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SURF4 has oncogenic potential in NIH3T3 cells

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ABSTRACT

SURF4, which is located in the Surfeit gene cluster, encodes for a conserved integral membrane protein containing multiple putative transmembrane regions. However, the physiological role of SURF4 has not been determined. We found that *SURF4* demonstrated aberrant amplification and increased expression in the tumor tissues of several human cancer patients. Overexpression of SURF4 led to increased cell proliferation, migration, and maintenance of anchorage-independent growth. In addition, NIH3T3 cells overexpressing SURF4 induced tumor growth in the mice. Collectively, our findings demonstrate that SURF4 has the potential for inducing cellular transformation and cell migration *in vitro* and has oncogenic transformation ability *in vivo*.

1. Introduction

The human Surfeit locus maps to 9q34.2 and has several unusual features including overlapping genes and a bidirectional transcriptional promoter [1–4]. The directions of transcription of five of these genes alternate in relation to neighboring genes, and the 5′ ends of each of these genes are associated with a CpG-rich island. The mouse Surfeit locus contains a cluster of six sequence-unrelated housekeeping genes in ~45 kb of genomic DNA [5,6]. One of the genes in this cluster, *SURF4*, is conserved between human and mouse and encodes an integral membrane protein [7]. ERV29p, the yeast homolog of *SURF4*, is enriched in COPII vesicles operates as a transport receptor for specific cargo in yeast [3,8,9]. SURF4 is known to interact with ERGIC53 and p25 proteins, and it interacts with STIM1 in ER lumina and modulates STIM1-mediated store-operated Ca^{2+} entry (SOCE) [4]. A recent study on the functions of *SURF4* in gastrointestinal stromal tumor-derived exosomes has highlighted new potential diagnostic biomarkers [10].

In previous studies, we characterized and described the tumor suppressive function of RASSF1A [11,12], and these findings prompted us to screen putative oncogenes for effects on cell proliferation using *in silico* screening for copy number alterations (CNA) in different cancer types [13,14]. In this study, we report that *SURF4* was amplified and highly expressed in the tumor tissues of several human cancer patients. Patients who tumors showed high *SURF4* expression had significantly shorter overall survival than those whose tumors had low *SURF4* expression. In addition, overexpression of SURF4 led to increased cell proliferation, migration, and maintenance of anchorage-independent growth *in vitro*. NIH3T3 cells overexpressing SURF4 induced tumor growth in mice. Collectively, our findings demonstrate that SURF4 can modulate cellular functions important for oncogenic transformation *in vivo* and suggest that SURF4 plays an oncogenic role in NIH3T3 cells.

2. Materials and methods

2.1. Plasmid construction

Human cDNA for *SURF4* (NM_033161) was cloned into HA-pCDNA3 (Invitrogen) or HA-pMSCV-Puro (Clontech) plasmids. To generate small interfering RNA (siRNA) against *SURF4* (si*SURF4*), annealed oligonucleotides containing *SURF4* sequences (sense 5′-

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GAT CCC CTC AGC ACC TTC CTG GAG GAT TCA AGA GAT CCT CCA GGA AGG TGC-3′ and antisense 5′-AGC TTT TCC AAA AAT CAG CAC CTT CCT GGA GGA TCT CTT GAA TTC TCC AGG AAG-3′) were ligated into pSUPER-retro vectors (Oligoengine) that had been digested with *Bgl* II and *Hind* III.

2.2. Cell culture

HEK293T cells were transiently transfected with plasmids by polyethyleneimine (PEI) precipitation, and retroviruses were produced by transfecting HEK293T packaging cells with the corresponding control or pMSCV-Puro_SURF4 vectors. After 48 h of transfection, virus-containing culture supernatants were used to infect NIH3T3 cells, and these cells were subsequently selected for 7 days in the presence of puromycin (3 μg/ml; Sigma).

2.3. Western blot

Western blot analysis was carried out following standard methods as previously described [15].

2.4. Reverse transcription PCR (RT-PCR)

Reverse transcription PCR was performed as previously described [15].

2.5. Cell proliferation assay

Cells were plated into a 6-well plate at a density of 10^5 cells/well and counted every other day.

