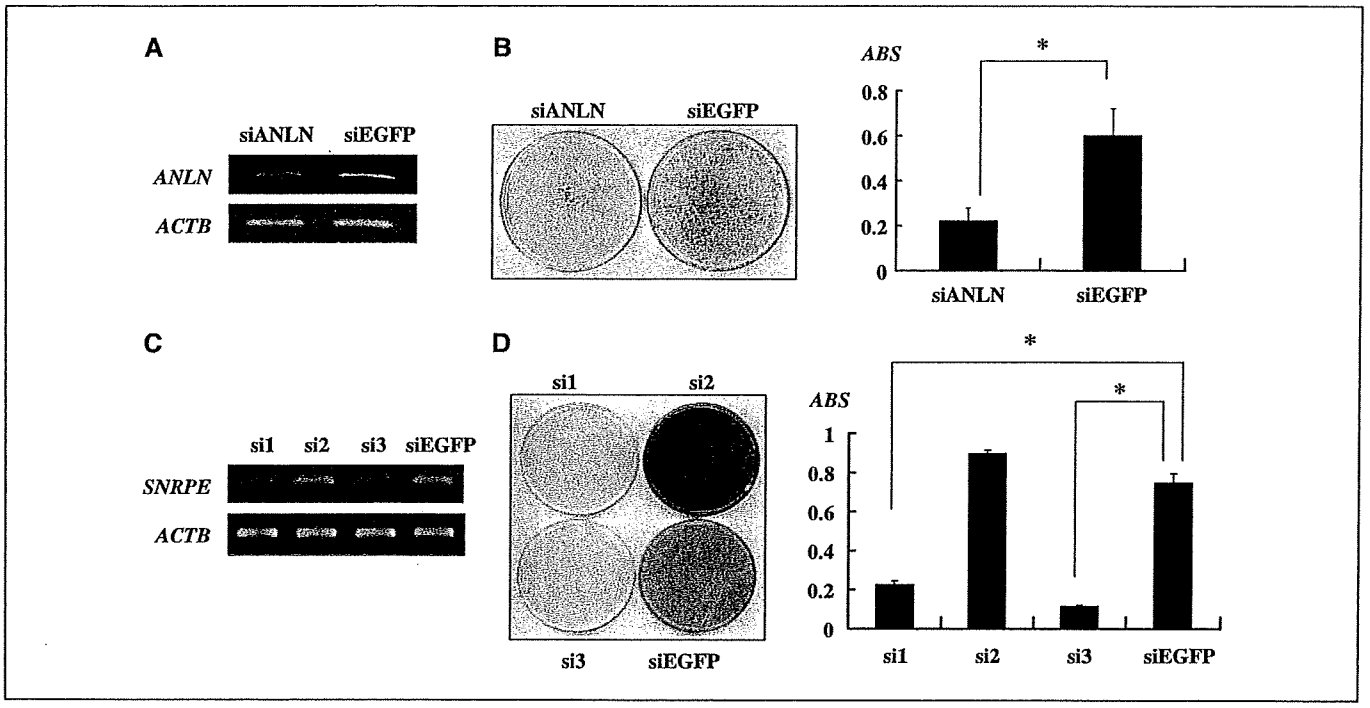

Figure 4. Knockdown of *ANLN* and *SNRPE* by siRNA in prostate cancer cells attenuated their growth and viability. *A* and *C*, knockdown effect of siRNA on *ANLN* and *SNRPE* in prostate cancer cell line 22Rv1 was evaluated by semiquantitative RT-PCR using cells transfected with each of siRNA-expressing vectors to *ANLN* (siANLN), *SNRPE* (si1-3), and a negative control vector (siEGFP). *ACTB* was used to quantify RNAs. Colony formation assay was assessed on 22Rv1 cells (*B* and *D, left*) transfected with each of indicated siRNA-expressing vectors to *ANLN* (siANLN), *SNRPE* (si1–si3), and a negative control vector (siEGFP). Cells were visualized with 0.1% crystal violet staining after 14-d incubation with geneticin. MTT assay was done for each of 22Rv1 (*B* and *D, right*) transfected with indicated siRNA-expressing vectors to *ANLN* (siANLN), *SNRPE* (si1–si3), or a negative control vector (siEGFP). *Columns*, average after 14-d incubation with geneticin; *bars*, SD. *ABS*, absorbance at 490 nm, and at 630 nm as reference, measured with a microplate reader (*Y-axis*). These experiments were carried out in triplicate. *, $P < 0.01$, Student's *t* test.

appropriate frozen HRPC samples (25, 26). Hence, we are confident that our precise genome-wide expression profiles of clinical HRPC cells are very valuable. The random permutation test comparing the expression profiles of 13 HRPCs with those of 10 HSPCs identified 36 up-regulated genes and 70 down-regulated genes in HRPCs (Tables 1 and 2). Some of such genes were considered to be associated with their androgen-independent growth and more aggressive phenotypes of clinical HRPCs. Among the 36 up-regulated genes in HRPCs, at first, we focused on *AR* overexpression. In spite of *AR* transactivation of mRNA in HRPC cells, the amount of stabilized AR protein in the nucleus and AR activity measured by the transcriptional levels of its downstream target genes (*PSA* and *NLX3.1*) in HRPC cells were similar to those in HSPC and normal prostate epithelial cells. Several reports suggested that even under low level of circulating testicular androgen, HRPCs still maintain some level of dependency to the AR pathway (10, 11, 26, 27) and our data also support this concept. However, of course, the retention of AR activity itself does not explain the more aggressive phenotype of clinical HRPCs, and apparently, the non-AR pathways should contribute to this clinical HRPC phenotype.

The list of up-regulated genes in HRPC (Table 1) included *ANLN* and *SNRPE* as well as *AR*. ANLN interacts with and activated RhoA and that this complex is likely to be essential for the growth-promoting pathway and aggressive features of lung cancers through phosphatidylinositol 3-kinase/Akt signaling (28), indicating that its overexpression in HRPCs can be involved with aggressive phenotype of clinical HRPCs. SNRPE may be involved with RNA splicing, but its function is unknown. Our siRNA experiments showed that overexpression of ANLN and SNRPE could play some important roles in prostate cancer cell viability and aggressive phenotype of HRPCs.

The list of the down-regulated genes in HRPC (Table 2) included *NR4A1, CYP27A1*, and *HLA-A* antigen. NR4A1 belongs to the steroid nuclear hormone receptor superfamily and its expression can cause apoptosis (29). NR4A1 expression is regulated by LH (30) and its down-regulation in HRPCs can reflect LH depletion in the patients under the treatment of LH-RH antagonist. CYP27A1 catalyses hydroxylations in the bioactivation of vitamin D3 (31). Epidemiologic evidence suggests an inverse relationship between prostate cancer and serum vitamin D levels (32), and active vitamin D3 inhibits growth and invasion of human prostate cancer cells (31). Down-regulation of NR4A1 and CYP27A1 can provide HRPC cells with some advantages for their survival and growth. Notably, HLA-A antigen, one of the MHC molecules, and many other HLA antigens (which were not listed in Table 2 because of their *P* value of 0.001–0.0001) were significantly down-regulated in clinical HRPCs, implicating that HRPC cells could acquire immunotolerance (33).

We attempted to identify the genes that were differentially expressed between HRPCs in metastatic site and those in the primary site (prostate). Because prostate cancer can preferentially metastasize to bone, several reports (34, 35) indicated that the microenvironment in bone marrow could promote prostate cancer growth and change their phenotype more aggressive. In comparing the gene expression patterns between metastatic tumors and primary tumors, it is critical to exclude the cells of the host organs of the metastatic tumors, and the expression profiles of bone metastasis of prostate cancer was very vulnerable

to contamination of bone marrow cells (26). In our study, the expression profiles of the microdissected cancer cells in bone metastasis was expected to reflect such inferences with the microenvironment in bone marrow and we did the supervised analysis using the expression profiles of 8 HRPC cells at bone metastatic lesions and 10 HRPC cells at the prostate. However, our analysis unexpectedly failed to distinguish them, and our supervised analysis did not clearly separate HRPC cells at the bone metastasis from those at the prostate. Taken together with the findings from the unsupervised hierarchical clustering analysis, our data indicated that the differences in expression patterns among the multiple metastatic loci derived from the individual patients were much smaller than the interindividual differences in the expression patterns.

In conclusion, our precise microarray analysis of clinical HRPC cells should provide useful information to understand the molecular mechanism of the hormone-refractory and more aggressive phenotypes of clinical HRPC and to identify molecular targets for the treatment of HRPCs.

Acknowledgments

Received 10/31/2006; revised 3/6/2007; accepted 3/16/2007.

Grant support: Japan Society for the Promotion of Science research grant 18590323 (H. Nakagawa).

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We thank Satomi Uchida and Noriko Ikawa for their technical assistance and other members in our laboratory for their helpful discussions.

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Expression of X-Linked Inhibitor of Apoptosis Protein Is a Strong Predictor of Human Prostate Cancer Recurrence

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Abstract

Purpose: The X-linked inhibitor of apoptosis protein (XIAP) is associated with cell survival by blocking caspase-mediated apoptosis. We examined the expression patterns of XIAP with regard to human prostate cancer, predicting that XIAP status may predict cancer recurrence and/or clinical outcome.

Experimental Design: Immunohistochemistry was done on tissue microarrays constructed from 226 primary prostate cancer specimen. The protein expression distribution was examined across the spectrum of epithelial tissues and its association with standard clinicopathologic covariates and tumor recurrence was examined in 192 outcome-informative patients.

Results: The mean XIAP expression was significantly higher in prostate cancer compared with prostatic intraepithelial neoplasia (PIN), normal, and benign prostatic hyperplasia. We observed that XIAP is an independent predictor of tumor recurrence in multivariate Cox proportional hazards analysis in all patients as well as after substratifying by Gleason score. Interestingly, patients with high XIAP levels had a much lower probability of tumor recurrence than those with lower XIAP expression. Even patients with high-grade tumors who had higher XIAP levels had a lower risk of recurrence compared with any patient whose tumors express lower XIAP.

