ANX7, a candidate tumor suppressor gene for prostate cancer

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The *ANX7* gene is located on human chromosome 10q21, a site long hypothesized to harbor a tumor suppressor gene(s) (TSG) associated with prostate and other cancers. To test whether *ANX7* might be a candidate TSG, we examined the *ANX7*-dependent suppression of human tumor cell growth, stage-specific ANX7 expression in 301 prostate specimens on a prostate tissue microarray, and loss of heterozygosity (LOH) of microsatellite markers at or near the *ANX7* locus. Here we report that human tumor cell proliferation and colony formation are markedly reduced when the wild-type *ANX7* gene is transfected into two prostate tumor cell lines, LNCaP and DU145. Consistently, analysis of ANX7 protein expression in human prostate tumor microarrays reveals a significantly higher rate of loss of ANX7 expression in metastatic and local recurrences of hormone refractory prostate cancer as compared with primary tumors ($P = 0.0001$). Using four microsatellite markers at or near the *ANX7* locus, and laser capture microdissected tumor cells, 35% of the 20 primary prostate tumors show LOH. The microsatellite marker closest to the *ANX7* locus showed the highest rate of LOH, including one homozygous deletion. We conclude that the *ANX7* gene exhibits many biological and genetic properties expected of a TSG and may play a role in prostate cancer progression.

cancer genetics | chromosome 10q21 | loss of heterozygosity

The gene for annexin 7 (*ANX7*‡‡, synexin; refs. 1–6) is located on human chromosome 10q21, where potential tumor suppressor genes (TSGs) have been hypothesized to exist for prostate and other cancers (5, 7–15). However, the specific relevance of the *ANX7* gene for cancer only became apparent after we created a knockout for this gene in the mouse (16). Although the homozygous *Anx7*(−/−) deletion is embryonically lethal, the phenotype of the *Anx7*(+/−) heterozygote includes calcium signaling deficits and growth defects such as gigantism, and selective organomegaly. As these mice aged, we also began to observe a profoundly increased frequency of disparate spontaneous tumors in both male and female *Anx7*(+/−) mutants (17).

Because of these observations, and the chromosomal location of the gene, we hypothesized that *ANX7* might be a candidate TSG associated with 10q21 locus. Commonly, TSGs can suppress growth of tumor cells, *in vitro*, and are frequently inactivated by mutations, deletions, or loss of expression in tumors, *in vivo*. In addition, loss of heterozygosity (LOH) often is observed for these genes in clinical tumor specimens. Therefore, to test this hypothesis for the *ANX7* gene, we analyzed the action of the *ANX7* gene on colony formation by human tumor cell lines. We also examined the expression of the ANX7 protein in hundreds of prostate cancers by using tumor tissue microarray technology. Finally, we tested a panel of primary and metastatic prostate cancers for evidence of LOH.

In this paper we show that the *ANX7* gene suppresses the growth of the prostate tumor cell lines DU145 and LNCaP. Consistently, in a prostate tissue microarray, we find significantly low frequencies of ANX7 protein in metastases and hormone-insensitive local recurrent cancers. In addition, using Ki67 immuno-staining as an index of tumor cell proliferation, we also find that a high Ki67 labeling index is positively correlated with lower levels of ANX7 expression. Finally, we find that allelic loss of the *ANX7* gene occurs in over one-third of carcinoma of the prostate (CaP) specimens, including an example of a homozygous gene deletion. We conclude that *ANX7* exhibits many properties expected of a TSG and suggest that the state of this gene may have significant prognostic potential in assessing the progression of human prostate cancer.

Materials and Methods

Assay of Tumor Cell Growth Suppression. Human tumor cells were obtained from the American Type Culture Collection and cultured as described by the supplier. Cells were plated in 6-well plates (35-mm wells) and grown in appropriate media to ≈70% confluency for transfection in media appropriate to the cell type. Transfection parameters initially were optimized by using a plasmid expressing β-galactosidase. These studies suggested that 2–4 μg plasmid DNA and 6 μl Lipofectamine would produce maximum transfection efficiency. Cells therefore were transfected for 5 h with various amounts (1–6 μg) of several plasmids (pcDNA3.1 alone or containing/expressing cDNA encoding human *ANX7*, p53, or *N*-methyl-D-aspartate receptor subunit 2C) and Lipofectamine (6 μl; Life Technologies, Grand Island, NY) in reduced serum medium (Optimem 1, Life Technologies) essentially as recommended by the supplier. Approximately 36 h later, selection with G418 (Geneticin, Life Technologies) at 800 μg/ml medium was initiated. Cells then were maintained with medium changes every 3–4 days, always containing G418. After ≈1 week of G418 selection, most nontransfected cells had died. After ≈2 weeks of selection, the cells were rinsed with PBS, fixed with 2% formaldehyde in PBS for 15 min, stained with 0.5% crystal violet in PBS for 15 min, and rinsed 1–2 times with distilled H_2O, dried, and stored for subsequent quantification of colonies. Colonies visible in each well without magnification

