Expression of SPANX proteins in normal prostatic tissue and in prostate cancer

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Abstract

The sperm protein associated with the nucleus in the X chromosome (*SPANX*) gene family encodes for proteins that are not only expressed in germ cells, but also in a number of tumors. In addition, *SPANX* genes map in an interval of the X chromosome (namely, Xq27), which has been found to be associated with familial prostate cancer by linkage analysis. The aim of this study was therefore to evaluate *SPANX* protein expression in normal prostate tissues and in prostate carcinoma. For this purpose, formalin-fixed and paraffin-embedded sections obtained from 15 normal (at autopsy) donors and 12 men with prostate cancer were analyzed by immunohistochemistry. About 40% of both normal and tumor prostate samples resulted SPANX positive. Signals were exclusively within the nucleus in normal prostate cells, whereas both nuclear and cytoplasmic positivity was observed in tumor cells. In conclusion, these findings showed that SPANX genes are expressed in both normal and tumor prostate gland, but the latter showed a peculiar cytoplasmic staining positivity. This suggests a possible association between *SPANX* over expression and prostate cancer development. Additional studies are needed to corroborate this hypothesis.

Introduction

Prostate cancer, one of the most commonly diagnosed male malignancies in Western countries, is now the leading cause of cancer-related death in men.[1] Over the years, genetic epidemiological evidence has accumulated in favor of a considerable hereditary component in prostate cancer susceptibility. Genetic linkage analysis in 360 hereditary prostate cancer pedigrees revealed the presence of a hereditary prostate cancer susceptibility gene(s) at Xq27-28 (*HPCX*).[2] In agreement with this finding, further data were obtained by analyzing linkage disequilibrium of molecular markers of X chromosome in a Finnish X haplotype.[3] Xq27 is a region containing a number of genes important in cancer and embryonic development, including the sperm protein associated with the nucleus in the X chromosome (*SPANX*) gene family[4,5] that consists of the two subfamilies *SPANX-N* and *SPANX-A/D* (Human genome build 37.1). *SPANX-A/D* genes map within segmental duplications,[6,7] which are regions involved in genomic rearrangements resulting in an abnormally high level of structural polymorphisms.[8] Accordingly, the *SPANX-B* and the *SPANX-C* genes were shown to be present in a variable number of copies (ranging from one to >11) in the normal population;[4,7] however, no association was found between SPANX copy number and the occurrence of hereditary prostate cancer by the genetic locus described by Xu,[2] thus leaving uncertain the possible identification of the aforementioned locus with *SPANX* gene cluster.[9]

SPANX proteins are normally expressed in germ cells;[4,10] however, their expression has also been detected in a number of tumors, including melanoma, myeloma, glyoblastoma, breast carcinoma, prostate cancer, and testicular germ cell tumors.[5,11-14] The present study was undertaken to evaluate the expression of SPANX proteins in normal prostate tissues and in prostate cancer by immunohistochemistry.

Materials and Methods

Patients

The analysis was carried out on 15 normal (at autopsy) donors and 12 men with prostate cancer following radical prostatectomy. Patients (71.7±1.8 years) and normal controls (74.5±1.3 years) had a similar mean age (Table 1). Patients' histological diagnosis, Gleason scores and pre-surgery serum PSA levels are shown in Table 1.

The protocol was approved by the internal Institutional Review Board and an informed written consent was obtained from each patient with prostate cancer or, if deceased, by his relatives.

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Immunohistochemical staining

Four μm formalin-fixed and paraffin-embedded prostate sections were processed following the standard protocol previously described.[5] We did not observe any time-related effect on immunostaining with the antibody used for this study nor with any other antibody used for diagnosis. A polyclonal serum against the common SPANX epitope TPTGDSDPQP, developed in mouse, was used, as reported in a previous study.[4] As negative control, anti-SPANX serum was pre-incubated with the immunizing peptide (100 ng) for 1 h. Tissues were visually scored at 20X magnification for SPANX positivity; the fraction of SPANX-positive cells was evaluated independently in a blinded fashion by two of us in microscopic fields where non prostatic cells (infiltrating leukocytes, fibroblasts, etc.) were as few as possible. Since no significant difference was observed between the two observers, a mean value was used.

Statistical analysis

Results were expressed as mean±SEM. Comparisons between the percentages of SPANX-positive cells were carried out by the Student's t test (SPSS 9.0 software package for Windows). $P<0.05$ were considered statistically significant.

Results

Normal tissues showed only a SPANX-positive nuclear signal in 6 samples out of the 15 examined (40%), with a mean of 21.7±7.2%

cells with SPANX-positive nuclei (Figure 1 A, C; Table 1). The expression of SPANX was also evaluated in 12 samples of prostate carcinoma with a mean Gleason score of 3.5±0.2 and 3.2±0.2 and a grading ranging from G1 to G3. Five samples exhibited a SPANX-positive nuclear signal (41.7%) with a mean of 32.9±11.8% positive nuclei (Figure 1 B, D; Table 1). Moreover, cytoplasmic staining for SPANX was observed in the 27.5±9.9% of the cells (range: 60-75). None of the controls, analyzed in parallel, exhibited cytoplasmic staining ($P<0.001$ vs. prostate carcinoma samples). No cytoplasmic staining was observed in the absence of nuclear staining in prostate cancer tissue (Table 1).