Conclusions: XIAP is expressed at higher levels in prostate cancers compared with matched normal tissues. High XIAP expression is strongly associated with a reduced risk of tumor recurrence and is not directly associated with Gleason score, tumor stage, capsular involvement, or preoperative prostate-specific antigen status, suggesting that it is a novel prognosticator and a potential target for prostate cancer diagnosis and therapy. Significantly, these findings provide important and extensive validation of previous results.

Prostate cancer is the most frequently diagnosed malignancy and ranks second among all cancers in men, with an estimated 232,090 new diagnoses and 30,350 deaths in the United States in 2005 (1). Most prostate cancers are clinically localized or regional upon diagnosis, and patients enjoy a 5-year survival rate approaching 100%.[9] Nonetheless, as evidence of the slow but steady nature of this disease, 30% to 40% will experience prostate-specific antigen (PSA) recurrence within 10 years following definitive surgery or radiation treatment (2). Patients with high risk or advanced disease on staging workup, or who have recurred, historically receive treatment with exogenous or endogenous androgen ablation, sometimes supplemented with chemotherapy and/or radiation (3). Unfortunately, progression of tumor cells to therapy resistance inevitably ensues, leaving few alternatives to care. As a result, the median survival in advanced disease is only 18 to 20 months, with an overall survival of 24 to 36 months.

Apoptosis (programmed cell death) is an important mechanism in tissue development, homeostasis, and response to stress factors. It relies on a concerted and tightly balanced signaling pathway involving pro- and antiapoptotic proteins. Dysregulation of apoptosis is a major contributor to tumorigenesis (4, 5), tumor growth (6), progression (4), metastases (7), and resistance to conventional therapies (8).

The mitochondrial pathway is activated by physiologic stress, including that induced by conventional cancer therapies, and is activated by p53 after DNA damage, ultimately resulting in increased mitochondrial membrane permeability and release of

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Received 4/23/07; revised 6/20/07; accepted 7/19/07.

Grant support: U.S. Department of Defense/U.S. Army DAMD 17-02-1-0023 (B. Bonavida), a grant from the University of California at Los Angeles Specialized Programs of Research Excellence in Prostate Cancer (B. Bonavida), the Jonsson Comprehensive Cancer Center Shared Resource Core Grant at UCLA NIH NCI 2 P30 CA16042-29 (D. Seligson), and the Early Detection Research Network NCI CA-86366 (L. Goodglick, D. Chia, and D. Seligson).

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doi:10.1158/1078-0432.CCR-07-0960

[9] American Cancer Society, http://www.cancer.org.

a variety of apoptogenic proteins, most notably cytochrome *c*, SMAC/DIABLO, and HtrA2/Omi (9, 10). Cytosolic cytochrome *c* forms an apoptosome complex (11) with procaspase-9 and APAF1, which in turn releases active caspase-9. Like the extrinsic pathway, the intrinsic pathway converges on activation of caspase-3 (12). Tight regulation of caspase activation is required to prevent unchecked cell death. To this end, members of the inhibitors of apoptosis protein (IAP) family provide an intrinsic layer of antiapoptotic regulation. IAPs are an evolutionarily conserved protein family that functions to block cell death by binding to and inhibiting caspases (13, 14).

Eight human IAPs have been reported, namely, X-linked IAP (XIAP), cIAP1, cIAP2, survivin, NAIP, Apollon, Livin, and ILP-2 (15). The X-linked inhibitor of apoptosis, XIAP, is the best characterized of the IAP family members in terms of its potent caspase inhibitory mechanisms and is considered the prototype

Table 1. Relationship of XIAP protein expression with clinicopathologic parameters in prostate adenocarcinomas

	All patients	Mean XIAP expression (SE)	*P* (XIAP: continuous variable)*	Low XIAP intensity ≤1.8 (% of total)	High XIAP intensity >1.8 (% of total)	*P* (XIAP: dichotomized variable)†
Total cases (*N* = 192)		1.28 (0.041)		158 (82)	34 (18)	
Age at surgery						0.26 (NS)*
Median (range)	65 (46-76)			65 (46-76)	63.0 (50-75)	
Mean	63.8			64.0	63.0	
Gleason score			0.99 (NS)			0.31 (NS)
2-6	112 (58)	1.28 (0.055)		89 (56)	23 (68)	
7-10	80 (42)	1.27 (0.063)		69 (44)	11 (32)	
Pathology pT stage‡			0.63 (NS)			0.21 (NS)
PT2-pT3a	158 (82)	1.28 (0.046)		127 (80)	31 (91)	
PT3b	34 (18)	1.24 (0.092)		31 (20)	3 (9)	
Lymph node status (*n* = 190)			0.47 (NS)			>0.99 (NS)
Positive	11 (6)	1.12 (0.202)		9 (6)	2 (6)	
Negative	179 (94)	1.29 (0.042)		147 (94)	32 (94)	
Surgical margins			0.36 (NS)			0.55 (NS)
Positive	62 (32)	1.22 (0.076)		53 (34)	9 (26)	
Negative	130 (68)	1.30 (0.049)		105 (66)	25 (74)	
Capsular involvement			0.016§			0.11 (NS)
No invasion	40 (21)	1.10 (0.094)		34 (21)	6 (18)	
Invasion	113 (59)	1.38 (0.052)		88 (56)	25 (73)	
Extension	39 (20)	1.16 (0.090)		36 (23)	3 (9)	
Organ confined‖			0.15 (NS)			0.15 (NS)
Yes	100 (52)	1.33 (0.058)		78 (49)	22 (65)	
No	92 (48)	1.22 (0.058)		80 (51)	12 (35)	
High risk¶ (*n* = 190)			0.62 (NS)			0.28 (NS)
Yes	38 (20)	1.24 (0.090)		34 (22)	4 (12)	
No	152 (80)	1.29 (0.046)		122 (78)	30 (88)	
PreOpPSA, ng/mL (*n* = 172)						0.80 (NS)*
Median (range)	9.2 (0.6-96.5)			9.8 (0.6-76.0)	8.9 (3.2-96.5)	
Mean	14.0			14.0	14.0	
<10	87 (51)	1.31 (0.063)	0.74 (NS)			0.48 (NS)
≥10	85 (49)	1.31 (0.061)				
Recurrence**			0.082 (NS)††			0.0010††
Yes	69 (36)	1.18 (0.059)		67 (42)	2 (6)	
No	123 (64)	1.33 (0.055)		91 (58)	32 (94)	
Overall follow-up‡‡ (mo)						
Median (range)	78.5 (0.1-182.0)			74.0 (0.1-182.0)	88.5 (6.0-152.0)	
Mean	74.5			72.4	84.2	0.085 (NS)*
Total follow-up§§ (mo)						
Median (range)	48.5 (0.1-163.0)			41.0 (0.1-163.0)	87.0 (6.0-152.0)	
Mean	52.3			46.1	81.3	<0.0001*

**P* value was determined by the Mann-Whitney *U* test unless otherwise specified.
†*P* value was determined by the Pearson χ^2 test with Yates continuity correction unless otherwise specified.
‡pT3b indicates seminal vesicle invasion. There are no pT4 cases.
§*P* value was determined by the Kruskal-Wallis test. With capsular involvement as a continuous variable, *P* = 0.45 using the Spearman correlation corrected for ties.
‖No capsular extension and/or seminal vesicle and/or lymph node involvement. Margins are negative.
¶High-risk seminal vesicle and/or nodal positivity.
**Recurrence PSA elevation raising >0.2 ng/mL status post-radical prostatectomy.
††XIAP mean intensity association with recurrence by logistic regression of continuous data; (*P* = 0.082; 0.63; 95% confidence interval, 0.37-1.06), and of dichotomized data (*P* = 0.0010; 11.78; 95% confidence interval, 2.73-50.88). XIAP expression was the independent variable.
‡‡Overall follow-up time from primary surgery to last PSA follow-up.
§§Total follow-up time to recurrence to last follow-up in nonrecurrence.

of the IAP protein family (14, 16, 17). Abundant XIAP protein expression has been reported in a number of human cancers, including leukemia (18, 19), lymphoma (20), and tumors derived from prostate (4, 7, 21, 22), colon (23), lung (24, 25), cervical (26), bladder (4), hepatocellular (27), and vascular cells (28).

Here, we report that XIAP is elevated in prostate cancer and prostatic intraepithelial neoplasia (PIN) and is an independent predictor of cancer recurrence. Significantly, our results validate and greatly expand upon results by Krajewska et al. (4), showing a similar pattern. This finding provides further evidence that XIAP expression produces a counterintuitive direct association between expression and favorable clinical outcome implicating an as-yet undetermined set of coregulated mechanisms in this disease model. Nonetheless, the strong associations of XIAP expression to prostate cancer recurrence identifies it as a key molecule for targeted therapeutic investigation.

Materials and Methods

Patients. The study cohort consisted of 226 randomly selected hormone-naive patients who underwent radical retropubic prostatectomy between 1984 and 1995 as previously described (29–31). All prostate tumors were staged according to the 1997 American Joint Committee on Cancer tumor-node-metastasis staging system (32) and histologically graded using the Gleason scoring system (33). All cases were of the histologic type "adenocarcinoma, conventional, not otherwise specified" (34). Of the 226, 192 were informative for both recurrence outcomes and marker expression data. Table 1 shows the clinicopathologic data for this cohort.

Prostate tissue microarray construction. Formalin-fixed, paraffin-embedded archival tumor specimens were obtained from the University of California at Los Angeles Department of Pathology under Institutional Review Board approval. Case material was reviewed for tissue array construction by a study pathologist (D.S.). At least three core tissue biopsies (each 0.6 mm in diameter) were taken from morphologically representative regions of each prostate tumor and precisely arrayed as previously described (28–30). Tumor samples were accompanied by matching benign (morphologically normal or hypertrophic) and *in situ* neoplastic lesions (PIN), when available. Case material was arrayed into three tissue microarray (TMA) blocks. For staining, sections (5 μm) were transferred to glass slides using an adhesive slide system (PSA-CS 4, Instrumedics Inc.) to support cohesion of the array elements.

