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Alpha-1-syntrophin protein is differentially expressed in human cancers

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Abstract

We studied the expression of α1-syntrophin (SNTA1) protein in histologically confirmed esophageal, stomach, lung, colon, rectal and breast cancerous tissue samples. Our results suggest a significant decrease in the expression level of SNTA1 protein in both esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) compared with their respective controls while a significant increase in expression of SNTA1 protein compared with the normal tissue was observed in breast carcinoma samples. No significant difference in expression of SNTA1 protein was observed in stomach, lung, colon and rectal cancers. Our results suggest that SNTA1 has a role in carcinogenesis and could possibly be used as a novel diagnostic or prognostic marker in esophageal and breast cancers.

Keywords: *Alpha-1-syntrophin; signal transduction; esophageal squamous cell carcinoma; adenocarcinoma; breast cancer*

Introduction

Syntrophins are a multigene family of cytoplasmic, peripheral membrane, scaffold proteins containing PDZ (i.e. postsynaptic density protein-95/disc large/zona occludens-1) domain (Sheng & Sala 2001). They function to link ion channels and signalling proteins to the dystrophin-associated protein complex (DAPC) via a direct interaction with dystrophin (Dys) (the protein product of Duchenne muscular dystrophy gene locus) (Campbell & Kahl 1989, Kramarcy et al. 1994, Hoffman et al. 1987) and dystrophin-related proteins, utrophin and dystrobrevin (Adams et al. 1993, Ahn et al. 1994, Yang et al. 1994). The syntrophin family consists of five known homologous protein isoforms (α1, β1, β2, γ1 and γ2) (Ahn et al. 1996, Piluso et al. 2000). The three syntrophin isoforms α1, β1 and β2 are found predominantly in muscles and are products of different genes (Ahn et al. 1994, Yang et al. 1994). Each syntrophin isoform has a unique tissue and developmental expression pattern and selectively pairs with different dystrophin family proteins *in vivo*, suggesting that complexes containing different isoforms serve distinct functional roles (Ahn et al. 1996).

α1-Syntrophin (SNTA1), also known as pro-transforming growth factor (TGF)-α cytoplasmic domain-interacting protein-1 (TACIP-1), is a 58-kDa dystrophin-associated protein-A1 acidic component-1 (pI 6.7) (Ahn et al. 1994), of 505 amino acids in length (unprocessed precursor). Interest in the protein first came from its location at the neuromuscular junction (Froehner 1984) and later from the demonstration that it is directly associated with dystrophin. SNTA1 regulates ARMS (ankyrin repeat-rich membrane spanning) localization at the neuromuscular junction and enhances EphA4 signalling in an ARMS-dependent manner (Luo et al. 2005). SNTA1 has also been shown to interact with the βγ complex of the heterotrimeric G protein through its PDZ domain and alters intracellular Ca^{2+} in muscles (Yan et al. 2004). In skeletal muscle, DAPC forms a link between the extracellular matrix

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and the actin cytoskeleton (Montanaro & Carbonetto 2003) and is thought to have a role in membrane stabilizing/contraction (Petrof et al. 1993). SNTA1 has been recently shown to be involved in a novel skeletal muscle signalling pathway through the dystrophin-glycoprotein complex (DGC) and Rac1 (Oak et al. 2003). Rac1 is a member of the small GTPases, which plays an important role in regulation of intracellular ROS (reactive oxygen species) (Sahai & Marshall 2002, Khanday et al. 2006a, 2006b). The DGC recruits Rac1 via syntrophin through a Grb2–Sos1 complex (Yan et al. 2007). Recently, syntrophin has been shown to bind Grb2, a SH2/SH3 adapter protein (Oak et al. 2001). Laminin by binding to α-dystroglycan through the DGC causes syntrophin to recruit Rac1 through the Grb2–Sos complex, which in turn binds PAK1, and results in JNK-p46 and c-Jun phosphorylation. Syntrophin antibody also prevents recruitment of Rac1, suggesting that the signalling complex requires syntrophin (Miguel et al. 2005). SNTA1 protein interacts with the tandem PH domain-containing protein 1 (TAPP1) and regulates the actin cytoskeletal organization in response to PDGF stimulation (Angela et al. 2004). The tumour suppressor phosphatase, PTEN, is a key regulator of the cell growth and apoptosis. The interaction of PTEN with PDZ domains has been shown to favour PTEN recruitment into high-molecular-weight molecular complexes and to enhance the PTEN-mediated downregulation of the cell survival and invasiveness activities of the protein kinase B/Akt oncogene (Vazquez et al. 2001, Wu et al. 2000a, Wu et al. 2000b, Subauste et al. 2005). Binding of PTEN to specific PDZ domains diminishes its degradation rate and facilitates its phosphorylation by microtubule associated Ser/Thr kinases. This suggests a regulatory role of the PDZ domain binding on PTEN function by controlling its stability and phosphorylation status. All the above-mentioned studies point towards a possible role of SNTA1 in the development and/or progression of cancers. The expression analysis of SNTA1 protein in different forms of human cancers has not yet been established in any form of human cancers. In the present study we examined the expression pattern of SNTA1 in esophageal, stomach, lung, breast, colon and rectal cancers.

