colorectal neoplasia (1), the HMW form of adiponectin may mediate the association between adiposity and colorectal neoplasia through its well-recognized influence on insulin resistance.

Our observations for colorectal adenoma agree with those for colorectal cancer from a case-control study nested in the Health Professionals Follow-up Study (13), in which a statistically significant inverse association was seen between plasma adiponectin level and the risk of colorectal cancer. Several clinical studies have also provided supportive evidence that patients with colorectal neoplasia had lower circulating levels of adiponectin than controls, although these studies were small (19–21). However, circulating adiponectin levels were not associated with risk in a case-control study of colorectal adenoma in a Japanese population (12) or in nested case-control studies of colorectal cancer in Norwegian and Swedish populations (14, 15). In contrast, the only epidemiologic investigation of HMW adiponectin in relation to the risk of colorectal neoplasia reported results inconsistent with ours (12). To date, epidemiologic evidence for the association of total and HMW adiponectin with colorectal neoplasm is both sparse and controversial, and further studies to corroborate our results are needed.

To our knowledge, this is the first study to provide epidemiologic evidence that adiponectin and leptin interact to modify the risk of colorectal adenoma separate to their profound involvement in insulin resistance. After adjusting for BMI and other potential confounders, an inverse association of adiponectin with colorectal adenoma was apparent in the highest two tertiles of leptin, particularly the middle, whereas a positive association of leptin was obvious in the lowest tertile of adiponectin. A recent basic research study in a model of preneoplastic colon epithelial cells analogously showed that adiponectin inhibited multiple signaling cascades associated with leptin-induced cell proliferation (8). These findings lead to the hypotheses that adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, and that leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. This interaction would be independent to their well-documented influences on insulin resistance. These hypotheses require further interdisciplinary examination.

Among the strengths of the present study, the provision of total colonoscopy to all study subjects likely decreased the possibility of misclassification between cases and controls. Also, the number of subjects was considerably larger than in previous studies of the association between circulating levels of adiponectin and colorectal neoplasia (12–15).

A major limitation of this study is its cross-sectional nature, and the observed associations might be due to reverse causality. In contrast to colorectal cancer, however, it is unlikely that colorectal adenoma affects the amount of adipose tissue, a major determinant of circulating adiponectin levels (3), because colorectal adenoma is an asymptomatic benign tumor. A second limitation is the relatively small body size of the study population: Given that median BMI for male and female controls was 23.4 and 21.8 kg/m^2, respectively, and the prevalence of overweight and obesity was 26% and 15%, respectively, our observations may not be directly applicable to severely obese populations, often found in North American and European countries, where more than half of adults are overweight or obese (22).

Table 5. Association of HMW adiponectin with colorectal adenoma according to tertiles of leptin and TNF-α

Measurement	Tertiles for HMW adiponectin*			*P* trend†
	Lowest	Middle	Highest	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	
Leptin‡ §				0.07‖
Highest tertile	1.00 (reference)	1.03 (0.70–1.53)	0.73 (0.47–1.15)	0.16
Middle tertile	1.00 (0.66–1.51)	0.93 (0.62–1.39)	0.60 (0.38–0.94)	0.05
Lowest tertile	0.49 (0.30–0.82)	0.87 (0.55–1.37)	0.77 (0.50–1.20)	0.13
TNF-α§ ¶				0.21‖
Highest tertile	1.00 (reference)	1.47 (0.96–2.26)	0.76 (0.47–1.22)	0.36
Middle tertile	1.46 (0.95–2.23)	1.39 (0.90–2.13)	1.46 (0.93–2.31)	0.62
Lowest tertile	1.45 (0.92–2.29)	1.37 (0.89–2.11)	1.03 (0.65–1.61)	0.06

*Cutoff points were 0.88 and 1.91 μg/mL for men and 2.19 and 3.90 μg/mL for women.
†Statistical tests for trend (two-sided) were assessed by assigning ordinal values to tertiles of each measurement.
‡Cutoff points were 1,756 and 3,842 pg/mL for men and 3,856 and 7,908 pg/mL for women.
§Adjusted for age, screening period, duration of fasting, sex, cigarette smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, and BMI.
‖Values are *P* interaction instead of *P* trend.
¶Cutoff points were 2.38 and 2.97 pg/mL for men and 2.22 and 2.79 pg/mL for women.

Further studies in populations with larger body sizes are thus required. Finally, the present study was based not on incident but on prevalent cases, meaning that the ORs of colorectal adenoma presented in this study did not necessarily indicate the risk of "developing" colorectal adenoma, but rather the risk of "having" colorectal adenoma at a point in time, and should therefore be interpreted with caution.

In summary, adiponectin may decrease the risk of colorectal neoplasia through mechanisms other than the indirect mechanism through insulin resistance. Taking recent evidence from basic research into account, we hypothesize that adiponectin may exert an anticarcinogenic effect on the large intestine by interfering with leptin, and that leptin could conversely exert a carcinogenic effect under conditions of a lower abundance of adiponectin. Our observations add to a growing body of evidence for the interactive effects of adiponectin and leptin in the early stage of colorectal tumorigenesis separate to their profound involvement in insulin resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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High Dietary Intake of Magnesium May Decrease Risk of Colorectal Cancer in Japanese Men[1,2]

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Abstract

Magnesium maintains genomic stability and is an essential cofactor for DNA synthesis and repair. Magnesium intake has been reported to be inversely associated with colorectal cancer (CRC) risk in Western populations. This study examined the association between dietary intake of magnesium and CRC risk in Japanese men and women aged 45–74 y. Data from 40,830 men and 46,287 women, at the 5-y follow-up of the Japan Public Health Center-based Prospective Study, who responded to a 138-item FFQ were used in this analysis. A total of 689 and 440 CRC events were observed during the mean follow-up of 7.9 and 8.3 y for men and women, respectively. When adjusted for potential confounders, the hazard ratio and 95% CI in the highest quintile of magnesium intake compared with the lowest quintile in men were 0.65 (95% CI, 0.40–1.03) for CRC (P-trend = 0.04), 0.48 (95% CI, 0.26–0.89) for colon cancer (P-trend = 0.01), and 0.97 (95% CI, 0.47–2.02) for rectal cancer (P-trend = 0.93). Borderline inverse associations were also observed in men who consumed alcohol regularly (P-trend = 0.07) or had a BMI <25 kg/m^2 (P-trend = 0.06). There were similar inverse associations for invasive colon cancer and distal colon cancer. There were no significant associations between magnesium intake and cancer risk in women. Higher dietary intake of magnesium may decrease the risk of CRC in Japanese men. J. Nutr. 140: 779–785, 2010.

Introduction

Magnesium maintains genomic stability and is an essential cofactor in almost all enzymatic systems involved in DNA synthesis and repair (1). Magnesium deficiency may increase membrane dysfunctions and susceptibility toward oxidative stress (1). Studies on supplemental magnesium in animals have demonstrated a reduced incidence of induced colon tumors by means of inhibition of oncogene expression in colon cancer cell

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proliferation (2–4). Prospective studies (5–8) in human populations of the association between magnesium intake and risk of colorectal cancer (CRC)[4] found inverse associations between magnesium intake and risk of colon cancer (5,6,8) and rectal cancer (6). In particular, 2 studies (6,8) indicated that magnesium intake may prevent colon cancer risk by improving insulin sensitivity in overweight or type 2 diabetes populations.

Calcium also has a beneficial effect against colon cancer and may share several metabolic pathways with magnesium (9–11). A recent large case-control study indicated that total magnesium consumption was linked to a significantly lower risk of colorectal adenoma, especially in those individuals with a low ratio of calcium:magnesium intake and a higher vitamin D intake (12).

Fewer observational studies of the association between magnesium intake and CRC incidence are available. In the Asian population, the association of dietary intake of magnesium with the incidence of CRC risk has not, to our knowledge, been investigated to date. In this article, we present an analysis for CRC, colon, and rectal cancer, which is based on data obtained through the Japan Public Health Center (JPHC)-based Prospective Study.

Materials and Methods

Study participants. The JPHC Study was initiated in 1990 and includes 11 public health center (PHC)-based areas throughout Japan. The study population was defined as all registered Japanese living in these PHC areas (13). In 1990, 5 PHC areas (Cohort I) were selected based on variation in the mortality rate of stomach cancer according to a previous ecological study; in 1993, 6 PHC areas (Cohort II) were added, which were selected according to geographical distribution and feasibility (14). The baseline survey was sent to a total of 140,420 participants, with an overall response rate of 79% in Cohort I and 84% in Cohort II (14). Previous reports (14,15) explained the details of the study design and baseline profiles. The study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Participants in Katsushika, Tokyo, were excluded from this analysis because of the lack of cancer incident data. Participants who responded to the 5-y follow-up survey of the JPHC study, a total of 46,034 men and 52,484 women aged 45–74 y, were used for this analysis. The 5-y follow-up survey conducted between 1995 and 1998 for 2 subcohorts (overall response rate was 81.3%) contained a self-administrated questionnaire on demographic characteristics, medical history, smoking habit, alcohol consumption, physical activity, occupation, and other factors, as well a 138-item FFQ to assess dietary intake.

Dietary intake assessment. In the FFQ, participants were asked how often they consumed individual food items and the representative size of their portions relative to the size of a standard portion. The 9 response choices for frequency were never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 time/d, 2–3 times/d, 4–6 times/d, and ≥7 times/d. Response choices for portion size were small (50% smaller than standard), medium (same as standard), and large (50% larger than standard). The details of the FFQ were described in a previous report (16). Daily food intake was calculated by multiplying the frequency by the standard portion size and the relative portion size for each food item in the FFQ; daily intake of nutrients was calculated by using the 5th revised and enlarged edition of the *Standard Tables of Food Composition in Japan* (17). The validity of dietary intake of magnesium estimated from the FFQ was evaluated in a subsample of cohorts by comparing the estimated intake with that in dietary records (18). Spearman correlation coefficients between energy-adjusted intakes estimated from the FFQ and dietary records for magnesium were 0.45–0.46 in men and 0.42–0.45 in women (18). In addition, although the FFQ had questions on supplement use, intake of magnesium and other nutrients from supplements was not included in this analysis because no comprehensive database for supplements was available (16).

