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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.

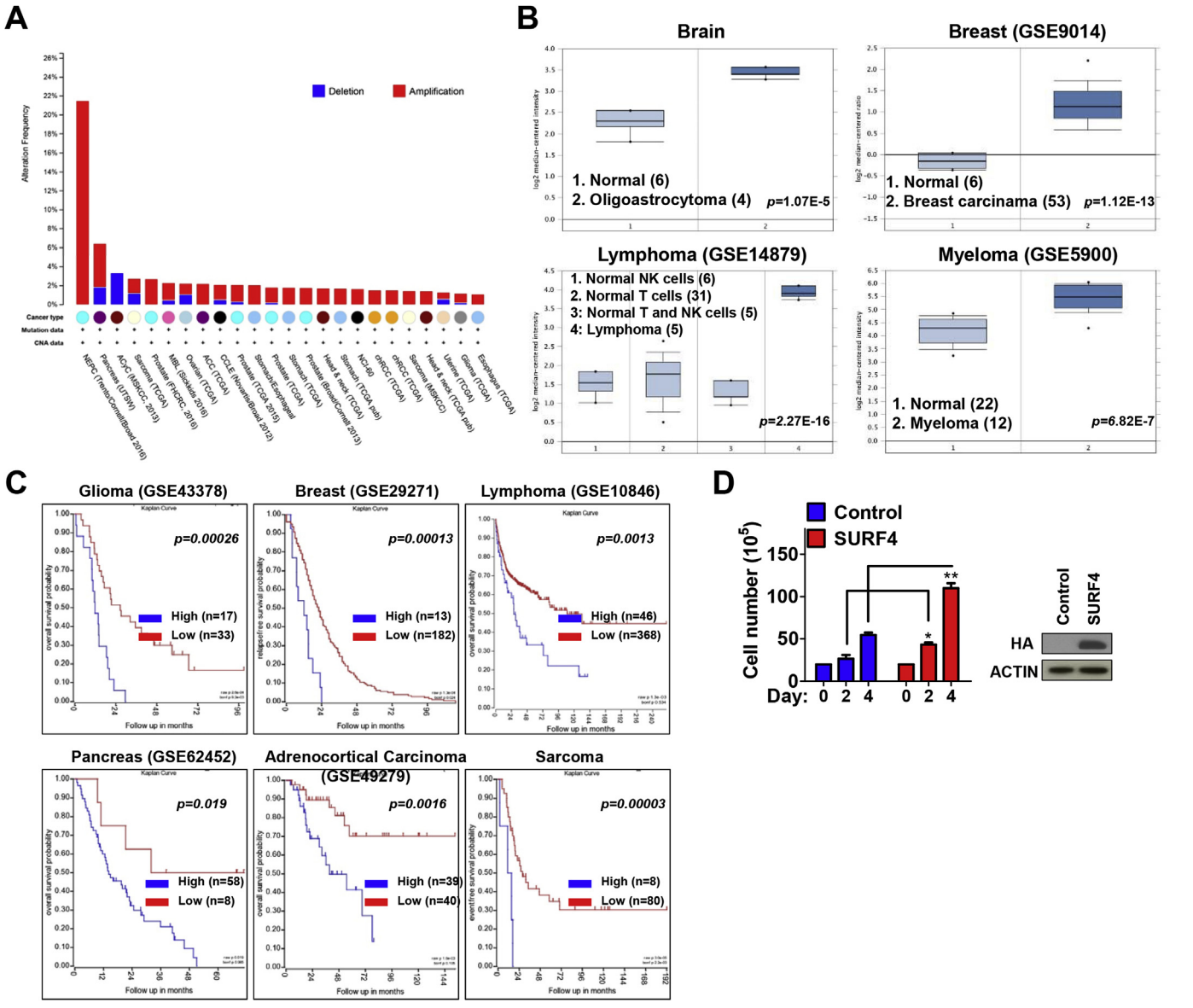

Fig. 1. *SURF4* expression in cancer patients. (A) Copy number alterations of *SURF4* in several cancers (from the cBioPortal for Cancer Genomics website (http://www.cbioportal.org)) [13,14]. (Neuroendocrine prostate cancer (NEPC), adenoid cystic carcinoma (ACC), mutational profiles of metastatic breast 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).