PROSTATE CANCER IN NATIVE JAPANESE AND JAPANESE-AMERICAN MEN: EFFECTS OF DIETARY DIFFERENCES ON PROSTATIC TISSUE

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ABSTRACT

Objectives. To investigate the relationship between diet and prostate cancer (CaP) among native Japanese (NJ) and second-generation or third-generation Japanese-American (J-A) men—focusing on the effects of animal fat and soy on prostatic tissues.

Methods. The subjects were 50 Japanese men undergoing radical prostatectomy, 25 NJ living in Nagoya, Japan and 25 U.S.-born J-A men, living in Los Angeles, California. A priori, the NJ men were believed to be a low-fat, high-soy group and the J-A men, a high-fat, low-soy group. The studies included postoperative measurements of diet (Block questionnaire), body fat (bioimpedance), blood, urine, and prostatic biomarkers in malignant and adjacent normal tissue, using a tissue microarray made from the original paraffin blocks.

Results. The NJ and J-A men were similar in age (65 to 70 years old; $P < 0.05$), prostate-specific antigen level (7.1 to 8.6 ng/mL), prostate volume (35 to 38 cm³), and Gleason score (5.6 to 6.6), but their body composition differed. J-A men had more body fat (24% versus 19%), higher serum triglyceride levels (245 versus 106 mg/dL), lower estradiol levels (27 versus 31 ng/mL), and much lower urinary soy-metabolite levels (1:3) than NJ men ($P < 0.02$). In both NJ and J-A groups, expression of numerous tissue biomarkers separated normal from CaP tissue, including markers for apoptosis (Bcl-2, caspase-3), growth factor receptors (epidermal growth factor receptor), racemase, 5-lipoxygenase, kinase inhibition (p27), and cell proliferation (Ki-67; all $P < 0.02$). Furthermore, within both normal and CaP tissues, caspase-3 and 5-lipoxygenase were expressed more in NJ than in J-A men ($P < 0.01$). Nuclear morphometry showed that the chromatin in each of the four groups (normal versus CaP, NJ versus J-A) was different (area under the curve 85% to 94%, $P < 0.01$), despite fundamental genetic homogeneity.

Conclusions. NJ and J-A men, products of similar genetics but differing environments, were shown to have differences in body composition that could influence CaP evolution. The CaP specimens from the NJ and J-A men were histologically similar, but tissue biomarker expression, especially of lipoxygenase and the caspase family, suggested differing mechanisms of carcinogenesis. Differences in nuclear morphometry suggested the additional possibility of gene-nutrient interactions. UROLOGY **64:** 765–771, 2004. © 2004 Elsevier Inc.

W hy prostate cancer (CaP) is rare in Asia but common in the West[1] has not yet been fully explained. A genetic explanation alone is not sufficient because the relative protection from CaP seen in Asian men begins to disappear when these men immigrate to the West. The more years and generations that men of Asian descent live in a Western country, the greater is their incidence and mortality

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TABLE I. *Clinical characteristics*

Characteristic	Native Japanese (n = 25)	Japanese-American (n = 25)	P Value
Age (yr)	65.4 ± 5.0	70.2 ± 5.4	<0.05
Height (in)	64.4 ± 2.2	66.0 ± 3.0	0.03
Weight (lb)	132.6 ± 17.0	145.6 ± 20.3	0.02
Waist (in)	32.7 ± 2.8	33.4 ± 2.5	0.35
Body fat (%)	19.3 ± 5.8	24.2 ± 4.7	<0.001
Prostate volume (g)	34.8 ± 13.6	38.5 ± 17.9*	0.43
Preoperative PSA (ng/mL)	11.7 ± 9.5	7.9 ± 5.7	0.09
Median PSA (ng/mL)	8.6	7.1	0.22
PSA density	0.4 ± 0.5	0.2 ± 0.2*	0.11
Interval from surgery to 3/02 (mo)	31.6 ± 19.9	59.8 ± 31.7	<0.001
Pathologic stage (n)			0.17
2a	9	5	
2b	12	12	
2c	0	2	
3a	4	6	
Gleason score (n)	5.6 ± 2.9	6.6 ± 1.5	0.15
2–6	18	17	
7	4	5	
8–10	3	3	

KEY: PSA = prostate-specific antigen.
Data presented as mean ± SD, unless otherwise noted.
Data collection from April to October 2001.
* n = 22.

of CaP.[2–4] Thus, environmental factors must account for some of the geographic differences.

