p66 Shc Tumor Levels Show a Strong Prognostic Correlation with Disease Outcome in Stage IIA Colon Cancer

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Abstract

Purpose: Most stage IIA colon cancer patients receive no adjuvant therapy despite an estimated 15% risk of disease-related death within 5 years of resection. Prognostication of disease outcome would benefit the clinician by categorizing patients with stage IIA disease by risk. The abundance of the signal transduction proteins p66 Shc and tyrosine-phosphorylated (PY)-Shc in tumor cells is a prognostic indicator of disease outcome in breast cancer, suggesting that Shc analysis may provide prognostic information in stage IIA colon cancer.

Experimental Design: Immunohistochemical staining of p66 Shc and PY-Shc was examined in resection specimens from 240 chemotherapy-naïve patients with stage IIA ($T_3N_0M_0$) colon cancer from two independent (130 and 110 cases, respectively) retrospective cohorts. Staining was scored on a 0 to 5 scale and correlated with relapse-free survival and disease-specific survival in a multivariate analysis to obtain hazard ratios (HR) for both outcomes.

Results: In a pooled analysis of both cohorts, p66 Shc score was a significant prognostic indicator of relapse-free survival (full-range HR, 13.0; $P = 0.012$) and disease-specific survival (full-range HR, 36.6; $P = 0.004$) when analyzed as a continuous variable in a multivariate Cox proportional hazards model stratified by study site and adjusted for age, sex, grade, and lymphovascular involvement. PY-Shc in this multivariate Cox model, however, did not achieve statistical significance for either outcome.

Conclusions: Measuring p66 Shc tumor levels provides a unique and simple tool for stratifying stage IIA colon cancer patients by risk of recurrence and disease-specific death and may assist in determining treatment strategies for these patients.

Colon cancer is the third most commonly diagnosed cancer in the United States, with 112,340 new cases estimated in the year 2007, and is the second most common cause of cancer-specific mortality, estimated to be ~49,000 deaths in 2007 (1, 2). Despite significant advances in recent years that have increased survival for both early-stage and late-stage patients (3), the optimal treatment of the second most common subset of early-stage patients—those with $T_{3\text{-}4}N_0M_0$ or stage II disease—remains unclear (4). For those patients who have had resection of a primary tumor but have no detectable metastatic disease, it is clear that adjuvant chemotherapy carries added benefit in those cases where malignant cells are found in regional lymph nodes ($T_xN_{1\text{-}3}M_0$, stage III; ref. 5). A number of regimens, including 5-fluorouracil/leucovorin, capecitabine, and oxaliplatin/leucovorin/5-fluorouracil (FOLFOX), all seem to increase disease-free and overall survival in stage III colon cancer (4, 6).

However, for patients who have had complete resection of stage II ($T_{3\text{-}4}N_0M_0$) tumors, in which the risk of disease-specific death following recurrence is still ≥15% (5, 7, 8), there remains a dilemma about whether adjuvant therapy provides benefit commensurate to its risk and inconvenience. A number of randomized studies and meta-analyses of those studies have addressed the specific role of adjuvant 5-fluorouracil–based chemotherapy as compared with observation alone in the treatment of stage II colon cancer, and none have shown a statistically significant benefit for adjuvant chemotherapy for increasing either disease-free survival or overall survival (4). More recently, in the MOSAIC study (6), wherein both stage II and stage III patients were randomized to receive oxaliplatin-based chemotherapy versus 5-fluorouracil/leucovorin chemotherapy, subset analysis of the stage II patients revealed a 3% increase in 3-year disease-free survival in those who received the oxaliplatin regimen, which achieved significance. For those

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stage II patients with adverse characteristics, such as T_4 lesion, perforation, or obstruction, the benefit was further increased to 5%, on par with the benefit seen in stage III patients for oxaliplatin-based therapy (4). Thus, the current consensus supports the use of adjuvant chemotherapy for those stage II patients with adverse characteristics but leaves open the question about the optimal management of those patients with $T_3N_0M_0$ tumors without adverse characteristics (9).