Follow-up and case ascertainment. Participants were followed until 31 December 2005. Residence status, relocation, and survival were confirmed annually by checking the residential registers. Under Japanese laws, resident and death registrations are required and inspection of resident registries is available to anyone (13). Information on the cause of death was obtained by examining the death certificates provided by the Ministry of Health, Labor, and Welfare, and the occurrence of cancer was identified by notification of active cancer patients through the major local hospitals in the study areas and by data linkage with population-based cancer registries. When local hospitals could not cover a sufficiently high proportion of cancer patients, the population-based registry (prefecture wide) was used as a supplemental data source (19). Death certificate notification and death certificate only have been clearly defined as indices of completeness and validity. In our cancer registry system, the proportion of cases with death certificate notification was 5.1%, in which information available from death certificates only was 2.4% for CRC. The site and histology of each cancer were coded according to the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) (codes C18–C20). Analyses of site-specific cancers were conducted: C18 for colon cancer (C180–C185 for proximal colon cancer and C186–C189 for distal colon cancer) and C199 and C209 for rectal cancer. In addition, CRC cases were further classified according to the depth of tumor invasion, i.e. invasive cancer [over a mucosal layer (malignant, primary site)] and noninvasive cancer [within a mucosal layer (carcinoma in situ)].

Statistical analysis. Exclusion of participants included those who reported a history of any cancer (1396 men and 1975 women) or had no nutrition data (567 men and 480 women). We also excluded participants who reported extreme values of height (<100 or >199 cm) and weight (<20 kg), did not provide information on dietary intake of magnesium, or reported extreme values (< or >2.5%) of total energy intake (lowest and highest cutoffs were 5192 and 14,150 kJ in men, respectively, and 3523 and 15,414 kJ in women, respectively) to remove some unreliable data and thus compute reasonable energy-adjusted nutrients. These exclusions left 40,830 men and 46,287 women eligible for this analysis.

Person-years of follow-up were counted from the date of the survey until the date of CRC diagnosis, the date of moving out of a study area, the date of death, or the end of 2005, whichever came first. Persons lost to follow-up were censored on the last confirmed date of their presence in the study area. On the basis of the sex-specific distribution of all study participants, dietary intakes of magnesium and other nutrients were adjusted for total energy intake with the residual model (20). BMI was categorized into 4 levels (<25, 25–<27, 27–<30, ≥30 kg/m^2) (21). Metabolic equivalent (MET) hours of physical activities were estimated by multiplying the reported time spent at each physical activity per day by its assigned MET intensity (22,23). Smoking habit categories consisted of never, former, and current smoking. Alcohol consumption was categorized into 4 groups (never; occasional; regular, <300 g/wk; and regular, ≥300 g/wk), in which regular drinker was calculated by multiplying the frequency per week by the usual daily intake of alcohol (15).

Hazard ratios (HR) and 95% CI for the development of CRC were explored by Cox proportional hazards regression analyses. In an analysis for site-specific events, cancer events in the other sites were considered censored cases (21). Testing of the associations between magnesium intake and CRC risk was first conducted with adjustment for age (continuous) and PHC, in which magnesium intake was categorized into a quintile category for analysis, with the lower quintile serving as the reference. On the basis of this model, other potential confounders were added, including alcohol consumption, smoking status, physical activities (MET-h/d) (continuous), CRC screening test (colonoscopy, barium enema, or fecal occult blood test), BMI, diabetes mellitus, vitamin supplement use, and menopausal status (for women). Further adjustment included dietary intake of total energy, energy-adjusted saturated fat, zinc, fiber, vitamin B-6, folate, and calcium (all in quintile categories).

[4] Abbreviations used: CRC, colorectal cancer; HR, hazard ratio; JPHC, Japan Public Health Center; MET, metabolic equivalent; PHC, public health center; TRP, transient receptor potential.

Stratified analysis was performed for alcohol consumption (never or occasional, regular), smoking status (never, ever), BMI (<25, ≥25 kg/m^2), and menopausal status (no, yes, for women). Tests for linear trend across quintiles were performed by using the median value of magnesium intake as a continuous variable. All *P*-values reported were 2-sided and the significance level was set at <0.05. All analyses were conducted with SAS version 9.1 (SAS Institute). Values in the text are means ± SD unless noted otherwise.

Results

As of December 2005, there were 689 newly diagnosed cases of CRC for men (172 proximal and 249 distal colon cancers, with 290 invasive cases; and 268 rectal cancers, with 224 invasive cases) and 440 for women (168 proximal and 127 distal colon cancers, with 230 invasive cases; and 145 rectal cancers, with 127 invasive cases). A total of 340,811.8 and 397,340.6 person-years were observed with a mean follow-up of 7.9 y for men and 8.3 y for women, respectively.

Magnesium intakes were 284.4 ± 105.3 mg/d in men and 279.4 ± 104.6 mg/d in women. Men and women who consumed more magnesium tended to be older, less likely to smoke and drink alcohol and more likely to undergo CRC screening and to have a higher prevalence of diabetes. For nutrients (except for total energy at the highest quintile of magnesium intake), both men and women who reported a higher intake of magnesium were more likely to have a higher intake of calcium, zinc, folate, fiber, vitamin B-6, and vitamin D; the men were more likely to have a higher intake of saturated fat, but women were not. In addition, the ratio of calcium:magnesium intake and the use of vitamin supplements in women were relatively higher than those in men (Table 1). The age- and PHC-adjusted model and other multivariate models for HR related to magnesium intake for CRC risk showed similar results (Table 2). With adjustment for all potential risk factors and relevant nutrient intakes, the HR and 95% CI in the highest quintile of magnesium intake in men, compared with the lowest quintile in men, were 0.65 (95% CI, 0.40–1.03; *P*-trend = 0.04) for CRC, 0.48 (95% CI, 0.26–0.89; *P*-trend = 0.01) for colon cancer, and 0.97 (95% CI, 0.47–2.02; *P*-trend = 0.93) for rectal cancer. Magnesium intake was inversely associated with risk of invasive colon cancer and distal colon cancer and tended to be negatively associated with risk of invasive CRC (*P* = 0.06), but it was not associated with risk of rectal cancer. Magnesium intake was not associated with CRC risk in women.

Compared with the lowest tertile, the highest tertile of magnesium intake was marginally associated with a reduction in CRC risk in men who consumed alcohol regularly (*P*-trend = 0.07) and in those with a BMI <25 kg/m^2 (*P*-trend = 0.06) (Table 3). There were no clear associations between combined BMI and alcohol consumption with CRC risk and the test for interaction was not significant in men. The restricted analysis was applied to postmenopausal women, nonsmoking women, women with a BMI <25 or ≥25 kg/m^2, and women who did not consume alcohol regularly. Associations were nonsignificant, similar to results obtained using data from all women (data not shown).

In addition, when participants who had diabetes (2674 men and 1497 women) or used vitamin supplements (4137 men and 6895 women) were excluded, associations observed for CRC, colon cancer, and rectal cancer were similar to those for total men or total women (data not shown). Moreover, when participants with CRC diagnosed within 2 y of follow-up were removed from the analysis, similar results were observed for both men and

Discussion

In this large population-based prospective study, we found significant inverse associations among dietary intake of magnesium and risk of CRC and colon cancer in men. These inverse associations were most evident for colon cancer.

Magnesium is abundant in vegetables, rice and wheat, soy and soy products, fish, and milk and other dairy products in the Japanese diet (24,25). The National Nutrition Survey in Japan in 2006 showed that intakes of magnesium among respondents >20 y of age were 274 ± 99 mg/d in men and 245 ± 92 mg/d in women (25), which is similar to our results. The daily intake of magnesium in our study was also similar to those reported in other populations (5,6,8). The interquintile range of total magnesium was 245–351 mg/d in the Iowa Women's Health Study (5) and 209–255 mg/d in the Swedish Mammography Cohort (6), both of which observed inverse associations between magnesium intake and colon cancer risk in women. The Netherland Cohort Study (8), with magnesium intakes of 286–373 mg/d in men and 256–326 mg/d in women, found weak, nonsignificant, inverse associations between total magnesium intake and CRC and colon cancer risk in both men and women and in participants with a BMI >25 kg/m^2. Compared with intakes in our study and others (5,6), the Women's Health Study (7) had a relatively higher intake of magnesium of 279–392 mg/d and did not observe any significant associations. This study pointed out that high magnesium intake may be related to reduction of CRC risk only among populations with relatively low intakes of magnesium and, therefore, populations who have sufficient intake of magnesium may obtain little benefit from increased intake.

