GAS-Luc2 reporter cell lines exhibit enhanced performance compared to the industry-standard interferon-gamma ELISA for immune activation studies and CAR-T evaluation

Abstract 14

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Abstract

Background: Interferon-gamma (IFN-γ) is a key cytokine involved in the activation of cellular immunity and the promotion of anti-tumor responses, and it serves as a critical biomarker for evaluating the efficacy of cancer immunotherapies. In CAR-T cell therapy research and development, testing for IFN-γ expression is a crucial aspect, particularly in assessing the potency and safety profile of the CAR-T cells. Although enzyme-linked immunosorbent assay (ELISA) is commonly used to detect IFN-γ, it has limited sensitivity in detecting low levels of the cytokine during the early stages of immune activation. In addition, ELISA is not well-suited for accurately quantifying paracrine IFN-γ signaling in three-dimensional (3-D) co-culture models.

Methods: To overcome these limitations and meet the growing need for effective immune activation monitoring in immunotherapy research, we developed several IFN-γ reporter cancer cell lines. These were engineered with a gamma-interferon activation site (GAS) upstream of a luciferase reporter gene. Upon activation of IFN-γ signaling, the cells express luciferase, enabling direct and quantifiable readout of immune activation. Three of the cell lines were selected through comprehensive protein expression profiling to ensure endogenous expression of immune checkpoint ligands such as PD-L1, CD155, and B7-H3, expanding their utility for immune checkpoint studies. Additional one was derived from the commonly used monocyte cell line THP-1, which recapitulates the function of monocyte and can be further differentiated into macrophage.

Results: To validate the system, reporter cells were exposed to graded doses of IFN-γ, treated with conditioned media from activated primary T cells, or co-cultured with IFN-γ-producing primary immune cells. They were also tested in both 2-D and 3-D co-culture formats alongside ELISA for comparison. Upon IFN-γ stimulation, the reporter cells exhibited a dose-dependent increase in bioluminescence intensity of approximately 100- to 250-fold. Conditioned media from T cells induced a 50- to 100-fold signal increase, while co-cultures with primary T or NK cells in the presence of immune checkpoint inhibitors produced 3- to 12-fold signal enhancements. Importantly, the reporter cells generated robust bioluminescent signals in both 2-D and 3-D systems, even at IFN-γ concentrations undetectable by ELISA, underscoring their superior sensitivity and adaptability. Testing for IFN-γ expressions of CAR-T cells were also conducted and compared between ELISA and reporter cell system in this study.

Conclusions: These reporter cell lines offer a sensitive, reliable, and user-friendly platform for early immune activation assessment and represent a valuable tool for evaluating cancer immunotherapy candidates.

Background

IFN-γ signaling pathway activation promotes luciferase expression in GAS-Luc2 cells

