Cathepsin B Expression and Survival in Colon Cancer: Implications for Molecular Detection of Neoplasia

Running Title: Cathepsin B and Colon Cancer Survival

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Abbreviations: BMI, body mass index; CI, confidence interval; CIMP, CpG island methylator phenotype; COX-2, cyclooxygenase-2; CTSB, cathepsin B; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable.

ABSTRACT

Background and Aims: Proteases play a critical role in tumorigenesis and are upregulated in colorectal cancer and neoplastic polyps. In animal models, cathepsin B activatable imaging agents demonstrate high enzyme activity within intestinal tumors.

Methods: We conducted a prospective cohort study of 558 men and women with colon cancer with tumors that were accessible for immunohistochemical assessment. We used Cox proportional hazards models, stratified by stage, to compute colon cancer-specific and overall mortality according to tumoral expression of cathepsin B.

Results: Among 558 participants, 457 (82%) had tumors that expressed cathepsin B (CTSB-positive) and 101 (18%) had tumors that did not express cathepsin B (CTSB-negative). Cathepsin B expression was not associated with disease stage (P=0.19). After a median follow-up of 11.6 years, there were 254 total and 155 colon cancer-specific deaths. Compared with participants with CTSB-negative tumors, participants with CTSB-positive tumors experienced a multivariate hazard ratio for colon cancer-specific mortality of 1.99 (95% CI, 1.19-3.34) and overall mortality of 1.71 (95% CI, 1.16-2.50). Cathepsin B expression was independently associated with *KRAS* (p=0.01) and *BRAF* mutation (p=0.04), but not MSI status, CIMP status, *PIK3CA* mutation, LINE-1 methylation, p53 expression, or COX-2 expression. Among 123 individuals with adenomas, 91% expressed cathepsin B.

Conclusions: As assessed by immunohistochemistry, cathepsin B is expressed in the vast majority of colon cancers, independent of stage, and is significantly associated with higher risk of colon cancer-specific and overall mortality.

Impact: These results support the potential of cathepsin B as a target for image detection of neoplastic lesions in humans.

Keywords: colon cancer; cathepsin B; near infrared

INTRODUCTION

Proteases play a critical role in tumorigenesis by facilitating rapid cell cycling, mediating local invasion, fueling angiogenesis, and promoting metastasis (1). Specifically, cathepsin B (CTSB, the HUGO-approved official gene symbol), a lysosomal cysteine protease, has been shown to be involved in tumor initiation, hyperproliferation, and de-differentiation and is up-regulated in early human colon adenomas, carcinomas and metastasic lesions (2-7). The central role of CTSB in carcinogenesis suggests that it is not only a promising target for therapy or chemoprevention, but for molecular detection of neoplasia (8).

In previous work, we developed a novel class of optical imaging agents that are "smart" near infrared (NIRF) protease-activatable agents that become brightly fluorescent in areas of increased CTSB expression, as is seen in colorectal neoplasia (9, 10). These agents offer high tumor to background ratio compared to non-specific agents, due to their selective activation. The agents are optically silent in their native (quenched) state and become highly fluorescent after enzyme-mediated release of fluorochromes, resulting in *in vitro* signal amplification of several hundred-fold. In $Apc^{Min/+}$ mice, immunohistochemistry and fluorescent antibody microscopy show that CTSB is expressed through the adenoma in epithelial and stromal cells (8). When mice were injected intravenously with the cathepsin-activatable agent, adenomas became highly fluorescent, indicative of high protease activity, and were easily visualized with a target-to-background ratio of 9:1 using NIRF imaging as opposed to 1:1 for standard white light imaging (8).

Given this promising preclinical data, we therefore examined the importance of CTSB in human colonic carcinogenesis by determining the overall prevalence of CTSB expression in

human colon tumors. Second, given the key role of CTSB in the pathogenesis of tumor growth and invasion, we specifically assessed the relationship between CTSB expression on prognosis in relation to other important tumoral molecular markers in colon cancer.

MATERIALS AND METHODS

Study Population

The Nurses' Health Study (NHS) was established in 1976 when 121,701 U.S. female registered nurses, 30-55 years of age, completed a mailed questionnaire. The Health Professionals Follow-up Study (HPFS) was established in 1986 as a parallel cohort of 51,529 U.S. male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians, who were 40-75 years of age at entry. In each cohort, with a follow-up rate of 92%, we have mailed biennial questionnaires to update information and identify new cases of cancer.