Shc is a signal transduction adaptor molecule that integrates the function of multiple growth control signaling pathways, most importantly the Ras-mitogen-activated protein kinase, phosphatidylinositol 3-kinase-AKT, and apoptotic pathways (10, 11). Tumor expression of the p66 Shc isoform has previously been reported to inversely correlate with an increased risk of disease recurrence in early-stage breast cancer (12) as well as response to chemotherapy.[9] Activated Shc is tyrosine phosphorylated, and this tyrosine-phosphorylated (PY)-Shc isoform has likewise been associated with breast cancer recurrence in early-stage patients (12). In the present study, we have performed a retrospective analysis of two independent cohorts of stage IIA colon cancer patients to determine if p66 Shc or PY-Shc staining intensity of the primary tumor is prognostic of disease recurrence or disease-specific survival (DSS). The results of these analyses are reported herein.

Materials and Methods

Cohorts. Formalin-fixed paraffin-embedded primary tumors from 130 patients diagnosed between 1983 and 1994 with stage IIA ($T_3N_0M_0$) colon cancer were obtained from the archives of the Pathology Department, Rhode Island Hospital (Providence, RI). All patients were treatment-naïve following surgical resection of the tumor. No rectal tumors were included. The samples were prepared in tissue microarray format as 1-mm cores, with typically three to six representative cores from each sample. Average follow-up time for this cohort was 7.2 years (median, 6.95 years; range, <1-17 years). Of the 120 patients for whom recurrence status was known, 18 (15%) relapses were reported, and of the 121 patients for whom vital status was known, 19 (15%) deaths from colon cancer were reported. Non-relapsing patients had a mean follow-up of 8.4 years and relapsing patients had a mean follow-up of 3.1 years.

A second cohort of formalin-fixed paraffin-embedded primary tumors from 110 patients diagnosed between 1995 and 2000 with stage IIA ($T_3N_0M_0$) colon cancer were obtained from the archives of the Pathology Department, University of Massachusetts Cancer Center (Worcester, MA). No rectal tumors were included in this cohort. Following surgical resection of the tumor, none of the patients in this cohort received adjuvant therapy. Whole tissue sections were used for this study. Average follow-up time for this cohort was 4.2 years (median, 4.1 years; range, <1-8.9 years). Twenty-six (23.6%) recurrences and 3 (2.7%) disease-specific deaths were reported from this 110-patient cohort. Of the 26 patients with a documented recurrence, a total of 8 (7.2%) died, although only 3 were officially characterized as actual disease-specific death. The cause of death in the other cases may have been related to disease recurrence or simply a result of the overall health of the patient before initial diagnosis, which may potentially be extrapolated from the official cause of death: cardiac arrest (ages 58 and 60 years), sepsis (age 90 years), liver failure due to cirrhosis (age 78 years), or acute respiratory failure (age 84 years). These five patients were coded as dead from other causes. Nonrelapsing patients had a mean follow-up of 4.6 years and relapsing patients had a mean follow-up of 2.8 years.

Antibodies and immunohistochemistry. Characterization of the affinity-purified antibodies to PY-Shc and p66 Shc, immunohistochemical staining procedure (OncoPlan, Catalyst Oncology, Inc.), and the scoring system based on a 0 to 5 continuous scale have previously been described (12, 13). The staining intensity on a scale of 0 to 5 was determined by two independent scorers (S.L. and R.L.) blinded to patient information and outcome. These two independent scorers have evaluated identical samples of invasive breast cancer from more than 500 patients and showed an exceptionally high degree of concordance ($r = 0.976$; $P < 0.0001$).[10] To calculate the final score for each sample, the intensity score (0-5) was multiplied by the fraction of tumor cells staining at each intensity level. These results were then added to arrive at a final score between 0 and 5. Zero is equivalent to no tumor cells staining and 5 equals all tumor cells stained at the maximum intensity level. This method of scoring was used to accurately capture the heterogeneous staining patterns that can be observed within each tumor. A negative tissue control was used to account for nonspecific staining in the assay. Only the staining characteristics of tumor epithelial cells were included in the scores reported by the scorers. Normal colonic mucosa was not evaluated.

