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ARTICLES

Fas Gene Mutations in Prostatic Intraepithelial Neoplasia and Concurrent Carcinoma: Analysis of Laser Capture Microdissected Specimens

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SUMMARY: Fas (Apo-1/CD95) is a cell-surface receptor involved in cell death signaling through binding of Fas ligand. Mutations of the *Fas* gene might be involved in proliferative diseases of the prostate by prolongation of programmed cell death of prostatic epithelial cells. Using the laser capture microdissection method, *Fas* gene mutations were examined on genomic DNA extracted from lesions with high-grade prostatic intraepithelial neoplasia (HGPIN), a possible precursor of prostatic cancer (PCA), and from PCA. A total of 193 lesions, 111 with HGPIN, 55 with PCA, and 27 benign glands, were microdissected from 27 patients with PCA. Polymerase chain reaction-amplified products were directly sequenced. Loss of heterozygosity (LOH) was examined at four sites of known polymorphisms. *Fas* gene mutations were detected in HGPIN: 4 of 27 (14.8%) cases or 4 of 111 (3.6%) lesions. All were point mutations: three missense and one nonsense in the death domain. Benign proliferative glands adjoining HGPIN and/or PCA, and PCA never showed mutations. LOH was found in 31.3% of PCA and 25% of HGPIN lesions, but was never found in benign glands. Exclusive occurrence of *Fas* mutations in HGPIN might underlie the development of these lesions. Occasional findings of LOH in HGPIN and PCA suggested that genetic instability might occur during the early phase of prostatic carcinogenesis. (*Lab Invest 2001, 81:283–288*).

Prostatic intraepithelial neoplasia (PIN) is characterized by intraluminar proliferation of epithelial cells in ducts and acini. PIN frequently coexists with prostatic carcinoma (PCA) (Bostwick, 1995; Bostwick and Brawer, 1987; Skjørten et al, 1997) and is commonly found in the nontransition zone, which is the predominant site for PCA (de la Torre et al, 1993; Qian et al, 1997). According to histologic and cytologic findings, PIN is divided into high-grade PIN (HGPIN) and low-grade PIN (LGPIN) (Montironi et al, 1996). Previous histopathologic study of whole-mount prostatectomy specimens showed the proximity of HGPIN lesions but not LGPIN lesions to PCA (Shin et al, 2000). Recent studies revealed that HGPIN and PCA share common cytogenetic features. Allelic loss of chromosome 8p is frequent in both HGPIN and invasive PCA (Emmert-Buck et al, 1995; Häggman et al, 1997). Mutations of the *H-ras* gene were closely associated with progression of HGPIN into invasive PCA in transgenic mice, although this was not confirmed in humans (Shibata et al, 1996). These findings indicated that HGPIN but not LGPIN is the most likely precursor lesion for PCA.

HGPIN lesions could be defined only at the microscopic level and therefore immunohistochemical procedures have been the main modalities used to analyze its biologic characteristics. Crowded cells in the HGPIN lesions were occasionally labeled with an antibody (MIB-1) against proliferating cells (Häussler et al, 1999). Meanwhile, foci of HGPIN were strongly immunoreactive with Bcl-2 (Häussler et al, 1999; Stattin et al, 1996), suggesting that programmed cell death (apoptosis) is prolonged in HGPIN.

Fas antigen is a 45-kDa transmembrane protein of the TNF receptor superfamily that can induce programmed cell death (apoptosis) through cross-linkage with Fas ligand (FasL) (Nagata, 1997; Suda et al, 1993). *Fas* is located on chromosome 10q24.1 and is comprised of 9 exons and 8 introns. The *Fas* extracellular domain contains three cysteine-rich motif repeats encoded by exons 2 to 5. Its transmembrane domain consists of the last 49 bp of exon 6 and the first 2 bp of exon 7. The remaining 81 bp in exon 7, the 25 bp in exon 8, and 329 bp in exon 9 comprise the cytoplasmic region (Cheng et al, 1995). Fas is expressed in a wide range of tissues including the thymus, liver, and activated T and B lymphocytes (Itoh et al, 1991;

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Watanabe-Fukunaga et al, 1992). A mutation in the death domain of the *Fas* gene leads to loss of its apoptosis function, a loss-of-function mutation, which might contribute to the pathogenesis of human malignancies. Indeed *Fas* gene mutations have been reported in both the lymphoid lineage and epithelial malignancies: approximately 10% of cases with multiple myeloma (Landowski et al, 1997) and sporadic non-Hodgkin's lymphoma (Grønbæck et al, 1998), 7.7% in lung cancer (Lee et al, 1999a), and 28% in urinary bladder cancer (Lee et al, 1999b).