On each biennial follow-up questionnaire, participants were asked whether they had had a diagnosis of colon cancer during the prior two years. When a participant reported a diagnosis of colon cancer, we asked for permission to obtain hospital records and pathology reports. Study physicians, blinded to exposure data, reviewed all medical records related to colon cancer, classifying disease stage according to the 6th version of the American Joint Committee on Cancer. We have previous described our procurement of colon cancer paraffin-embedded specimens in detail (**Supplementary Materials**) (11). For this analysis, we included the 558 participants (366 women from NHS and 192 men from HPFS) with pathologically-confirmed colon adenocarcinoma that were diagnosed through 2004 and for whom we were able to obtain sufficient amounts of tumor tissue for immunohistochemistry. Baseline characteristics among participants with colon cancer with available tissue for analysis were largely similar to those without available tissue. We excluded participants if they had reported any cancer (other than non-melanoma skin) previous to colon cancer diagnosis. We also requested paraffin embedded tissue samples of colorectal polyps that were greater than or equal to 1 cm in diameter on

endoscopy among a separate cohort of 123 men and women enrolled in these cohorts. The institutional review boards at the Brigham and Women's Hospital and the Harvard School of Public Health approved this study.

Immunohistochemical Assessment and Molecular Assays

We performed immunohistochemistry of CTSB, COX-2, and p53 from tissue microarrays of our tumor specimens (**Supplementary Methods**) (**Figure 1**) (11, 12). A pathologist (Y.B.), blinded to any other participant data, recorded cytoplasmic CTSB expression as absent, weak, moderate or strong expression. Among the 558 tumors, 101 tumors showed no CTSB expression, 253 showed weak expression, 184 showed moderate expression, and 20 showed strong expression. In our initial exploratory analysis, we did not observe a significant relationship between CTSB levels (negative, weak, moderate, or strong) and other molecular and clinical features ($P>0.05$). In our previous data from murine models, we demonstrated the ability of NIRF cathepsin-specific molecular agents to identify tumors with weak to strong levels of CTSB expression using immunohistochemistry. Thus, for further analysis in this study, we defined tumors with weak to strong cytoplasmic expression of CTSB as CTSB-positive and tumors with absent cytoplasmic expression of CTSB as CTSB-negative (**Figure 1**). A random sample of cancers were reread by a second pathologist and the concordance between readers was 0.92 ($\kappa=0.62$, $p<.001$, $N=108$) for COX-2, 0.87 ($\kappa=0.75$, $p<.0001$, $N=108$) for p53, and 0.87 ($\kappa=0.62$, $p<.001$, $N=364$) for CTSB. Methylation analyses, sequencing of *KRAS*, *BRAF* and *PIK3CA,* and microsatellite instability (MSI) analysis have each been previously described (**Supplementary Materials**) (13-21).

Ascertainment of Death

We included deaths that occurred after diagnosis of colon cancer and before June 1, 2008. We identified deaths through the National Death Index and next-of-kin. Mortality follow-up was more than 98 percent complete (22). For all deaths, we seek information to determine the cause, including death certificates and, when appropriate, request permission from next-of-kin to review medical records.

Statistical Analysis

As in our prior analysis (11), we pooled data from both cohorts and tested for heterogeneity using the Q statistic. We observed no heterogeneity between the cohorts regarding the association of CTSB and colon cancer-specific survival (p=0.75 for Cochran's Q test) (23). For categorical data, the chi-square test was performed. To assess independent relations of CTSB expression with other variables, a multivariate logistic regression analysis was performed (**Supplementary Materials**). For survival analyses, participants eligible for analysis accrued follow-up time beginning on the month of their diagnosis of colon cancer and ending on the month of death from colon cancer, death from any cause, or June 1, 2008, whichever came first. We categorized participants according to CTSB-positive vs. CTSB-negative. We used Kaplan-Meier curves and the log-rank test to compare colon cancer-specific and overall mortality according to CTSB expression. To assess the effect of CTSB independent of stage, we used Cox proportional hazards modeling with tumor stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown) as a stratifying variable, which enabled us to avoid residual confounding and overfitting. To further adjust for other potential confounding variables, we constructed a multivariate, stage-matched Cox proportional hazards model (**Supplementary Materials**). For all analyses, we used SAS

version 9.1.3 (Cary, NC). All p values are two-sided and a level of significance <0.05 was considered statistically significant.