The absorption of magnesium is directly or indirectly affected by calcium (26,27), whereas the absorption of calcium is closely related to and regulated by vitamin D (16,28). Ionized magnesium (Mg^{2+}) is a chronic regulatory agent, as opposed to ionized calcium (Ca^{2+}), because Mg^{2+} shares the transient receptor potential (TRP) channels with Ca^{2+} in the paracellular pathway, the epithelial Ca^{2+} (TRPV5/6) and Mg^{2+} (TRPM6/7) channels (27). In particular, the TRPM7 receptor possesses a higher affinity for Mg^{2+} than Ca^{2+} and is expressed and implicated in cellular Mg^{2+} homeostasis (12,27,29). Facilitated by these channels, a high calcium intake may interfere with magnesium absorption and vice versa. On the other hand, magnesium absorption is vitamin D independent, but repletion of vitamin D is associated with increments in magnesium absorption (26). The interactions among magnesium, and calcium, as well as vitamin D are important in intestinal magnesium transport and absorption (26,27). Our previous study (15) reported the potential inverse association between dietary intake of calcium and CRC risk as well as the potential effect modification between calcium and vitamin D against CRC risk in Japanese men. Another American study (12) identified inverse associations between total magnesium intake and colorectal adenoma in total participants and participants with a low ratio of calcium: magnesium intake (<2.78); magnesium intake (301.4 ± 128.6 mg/d in adenoma cases and 321.2 ± 122.1 mg/d in controls) in this study was also greater than that in our study. The American study (12) also indicated that the absorption of magnesium might be significantly elevated when vitamin D intake was high and that the ratio of calcium:magnesium intake was low, because the absorption of magnesium could be significantly depressed when the calcium concentration was high (26). In our previous study (15), the amount of dietary intake of calcium was considered to be relatively lower; accordingly, the ratio of

TABLE 1 Characteristics of study population according to magnesium intake at 5-y follow-up (JPHC-based Prospective Study)[1]

	Quintiles of energy-adjusted magnesium intakes, range (median), *mg/d*				
	Q1 (lower)	Q2	Q3	Q4	Q5
Men	<238 (216)	238–<267 (254)	267–<294 (280)	294–<327 (308)	≥327 (355)
Participants, *n*	8166	8166	8166	8166	166
Age, *y*	55.4 ± 7.6	55.9 ± 7.7	56.6 ± 7.7	57.5 ± 7.8	58.7 ± 7.8
BMI, *kg/m²*	23.6 ± 3.3	23.5 ± 3.1	23.5 ± 3.0	23.6 ± 3.1	23.6 ± 3.2
Current smoker, %	52.8	47.1	45.4	41.2	37.0
Regular alcohol consumption, %	79.2	70.6	65.7	62.9	55.4
Physical activity, *MET-h/d*	26.5 ± 7.1	26.3 ± 7.0	26.3 ± 6.9	26.3 ± 6.9	26.0 ± 6.9
Diabetes melitus, %	5.7	5.8	6.2	7.7	10.9
Colonscopy or barium enema or fecal occult blood test, %	25.8	31.6	33.6	35.4	36.1
Vitamin supplement use, %	8.1	9.5	10.4	11.0	11.5
Nutrient intakes[2]					
Total energy, *kJ/d*	2159.6 ± 644.9	2173.6 ± 633.9	2186.4 ± 636.1	2188.4 ± 635.6	2148.5 ± 648.7
Saturated fat, *g/d*	15.4 ± 7.0	17.0 ± 6.2	17.4 ± 6.1	17.6 ± 5.9	17.5 ± 5.8
Dietary fiber, *g/d*	7.6 ± 2.3	9.7 ± 2.4	11.3 ± 2.7	13.2 ± 3.1	17.1 ± 4.9
Calcium, *mg/d*	336.5 ± 142.2	447.6 ± 178.1	514.9 ± 204.5	576.2 ± 217.1	679.5 ± 233.4
Calcium:magnesium	1.6 ± 0.6	1.8 ± 0.7	1.8 ± 0.7	1.9 ± 0.7	1.9 ± 0.6
Zinc, *mg/d*	7.9 ± 1.5	8.6 ± 1.2	8.8 ± 1.1	9.0 ± 1.1	9.5 ± 1.2
Folate, *μg/d*	250.9 ± 77.6	318.9 ± 83.5	365.8 ± 88.1	424.7 ± 102.7	544.2 ± 158.3
Vitamin B-6, *mg/d*	1.3 ± 0.3	1.4 ± 0.2	1.5 ± 0.2	1.7 ± 0.3	1.9 ± 0.3
Vitamin D, *μg/d*	7.2 ± 4.6	9.2 ± 5.4	10.3 ± 5.8	11.5 ± 6.6	13.0 ± 8.1
Women	<237 (219)	237–<262 (250)	262–<286 (274)	286–<316 (299)	≥316 (342)
Participants, *n*	9257	9257	9258	9257	9258
Age, *y*	55.4 ± 8.1	56.2 ± 8.0	57.0 ± 7.8	57.8 ± 7.6	58.9 ± 7.5
BMI, *kg/m²*	23.5 ± 3.6	23.4 ± 3.5	23.4 ± 3.4	23.5 ± 3.5	23.7 ± 3.7
Current smoking, %	7.3	5.6	4.5	4.3	4.6
Regular alcohol consumption, %	14.1	13.0	13.1	11.8	10.9
Physical activity, *MET-h/d*	25.4 ± 6.0	25.6 ± 6.0	25.9 ± 5.9	25.8 ± 5.9	25.7 ± 5.9
Diabetes melitus, %	2.7	2.8	3.3	4.0	5.5
Postmenopausal, %	63.0	68.3	72.9	77.0	79.8
Colonscopy or barium enema or fecal occult blood test, %	23.8	29.6	32.4	34.7	35.2
Vitamin supplement use, %	14.3	14.5	15.6	14.9	15.2
Nutrient intake[2]					
Total energy, *kJ/d*	1844.8 ± 594.8	1856.9 ± 559.8	1894.5 ± 550	1899.8 ± 540.8	1838.3 ± 554.1
Saturated fat, *g/d*	18.6 ± 6.3	17.9 ± 5.3	17.4 ± 5.1	16.8 ± 5.1	16.3 ± 5.3
Dietary fiber, *g/d*	9.4 ± 2.4	11.5 ± 2.4	13.1 ± 2.6	15.0 ± 2.9	18.8 ± 4.5
Calcium, *mg/d*	412.1 ± 172.2	516.3 ± 193.9	570.3 ± 199.4	618.2 ± 198.0	705.6 ± 213.5
Calcium:magnesium	1.9 ± 0.8	2.1 ± 0.8	2.1 ± 0.7	2.1 ± 0.7	2.0 ± 0.6
Zinc, *mg/d*	7.9 ± 1.0	8.1 ± 0.8	8.2 ± 0.8	8.3 ± 0.8	8.7 ± 0.9
Folate, *μg/d*	280.0 ± 76.6	351.4 ± 80.7	403.7 ± 91.5	459.6 ± 102.0	579.9 ± 157.2
Vitamin B-6, *mg/d*	1.2 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.3
Vitamin D, *μg/d*	7.3 ± 4.4	9.3 ± 5.2	10.5 ± 5.8	11.2 ± 6.1	12.2 ± 7.5

[1] Values are mean ± SD or %.
[2] Energy-adjusted intake.

with those reported in Western studies (5,6,12). On the other hand, vitamin D intake in our study was higher than that reported in this American study (3.4 ± 2.2 μg/d in adenoma cases and 3.7 ± 2.5 μg/d in controls). Our findings also provided a reasonable explanation that a higher intake of magnesium in persons at the lower ratio of calcium:magnesium intake level may have a reduction in their CRC risk through the balance of nutrients including magnesium and calcium.

We think that the lack of an inverse relationship in women may be due to the different risk profiles in men and women in the Japanese population. In Japanese men, physical activity was associated with decreased risk of CRC, whereas obesity, diabetes, and C-peptide were associated with increased risk of CRC (13,21,22,30). In Japanese women, however, these associations were not significant and were weaker than in Western populations (31). Therefore, magnesium likely did not have a protective effect in women via an improvement in insulin sensitivity. In addition, the increased level of female hormones as the increment of internal fattiness may reduce CRC risk (31). The difference between men and women in CRC risk may also be explained by differences in alcohol consumption. About 75% of Japanese men and only 20% of Japanese women consume alcohol; this rate of alcohol consumption in Japanese men is higher than that in other populations (16,32). Alcohol may increase CRC risk by disturbing DNA synthesis and methylation in the one-carbon metabolism pathway (32,33). Animal studies suggest that marginal magnesium deficiency is more likely to result in pathological signs in the presence of increased oxidative

TABLE 2 HR and 95% CI of CRC according to magnesium intake (JPHC-based Prospective Study, 1995–2005)[1]