Abbreviations: LOH, loss of heterozygosity; TSG, tumor suppressor gene; CaP, carcinoma of the prostate.

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‡‡Nomenclature: Italics are used to denote genes, while roman text is for cognate proteins. Uppercase letters are for human genes (e.g., *ANX7*); uppercase first letters denote mouse genes (e.g., *Anx7*); lowercase letters denote a gene from other species (e.g., *anx7*).

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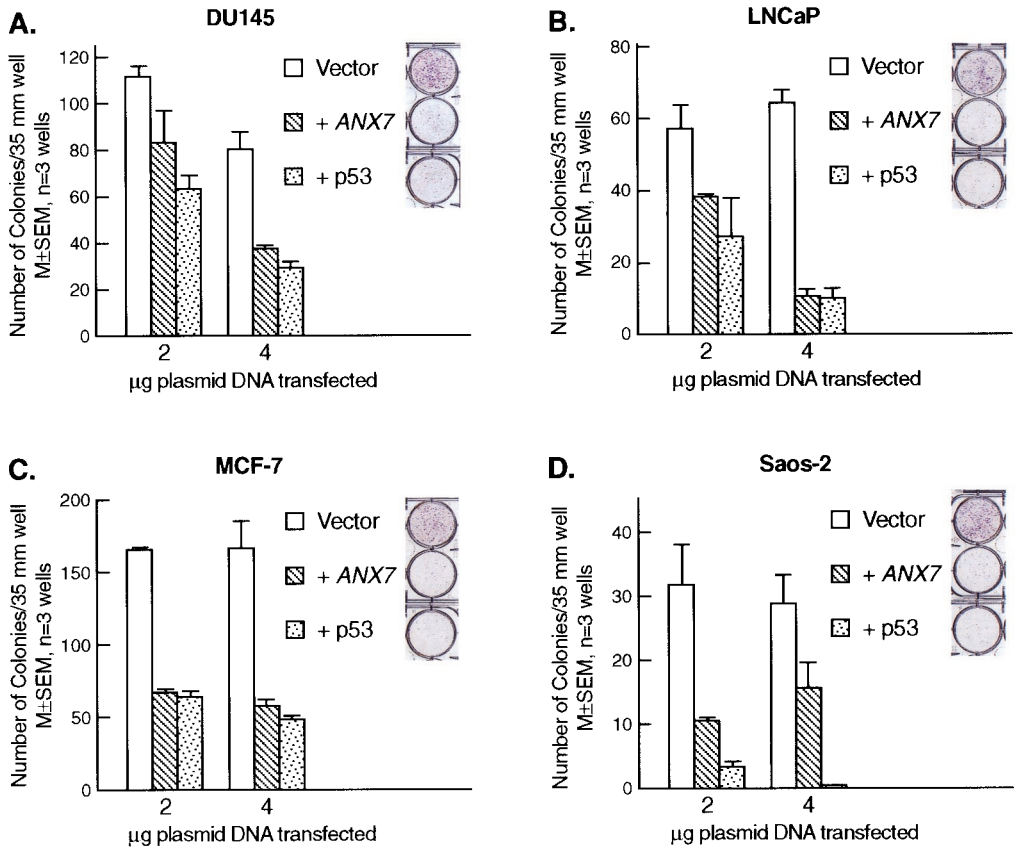

Fig. 1. Growth suppression of tumor cells by *ANX7* and *p53*. (*A*) DU145 prostate cancer cells are transfected with pcDNA3.1 alone (vector) or vector expressing *ANX7* (+*ANX7*) or p53 (+p53) and selected for plasmid-containing cells with G418 for ≈2 weeks. Surviving cells then were fixed and stained with crystal violet. Colonies were counted and displayed as histograms. For each cell line, a photograph of one well of cells treated with 4 μg of each plasmid is also shown. (*B*) LNCaP prostate cancer cells are treated exactly as indicated in *A*. (*C*) MCF-7 breast cancer cells are treated exactly as indicated in *A*. (*D*) Saos-2 osteosarcoma cells are treated exactly as indicated in *A*.

were counted, and average values (mean + SEM) were determined for wells transfected with each concentration of each plasmid. Transfectants were cloned, and levels of ANX7 protein were measured by Western blot analysis.