RESULTS

Among the 558 eligible participants with colon cancer, we documented 254 total deaths with 155 deaths due to colon cancer. For participants who are alive, the median time of follow-up from date of diagnosis was 11.6 years (interquartile range, 3.9-26.3 years). Four hundred and fifty-seven (82%) of participants had cancers that expressed CTSB (CTSB-positive) and 101 (18%) had cancers that did not express CTSB (CTSB-negative). Baseline clinical characteristics of the participants are shown in **Table 1**. Compared to participants with CTSB-negative tumors, participants with CTSB-positive tumors were more likely to be male. Of note, CTSB expression did not vary according to stage (p=0.19). The number and percentage with CTSB expression are as follows: stage I, 87/108 (81%); stage II, 166/194 (86%); stage III, 114/150 (76%); stage IV, 69/81 (85%).

A comparison of other molecular features in colon cancers according to expression of CTSB is shown in **Table 2**. Compared with participants with CTSB-negative tumors, participants with CTSB-positive tumors were more likely to have an activating *KRAS* mutation. In contrast, there did not appear to be a significant difference in microsatellite instability (MSI) status, CpG island methylator phenotype (CIMP) status, *BRAF* mutation, *PIK3CA* mutation, LINE-1 level, p53 expression, or COX-2 expression. In a multivariate model adjusting for both clinical and molecular features, participants with CTSB-positive cancers had 2.47 (95% CI, 1.25-4.88) higher risk of having a *KRAS* mutation and 2.47 (95% CI, 1.00-5.92) higher risk of having a *BRAF* mutation (**Table 3)**.

CTSB expression was associated with a significant increase in risk colon-cancer specific mortality (log-rank p=0.02; **Figure 2A**) and an increase in overall mortality (log-rank p=0.005;

Figure 2B). This relationship remained largely unchanged even after adjusting for stage or other predictors of cancer recurrence (**Table 4**). Compared with participants who had CTSB-negative cancers, the multivariate HR associated with having a CTSB-positive cancer was 1.99 (95% CI, 1.19-3.34) for colon-cancer specific mortality and 1.71 (95% CI, 1.16-2.50) for overall mortality. Additionally adjusting for MSI status, CIMP status, *BRAF* mutation, *PIK3CA* mutation, LINE-1 level, p53 expression, or COX-2 expression did not materially alter these findings (multivariate HR, 2.20, 95% CI, 1.15-4.20 for colon-cancer specific mortality and multivariate HR, 1.89, 95% CI, 1.18-3.00 for overall mortality).

We examined whether the influence of CTSB expression on colorectal cancer-specific survival was modified by any of the clinical, pathologic and molecular variables. We did not observe a significant interaction between CTSB expression and any of the covariates (all $P_{interaction}>0.16$). Notably, the effect of CTSB did not significantly differ between the two independent cohort studies ($P_{interaction}=0.71$). In addition, there was no significant interaction between CTSB and tumor stage ($P_{interaction}=0.98$) or tumor location ($P_{interaction}=0.85$).

Because CTSB is a promising target for molecular imaging agents in murine models of adenomatous polyps, we also examined the prevalence of CTSB expression in human adenomas. Among a separate group of 123 patients enrolled in these cohorts who had polyps greater than 1 cm in diameter on endoscopy, we found 112/123 (91%) cases expressed CTSB. Among the 101 adenomas in which we had complete size and histological data, we found that CTSB expression was independent of adenoma size (p=0.45) and histology (p=0.28). The number and percentage that were CTSB-positive according to size are as follows: 1.0 to 1.5 cm in diameter, 31/35 (89%); 1.6 cm to 2.0 cm in diameter, 21/26 (81%); greater than 2.0 cm in diameter, 31/40 (78%). The

number and percentage that were CTSB-positive according to histology are as follows: tubular, 54/69 (78%); tubulovillous, 24/26 (92%); villous, 5/6 (83%).

DISCUSSION

In summary, we observed that the CTSB proteases are overexpressed in the vast majority of human colon adenoma and cancers, independent of stage. CTSB expression was significantly associated with an increased risk of colon cancer-specific and overall mortality. Overall, CTSB was not associated with other molecular features of colon cancer except for the presence of *KRAS* and *BRAF* mutations. Our data support the potential for CTSB as a target for molecular detection of neoplasia and therapeutic intervention.