	Quintiles of energy-adjusted magnesium intake					
	Q1 (lower)	Q2	Q3	Q4	Q5	*P*-trend
Men						
CRC cases, *n*	163	131	118	136	141	
HR (95% CI)	1.00	0.79 (0.59–1.04)	0.66 (0.47–0.92)	0.71 (0.48–1.04)	0.65 (0.40–1.03)	0.04
Invasive CRC						
Cases, *n*	118	102	85	102	107	
HR (95% CI)	1.00	0.82 (0.59–1.13)	0.65 (0.44–0.96)	0.69 (0.44–1.07)	0.59 (0.34–1.02)	0.06
Colon cancer						
Cases, *n*	105	74	82	78	82	
HR (95% CI)	1.00	0.68 (0.47–0.98)	0.71 (0.47–1.09)	0.62 (0.37–1.01)	0.48 (0.26–0.89)	0.01
Proximal colon cancer						
Cases, *n*	42	31	33	33	33	
HR (95% CI)	1.00	0.78 (0.44–1.40)	0.85 (0.43–1.66)	0.84 (0.39–1.84)	0.55 (0.21–1.46)	0.25
Distal colon cancer						
Cases, *n*	63	43	49	45	49	
HR (95% CI)	1.00	0.61 (0.38–0.97)	0.62 (0.36–1.08)	0.49 (0.25–0.93)	0.43 (0.19–0.95)	0.02
Invasive colon cancer						
Cases, *n*	88	56	55	54	57	
HR (95% CI)	1.00	0.72 (0.46–1.12)	0.72 (0.43–1.20)	0.60 (0.33–1.10)	0.44 (0.21–0.92)	0.02
Rectal cancer						
Cases, *n*	58	57	36	58	59	
HR (95% CI)	1.00	0.97 (0.62–1.51)	0.54 (0.31–0.97)	0.86 (0.47–1.59)	0.97 (0.47–2.02)	0.93
Invasive rectal cancer						
Cases, *n*	50	46	30	48	50	
HR (95% CI)	1.00	0.95 (0.58–1.54)	0.54 (0.29–1.01)	0.80 (0.41–1.57)	0.85 (0.38–1.90)	0.81
Women						
CRC						
Cases, *n*	60	93	89	93	105	
HR (95% CI)	1.00	1.72 (1.12–2.63)	1.46 (0.89–2.39)	1.37 (0.78–2.39)	1.15 (0.60–2.21)	0.69
Invasive CRC						
Cases, *n*	49	75	75	72	86	
HR (95% CI)	1.00	1.64 (1.03–2.61)	1.49 (0.87–2.55)	1.36 (0.74–2.49)	1.19 (0.58–2.44)	0.94
Colon cancer						
Cases, *n*	39	59	61	63	73	
HR (95% CI)	1.00	1.72 (1.01–2.94)	(0.86–2.92)	1.49 (0.75–2.97)	1.29 (0.57–2.89)	0.92
Proximal colon cancer						
Cases, *n*	21	32	41	37	37	
HR (95% CI)	1.00	1.46 (0.71–3.01)	1.64 (0.73–3.68)	1.38 (0.56–3.44)	0.85 (0.28–2.53)	0.41
Distal colon cancer						
Cases, *n*	18	27	20	26	36	
HR (95% CI)	1.00	2.03 (0.92–4.49)	1.39 (0.53–3.61)	1.55 (0.54–4.45)	2.01 (0.60–6.71)	0.44
Invasive colon cancer						
Cases, *n*	31	46	50	46	57	
HR (95% CI)	1.00	1.63 (0.90–2.95)	1.59 (0.81–3.14)	1.43 (0.66–3.09)	1.34 (0.54–3.32)	0.86
Rectal cancer						
Cases, *n*	21	34	28	30	32	
HR (95% CI)	1.00	1.70 (0.83–3.46)	1.24 (0.53–2.88)	1.16 (0.45–2.99)	0.93 (0.31–2.86)	0.59
Invasive rectal cancer						
Cases, *n*	18	29	25	26	29	
HR (95% CI)	1.00	1.65 (0.78–3.49)	1.32 (0.55–3.20)	1.27 (0.47–3.43)	1.00 (0.31–3.25)	0.77

[1] Adjusted for age (continuous) and PHC, BMI (<25, 25–<27, 27–<30, ≥30 kg/m^2), smoking status (never, former, current), alcohol consumption (never; occasional; regular, <300 g/wk; regular, ≥300 g/wk), screening test (no, yes), vitamin supplement use (no, yes), diabetes (no, yes), menopausal status (no, yes, for women), physical activities (continuous), total energy intake, energy-adjusted intakes of saturated fat, dietary fiber, calcium, zinc, folate, vitamin D, and vitamin B-6 (all in quintiles).

or chronic inflammatory stress (1), which may have been caused by alcohol consumption. Significant associations in our study suggest that magnesium intake may provide a prominent benefit for men who regularly consume alcohol.

The Netherland Cohort Study reported that higher total magnesium intake reduced CRC risk in overweight women as a result of decreased insulin resistance (8). However, in our study, we did not observe significant associations in either overweight

TABLE 3 HR and 95% CI of CRC according to magnesium intake with stratified by risk factors in men (JPHC-based Prospective Study, 1995–2005)[1]

	Tertiles of energy-adjusted magnesium intake			
	T1 (lower)	T2	T3	*P*-trend
No or occasional alcohol consumption				
Cases, *n*	46	52	83	
Age- and PHC-adjusted HR (95% CI)	1.00	0.71 (0.47–1.06)	0.83 (0.56–1.22)	0.43
Multivariate HR (95% CI)	1.00	0.71 (0.42–1.20)	0.97 (0.49–1.93)	0.66
Regular alcohol consumption				
Cases, *n*	201	152	142	
Age- and PHC-adjusted HR (95% CI)		0.80 (0.65–0.99)	0.79 (0.63–0.99)	0.04
Multivariate HR (95% CI)	1.00	0.81 (0.61–1.07)	0.69 (0.47–1.03)	0.07
No smoking				
Cases, *n*	66	65	65	
Age- and PHC-adjusted HR (95% CI)	1.00	0.78 (0.55–1.11)	0.65 (0.45–0.92)	0.02
Multivariate HR (95% CI)		0.82 (0.51–1.32)	0.68 (0.36–1.29)	0.35
Smoking	201			
Cases, *n*	1.00	137	149	
Age- and PHC-adjusted HR (95% CI)	1.00	0.75 (0.60–0.94)	0.91 (0.64–1.01)	0.06
Multivariate HR (95% CI)	1.00	0.85 (0.63–1.14)	0.86 (0.57–1.31)	0.21
BMI <25 kg/m^2				
Cases, *n*	171	155	156	
Age- and PHC-adjusted HR (95% CI)	1.00	0.79 (0.64–0.99)	0.74 (0.59–0.93)	0.01
Multivariate HR (95% CI)	1.00	0.90 (0.66–1.21)	0.68 (0.45–1.04)	0.06
BMI 25 kg/m^2				
Cases, *n*	79	53	75	
Age- and PHC-adjusted HR (95% CI)	1.00	0.65 (0.45–0.92)	0.79 (0.57–1.11)	0.26
Multivariate HR (95% CI)	1.00	0.71 (0.44–1.13)	1.17 (0.64–2.15)	0.78

[1] Multivariate HR are adjusted for age (continuous), PHC, BMI (<25, 25–<27, 27–<30, ≥30 kg/m^2), smoking status (never, former, current), alcohol consumption (never; occasional; regular, <300 g/wk; regular, ≥300 g/wk), diabetes (no, yes), screening test (no, yes), vitamin supplement use (no, yes), physical activities (continuous), total energy intake, energy-adjusted intakes of saturated fat, dietary fiber, calcium, zinc, folate, vitamin D, and vitamin B-6 (all in quintiles).

men or women. In contrast, we found an inverse association between magnesium intake and CRC risk in men with a BMI < 25 kg/m^2. The Japanese population has a higher proportion of lean people (BMI ≥ 27 kg/m^2 was ~11.5% in men and 13.4% in women) compared with populations in Western countries (8,32). This may be a reason for the different findings between Japanese women and Western women regarding the modified risk associations by BMI. Interestingly, the incidence of diabetes in the Japanese population is also higher in lean people, in contrast to Western populations (34). Given their relatively moderate magnesium status, it is possible that lean people might benefit substantially from magnesium intake through improvement of insulin sensitivity in the Japanese population. Diabetes was a risk factor of colon cancer in the Japanese population (13), however, the limited number of participants with diagnosed diabetes during follow-up restricted further analysis.

Our study has several limitations. First, this analysis was based on a single measurement of intake of dietary nutrients obtained through the self-administered FFQ; therefore, inevitable misclassification of magnesium intake might attenuate the true relationship with CRC risk (6). Second, magnesium intake from drinking water was not considered and intake of magnesium supplements could not be computed; hence, the total magnesium intake of each participant from different sources may be underestimated in this study. However, a Japanese study (35) on trace element levels in drinking water reported that the magnesium concentration was only 3.83 ± 3.29 mg/L in 34 municipalities. Furthermore, the observed similar inverse associations in participants who did not report supplemental magnesium intake indicate that it is unlikely that data fo individuals who consumed magnesium supplements could hav caused notable fluctuations of this study's results (12,24) Nevertheless, further studies with detailed information o drinking water and supplement use may be helpful in examinin these associations (36). Third, we could not rule out th possibility of biases from unmeasured confounders, althoug the multivariate analysis showed results similar to those from th age- and PHC-adjusted analyses. Because relevant data wer unavailable in this population, we could not test the associatio between the potential relevant genotype(s) and CRC risk and th interaction effect with magnesium intake related to CRC risk (12).

One advantage of this study is that the quality of the cance registry in this population was satisfactory according to an international comparison (37). In addition, participants in this large-scale population-based prospective study had a highe compliance rate, with only 0.7% men and 0.8% women lost to follow-up up to the end of the analysis.

It should be noted that many findings in this study were borderline or not significant; thus, further evidence is needed. In Japan, the recommended dietary allowances for magnesium intake are 370, 350, and 310 mg/d for men aged 30–49, 50–69, and >70 y, respectively, and 280, 290, and 270 mg/d for women aged 30–49, 50–69, and >70 y, respectively (38). In summary, higher dietary intake of magnesium may reduce CRC risk in Japanese men. Increased intake of magnesium-rich foods is recommended if other studies, including randomized controlled trials, confirm our findings.

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Original contribution

Metaplastic carcinoma of the breast☆

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Summary The purposes of this study were to investigate whether the biological characteristics or outcomes of patients with metaplastic carcinoma, invasive ductal carcinoma, or invasive lobular carcinoma of the breast differ; to determine whether the metaplastic carcinoma subtypes have similar malignant potentials; and to identify accurate predictors of outcome in patients with metaplastic carcinoma. The subject comprised 6137 invasive ductal carcinoma patients, 301 invasive lobular carcinoma patients, and 46 metaplastic carcinoma patients of the breast. The metaplastic carcinomas were classified according to the World Health Organization classification. Multivariate analyses clearly demonstrated that the metaplastic carcinoma patients had a significantly poorer outcome than the invasive ductal carcinoma patients or the invasive lobular carcinoma patients independent of the nodal status or age not exceeding 39 years, whereas patients with triple-negative metaplastic carcinomas or triple-negative invasive lobular carcinomas had a poorer outcome than those with triple-negative invasive ductal carcinomas. Although no significant differences in clinical outcome were observed among the metaplastic carcinoma subtypes in multivariate analyses, an age not exceeding 39 years, the presence of skin invasion, and the presence of a squamous cell carcinoma component in nodal tumors were significant outcome predictors for metaplastic carcinoma patients. In conclusion, the results of this study clearly demonstrated that metaplastic carcinoma is more aggressive than invasive ductal carcinoma or invasive lobular carcinoma. Although the metaplastic carcinoma subtypes had no prognostic significance, an age not exceeding 39 years, the presence of skin invasion, and the presence

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of a squamous cell carcinoma component in nodal tumors were significant predictors of outcome among metaplastic carcinoma patients.