Immunohistochemistry. Immunohistochemical staining was done using an affinity-purified polyclonal rabbit anti-human/mouse XIAP antibody (R&D Systems, Inc.; Immunogen: aa 244-263 of human XIAP). A standard two-step indirect avidin-biotin complex (ABC) method was used (Vector Laboratories) as previously described (29, 30). PC-3 cells were used as a positive staining control for XIAP and were prepared as previously described (29). As a negative assay control, pooled nonimmune rabbit immunoglobulin G was applied at the same concentration as the anti-XIAP antibody.

Scoring of immunohistochemistry. Semiquantitative assessment of antibody on the TMAs was done by a study pathologist (H.Y.) blinded to the clinopathologic variables. The TMA was spot checked by a second pathologist (D.B.S.) for consistency of scoring. The target tissue for scoring was the glandular prostatic epithelium; scoring of benign tissues did not include basal cells. Tissue spot histology and grading were confirmed on the counterstained study slides. XIAP cytoplasmic expression was scored using two measures, intensity on a 0 to 3 scale (0 = negative, 1 = weakly positive, 2 = moderately positive, 3 = strongly positive) and percentage of positively stained target cells (range, 0-100% positive) staining at each intensity. To better represent overall protein levels, we combined the frequency and the intensity measures into an integrated intensity using the following formula: (% staining at intensity 3) × 3] + [(% staining at intensity 2) × 2] + [(% staining at intensity 1) × 1/100. To represent expression within cases, the mean pooled integrated intensity of the invasive tumor spots was used.

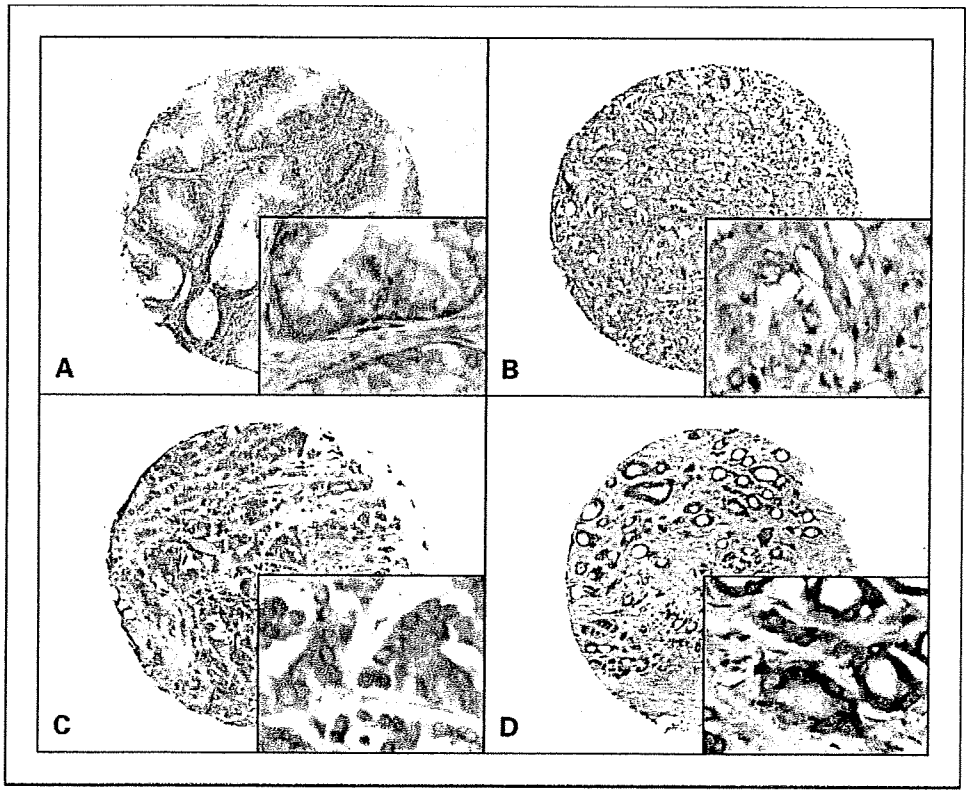

Fig. 1. XIAP protein expression in morphologically normal prostate and prostate cancer on tissue microarrays. Immunohistochemical staining for XIAP protein is seen on representative prostate tissue samples. *A*, normal tissue showing weak cytoplasmic epithelial staining of glandular cells. Staining in basal cells is frequently higher than that seen in glandular cells; scoring is from glandular cells. Invasive prostate cancers are shown demonstrating weak (*B*), moderate (*C*), and strong (*D*) cytoplasmic staining. Magnification, 100×, with 400× inserts.

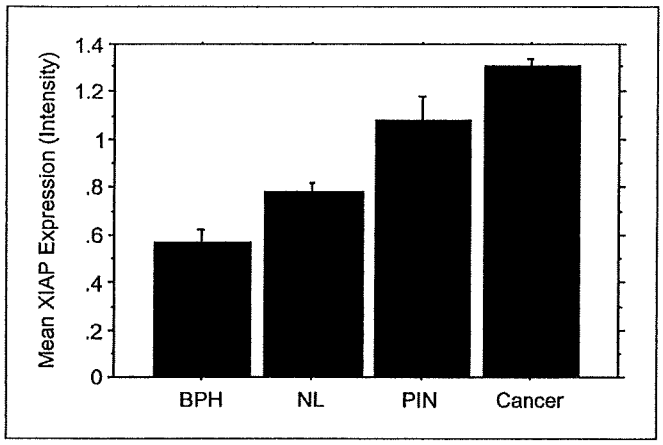

Fig. 2. XIAP protein expression distribution on the prostate tissue microarray stratified by histologic category. The intensity of XIAP protein expression in cells staining by immunohistochemistry as seen in 1,107 informative tissue microarray spots containing benign prostatic hyperplasia (*BPH*; $n = 122$), morphologically normal prostate (*NL*; $n = 252$), prostatic intraepithelial neoplasia (*PIN*; $n = 48$), and invasive prostate cancer (*Cancer*; $n = 685$) are shown as mean bar graphs. The mean XIAP expression was significantly higher in cancer (intensity = 1.32) compared with PIN (intensity = 1.08; $P = 0.019$), normal (intensity = 0.78; $P < 0.0001$), and BPH (intensity = 0.57; $P < 0.0001$). XIAP expression in PIN was significantly higher than normal ($P = 0.010$) and BPH ($P < 0.0001$), and expression in normal epithelium was significantly higher than that seen in BPH ($P = 0.0006$). The Mann-Whitney *U* test was used for two-group comparisons. Bars, 1 SE.

Statistical analysis. The Kruskal-Wallis and Mann-Whitney *U* tests were used to determine the significance of XIAP protein expression differences between categorical clinicopathologic prognostic variables. Associations of XIAP expression with continuous covariates were tested with the Spearman correlation. We used the Pearson χ^2 test to examine the association of dichotomized XIAP expression groups versus categorical variables. Recurrence was defined as a rising total PSA >0.2 ng/mL status post-prostatectomy, and time to recurrence was calculated from the date of the primary surgery. Patients without recurrence at last follow-up were censored. Kaplan-Meier plots were used to visualize recurrence-free time distributions, and the log-rank test was used to test for differences between them. We determined the optimal cut-point for dichotomized XIAP expression data using recursive partitioning, regression trees (rpart package), and plotting log-rank *P* values versus hazard ratios as previously described (35–37). An integrated intensity value of 1.8 gave a maximum hazard ratio and a minimal *P* value.

To assess which covariates associate with recurrence-free time, we fit both univariate and multivariate Cox proportional hazards regression models. The proportional hazards assumption was verified using Schoenfeld residuals (38). All *P* values were two-sided, and $P < 0.05$ was considered significant. All statistical analyses were done using R statistical software[10] and StatView version 5 (SAS Institute Inc.).

Results

XIAP protein expression in human prostate tissues. Using immunohistochemical techniques, we examined XIAP expression in human prostate tissue samples. Expression of XIAP in human prostate tissue was observed in the normal and malignant glandular epithelium, basal cells, and occasionally in stromal fibromuscular cells (Fig. 1). The human prostate cancer cell line, PC3, was used as a positive control for XIAP expression (data not shown). XIAP is typically expressed diffusely in the cytoplasm, but occasionally, discrete supranuclear staining in coarse clusters is additionally seen (Fig. 1).