Laser capture microdissection under direct microscopic visualization enables rapid one-step procurement of selected human cell populations from histologic sections. This method facilitates microdissection of selected cells, and thus extensive studies of the objective lesions are now possible. Using this method on whole-mount samples, we selectively microdissected numerous PIN and PCA lesions from 27 patients with PCA. Genomic DNA extracted from each lesion was analyzed for *Fas* mutations.

Results

Fas *Gene Mutations*

As shown in Table 1, four mutations of the *Fas* gene were detected in four HGPIN lesions from four cases. There were no prominent differences in frequency of mutations or loss of heterozygosity (LOH) in HGPIN or PCA lesions between noncastrated and castrated cases. All mutations were point mutations: three missense and one nonsense mutation detected in exon 9, which encodes the death domain region of the Fas receptor (Nagata, 1997). Substitutions at codon 260 of the Fas cDNA sequence (Gene Bank accession No. M67454), Gln to Stop (Case 10) and Gln to Arg (Case 21), respectively, were found in the HGPIN lesions. All of the mutations were transitions: C to T and A to G. No G to C or C to A transversions were found. All of the HGPIN lesions with mutations were present in the nontransition zone. Neither PCA nor benign proliferative glands adjoining HGPIN and/or PCA showed *Fas* mutations.

Allelic Status

Sixteen of 27 (59.6%) cases were heterozygous for one or more sites of the known biallelic polymorphisms, ie, at positions −1377, −670, 416, and 836. Of these 16 cases, 5 (31.3%) with PCA and 4 (25%) with HGPIN showed LOH in the promoter region (−670) or exon 7. The HGPIN lesion in Case 24 had a missense mutation at position 1099 and LOH at −670. In Case 21, the HGPIN lesion had a mutation at exon 9 but no LOH, and the PCA lesion had LOH but no mutation.

Immunohistochemistry

Results of immunohistochemical study in lesions with *Fas* gene mutations and LOH are summarized in Table 1. Fas protein was expressed in 11 of 15 (73.3%) HGPIN lesions and 5 of 6 PCA (83.3%) lesions. No relationship was found between immunoreactivity for anti-Fas antibody and mutation or LOH in HGPIN and PCA lesions.

Discussion

Information regarding the molecular genetic characteristics of PIN was quite limited until development of the microdissection technique. Previous studies showed that allelic loss of chromosomes 8p, 10q, and 16q was frequent in both HGPIN and invasive PCA, suggesting the involvement of tumor suppressor genes or oncogenes at these loci (Emmert-Buck et al, 1995; Gray et al, 1995; Häggman et al, 1997; Strup et al, 1999). Through construction of a detailed deletion map spanning 10q23–25, Gray et al (1995) suggested the presence of prostate tumor suppressor genes near the 10q23–24 boundary, which is close to the location of the *Fas* gene, 10q24.1. Using the laser capture microdissection method, we analyzed the *Fas* gene mutations in numerous HGPIN lesions; *Fas* gene mutations were detected in 4 of 27 (14.8%) cases or 4 of 111 (3.6%) lesions with HGPIN. In contrast, none of 55 lesions with PCA had *Fas* gene mutations, indicating that PCA develops in HGPIN without *Fas* gene mutations. *Fas* gene mutations were never detected in benign proliferative glands adjoining HGPIN and/or PCA. Taken together, these observations suggest that *Fas* gene mutation might not contribute to the pathogenesis of PCA, in contrast to lung cancer, hematologic malignancies, and bladder cancer (Lee et al, 1999a, 1999b).