Chief among the suspect factors in the environment is diet. Major East-West differences in animal fat and soy consumption are known to exist,[5–7] but a clear linkage relating diet to carcinogenic change in prostatic tissue has not yet been established. If such a relationship were shown to exist, dietary modification would become an important public health issue and interest in nutrition-related treatment methods might evolve.

Most evidence to date relating diet and CaP has been epidemiologic.[8–10] In contrast, the present study was conceived to help determine whether factors exist in the Western diet that translate into carcinogenic effects in prostatic tissue. Thus, we examined dietary patterns, body composition, and tissue characteristics in native Japanese (NJ) men undergoing radical prostatectomy in Japan and American-born Japanese (J-A) men undergoing radical prostatectomy in the United States.

MATERIAL AND METHODS

A retrospective study of 50 Japanese men undergoing radical prostatectomy for cure of localized CaP between 1994 and 2001 was performed. All 50 men had typical Japanese surnames, and each regarded himself as of pure Japanese ancestry. Of the 50 men, 25 were Japanese-born men undergoing surgery in Nagoya, Japan, and 25 were second-generation or third-generation American-born Japanese men undergoing surgery in Los Angeles, California. Each series was consecutive and included all qualifying patients from the two sites

from 1994 to 2001, excluding other men who had received any form of androgen-deprivation therapy (n = 6), who had received preoperative radiotherapy (n = 2), who were lost to follow-up (n = 2), or for whom tissue blocks could not be obtained (n = 8). The clinical characteristics are shown in Table I.

All men were interviewed postoperatively between April and October 2001. At the interview, the 50 men in the study all had a serum prostate-specific antigen level of 0.4 ng/mL or less and no clinical evidence of cancer. At the interview, each man signed a consent form approved by the institutional review boards of both the University of California, Los Angeles (UCLA) Medical Center and the Nagoya Urology Hospital and then completed clinical and dietary questionnaires,[11] provided blood and urine samples, and underwent measurement of height, weight, and a bioimpedance test for body fat determination. Tissue blocks from the prostatectomy were gathered from Nagoya Urology Hospital and from several hospitals in Los Angeles. For the Nagoya men, forms and questionnaires were translated into Japanese, and the results translated back into English for compilation by one of us (M.K.). All specimens and questionnaires were processed through the offices of the Urological Sciences Research Foundation and then forwarded to various laboratories for study. The nutritional and hormonal studies, along with the blood counts and multiphasic serum testing, were completed at the UCLA Center for Human Nutrition.

A tissue microarray was constructed at the Johns Hopkins Medical Institutions under the direction of one of us (A.D.), using the method of Kononen et al.[12] The representative areas of tumor and adjacent normal tissue were demarcated on the hematoxylin-eosin slides. The cancerous areas were assigned a Gleason grade by a single uropathologist (M.P.), and regions of representative tumor and normal tissue (four cores each 0.6 mm in diameter) were used for construction of the tissue microarray. The antigenic integrity of the tissues was confirmed by preliminary staining for p27. Biomarker analysis was per-

formed in five different laboratories (A.D., J.L.M., J.Q., A.W.P, and R.W.V.), using commercially available antibodies, standard immunohistochemistry techniques, and quantitative image analysis. Quantitative grading of the prostatic epithelial nuclei (QNG) was performed as previously described using computer-assisted image analysis of Feulgen-stained tissue.[13,14] Receiver operating characteristic curves were generated from the QNG data, using 60 nuclear morphometric descriptors of size, shape, DNA content, and chromatin textural features. All tissue studies were performed without reader knowledge of tissue origin.

One of us (F.J.D.) coordinated data handling and statistical analysis using IBM-compatible personal computers, Excel spreadsheets, and Stata software. Various *t* tests (simple, paired, two-sample) and linear regression analysis were used to compare group differences.