Statistical analyses. Patient's age, sex, tumor grade, lymphovascular involvement (LVI), PY-Shc score, and p66 Shc score were collected as study variables. Statistical calculations were done, stratified by cohort, using Intercooled Stata version 9.0 (StataCorp) or SAS for Windows version 9 (SAS Institute, Inc.). End points were defined as the time from surgical removal of the tumor until first clinical recurrence or death from disease. Patients lost to follow-up or without evidence of disease by the last time point were censored in these analyses. Subjects with missing data on any variables in an analysis were excluded from that analysis. The distribution of each variable was compared between two study sites with the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. Relapse-free survival (RFS) and DSS were estimated by the Kaplan-Meier method and evaluated with the use of log-rank test for categorical PY-Shc score and p66 Shc score. Cutoff points for risk stratification were determined by the Monte Carlo method using X-Tile version 3.5.0 (Yale University, New Haven, CT). The Cox proportional hazards model stratified by cohort was used to assess the simultaneous contribution of age, sex, tumor grade, and LVI with PY-Shc and p66 Shc. A two-sided P value of <0.05 was considered to indicate statistical significance.

Results

Immunohistochemical analysis of p66 Shc and PY-Shc in stage IIA colon cancer samples. Two separate cohorts of tumors from patients with resected stage IIA ($T_3N_0M_0$) colon cancer were analyzed by immunohistochemical staining for p66 Shc and PY-Shc, and a Shc score was assigned to each as described in Materials and Methods. Stage IIA colon cancer samples from these two cohorts showed similar heterogeneity in p66 Shc and PY-Shc staining patterns, as has been observed in other tumor types such as breast cancer (12), prostate and gastric cancer, and malignant melanoma.[11] The mean p66 Shc score for these combined cohorts was 1.9 ± 0.8 (range, 0.1-4.7). The mean PY-Shc score for the combined cohorts was 3.0 ± 1.1 (range, 0.2-4.9). Only tumor epithelial cells were scored in each patient case; normal colonic mucosa was not evaluated. The scoring scale for p66-Shc and PY-Shc as derived from a previous study

[9] A.R. Frackelton, Jr., L.J. Hafer, unpublished data.

[10] L.J. Hafer, S. Lyle, R.T. Lis, G. Bhat, A.R. Frackelton, Jr., unpublished observations.

[11] A.R. Frackelton, Jr., L.J. Hafer, R. Lis, E. Sabo, M.B. Resnick, S. Lyle, unpublished observations.

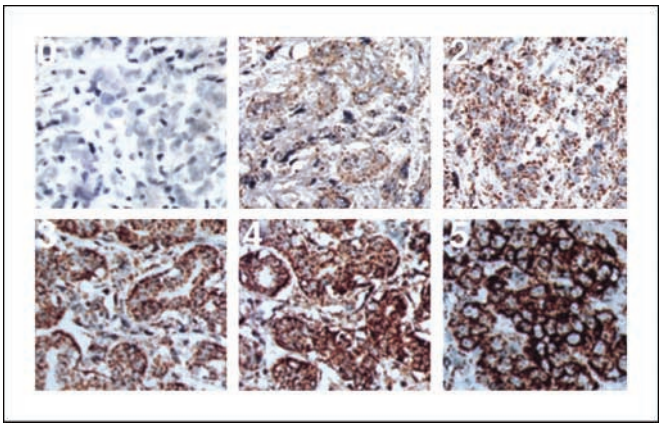

Fig. 1. Representative staining patterns of the 0 to 5 scoring system for p66 (shown) and PY-Shc on breast cancer samples. The score is inset into the top left corner of each photomicrograph. Magnification, ×200. Reprinted with permission from Davol PA. (12). Copyright ©2003 AACR. All rights reserved.

of breast cancer samples (12) is shown in Fig. 1. Representative p66 Shc and PY-Shc staining of two stage IIA colon cancer tumors—one from a patient who relapsed and the other from a patient who did not relapse—are shown in Fig. 2. As can be discerned from the representative photomicrographs in Fig. 2, there is a clear distinction in p66 Shc and PY-Shc staining intensities between these patients' tumors, otherwise matched for age, LVI, and tumor grade, which correlates with their respective clinical outcomes.