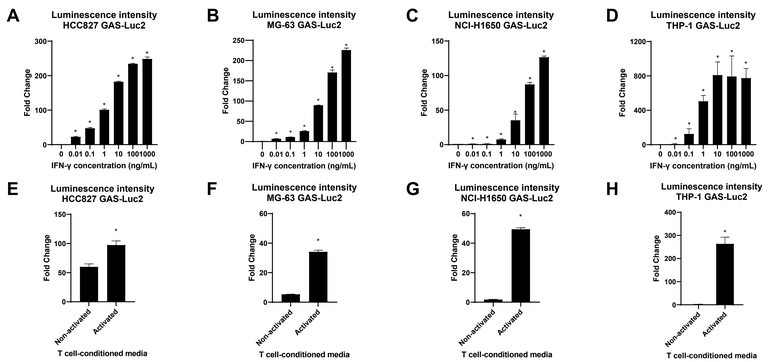


Figure 1: Stimulation of established GAS-Luc2 cell lines with IFN-γ or T cell-conditioned media. (A-D) The following reporter cell lines were stimulated overnight with varying concentrations of IFN-γ. (A) HCC827 GAS-Luc2 (ATCC® CRL-2868-GAS-LUC2™), (B) MG-63 GAS-Luc2 (ATCC® CRL-1427-GAS-LUC2™), (C) NCI-H1650 GAS-Luc2 (ATCC® CRL-5883-GAS-LUC2™), and (D) THP-1 GAS-Luc2 (ATCC® TIB-202-GAS-LUC2™). (E-H) The following reporter cell lines were administered with the conditioned media collected from non-activated or activated human primary CD8+ cytotoxic T cells. The activated conditioned media were harvested 3 days post-activation with anti-CD2/CD3/CD28 beads. (E) HCC827 GAS-Luc2, (F) MG-63 GAS-Luc2, (G) NCI-H1650 GAS-Luc2, and (H) THP-1 GAS-Luc2. 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.

Results

Co-culture with adaptive or innate immune cells yields luciferase expression in GAS-Luc2 cells

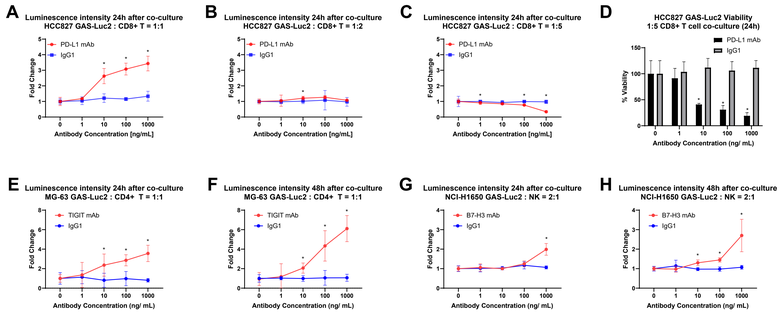


Figure 2: Co-culture of immune checkpoint-expressing GAS-Luc2 cell lines with primary human immune cells at varying cell ratios and co-culture durations in the presence of a corresponding immune checkpoint inhibitor antibody. (A-C) Luminescence intensity from HCC827 GAS-Luc2 after 24-hour co-culture with CD8+ cytotoxic T cells at a (A) 1:1, (B) 1:2, or (C) 1:5 ratio of target to effector cells in the presence of a PD-L1 mAb or isotype control (1-1,000 ng/mL). (D) Percent viability of HCC827 GAS-Luc2 after co-culture with CD8+ cytotoxic T cells for 24 hours in the presence of a PD-L1 mAb or isotype control (1-1,000 ng/mL). (E-F) Luminescence intensity from MG-63 GAS-Luc2 after co-culture at a 1:1 ratio with CD4+ helper T cells for (E) 24 hours or (F) 48 hours in the presence of a TIGIT mAb or isotype control (1-1,000 ng/mL). (G-H) Luminescence intensity from NCI-H1650 GAS-Luc2 after co-culture with CD56+ NK cells at a 2:1 ratio of target to effector cells for (G) 24 hours or (H) 48 hours in the presence of a B7-H3 ADCC mAb or isotype control (1-1,000 ng/mL). 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.

GAS-Luc2 cells demonstrate superior sensitivity to ELISA in 2-D and 3-D co-culture with T cells

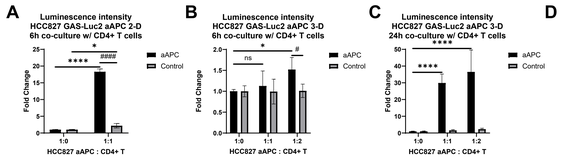
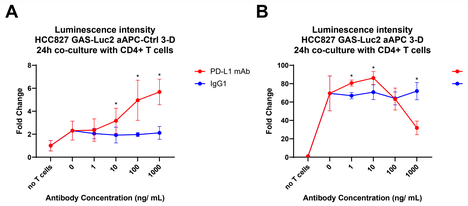