[10] http://www.r-project.org/

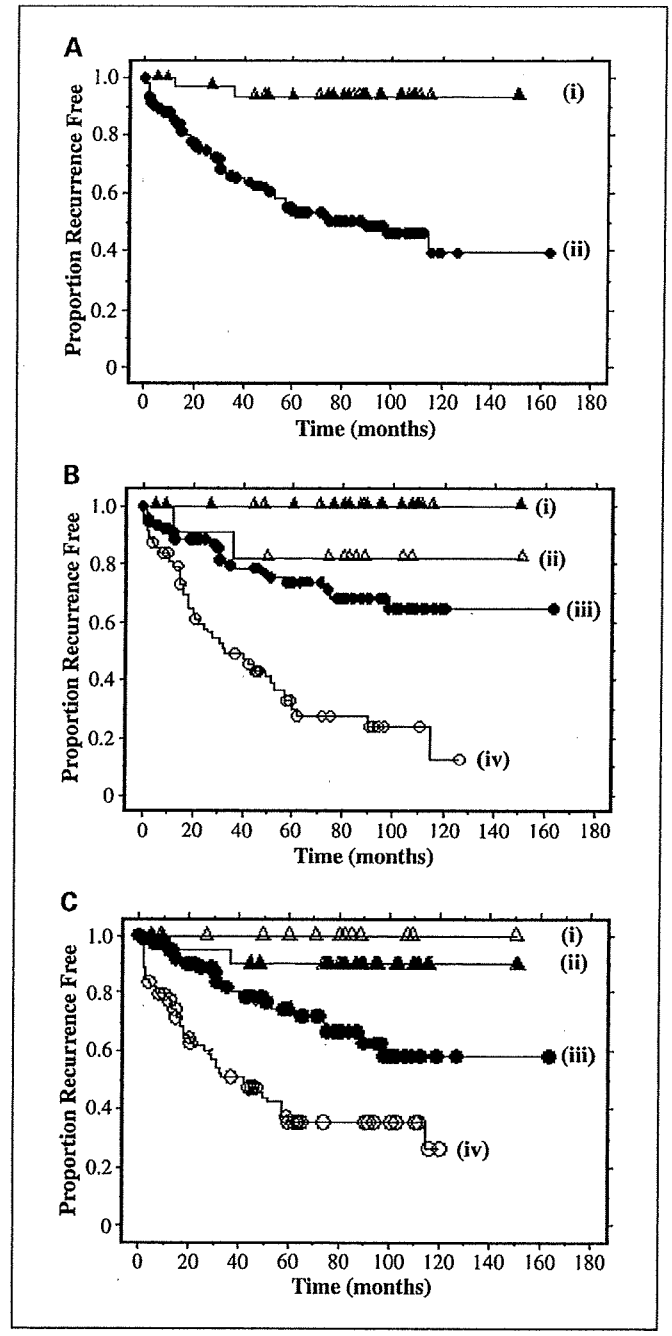

Fig. 3. Kaplan-Meier curves for time to prostate cancer recurrence. The high cytoplasmic XIAP expression phenotype is consistently associated with a lower risk of developing recurrent prostate cancer. For all figures, XIAP expression intensities of >1.8 and ≤1.8 are considered high and low XIAP, respectively. *A,* Kaplan-Meier curves for time to tumor recurrence stratified by cytoplamsic XIAP protein expression status ($n = 192$ patients) are seen in all patients. (*i*) High XIAP expression ($n = 34$); (*ii*) low XIAP expression ($n = 158$). Log-rank $P < 0.0001$. *B,* Kaplan-Meier curves in patients stratified by tumor grade. Gleason scores of 7 to 10 and 2 to 6 are considered high and low grade, respectively. (*i*) High XIAP, low grade ($n = 23$); (*ii*) high XIAP, high grade ($n = 11$); (*iii*) low XIAP, low grade ($n = 89$); (*iv*) low XIAP, high grade ($n = 69$). Log-rank $P < 0.0032$ for (*ii*) versus (*iv*). Log-rank $P < 0.0001$ for (*iii*) versus (*iv*) and for (*i*) versus (*iv*). There is no statistically significant difference between (*ii*) and (*iii*). *C,* Kaplan-Meier curves in patients stratified by their disease being organ confined with negative surgical margins ("confined") versus "not confined" (capsular extension and/or seminal vesicle involvement and/or lymph node involvement). (*i*) High XIAP, not confined ($n = 12$); (*ii*) high XIAP, confined ($n = 22$); (*iii*) low XIAP, confined ($n = 78$); (*iv*) low XIAP, not confined ($n = 80$). Log-rank $P < 0.041$ for (*ii*) versus (*iii*). Log-rank $P < 0.0001$ for (*ii*) versus (*iv*), (*i*) versus (*iv*), and (*iii*) versus (*iv*). For all figures, censored times are marked by either circles or triangles.

Table 2. Cox proportional hazards analysis for time to PSA recurrence

Variable	Univariate* (all patient†, *n* = 192)	Multivariate* (all patients‡, *n* = 172)		Univariate* (low Gleason§, *n* = 112)
		Continuous	Dichotomized	
Gleason score >7	<0.0001 3.70 (2.23-6.11)	0.0011 2.81 (1.51-5.24)	0.0014 2.80 (1.49-5.26)	NA
Seminal vesicle invasion (stage = pT3b)	<0.0001 4.10 (2.47-6.81)	0.0035 2.46 (1.35-4.51)	0.0032 2.46 (1.35-4.47)	0.0065 5.52 (1.61-18.89)
Capsular invasion	0.0038 1.73 (1.19-2.52)	0.019 1.67 (1.09-2.57)	0.036 1.55 (1.03-2.35)	0.014 2.21 (1.17-4.16)
Preoperative PSA	0.015 1.02 (1.00-1.03)††	0.60 1.00 (0.99-1.02)	0.70 1.00 (0.99-1.02)	0.024 1.04 (1.01-1.07)‡‡
XIAP intensity (continuous)‖‖	0.033 1.54 (1.04-2.29)	0.077 1.49 (0.96-2.33)	NA	0.028 2.20 (1.09-4.44)
XIAP intensity ≤1.8 (dichotomized)¶¶	0.0010 10.69 (2.61-43.73)	NA	0.0025 8.92 (2.16-38.86)	**

*P value; hazard ratio; (95% confidence interval) provided.
†64% of cases are censored.
‡67% of cases are censored.
§Gleason score 2 to 6; 79% of cases are censored.
‖Gleason score 2 to 6; 83% of cases are censored.
¶Gleason score 7 to 9 (no Gleason score 10 cases are present); 43% of cases are censored.
**High XIAP group has no events (all patients are censored).
††n = 172.
‡‡n = 103.
§§n = 69.
‖‖Pooled mean XIAP intensity. Used formula (3 − continuous XIAP intensity) to reverse hazard ratio to compare directly to other covariates. A high XIAP carries a reduced risk of recurrence.
¶¶Pooled mean XIAP intensity dichotomized: ≤1.8 (n = 158); >1.8 (n = 34).

Basal cells in normal glands are frequently stained more strongly than the glandular cells. Our scoring of benign epithelium was limited to these glandular cells.

We examined the XIAP expression distribution stratified by histologic category (Fig. 2). Notably, XIAP is elevated in prostate cancer versus matching benign tissues; this increase can be seen starting in PIN lesion. Regions of benign prostatic hyperplasia (BPH) showed the lowest expression. The intensity of XIAP staining are shown in Fig. 2. The mean XIAP expression was significantly higher in cancer (intensity = 1.32) compared with PIN (intensity = 1.08; P = 0.019), normal (intensity = 0.78; P < 0.0001), and BPH (intensity = 0.57; P < 0.0001). In addition, XIAP expression in PIN was significantly higher than normal (P = 0.010) and BPH (P < 0.0001), and expression in normal epithelium was significantly higher than that seen in BPH (P = 0.0006). We found no significant difference in XIAP expression when broken down by tumor grade or Gleason score (data not shown).

XIAP expression and cancer recurrence. We next examined the potential association XIAP protein expression with tumor recurrence following radical prostatectomy. Recurrence data were available for 192 XIAP-informative cases. Case-level expression was derived by pooling the mean integrated intensities of the spots as previously reported (38). Supervised survival tree analysis was applied to pooled data, and a dichotomized population was defined with an optimal cut-point of 1.8 mean integrated intensity representing individuals with higher versus lower XIAP expression. Specifically, an expression intensity of >1.8 was considered "Higher XIAP expression", and ≤1.8 was considered "Lower XIAP expression".

We examined the association of XIAP as either a continuous or dichotomized variable with established prognostic factors and found that expression of XIAP was associated with disease recurrence (Table 1). Figure 3A shows a Kaplan-Meier estimate of cancer recurrence-free time stratified by XIAP expression. Significantly, the median recurrence-free time was 75 months for cases with low XIAP, compared with >152 months for cases with high XIAP (P < 0.0001).

Cox proportional hazards analyses were done for established prognostic factors and time to PSA recurrence (Table 2). Of particular note is the strength of XIAP predictive power as a dichotomized variable, which was higher in all cases than the conventional prognosticators. Higher XIAP expression predicted a reduced risk of tumor recurrence both as a continuous (P = 0.033) and a dichotomized (P = 0.0010) variable in univariate analysis. The dichotomized XIAP remains highly significant in multivariate analysis in this category (P = 0.0025), as well as after substratifying by Gleason score (P = 0.010 for high-grade cases). Significantly, in patients with primary low-grade cancer, no individuals who had high levels of XIAP had tumor recurrence (n = 23). In contrast, 26% of individuals with low-grade cancer who had low levels of XIAP had tumor recurrence (n = 89). Figure 3B shows XIAP expression further substratified by Gleason score, and Fig. 3C shows XIAP expression further substratified by whether or not the tumor is organ confined. Significantly, higher XIAP portends a good outcome regardless of the grade or organ confinement status; patients with higher grade or non-organ-confined tumors with higher XIAP expression do better as a group than any patient whose tumors express

Table 2. Cox proportional hazards analysis for time to PSA recurrence (Cont'd)

Multivariate* (low Gleason‖, *n* = 103)		Univariate* (high Gleason¶, *n* = 80)	Multivariate* (high Gleason¶, *n* = 69)	
Continuous	**Dichotomized**		**Continuous**	**Dichotomized**
NA	NA	NA	NA	NA
0.037	**	0.0086	0.012	0.0089
4.07 (1.09-15.20)		2.21 (1.22-3.98)	2.36 (1.21-4.60)	2.45 (1.25-4.80)
0.0049	**	0.42	0.52	0.69
3.08 (1.41-6.73)		1.23 (0.75-2.04)	1.20 (0.69-2.09)	1.11 (0.66-1.86)
0.011	**	0.95	0.84	0.67
1.04 (1.01-1.08)		1.00 (0.98-1.02)§§	1.00 (0.98-1.02)	1.00 (0.98-1.02)
0.17	NA	0.25	0.19	NA
1.85 (0.77-4.43)		1.33 (0.82-2.17)	1.42 (0.84-2.41)	
NA	**	0.011	NA	0.010
		6.37 (1.54-26.43)		6.61 (1.57-27.89)

low XIAP, even those of low grade or that are organ confined (Fig. 3B and C).

Of note, the high predictive value of XIAP in the specific substrata described above generate subgroups in which 100% of the population was without tumor recurrence (Fig. 3B and C). Because of this, no Cox *P* values can be calculated in these statistical models. However, Table 3 shows how effectively XIAP stratification can isolate low-recurrence groups in all patient substrata examined. For example, in patients whose tumors were not organ confined (n = 92), 50% experienced disease recurrence. However, within this group, none of the 12 patients with high XIAP expression tumors experienced recurrence.

Discussion

The IAP family member XIAP is the strongest direct inhibitor of caspases and is therefore a significant downstream anti-apoptotic protein. Aberrant expression of XIAP has been implicated in the pathology of a number of human cancers; however, few large-scale *ex vivo* studies have been done, and fewer provide translational associations of XIAP expression levels to clinical outcomes.