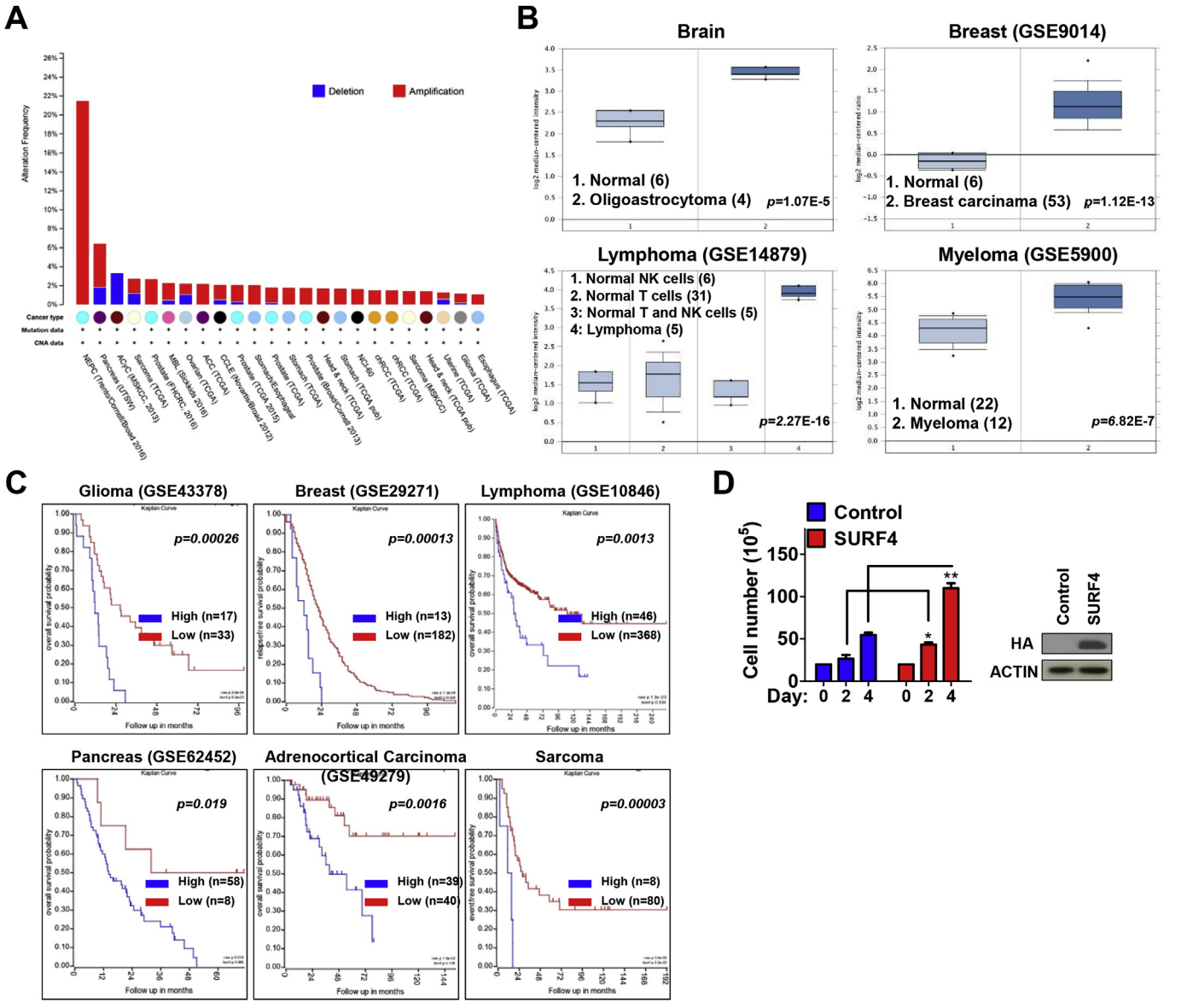

Fig. 1. *SURF4* expression in cancer patients. (A) Copy number alterations of *SURF4* in several cancers (from the cBioPortal for Cancer Genomics website (http://www.cbioportal.org)) [13,14]. (Neuroendocrine prostate cancer (NEPC), adenoid cystic carcinoma (ACC), mutational profiles of metastatic breast cancer (MBL), adrenocortical carcinoma (ACC), Cancer Cell Line Encyclopedia (CCLE), kidney chromophobe (chRCC). **(B)** The *SURF4* expression in tumors from primary cancer patients was assessed using human genome CGH microarray data obtained from the Oncomine database. C panel corresponds to data from Refs. [19–22]. **(C)** Kaplan-Meier survival curves for *SURF4* were obtained using tools found at http://www.canevolve.org [17]. The survival curve relative to the median expression of *SURF4* (high expression and low expression) in individuals affected by different cancer types (log-rank test). **(D)** Overexpression of SURF4 promotes cell proliferation. Error bars indicate the S.E.M. ($^{**}p < 0.01$, $^{*}p < 0.05$, n = 3).

