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Proteogenomic analysis of NCC-S1M, a gastric cancer stem cell-like cell line that responds to anti-PD-1

Jun Won Park [a, b, 1], Hyejin Um [a, 1], Hanna Yang [a], Woori Ko [a], Dae-Yong Kim [b, **], Hark Kyun Kim [a, *]

[a] National Cancer Center, 323 Ilsanro, Goyang, Gyeonggi 10408, Republic of Korea
[b] College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea

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ABSTRACT

To elucidate signaling pathways that regulate gastric cancer stem cell (CSC) phenotypes and immune checkpoint, we performed a proteogenomic analysis of NCC-S1M, which is a gastric cancer cell line with CSC-like characteristics and is the only syngeneic gastric tumor cell line transplant model created in the scientific community. We found that the NCC-S1M allograft was responsive to anti-PD-1 treatment, and overexpressed *Cd274* encoding PD-L1. PD-L1 was transcriptionally activated by loss of the TGF-β signaling. Il1rl1 protein was overexpressed in NCC-S1M cells compared with NCC-S1 cells that are less tumorigenic and less chemoresistant. *Il1rl1* knockdown in NCC-S1M cells reduced tumorigenic potential and *in vivo* chemoresistance. Our proteogenomic analysis demonstrates a role of Smad4 loss in the PD-L1 immune evasion, as well as Il1rl1's role in CSC-like properties of NCC-S1M.

1. Introduction

Signaling pathways that regulate gastric cancer stem cell phenotypes, such as self-renewal potential, are not fully known. We previously reported NCC-S1M, a gastric cancer cell line with cancer stem cell (CSC)-like properties, including increased tumor-initiating potential. NCC-S1M is a metastatic derivative of NCC-S1, a primary cultured cell line from a gastric cancer that was formed in a *Villin-cre;Smad4*$^{F/F}$*;Trp53*$^{F/F}$*;Cdh1*$^{F/+}$ mouse [1,2]. NCC-S1M is more chemoresistant, and exhibits a higher degree of tumor-initiating and metastatic potentials, than its parental cell line NCC-S1 [1].

NCC-S1M is a syngeneic gastric tumor cell line transplant model, which is a rare and unique resource in the scientific community [1]. NCC-S1M proved useful in testing the efficacy of anti-4-1BB, an immunotherapeutic agent [1]. In the era of cancer immunotherapy, there is a strong need for syngeneic mouse tumor models, which are crucial for testing the efficacy of immunotherapeutic agents in immunocompetent mice. Anti-PD-1/PD-L1 antibodies are emerging as promising therapeutic options for gastric cancer, demonstrating promising efficacy in patients with chemorefractory metastatic gastric cancers [3]. Regulatory mechanisms for immune checkpoint signaling in gastric cancer are under active investigation, but have not been fully elucidated. We have found that NCC-S1M allograft is responsive to anti-PD-1 treatment, thereby serving as a unique syngeneic gastric tumor transplant model that is useful for developing immune checkpoint inhibitors. In this paper, we sought to identify key genes that may determine phenotypes of NCC-S1M, including anti-PD1 responsiveness and CSC-like features, through a proteogenomic analysis.

2. Materials and methods

Anti-mouse-PD-1 (10mg/kg, BioLegend, San Diego, CA) was intraperioneally administered to mice twice a week. For *in vivo* metastasis study, the liver was harvested 6 weeks after injecting 5×10^5 cells in the spleen. RNA sequencing was conducted using 1 μg of total RNAs isolated from normal gastric epithelium and NCC-S1M cells with a HiSeq 2000 sequencer and TruSeq protocol (Illumina, San Diego, CA). Array comparative genomic hybridization (CGH) analysis was performed using Mouse GE 4 × 44 K v2 Microarrays (Agilent Technologies, Santa Clara, CA). For proteomic

Abbreviations: CSC, cancer stem cell; CGH, comparative genomic hybridization; LC, liquid chromatography; MS, mass spectrometry; NGE, normal gastric epithelium; TGF, transforming growth factor; TMA, tumor tissue microarray.

* Corresponding author.
** Corresponding author.
E-mail address: hkim@ncc.re.kr (H.K. Kim).
[1] These authors equally contributed to this work.