In support of the role of XIAP as an apoptosis inhibitor, we find that the level of XIAP expression is higher overall in prostate cancer as compared with matched benign tissues, with an intermediate expression observed in PIN. These findings are in agreement with other studies, suggesting that XIAP helps to promote tumor cell survival. Pathologically elevated XIAP levels have been found in a number of hematologic (19, 20, 40–42), vascular (28), and epithelial (4, 23–25, 27) malignancies, as well as in most cell lines of the NCI-60 tumor screening panel (40, 43). Only rare exceptions to this pattern have been reported (26).

We further examined the potential association of XIAP expression with clinicopathologic parameters. Paradoxically, when dichotomized optimally, lower levels of XIAP expression were a strong predictor of recurrence, whereas higher expression strongly predicted a substantially reduced risk of recurrence. In fact, XIAP generated a larger hazard ratio (i.e., stronger predictive power) than those seen from conventional prognostic indicators, including Gleason score, tumor stage capsular invasion, and preoperative PSA. As demonstration of its predictive power, patients with high-grade metastatic tumors and high XIAP had a lower risk of recurrence than patients with low-grade nonmetastatic tumors and with low XIAP. Strikingly, no patients with low-grade tumors plus high XIAP levels had tumor recurrence. In contrast, more than 25% of patients with low XIAP expression experienced recurrences. Despite having a longer overall PSA follow-up, 94% of all patients with high XIAP expression were recurrence-free at the end of follow-up, versus 58% of patients with low XIAP tumors. These findings,

Table 3. Prostate cancer recurrence status in patient groups and substratified by XIAP protein expression category

Patient group	Total count (*n*)	Total % censored*	Low XIAP† % censored (count, *n*)	High XIAP† % censored (count, *n*)
All patients	192	64	58 (158)	94 (34)
Low grade‡	112	79	74 (89)	100 (23)
High grade	80	43	36 (69)	82 (11)
Organ confined§	100	77	73 (78)	91 (22)
Not confined	92	50	42 (80)	100 (12)

*Proportion of patients who reach the end of PSA follow-up without evidence of recurrence. Recurrence = PSA elevation raising >0.2 ng/mL status post–radical prostatectomy.
†Pooled mean XIAP intensity dichotomized: low ≤1.8; high >1.8 on a 0 to 3 scale.
‡Low grade = Gleason score of 2 to 6; high grade = Gleason score of 7 to 9 (there are no cases of Gleason 10 in this cohort).
§Organ confined = no capsular extension and/or seminal vesicle and/or lymph node involvement. Margins are negative.

coupled to the lack of direct association with any of the clinicopathologic variables tested, shows the independence XIAP and its widespread applicability as a prognostic indicator.

Our current study confirms the work of Krajewska et al., who also found that high levels of XIAP were associated with a reduced risk of recurrence in prostate cancer patients (4). The importance of independent validation for tumor biomarkers cannot be overemphasized because such verification is an absolute requirement for differentiating biomarkers, which have the potential to be meaningful clinical predictors from those that demonstrate merely idiosyncratic expression (44–46). In addition, such validation studies are also critical to minimize overfitting of statistical data. Therefore, that the predictive power of XIAP was observed in two separate and independent patient populations is highly significant.

The results shown here not only validate the findings of Krajewska et al., but it also extends their work (4). To our knowledge, our study is the largest study to date examining the association of XIAP protein to clinical outcomes. Moreover, the patient cohort for clinical outcomes in the aforementioned study (4) consisted of needle core biopsies from 64 $T_2N_0M_0$ radiation-treated patients. Here, we provide an expanded and unrelated patient population on tissue microarrays to include 192 informative patients with a spectrum of disease stages. The only other major difference between the two studies is that our results suggest that XIAP is an independent predictor of outcome, whereas Krajewska et al. found a significant inverse correlation of XIAP with preoperative PSA level; they offered this as a potential link to the positive outcome seen in high XIAP-expressing patients.

XIAP expression in other malignancies. The association of high XIAP expression with a positive clinical outcome is counterintuitive to expectations that IAPs promote tumor cell survival. Nevertheless, some recent studies of lung cancer have shown that increased levels of XIAP are associated with an improved prognosis (4, 47). For example, Ferriera et al. (47) found that higher levels of XIAP correlated with longer survival in early-stage non–small cell lung cancer (NSCLC) patients. Surprisingly, the same group found that XIAP was not associated with survival in advanced NSCLC (24).

Conversely, several studies have shown a negative association of XIAP levels to outcomes (cancer recurrence/remission and/or death) in other types malignancies. For example, XIAP expression was found in 95% of clear cell renal cell carcinomas (48). A significant increase was observed from well to poorly differentiated tumors. Tamm et al. and Carter et al. (18, 40–42) found that in patients with acute myelogenous leukemia, higher levels of XIAP correlated with a slightly shorter remission durations and a decreased survival time. Several other studies failed to find associations between XIAP levels and survival, including those focusing on colon (23), cervical (26), and bladder cancers (4); the latter two studies also noted a lack of association of XIAP with tumor grade and stage.

Potential mechanism of action. The observation that XIAP is elevated in primary prostate tumor cells, yet also high levels ultimately predict a lower probability of tumor recurrence, is intriguing. There are a number of possible explanations for these observations. XIAP has been reported to mediate cell cycle arrest via down-regulation of cyclins A and D1 and induction of cyclin-dependent kinase inhibitors p21Cip1/Waf1 and p27Kip1 (28). Thus, although XIAP may provide a selective antiapoptotic survival advantage, it may simultaneously impair the proliferation of cancer cells. It is possible that these two properties function with some degree of independence.

XIAP is itself regulated by antagonists such as SMAC/DIABLO, which is released from the mitochondria upon apototic stimuli (12, 49–51). Recent studies have shown that the relative proportion of XIAP compared with SMAC/DIABLO is the factor that dictates life versus death decisions. Therefore, it is possible that the high levels of XIAP expression seen in our study are counteracted by higher levels of anti-IAPs. We are currently exploring this possibility.

Finally, as is the case with all studies involving immunohistochemistry on archival paraffin-embedded sections, the overall activity of XIAP cannot be assessed. Whether XIAP functions differently in a progressing tumor cell and/or interacts with alternate target molecules in an evolving malignant cell is an intriguing possibility that warrants further study.

Malignant prostate cancer remains a disease with few useful outcome measures and no current consistently effective therapies. Therefore, informative biomarkers are urgently needed to guide patient surveillance and clinical intervention. This study reports the overexpression of XIAP in primary human prostate cancers and provides strong evidence for its beneficial prognostic association.

Acknowledgments

We thank Stephanie Hana and Greg Kanter for technical assistance and Rebecca Schatz for constructive comments.

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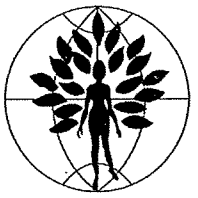

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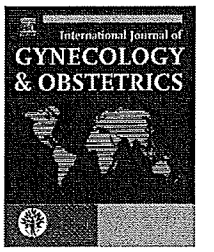

CLINICAL ARTICLE

Surgical treatment for neuroendocrine carcinoma of the uterine cervix

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Received 5 May 2007; received in revised form 6 June 2007; accepted 7 June 2007

KEYWORDS
Neuroendocrine carcinoma;
Radical hysterectomy;
Recurrent sites;
Uterine cervix

Abstract

Objective: To identify the best operative approach for neuroendocrine cervical carcinoma (NECC). *Methods:* The records of surgically treated patients with stages IB to IIB NECC were reviewed. *Results:* Of 10 patients who met the study criteria for NECC and underwent radical hysterectomy, 4 had pT1bN0, 4 had pT1bN1, 1 had pT2aN0, and 1 had pT2bN1 disease. Those with pT1bN1 or pT2bN1 disease received postoperative adjuvant radiotherapy and/or chemotherapy, and recurrence occurred in 7 patients (70%). Among these 7 patients, 5 (71%) had a primary NECC tumor with deep stromal invasion and 5 (71%) had extrauterine disease (parametrium and/or lymph node). The recurrences in 6 patients (86%) were located outside the pelvis (lung, liver, or brain). Stromal invasion was 6 mm or less in the 3 patients who did not experience disease recurrence. *Conclusions:* Pelvic control by radical hysterectomy may not be beneficial for patients with NECC except for those with an early invasive lesion.

1. Introduction

Neuroendocrine carcinoma arising from the uterine cervix is an uncommon malignancy comprising less than 5% of all cervical malignancies [1]. Histopathologically, neuroendocrine cervical carcinoma (NECC) resembles small cell carcinoma of the lung and is classified as small cell carcinoma of the cervix in the World Health Organization International Histologic Classification of Tumors. It is noted for its very aggressive behavior and has the poorest prognosis of the various cervical carcinomas, even after multimodal therapy. In a recent study, the 5-year survival rate of patients with International Federation of Gynecologists and Obstetricians (FIGO) stage IB1 disease was between 50% and 60%, which was significantly poorer than the 90% rate for patients with stage IB1 squamous cell carcinoma [2]. In that study, none of the patients whose disease was more extensive than stage IB1 or who had clinical evidence of lymph node metastasis survived

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0020-7292/$ - see front matter
doi:10.1016/j.ijgo.2007.06.051

their disease. It has been suggested that the poor outcome in patients with NECC is due to early and frequent metastasis.

Owing to the rarity of NECC, no multicenter study has been conducted on the disease and the optimal initial therapeutic approach has not been clarified. On the other hand, in patients with stages IB to II cervical carcinoma of the ordinary histologic type, radical hysterectomy followed by adjuvant pelvic radiation is the standard surgical approach. Radical hysterectomy has also been employed for the treatment of NECC. The present retrospective study was carried out to assess the efficacy of surgical treatment for NECC; establish a framework for designing new therapeutic strategies; and improve prognosis.