RESULTS

CLINICAL CHARACTERISTICS

The men in this study were mostly in their mid to late 60s with moderately differentiated, organ-confined CaP (Table I). The J-A men were, on average, 5 years older (70 years versus 65 years), had a greater percentage of body fat (24.2% versus 19.3%), and underwent surgery 28.2 months earlier than the NJ men ($P < 0.002$). Otherwise, the two groups were similar in terms of prostate weight (38.5 to 34.8 g), serum prostate-specific antigen level (7.1 to 8.6 ng/mL), pathologic tumor stage (mostly organ confined), and Gleason tumor grade (5.6 to 6.6).

FOOD FREQUENCY CHARACTERISTICS

Using the Block Food Frequency Questionnaire,[11] few statistically significant dietary differences were detected between the NJ and J-A subjects (Table II). Of the 21 dietary components measured, 5 (cholesterol, vitamin D, calcium, alcohol, and selenium) were significantly greater in the J-A men than in the NJ men. The other 16 nutritional variables showed no statistically significant differences between groups. Of note were the extremely low calorie values for both groups (~1280 kcal/day). During the interview with each patient, none reported a history of any major dietary modification; thus, we believe the reported food frequencies were reflective of long-standing patterns.

NUTRITIONAL AND HORMONAL CHARACTERISTICS

The estradiol serum levels were somewhat greater in the NJ men than in the J-A men (32 versus 27 pg/mL, $P < 0.01$), but the differences in the testosterone (free and total) and sex hormone binding globulins were not statistically significant between groups (Table II). The serum triglyceride levels were considerably greater in the J-A men than in the NJ men (245 versus 106 mg/dL, $P < 0.01$), but other lipid components, including various cholesterols, showed no statistically significant differences (data not shown). Excretion of

the soy metabolites daidzein and genistein was threefold to fourfold greater in the NJ than in the J-A men ($P < 0.02$). Aside from the above differences, the groups were matched regarding blood counts and multiphasic serum testing findings.

PROSTATIC TISSUE CHARACTERISTICS

All tissue studies were performed on the tissue microarray. When stratified by Gleason grade, the cancers were similar between the NJ and J-A men (Table I). The tumors in both NJ and J-A men were largely organ confined and moderately well-differentiated (Table I), and the androgen receptors and pro-prostate-specific antigen were evenly expressed across all tissues (Table III). The stroma/epithelial ratio was similar in the normal peripheral zone tissues of the two groups (3.2 to 3.4), and it was relatively diminished in the cancer tissues of the two groups.

In comparing the benign and malignant tissue, the expression of racemase, intact caspase-3, epidermal growth factor receptor, lipoxygenase, histone H3 mitosis marker (H3), Bcl-2 (Bostwick Labs) (BAD), and poly(ADP-ribose) polymerase (PARP) was significantly greater statistically in the cancer tissues than in the normal peripheral zone; this finding was noted in both groups. p27 expression was significantly greater statistically in the normal peripheral zone tissue than in the cancer tissue, and this finding was also noted in both groups. Ki-67, a marker of cell proliferation, was minimally expressed in all tissues, but was significantly greater statistically in the malignant tissues compared with benign prostatic hyperplasia. Racemase expression showed the greatest differential between benign and malignant tissue, with a ratio of 1:5 in the NJ men and 1:3 in the J-A men.

Lipoxygenase and intact caspase-3 were more abundantly expressed in both tissues of the NJ men than the tissues of the J-A men. Lipoxygenase was expressed to a greater degree than any other marker, with such expression appearing in 79.5% of cells in the cancer tissues of the NJ men. Of the individual biomarkers studied, only lipoxygenase permitted four-way discrimination, showing greater expression in cancer than benign tissues and also greater expression in NJ than in J-A men.

NUCLEAR MORPHOMETRY

With QNG, receiver operating characteristic curves were generated to separate benign versus malignant nuclei within each group and NJ versus J-A nuclei within each diagnosis. Of the 60 morphometric features (descriptors) used in the schema to characterize the nuclei, only five or fewer (Fig. 1) were needed to form the receiver