Analysis of ANX7 Protein Expression in Human Prostate Specimens by Immunohistochemistry on Tissue Microarrays. The prostate tissue microarray was constructed as described (18–20). Formalin-fixed and paraffin-embedded tumor and benign control specimens were obtained from the archives of the Institutes of Pathology, University of Basel and the Tampere University Hospital. The tissue array contained 301 specimens from all stages of tumor progression including benign prostatic hyperplasia as control (22 specimens), high-grade prostatic intraepithelial neoplasia (17 specimens), primary tumors with stage T2 and stage T3/4 (97 specimens) as defined by the International Union Against Cancer (21), distant metastases (35 specimens), and local recurrences from patients with hormone-refractory disease (108 specimens). Original tumor grading was performed according to Gleason (18–20). Standard indirect immunoperoxidase procedures were used for immuno-histochemistry (ABC-Elite, Vector Laboratories). The ANX7 protein was imaged by using a mouse mAb against human ANX7 (1:1,000, Transduction Laboratories, Lexington, KY), and compared with our proprietary rabbit polyclonal antibody against recombinant human ANX7. The intensity of the cytoplasmic staining was classified into four groups (negative, weak, intermediate, and strong). To construct the statistical contingency table, tumors with negative immunostaining were compared with those showing any degree of positive staining. The mAb MIB 1 (1:800; Dianova, Hamburg, Germany) was applied for visualization of Ki67 protein. Ki67 is expressed in all proliferating cells (G_1, S, and G_2 M phase), but not in quiescent cells (G_0 phase). The proportion of Ki67-positive tumor cell nuclei was estimated on a scale from 0 to 6 (0 = negative, 1 = 0–5%, 2 = 5–10%, 3 = 10–25%, 4 = 25–50%, 5 = 50–75%, and 6 = 75–100%). For statistical analysis, 0–10% positive nuclei were defined as a low growth fraction and >10% as a high growth fraction.

Tumor Tissue Dissection and DNA Extraction. Matched CaP and adjacent normal prostate tissues were obtained under an Institutional Review Board-approved protocol from 20 patients who had undergone radical prostatectomy at Walter Reed Army Medical Center, Washington, DC. The tissues were immediately embedded in Tissue-Tek OCT (Miles) and frozen at −70°C. A laser-gene capture microdissection (LCM) instrument was used to microdissect tumors from 1-μm frozen sections. Initial sections were stained by hematoxylin and eosin, and these stained sections were used as optical templates for identification and isolation of tumor and normal cells from serial unstained sections from the same block. Normal cells and tumor cells dissected by LCM were digested with proteinase K and extracted with phenol/chloroform, followed by ethanol precipitation. Furthermore, to ensure the DNA integrity, all DNA samples were analyzed by PCR for β-actin gene amplification.

PCR and Microsatellite Polymorphism Analysis. We used PCR primers for four microsatellite markers spanning the *ANX7* locus on chromosome 10q21, encompassing 4cM. The primer sequences for some of the markers were obtained from the Genome Database (http://gdb.www.gdb.org/). The microsatellite markers used in our study include AFMa299ya5 (D10S1688 dinucleotide repeat), AFM200wf4 (D10S535 dinucleotide repeat), AFM220xe5 (D10S218 dinucleotide repeat), and AFM063xc5 (D10S188 dinucleotide repeat). The primers were obtained from Applied Biosystems (Perkin–Elmer). The order of the markers used for LOH