Materials and methods

Cases and controls

Fresh human tissue samples ($n = 187$) of different cancers together with adjacent non-cancerous normal tissue (controls) were obtained from the department of Surgery, Shere-Kashmir Institute of Medical Sciences, Soura, Jammu and Kashmir, India. The patients were diagnosed with histologically confirmed carcinomas and were not given any radiotherapy or chemotherapy before surgery. Fifty-one patients were confirmed as esophageal squamous cell carcinoma (ESSC), while twenty-five samples were from patients with esophageal adenocarcinoma (EAC) (Table 1). Thirty-two cases had a first-time diagnosis of histologically confirmed colorectal adenomas (17 cases of colon cancer and 15 of rectal cancer). Among the forty-four breast cancer samples, eight were recurrent breast cancer cases and 34 cases had a first-time diagnosis of histologically confirmed breast cancer. Nineteen cases had a diagnosis of histologically confirmed stomach cancer, while 16 cases for confirmed lung cancer were considered for the study. The adjacent normal tissues (controls) taken for the study were about 3 cm away from the tumorigenic area. Samples were obtained after approval by the institutional review board and grant of ethical permission (no. SIMS 131 IEC/2009-1982) from the research institute. Tissues were frozen within 10 min of surgery, placed in sealed cryovials and were subsequently snap frozen by immersion in liquid nitrogen N_2 (l) and stored in liquid nitrogen N_2 (l) until use.

Table 1. General characteristics of the study subjects and percentage distribution of cancer cases.

	Males (52.4%)		Females (47.59%)	
	n	%	*n*	%
Total ($n = 187$)	98	100	89	100
Age (years), mean ± SD	48.8 ± 11.5		36.8 ± 11.5	
Smoking				
Never	31	31.63	54	60.67
Ex-smoker	19	19.38	19	21.34
Current	48	48.97	16	17.97
Esophageal squamous cell carcinoma	34	34.69	17	19.10
Well differentiated	11	11.22	6	6.74
Moderately differentiated	16	16.32	7	7.86
Poorly differentiated	7	7.14	4	4.49
Esophageal adenocarcinoma	12	12.24	13	14.60
Stomach cancer	14	14.28	5	5.61
Lung cancer	13	13.26	3	3.37
Small cell	9	9.01	1	1.3
Non-small cell	4	4.25	2	2.07
Breast cancer	2	2.04	42	47.19
Recurrent	–	–	8	8.98
Non-recurrent	2	2.04	34	38.20
Colon cancer	13	13.26	4	4.49
Rectal cancer	10	10.20	5	5.61

Chemicals

A Bradford microprotein estimation kit was purchased from Genei Laboratories (Bangalore, India). PVDF membrane was purchased from Whatman GmbH, (Dassel Germany). Prestained protein molecular weight markers were from Fermentas (Burlington, ON, Canada). All electrophoresis reagents were obtained from Sigma-Aldrich (St Louis, MO, USA), Qualigens (Mumbai, India) and Spectrochem (Mumbai, India). All the chemicals for carrying out protein extraction and Western blotting were of analytical grade and were acquired from Sigma-Aldrich.

Protein extraction and estimation

Tissue was disintegrated using 0.5% trypsin-EDTA at 37°C for 5 min, centrifuged at 12 000 rpm for 5 min, rinsed twice with ice-cold PBS, pH 7.4. NP-40 lysis buffer containing protease and phosphatase inhibitors (20 mM Tris–HCl, pH 8.0, 137 mM NaCl, 1% Nonidet P-40, 1% glycerol, 2 mM EDTA, 10 mM NaF, 1 mM PMSF, protease inhibitor cocktail 10 µl per 1 ml of lysis buffer) was used to lyse cells, incubated on ice for 45 min and centrifuged at 12 000 rpm for 10 min at 4°C to obtain the extract. Protein concentration was determined spectrophotometrically (Shimadzu, Kyoto, Japan) at 595 nm with the Bradford assay kit.