TABLE II. *Food frequency and nutritional and hormonal characteristics*

Characteristic	Native Japanese (n = 25)	Japanese-American (n = 25)	*P* Value
Daily food frequency			
Kilocalories (kcal)	1282.0 ± 436.0	1277.0 ± 926.6	NS
Carbohydrates (g)	161.0 ± 49.2	153.5 ± 105.0	NS
Protein (g)	56.4 ± 23.6	50.4 ± 33.4	NS
Fat (g)	42.4 ± 19.3	50.4 ± 35.0	NS
Saturated fat total (g)	14.8 ± 6.7	16.0 ± 10.3	NS
Monounsaturated, total (g)	15.0 ± 6.9	19.0 ± 14.0	NS
Polyunsaturated, total (g)	8.9 ± 5.5	11.5 ± 8.8	NS
Fiber total (g)	11.1 ± 5.0	10.8 ± 7.2	NS
Soluble (g)	3.9 ± 1.8	3.7 ± 2.4	NS
Insoluble (g)	7.1 ± 3.1	7.0 ± 4.9	NS
Cholesterol (mg)	271.0 ± 129.0	205.0 ± 177.7	0.02
Vitamins			
A (μg)	931.0 ± 592.0	728.6 ± 462.2	NS
C (mg)	57.4 ± 21.0	65.3 ± 28.3	NS
D (μg)	4.9 ± 2.5	3.4 ± 2.7	0.01
E (mg)	5.3 ± 2.6	5.9 ± 3.9	NS
Calcium (mg)	519.0 ± 290.0	384.3 ± 289.2	0.03
Iron (mg)	9.1 ± 3.2	9.2 ± 5.8	NS
Betacarotene (mg)	3686.0 ± 2884.0	2582.0 ± 1483.0	NS
Retinol (μg)	317 ± 354.0	298.0 ± 358.5	NS
Alcohol (g)	5.3 ± 5.4	3.9 ± 16.6	0.001
Selenium (μg)	101 ± 39.0	81.9 ± 52.1	0.05
Nutritional and hormonal			
Soy metabolites (urine, nmol/mg creatinine)			
Daidzein	13.0 ± 12.8	3.9 ± 4.9	0.02
Genistein	11.1 ± 11.7	3.5 ± 4.1	0.02
Carotenoids (plasma, μmol/L)			
Lutein	0.5 ± 0.1	0.3 ± 0.1	0.01
Zeaxanthin	0.2 ± 0.1	0.1 ± 0.0	0.01
Alpha-tocopherol	26.1 ± 10.9	38.0 ± 24.4	0.01
Triglycerides (serum, mg/dL)	106.1 ± 53.2	245.0 ± 266.2	0.01
Total testosterone (ng/dL)	3.5 ± 1.8	3.3 ± 1.3	0.69
Free testosterone (pg/mL)	4.4 ± 2.2	4.6 ± 1.6	0.67
Sex hormone binding globulin (nmol/L)	32.7 ± 15.8	28.3 ± 16.4	0.34
Estradiol (serum, pg/mL)	31.9 ± 6.6	27.3 ± 5.4	0.01

KEY: NS = not statistically significant.
Data given as mean ± SD.

operating characteristic curves. The area under these curves ranged from 73% to 91%, all serving to differentiate the source of the nuclei significantly ($P <0.01$). Thus, four-way discrimination was also possible by studying the genetic material in the prostatic epithelial nuclei.

COMMENT

The main hypothesis of the present study was that Western diet leads to tissue changes associated with malignant transformation in the prostate. Thus, two groups of men were studied, all with CaP and all from the same gene pool (all Japanese), but ingesting diets traditionally known to be markedly different. Although the dietary questionnaire did not reveal dramatic differences between the NJ and J-A men, their body composition was found to be different in several important ways, and related prostatic tissue changes were also discovered. Differences in the genetic material, seen in the study of QNG, suggested a possible gene-nutrient interaction.

Animal or saturated fat (rich in U.S. diets) and soy (rich in Asian diets) are perhaps the most implicated dietary factors that could explain the disparate incidence of CaP in the two populations. A positive relationship between CaP incidence and saturated or animal fat in the diet has been supported by epidemiologic data,[5,9] although not all authorities agree.[15] Conversely, a negative relationship may exist for CaP and soy consump-