1. Introduction

The World Heath Organization (WHO) classifies metaplastic carcinoma (MPC) into (1) epithelial type and (2) mixed type [1]. Epithelial-type MPC is in turn classified into (1) squamous cell carcinoma, (2) adenocarcinoma with spindle cell differentiation, and (3) adenosquamous carcinoma, whereas mixed type of MPC is classified into (1) carcinoma with chondroid metaplasia, (2) carcinoma with osseous metaplasia, and (3) carcinosarcoma [1]. Several studies have investigated whether the biological characteristics of these MPC subtypes differ [2-19] and whether outcome predictors exist for patients with these MPC subtypes [2-7,9,10,13-15,18]. However, almost all these studies involved survival analyses performed for a small number of MPC cases or that only used univariate analyses [2-7,9,10,13-15,18]. Thus, whether the presently used subtype classification reflects the malignant potential of these lesions remains uncertain; and which factors are the most important predictors of outcome in patients with MPC remains controversial.

Furthermore, although patients with MPC are though to exhibit a poorer outcome than patients with invasive ductal carcinoma (IDC) or invasive breast carcinoma [15,18], the survival periods of patients with MPC, IDC, or invasive lobular carcinoma (ILC) have not been compared using consecutive cases treated during the same period. Thus, no direct evidence indicating that MPC is more aggressive than IDC or ILC presently exists.

The purposes of this study were (1) to investigate whether the biological characteristics or outcomes of patients with MPC, IDC, or ILC of the breast differ; (2) to determine whether the MPC subtypes have similar malignant potentials; and (3) to identify accurate predictors of outcome in patients with MPC. The results of this study clearly demonstrated that patients with MPC have a significantly poorer outcome than patients with IDC or ILC; that the MPC subtype has no prognostic significance; and that an age not exceeding 39 years, the presence of skin invasion, and the presence of a squamous cell carcinoma component in nodal tumors were significant predictors of outcome among patients with MPC.

2. Materials and methods

2.1. Cases

The subject comprised 6137 consecutive cases of IDC, 301 consecutive cases of ILC, and 46 consecutive cases of MPC of the breast; all the subjects had undergone surgery at the National Cancer Center Hospital between January 1982 and March 2007. For the MPC cases, all the breast carcinomas diagnosed as squamous cell carcinoma, epidermoid carcinoma, MPC, carcinosarcoma, carcinoma with spindle cell metaplasia, carcinoma with chondroid metaplasia, or carcinoma with osteoid metaplasia at the National Cancer Center Hospital between January 1982 and March 2007 were reviewed; 46 cases of MPC were subsequently identified.

Clinical information was obtained from the patients' medical records. All the patients were Japanese women, ranging in age from 20 to 98 years (median, 53 years). Overall, 2094 patients were premenopausal and 3056 were postmenopausal. A partial mastectomy had been performed in 1208 patients, a modified radical mastectomy had been performed in 3340, and a standard radical mastectomy had been performed in 1139. A level I and II axillary lymph node dissection had been performed in all the patients, and some of the patients had been received a level III axillary lymph node dissection.

The protocol (20-112) for this study was approved by the Institutional Review Board of the National Cancer Center.

2.2. Neoadjuvant therapy and adjuvant therapy

Because standardized neoadjuvant therapy and adjuvant therapy for patients with breast cancer were started in the 1990s at the National Cancer Center Hospital, the effect of neoadjuvant therapy or adjuvant therapy was examined in patients with IDCs, ILCs, or MPCs that had been surgically treated since January 1990. Neoadjuvant therapy was performed in 467 out of 2039 patients with IDC, 28 out of 111 patients with ILC, and 4 out of 46 patients with MPC, whereas adjuvant therapy was performed in 1756 out of 2303 patients with IDC, 101 out of 122 patients with ILC, and 18 out of 46 patients with MPC. Among these patients, 378 received chemotherapy, 749 received endocrine therapy, and 693 received combined chemoendocrine therapy. In the 1980s, the main chemotherapy regimens in use were anthracycline based; but nonanthracycline-based regimens were used in some patient populations. In the 1990s, the chemotherapy regimens in use were anthracycline based and were combined with or without taxane. In the 1980s, the endocrine therapy regimens in use were tamoxifen combined with or without a gonadotropin-releasing hormone agonist, whereas an aromatase inhibitor was additionally used in the 1990s.

2.3. Histologic examination of IDCs and ILCs

The following IDC and ILC characteristics were obtained from the pathologic diagnostic records, which

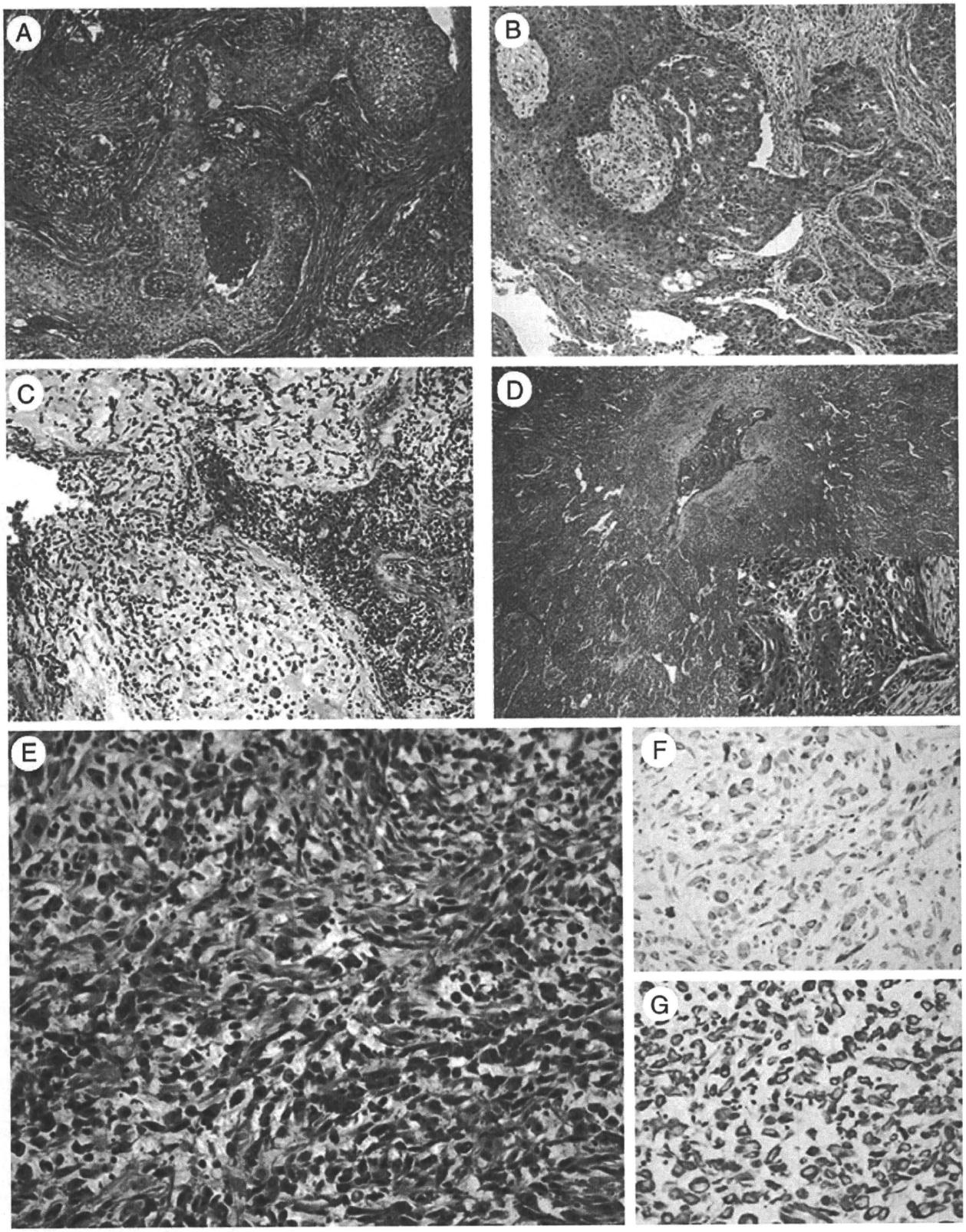

Fig. 1 Histologic features of MPC. A, Squamous cell carcinoma. The carcinoma cells invade as irregularly shaped solid nests showing squamous features with hyalinization. B, Adenosquamous carcinoma. The carcinoma cells invade as irregularly shaped solid nests and show squamous and tubular features. C, Carcinoma with chondroid metaplasia. The carcinoma cells invade as strands or solid nests with chondroid stroma. D, Squamous cell carcinoma in lymph node. Carcinoma cells metastasizing to the lymph node show squamous differentiation. E-G, Carcinosarcoma. Carcinosarcoma consists mainly of spindled tumor cells and epithelioid tumor cells admixed with pleomorphic tumor cells (E). Carcinosarcoma shows positive staining both for keratin (AE1/3) (F) and vimentin (G).