With regard to the sites and patterns of mutations in the *Fas* gene, a point mutation at codon 253 was reported in two patients with multiple myeloma (Landowski et al, 1997). In non-Hodgkin's lymphomas, mutations at codons 248 and 251 were identified (Grønbæck et al, 1998). Lee et al (1999b) reported that 8 of 12 mutations found in bladder cancer showed a G to A transition at codon 251, suggesting that this might be a mutational hotspot. Two of the current HGPIN lesions showed mutations at codon 260. All of the mutations in the current series were transitions, suggesting that some "endogenous" mutagens act in the pathogenesis of HGPIN.

Missense mutations in the death domain have been suggested to affect receptor function in a dominant-negative fashion (Fisher et al, 1995), ie, mutant Fas protein derived from one mutated *Fas* allele might bind with normal Fas protein derived from the other normal allele to construct a structurally abnormal Fas trimer, which might have a defect in binding to adapter proteins. Among four HGPIN lesions with *Fas* gene mutations, one informative case (Case 24) had LOH at position −670. As the distance between the site of LOH and the mutation was approximately 10 kb, LOH might involve the mutation site in the same allele. Therefore, it is reasonable to consider that the LOH and mutation found in the HGPIN lesion in Case 24 occurred in different alleles, thus resulting predominantly in production of mutant Fas protein. The occur-

Table 1. Mutations and LOH of the *Fas* Gene in High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) and Prostatic Cancer (PCA)

Case	Lesion	Castration	Stage	Immunohistochemistry for Fas protein	Mutation					LOH analysis			
					Sites	Codon	Position	Nucleotide	Amino acid	−1377	−670	Exon3	Exon7
3	HGPIN	+	pT2bNxMx	positive	Exon 9	256	1002	ACA/GCA	Thr/Ala	NI	NI	NI	NI
10	HGPIN	+	pT2bNxMx	negative	Exon 9	260	1020	CAA/TAA	Gln/Stop	NI	NI	NI	NI
21	HGPIN	−	pT3aNxMx	negative	Exon 9	260	1021	CAA/CGA	Gln/Arg	NI	HET	NI	NI
	PCA	−	pT3aNxMx	negative						NI	LOH	NI	NI
23	HGPIN1	+	pT3aNxMx	positive						NI	LOH	NI	HET
	HGPIN2	+	pT3aNxMx	positive						NI	LOH	NI	LOH
	PCA1	+	pT3aNxMx	positive						NI	LOH	NI	HET
	PCA2	+	pT3aNxMx	positive						NI	LOH	NI	LOH
24	HGPIN1	−	pT2bNxMx	positive	Exon 9	286	1099	AAT/AGT	Asn/Ser	NI	LOH	NI	NI
	HGPIN2	−	pT2bNxMx	positive						NI	LOH	NI	NI
	HGPIN3	−	pT2bNxMx	positive						NI	LOH	NI	NI
	HGPIN4	−	pT2bNxMx	positive						NI	LOH	NI	NI
	HGPIN5	−	pT2bNxMx	positive						NI	LOH	NI	NI
	PCA	−	pT2bNxMx	positive						NI	LOH	NI	NI
25	HGPIN1	−	pT3aNxMx	positive						NI	LOH	NI	NI
	HGPIN2	−	pT3aNxMx	negative						NI	LOH	NI	NI
	HGPIN3	−	pT3aNxMx	negative						NI	LOH	NI	NI
	HGPIN4	−	pT3aNxMx	positive						NI	LOH	NI	NI
	PCA	−	pT3aNxMx	positive						NI	LOH	NI	NI
27	HGPIN	+	pT3aNxMx	positive						NI	LOH	NI	NI
	PCA	+	pT3aNxMx	positive						NI	LOH	NI	NI

NI, not informative; HET, retention of heterozygosity; LOH, loss of heterozygosity.

rence of LOH was unknown in the remaining three cases (Case 3, 10, 21) with *Fas* mutations. Fas function might have been lost or reduced due to a dominant-negative effect of mutant Fas protein in cases without LOH or because of predominant production of mutant Fas protein in cases with LOH. Fas-mediated apoptosis was suggested to be disrupted in these four HGPIN lesions.