TABLE III. *Prostate tissue characteristics* *

Characteristic	Native Japanese (n = 25)			Japanese-American (n = 25)			P Value (NJ vs. J-A)	
	Benign	Cancer	P Value	Benign	Cancer	P Value	Benign	Cancer
Androgen receptors	47.2	44.1	0.63	53.2	50	0.99	0.82	0.54
PARP	61.9	66.7	0.02	60.1	68.2	0.01	0.91	0.85
Bcl-2	16.3	0	0.01	8.4	1.6	0.01	0.91	0.85
Caspase-3 (intact)	29.2	30.9	0.09	7.1	14.6	0.16	0.01	0.01
EGFR	68.9	75	0.01	49.9	64.5	0.01	0.26	0.1
BAD	30.7	45.5	0.01	11.6	37.4	0.01	0.26	0.4
H3	67.5	75.1	0.01	78.2	70.7	0.06	0.25	0.61
Lipoxygenase	45	79.5	0.01	27.7	60.6	0.01	0.01	0.01
p27	71.1	27.3	0.01	64	36.5	0.01	0.42	0.26
Racemase 3[†]	42.4	202.6	0.01	62.5	180.4	0.01	0.12	0.36
Ki-67[‡]	0.4	1.6	0.01	0.5	1.5	0.01	0.67	0.5
Pro-PSA[§]	201.87	190.04	>0.10	201.17	202.19	>0.10		
Stroma/epithelial ratio	3.2	2.1	0.01	3.4	3	0.09	0.4	0.04

KEY: NJ = native Japanese; J-A = Japanese-American; PARP = poly(ADP-ribose) polymerase; EGFR = epidermal growth factor receptor; BAD = Bcl-2 (Bostwick Labs); H3 = histone H3 mitosis marker; PSA = prostate-specific antigen.
Results presented as average of percentage of cells staining positive, unless otherwise noted.
Biomarker analysis was performed under direction of Drs. Demarzo and Putzi (p27, racemase, Ki-67), Drs. Bostwick and Qian (PARP, Bcl-2, caspase, EGFR, BAD, H3, lipoxygenase), and Dr. Partin (SER, Pro-PSA).
* Four spots (0.6 mm/spot) analyzed for each entry, with 50–100 cells counted per spot; although fixation techniques probably differed between sites, only spots staining positive for p27 in normal or adjacent normal tissue were used in the tissue studies, confirming intact antigenicity.
† Average of score (percentage weak + 2 × percentage moderate + 3 × percentage strong).
‡ Weighted average of score (0 = no staining; 3 = extensive staining).
§ Average staining intensity.

tion.[6,8,16] Oxidative damage from saturated fat[10] and a protective effect from soy phytoestrogens[17,18] are possible mechanisms.

In the present attempt to examine the effects of different diets on genetically similar people, the Block questionnaire[11] was used to quantify the expected dietary differences. However, the anticipated differences were not found to be as marked as expected (Table II). The reasons for the lack of demonstrable differences could be that Western diets have penetrated Japan more than appreciated, that the J-A men continued to ingest a traditional Japanese diet in the United States, that the sample size was too small, that the 3-month focus of the questionnaire did not reflect dominant lifelong patterns, or that the translated questionnaire was not sensitive enough to detect real differences. The latter explanation is likely, because the low calorie values probably indicate that the questionnaire failed to include traditional Japanese foodstuffs, which may have accounted for a considerable part of the daily caloric intake in both groups.

Despite the similarities in the questionnaire responses, important group differences were found in body composition, verifying that we had studied two nutritionally different groups of Japanese men. Serum triglycerides and the percentage of body fat were significantly greater in the J-A men than in the NJ men. The excretion of the soy metabolites genistein and daidzein were significantly greater (threefold to fourfold) in the NJ men than in the J-A men, and the serum estradiol levels were also elevated in the NJ men. These differences support the concept that the NJ men were the product of a diet relatively low in fat and high in soy compared with the J-A men.

Histologically, the cancerous and normal tissues appeared similar in the two groups, but several biomarker differences were noteworthy. First, the increase of p27 staining in the benign tissue and of racemase staining in the malignant tissue are in consonance with previous reports.[12,19,20] The p27 and racemase staining differentials were similar in the two groups, indicating that diet did not affect the expression of these biomarkers. Second, staining for lipoxygenase, an enzyme believed to help protect cells from apoptosis, was increased in the cancer cells of both groups, supporting the hypothesis that the activity of this enzyme—stimulated by way of dietary fat—plays a critical role in the survival of cancer cells.[21] Why lipoxygenase expression was greater in NJ men than in J-A men is not clear. Third, intact caspase-3, although not differentially expressed in benign versus malignant tissues, was significantly increased in the tissues of the NJ men. Because the androgen-sensitive caspase family of enzymes is known to be central to the initiation and direct induction of apoptosis,[22,23] this finding suggests differences in the pathogenesis of CaP in the two groups.