To assess the effect of tissue microarray core redundancy on this study and of sampling distribution of PY-Shc and p66 Shc on variability, we conducted a bootstrap resampling analysis for one to four cores of p66 Shc and PY-Shc variance, hazard ratio (HR), and *P* values in Cox proportional hazards models. For p66 Shc, three cores were optimal for HR and *P* value as well as for variance. Considering only two cores per patient, the HR decreased by ~70% and the *P* value decreased from 0.05 to 0.57. When decreased to a single core per patient sample, both the HR and *P* value approached 1.0. No added benefit was observed when the core numbers exceeded three for p66 Shc. With regard to the variability of PY-Shc, no effect of core number was observed in this analysis. This suggests that there was no improvement in variability of PY-Shc regardless of number of samples drawn, and may also imply that PY-Shc levels are more homogeneously distributed in colon cancer tissue as compared with p66 Shc.

Baseline characteristics of stage IIA colon cancer specimens. In each cohort, the incidence of disease recurrence was within the reported range for stage IIA colon cancer (7). With respect to disease-specific death, the University of Massachusetts Memorial Cancer Center (UMMC) cohort as compared with the Rhode Island Hospital (RIH) cohort showed a marked decrease in incidence. This difference, however, may be explained, in part, by the shortened follow-up time available for the UMMC cohort, a potentially robust effect of treatment on disease recurrence, and/or the censoring of 5% of the patients (5% of the UMMC cohort relapsed within the study period but died of other causes before succumbing to colon cancer).

Each cohort was independently analyzed and achieved trending or marginal significance for p66 Shc. PY-Shc did not achieve statistical significance in either cohort. A power analysis incorporating a clinically relevant HR of 0.25 for a protective effect or a HR of 4 for a hazardous effect, a high variability in HR (SD, 0.063), and an event rate of 10% determined that a sample size of 140 patients would be required to detect a significant result ($P < 0.05$) for disease recurrence or disease-specific death with 95% power (NCSS PASS 2002 Software, STATCON).

Statistical comparison of the two retrospective case series showed significant differences in some of the baseline characteristics between the two cohorts, as shown in Table 1. Despite these differences in characteristics between the UMMC and RIH samples, pooling of the data for the purpose of statistical analysis was found to be possible because the effect of p66 Shc was similar in each cohort when independently analyzed within a full multivariate Cox proportional hazards model. In both analyses, a positive association was observed between p66 Shc and disease outcome (hazardous effect).

Additionally, to account for the significant differences in baseline characteristics, all statistical analyses done on the pooled data were stratified by cohort. Stratification of the combined data set by cohort adjusted each individual cohort to its baseline characteristics before proceeding with any statistical analysis of the pooled data, therefore eliminating bias in the analysis.

Univariate analyses of RFS and DSS. As expected from an analysis of the individual cohorts, no significant correlation of the clinicopathologic characteristics (sex, age, LVI, or tumor grade) of the tumors as a pooled data set with PY-Shc or p66 Shc was observed (data not shown). Consistent with observations of the clinical factors from the individual analyses of the cohorts, univariate log-rank analysis of RFS identified significant prognostic values for tumor grade and LVI; however, for DSS, only LVI was identified as having significant prognostic value (Table 2). p66 Shc, as both a continuous and a categorical variable, was a significant prognostic indicator of RFS and DSS. PY-Shc was not statistically significant for either RFS or DSS (data not shown).

Visualization of the relationship between the tumor levels of p66 Shc and the risk of disease recurrence and disease-specific death can be observed using the Kaplan-Meier method (Fig. 3).