Our data are supported by other studies that have shown that synthesis and secretion of CTSB is increased in the extracellular environment of colon cancers (2, 24). CTSB has been shown to play an essential role in disrupting the extracellular matrix barriers between tumors and surrounding tissue, thereby facilitating invasion and metastasis (8). A number of observations implicate CTSB in tumor progression: 1) inhibitors of CTSB retard metastases and *in vitro* growth (25); 2) genetic modulation of CTSB favorably alters the invasive properties of tumor cells (26); 3) CTSB levels correlate with tumor aggressiveness and angiogenesis (8, 27); 4) serum levels of CTSB are significantly higher in patients with colorectal cancer and adenoma than in tumor-free controls (4, 28, 29). Our results are generally consistent with two previous smaller studies that also demonstrated that increased tissue expression or antigen activity levels of CTSB in colorectal cancer was associated with significantly shorter patient survival (7, 30). In another small study of 60 patients with colorectal cancer, there was a trend toward worsened survival associated with tumor antigen levels of CTSB (5).

Prior studies have observed that CTSB antigen levels or immunohistochemical staining does correlate with advancing Dukes' stage and progression from adenoma to adenocarcinoma (2, 5, 6, 30). However, each of these studies used varying techniques to assay for cathepsin levels

as well as analytic methodology to relate levels to clinicopathological parameters. Thus, it is difficult to directly compare these findings with our study, in which we observed a high prevalence of CTSB expression in all stages of disease, including premalignant adenomas. Previous studies are consistent with our results, demonstrating high levels of CTSB in human colon cancer irrespective of stage (3, 7), as well as in adenomas (2). Moreover, other studies suggest that expression or activity levels may actually peak in early stage cancer and decline with advanced disease (7, 31, 32). The importance of CTSB across stages of neoplasia are also validated by prior data showing uniform CTSB expression and high-enzyme activity in intestinal adenomas generated in mouse models. In a previous study of $Apc^{Min/+}$ mice, we observed, using immunohistochemistry and fluorescent antibody microscopy, that CTSB is ubiquitously expressed in intestinal adenomas, even in microscopic lesions that are difficult to visualize through standard visual inspection. Compared to adjacent normal tissue, adenomas had a 36% higher level of CTSB protein by Western blot and 35% higher level of CTSB mRNA by reverse transcriptase-polymerase chain reaction (8). Similarly, in a comprehensive proteomic screening study, we identified CTSB as one of 6 proteins upregulated in the plasma of tumor-bearing $APC^{\Delta 580}$ mice with concomitant overexpression at the RNA and protein level in adenoma tissue (24). Finally, recent data has shown that genetic ablation of CTSB attenuates polyposis in a hemizygous $APC^{\Delta 468}$ mouse model (33). Taken together, these data support emerging evidence that cathepsins have a role not only in facilitating cancer invasion and metastasis, but also in mediating early, premalignant processes, such as tumor initiation, hyperproliferation and de-differentiation (8, 34).

Interestingly, we found significant relations of CTSB expression with *KRAS* and *BRAF* mutations. Experimental studies have shown a correlation between mutations in *KRAS* and

upregulation in CTSB expression (35, 36), which are in agreement with our findings. *KRAS* and *BRAF* mutations are not only critical events during carcinogenesis, but also important prognostic and/or predictive markers in colon cancer patients. *KRAS* mutational status of stage IV colorectal cancer is a predictive biomarker for anti-EGFR treatment and BRAF mutation identifies a subgroup of patients with unfavorable prognosis (13, 37-41). Further understanding of the relationship between CTSB and mutations in KRAS and BRAF could potentially provide useful information for refinement of therapeutic strategies. In this respect, our findings may be of clinical interest if confirmed by additional studies.

To exploit the potential role of cathepsins in colonic neoplasia, we have developed biocompatible, optically quenched, NIRF imaging agents that release fluorochromes after enzyme activation by tumor-associated cathepsin proteases (9). In our previous study, when we injected $APC^{Min/+}$ mice with this cathepsin-activatable reporter agent and imaged them *ex vivo* with NIRF, intestinal adenomas became highly fluorescent, with a signal intensity consistently higher than background (target-to-background=9:1 with NIRF imaging as compared to 1:1 with standard white light imaging) (8). We have confirmed these results with *ex vivo* imaging in tumor-bearing $APC^{\Delta 580}$ mice (24). Using a novel NIRF microendoscope, we and others have demonstrated the ability of cathepsin-activatable agents to image adenoma and carcinomas *in vivo* among $APC^{Min/+}$ mice, hemizygous $APC^{\Delta 468}$ mice, mice with orthotopically implanted tumors, and APC conditional knockout mice in which an adenovirus expressing cre recombinase infection is focally delivered to the distal colon**.** Several microscopic lesions that were not obviously detectable by white light imaging were visualized in these animal models using cathepsin-activatable agents with NIRF imaging (33, 42-46). Taken together, these data provide proof-of-principle of the potential for targeting cathepsins for early detection of colonic

neoplasia using NIRF endoscopy (8). Thus, human translation of cathepsin-activatable imaging platforms may provide a unique opportunity to improve the broad detection of adenomas.