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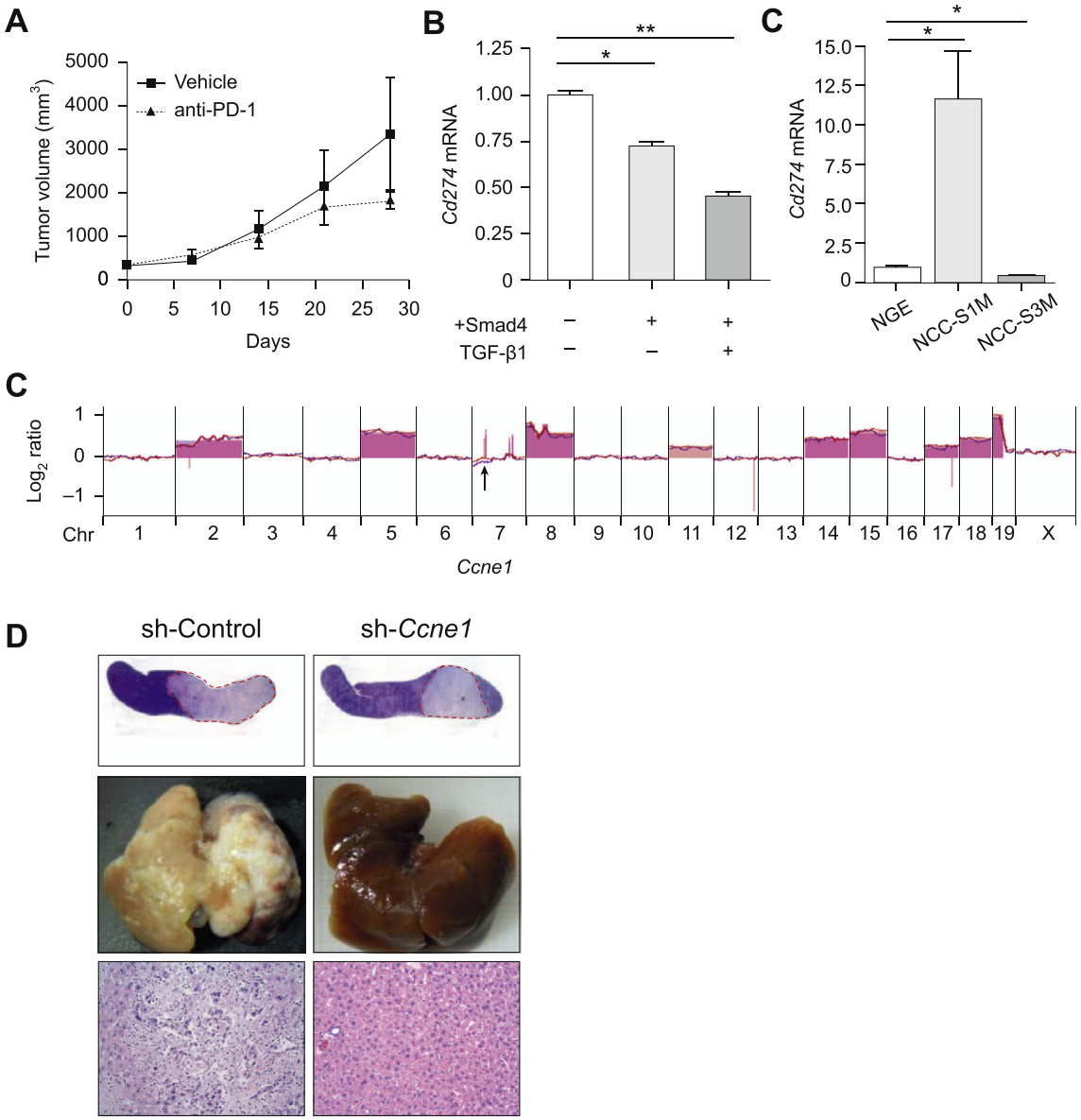

Fig. 1. **P < 0.001; *P < 0.01. (A) *In vivo* growth rate of NCC-S1M allograft decreased after treatment with anti-PD-1. (B) Real-time PCR for *Cd274* mRNA after re-expression of *Smad4* in *Pdx1-cre;Smad4*$^{F/F}$*;Trp53*$^{F/F}$*;Cdh1*$^{F/+}$ cells; 16-h exposure of 5 ng/ml of TGF-β ($P = 7.2 \times 10^{-5}$) (C) Real-time PCR for *Cd274* mRNA in NCC-S1M and NCC-S3M (D) Copy number profiles of NCC-S1M; arrow, focal amplification in *Ccne1* (E) Splenic injection of NCC-S1M; metastasis in the liver decreased after knockdown for *Ccne1*; Top panel, red lines delineate tumor areas in the spleen; middle panel, gross morphology of the liver; bottom panel, microscopic photograph of the liver (magnification, 200×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

determination, the tryptic peptides were analyzed nano-liquid chromatography (LC)-tandem mass spectrometry (MS/MS).

3. Results

Anti-PD-1 treatment suppressed *in vivo* growth of NCC-S1M allografts in syngeneic mice compared with control rat IgG (Fig. 1A). Consistent with previous clinical reports that a PD-L1 overexpression in tumor and stroma significantly correlates with anti-PD-1 responsiveness [4], our RNA sequencing analysis indicated that NCC-S1M cells overexpress *Cd274* (encoding PD-L1) compared with normal gastric tissue (Table 1).

The molecular basis for PD-L1 overexpression in tumors is mostly unclear, although *CD274* gene rearrangements and amplifications have been reported in lymphoma and gastric cancer, especially EBV-positive gastric cancer [5,6]. Since NCC-S1M is primary cultured from a gastric tumor formed in mice deficient in *Smad4* and *Trp53*, we first investigated if *CD274* is under the

Table 1
RNA and protein expression levels of selected genes.

Gene name	FPKM[a]		FC[b], proteomic determination (NCC-S1M *vs.* normal)
	Normal	NCC-S1M	
Cd274	0.2	2.0	N/D[c]
Ccne1	0.3	5.3	N/D
Il1rl1	0	200.7	6.2

[a] FPKM, Fragments Per Kilobase of exon per Million fragments mapped.
[b] FC, fold change in $\log_2$ of NCC-S1M *vs.* normal gastric epithelium of protein expression levels quantified based on the spectral count data.
[c] N/D, not determined.

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