Table 1 Univariate analyses for identifying factors that are significantly different among patients with IDC, ILC, or MPC

No. of patients (%)			
Factors	IDC	ILC	*P* values; a, b, c
			MPC
Age, y			a, .002; b, .605; c, .033
≤39	655 (11)	15 (5)	6 (13)
>39	5481 (89)	286 (95)	40 (87)
Total	6136	301	46
Neoadjuvant therapy			a, .572; b, .023; c, .019
No	1572 (77)	83 (75)	42 (91)
Yes	467 (23)	28 (25)	4 (9)
Total	2039	111	46
Adjuvant therapy			a, .097; b, <.001; c, <.001
No	547 (24)	21 (17)	28 (61)
Yes	1756 (76)	101 (83)	18 (39)
Total	2303	122	46
ER			a, .085; b, <.001; c, <.001
Negative	615 (28)	25 (21)	21 (100)
Positive	1577 (72)	95 (79)	0
Total	2192	120	21
PR			a, .725; b, <.001; c, <.001
Negative	715 (33)	41 (34)	21 (100)
Positive	1477 (67)	79 (66)	0
Total	2192	120	21
HER2 category (0, 1 *vs* 2, 3)			a, .017; b, .052; c, .313
0 or 1	1799 (81)	107 (90)	25 (96)
2	189 (9)	6 (5)	0
3	226 (10)	6 (5)	1 (4)
Total	2214	119	26
Invasive tumor size (mm)			a, <.001; b, .090; c, .804
≤20	2214 (41)	83 (30)	13 (28)
>20	3242 (59)	193 (70)	33 (72)
Total	5456	276	46
Skin invasion			a, .069; b, .037; c, .292
Absent	5002 (92)	247 (89)	37 (84)
Present	407 (8)	29 (11)	7 (16)
Total	5409	276	44
Lymph vessel invasion			a, <.001; b, <.001; c, .001
Absent	2848 (47)	178 (60)	41 (89)
Present	3160 (53)	118 (40)	5 (11)
Total	6008	296	46
Blood vessel invasion			a, .051; b, .230; c, .597
Absent	5589 (93)	285 (96)	45 (98)
Present	393 (7)	11 (4)	1 (2)
Total	5983	296	46
Lymph node metastasis			a, .963; b, .461; c, .499
Absent	3716 (60)	183 (61)	29 (66)
Present	2430 (40)	119 (39)	15 (34)
Total	6147	302	44

NOTE. *P* value a, IDC *vs* ILC; *P* value b, IDC *vs* MPC; *P* value c, ILC *vs* MPC.

were completed by 2 or 3 pathologists per case at the time of treatment: (1) skin invasion (absent, present), (2) lymph vessel invasion (absent, present), (3) blood vessel invasion (absent, present), and (4) lymph node metastasis (absent, present).

2.4. Histologic examination of MPCs

Serial sections of each MPC tumor were cut from paraffin blocks. One section from each tumor was stained with hematoxylin and eosin and examined histologically to confirm the diagnosis, and another section was subjected to immunohistochemistry. The following 14 histologic features of primary invasive MPCs were evaluated, and several of these histologic features (numbers 7 to 14) were evaluated according to the WHO classification [1] : (1) invasive tumor size (≤20, >20 to ≤50, >50 mm), (2) skin invasion (absent, present), (3) histologic grade (1, 2, 3; only for carcinoma component) [20], (4) number of mitotic figures in 10 high-power-fields, (5) lymph vessel invasion (absent, present), (6) blood vessel invasion (absent, present), (7) tumor necrosis (absent/≤30%, >30%), (8) MPC type (epithelial, mixed), (9) squamous cell carcinoma versus other types of carcinoma (Fig. 1A), (10) adenocarcinoma with spindle cell differentiation versus other types of carcinoma, (11) adenosquamous carcinoma versus other types of carcinoma (Fig. 1B), (12) carcinoma with chondroid metaplasia versus other types of carcinoma (Fig. 1C), (13) carcinoma with osseous metaplasia versus other types of carcinoma, and (14) carcinosarcoma versus other types of carcinoma (Fig. 1E). The following 7 histologic features of MPCs metastasizing in lymph nodes were evaluated: (1) histologic grade (1, 2, 3; only for

carcinoma component) [20], (2) extranodal invasion (absent, present), (3) squamous cell carcinoma in lymph node-metastatic tumors (absent, present) (Fig. 1D), (4) adenocarcinoma with spindle cell differentiation in lymph node-metastatic tumors (absent, present), (5) adenosquamous cell carcinoma in lymph node-metastatic tumors (absent, present), (6) carcinosarcoma in lymph node-metastatic tumors (absent, present), and (7) tumor stroma in lymph node-metastatic tumors (none, mild, moderate, severe). *Extranodal invasion* was defined as the extension of tumor cells through the capsule of at least one lymph node into the perinodal adipose tissue. Nuclear atypia, structural atypia, and the number of mitotic figures were evaluated in the same manner as for the primary invasive tumors.

One author (N. O.) assessed all the characteristics of the primary tumors and the nodal metastatic tumors, and another author (T. H.) identified the characteristics of all the IDCs to confirm the tumor cell characteristics in these tumor components recorded by N. O. without knowledge of the outcome of the patients with MPC. Whenever a discrepancy occurred, the authors reexamined the slides to reach a consensus.

2.5. Immunohistochemistry

We used the immunohistochemistry records for estrogen receptor (ER), progesterone receptor (PR), and HER2 for the IDCs, ILCs, and MPCs diagnosed by 2 or 3 pathologists at the time of routine examination. A tumor with nuclear staining for ER or PR in 10% or more of its tumor cells was assessed as ER-positive or PR-positive. HER2 cell membrane expression was categorized as follows: (1) HER2 category 0, negative; (2) HER 2 category 1, weakly positive (faintly stained cell membrane and $\leq$10% of overall tumor area); (3) HER2 category 2, moderately positive (moderately stained cell membrane and >10% of overall tumor area); and (4) HER2 category 3, strongly positive (strongly stained cell membrane and >10% of overall tumor area). Tumors classified as HER2 category 0 or 1 were considered negative for HER2 expression. All the MPCs were immunohistochemically studied using commercially available monoclonal antibodies to keratins (AE1/3) (Fig. 1F) and vimentin (Fig. 1G) and were confirmed to be positive for both keratins and vimentin.

2.6. Patient outcome and statistical analysis

Survival was evaluated using a median follow-up period of 153 months (range, 1-304 months) until February 2007. Of the 6138 IDC patients, 1019 developed tumor recurrences; and 771 died of their disease. Of the 302 ILC patients, 55 developed tumor recurrences; and all of them died of their disease. Of the 46 MPC patients, 15 developed tumor recurrences; and 11 died of their disease. The recurrence-free and overall survival periods were determined beginning at the time of surgery. Tumor relapse was considered to have occurred whenever evidence of metastasis was first observed.

The χ^2 test was used to analyze whether significant differences existed in the frequencies of the clinicopathologic factors among the patients with IDC, ILC, or MPC.

We analyzed the outcome predictive power of tumor histology (IDC, ILC, MPC) and clinicopathologic factors for tumor recurrence and tumor-related death using multivariate analyses performed according to the Cox proportional hazard regression model as follows: model 1 included tumor histology, age, invasive tumor size, skin invasion, lymphatic invasion, blood vessel invasion, and nodal status; and model 2 included the above 7 factors plus neoadjuvant therapy, adjuvant therapy, ER/PR expression, and HER2 expression.

For the MPCs, the 14 histologic factors examined in the primary MPCs plus the 7 histologic factors examined in the MPCs located in the lymph nodes as well as age, neoadjuvant therapy, adjuvant therapy, and HER2 expression were entered into the univariate analyses; the factors that were significantly associated with tumor recurrence or tumor-related death were then entered into the multivariate analyses performed using the Cox proportional hazard regression model.

The multivariate analyses were performed using a casewise and step-down method that was applied until all the remaining factors were significant at a P value $< .05$. Survival curves were drawn using the Kaplan-Meier method. All the analyses were performed using Statistica/Windows software (StatSoft, Tulsa, OK).

3. Results

3.1. Univariate analyses of factors with significant differences among patients with IDC, ILC, or MPC

Patients with MPC showed significantly lower frequencies of neoadjuvant therapy, adjuvant therapy, and lymph vessel invasion than patients with IDC or ILC and a significantly higher frequency of skin invasion than patients with IDC (Table 1). Furthermore, all the patients with MPC exhibited negative immunostaining for ER and PR. Patients with ILC were significantly older than patients with IDC or MPC and had a significantly larger tumor size, a significantly lower HER2 category, and a significantly lower frequency of lymph vessel invasion than patients with IDC (Table 1). No significant differences in any other factor were observed among the 3 groups.

3.2. Multivariate analyses of outcome among patients with IDC, ILC, or MPC

In model 1 and model 2, the patients with MPC had significantly higher hazard rates (HRs) for tumor recurrence

(model 1: HR, 5.5; 95% confidence interval [CI], 3.2-9.6; model 2, HR, 6.6; 95% CI, 2.5-17.1) and tumor death (model 1: HR, 4.2; 95% CI, 2.2-8.1; model 2, HR, 12.4; 95% CI, 3.2-46.2) (Fig. 2A) than the patients with IDC in the multivariate analyses, although no significant differences in the HRs for tumor recurrence and tumor death were observed between patients with IDC and those with ILC in the multivariate analyses (data not shown). Furthermore, the patients with MPC had significantly higher HRs for tumor recurrence and tumor death that the patients with IDC independent of nodal metastasis in the multivariate analyses (Table 2). No significant differences in the HRs for tumor recurrence and tumor death were observed between patients with IDC and those with ILC among patients with or without nodal metastasis in the multivariate analyses (Table 2). Meanwhile, among patients not older than 39 years, the patients with MPC had significantly higher HRs for tumor recurrence and tumor death in model 1 of the multivariate analysis (Table 3); but model 2 could not be examined because of the small numbers of patients with ILC (2 cases) and MPC (3 cases). In patients with triple-negative carcinomas, the patients with MPC and the patients with ILC had significantly higher HRs for tumor recurrence and tumor death than the patients with IDC in multivariate analyses (Table 3).