Figure 3: Co-culture of 2-D and 3-D HCC827 GAS-Luc2 aAPC (artificial Antigen Presenting Cells) with primary CD4+ T cells. HCC827-GAS-Luc2 aAPC was generated from HCC827 GAS-Luc2 to express anti-CD3/CD28 to enhance the tumor cell recognition by T cells. (A) HCC827 GAS-Luc2 aAPC and aAPC transduction control cells (no anti-CD3/CD28) cultured in 2-D were co-cultured with primary CD4+ T cells (T:E = 1:0 or 1:1) for 6 hours. (B-C) HCC827 GAS-Luc2-aAPC cells and control cells cultured as 3-D spheroids were co-cultured with primary CD4+ T cells (T:E = 1:0, 1:1, or 1:2) for (B) 6 hours or (C) 24 hours. After the co-culture, the conditioned media were collected for ELISA and the cells were harvested for luciferase assay. The luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). (D) IFN-γ concentrations in the cell-conditioned media were quantified by Human IFN-γ Quantikine ELISA Kit (R&D Systems) N=3 in all experiments. **** or ####, P < 0.0001. * or #, P < 0.05; ns, P ≥ 0.05.

Condition	Cell Type	T:E	OD	IFN-γ conc. (pg/ mL)
2-D / 6h	aAPC	1:0	0.013275	Below MDD*
		1:1	0.27545	59.662791
	aAPC Ctrl	1:0	0.01515	Below MDD*
		1:1	0.0242	Below MDD*
3-D / 6h	aAPC	1:0	0.00815	Below MDD*
		1:1	0.01005	Below MDD*
		1:2	0.04655	Below MDD*
	aAPC Ctrl	1:0	0.01735	Below MDD*
		1:1	0.0089	Below MDD*
		1:2	0.0084	Below MDD*
3-D / 24h	aAPC	1:0	0.0089	Below MDD*
		1:1	0.03945	Below MDD*
		1:2	0.0599	1.643478**
	aAPC Ctrl	1:0	0.0158	Below MDD*
		1:1	0.0106	Below MDD*
		1:2	0.0102	Below MDD*

* MDD (minimum detectable dose) = 1.28 pg/ mL
** Below assay range of 15.6 – 1,000 pg/ mL

Comparison of GAS-Luc2 cells and ELISA in immune checkpoint inhibitor evaluation



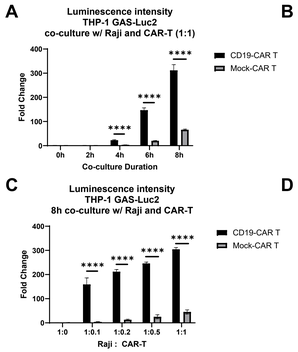
C

Condition	Cell Type	PD-L1 mAb (ng/ mL)	OD	IFN-γ conc. (pg/ mL)
3-D / 24h	aAPC Ctrl	No T cells	0.00775	Below MDD*
		0	0.0076	Below MDD*
		1	0.00865	Below MDD*
		10	0.0082	Below MDD*
		100	0.0079	Below MDD*
		1000	0.008	Below MDD*
	aAPC	No T cells	0.0107	Below MDD*
		0	0.02165	5.357143**
		1	0.0219	5.47619**
		10	0.02825	8.5**
		100	0.04305	15.54762**
		1000	0.15125	67.07143

* MDD (minimum detectable dose) = 1.28 pg/ mL
** Below assay range of 15.6 – 1,000 pg/ mL

Figure 4: 3-D co-culture of HCC827 GAS-Luc2 aAPC or aAPC-control cells with primary CD4+ T cells in the presence of an immune checkpoint inhibitor antibody. (A) HCC827 GAS-Luc2-aAPC transduction control cells and (B) aAPC cultured as 3-D spheroids were co-cultured with primary CD4+ T cells (T:E = 1:1) for 24 hours in the presence of a PD-L1 mAb or isotype control (1-1,000 ng/mL). After the co-culture, the conditioned media were collected for ELISA and the cells were harvested for luciferase assay. The luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). (C) IFN-γ concentrations in the conditioned media were quantified by Human IFN-γ Quantikine ELISA Kit (R&D Systems) N=3 in all experiments. *, P < 0.05.

