Luciferase Reporter Cell Lines Allow Simultaneous Incorporation of Tumor Cells, Innate Immune Cells, and Adaptive Immune Cells for In-depth Immune Checkpoint Studies

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Abstract 75

Abstract

Despite the considerable success of immune checkpoint therapies targeting T cells, a sizable proportion of patients experience resistance or relapse due to the immunosuppressive nature of the tumor microenvironment. Myeloid cells, a major component that suppresses effector lymphocytes, have emerged as an alternative and promising therapeutic target. However, there is a deep lack of widely accessible immunological models capable of representing the intricate three-way interaction between tumor cells, T cells, and myeloid cells. To address this need, we conducted a comprehensive protein profiling of human tumor cell lines and RNA-seq profiling of human myeloid cell lines available at ATCC® for various established and novel immune checkpoint molecules. Cell lines with high endogenous expression of the immune checkpoints, such as programmed death-ligand 1 (PD-L1), cluster of differentiation 155 (CD155), B7 homolog 3 (B7-H3), sialic acid-binding Ig-like lectin 10 (Siglec-10), or signal-regulatory protein alpha (SIRPα), were selected and constructed into luciferase reporter cell lines. For tumor reporter cell lines, a gamma interferon activation site (GAS) response element was placed upstream of the luciferase gene in the lentiviral vector, enabling the activation of the JAK-STAT signaling pathway within tumor cells to induce luciferase expression. In myeloid reporter cell lines, a nuclear factor kappa B (NF-κB) response element replaced GAS to monitor the activation of the NF-κB signaling pathway. In the presence of corresponding immune checkpoint inhibitors that enhance T cell-mediated anti-tumor activity, these reporter cell lines produce a bioluminescent signal based on luciferase expression. This signal can be easily detected and quantified to assess the efficacy of the inhibitor. Our data revealed that bioluminescence intensity in the tumor and myeloid reporter cell lines increased by >100-fold in a dose-dependent manner in response to interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) stimulation, respectively, and by >50-fold in response to the conditioned media collected from activated primary T cells. Moreover, in co-culture assays involving various combinations of tumor cell lines and adaptive immune cell types with corresponding immune checkpoint inhibitors, these reporter cell lines demonstrated a significant increase in luciferase expression. Subsequent development into an artificial antigen-presenting cell line (aAPC) further enhanced T cell–induced signaling activation, yielding an even higher bioluminescence intensity. In conclusion, these newly established luciferase reporter cell lines offer an excellent ex vivo model for cancer immunotherapy. These cell lines naturally express immune checkpoint proteins, enabling the sensitive and reproducible monitoring of combinatorial responses from various immune cell types.

Background

Immune checkpoint molecule expression profiling of cancer cell lines and myeloid cell lines

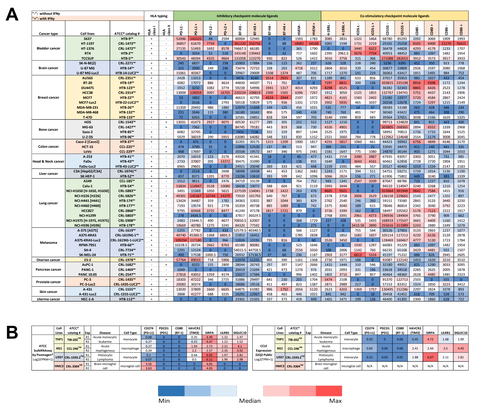

Figure 1: Heat maps based on protein profiling data of selected cancer cell lines and RNA-seq profiling data of selected myeloid cell lines for immune checkpoint molecule expression. (A) Immune checkpoint molecule expression levels in cancer cell lines under basal (-) and 100 ng/mL IFN-γ stimulated (+) 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) Immune checkpoint gene expression levels in myeloid cell lines were profiled by RNA sequencing (Psomagen®) and the results were compared with CCLE (Cancer Cell Line Encyclopedia, Broad Institute) RNA-seq expression data. (C) Mechanism of action of the GAS-Luc2 cancer cell lines for immune checkpoint studies. (D) Mechanism of action of the NF-κB-Luc2 myeloid cell lines for myeloid checkpoint studies. Created with BioRender.com.

Results

Luciferase expression in GAS-Luc2 tumor cells upon JAK-STAT signaling pathway activation

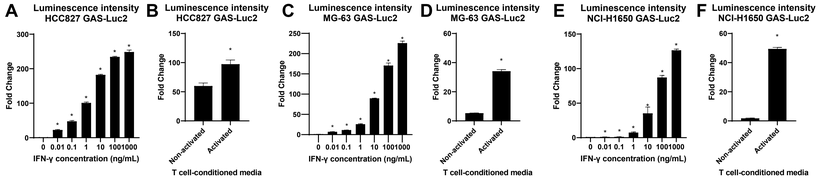

Figure 2: Evaluation of monoclonal GAS-Luc2 cell lines as immune checkpoint reporters. (A-B) HCC827 GAS-Luc2 cell line with high endogenous PD-L1 expression was stimulated overnight with (A) IFN-γ or (B) T cell–conditioned media. (C-D) MG-63 GAS-Luc2 cell line with high endogenous CD155 expression was 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. N=3 in all experiments. *, P < 0.05.