The normal DNA repair mechanism is important for maintaining the integrity of the genome. Humans are frequently exposed to naturally occurring DNA-damaging agents, and the combined occurrence of DNA damage and impaired DNA repair function results in development of neoplasia. Indeed, replication errors, as revealed by microsatellite instability (MSI), were reported in cases with PCA (Dahiya et al, 1997; Rohrbach et al, 1999). The occurrence of LOH also indicates the underlying genetic instability in lesional proliferating cells. Rohrbach et al (1999) reported that MSI and LOH were found in 35% and 16%, respectively, of their PCA cases. In the present study, LOH at four sites in the *Fas* gene was found in 31.6% of PCA and 25% of HGPIN lesions. LOH of the *Fas* gene was never found in the benign lesions. Previous studies suggested that HGPIN is the precursor lesion of PCA (Bostwick, 1995; Bostwick and Brawer, 1987; Skjørten

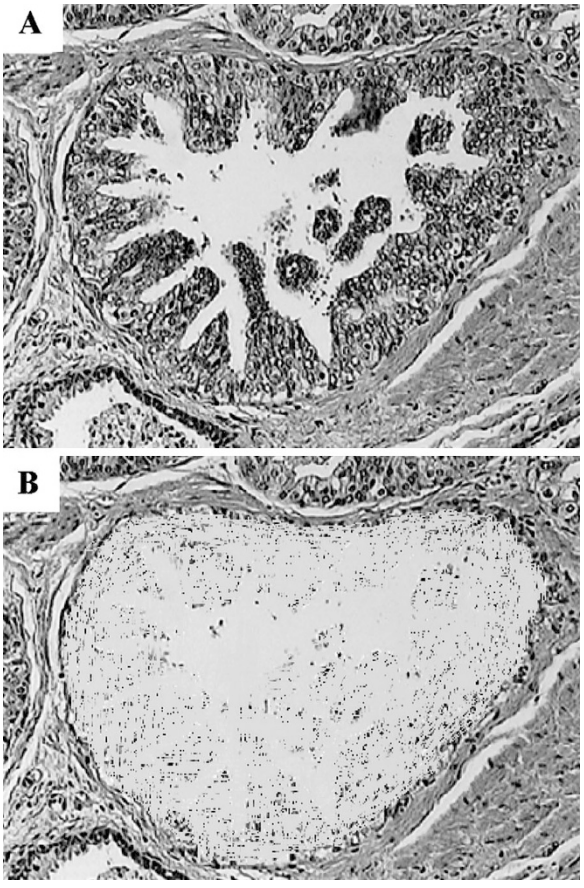

Figure 1.
High-grade prostatic intraepithelial neoplasia (HGPIN) lesion (A) in a representative case was successfully microdissected (B). Note successful resection of intraductal epithelial cells. Hematoxylin and eosin staining; original magnification, ×200.

Table 2. Amplification Primers for Mutation Analysis of the *Fas* Gene

	First PCR			Second PCR		
Exon/Primer	Sequence (5′→3′)	Size of PCR product (bp)	Annealing temperature (°C)	Sequence (5′→3′)	Size of PCR product (bp)	Annealing temperature (°C)
PA-F(promoter)	5′-gccctataccatcctccttat-3′	172	55	5′-gccctataccatcctccttat-3′	145	55
PA-R	5′-ctgtcactgcacttaccacc-3′			5′-gtaggtgttgataggcttgtct-3′		
PB-F(promoter)	5′-cctcttgaaaataaaaact-3′	242	56	5′-cctcttgaaaataaaaact-3′	228	56
PB-R	5′-tcactcagagaaagacttgcgg-3′			5′-gacttgcggggcatttgac-3′		
3-F	5′-acttcccacctgttacctg-3′	310	55	5′-acttcccacctgttacctg-3′	250	55
3-R	5′-acttcccacctgttacctg-3′			5′-tgtgtgtcaacatagcaccac-3′		
7-F	5′-tcttagtgtgaaagtatgttctc-3′	223	46	5′-ctacaaggctgagacctgagtt-3′	203	55
7-R	5′-caaatcactaatttctctatttt-3′			5′-aggaagtaacaaaaagccaaatc-3′		
8-F	5′-attaaggaaaaattagaagttcacat-3′	217	50	5′-attaaggaaaaattagaagttcacat-3′	181	50
8-R	5′-atcccataatatgtcactgaaa-3′			5′-atcccataatatgtcactgaaa-3′		
9-F	5′-ggttttcactaatgggaatttca-3′	536	50	5′-ggttttcactaatgggaatttca-3′	443	50
9-R	5′-tatgttggctcttcagcgcta-3′			5′-ctaattgcatatactcaggaa-3′		