We acknowledge several limitations of our study. Beyond causes of mortality, data on cancer recurrences were not available in this cohort. Nonetheless, since median survival for recurrent (metastatic) colon cancer was approximately 10 to 12 months during much of the time period of this study (47), colon cancer-specific mortality should be a reasonable surrogate for cancer-specific outcome. In this cohort, we also had limited data on chemotherapy. However, it is unlikely that differential receipt of chemotherapy could explain the observed findings. First, it is unlikely that chemotherapy use differed according to tumoral CTSB expression since such data were not available to patients or treating physicians. Second, the association of CTSB and survival was similar among participants with stage 1 or 2 disease, for which surgery alone would be represent a standard of care, and among those with stage 3 cancer, for which adjuvant chemotherapy would represent a routine approach. Third, since our cohort consisted of health professionals, considerable heterogeneity in use of adjuvant chemotherapy would be unlikely.

We were unable to obtain tumor tissue on all cases of confirmed colon cancer over follow-up. However, it is unlikely that CTSB or mortality would be differential according to retrieval success. Moreover, an assessment of risk factors did not appreciably differ among those participants for whom we were unable to obtain tumor tissue (11). Finally, we did not assay other proteases which may also be important in colorectal cancer, such as cathepsin L (5). We focused on CTSB given the greater near-term potential to exploit targeting of this specific enzyme using an already developed molecular imaging probe.

Consistent with the well-established role of proteases in facilitating tumor invasiveness and spread, our findings demonstrate that CTSB expression is significantly associated with an

increased risk of colon cancer-specific and overall mortality. In addition, we demonstrate that CTSB is expressed in the vast majority of human colon adenoma and cancers of all stages, supporting the possible role of CTSB in early alterations leading to tumor formation. These results support the potential for exploiting CTSB as a target for imaging agents with specificity for their enzymatic activity. The feasibility of using such CTSB-specific probes with fluorescent endoscopy has been validated in multiple animal models; adapting this technology for humans may be a promising adjunct to current endoscopic approaches to colon cancer screening and surveillance.

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Table 1. Clinical and pathologic features of colon cancer according to cathepsin B (CTSB) expression

Clinical or pathologic feature	Total N	CTSB (-)	CTSB (+)	P value
All cases	558	101	457	
Gender				0.02
Men	192 (34%)	25 (25%)	167 (37%)	
Women	366 (66%)	76 (75%)	290 (63%)	
Mean age ± SD	67.2 ± 8.2	67.8± 8.8	67.1± 8.1	0.43
Body mass index (BMI, kg/m^2)				0.58
<30	459 (82%)	85 (84%)	374 (82%)	
≥30	99 (18%)	16 (16%)	83 (18%)	
Family history of colorectal cancer in any first degree relative				0.27
(-)	418 (75%)	80 (79%)	338 (74%)	
(+)	140 (25%)	21 (21%)	119 (26%)	
Tumor location				0.87
Right	344 (62%)	63 (62%)	281 (61%)	
Left	214 (38%)	38 (38%)	176 (39%)	
Stage				0.19
I	108 (19%)	21 (21%)	87 (19%)	
II	194 (35%)	28 (28%)	166 (36%)	
III	150 (27%)	36 (36%)	114 (25%)	
IV	81 (15%)	12 (12%)	69 (15%)	
missing	25 (4.5%)	4 (4.0%)	21 (4.6%)	
Tumor grade				0.28
Low	500 (90%)	87 (87%)	413 (91%)	
High	56 (10%)	13 (13%)	43 (9.4%)	
Mucinous component				0.54
0%	269 (52%)	45 (51%)	224 (53%)	
>0%	245 (48%)	46 (49%)	199 (47%)	
Signet ring cell component				0.24
0%	428 (88%)	76 (84%)	352 (89%)	

>0%	58 (12%)	14 (16%)	44 (11%)