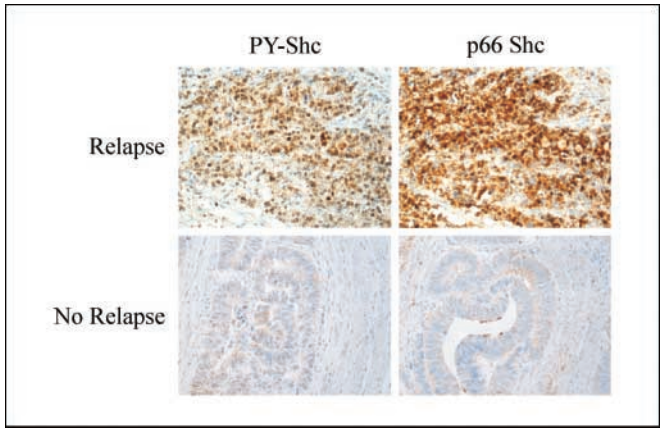

Fig. 2. Representative staining from matched stage IIA colon cancer patients. PY-Shc and p66 Shc staining of colon cancer from a male patient, 79 y of age with a grade 2 tumor and negative for LVI who relapsed (PY-Shc score, 2.5; p66 Shc score, 3.8), and a male patient, 78 y of age with a grade 2 tumor and negative for LVI who did not relapse (PY-Shc score, 1.5; p66 Shc score, 1.0).

Table 1. Clinicopathologic characteristics of patients in the two cohorts

Characteristic	RIH (n = 130)	UMMC (n = 110)	P*
Sex			
Male	62	40	0.09
Female	68	70	
Tumor grade			
Low (1 and 2)	90	92	**0.02**
High (3 and 4)	37	17	
LVI			
No	115	68	**<0.0001**
Yes	12	36	
Age, mean ± SD (y)	74 ± 11	72 ± 12	0.11
p66 Shc, mean ± SD	1.9 ± 0.9	1.9 ± 0.7	0.69

*P is given for comparing the distribution of characteristics of patients between two cohorts. Statistically significant values (P < 0.05) are in boldface.

Optimal cutoff points of the p66 Shc score for predicting disease recurrence and disease-specific death were derived from Monte Carlo analyses using X-tile (see Materials and Methods). Both the binary and ternary variables derived for p66 Shc were analyzed by this method. The binary variable was defined as low risk (p66 Shc score, ≤2.5) and high risk (p66 Shc score, >2.5). Seventy-two percent of the patients in the combined cohorts were classified as low risk and 18% as high risk. A clear and statistically significant separation of the low-risk and high-risk groups was noted for both RFS (83% versus 64% RFS at 6 years; P = 0.006; Fig. 3A) and DSS (92% versus 63% DSS at 6 years; P = 0.0004; Fig. 3B). The ternary variable was defined as low risk (p66 Shc score, ≤1.7), intermediate risk (p66 Shc score, 1.8-2.5), and high risk (p66 Shc score, >2.5). Thirty-seven percent of the patients in the combined cohorts were classified as low risk, 45% as intermediate risk, and 18% as high risk. The ternary model also showed a statistically significant divergence of the Kaplan-Meier plots of the low-risk, intermediate-risk, and high-risk groups for both RFS (87%, 80%, and 64% RFS at 6 years; P = 0.008; Fig. 3C) and DSS (94%, 92%, and 75% DSS at 6 years; P = 0.001; Fig. 3D).

Multivariate analysis for RFS and DSS. The independent ability of the Shc proteins to prognose risk of disease recurrence and disease-specific death for stage IIA colon cancer patients was tested within a base multivariate Cox proportional hazards model (Table 3). p66 Shc retained its prognostic value and strength when tested within the base model, with tumor grade and LVI remaining as significant covariates for RFS and with LVI remaining as a significant covariate for DSS. By partial likelihood ratio analysis, p66 Shc significantly contributed to the prognostic value of the model for RFS and DSS and significantly improved model fit (RFS: partial likelihood ratio $\Delta\chi^2$ = 6.1 Δdf = 1, P = 0.014; DSS: partial likelihood ratio $\Delta\chi^2$ = 8.4 Δdf = 1, P = 0.004).

Controlling for cohort, sex, age, tumor grade, and LVI, a significantly increased risk of disease recurrence and disease-specific death was associated with increasing levels of p66 Shc (as a continuous variable). The full-range HR was 13.0 [95% confidence interval (95% CI), 1.7-97] for RFS and 36.6

Table 2. Univariate analysis of clinicopathologic characteristics in the combined cohorts

