GAS-Luc2 reporter cancer cell lines demonstrating superior performance to the industrial standard interferon-gamma ELISA in 2-D and 3-D ex vivo immune activation and drug screening

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Abstract #3959

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Abstract

Interferon-gamma (IFN-γ), a cytokine critical for activating cellular immunity and facilitating anti-tumor responses, has become a key cytokine for assessing the efficacy of cancer immunotherapy drugs. While enzyme-linked immunosorbent assay (ELISA) is widely employed for the detection of IFN-γ, it has shown significant limitations in detecting low levels of IFN-γ during early-stage immune activation. Moreover, it presents serious challenges in accurately quantifying paracrine signaling of IFN-γ secreted by immune cells within 3-D co-culture models. To address the rapidly growing demand for more effective monitoring of immune activation for cancer immunotherapy research and better assessment of immunotherapy drug candidates, we developed immune activation reporter cancer cell lines engineered with a gamma-interferon activation site (GAS)-response element positioned upstream of luciferase gene. Upon activation of IFN-γ signaling pathway, these cells express luciferase, allowing for simple detection and quantification to measure immune activation. The cell lines were selected based on a comprehensive protein profiling to ensure endogenous expression of immune checkpoint ligands, such as PD-L1, CD155, or B7-H3, for additional benefit in application in immune checkpoint research. To evaluate the system, the reporter cells were stimulated with varying concentrations of IFN-γ, treated with conditioned media from primary T cells, or co-cultured with IFN-γ-producing primary immune cells. Additionally, the reporter cells were co-cultured in 2-D or 3-D systems for the comparison with ELISA. Our data revealed that bioluminescence intensity from the reporter cells increased by approximately 100- to 250-fold in a dose-dependent manner following IFN-γ stimulation. Primary T cell-conditioned media stimulation resulted in a 50- to 100-fold increase in bioluminescence intensity. In co-culture assays with primary T cells or NK cells in the presence of immune checkpoint inhibitors, the reporter cell lines exhibited a 3- to 12-fold increase in bioluminescence intensity. Notably, in both 2-D and 3-D co-culture systems, the reporter cells generated a robust bioluminescent signal even at the IFN-γ concentrations below the detection range of conventional ELISA, highlighting their superior sensitivity and versatility. These reporter cell lines can serve as a valuable standard for early-stage monitoring of immune activation and offer a convenient and highly sensitive platform for evaluating cancer immunotherapy drug candidates.

Background

Protein profiling of immune checkpoint expression in popular cancer cell lines

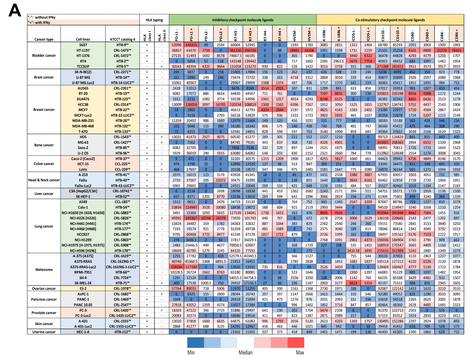

Figure 1: Protein profiling data of selected cancer cell lines for immune checkpoint molecule expression. (A) Immune checkpoint molecule expression levels in cancer cell lines under basal (-) and 100 ng/mL IFN-γ stimulated (+) conditions were profiled by flow cytometry. Table values represent median fluorescence intensity (MFI) of sample subtracted by isotype control MFI. Each column was color-coded separately to avoid cross comparison. (B) Mechanism of action of the GAS-Luc2 luciferase reporter cell lines for cancer immune checkpoint studies. Activation of JAK/STAT signaling pathway in these reporter cells in response to IFN-γ receptor binding leads to luciferase expression which can be readily quantified and analyzed. Created with BioRender.com.

Results

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

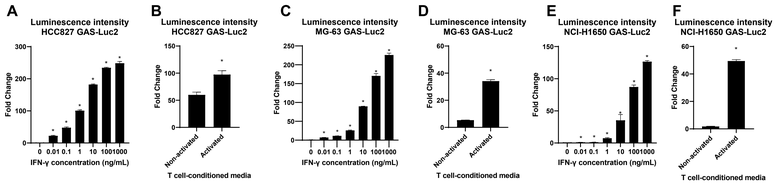

Figure 2: Evaluation of monoclonal GAS-Luc2 cell lines as immune checkpoint reporters. (A-B) HCC827 GAS-Luc2 cell line with high endogenous PD-L1 expression was stimulated overnight with (A) IFN-γ or (B) T cell–conditioned media. (C-D) MG-63 GAS-Luc2 cell line with high endogenous CD155 expression was stimulated overnight with (C) IFN-γ or (D) T cell–conditioned media. (E-F) NCI-H1650 GAS-Luc2 cell line with high endogenous B7-H3 expression was stimulated overnight with (E) IFN-γ 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 CD8+ cytotoxic 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.