2. Patients and methods

The medical records and pathologic materials of 2096 patients with cervical carcinoma who were treated at the Gynecology Division and Diagnostic Pathology Division of the National Cancer Center Hospital in Tokyo, Japan, between 1980 and 2004 were reviewed. The study criteria were the following: having a lesion that fulfilled the histologic criteria for neuroendocrine carcinoma according to the WHO International Histologic Classification of Tumors and the Armed Forces Institute of Pathology; having stage IB to IIB disease; and having undergone primary radical hysterectomy. Patients in whom only a portion of the tumor showed neuroendocrine features were excluded. The biopsy materials were immunohistochemically stained for keratin, carcinoembryonic antigen, chromogranin, synaptophysin, and neuron-specific enolase (NSE). For this study, a gynecologic pathologist re-examined all hysterectomy materials. All patients were staged according to the 1994 FIGO staging system. Those treated before 1994 were retrospectively restaged based on their clinical records and pathologic findings.

Every 2 to 6 months, the patients found to be asymptomatic after the primary radical hysterectomy underwent a pelvic examination and a chest radiograph, and had a cervical smear taken and tumor markers measured (chiefly, NSE). Symptomatic patients then underwent appropriate examinations by ultrasound, computed tomography, and/or magnetic resonance imaging. Follow-up continued through March 2006. Survival curves were obtained by the Kaplan–Meier method.

3. Results

Among the 2096 patients with cervical carcinoma, 10 met the study criteria and were diagnosed as having pure NECC. Their median age was 41 years (range, 28–61 years) and median follow-up time (or time to death) was 25 months (range, 8–204 months). No patient was lost to follow-up. Eight patients had FIGO stage IB1 disease, one had stage IB2, and one had stage IIB. Table 1 shows the clinical characteristics of the 10 patients. All underwent radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy. All tumors were completely removed. Adjuvant radiotherapy or chemotherapy was administered to the 5 patients in whom lymph node metastasis or parametrial invasion was diagnosed from the surgically resected materials. Two of these 5 patients received radiotherapy to the whole pelvis and the para-aortic field, for a total dose of 45 to 50 Gy; 1 received radiotherapy to the whole pelvis alone; and the remaining 2 were treated with a chemotherapy regimen (one was treated with a combination of cisplatin, doxorubicin, and cyclophosphamide and the other with cisplatin and etoposide).

Eight patients had stage pT1b disease and 2 patients had stage pT2 disease. Lymph node metastasis was found in 4 (50%) and lymph-vascular space invasion was found in 7 (88%) of the 8 patients found from the surgically resected materials to have stage pT1b disease. The primary lesions of the 2 patients with stage pT2 disease showed lymphovascular invasion.

Disease recurred in 7 patients (70%) at a median interval of 8 months following initial surgery (range, 4–26 months). Of these 7 patients, 6 (86%) died at a median interval of 16 months after the onset of recurrence despite aggressive multimodal therapy (systemic chemotherapy, radiation, and surgery). For the 10 patients, the cumulative 5-year survival rate was 43% and the median survival time was 29 months. The disease-free survival rate was 50% at 24 months and 30% at 36 months.

In the 3 patients in whom disease did not recur, the primary tumor was an early invasive lesion of 6 mm or less (3, 5, and 6 mm, respectively). In the 7 patients in whom disease recurred, it was a deeply invasive lesion (median, 19 mm [range, 6–40 mm]). Pelvic lymph node metastasis was found in the surgically resected materials of 1 (33%) of the 3 patients with no recurrence and in 4 (57%) of the 7 patients with recurrence.

Table 1 Clinical characteristics of the patients with NECC and their status

Patient no.	Postsurgical stage	Tumor size, mm		Adjuvant therapy	Initial failure sites	Status (no. of months)
		Depth	Length			
1	pT1b1N0	3	14	None	NA	NED (29)
2	pT1b1N0	5	40	None	NA	NED (122)
3	pT1b1N0	15	28	None	Liver, lung	DOD (65)
4	pT1b1N0	18	29	None	Pelvic wall	DOD (18)
5	pT1b1N1	6	20	Radiotherapy	Liver, lung	DOD (29)
6	pT1b1N1	6	38	Chemotherapy	NA	NED (204)
7	pT1b1N1	20	24	Radiotherapy	Liver	DOD (8)
8	pT1b2N1	40	80	Radiotherapy	Liver, lung	DOD (22)
9	pT2aN0	10	20	None	Pelvic wall, PALN	DOD (22)
10	pT2bN1	25	50	Chemotherapy	Pelvic wall, Brain	AWD (21)

Abbreviations: AWD, alive with disease; DOD, dead of disease; NA, not applicable; NED, no evidence of disease; PALN, para-aortic lymph node.

The initial recurrence sites were located outside the pelvis in 6 (86%) of these 7 patients and in the pelvic sidewall of the remaining patient. In the 9 patients with distant metastasis the most frequent site was the liver (in 4 patients [44%]), followed by the lung (in 3 [33%]), the brain (in 1 [11%]), and para-aortic lymph nodes (in 1 [11%]).

4. Discussion

NECC has the poorest prognosis of the various cervical carcinomas owing to early and frequent metastasis. Because of the rarity of the disease, no large-scale multicenter study has been performed and the optimal initial therapeutic approach to NECC has not been determined. Radical hysterectomy, which is the standard surgical procedure for stages IB to II cervical carcinoma of the ordinary type, has been adopted for the treatment of NECC. Sevin et al. [3] reported a 5-year survival rate of 36.5% for patients with stages IB to IIA NECC who underwent radical hysterectomy followed by adjuvant chemotherapy, compared with 71.6% for patients with cervical carcinoma of other histologic subtypes. At our institute, the cumulative 5-year survival rate was 43% for patients with stages IB to IIB NECC, whereas it was 84%, 78%, and 65%, respectively, for stages IB, IIA, and IIB cervical carcinoma of the ordinary type [4]. The traditional surgical approach therefore does not appear to be effective in patients with NECC.

Using as search words *small cell carcinoma* and *uterine cervix* as well as *neuroendocrine carcinoma*, we conducted a Medline search of the articles on NECC published in English from January 1976 to July 2006, selecting those reporting on more than 5 patients and specifying both sites of recurrence and outcomes. This literature provided information on a total of 49 patients, including our own, who underwent radical hysterectomy for stages IB to IIB disease. The clinical

Table 2 Outcome and patterns of recurrence in patients with neuroendocrine cervical carcinoma who underwent radical hysterectomy

Author	No. of patients	Adjuvant therapy (no. of patients)	No. of recurrent sites	Status (no. of patients)
Perrin and Ward [12]				
IB	4	Chemoradiotherapy (n=4)	Locoregional (n=2)	NED (n=1)
IIA	1	None (n=1)	Lung (n=2)	DOD within 12 months (n=4)
			Liver (n=1)	
			Brain (n=1)	
			Thoracic spine (n=1)	
Chang et al. [13]				
IB	19	Chemotherapy (n=23)	Locoregional (n=5)	NED (n=13)
			Lung (n=5)	DOD within 10 months of recurrence
II	4		Liver (n=5)	(n=10)
			Brain (n=3)	
			Distant node (n=3)	
			Bone (n=2)	
			Kidney (n=2)	
			Breast (n=1)	
			Spleen (n=1)	
			Adrenal gland (n=1)	
Viswanathan et al. [2]				
IB	6	Chemotherapy (n=4)	Locoregional (n=2)	NED (4)
		None (n=2)	Distant node (n=1)	DOD (2)
			Liver (n=1)	
			Bone (n=1)	
			Breast (n=1)	
Tsunoda et al. [14]				
IB	3	Chemotherapy (n=2)	Locoregional (n=1)	NED (n=2)
IIB	2	Radiotherapy (n=2)	Lung (n=1)	DOD within 16 months (n=3)
		None (n=1)	Liver (n=1)	
			Brain (n=1)	
			Kidney (n=1)	
Present study				
pT1b	8	Chemotherapy (n=2)	Locoregional (n=3)	NED (n=3)
pT2	2	Radiotherapy (n=3)	Liver (n=4)	AWD (n=1)
		None (n=5)	Lung (n=3)	DOD within 65 months (n=6)
			Distant node (n=1)	
			Brain (n=1)	

Abbreviations: AWD, alive with disease; DOD, dead of disease; NED, no evidence of disease.

characteristics of these 49 patients are summarized in Table 2. Forty patients (82%) had stage IB or pT1b disease; 31 (63%) received adjuvant chemotherapy; 9 (18%) received radiotherapy or chemoradiotherapy; and the remaining 10 (20%) did not receive adjuvant therapy. Fourteen patients were treated with combination chemotherapy using vincristin, doxorubicin, and cyclophosphamide alternating with cisplatin and etoposide; 8 were treated with cisplatin, vinblastin, and bleomycin; 3 were treated with cisplatin and etoposide; and 3 were treated with cisplatin, doxorubicin, and etopocide. Recurrence occurred in 26 patients (53%) and 25 patients (51%) died of the disease. Among those in whom disease recurred, 23 (88%) had extrapelvic metastasis. Of the 45 distant sites of recurrence, the most frequently reported were the lung (27%) and liver (27%), followed by a distant node (11%), and the brain (13%). Based on these findings, the development of widespread hematogenous metastasis is the most important pattern in NECC, and controlling hematogenous spreading should be a top priority in the attempt to improve the survival of patients with this type of cervical carcinoma.

In comparison, the prognosis for patients with cervical squamous cell carcinoma who are treated with radical hysterectomy is good. Recurrence develops in 10% to 15% of patients with stages IB or IIA disease who undergo radical hysterectomy, with or without postoperative radiation of the whole pelvis [5]. Following radical hysterectomy, the difference in outcome among patients with squamous cell carcinoma and those with NECC may be due to differences in the biologic behavior of the carcinomas. In patients with NECC, pelvic control alone usually does not lead to a good outcome because of the high incidence of distant metastasis in the early stage.

Lymphedema and bladder dysfunction develop in almost all patients who undergo radical hysterectomy [6]. Morbidities associated with radical hysterectomy include chronic bladder dysfunction (in 3% of patients), ureterovaginal or vesicovaginal fistula (in 1%–2%), lymphocele formation (in 5%), small bowel obstruction (in 1%), pulmonary embolism (in 1%–2%), injury to the obturator or genitofemoral nerve, and blood loss requiring transfusion [7]. These complications may interfere with systemic postoperative adjuvant therapy for the control of distant metastasis. Thus, radical hysterectomy does not appear to be beneficial in patients with NECC, and indications for this treatment should be limited.