Characteristic	RFS*			DSS*		
	n †	6 y (%)	P ‡	n	6 y (%)	P
All patients	44/230	80		22/231	89	
Sex						
Male	22/96	75	0.17	13/97	86	0.19
Female	22/134	83		9/134	91	
Age (y)						
<50	3/7	48	0.18	1/7	100	0.57
≥50	41/223	81		21/224	89	
Tumor grade						
Low (1 and 2)	30/176	81	**0.032**	14/176	92	0.34
High (3 and 4)	14/51	73		8/52	81	
LVI						
No	27/174	85	**0.003**	16/175	91	**0.021**
Yes	16/48	64		6/48	79	
p66 Shc						
Continuous	44/230	ND	**0.002**	22/231	ND	**<0.0001**
p66 Shc						
Category 1§	30/188	83	**0.006**	12/189	92	**0.0004**
Category 2	14/42	64		10/42	73	
p66 Shc						
Category 1§	11/84	87		4/85	94	
Category 2	19/104	80	**0.008**	8/104	92	**0.001**
Category 3	14/42	64		10/42	75	

Abbreviation: ND, not done.
*RFS and DSS from Kaplan-Meier functions are given as a percent at 6 y after initial surgery.
† n = 39/218 indicates that there were 39 recurrences in 218 patients with known values for the clinical characteristic.
‡ P is given for log-rank or log-rank trend univariate analysis of patients. Statistically significant values (P < 0.05) are in boldface.
§The reference category for each analysis is defined as category 1 and equals the lowest scores of p66 Shc.

(95% CI, 3.5-411) for DSS, in which the full-range HR reflects the relative hazard comparing patients with the highest observed score to those with the lowest observed score. This was also observed when levels of p66 Shc were partitioned into a binary or ternary variable. Comparing p66 Shc scores >2.5 and ≤2.5, the HR was 2.7 (95% CI, 1.4-5.6) for RFS and 5.0 (95% CI, 2.0-12) for DSS. Significant dose-response relations were also observed between the ternary p66 Shc variable and outcomes: HRs for RFS were 1.0, 1.7, and 3.7 (P_{trend} = 0.003) for p66 Shc scores of ≤1.7, 1.8-2.5, and >2.5, respectively, and for DSS were 1.0, 2.0, and 7.5 (P_{trend} = 0.001). PY-Shc did not achieve statistical significance for either outcome (data not shown).

The ternary multivariate Cox models for RFS and DSS were next used to calculate the risks of RFS and DSS 6 years after resection for patients with p66 Shc scores across the three categories, with the LVI and tumor grade covariates assigned values equal to the average of each across the combined cohorts. The risk of disease recurrence was calculated to be 7% for patients whose tumors had the lowest possible p66 Shc score (0), 18% for patients with an intermediate score (2.1), and 57% for patients whose tumors had the highest possible p66 Shc score (5). The comparable risks for disease-specific death were 2%, 10%, and 59% for patients whose tumors had a low, intermediate, or high p66 Shc score, respectively.

Discussion

The treatment of stage IIA colon cancer presents a conundrum. Surgery is mostly successful at achieving cure, yet a substantial portion (≥15%) of these patients will recur and succumb to the disease (7). Whereas those stage IIA patients who have a clinical history or pathologic findings that confer adverse risk may benefit from adjuvant chemotherapy akin to stage III patients, those with $T_3N_0M_0$ tumors without a history of perforation or obstruction as a group do not experience any major benefit from currently available regimens (4). This lack of perceived benefit arises, in part, due to the relatively low rate of recurrence and the resulting lack of statistical power in the trials done to date. The first step in developing an effective adjuvant therapy for stage IIA colon cancer patients will therefore be the ability to identify those patients destined to recur and in presumed need of adjuvant treatment. Shc staining intensity of the primary tumor in 240 cases of resected, chemotherapy-naïve, stage IIA ($T_3N_0M_0$) colon cancers revealed that the p66 Shc staining score could differentiate those patients with low, moderate, and high risks of recurrence and disease-specific death.