3.3. Outcome predictive factor for patients with MPC

A patient age not exceeding 39 years (Fig. 2C), the use of neoadjuvant therapy, the presence of skin invasion (Fig. 2B), the presence of squamous cell carcinoma in a lymph node (Fig. 2D), and the International Union Against Cancer

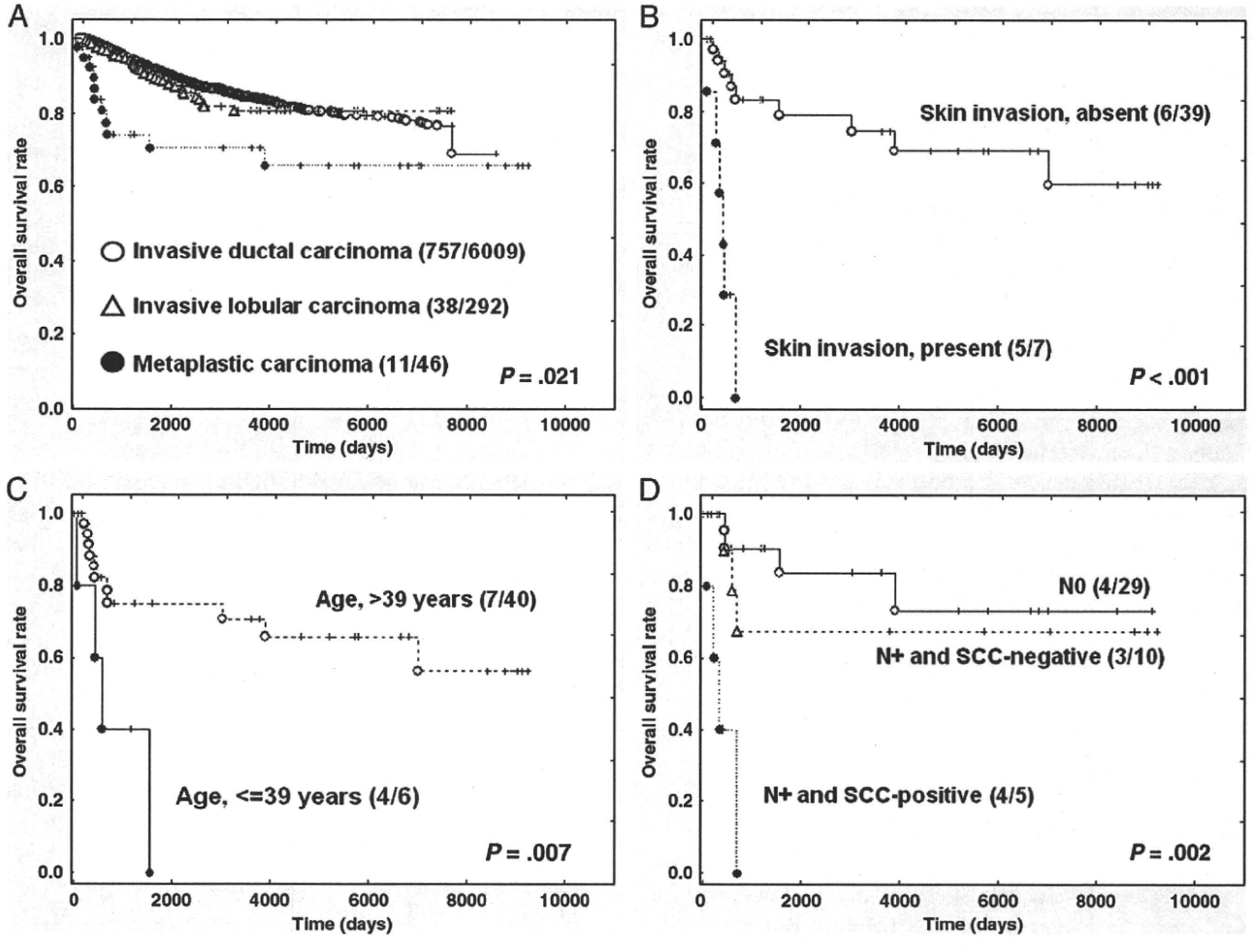

Fig. 2 Overall survival curves. A, Patients with MPC show a significantly shorter overall survival period than patients with IDC and patients with ILC, and no significant difference in overall survival period is present between patients with IDC and patients with ILC. B, MPC patients with skin invasion show a significantly shorter overall survival period than those without skin invasion. C, MPC patients 39 years and younger show a significantly shorter overall survival period than those older than 39 years. D, MPC patients with squamous cell carcinoma in lymph nodes show a significantly shorter overall survival period than those without nodal metastasis or those with nodal metastasis but with no squamous cell carcinoma in their lymph nodes.

Table 2 Multivariate analyses for tumor recurrence and tumor-related death in patients with IDC, ILC, or MPC according to nodal status

No. of patients (%)					
Model 1					
Patients without nodal metastasis (n = 3915)					
		Tumor recurrence		Tumor-related death	
	Cases	Cases	HR (95% CI) *P* value	Cases	HR (95% CI) *P* value
IDC	3703	403 (11)	Referent	261 (7)	Referent
ILC	183	22 (12)	1.2 (0.7-2.1) .425	12 (7)	1.0 (0.5-2.0) .943
MPC	29	7 (24)	6.0 (2.8-12.9) <.001	4 (14)	3.5 (1.3-9.8) .016
Patients with nodal metastasis (n = 2558)					
IDC	2424	614 (25)	Referent	510 (21)	Referent
ILC	119	33 (28)	1.2 (0.8-1.9) .336	29 (25)	1.3 (0.9-2.0) .163
MPC	15	7 (47)	4.9 (2.3-10.5) <.001	7 (47)	4.0 (1.8-9.2) <.001
Model 2					
Patients without nodal metastasis (n = 1852)					
IDC	1737	163 (9)	Referent	42 (2)	Referent
ILC	101	10 (10)	1.2 (0.6-2.4) .690	3 (7)	1.4 (0.4-4.6) .627
MPC	14	4 (29)	5.2 (1.2-22.7) .028	4 (29)	4.4 (1.2-15.9) .023
Patients with nodal metastasis (n = 412)					
IDC	391	94 (24)	Referent	30 (8)	Referent
ILC	16	5 (31)	2.0 (0.7-5.9) .187	3 (19)	3.0 (0.7-13.9) .164
MPC	5	3 (60)	8.6 (2.3-32.9) .001	3 (60)	28.9 (4.6-123.5) <.001

NOTE. Patients without nodal metastasis—Model 1 (tumor recurrence and tumor-related death): adjusted for tumor histology, age, skin invasion, lymphatic invasion, blood vessel invasion, and tumor size. Model 2 (tumor recurrence and tumor-related death): adjusted for the above factors (in model 1) as well as neoadjuvant therapy, adjuvant therapy, HER2 category, and ER and PR statuses. Patients with nodal metastasis—Model 1 (tumor recurrence and tumor-related death): adjusted for tumor histology, age, skin invasion, lymphatic invasion, blood vessel invasion, and tumor size. Model 2 (tumor recurrence and tumor-related death): adjusted for the above factors (in model 1) as well as neoadjuvant therapy, adjuvant therapy, HER2 category, and ER and PR statuses.

Abbreviation: n, number of cases that were examined in the multivariate analyses.

(UICC) pTNM stage were significantly associated with tumor recurrence and tumor-related death in the univariate analyses (Table 4). A tumor necrosis percentage of more than 30% of the primary tumors, the UICC pN category, the histologic grade of the tumors in the lymph nodes, the presence of extranodal invasion, the presence of adenocarcinoma with spindle cell differentiation in tumors in the lymph nodes, and the presence of tumor stroma in tumors in the lymph nodes were significantly associated with tumor-related death in the univariate analyses (Table 4). Other clinicopathologic factors, including MPC subtype, were not significantly associated with tumor recurrence or tumor death in the univariate analyses (Table 4).

In the multivariate analyses, the presence of skin invasion and an age not exceeding 39 years significantly increased the HRs for tumor recurrence and tumor death, whereas the presence of squamous cell carcinoma in tumors in the lymph nodes significantly increased the HR for tumor death (Table 5).

4. Discussion

In this study, none of the MPCs was positive for ER and PR; and only one MPC was positive for HER2. Furthermore, the presence of lymph vessel invasion, the presence of blood vessel invasion, and the UICC pN status did not exhibit any prognostic significance in patients with MPC, confirming the results of previous studies [7,9,14]. Because these factors are well-known outcome predictors

Table 3 Multivariate analyses for tumor recurrence and tumor-related death in patients with IDC, ILC, or MPC according to age and triple-negative status

No. of patients (%)					
Model 1 (n = 674)					
Patients ≤39 y old					
		Tumor recurrence		Tumor-related death	
	Cases	Cases	HR (95% CI) *P* value	Cases	HR (95% CI) *P* value
IDC	654	159 (24)	Referent	114 (17)	Referent
ILC	15	2 (13)	1.0 (0.3-6.3) .952	1 (7)	0.7 (0.1-5.0) .712
MPC	6	4 (67)	32.4 (11.1-99.2) <.001	4 (67)	55.5 (17.1-173.5) <.001
Patients whose carcinomas were negative for ER, PR, and HER2 (triple-negative IDC) (n = 304)					
IDC	271	42 (16)	Referent	19 (7)	Referent
ILC	14	4 (29)	3.6 (1.2-11.1) .023	2 (14)	4.6 (0.9-21.9) .059
MPC	19	6 (32)	9.4 (1.8-15.0) .002	3 (16)	5.1 (1.3-19.4) .017

NOTE. Patients not older than 39 years—Model 1: adjusted for tumor histology, skin invasion, lymphatic invasion, blood vessel invasion, tumor size, and nodal status. Triple-negative IDC patients—Tumor recurrence: adjusted for tumor histology, age, skin invasion, lymphatic invasion, and nodal status. Tumor-related death: adjusted for tumor histology, age, skin invasion, and nodal status.