Discussion

This is the first demonstration that *SPANX* genes are expressed in a normal somatic tissue, apart the already known expression in germ cells.[4,5] The percentage of cells showing a SPANX-positive nuclear staining was comparable both in normal and in pathologic tissues; prostate cancer cells showed also a cytoplasmic staining. This peculiar feature of SPANX staining, if confirmed by larger cohort studies, may be of clinical usefulness for the immunohistochemical differential diagnosis of prostate carcinoma. Cytoplasmic SPANX labeling is a common finding in malignant cancer cells, such as embryonal carcinomas[5] and melanomas.[15,16] In addition, evidences of cytoplasmic labeling in melanomas has been shown by Westbrook *et al*.[17] On the basis of extensive observations of histological samples, we believe that the cytoplasmic signal represents a leakage of SPANX proteins from the nucleus. A definitive answer will require the study of tumor cells lines expressing SPANX. It is conceivable that similar events, which have the potential of altering the regulation of gene expression, may also be present in malignant cells. The similar fraction of SPANX positive cells in normal and cancer

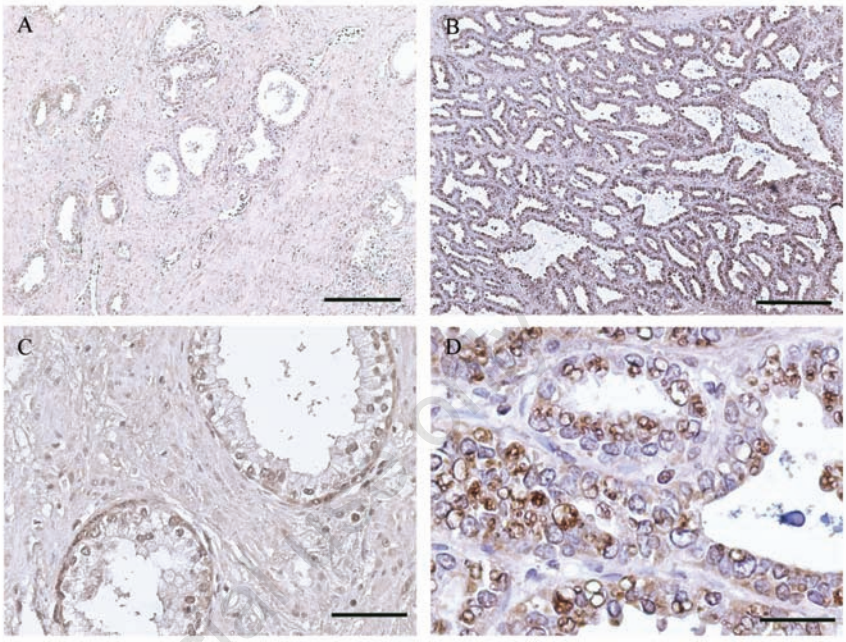

Figure 1. Immunohistochemistry of prostate. (A) Normal prostatic tissue; (B) prostate carcinoma (A, B Hematoxylin counterstain; bar = 800 μm); (C) normal prostatic tissue (Hematoxylin counterstain; bar = 200 μm); (D) prostate carcinoma (Hematoxylin counterstain; bar = 80 μm).

Table 1. Characteristics of healthy prostate tissue donors and of patients who underwent radical prostatectomy for prostate carcinoma.

Normal prostate (autopsy)				Prostate carcinoma (radical prostatectomy)								
ID	Age (years)	Positive cell nuclei (%)	Positive cell cytoplasm (%)	ID	Age (years)	PSA (ng/mL)	pT	N	Gleason score	G	Positive cell nuclei (%)	Positive cell cytoplasm (%)
0.1	63	0	0	1	72	7.8	pT3	0	4+3	G2	80	70
0.2	77	0	0	2	63	9.2	pT3	0	4+4	G3	0	0
0.3	72	55	0	3	79	8.7	pT3	NA	4+4	G3	0	0
0.4	71	60	0	4	70	7.6	pT3	0	3+3	G2	75	65
0.5	68	0	0	5	72	11.6	pT3	NA	4+3	G2	80	60
0.6	80	0	0	6	79	6.8	pT2b	NA	3+3	G2	0	0
0.7	79	55	0	7	72	8.2	pT3	0	3+2	G1	75	60
0.8	77	0	0	8	77	9.6	pT2b	0	3+4	G2	0	0
0.9	76	50	0	9	74	10.1	pT3	NA	4+4	G2	0	0
0.10	80	0	0	10	73	9.9	pT3	0	4+3	G2	0	0
0.11	71	60	0	11	57	8.7	pT3	0	4+3	G2	85	75
0.12	74	0	0	12	72	9.5	pT2b	0	2+2	G1	0	0
0.13	79	0	0									
0.14	72	0	0									
0.15	78	45	0									

ID, identification; PSA, prostate specific antigen; pT, tumor stage; N, lymphonodes; NA, lymphonodes not excissed; G, grading.

tissues suggests that the expression of SPANX genes in prostate carcinomas correlates with their expression in normal cells from which the tumor originates: a finding similar to what previously described in seminomas,[5] where nonetheless the nuclear positivity involves approximately 100% of the cells. Since our antibody was generated by immunizing mice with a synthetic peptide, which sequence is shared by almost all known SPANX proteins,[4] we cannot differentiate SPANX-B form SPANX-A-like subfamilies. Although this is a limiting aspect, we believe that, given the high homology among SPANX gene family members, it would be difficult to assess the specificity of any antibody for a SPANX subfamily or for a single member, especially for immunohistochemical purposes. In conclusion, the present findings showed that SPANX expression is physiological in 40% of normal individuals, at least in the Sicilian sample examined in this study. This might relate to predisposing haplotypes of SPANX locus.[3] The similar incidence of SPANX-positive cells in normal and neoplastic tissues, although suggestive, is not sufficient to establish a causative relationship between SPANX expression and prostate cancer tissues, and deserves more detailed studies in additional patients.

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