Antibodies

Antibody against SNTA1 (1:1200 dilution) was from Sigma and Abcam Inc., Odyssey-IR-Dye-labelled antirabbit IgG secondary antibody (1: 5000 dilution) was purchased from Li Cor (Lincoln, NE, USA) while Vinculin antibody was purchased from Abcam inc.

Western blotting

Forty micrograms of protein extract preheated at 100°C for 3 min in reducing sample buffer containing 50 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 0.1% bromophenol blue, 100 mM β-mercaptoethanol was run on 10% SDS-polyacrylamide gel. A standardized protocol, as provided by the manufacturer of Odyssey (Li Cor), was followed for Western blotting and quantitation of the bands was carried out with Odyssey (Li Cor) quantitation software and independently confirmed using quantity one (4.5.0) 1D analysis.

Statistical analysis

All experiments were performed in triplicate, and results were calculated as mean ± SD. Statistical analyses were performed by the *t*-test (Microsoft Excel), and $p < 0.05$ was considered statistically significant.

Results

Alpha 1 syntrophin protein is downregulated in esophageal cancers

Western blot analysis of SNTA1 in ESCC and EAC from different patients indicated a consistent decrease in SNTA1 protein levels in both carcinomas when compared with their adjacent normal controls (Figure 1A, B). The controls of adenocarcinoma (EAC) showed the same level of SNTA1 expression when compared with the controls of ESCC (Figure 1B). Results of the expression analysis on poorly, moderately and well-differentiated tumour samples indicate (Figure 1A, B) a consistent decrease in SNTA1 expression compared with the respective normal tissue. Densitometric analysis indicated a 2–5-fold decrease in the level of expression of SNTA1 in ESCC and EAC (Figure 1E). Decrease in SNTA1 protein expression in esophageal cancerous tissues was independent of age, gender and smoking habits of the subjects. We did not observe any difference in SNTA1 protein expression in stomach and lung cancers when compared with their respective normal controls (Figure 2A, B).

Alpha 1 syntrophin protein is upregulated in breast cancer

Western blot analysis in samples of breast cancer tissue from independent patients diagnosed with breast cancer indicated a consistent increase in SNTA1 expression compared with the adjacent normal controls (Figure 1C, D). We observed an increase of SNTA1 protein in both recurrent and non-recurrent cancers compared with their respective normal control tissue (Figure 1C, D). The controls for recurrent breast cancers showed a higher basal level of SNTA1 expression compared with the levels found in the controls of non-recurrent carcinoma samples (Figure 1C, D). Densitometric analysis indicate a 5–6-fold increase in the level of expression of SNTA1 in recurrent samples as against a 2–3-fold increase found in non-recurrent samples compared with their respective normal controls (Figure 1F). Also, the increase in expression of SNTA1 was found to be consistent in cases of male breast cancer samples (data not shown). We did not observe any difference in SNTA1 protein expression in colon and rectal cancers when compared with their normal controls (Figure 2C, D).

Discussion

Our results suggest that SNTA1 protein is differentially expressed in different forms of human cancer. We found

