrate NF-κB signaling pathway inhibition via decreased luciferase expression

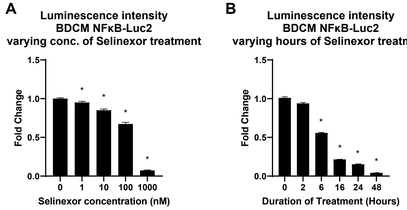

Figure 3: Inhibition of the NF-κB signaling pathway by Selinexor treatment results in a decrease in luciferase expression in the B reporter cell line. BDCM NFκB-Luc2 cells (ATCC® CRL-2740-NFκB-LUC2™) that endogenously express high levels of checkpoint molecules LILRB1 and B7-1 were incubated with (A) various concentrations of Selinexor for 24 hours or (B) 1 μM Selinexor for different lengths of time. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

T reporter cells demonstrate NFAT signaling pathway activation via luciferase expression

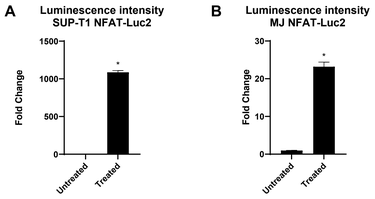

Figure 4: Activation of the NFAT signaling pathway leads to an increase in luciferase expression in T reporter cell lines. (A) The SUP-T1 NFAT-Luc2 cell line (ATCC® CRL-1942-NFAT-LUC2™) with high endogenous expression of PD-1 and (B) MJ NFAT-Luc2 cell line (ATCC® CRL-8294-NFAT-LUC2™) with high endogenous expression of TIGIT and GITR were stimulated for 6 hours with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 10 ug/mL ionomycin. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

Co-culture with primary T cells increases luciferase expression in myeloid reporter cell lines

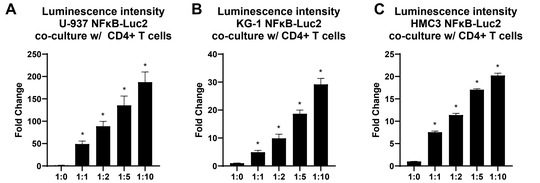

Figure 5: Activation of NF-κB signaling upon co-culture with primary CD4+ T cells results in increased luciferase expression in myeloid reporter cell lines. (A) U-937 NFκB-Luc2 cells, (B) KG-1 NFκB-Luc2 cells, and (C) HMC3 NFκB-Luc2 cells were co-cultured with human primary CD4+ helper T cells for 6 hours. The ratios of myeloid reporter cells to CD4+ T cells were 1:0, 1:1, 1:2, 1:5, and 1:10. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

Reporter cell lines display effects of triple co-culture of CAR-T, myeloid, and cancer cells on signaling pathways

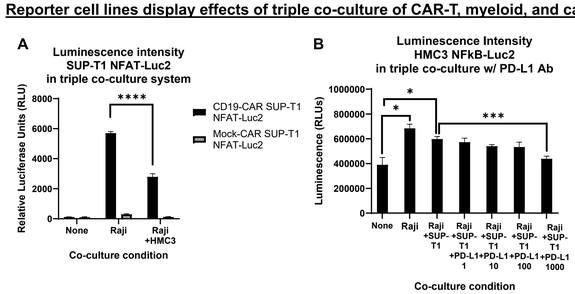

Figure 6: Triple co-culture of CD19-CAR T, myeloid, and cancer cells demonstrates signaling activation or suppression in the reporter cell lines. CD19-CAR (Chimeric Antigen Receptor) SUP-T1 cells were generated from SUP-T1 parental and reporter cells to express a CAR that targets CD19 protein expressed on Raji cells. (A) CD19-CAR or mock-CAR SUP-T1 NFAT-Luc2 reporter cells were co-cultured with Raji and HMC3 parental myeloid cells for 6 hours. (B) HMC3 NFκB-Luc2 myeloid reporter cells were co-cultured with CD19-CAR SUP-T1 parental cells and Raji cells for 6 hours in the presence of PD-L1 mAb (1-1,000 ng/ mL). Cell ratios were 1:1:0.5 (CAR-T : Raji : HMC3). Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. ****, P < 0.0001. *, P < 0.05.

Conclusion

- Three myeloid reporter cell lines, U-937 NFκB-Luc2 (ATCC® CRL-1593.2-NFkB-LUC2™), KG-1 NFκB-Luc2 (ATCC® CCL-246-NFkB-LUC2™), and HMC3 NFκB-Luc2 (ATCC® CRL-3304-NFkB-LUC2™), with high endogenous expression of SIPRα, siglec-10, and PD-L1/SIRPα, respectively; one B reporter cell line, BDCM NFκB-Luc2 (ATCC® CRL-2740-NFkB-LUC2™), with high endogenous expression of LILRB1 and B7-1; and two T reporter cell lines, SUP-T1 NFAT-Luc2 (ATCC® CRL-1942-NFAT-LUC2™) and MJ NFAT-Luc2 (ATCC® CRL-8294-NFAT-LUC2™), with high endogenous expression of PD-1 and TIGIT/GITR, respectively, were successfully developed based on the protein and RNAseq profiling data for endogenous immune checkpoint expression.
- These immune reporter cell lines exhibit strong, sensitive, and reproducible luciferase signal upon NF-κB signaling activation in myeloid and B cells or NFAT signaling activation in T cells. When pro-inflammatory signal triggers activation of the above signaling pathway, this system enables reliable quantification of the respective signaling activation, offering a convenient means for measuring the level of immune responses from these reporter cells.
- Endogenous expression of immune checkpoints in the reporter cell lines allows preservation of physiological relevance and stable expression while effectively eliminating the donor variability issue commonly associated with using primary immune cell models, providing a more practical alternative to the conventional models.
- The co-culture of CAR-T cells, myeloid cells, and cancer cells using reporter cell lines demonstrated the negative effect of the presence of myeloid cells on the activation of CAR-T cells. Interestingly, the addition of PD-L1 antibody to the triple co-culture decreased the NF-κB signaling activation in the myeloid reporter cells, displaying the intricate role of NF-κB signaling pathway in myeloid cells in response to the immune checkpoint blockade.
- The co-culture experiments combining myeloid reporter cells with T cells and cancer cells demonstrated the value of these novel immune reporter cell lines for investigating complex, multi-directional interactions among cancer cells, innate and adaptive immune cells, and other tumor microenvironmental components—an essential step toward understanding the intricate immune landscape of the tumor microenvironment.

Key References

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