In the present study, the stromal invasion of the primary tumor was 6 mm or less in patients who did not experience recurrence and a median of 19 mm in those who experienced recurrence. The incidence of pelvic lymph node metastasis was higher among patients who experienced recurrence than in those who did not. And 2 series of meta-analyses demonstrated that the presence of lymph node metastasis was the most important factor for a poor prognosis [8,9]. Viswanathan et al. [2] reported that none of their patients with clinical evidence of lymph node metastasis survived their disease. In patients with NECC, radical hysterectomy may be indicated only in cases of early invasive lesion with no lymph node metastasis.

Theoretically, to reduce the incidence of widespread distant metastasis after hysterectomy, adjuvant systemic chemotherapy is indicated. As no large-scale, multicenter study has been conducted with patients diagnosed as having NECC, no optimal regimen has been established for treating the disease. A regimen originally developed for the treatment of small cell carcinoma of the lung, which includes cisplatin and etoposide, has been tried. Although several studies have suggested the combination of cisplatin and etoposide to be beneficial, they reported on small numbers of patients [8,10,11]. Multicenter randomized controlled trials are needed.

In conclusion, pelvic control by radical hysterectomy does not appear to be generally beneficial for patients with NECC, and it should be limited to those with an early invasive lesion without obvious lymph node metastasis. Rather, nonradical hysterectomy followed by new, aggressive adjuvant chemotherapy may be considered following surgery.

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The American Journal of Surgery®

The American Journal of Surgery 191 (2006) 225–229
Scientific papers–International

Sentinel lymph node biopsy examination for breast cancer patients with clinically negative axillary lymph nodes after neoadjuvant chemotherapy

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Manuscript received February 10, 2005; revised manuscript June 22, 2005

Abstract

Background: The feasibility and accuracy of sentinel lymph node (SLN) biopsy examination for breast cancer patients with clinically node-negative breast cancer after neoadjuvant chemotherapy (NAC) have been investigated under the administration of a radiocolloid imaging agent injected intradermally over a tumor. In addition, conditions that may affect SLN biopsy detection and false-negative rates with respect to clinical tumor response and clinical nodal status before NAC were analyzed.

Methods: Seventy-seven patients with stages II and III breast cancer previously treated with NAC were enrolled in the study. All patients were clinically node negative after NAC. The patients then underwent SLN biopsy examination, which involved a combination of intradermal injection over the tumor of radiocolloid and a subareolar injection of blue dye. This was followed by standard level I/II axillary lymph node dissection.

Results: The SLN could be identified in 72 of 77 patients (identification rate, 93.5%). In 69 of 72 patients (95.8%) the SLN accurately predicted the axillary status. Three patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 11.1% (3 of 27). The SLN identification rate tended to be higher, although not statistically significantly, among patients who had clinically negative axillary lymph nodes before NAC (97.6%; 41 of 42). This is in comparison with patients who had a positive axillary lymph node before NAC (88.6%; 31 of 35).

Conclusions: The SLN identification rate and false-negative rate were similar to those in nonneoadjuvant studies. The SLN biopsy examination accurately predicted metastatic disease in the axilla of patients with tumor response after NAC and clinical nodal status before NAC. This diagnostic technique, using an intradermal injection of radiocolloid, may provide treatment guidance for patients after NAC.

Keywords: Sentinel node biopsy; Neoadjuvant chemotherapy; Clinically node negative; Intradermal injection

Currently, the status of the axillary lymph nodes remains the most important prognostic indicator for breast cancer and helps the physician in guiding adjuvant therapy. More than 40 peer-reviewed pilot studies published between 1993 and 1999 have established the validity of sentinel lymph node (SLN) biopsy examination technique for clinically node-negative breast cancer [1], and the SLN biopsy procedure has become the standard of care for axillary staging in these patients.

Recent studies report identification rates of more than 90%, with false-negative rates ranging from 2% to 10% [2,3]. To ensure a high SLN identification rate and a low false-negative rate, some relative contraindications for SLN biopsy examination have been established: these include T3 or T4 tumors, multicentric or multifocal lesions, a large biopsy cavity, previous axillary surgery, previous chest-wall irradiation, and neoadjuvant chemotherapy (NAC) [4,5].

The application of SLN biopsy examination in NAC-treated patients may, as in nonneoadjuvant chemotherapy groups, identify patients who do not necessarily require an axillary lymph node dissection (ALND). Several studies

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0002-9610/06/$ – see front matter
doi:10.1016/j.amjsurg.2005.06.049

Table 1
Patient demographics

	Number of patients
Age, y	
Mean	51.1
Range	27–75
Clinical tumor size, cm*	
Mean	4.82
Range	2.7–12
Tumor classification*	
T2	50 (65.0%)
T3	24 (31.2%)
T4	3 (3.8%)
Lymph node status*	
N0	42 (54.5%)
N1	28 (36.4%)
N2	7 (9.1%)
Tumor type	
Invasive ductal	74 (96.1%)
Invasive lobular	3 (3.9%)
Type of NAC	
FEC plus paclitaxel	73 (94.9%)
Paclitaxel alone	4 (5.1%)
Clinical response of the tumor	
CR	41 (53.2%)
PR	28 (36.4%)
SD	8 (10.4%)
Pathologic response of the tumor	
pCR	17 (22.1%)
pINV	60 (77.9%)
Pathologic nodal status	
Negative	47 (61.0%)
Positive	30 (39.0%)

CR = complete response; FEC = fluorouracil/epirubicin/cyclophosphamide; PR = partial response; SD = stable disease; pCR = pathologic complete response; pINV = pathologic invasive.
* Before NAC.

have evaluated the use of SLN biopsy examination in patients with breast cancer after NAC but results are varied and inconclusive [6–14].

Recently, several studies have shown the feasibility and accuracy of SLN biopsy examination using peritumoral injection of radiocolloid for patients with NAC-treated breast cancer. However, false-negative rates varied considerably among these studies [6–13]. It is possible that tumor response to chemotherapy may alter or interrupt the lymphatic drainage, thus causing the lower SLN identification rates and higher false-negative rates as opposed to nonneoadjuvant studies. Our hypothesis is that the lymphatic flow within the skin lesion overlying the tumor is less damaged by the chemotherapy than that in the parenchyma surrounding the tumor, except in T4 tumors. Thus, the usefulness of SLN biopsy examination with intradermal injection of radiocolloid for patients with NAC-treated breast cancer has yet to be established.

The aim of this study was to determine the feasibility and accuracy of the SLN biopsy procedure using intradermal injection of radiocolloid over the tumor in clinically node-negative NAC-treated breast cancer patients.

Methods

Between May 2003 and January 2005, 77 patients with T2-4N0-2 breast cancer underwent NAC with SLN biopsy examination plus ALND performed by a single surgeon. The pathologic diagnosis was established by core needle biopsy examination in all patients.

Patients younger than 65 years of age received 4 cycles of 5-fluorouracil (500 mg/m^2)/epirubicin (100 mg/m^2)/cyclophosphamide (500 mg/m^2) plus 12 weekly cycles of paclitaxel (80 mg/m^2), and patients older than 65 years of age received 12 weekly cycles of paclitaxel (80 mg/m^2) alone. After NAC, we enrolled the 77 clinically node-negative patients in this study.

Lymphatic mapping was performed using a 3-mL combination of blue dye (Patent blue V; TOC Ltd, Tokyo, Japan) and 30 to 80 MBq of technetium-99m–labeled Phytate (Daiichi RI Laboratory, Ltd, Tokyo, Japan). The day before surgery, the radiotracer was injected intradermally into the area overlying the tumor, and blue dye was injected into the subareolar site intraoperatively. For nonpalpable lesions, injections were performed under mammographic or ultrasonic needle localization. Sentinel lymph nodes were identified as being stained blue, radioactive, or both. The SLN biopsy procedure then was followed by a standard level I/II ALND.

All sentinel nodes were evaluated histologically by submitting each node as a 3-mm to 5-mm serial section stained with hematoxylin-eosin. Lymph nodes submitted as part of the axillary dissection were totally submitted and evaluated using standard hematoxylin-eosin staining.

Results

Patient characteristics, type of chemotherapy, clinical response of the tumor, and pathologic findings are summarized in Table 1. All patients underwent breast-conserving therapy or mastectomy and were clinically node negative at the time of surgery.

As shown in Table 2, the overall SLN identification rate was 93.5% (72 of 77). Of the 72 patients in whom an SLN could be identified, 24 (33.3%) had positive SLNs. Within

Table 2
Results of sentinel node biopsy examination

	Number of patients
Total number of patients	77
SLN identified	72 (93.5%)
SLN positive	24 (33.3%)
SLN was only positive lymph node	11 (45.8%)
SLN identification method	
Radiocolloid and blue dye	53 (73.6%)
Radiocolloid only	11 (14.3%)
Blue dye only	8 (11.1%)

Table 3
Comparison of lymph node status of SLNs and non-SLNs

SLN status	Non-SLN status	
	Positive	Negative
Positive	13	11
Negative	3	45

False-negative rate = 11.1%.

11 of these patients (45.8%), the SLN was the only positive node. SLNs were identified by both radiocolloid and blue dye in 53 patients (73.6%), by radiocolloid alone in 11 patients (14.3%), and by blue dye alone in 8 patients (11.1%).

The pathologic status of the SLNs and non SLNs is shown in Table 3.

The SLNs accurately predicted the axillary status in 69 of 72 patients (95.8%). Three patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 11.1% (3 of 27). Forty-five patients had pathologically negative SLNs and non-SLNs.

The pathologic status of the SLNs and non-SLNs were analyzed according to tumor classifications before NAC, clinical lymph node status before NAC, and response of the tumor after NAC, respectively.

In T2 tumors before NAC, the SLN identification rate was 94% (47 of 50), and 2 patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 14.3%. In T3 and T4 tumors, results were 92.6% (25 of 27) and 7.7% (2 of 27), respectively (Table 4). For the results of SLN biopsy examination, there was no significant difference between T2 and T3/T4 tumors before NAC.