Table 2. Molecular features of colon cancer according to CTSB expression

Molecular feature	Total N	CTSB (-)	CTSB (+)	P value
MSI status				0.84
MSI-low/MSS	319 (79%)	45 (78%)	274 (79%)	
MSI-high	87 (21%)	13 (22%)	74 (21%)	
CIMP status				0.49
CIMP-low/0	326 (81%)	45 (78%)	281 (81%)	
CIMP-high	77 (19%)	12 (22%)	64 (19%)	
BRAF mutation				0.43
(-)	340 (84%)	50 (88%)	290 (84%)	
(+)	64 (16%)	7 (12%)	57 (16%)	
KRAS mutation				0.01
(-)	254 (63%)	44 (77%)	210 (60%)	
(+)	152 (37%)	13 (23%)	139 (40%)	
PIK3CA mutation				0.76
(-)	300 (84%)	42 (86%)	258 (84%)	
(+)	56 (16%)	7 (14%)	49 (16%)	
LINE-1 methylation level (Mean ± SD)	60.4 ± 9.2	61.7 ± 9.4	60.2 ± 9.2	0.25
p53 expression				0.63
(-)	264 (65%)	40 (68%)	224 (65%)	
(+)	142 (35%)	19 (32%)	123 (35%)	
COX-2 expression				0.72
(-)	156 (38%)	21 (36%)	135 (39%)	
(+)	251 (62%)	37 (64%)	214 (61%)	

CTSB, cathepsin B; CIMP, CpG island methylator phenotype; COX-2, cyclooxygenase-2; MSI, microsatellite instability; MSS, microsatellite stable; LINE-1, long interspersed nuclear elements-1.

Table 3. Multivariate analysis of the relationship of other molecular features with CTSB expression in colon cancer

Variables in the final model for CTSB	Multivariate OR (95% CI)	P value
KRAS mutation	2.47 (1.25-4.88)	0.009
BRAF mutation	2.47 (1.00-5.92)	0.049
Male gender	1.85 (0.96-3.56)	0.065
LINE-1 hypomethylation (for a 30% decrease)	2.35 (0.85-6.49)	0.098

Multivariate logistic regression analysis initially included age, sex, body mass index, tumor location, stage, tumor grade, mucinous component, signet ring cell component, MSI, CpG island methylator phenotype, COX-2, p53, LINE-1 methylation, *KRAS, PIK3CA*, and *BRAF*. Backward stepwise elimination with threshold of P=0.20 was used to select variables in the final model. CTSB, cathepsin B; COX-2, cyclooxygenase-2; MSI, microsatellite instability; MSS, microsatellite stable; LINE-1, long interspersed nuclear elements-1; OR, odds ratio; CI, confidence interval;

Table 4. CTSB expression in colon cancer and overall mortality

CTSB expression	Total N	Colon cancer-specific mortality				Overall mortality			
		Deaths / person-years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)	Deaths / person-years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)
CTSB (-)	101 (18%)	18/936	1 (referent)	1 (referent)	1 (referent)	32/936	1 (referent)	1 (referent)	1 (referent)
CTSB (+)	457 (82%)	137/3787	1.82 (1.11-2.97)	1.69 (1.03-2.79)	1.99 (1.19-3.34)	222/3787	1.69 (1.17-2.45)	1.56 (1.07-2.27)	1.71 (1.16-2.50)
P value			0.018	0.038	0.009		0.0057	0.021	0.0063

The multivariate, stage-matched conditional Cox regression model initially included age, year of diagnosis, sex, family history of colorectal cancer, body mass index (BMI), tumor location, grade, mucinous component, and signet ring cell. Backward stepwise elimination with threshold of P=0.20 was used to select variables in the final model. HR, hazard ratio; CI, confidence interval;

Figure 1. CTSB expression in colon cancer.

Figure 1. CTSB expression in colon cancer.
A, B Strong expression of CTSB in colon cancer cells (white arrows).
C, D Weak expression of CTSB in colon cancer cells (white arrowheads).
E, F Negative expression of CTSB in colon cancer cells (black arrows). Stromal cells serve as an internal positive control for CTSB expression (arrowhead).
(**A**,**C**,**E**: low magnification, **B**,**D**,**F**: high magnification)

Figure 2. Survival of colon cancer patients according to CTSB expression status.