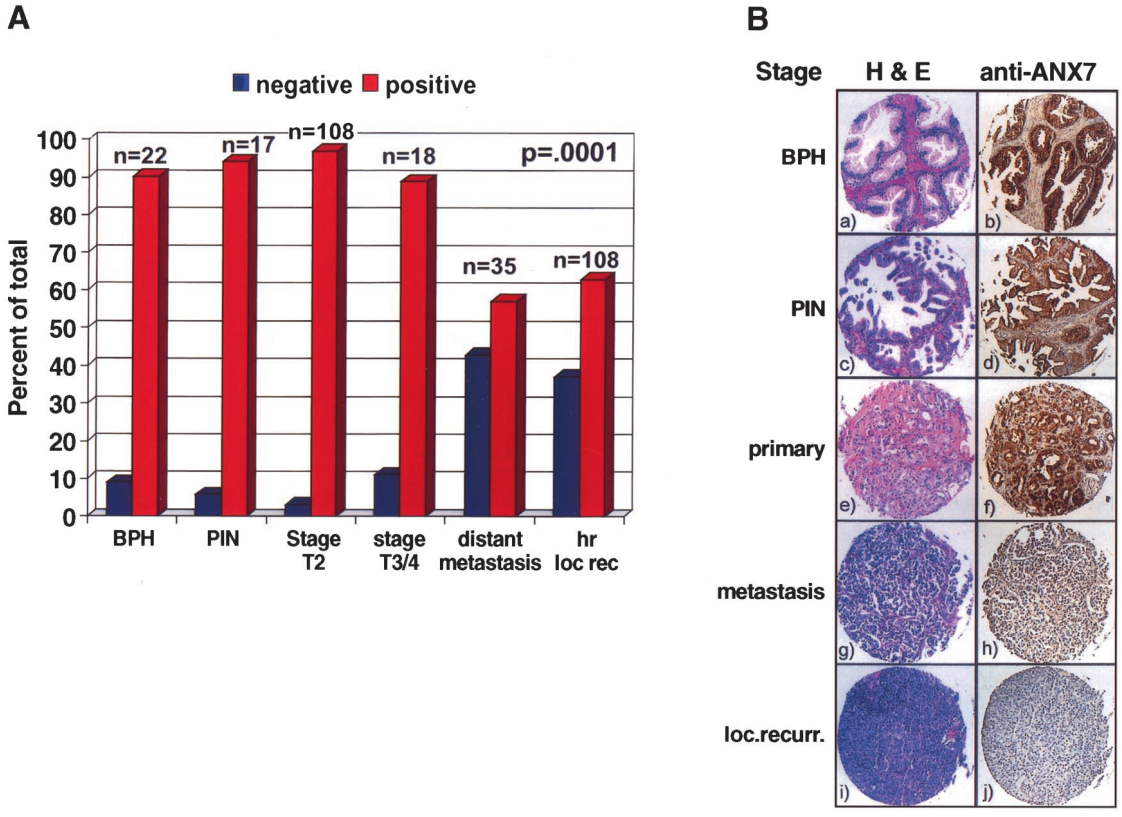

Fig. 2. Levels of ANX7 protein expression in a prostate cancer tissue microarray. (*A*) Loss of expression of ANX7 during prostate cancer progression. BPH: benign prostatic hyperplesia; PIN: high-grade prostatic intraepithelial neoplesia; stage T2: clinically localized primary cancer; stage T3/T4: locally advanced primary cancer; Hr loc re: hormone refractory local recurrence. (*B*) Representative sections of the hemotoxylin-eosin (H&E) and immunohistochemical staining of ANX7 protein using monoclonal anti-ANX7 antibody on the prostate cancer tissue microarray (original magnification: ×100).

analysis and their distance in cM from the centromere were based on the information from both the Genome Database and the Whitehead/Massachusetts Institute of Technology databases. Most of these polymorphic markers had heterozygosity frequencies of 0.6–0.9. PCR was performed on the genomic DNA samples using the following conditions: 5 ng of DNA template, 50 ng of each primer, 0.5 unit of AmpliTaq Gold (Perkin–Elmer), 1 × PCR buffer, 200 μM dNTP mix in a 50 μl final volume. PCR conditions were identical for all primers used. PCR cycles included one cycle of 95°C for 10 min followed by 25 cycles of 95°C for 30 sec, 55°C for 45 sec and 72°C for 1 min. Four markers were analyzed by using fluorescent-labeled primers in a Perkin–Elmer Applied Biosystems Prism 310 Genetic analyzer. Each locus exhibiting allelic loss or gain was coamplified with β-actin to ascertain that we used similar amounts of the input DNA in the PCRs. Human placental DNA was used as a positive control for all PCRs. LOH was analyzed by using GENESCAN and GENOTYPE software.