2.6. In vitro transforming assay

Control or SURF4-expressing NIH3T3 cells were resuspended in 7 ml of complete Dulbecco's modified Eagle's medium (DMEM) containing 0.1% agarose and then seeded onto 10 cm plates containing a 7 ml layer of solidified 0.6% agar in complete medium. Foci were stained with crystal violet and counted.

2.7. In vitro cell migration assay

The lower well of a Neuro Probe 48 well micro chemotaxis chamber was filled with serum-free conditioned media and used as a source of chemoattractants. Approximately, 4×10^5 cells were added to the upper compartment and incubated for 4 h, at which time filters were fixed in methanol and stained with Giemsa (Sigma).

2.8. In vivo tumor implantation assay

Control or SURF4-expressing NIH3T3 cells were inoculated subcutaneously (sc) into a posterior flank of athymic nude mice. Tumor growth was monitored twice a week using an electronic caliper, and tumor volume was calculated using the following formula: (length X width^2) X 2^{-1}, where length is greater than the width. All animal experiments were conducted with the approval of the Korea Advanced Institute of Science and Technology (KAIST).

2.9. In silico analysis of SURF4 expression in cancer patients

Kaplan-Meier survival curves associated with high and low levels of *SURF4* median expression were obtained using tools found at http://www.canevolve.org, probe set 222978_a_at [17]. Cancer patients were subdivided based on the median expression of *SURF4* (high and low expression) in tumor cells. Copy number alteration (CNA) array analyses were conducted using the publicly available database at the cBioPortal for Cancer Genomics website (http://www.cbioportal.org) [13,14].

2.10. Statistical analyses

Sample sizes required for the experiments were estimated using preliminary results. *In vitro* and *in vivo* data were analyzed using a two-tailed unpaired Student's T-Test (GraphPad Prism (GraphPad Software Inc.) and SigmaPlot software (SPSS Inc.)). *P* values of <0.05 were considered statistically significant ($^*p < 0.05$; $^{**}p < 0.01$). The Kaplan-Meier log-rank test was used to analyze mouse survival data using GraphPad Prism (GraphPad Software Inc.). No blinding or randomization was performed for any of the experiments.

3. Results and discussion

3.1. SURF4 expression in different cancer types

To investigate whether SURF4 affects human malignancies, we conducted *in silico* screening using the copy number alterations (CNA) array database at the cBioPortal for Cancer Genomics (http://www.cbioportal.org) [13,14] and Oncomine website [18] (Fig. 1A and B). These studies enabled us to explore *SURF4* expression in patient samples [19–22]. Interestingly, *SURF4* expression was found to be profoundly increased in brain, breast, lymphoma and myeloma cancers. We next explored the median overall survival (OS) of patients based on relative levels of *SURF4* expression (Fig. 1C). Glioma [23], breast cancer [24], lymphoma [25], pancreatic cancer [26], adrenocortical carcinoma [27], and sarcoma [28] patients showing high *SURF4* expression had significantly shorter overall survival than those with low expression. The observation that the copy number and expression of *SURF4* were significantly increased in various patients suggests that SURF4 is relevant to the pathogenesis of human malignancies.

Given that the *SURF4* gene is aberrantly amplified and its expression is increased in human malignancies (Fig. 1A and B), we further investigated the biological function of SURF4. We assessed cell growth from 293T cells expressing SURF4 (Fig. 1D). 293T cells expressing SURF4 had markedly increased proliferation compared to control cells. Furthermore, siRNA deletion of *SURF4* significantly decreased 293T cellular growth (Fig. S1). These data demonstrate the ability of SURF4 modulation to alter cancer cell growth.