et al, 1997), and the present results showed that genetic instability occurred during the early phase of prostatic carcinogenesis.

In conclusion, *Fas* gene mutations were exclusively found in the HGPIN lesions, which might underlie the development of these lesions.

Materials and Methods

Patients

Twenty-seven patients, who had received total prostatectomy under the diagnosis of PCA, were selected for the present study. All of the patients were admitted to our hospitals between 1996 and 1998. Fourteen patients received androgen deprivation therapy (castration): LH-RH agonist (leuprolide) for 2 to 6 months (mean 3.4 months) together with the antiandrogen agents (flutamide or chlormadinone). The remaining 13 patients did not receive this treatment (noncastrated). The present cases were conventionally subdivided into the preoperatively noncastrated group (13 cases) and medically castrated group (14 cases). None of the 27 patients received preoperative chemotherapy or radiation therapy.

Histologic findings in a subpopulation of these cases were reported previously (Shin et al, 2000). Based on the American staging system (modified by Whitmore-Jewett) (Bostwick et al, 1994), pathologic stage was determined as follows: 11 (41%) cases were in stage T2 and 16 (59%) were in stage T3. Histologic specimens were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Serial sections 5-μm thick were cut and stained with hematoxylin and eosin, and reviewed independently by two pathologists (HT and KA). The mean number of sections examined was 9.3 per case. In problematic cases, preservation of the basal cell layer, a reliable criterion for benign prostatic lesions, was immunohistochemically examined using basal cell–specific monoclonal anti-high molecular weight cytokeratin antibody (34βE12; Enzo Diagnostics, New York). Diagnosis of HGPIN was made according to histologic and cytologic features; ie, intraluminal proliferation of glandular epithelial cells with large and prominent nuclei. The basal layer was partially disrupted in HGPIN.

Laser Capture Microdissection and DNA Extraction

Microdissection of each lesion was performed using a PixCell laser capture microscope (Arcturus Engineering, Santa Clara, California) according to previously described methods with some modifications (Bonner et al, 1997; Emmert-Buck et al, 1996). Briefly, histologic sections were dehydrated, and then the histologic fields of interest were selected, overlaid with thermoplastic film mounted on transparent cap, and captured on the film through laser energy (Fig. 1). The dissected pieces were allowed to adhere to the transparent cap and were collected in 0.5 ml Eppendorf tubes. The cells were subsequently resuspended in 20 to 50 μl of extraction buffer containing 10 mM Tris (pH 8.0), 2 mM EDTA, 0.2% Tween 20, and 200 μg/ml proteinase K, and incubated overnight at 37° C. The mixture was heated at 100° C for 10 minutes to inactivate proteinase K, and 3% to 5% of the solution was used as a template for each PCR. The total number of microdissected lesions from 27 cases was 193: 111 lesions from HGPIN, 55 from PCA, and 27 from benign glands.

Detection of Mutations and LOH

The death domain is necessary for transduction of the apoptotic signal (Cheng et al, 1995; Itoh and Nagata, 1993; Itoh et al, 1991). Therefore, we examined mutations in exons 7, 8, and 332 bp of exon 9. The primers used for PCR analysis are listed in Table 2. DNA was subjected to first-round PCR consisting of 10 cycles with oligonucleotide primers, followed by a second PCR consisting of 35 cycles using 0.1% of the first-round PCR products as the template, then denaturation for 30 seconds at 95° C, annealing for 30 seconds at variable temperatures, and extension for 30 seconds at 72° C in a 9700 Applied Biosystems Thermocycler (Foster City, California). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, California), and were sequenced by the dideoxy chain termination method using a DNA sequencing kit (Applied Biosystems). The samples were analyzed using a Genetic Analyzer (ABI PRISM 310'; Applied Biosystems). PCR products with suspected mutations were cloned in pCR 2.1-TOPO (Invitrogen, Carlsbad, California), and sequenced to confirm the mutation.

