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［投稿論文：研究ノート］

Synthetic Tumor Recruited Immuno-Cellular Therapy (STRICT)
合成腫瘍リクルート免疫細胞療法

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Abstract: Synthetic Tumor Recruited Immuno-Cellular Therapy (STRICT) enables tumor-localized therapeutic payload by recruitment and activation of immune cells to improve existing treatment regiments for metastatic cancer and alleviate triple-negative breast cancer (TNBC).

合成腫瘍リクルート免疫細胞療法（STRICT）は、腫瘍に限局したペイロードの産生を可能にし、免疫細胞の勧誘と活性化により転移性癌の既存の治療レジメンを改善、トリプルネガティブ乳癌（TNBC）の治療に有望な治療法である。

Keywords: breast cancer, cancer immunotherapy, gene circuits
乳がん、がん免疫療法、遺伝子回路

1 Introduction

Breast cancer is a disease where cells in the lobules in milk glands or ducts that connect the lobules to the nipple undergo uncontrolled division, resulting in the formation of abnormal lumps or masses. In the United States, breast cancer is a disease that most commonly affects women. Approximately one in eight women in the United States will experience the development of invasive breast cancer during

their lifetime. Among all breast cancer subtypes, triple-negative breast cancer (TNBC) has comparably worse prognosis and often relapses after treatment. Traditional cancer immunotherapies have demonstrated potential, but significant challenges persist in treating solid tumors, such as breast cancer. Despite remarkable progress with multiple novel agents targeting ER+ or HER2+ breast cancers, treatment is limited to cytotoxic chemotherapy, and few options have developed in the past decades. The overall response rate of several early-phase clinical trials of advanced TNBC therapies was lower than 10%. Due to the low mutation burden of breast cancer, it forms hard-to-penetrate tumor masses. It also creates an immunosuppressive tumor microenvironment (TME) that limits immune cell recruitment, infiltration, and activation.

Gene therapies are increasingly applied to deliver payload genes into cancer cells to trigger anti-tumor immunity. Examples include intra-tumoral injection of viral vectors[1)] developed to produce chemokines to enhance immune cell recruitment. Despite intra-tumoral injection, the possibility for non-specific infection of surrounding normal cells remains, and breast cancer patients often do not respond to a single immunotherapy agent such as a checkpoint inhibitor. Therefore, combination therapies have been used to enhance the response rate. However, combination therapies generally trigger significantly higher systemic toxicity than monotherapy and are challenging to administer. As the breast cancer microenvironment strongly inhibits functional immune cell recruitment and activation, chimeric antigen receptor (CAR)-T cells have shown limited efficacy to date. To combat this challenge, we are constructing Synthetic Tumor Recruited Immuno-Cellular Therapy (STRICT), a second-generation immunotherapy system to enhance specificity and efficacy. STRICT utilizes gene circuits that are encoded in viruses and delivered to the target sites to enable the production of therapeutic payloads for the recruitment and activation of immune cells.

2 Research Question

STRICT gene circuits are designed to sense the activities of two cancer-associated transcription factors (TFs) within the cells. These circuits use Boolean-logic computation to determine if the cell is cancerous, and are programmed to detect pre-defined intracellular tumor signatures. Only when the activities of both TFs are high, the circuits will trigger tumor-localized combinatorial immunotherapy, while keeping normal cells from harm. If the cell is considered cancerous, the circuit will hijack and command the cell, producing an effective tumor-localized combinatorial immunotherapy. Thus, we leveraged STRICT to induce a more effective tumor-localized anti-TNBC immunity with less systemic toxicity and evaluated combinatorial immunotherapies to overcome TNBC resistance for other challenging cancer types.

STRICT was validated in TNBC cell lines and primary patient tumors, but not in normal mammary epithelium in vitro. STRICT will be validated in the whole mouse body in vivo to minimize any potential off-target toxicity when administered systematically. To achieve maximal delivery and therapeutic output combinations, we examined STRICT's ability to express tumor-localized combinatorial immunotherapy and its efficacy in triggering an immune response to reduce TNBC cells in vitro and in vivo. We measured T-cell-mediated cytotoxicity and cytokine production in vitro. We will continue to measure tumor burden and survival benefit and identify the minimal delivery requirement for achieving robust efficacy in vivo.

3 Discussion

STRICT may trigger systemic toxicity in advanced metastatic breast cancer because high-level production of cytokines may result in systemic exposure. However, we aim to address this challenge by utilizing a small molecule inducible transcription factor to replace GAD (a fusion protein consisting of the yeast GAL4 DNA-binding domain and the viral VP16 transcriptional activation domain), which drives output production in STRICT. This will allow fine-tuning of output level by

adjusting the dosage of small molecule inducers. We will also measure systemic cytokine levels and signs of systemic toxicity to validate the safety profile. Furthermore, we are developing composite sensors that identify TNBC-specific TFs through screening to enhance the detection of heterogenous TNBC samples. Additionally, as one-third of invasive breast cancer patients relapse after therapy, we hope to leverage STRICT to eliminate systemic metastasis and establish long-term immune memory[2] to obviate tumor replacement and morbid systemic chemotherapy.

4 Conclusion

We demonstrated that STRICT mediates robust therapeutic efficacy in TNBC in vitro, even when only a small fraction of tumor cells was delivered with the circuit. To further accomplish the clinical translation of STRICT, we continue to build breast cancer-specific circuits that efficiently detect highly heterogeneous patient tumors, and optimize tumor-targeting efficiency, specificity, and therapeutic output combinations to encode these circuits into FDA-approved viral vehicles[3]. We hope to utilize STRICT to achieve maximal anti-tumor immunity, prevent relapse, and elicit a robust host immune response against metastatic TNBC.

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Conflict of interest

There are no potential conflicts of interest.

Endnote

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