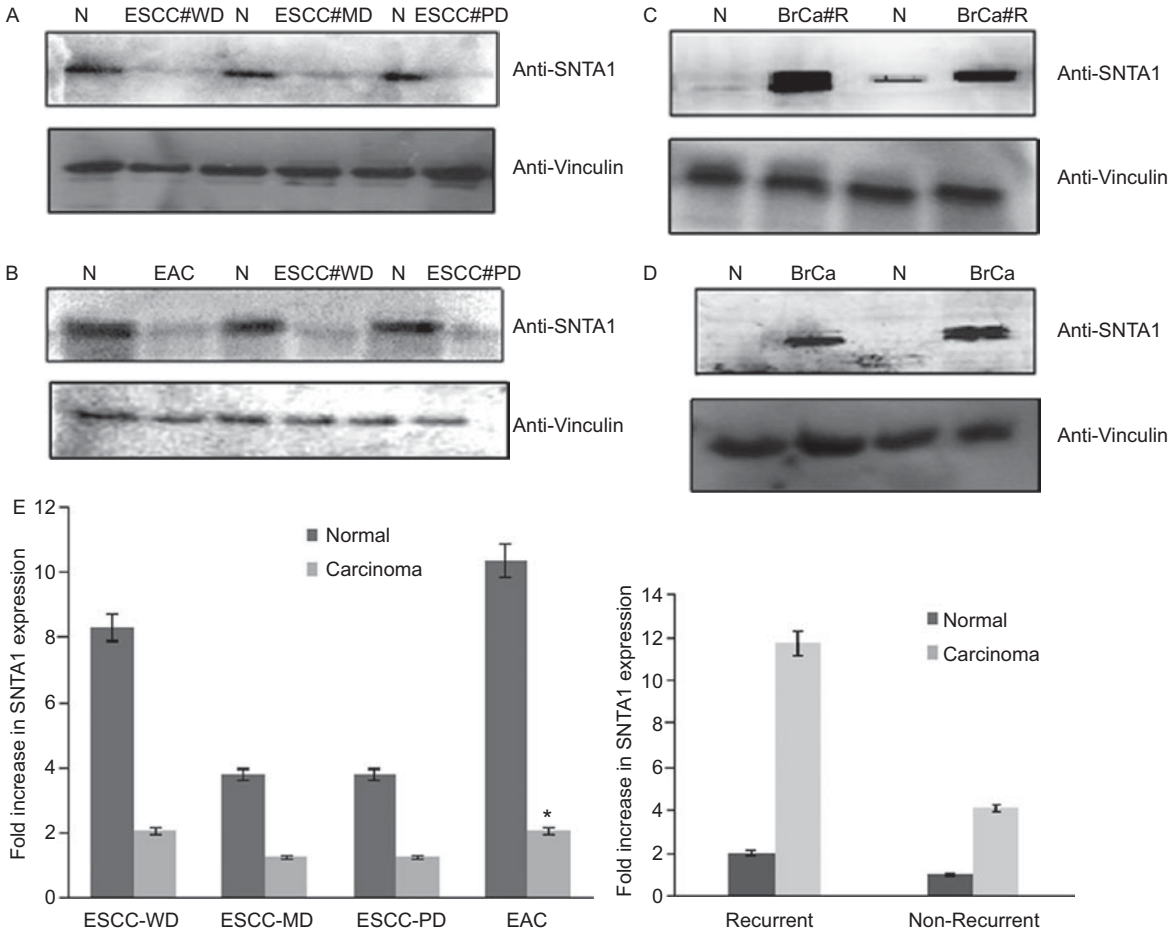

Figure 1. Immunoblot showing: (A) downregulation of α1-syntrophin (SNTA1) protein in well-differentiated (WD), moderately differentiated (MD) and poorly differentiated (PD) esophageal squamous cell carcinoma (ESSC) with vinculin protein as loading control (lower panel); and (B) downregulation of SNTA1 protein in esophageal adenocarcinoma (EAC). (C, D) Elevated expression of SNTA1 protein in breast recurrent and non-recurrent cancer samples, with vinculin protein as loading control. (E) Bar chart comparing the expression level of SNTA1 proteins in esophageal and breast cancers with respect to adjacent normal tissue. Values are expressed as fold increase in expression compared with the respective controls, bars ± SE. *Statistically significant $p < 0.05$, differences compared with adjacent normal tissue as control using *t*-test.

a consistent decrease in its expression in esophageal cancers (ESSC and EAC samples), irrespective of the age and sex of subjects involved. However, we could not detect any difference in SNTA1 protein levels between cancerous and adjacent normal tissue in stomach, lung, colon and rectal cancer samples. Also, our results indicate a higher level of SNTA1 protein expression in breast cancer samples compared with their respective normal tissue in both the recurrent and non-recurrent samples. The study was carried on an ethnic Kashmiri population, in whom a very high incidence of esophageal and breast cancers is observed compared with other forms of cancers. The higher incidence of these two cancers is possibly attributed to the unique food habits like high intake of spices, salt and sundried foodstuffs. We have observed a reciprocal (decrease in esophageal and increase in breast carcinoma) expression of SNTA1 protein in esophageal and breast carcinoma. This reciprocal expression pattern is common with many proteins, particularly those that are part of membrane complexes and or act as signal transducers and have a role to play in both cell proliferation and/or suppression of cell growth. The membrane associated proteins annexins and claudins have been shown to be differentially expressed in colorectal cancers (Grone et al. 2006, Duncan et al. 2008). Previously, in breast cancer cell lines, elevated expression of p66shc protein has been observed in those with a high metastatic ability, and a similar phenomenon was observed in lymph node-positive breast tumours (Jackson et al. 2000). We have also recently reported an increase in p66shc expression in esophageal cancer tissue samples (Bashir et al. 2010). However, decrease or absence of p66shc expression in stomach cancers has been reported (Yukimasa et al. 2005). The antagonism of p66shc by melanoma inhibitory activity has also been reported (Kasuno et al. 2007). The reciprocal/differential expression has also been observed with p53 protein. The overexpression of the tumour suppressor gene p53 has been reported in colorectal cancers