LOH was examined at four sites of known polymorphisms, ie, at position −1377, −670 (promoter region), 416 (exon 3), and 836 (exon 7). DNA was amplified using primers flanking the four polymorphic sites (Table 1). Polymorphisms at −1377, 416, and 836 were examined by direct sequencing, and that at position −670 by restriction fragment length polymorphism by digestion with *Mva I* (Fermentas, Vilnius, Lithuania) (Huang et al, 1997).

Immunohistochemistry

Immunohistochemical study of paraffin sections was carried out using the avidin-biotin peroxidase complex method. For detection of Fas protein, mouse antihuman Fas antibody (4B4-B3) that recognizes extracellular domain of Fas was prepared by Dr. S. Nagata (unpublished data). The histologic sections were deparaffinized and rinsed in three changes of PBS. Then the antibody, diluted 1:200, was applied at 37° C for 45 minutes. Positive cells showed an intracytoplasmic dot-like staining. Staining manner in each case was distinct, ie, almost all cells were positive in positive cases and negative in negative cases.

References

Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, and Liotta LA (1997). Laser capture microdissection: Molecular analysis of tissue. Science 278: 1481–1483.

Bostwick DG (1995). High grade prostatic intraepithelial neoplasia: The most likely precursor of prostatic cancer. Cancer 75:1823–1836.

Bostwick DG and Brawer MK (1987). Prostatic intra-epithelial neoplasia and early invasion in prostate cancer. Cancer 59:788–794.

Bostwick DG, Myers RP, and Oesterling JE (1994). Staging of prostate cancer. Semin Surg Oncol 10:60–73.

Cheng J, Liu O, Koopman WJ, and Mountz JD (1995). Characterization of human *Fas* gene: Exon/intron organization and promoter region. J Immunol 154:1239–1245.

Dahiya R, Lee C, McCarville J, Hu W, Kaur G, and Deng G (1997). High frequency of genetic instability of microsatellites in human prostatic adenocarcinoma. Int J Cancer 72:762–767.

de la Torre M, Häggman MJ, Brändstedt S, and Busch C (1993). Prostatic intraepithelial neoplasia (PIN) and invasive carcinoma in total prostatectomy specimens: Distribution, volumes, and DNA ploidy. Br J Urol 72:207–213.

Emmert-Buck MR, Boner RF, Smith PD, Chuaqui R, Zhaung Z, Goldstein SR, Weiss RA, and Liotta LA (1996). Laser capture microdissection. Science 274:998–1001.

Emmert-Buck MR, Vocke CD, Pozzatti RO, Duray PH, Jennings SB, Florence CD, Zhuang Z, Bostwick DG, Liotta LA, and Linehan WM (1995). Allelic loss on chromosome 8p12–21 in microdissected prostatic intraepithelial neoplasia. Cancer Res 55:2959–2962.

Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, and Puck JM (1995). Dominant interfering *Fas* gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81:935–946.

Gray IC, Phillips SMA, Lee SL, Neoptolemos JP, Weissenbach J, and Spurr N (1995). Loss of the chromosomal region 10q23–25 in prostate cancer. Cancer Res 55:4800–4803.

Grønbæck K, Straten PT, Ralfkiaer E, Ahrenkiel V, Andersen MK, Hansen NE, Zeuthern J, Hou-Jensen K, and Guldberg P (1998). Somatic *Fas* mutations in non-Hodgkin's lymphoma: Association with extranodal disease and autoimmunity. Blood 92:3018–3024.

Häggman MJ, Wojno KJ, Pearsall CP, and Macoska JA (1997). Allelic loss of 8p sequences in prostatic intraepithelial neoplasia and carcinoma. Urology 50:643–647.

