GAS-Luc2 Reporter Cell Lines for Immune Checkpoint Drug Screening in Solid Tumors

Hyeoung Chang, PhD; Kevin M. Tyo, PhD; John G. Fouke, MS; Lumping Chen, BS; Fang Tian, PhD; Zhizhuang Gu, MD, PhD

ATCC®, Manassas, VA 20110-2209

Abstract

Cancer immunotherapies that target immune checkpoints, such as immune checkpoint inhibitors (ICIs), antibody-dependent cellular cytotoxicity (ADCC), and antibody drug conjugates (ADCs), have shown tremendous success in the treatment of solid tumors, including skin, lung, breast, renal, and liver cancers. However, the built-in complexity of immunological models and the variable drug responses among different cancer types have challenged the development and application of these novel immunotherapies. To facilitate large-scale drug discovery for this growing class of immunomodulators, we conducted a comprehensive cell surface protein profiling of ATCC®'s vast portfolio of human tumor and immune cell lines for established and novel immune checkpoint molecules as well as their drug targets. Based on this protein profiling data, we generated three immune checkpoint reporter cell lines HCC827-GAS-Luc2 (ATCC® CRL-2889-GAS-LUC2™), MG-63-GAS-Luc2 (ATCC® CRL-1247-GAS-LUC2™), and NCI-H520-GAS-Luc2 (ATCC® CRL-834-GAS-LUC2™), which endogenously express high levels of programmed death-ligand 1 (PD-L1), cluster of differentiation 195 (CD195), and PD-L2, respectively. These reporter cell lines were engineered to contain a gammalineron activation site (GAS)-response element upstream of a luciferase gene. The luciferase expression is suppressed when the relevant immune checkpoint molecule on the cancer cell binds to the corresponding checkpoint molecule on the T cells. In the presence of a relevant immune checkpoint inhibitor, the GAS-Luc2 reporter cell senses the IFNγ from the activated T cells to produce a luciferase expression-based bioluminescent signal. This signal can be readily detected and quantified to evaluate the efficacy, potency, and dynamic of the checkpoint inhibitor. In addition to drug screening for immune checkpoint inhibitors, these GAS-Luc2 reporter tumor cell lines have also been demonstrated to be effective in detecting paracrine IFNγ signaling for immune checkpoint-targeted ADCC drug development.

Background

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Figure 1: Schematics of the immune checkpoint molecule-expressing GAS-Luc2 reporter system

Figure 2: Heat map based on protein profiling data of selected cancer cell lines for immune checkpoint molecule expression by flow cytometry. Immune checkpoint molecule high expression levels in cancer cell lines under test (■) and >100, high (□) conditions were profiled. HLA class was defined by either low expression (-) or high expression (+). Table values represent median Fluorescence intensity samples validated by luciferase-activated MRI. Each column was color-coded separately to avoid cross contaminations.

Results

Figure 3: Cytokine stimulation of monoclonal GAS-Luc2 cell lines with IFNγ or CD80/CD86-APC.

Conclusion

- The expandable protein profiling of cancer cell lines for numerous immune checkpoint molecules and their ligands provides crucial information that facilitates immune checkpoint molecule interaction studies, checkpoint assay development, and cancer immunotherapy screening.
- Based on the protein profiling data, we developed three cancer reporter cell lines with a high endogenous expression of PD-L1, CD80, or CD86.
- The naturally high expression of the immune checkpoint molecules on the surface of the reporter cell facilitates the immune checkpoint inhibitor drug variable binding, promoting the immune cell activation and subsequent release of IFNγ by the immune cells, resulting in the introduction IFNγ and ADCC/GAS signaling activation and luciferase expression by the reporter cells.
- The reporter cell lines present robust, quantitative, and reproducible luciferase expression upon signaling activation that enables reliable measurement of the potency and stability of the relevant immune checkpoint inhibitors that trigger immunomodulatory microenvironmental-mediated immune responses.
- By maintaining physiological relevance and stable expression of the checkpoint ligand using the endogenous expression, these reporter cell lines effectively eliminate the donor variability issue commonly experienced by using primary cell lines.

Figure 4: Cytokine stimulation of monoclonal GAS-Luc2 cell lines with IFNγ or CD80/CD86-APC.