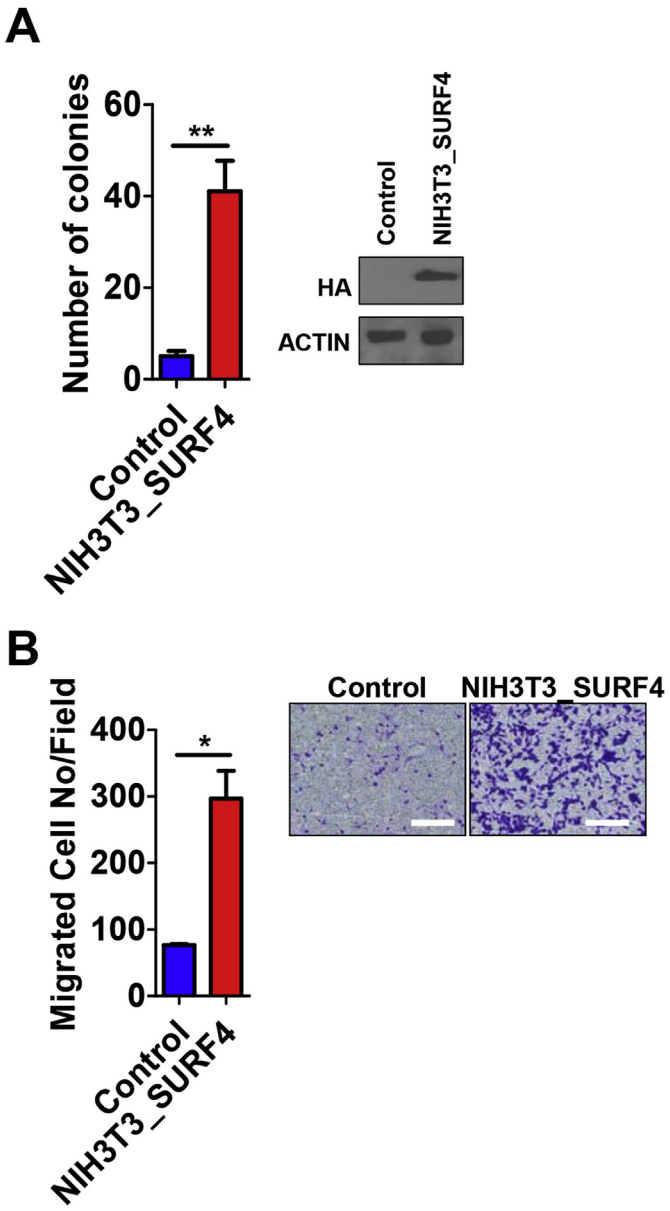

Fig. 2. The cellular transformation effect of SURF4 in NIH3T3 cells *in vitro*. **(A)** A colony forming assay showed an increased colony cell number in HA-SURF4 expressing NIH3T3 cells (NIH3T3_SURF4). Error bars indicate the S.E.M. ($^{**}p < 0.01$, n = 3). **(B)** A two chamber invasion assay showed the increased cell migration ability of NIH3T3_-SURF4 cells. Migrated cells were counted in randomly selected fields. Scale bar; 200 μm. Error bars indicate the S.E.M. ($^*p < 0.05$, n = 4).