Results

Suppression of Human Tumor Cell Proliferation and Colony Formation by *ANX7*. To begin evaluating the possibility that *ANX7* might be a TSG, our immediate approach was to test the ability of the gene to suppress tumor cell growth. For this study we used the metastatic prostate cancer cells lines, DU145 and LNCaP, which differ in terms of androgen sensitivity. We subsequently extended this experiment to include the metastatic breast cancer cell line MCF-7 and an osteosarcoma cell line, Saos-2. As shown in Fig. 1, colony formation by all four tumor cell lines is suppressed in a DNA dose-dependent manner by both *ANX7* and *p53*, but not by the vector controls. In each case the efficacy and potency of *ANX7* nearly equals *p53* in terms of suppressing tumor cell proliferation. The well-known TSG *p53* was used as a positive control because these tumor cell lines all differ from each other in terms of mutational state of both *p53* and *RB*.

Levels of ANX7 Protein Expression in Human Prostate Tumor Tissue Microarrays. Because ANX7 expression suppresses colony formation by prostate tumor cells of metastatic origin, it was hypothesized that the levels of ANX7 protein might be reduced in late-stage prostate cancers. We therefore determined the frequency of ANX7 protein expression in a prostate tissue microarray containing 301 specimens from all stages of human prostate tumor progression. As shown in Fig. 2*A*, significant reductions in ANX7 expression are found to occur in a stage-specific manner. ANX7 expression is completely lost in a high proportion of metastases (57%) and in local recurrences of hormone refractory prostate cancer (63%). By contrast, ANX7 remains high in the vast majority of benign prostate glands, high-grade prostatic intraepithelial neoplasias, and stage T2 and T3/4 primary tumors (all in the range of 89% to 96%).

Typical examples of the original data from the human tissue microarray are shown in Fig. 2*B*. The images on the left side of Fig. 2*B* are hemotoxylin and eosin-stained sections, while images on the right show the brown diaminobenzidine stain from a monoclonal anti-ANX7 antibody. The top three sections are heavily stained, while the bottom two sections, representing metastatic and locally recurrent tumors, respectively, are negative. The *P* value for stage-specific loss is $P = 0.0001$. This visual comparison illustrates the statistically significant lack of ANX7 in the two worst prognostic situations.

Serial sections of the same tissue microarray used for Fig. 2 were also used to explore the relationship between ANX7

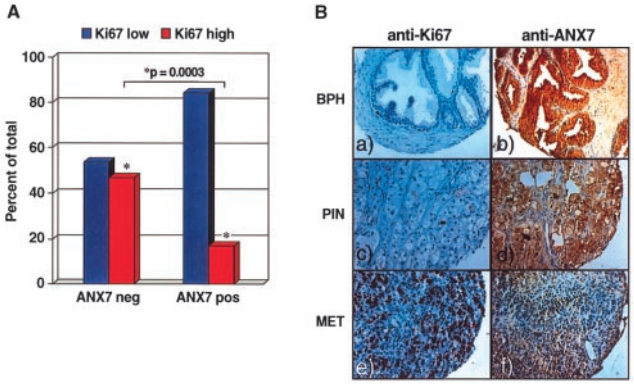

Fig. 3. Levels of ANX7 protein expression and tumor cell proliferation. (*A*) Relationship between ANX7 expression and Ki67 tumor growth fraction. (*B*) Representative sections of immunohistochemical staining of Ki67 (*Left*) and ANX7 (*Right*) on the prostate tissue microarray (original magnification: ×200). (*a* and *b*) Benign prostatic hyperplasia (BPH). (*c* and *d*) Primary untreated prostate cancer with only a few scattered Ki67 positive nuclei, but strong ANX7 immunoreactivity. PIN, prostatic intraepithelial neoplasias. (*e* and *f*) Distant metastasis (MET) with a high fraction of Ki67-positive tumor cell nuclei and lack of ANX7 immunostaining.

expression and tumor cell proliferation. As shown in Fig. 3*A*, a high tumor growth fraction (>10% of Ki67-positive tumor cell nuclei) was significantly more frequent in the 56 ANX7-negative tumors than in the 248 ANX7-positive tumors (53.6% vs. 28.3%, $P = 0.0003$). A comparison of representative data from serial sections is shown in Fig. 3*B*.