Abbreviation: n, number of cases that were examined in the multivariate analyses.

Table 4 Association of clinicopathologic factors with tumor recurrence and tumor-related death in patients with MPC

Factors	Cases	No. of patients (%)					
	46	Cases with tumor recurrence		*P* value	Cases with tumor-related death		*P* value
Age, y							
≤39	6	4	(67)	.009	4	(67)	.007
>39	40	11	(28)		7	(18)	
Neoadjuvant therapy							
No	42	11	(26)	<.001	8	(19)	.002
Yes	4	4	(100)		3	(75)	
Adjuvant therapy							
No	37	10	(27)	.505	7	(19)	.474
Yes	9	5	(56)		4	(44)	
Invasive tumor size (mm)							
≤20	13	3	(23)	.352	1	(8)	.072
>20 to ≤50	26	9	(35)		7	(27)	
>50	7	3	(43)		3	(43)	
Skin invasion							
Absent	39	9	(20)	<.001	6	(13)	<.001
Present	7	6	(86)		5	(71)	
Histologic grade							
Grade 1	1	0		.558	0		NA
Grade 2	5	1	(20)		0		
Grade 3	40	14	(35)		11	(28)	
No. of mitotic figures in 10 high-power fields.							
≤32	24	8	(33)	.878	5	(21)	.483
>32	22	7	(32)		6	(27)	
Lymph vessel invasion							
Absent	41	12	(29)	.398	9	(22)	.498
Present	5	3	(60)		2	(40)	
Blood vessel invasion							
Absent	45	15	(33)	NA	11	(24)	NA
Present	1	0			0		
Area (%) occupied by of tumor necrosis within the tumor							
Absent/≤30	38	11	(29)	.119	7	(18)	.031
>30	8	4	(50)		4	(50)	
Types of MPC							
Epithelial	34	12	(35)	.813	8	(24)	.828
Mixed	12	3	(25)		3	(25)	
Squamous cell carcinoma *vs* other types of carcinoma							
Squamous	7	4	(57)	.134	3	(43)	.136
Other types	39	11	(28)		8	(21)	
Adenocarcinoma with spindle cell differentiation *vs* other types of carcinoma							
Adenoca with spindle	8	4	(50)	.422	2	(25)	.938
Other types	38	11	(29)		9	(23)	
Adenosquamous carcinoma *vs* other types of carcinoma							
Adenosquamous ca	19	4	(21)	.150	3	(16)	.264
Other types	27	11	(41)		8	(30)	
Carcinoma with chondroid metaplasia *vs* other types of carcinoma							
Ca with chondroid	4	1	(25)	.659	1	(25)	.835
Other types	42	14	(33)		10	(24)	
Carcinoma with osseous metaplasia *vs* other types of carcinoma							
Ca with osseous	1	0		NA	0		NA
Other types	45	15	(33)		11	(24)	
Carcinosarcoma *vs* other types of carcinoma							
Carcinosarcoma	7	2	(29)	.660	2	(29)	.432
Other types	39	13	(33)		9	(23)	

(*continued on next page*)

Table 4 *(continued)*

Factors	Cases	No. of patients (%)					
	46	Cases with tumor recurrence		*P* value	Cases with tumor-related death		*P* value
UICC pN category (n = 44)							
N0	29	7	(24)	.255	4	(14)	.049
N1	11	4	(36)		4	(36)	
N2	2	2	(100)		2	(100)	
N3	2	1	(50)		1	(50)	
Histologic grade of lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.195	4	(14)	.032
Grade 1	1	0			0		
Grade 2	2	1	(50)		1	(50)	
Grade 3	12	6	(50)		6	(50)	
Extranodal invasion of lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.214	4	(14)	.035
Absent	7	3	(43)		3	(43)	
Present	8	4	(50)		4	(50)	
Squamous cell carcinoma in lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.024	4	(14)	.002
Absent	10	3	(30)		3	(30)	
Present	5	4	(80)		4	(80)	
Adenocarcinoma with spindle cell differentiation in lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.159	4	(14)	.020
Absent	14	6	(43)		6	(43)	
Present	1	1	(100)		1	(100)	
Adenosquamous carcinoma in lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.554	4	(14)	.163
Absent	12	6	(50)		6	(50)	
Present	3	1	(33)		1	(33)	
Carcinosarcoma in lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.610	4	(14)	.199
Absent	13	7	(54)		7	(54)	
Present	2	0			0		
Tumor stroma in lymph node-metastatic tumors (n = 44)							
N0	29	7	(24)	.061	4	(14)	.032
None	7	3	(42)		3	(43)	
Mild	1	1	(100)		1	(100)	
Moderate	3	0			0		
Severe	2	2	(100)		2	(100)	
UICC pTNM stage (n = 44)							
I	11	2	(18)	.044	0		.003
IIA	16	4	(25)		3	(19)	
IIB	6	2	(33)		2	(33)	
IIIA	4	1	(25)		1	(25)	
IIIB	5	4	(80)		4	(80)	
IIIC	2	1	(50)		1	(50)	

Abbreviations: NA, not available; Squamous, squamous cell carcinoma; Adenoca with spindle, adenocarcinoma with spindle cell differentiation; Adenosquamous ca, adenosquamous carcinoma; Ca with chondroid, carcinoma with chondroid metaplasia; Ca with osseous, carcinoma with osseous metaplasia; pN, pathologic regional lymph node; N0, no nodal metastasis; N1, 1 to 3 nodal metastases; N2, 4 to 9 nodal metastases; N3, 10 or more nodal metastases; pTNM, pathologic TNM.

for patients with IDC or patients with ILC, these findings strongly suggest that the biological characteristics of MPCs are quite different from those of IDCs or ILCs [16,21-25]. Four previous studies have investigated whether a significant difference in the survival period exists between patients with MPC and those with IDC [8,15,18,19]. The statistical analyses for survival in these studies, which produced controversial results regarding the survival of patients with MPC, were performed using a matched control case analysis, not a consecutive case analysis; and

Table 5 Multivariate analyses for tumor recurrence and tumor-related death in patients with MPC

	Tumor recurrence		Tumor-related death	
	HRs 95% CI	*P* value	HRs 95% CI	*P* value
Skin invasion				
Absent	Referent		Referent	
Present	24.8 5.4-112.1	<.001	39.1 5.0-309.2	<.001
Age, y				
>39	Referent		Referent	
≤39	14.1 3.1-65.3	<.001	34.4 4.4-269.9	<.001
Squamous cell carcinoma in lymph node-metastatic tumors				
N0 and absent	Referent		Referent	
Present	2.2 0.9-5.3	.087	5.6 1.6-19.4	.006

NOTE. Tumor recurrence: adjusted for skin invasion, age, neoadjuvant therapy, and squamous cell carcinoma in lymph node-metastatic tumors. Tumor-related death: adjusted for skin invasion, age, squamous cell carcinoma in lymph node-metastatic tumors, neoadjuvant therapy, occupied area of tumor necrosis, UICC pN category, histologic grade of lymph node-metastatic tumors, extranodal invasion, adenocarcinoma with spindle cell differentiation in lymph node-metastatic tumors, and tumor stroma in lymph node-metastatic tumors.

the periods during which the patients with MPC and the patients with IDC were operated on also differed [8]. The results of the present study were obtained using consecutive cases treated during the same period; our findings clearly demonstrated that MPCs are associated with a significantly higher rate of tumor recurrence or tumor death than IDCs or ILCs, independent of the nodal status, age not exceeding 39 years, adjuvant therapy status, or neoadjuvant therapy status. Thus, we can conclude that MPCs have a greater malignant biological potential than IDCs or ILCs. Furthermore, the triple-negative MPCs observed in this study had more aggressive characteristics than the triple-negative IDCs and the triple-negative ILCs, whereas the triple-negative ILCs had greater malignant biological characteristics than the triple-negative IDCs; these findings strongly suggest that studies on outcome predictors or targeted therapies for triple-negative breast carcinoma should be performed according to the specific type of triple-negative breast carcinoma. Because some genes are selectively expressed in patients with MPC but not in patients with other types of breast carcinomas [13,16,24,25], the development of neoadjuvant therapy or adjuvant therapy targeting such genes may improve the outcome of patients with MPC.

At the beginning of this study, we speculated that the MPC type, such as epithelial versus mixed or squamous versus others, might be significantly associated with the outcome of patients with MPC. However, the results of this study clearly demonstrated that the MPC subtype had no significant effect on the outcome of patients with MPC, confirming the results of previous studies [8,11,13,26]; instead, the most important outcome predictors for patients with MPC were the presence of skin invasion, an age not exceeding 39 years, and the presence of a squamous cell carcinoma component in tumors in the lymph nodes. Consequently, these 3 factors appear to be important prognostic factors for patients with MPC; and the results of this study confirm that the WHO classification for MPC, which contains both epithelial and mixed types of MPC [1], is a reasonable classification for patients with MPC from the viewpoint of patient outcome. Because of the relatively small number of cases of each MPC subtype, however, this study was unable to investigate whether important clinicopathologic predictors of outcome exist for specific MPC subtypes, such as low-grade adenosquamous carcinoma versus high-grade adenosquamous carcinoma and fibromatosis-like low-grade carcinosarcoma versus high-grade carcinosarcoma. Therefore, the clinicopathologic outcome predictors for each MPC subtype should be separately investigated in the future.

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