In the patients with clinically negative lymph nodes (N0) before NAC, the SLN identification rate was 97.6% (41 of 42), and 1 patient had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 10%. In the patients with clinically positive lymph nodes (N1/N2), the results were 88.6% (31 of 35) and 11.2% (4 of 35), respectively (Table 5). The SLN identification rate tended to be higher, although not statistically significantly, among patients who had clinically negative lymph nodes before NAC compared with patients who had positive axillary lymph nodes before NAC.

Table 4
Comparison of lymph node status of SLNs and non-SLNs among tumor classifications before NAC

SLN status	Non-SLN status			
	T2 (n = 50)		T3/T4 (n = 27)	
	Positive	Negative	Positive	Negative
Positive	6	6	7	5
Negative	2	33	1	12
Total number of SLNs identified	47 (94%)		25 (92.6%)	
False-negative rate	14.3%		7.7%	

Table 5
Comparison of lymph node status of SLNs and non-SLNs among nodal status before NAC

SLN status	Non-SLN status			
	N0 (n = 42)		N1/N2 (n = 35)	
	Positive	Negative	Positive	Negative
Positive	3	6	10	5
Negative	1	31	2	14
Total number of SLNs identified	41 (97.6%)		31 (88.6%)	
False-negative rate	10%		11.2%	

For patients with complete tumor response after NAC, the SLN identification rate was 92.0% (37 of 41), with 1 patient having a false-negative SLN biopsy examination result, resulting in a false-negative rate of 12.5%. For patients with a partial tumor response and stable disease, the results were 97.2% (35 of 36) and 10.5% (1 of 36), respectively (Table 6). The SLN identification rate tended to be lower, although not statistically significantly, among patients with complete tumor response after NAC, compared with partial tumor response and patients with stable disease after NAC.

There was no significant difference in the false-negative rate according to tumor classifications before NAC, clinical lymph node status before NAC, and response of the tumor after NAC.

Comments

ALND is the surgical standard for treatment of the axilla in breast cancer patients. The rationales for ALND are exact staging and prognosis, regional control of the axilla, and the possibility of improved survival. The extent of axillary lymph node involvement is one of the most important independent prognostic factors for recurrence and survival. The SLN biopsy procedure is an accurate minimally invasive method for axillary staging in early breast cancers. In many clinics the SLN biopsy examination is replacing standard ALND because of minimal morbidity. However, with the increasing size of tumors, lymphatic mapping becomes

Table 6
Comparison of lymph node status of SLNs and non-SLNs among clinical response after NAC

SLN status	Non-SLN status			
	CR (n = 41)		PR/SD (n = 36)	
	Positive	Negative	Positive	Negative
Positive	3	4	10	7
Negative	1	29	2	16
Total number of SLNs identified	37 (90.2%)		35 (97.2%)	
False-negative rate	12.5%		10.5%	

Table 7
Studies of SLN biopsy procedures after NAC

	Number of patients	Stage	Tumor size, cm	Number (%) of successful SLN biopsy procedures	False negative (%)
Breslin et al [6], 2000	51	II or III	5.0	43 (84.3)	3 (12)
Miller et al [7], 2002	35	T1-3N0	3.5	30 (86.0)	0 (0)
Stearns et al [8], 2000	34	T3-4, any N	5.0	29 (85.0)	3 (14)
Haid et al [9], 2001	33	T1-3, any N	3.3	29 (88.0)	0 (0)
Julian et al [11], 2002	31	I or II	NS	29 (93.5)	0 (0)
Tafra et al [12], 2001	29	Any T, N0	NS	27 (93.0)	0 (0)
Nason et al [13], 2000	15	T2-4, N0	NS	13 (87.0)	3 (33)
Shimazu et al [14], 2004	47	II or III	4.5	44 (93.6)	4 (12)
Current study	77	T2-4, any N	4.8	72 (93.5)	3 (11)

NS = not specified.

less accurate [15,16]. NAC can reduce tumor size and significantly increase the ability to perform breast-conserving therapy [17,18]. After NAC, axillary downstaging is affected similarly. NAC with anthracycline/cyclophosphamide-containing regimens has been shown to neutralize involved axillary nodes in about 30% of patients [17]. The addition of taxanes to anthracycline/cyclophosphamide-containing regimens has increased the conversion rate to around 40% [19,20]. With the increasing number of patients receiving NAC, the question arises of whether the SLN biopsy examination is an option for these patients. We summarized the studies concerning SLN biopsy examination after NAC in Table 7, but they are inconclusive [6–14]. Breslin et al [6] reported a study of 51 patients who underwent an SLN biopsy examination after NAC and concluded that an SLN biopsy examination is accurate after NAC. They had an identification rate of 84.3% and a false-negative rate of 12.0%. Nason et al [13] reported on a smaller number of patients who received NAC. Their identification rate was 87.0% and their false-negative rate was 33.3%, concluding that the SLN biopsy examination resulted in an unacceptably high false-positive rate. We have to understand that in most of these small series, even 1 or 2 patients with a false-negative SLN node can sway the conclusions in a different direction. We report a study of 77 patients who received NAC, and had an identification rate of 93.5% and a false-negative rate of 11.1%. We conclude in our study that an SLN biopsy examination after NAC is accurate even for large tumors and positive axillary nodal status before NAC without inflammatory breast cancer.

It has been speculated that among patients who have their axillary lymph node status downstaged by NAC, tumors also typically respond to NAC and shrink, so that damage to and alteration of the lymphatic flow from tumor tissues to the axillary basin are more likely to occur. This may cause an increase in the false-negative rate for SLN biopsy examination and a decreasing identification rate for SLN biopsy examination. Our hypothesis is that the lymphatic flow around the skin lesion is rich and less influenced by the effect of chemotherapy and tumor size than that in the parenchyma around the tumor. Our results were not significantly influenced by tumor size, tumor response, or nodal status before NAC.

In conclusion, the results of our study suggest that an SLN biopsy procedure after NAC using intradermal injection of radiocolloid is feasible and can predict axillary lymph node status with high accuracy for patients with clinically negative lymph node status after NAC. This procedure could make patients who have had their axillary lymph node status downstaged from positive to negative and patients with large tumors appropriate candidates for an SLN biopsy examination.

Further studies involving a larger number of patients will be required to establish fully the feasibility and accuracy of the SLN biopsy procedure for patients with breast cancer who have been treated with NAC.

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Prognosis of Resected Non-Small Cell Lung Cancer Patients with Intrapulmonary Metastases

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Background: In the current TNM staging system revised in 1997 for lung cancer, intrapulmonary metastases (PM) are classified into two categories: PM1 (in the same lobe of the primary tumor), designated as T4; and PM2 (in a different lobe), as M1. There have been no large-scale analyses on PM in non-small cell lung cancer (NSCLC) patients. We collected data nationwide in Japan for 7408 lung cancer patients undergoing surgical resection during a single year, 1994. We analyzed the long-term survival of NSCLC patients to evaluate the prognostic impact of PM in relation to other prognostic factors.

Method: Medical records of 6525 NSCLC patients undergoing surgical resection during a single year, 1994, were analyzed as a subset work of the Japanese Joint Committee of Lung Cancer Registry. The committee sent a questionnaire on outcome and clinicopathological profiles to 303 institutions.

Results: There were 6080 PM0 (no PM), 317 PM1, and 128 PM2 patients. The 5-year survival rates were 55.1% for PM0 patients, 26.8% for PM1, and 22.5% for PM2 patients, respectively. The differences in survival between patients with PM0 and PM1 and between patients with PM0 and PM2 were significant ($p < 0.001$, respectively); the difference in survival was not significant between patients with PM1 and PM2 ($p = 0.298$). In R0 and N0 patients, survival differences were similar for PM0, PM1, and PM2 patients. Significant survival difference was detected between T3 and PM1 ($p = 0.0317$) and between PM1 patients and T4 patients excluding PM1 ($p = 0.0083$). The 5-year survival rates of PM2 patients and M1 patients excluding PM2 were 22.5% and 20.5%, respectively, and there was no significant difference between the groups ($p = 0.434$).

Conclusion: There was no significant survival difference between NSCLC patients with PM1 and PM2. The survival of patients with PM1 was between that of the T3 and T4 patients excluding PM1.

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Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864/07/0204-0282

Key Words: Non-small cell lung cancer, Intrapulmonary metastases, Prognosis, TNM staging system.

(*J Thorac Oncol.* 2007;2: 282–286)

In 1989, Deslauriers et al.[1] described intrapulmonary metastasis (PM) in patients with non-small cell lung cancer (NSCLC) as satellite nodules, concluding that patients with these lesions should be classified as stage IIIA in the TNM staging system. In 1992, the Union Internationale Contre le Cancer (UICC [International Union Against Cancer]) and the American Joint Committee on Cancer revised the TNM classification, and the T factor of lung cancer with PM was upstaged as a local progression.[2,3] T factor was upgraded by a single unit if PM was located in the primary lobe, and it was classified as T4 if the PM was located in other lobes of the ipsilateral lung. When the TNM staging system was revised in 1997 for lung cancer, PM was designated as T4 if it was in the same lobe of the primary tumor (PM1) and as M1 if it was in a different lobe (PM2).[4]

Since then, there have been no large-scale survival analyses on NSCLC patients with PM. We collected data nationwide in Japan for 7408 lung cancer patients undergoing surgical resection during a single year, 1994.[5] We retrospectively analyzed the survival of these patients to evaluate the prognostic impact of PM in relation to other prognostic factors.

PATIENTS AND METHODS

As described previously,[5] the Japanese Joint Committee of Lung Cancer Registry sent a questionnaire in 1995 to 320 Japanese institutions, asking them to report outcomes and clinicopathological profiles for patients who underwent primary lung cancer resection in 1994. Data for 7408 patients were collected from 303 institutions. In 2001, the joint committee sent a questionnaire to these institutions to acquire clinicopathological profiles and outcome. The following 27 items were included in the questionnaire: gender, age, clinical (c-) T, c-N, c-M, c-stage, preoperative treatment, surgical procedure, extent of lymph node dissection, curability, residual tumor, primary site by lobe, tumor diameter, histology, organ invasion, pleural involvement, pleural dissemination, PM, pleural cytology, pathological (p-) T, p-N, p-M, p-stage, location of nodal metastasis, survival time recurrence, and cause of death. Recurrent or multiple lung cancers were not