Assessment of LOH by Polymorphic Microsatellite Marker Analysis. To determine the genetic basis for possible tumor suppressor activity by the ANX7 gene, we searched for LOH at the ANX7 locus in prostate cancers. For this purpose we isolated matched genomic DNA from tumor and normal prostate tissues by laser capture microdissection from radical prostatectomy specimens of 20 patients. We analyzed these samples by PCR for four polymorphic microsatellite markers on 10q21 at or near the *ANX7* locus. The fluorescently labeled PCR products were analyzed by using the Applied Biosystems Prism 310 genetic analyzer.

Representative experiments exhibiting deletions on various patient tumor samples at the 10q21 locus are shown in Table 1 and Fig. 4*A*. Seven of 20 tumor DNAs (35%) exhibited LOH of at least one or more of these polymorphic markers. Tumor DNA from patient 15 exhibited LOH at multiple microsatellite markers between the AFM220xe5 and AFM063xc5 locus. Among the DNAs exhibiting any deletions, the highest frequency of losses were noted at the AFM220xe5 marker, the closest site to the *ANX7* locus at chromosome 10q21. One tumor sample from patient 17 demonstrated bi-allelic loss, indicating a homozygous deletion at this locus (Fig. 4*B*). LOH and homozygous loss at the same site lend genetic credence to the hypothesis that *ANX7* may be acting as a TSG involved in prostate cancer.

Discussion

Biological function of ANX7 in prostate cancer cells and its loss of expression during prostate cancer progression strongly suggest that *ANX7* appears to play a major role in prostate cancer. These findings, along with localization of *ANX7* to chromosome 10q21 and demonstration of increased frequency of LOH and homozygous deletion near the *ANX7* locus, further supports our hypothesis that ANX7 may function as a candidate TSG. For example, we find that transfection of the human *ANX7* gene into any of four different types of human tumor cells results in profound suppression of tumor cell proliferation and tumor cell colony formation. Interestingly, the data show that the human *ANX7* gene is at least as potent and efficacious as p53. In addition, this experimental finding is also strongly supported by

Table 1. Summary of LOH/homozygous deletion data for four loci mapped to chromosome 10q21 in clinically localized CaP

		Markers			
Patient	β-Actin	AFMa299ya5	AFM220xe5	AFM 063xc5	AFM200wf4
1	ok	Normal	Normal	ND	ND
2	ok	Normal	Normal	Normal	R
3	ok	Normal	NI	NI	NI
4	ok	Normal	NI	Normal	NI
5	ok	Normal	Normal	Normal	Normal
6	ok	Normal	NI	ND	ND
7	ok	NI	NI	NI	LOH
8	ok	NI	NI	Normal	Normal
9	ok	NI	LOH	NI	NI
10	ok	Normal	LOH	Normal	Normal
11	ok	Normal	Normal	Normal	Normal
12	ok	NI	Normal	Normal	NI
13	ok	NI	NI	NI	NI
14	ok	Normal	Normal	Normal	LOH
15	ok	NI	LOH	LOH	Normal
16	ok	Normal	LOH	NI	Normal
17	ok	Normal	Homozygous deletion	Normal	Normal
18	ok	Normal	R	Normal	Normal
Number of informative cases		12/18	11/16	11/16	10/15
Number of LOH		0/12	5/11	1/11	2/10

Matching samples from 20 patients containing normal and tumor specimens were analyzed by four different microsatellite markers located on chromosome 10q21 encompassing *ANX7* locus. Samples from 1–16 were derived from patients with primary prostate cancers. Samples 17 and 18 were from metastatic tumor patients. Only cases demonstrating LOH are illustrated. NI, non-informative; ND, not detected; R, to be repeated. β-actin was used for ascertaining similar amounts of input DNA. The microsatellite marker that is closest to ANX7 is AFM220xe5.