Comparison of GAS-Luc2 cells and ELISA in CAR-T functionality evaluation



B

Cell Type	Time	OD	IFN-γ conc. (pg/ mL)
CD19-CAR T	0 h	0.0329	9.142857**
	2 h	0.045	14.90476**
	4 h	0.30075	136.6905
	6 h	1.2567	591.9048
	8 h	2.1615	1022.762
Mock-CAR T	0 h	0.0182	2.142857**
	2 h	0.0175	1.809524**
	4 h	0.0218	3. 857143**
	6 h	0.0283	6.952381**
	8 h	0.0297	7.619048**

D

Cell Type	T:E	OD	IFN-γ conc. (pg/ mL)
CD19-CAR T	1:0	0.01595	Below MDD*
	1:0.1	0.0921	42.33333
	1:0.2	0.2342	121.2778
	1:0.5	0.40935	218.5833
	1:1	0.89765	489.8611
Mock-CAR T	1:0	0.0167	Below MDD*
	1:0.1	0.01785	Below MDD*
	1:0.2	0.02055	2.583333**
	1:0.5	0.0251	5.111111**
	1:1	0.03805	12.30556**

* MDD (minimum detectable dose) = 1.28 pg/ mL
** Below assay range of 15.6 – 1,000 pg/ mL

Figure 5: Co-culture of CD19-expressing Raji with CD19-CAR or mock-CAR T cells evaluated by THP-1 GAS-Luc2 cells for CAR-T function. Raji cells were co-cultured with CD19-CAR or mock-CAR T cells (ProMab) in the presence of THP-1 GAS-Luc2 reporter cells. After the co-culture, the conditioned media were collected for ELISA and the cells were harvested for luciferase assay. (A-B) The cells were co-cultured at T:E = 1:1 for 0, 2, 4, 6, or 8 hours. (C-D) The cells were co-cultured for 8 hours at T:E = 1:0, 1:0.1, 1:0.2, 1:0.5, or 1:1 ratio. The activation of CAR-T was measured by (A,C) luciferase assay and (B,D) ELISA. The luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). IFN-γ concentrations in the cell-conditioned media were quantified by Human IFN-γ Quantikine ELISA Kit (R&D Systems). N=3 in all experiments. ****, P < 0.0001.

Conclusion

- GAS-Luc2 reporter cancer cell lines were engineered with GAS-response element upstream of luciferase gene, allowing robust and reproducible luciferase expression upon IFN-γ signaling activation. They enable reliable quantification of diverse immune cell-mediated pro-inflammatory response while preserving physiological relevance and stable expression, eliminating donor variability issues commonly associated with primary cell models.
- In comparison to the conventional ELISA, GAS-Luc2 reporter cells displayed superior assay sensitivity and adaptability, producing strong luminescence signal in response to IFN-γ signaling activation even at concentrations below the minimum detectable dose by ELISA. This technology provides a rapid, cost-effective, and convenient means to assess early-stage immune activation or immune activation in 3-D culture system where traditional methods fall short.
- Artificial expression of anti-CD3/CD28 on HCC827 GAS-Luc2 aAPC for bypassing T-cell receptor (TCR) recognition and directly activating TCR signaling resulted in faster and heightened responses in contrast to aAPC-control cells in 2-D and 3-D co-culture with primary T cells. This highlights the growing potential of the reporter cell lines and their broad applications in cancer immunology research.
- Furthermore, the reporter cell lines were successfully applied to evaluate the functionality of CAR-T cells in co-culture with their target cells. The increased sensitivity and robustness of the method allowed for the assessment to take place at an earlier time (8 hours) in comparison to the standard co-culture duration (24+ hours) utilizing considerably smaller number of CAR-T cells at 1:0.1 T:E ratio compared to the standard ratio of 1:10, providing an indispensable tool for CAR-T functionality evaluation.

Key References

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