A. Colon cancer-specific survival **B.** Overall survival

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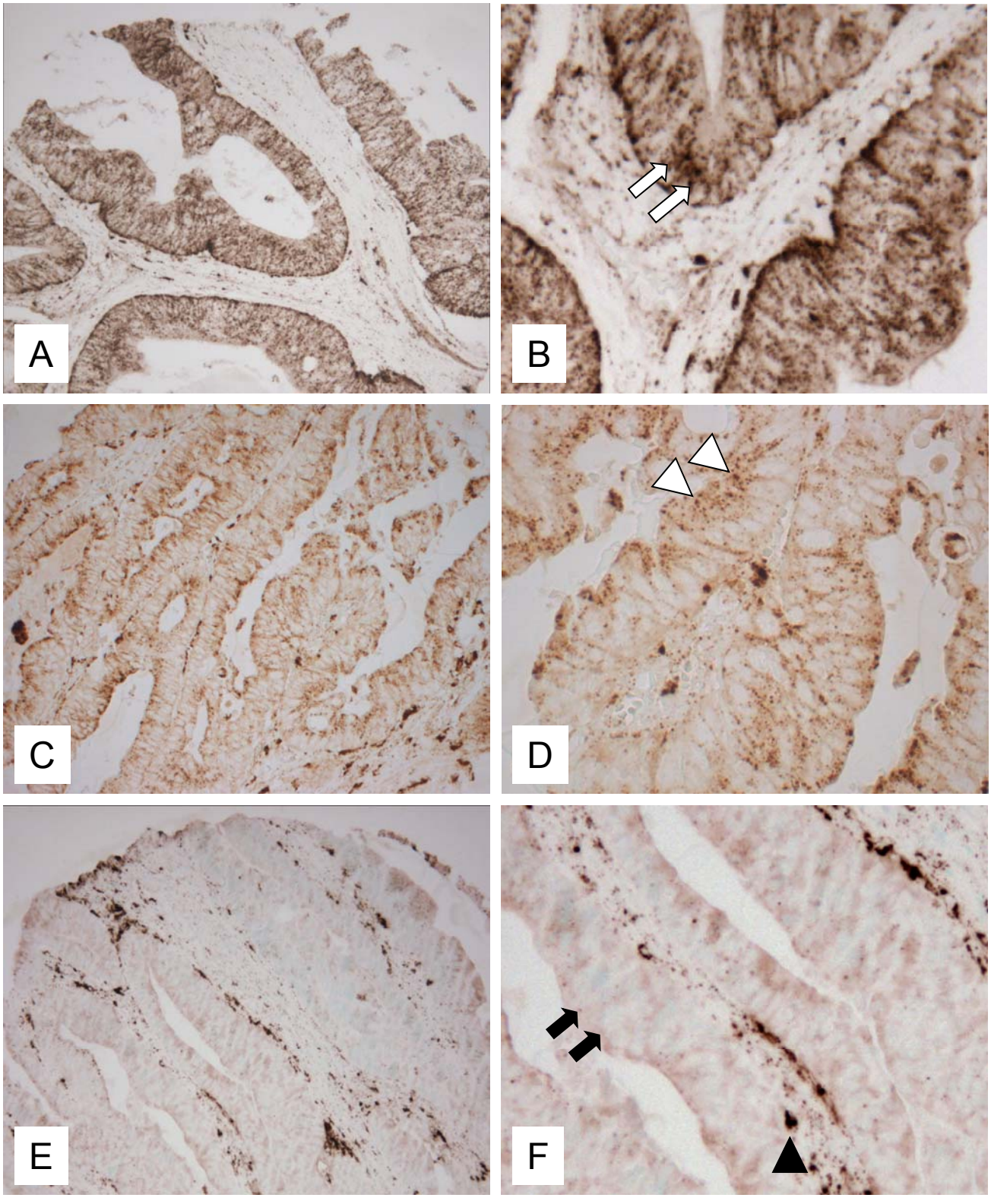