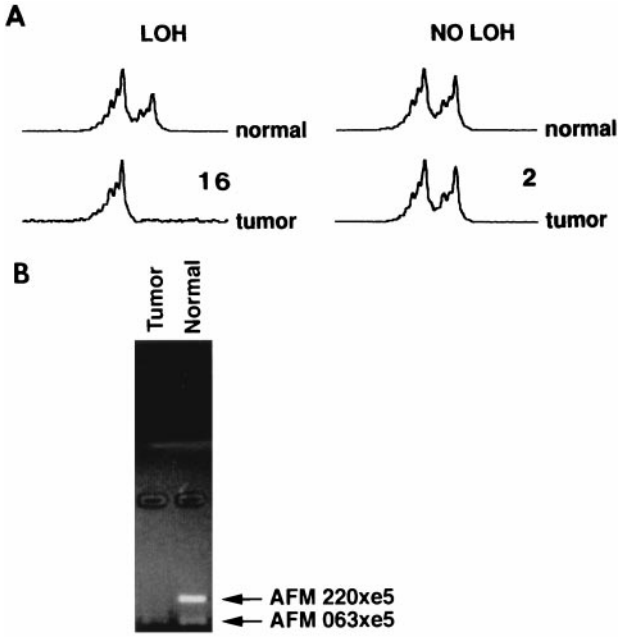

Fig. 4. LOH analysis using microsatellite markers on the chromosome 10q21 locus. (*A*) LOH analysis by microcapillary electrophoresis and data analysis with the Applied Biosystems 310 genetic analyzer in representative samples. LOH was scored by comparing the peak heights of tumor DNA and matched normal DNA. (*B*) Tumor and normal tissue DNA were prepared from 20 patients and analyzed for LOH as described in *Materials and Methods*. Arrows indicate alleles showing loss. Tumor of patient 17 shows homozygous deletion with AFM220xe5. No such loss was noted when the same sample was coamplified with another microsatellite marker AFM063xc5.

the association between lack of ANX7 expression and increased tumor cell proliferation in clinical specimens on human prostate tissue microarray. Finally, analysis of ANX7 protein expression in the prostate tumor microarray indicates that ANX7 is selectively reduced in metastases and in locally recurrent hormone insensitive tumors. Taken together, these results strongly suggest that *ANX7* may act as a TSG, not only in prostate cancer cell lines, but also in clinical prostate cancer, where loss of anx7 expression appears to be highly correlated with late-stage prostate cancer.

Hints from Dictyostelium of *ANX7* Involvement in Cell Proliferation. In retrospect, there may have been experimental suggestions regarding the possible involvement of the *ANX7* gene in proliferation. For example, in the case of the slime mold *Dictyostelium discoidum*, such a role has been emphasized by a gene termed *anx7* in the original literature, and now known to be the closely related *anxc1*. This primitive eukaryotic organism can switch from a growth phase, in which the cells are proliferating, to a differentiated phase, in which the cells form multicellular aggregates and fruiting bodies. Studies of *anx7* gene disruption mutants in this organism have shown that these mutants lose many properties related to growth, differentiation, motility, and chemotaxis, especially in Ca^{2+}-limiting conditions (23–25). Bonfils *et al.* (24) have further shown that the proliferating form of *Dictyostelium* has only 20% of *anx7*-mRNA and only 1.6% of anx7 protein when compared with the differentiated form. The mechanism of this transition involves synthesis by the organism of *anx7* antisense mRNA from the complementary strand (26). Thus, the *Dictyostelium anx7* gene seems to control differentiation by a mechanism in which a relative decrease in anx7 protein enhances growth and proliferation at the expense of Ca^{2+}-dependent differentiated functions. In summary, the apparent parallels between proliferation in the *Dictyostelium* system and prostate cancer in humans are remarkable.

Relationship of *ANX7* Action to Presence of Other TSGs. In as much as suppression of tumor cell growth is a property of many well-known TSGs (27–32), we had decided to test whether the *ANX7* gene could suppress tumor cell growth systematically on four different cell lines. As a positive control, we performed a detailed comparison between *ANX7* and the classical TSG p53. In all four human tumor cell lines, the *ANX7* gene was found to express virtually the same potency and efficacy range as the p53 control vector in suppressing tumor cell proliferation and colony formation. We initially had reasoned that susceptibility to growth suppression by ANX7 might be related to the states of Rb or p53. However, these tumor cell lines all differ from one another in terms of the endogenous state of the *p53* and *RB* genes. For example, whereas LNCaP cells and MCF-7 cells have wild-type *p53* genes, the DU145 cells and Saos-2 cells have mutant *p53* genes (http://perso.curie.fr/Thierry.Soussi/p53_databaseWh.htm. Interestingly, the DU145 cells also have mutant *RB* genes (http://perso.curie.fr/Thierry.Soussi/p53_databaseWh.htm). Thus the state of these classical TSGs in these cell lines appear to have little consequence for the sensitivity of these tumor cells to the *ANX7* gene.