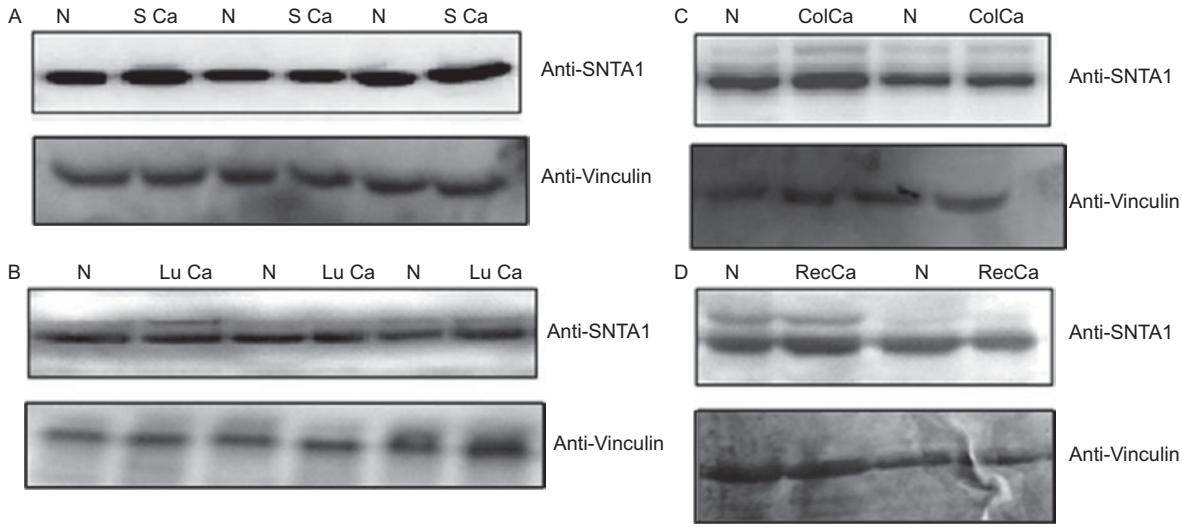

Figure 2. (A, B) Immunoblot showing expression of α1-syntrophin (SNTA1) in stomach carcinomas and lung cancers with vinculin protein as loading control (lower panels). (C, D) Immunoblot analysis of SNTA1 protein in colon and rectal cancers with vinculin protein as loading control (lower panels).

(Hirotsugu & Yoshihiro 2006, Scott et al. 1991). However, loss of p53 expression correlating with a metastatic phenotype has been observed in head and neck cancers (Ku et al. 2007). Based on our observations we propose that SNTA1 is a multidomain protein that might be needed by certain factors within the cells for proliferation and by others for suppression of the growth of cells, possibly explaining its decrease in one form of cancer compared with an increase in another form. As cancer is an extraordinarily complex disease, such an expression scenario might be due to the effective role of other signal transduction proteins associated with the protein. Nevertheless our results pave the way for further investigations into the role of SNTA1 protein in cancer development and progression and point towards a possible use of SNTA1 protein as a marker in esophageal and breast cancers.

In conclusion, our results indicate that syntrophin protein is downregulated in esophageal cancers compared with normal control samples. We observed a consistent increase of syntrophin protein in breast cancers compared with normal tissues. We did not find any difference in protein expression levels in cases of stomach, lung, colon and rectal cancers compared with their respective normal tissue. Overall our results suggest that there is a differential pattern of expression for syntrophin protein in different cancers and thus it can act as a novel diagnostic or prognostic marker in esophageal and breast cancers.

Declaration of interest

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