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

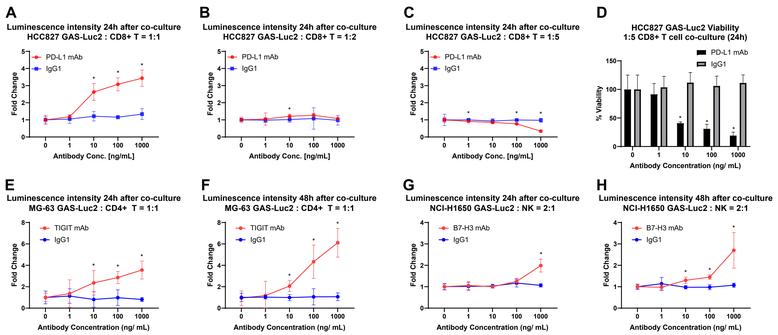

Figure 3: Co-culture of monoclonal GAS-Luc2 cell lines with primary human immune cells at varying cell ratios and co-culture durations in the presence of a respective blocking antibody. (A-C) The luminescence intensity from HCC827 GAS-Luc2 after 24-hour co-culture with CD8+ cytotoxic T cells at a (A) 1:1, (B) 1:2, or (C) 1:5 ratio of target cells to effector cells in the presence of a PD-L1 mAb or isotype control IgG (1-1,000 ng/mL). (D) The 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 IgG (1-1,000 ng/mL). (E-F) The 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 IgG (1-1,000 ng/mL). (G-H) The 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 IgG (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.

Key References

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- Larson RC, et al. CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature 604(7906): 563-570, 2022. PubMed: 35418687
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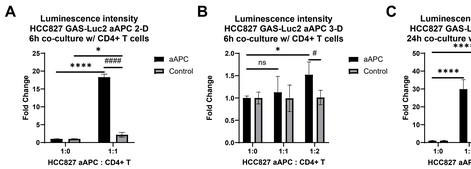
GAS-Luc2 cells demonstrate superior sensitivity to ELISA in 2-D and 3-D co-culture with T cells

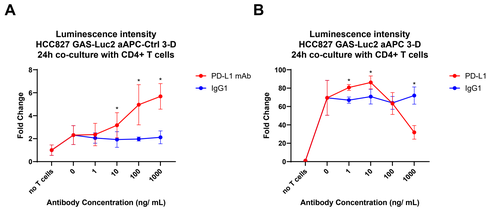


Condition	Cell Type	T:E	OD	IFN-γ conc. (pg/ mL)
2-D / 6h	aAPC	1:0	0.013275	Below MDD*
		1:1	0.27545	59.662791
	aAPC Ctrl	1:0	0.01515	Below MDD*
		1:1	0.0242	Below MDD*
3-D / 6h	aAPC	1:0	0.00815	Below MDD*
		1:1	0.01005	Below MDD*
		1:2	0.04655	Below MDD*
	aAPC Ctrl	1:0	0.01735	Below MDD*
		1:1	0.0089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

Figure 5: Co-culture of 2-D and 3-D HCC827 GAS-Luc2 aAPC (artificial Antigen Presenting Cells) with primary CD4+ T cells evaluated by luciferase assay and ELISA. The aAPC cell line 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 and 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 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.

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



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 6: 3-D co-culture of HCC827 GAS-Luc2 aAPC or aAPC-control cells with primary CD4+ T cells in the presence of a PD-L1 blocking antibody evaluated by luciferase assay and ELISA. (A-B) HCC827 GAS-Luc2-aAPC (A) 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 IgG (1-1,000 ng/mL). After 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 cell-conditioned media were quantified by Human IFN-γ Quantikine ELISA Kit (R&D Systems) N=3 in all experiments. *, P < 0.05.

Conclusion

- Utilizing comprehensive protein profiling data of cancer cell lines for various immune checkpoint molecules, we established the luciferase reporter cancer cell lines HCC827 GAS-Luc2 (ATCC® CRL-2868-GAS-LUC2™), MG-63 GAS-Luc2 (ATCC® CRL-1427-GAS-LUC2™), and NCI-H1650 GAS-Luc2 (ATCC® CRL-5883-GAS-LUC2™), which exhibit high endogenous expression of PD-L1, CD155, and B7-H3, respectively.
- These 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 in cancer cells. They enable reliable quantification of diverse immune cell-mediated pro-inflammatory response induced by immune checkpoint inhibition while preserving physiological relevance and stable expression, eliminating donor variability issues commonly associated with primary cell models.
- Compared to the conventional ELISA method, GAS-Luc2 reporter cells demonstrated superior assay sensitivity and adaptability, producing strong luminescence signal in response to IFN-γ signaling activation at concentrations below the minimum detectable dose by ELISA. This method provides a cost-effective and convenient approach for detecting early-stage immune activation and supporting applications in 3-D cell culture models.
- 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 compared to aAPC-control cells in 2-D and 3-D co-culture with primary T cells. This highlights the potential of reporter cell lines and their broad applications in cancer immunology research.