Nuclear morphometric features were studied to determine a possible relationship among diet, cancer, and the genetic material. Using the QNG technique, the chromatin was found to be significantly different
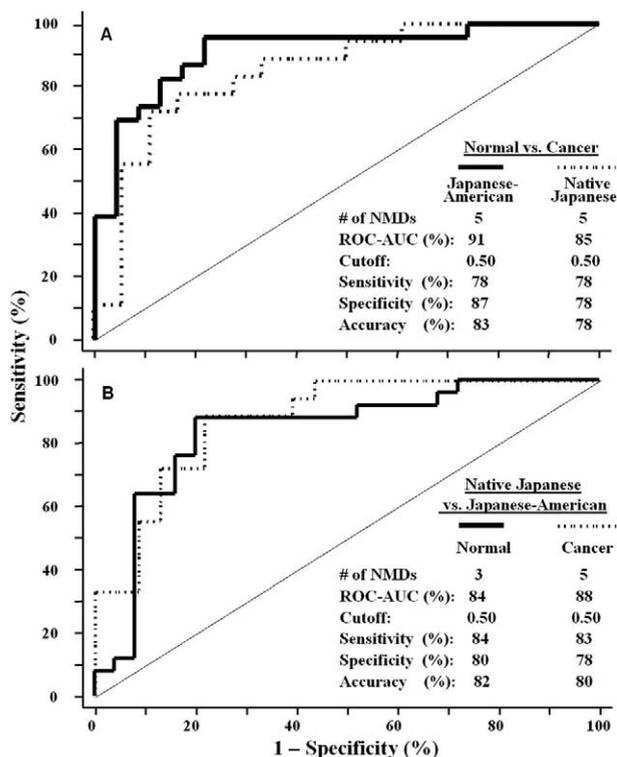
Normal vs. Cancer

	Japanese-American	Native Japanese
# of NMDs	5	5
ROC-AUC (%):	91	85
Cutoff:	0.50	0.50
Sensitivity (%):	78	78
Specificity (%):	87	78
Accuracy (%):	83	78

Native Japanese vs. Japanese-American

	Normal	Cancer
# of NMDs	3	5
ROC-AUC (%):	84	88
Cutoff:	0.50	0.50
Sensitivity (%):	84	83
Specificity (%):	80	78
Accuracy (%):	82	80

FIGURE 1. *Use of QNG to discriminate source of origin of prostatic epithelial nuclei. Receiver operating characteristic curves generated from various nuclear morphometric descriptors (NMDs), including measurements of size, shape, DNA content, and chromatin textural features.[13,14,24] No more than five NMDs were needed to discriminate accurately among the four sources of nuclei. (A) Normal versus cancer. (B) NJ versus J-A. Note that by using QNG, nuclei source could be identified with high degree of accuracy. ROC-AUC = receiver operating characteristic-area under curve.*

statistically in cancer versus normal cells within each group (ie, NJ or J-A) and for each diagnosis compared across groups (Fig. 1). Hence, CaP was associated with changes in the genetic material in the prostatic epithelial cells, as shown elsewhere,[24] and diet was also associated with such changes. Because the chromatin features were consistent within the normal cells of each group, but altered in the CaP cells of each group, the possibility of a gene-nutrient interaction is suggested. Whether such an interaction is mediated by dietary fat by way of the lipoxygenase pathway or by soy phytoestrogens by way of the caspase pathway (or by some other mechanism) is not clear from these data.

The limitations of the present study included the retrospective nature of the data collection, the small sample size, the time lapse between surgery and data collection, differences in tissue processing, and the lack of dramatic differences in the diet recorded between the groups. The latter limitation served to bias the data against the hypothesis in the present study, but important differences were seen

nonetheless. The differences might be even more pronounced in a study using fresh tissue and comparing groups having greater dietary differences than seen here. However, such a study may prove difficult because people ingesting a "highly protective" diet (ie, rural groups subsisting largely on fresh fruits and vegetables) rarely undergo CaP tissue collection.

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