Luciferase expression in GAS-Luc2 cells upon co-culture with primary adaptive immune cells

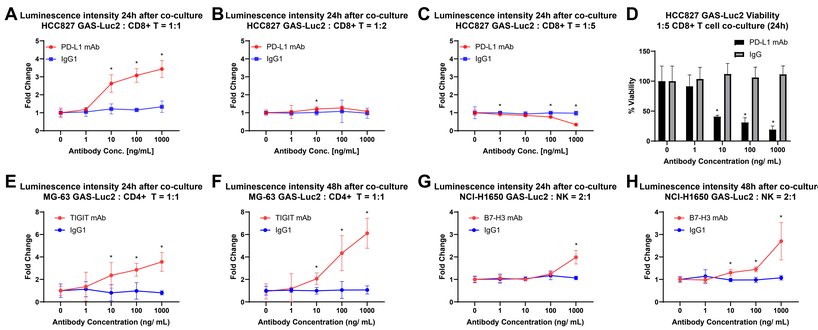

Figure 3: Co-culture of monoclonal GAS-Luc2 cell lines with primary human immune cells at varying cell ratios and co-culture durations in the presence of a respective blocking antibody. (A-C) The luminescence intensity from HCC827 GAS-Luc2 after 24-hour co-culture with CD8+ cytotoxic T cells at a (A) 1:1, (B) 1:2, (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). N=3 in all experiments. *P < 0.05.

Comparison of luciferase expression in non-aAPC and aAPC upon co-culture with primary T cells

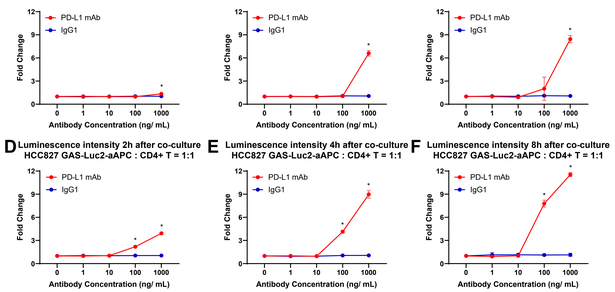

Figure 4: HCC827 GAS-Luc2 and HCC827 GAS-Luc2-aAPC in co-culture with primary human CD4+ T cells in the presence of a PD-L1 blocking antibody. The aAPC cell line was generated from HCC827 GAS-Luc2 to express anti-CD3/CD28 to enhance the tumor cell recognition by T cells. (A-C) The luminescence intensity from HCC827 GAS-Luc2 after co-culture with 1:1 CD4+ helper T cells for (A) 2 hours, (B) 4 hours, or (C) 8 hours. (D-F) The luminescence intensity from HCC827 GAS-Luc2-aAPC after co-culture with 1:1 CD4+ helper T cells for (D) 2 hours, (E) 4 hours, or (F) 8 hours. During the co-culture, the cells were administered with either PD-L1 mAb or isotype control IgG. N=3 in all experiments. *P < 0.05.

Luciferase expression in NFκB-Luc2 myeloid cells upon NF-κB signaling pathway activation

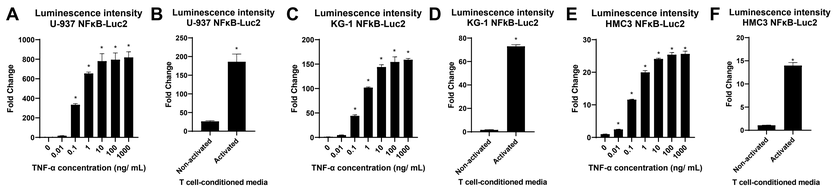

Figure 5: Evaluation of monoclonal NFκB-Luc2 cell lines as immune checkpoint reporters. (A-B) U937 NFκB-Luc2 cell line with high endogenous SIRPα expression was stimulated for 6 hours with (A) TNF-α or (B) T cell–conditioned media. (C-D) KG-1 NFκB-Luc2 cell line with high endogenous Siglec-10 expression was stimulated for 6 hours with (C) TNF-α or (D) T cell–conditioned media. (E-F) HMC3 NFκB-Luc2 cell line 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. N=3 in all experiments. *, P < 0.05.

Conclusion

- Expansive protein profiling data for cancer cell lines and RNA-seq profiling data for myeloid cell lines for numerous immune checkpoint molecules enabled us to develop luciferase reporter cancer cell lines with a high endogenous expression of PD-L1, CD155, or B7-H3 and luciferase reporter myeloid cell lines with a high endogenous expression of SIRPα, Siglec-10, or PD-L1.
- These immune checkpoint reporter cell lines produce robust, responsive, and reproducible luciferase expression upon JAK-STAT signaling or NF-κB signaling activation in cancer cells or in immune cells, respectively, allowing reliable quantification of various types of immune cell-mediated pro-inflammatory responses triggered by relevant immune checkpoint inhibition.
- Artificial expression of anti-CD3/CD28 on HCC827 GAS-Luc2-aAPC to circumvent TCR (T-cell receptor) recognition and trigger TCR signaling elicited more rapid and elevated responses compared to non-aAPC in a co-culture experiment with primary T cells, demonstrating further potential of the reporter cell lines and their broad applications in cancer immunology studies.
- By maintaining physiological relevance and stable expression of the checkpoint molecules owing to the endogenous expression, these reporter cell lines effectively eliminate the donor variability issue commonly experienced by using primary cell models.
- The co-culture experiments with tumor reporter cell lines and various primary immune cells also show the possibility of more intricate three-way immune cell modeling for the in-depth study of tumor cell, T cell, and myeloid cell interactions.

Key References

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