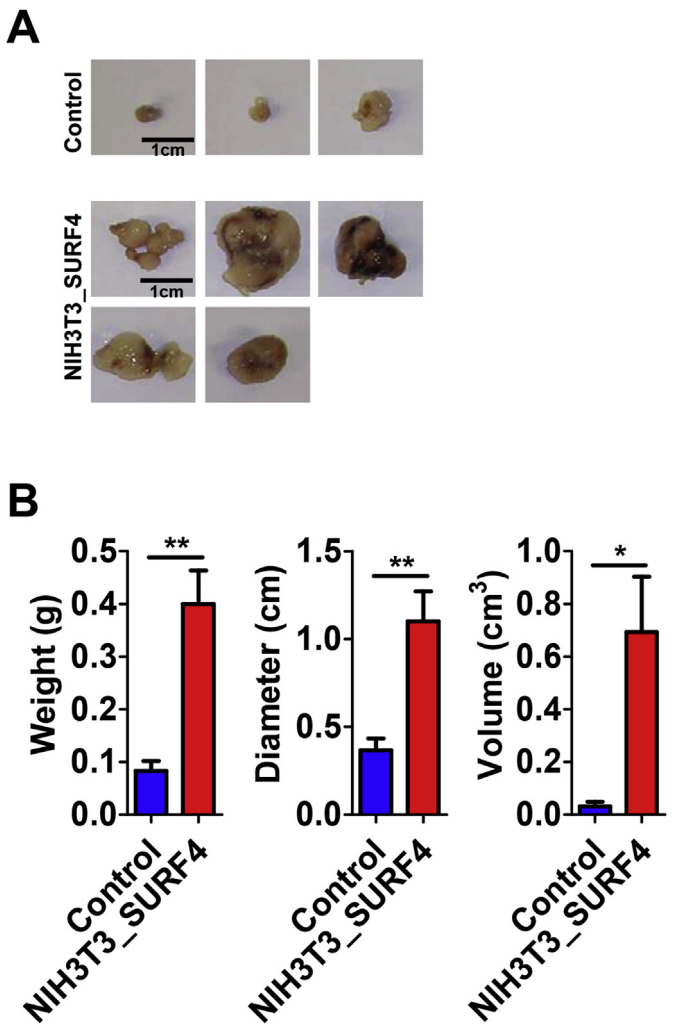

Fig. 3. The oncogenic effect of SURF4 in NIH3T3 cells *in vivo*. **(A and B)** NIH3T3_SURF4 cells were subcutaneously (sc) injected into a posterior flank of female nude mice, which were then checked daily for tumor formation at the sites of inoculation. Representative examples of tumor masses are shown. Error bars indicate the S.E.M. (**$p < 0.01$, *$p < 0.05$, n = 3–5).

3.2. *SURF4 is a potent oncogene when overexpressed in NIH3T3 cells*

Anchorage independence is a major phenotypic hallmark of neoplastic transformation, and local invasive growth is a key feature of primary malignant tumors [29]. To investigate the ability of SURF4 to induce cellular transformation *in vitro*, we assayed the effects of retroviral infection with SURF4 on NIH3T3 cells that formed colonies in soft agar (Fig. 2A). SURF4-expressing NIH3T3 (NIH3T3_SURF4) cells were found to produce greater numbers of colonies than control cells. Next, to determine whether SURF4 promotes migration *in vitro*, we performed a transwell migration assay using NIH3T3_SURF4 cells (Fig. 2B). Compared with control cells, NIH3T3_SURF4 cells showed an enhanced capacity for cell migration. The greater growth observed for NIH3T3_SURF4 cells *in vitro* (Fig. 2) suggests that SURF4 might influence tumor formation *in vivo*. NIH3T3 cells overexpressing SURF4 induce increased tumor growth in nude mice (Fig. 3). Collectively, these results show that SURF4 has the potential to induce cellular transformation and cell migration *in vitro* and promotes oncogenic transformation *in vivo*.

Our results demonstrate that SURF4 induces anchorage-independent growth and cellular transformation *in vitro* and tumor formation *in vivo*. Loss of contact inhibition leads to phenotypic changes and promotes foci formation *in vitro* [30]. Specifically, NIH3T3 cells overexpressing SURF4 induce anchorage-independent growth and cellular transformation *in vitro* and increased tumor size *in vivo*. Our analysis of patient data indicates that the *SURF4* copy number is amplified and gene expression is increased in the tumor tissues of several human cancer patients. Given the impact of SURF4 on oncogenic transformation *in vivo* as demonstrated by our tumor implantation studies, we suggest that upregulating SURF4 advantages cancer cells and/or normal cells. We, therefore, propose SURF4 as a key and previously unrecognized participant in the pathophysiologic process of oncogenic transformation. Although the molecular mechanisms underlying the aberrant deregulation of *SURF4* in cancer require further elucidation, our findings suggest that *SURF4* expression is associated with tumor invasion and clinical outcome in cancer patients. Therefore, *SURF4* is a molecular contributor to cancer progression and may represent a target for drug discovery. Our work points to the value of models examining cellular oncogenic transformation and provides a previously unrecognized modulator with therapeutic implications for cancer.

Conflicts of interest

The authors declare no conflicts of interest.

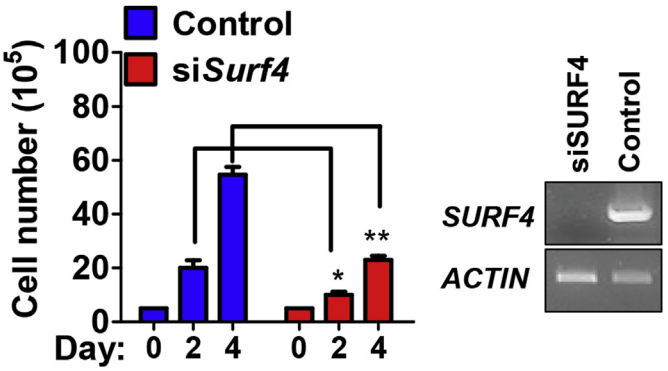

Fig. S1. 293T cells depleted of *SURF4* via siRNA were placed into liquid culture, and cells were counted every other day. Error bars indicate the S.E.M. (**$p < 0.01$, *$p < 0.05$, n = 3).

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