***ANX7* as a Potential TSG at Chromosome 10q21.** The 10q21 site is an interesting and provocative locus for human tumor genetics because it has been hypothesized to contain multiple potential TSGs. The human *ANX7* gene is located on chromosome 10q21 (5), along with many other potential genes of interest (33–38). Loss of DNA sequences at this site have been described in various tumor types including myxoid chondrosarcoma (10q21.1) (7), sporadic nonmedullary thyroid carcinoma (10q21.1) (8), renal cell carcinoma (10q21–23) (9), chronic myelogenous leukemia (10q21) (10), glioma (10q21–26) (11), glioblastoma (two independent regions: 10pter-q11 and 10q24-q26) (12), colonic adenocarcinoma (inverted, nonret duplication of 10q11 to 10q21) (13), lung carcinoma (10q21–10qter) (14), and prostate cancer (two independent loci: 10q21 and 10q23–24) (15). The possibility of the presence of other TSGs also exist. In our studies using four microsatellite markers, 35% of the 20 primary tumors showed LOH at or near the 10q21 locus of ANX7. Our data also revealed homozygous deletion at this site in one of the specimens. Our data showing LOH and homozygous deletion at the *ANX7* locus in prostate cancer thus strongly suggest that *ANX7* has the likelihood of being a candidate TSG.

Clinical Significance of *ANX7* Compared with Other TSGs for Prostate Cancer. Loss of ANX7 expression appears to be a biomarker for tumor cell proliferation and progression to late-stage prostate cancer. To evaluate the clinical significance of candidate genes emerging from model systems and functional *in vivo* experiments, we needed the statistical power of being able to analyze large numbers of clinical specimens. Tissue microarray technology has been shown to be such a powerful tool for the analysis of molecular alterations in hundreds of tumors at a time (18–20). As indicated above, prostate tissue microarray data show that reductions in ANX7 expression are confined to metastases and hormone refractory primary recurrences. The relationship is statistically powerful ($P = 0.0001$).

The tissue microarray data also suggest that loss of ANX7 expression occurs late in the progression of prostate cancer. Similar correlations have been found for other TSGs. For example, mutation or low expression of *p53* has been suggested as a late-stage event in prostate cancer (39). Similar results have been reported for CD44 (40) and KAI-1 (41). In the case of PTEN/MMAC1, reduced expression levels have been correlated with Gleason score and poor prognosis, and loss of PTEN expression has been emphasized in metastases (42, 43). By contrast, the expression levels

of *p27*, another possible TSG associated with prostate cancer, have not found to be associated with the pathologic stage (44). Thus, compared with other TSGs like *PTEN/MMAC1* in prostate cancer, reduced expression of the *anx7* gene is strongly correlated with the most clinically compromising forms of this cancer. Future large-scale studies on tissue microarrays hopefully will elucidate the correlation among these multiple molecular markers with prostate cancer progression and comprehensively illuminate the complex relationships among these genes.

Conclusions

The data described in this study correlate prostate cancer with alterations of *ANX7* expression and deletions of chromosomal region harboring ANX7. This is an important insight because the mammalian *ANX7* gene had never been thought to play a role in cancer. Rather, *ANX7* had only been known previously from the perspective of exocytosis as a highly conserved gene defining a calcium binding protein with Ca^{2+} channel (22) and Ca^{2+}-activated GTPase (4) activities. However, ANX7 is known to be located in both the nucleus and the cytoplasm (6). ANX7 is also a substrate for protein kinase C and other kinases associated with proliferation (45). In summary, our data show that, *in vitro*, *ANX7* suppresses proliferation of human tumor cells from prostate, as well as other sources. The study also provides evidence that LOH occurs at or very near the ANX7 locus at 10q21 in 35% of primary CaP patients. In a large retrospective study, ANX7 protein expression is significantly reduced in androgen-insensitive metastatic and locally recurrent hormone-insensitive prostate cancers. Taken together these data suggest that the study of *ANX7* action in cancer cells and prostate cancer specimens has great potential importance for not only understanding human prostate cancer progression, but also for development of novel diagnostic and therapeutic approaches.

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