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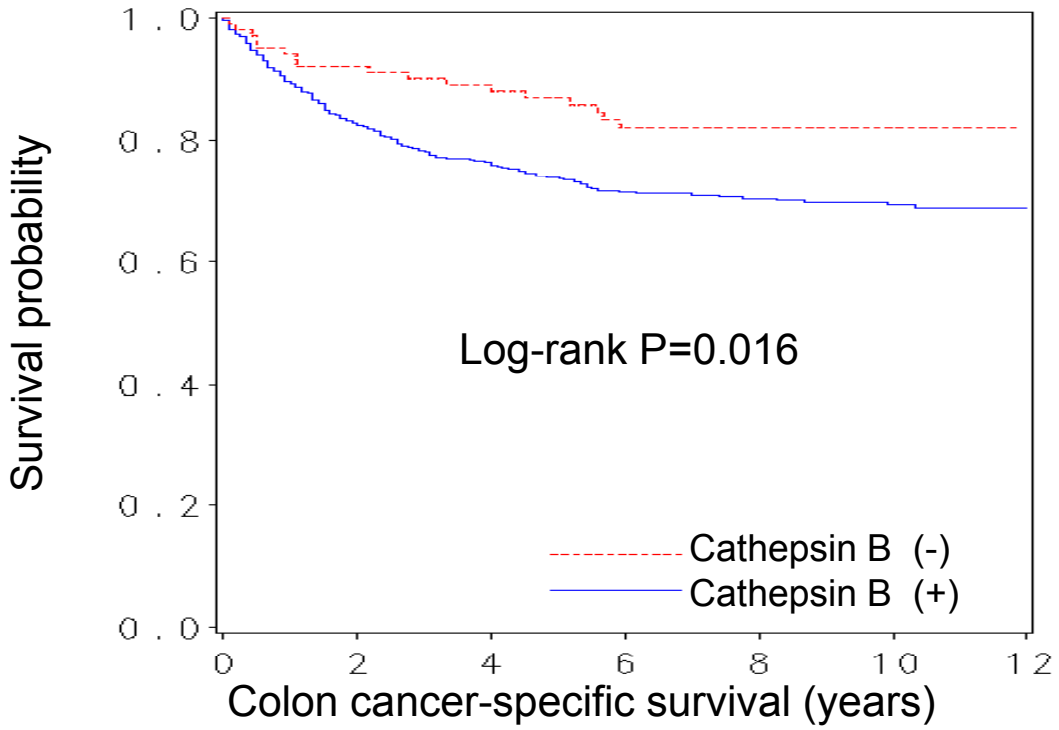

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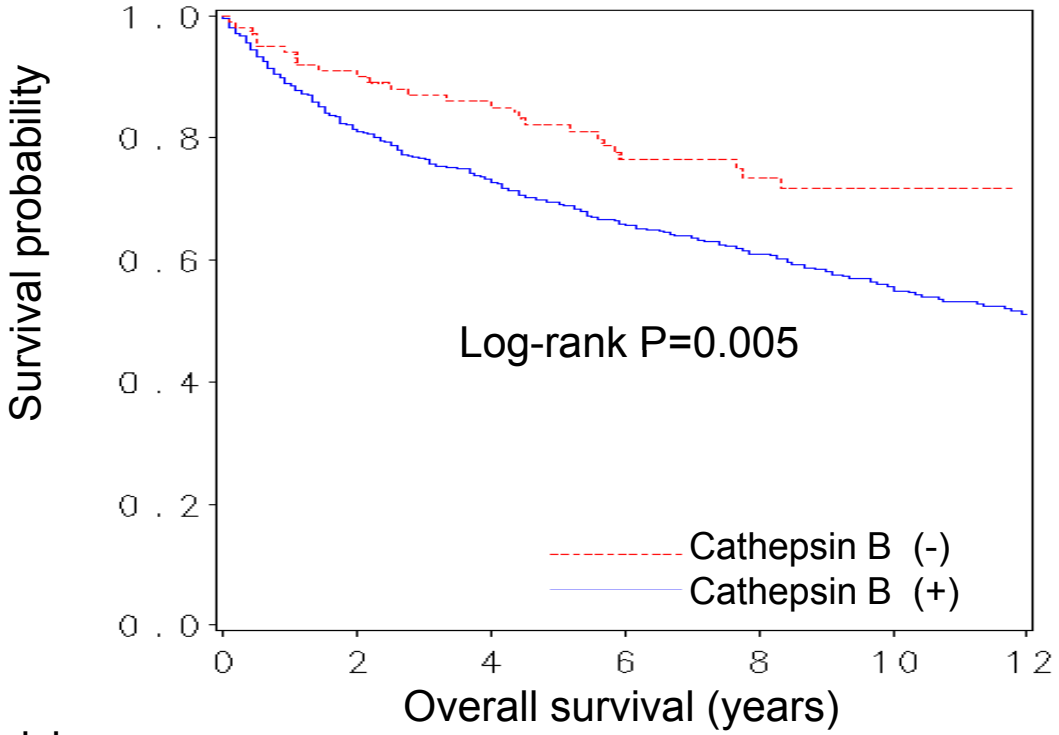

Number at risk

Year	0	2	4	6	8	10
Cathepsin B (-) ·····	101	91	85	63	45	39
Cathepsin B (+) ——	457	371	334	284	230	164

Cancer Epidemiology, Biomarkers & Prevention

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Cathepsin B Expression and Survival in Colon Cancer: Implications for Molecular Detection of Neoplasia

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