Häussler O, Epstein JI, Amin MB, Heitz PU, and Hailemariam S (1999). Cell proliferation, apoptosis, oncogene, and tumor suppressor gene status in adenosis with comparison to benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and cancer. Human Pathol 30:1077–1086.

Huang QI, Morris D, and Manollos N (1997). Identification and characterization of polymorphisms in the promoter region of the human *Apo-1/Fas (CD95)* gene. Mol Immunol 34:577–582.

Itoh N and Nagata S (1993). A novel protein domain required for apoptosis: Mutational analysis of human Fas antigen. J Biol Chem 268:10932–10937.

Itoh N, Yonehara S, Ishii M, Yonehara S, Mizushima M, Sameshima A, Hase A, Seto Y, and Nagata S (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66:233–243.

Landowski TH, Qu N, Buyuksal I, Painter S, and Dalton WS (1997). Mutations in the Fas antigen in patients with multiple myeloma. Blood 90:4266–4270.

Lee SH, Shin MS, Park WS, Kim SY, Kim HS, Han JY, Park GY, Dong SM, Pi JH, Kim CH, Kim SH, Lee JY, and Yoo NJ (1999a). Alterations of *Fas (Apo/CD95)* gene in non-small cell lung cancer. Oncogene 18:3754–3760.

Lee SH, Shin MS, Park WS, Kim SY, Kim HS, Han JY, Park GY, Dong SM, Pi JH, Kim CH, Kim SH Lee JY, and Yoo NJ (1999b). Alterations of *Fas (Apo/CD95)* gene in transitional cell carcinoma of urinary bladder. Cancer Res 59:3068–3072.

Montironi R, Bostwick DG, Bonkhoff H, Cockett ATK, Helpap B, Troncoso P, and Waters D (1996). Origins of prostate cancer. Cancer 78:362–365.

Nagata S (1997). Apoptosis by death factor. Cell 88:355–365.

Qian J, Wollan P, and Bostwick DG (1997). The extent and multi-centricity of high-grade prostatic intraepithelial neoplasia in clinically localized prostatic adenocarcinoma. Hum Pathol 28:143–148.

Rohrbach H, Hass CJ, Baretton GB, Hirschmann A, Diebold J, Behrendt RP, and Löhrs U (1999). Microsatellite instability and loss of heterozygosity in prostatic carcinomas: Comparison of primary tumors, and of corresponding recurrences after androgen-deprivation therapy and lymph-node metastases. Prostate 40:20–27.

Shibata M, Ward JM, Devor DE, Liu ML, and Green JE (1996). Progression of prostatic intraepithelial neoplasia to invasive carcinoma in C3(1)/SV40 large T antigen transgenic mice: Histopathological and molecular biological alterations. Cancer Res 56:4894–4903.

Shin M, Takayama H, Nonomura N, Wakatsuki A, Okuyama A, and Aozasa K (2000). Extent and zonal distribution of prostatic carcinoma in Japan: Analysis of whole-mounted prostatectomy specimens. Prostate 42:81–87.

Skjørten FJ, Berner A, Harvei S, Robsahm TE, and Tretli S (1997). Prostatic intraepithelial neoplasia in surgical resections. Cancer 79:1172–1179.

Stattin P, Damber JE, Karlberg L, Nordgren H, and Bergh A (1996). Bcl-2 immunoreactivity in prostate tumorigenesis in relation to prostatic intraepithelial neoplasia, grade, hormonal status, metastatic growth and survival. Urol Res 24:257–264.

Strup SE, Pozzatti RO, Florence CD, Emmert-Buck MR, Duray PH, Liotta LA, Bostwick DG, Linehan WM, and Vocke CD (1999). Chromosome 16 allelic loss analysis of a large set of microdissected prostate carcinomas. J Urol 162:590–594.

Suda T, Takahashi T, Goldstein P, and Nagata S (1993). Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 75:1169–1178.

Watanabe-Fukunaga R, Brannan C, Itoh N, Yonehara S, Copeland NG, Jenkins NA, and Nagata S (1992). The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J Immunol 148:1274–1279.