A full multivariate analysis revealed that the prognostic value of p66 Shc score was entirely independent of other ostensible

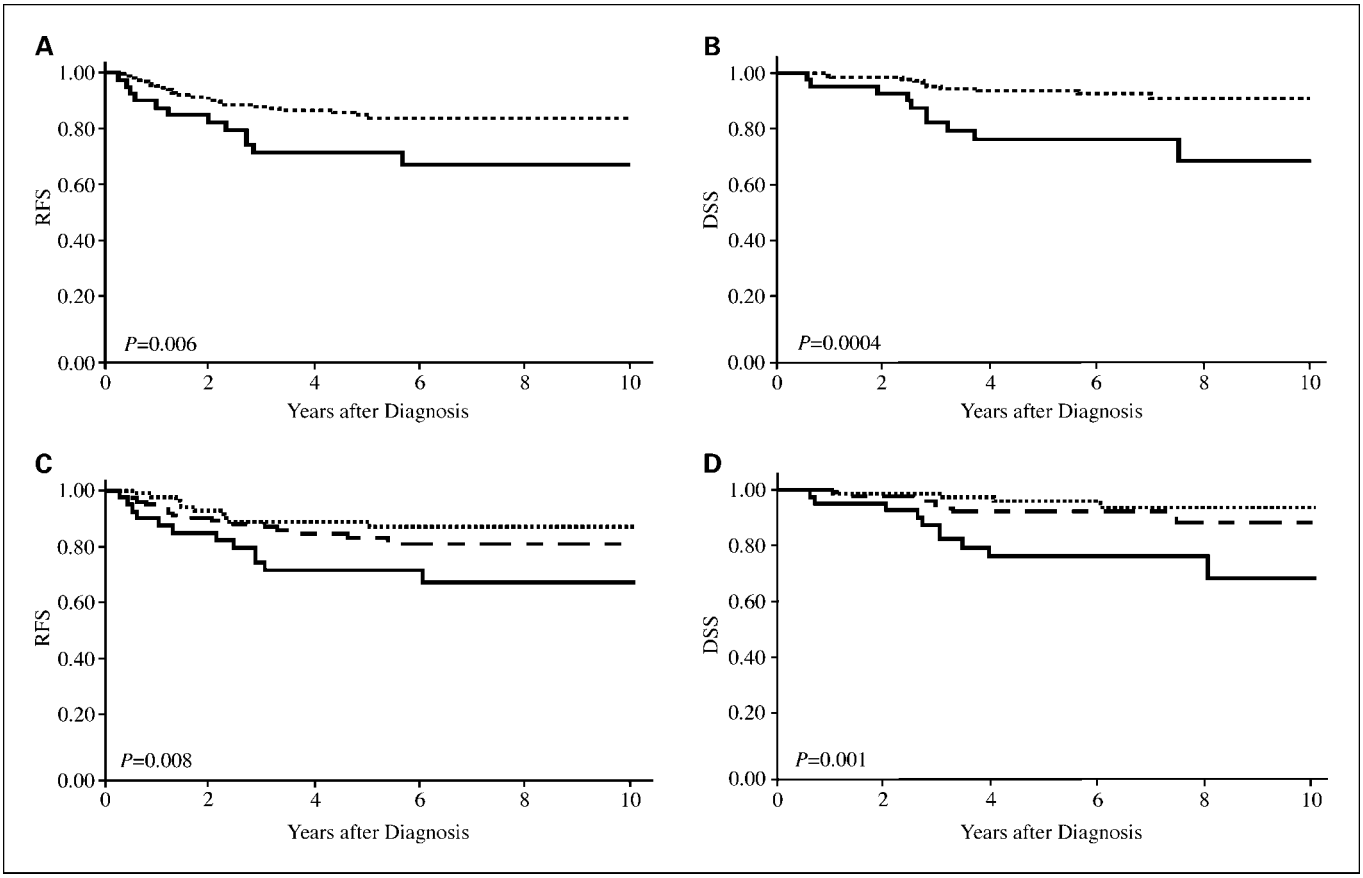

Fig. 3. Disease outcome by p66 Shc score in stage IIA colon cancer patients. *A,* Kaplan-Meier graphical analysis of RFS in patients from the combined cohorts partitioned on p66 Shc scores by Xtile (see Materials and Methods) into low-risk (p66 Shc score, ≤2.5; ·····) and high-risk (p66 Shc score, ›2.5; —) groups (P = 0.006). *B,* Kaplan-Meier graphical analysis of DSS in patients from the combined cohorts partitioned on p66 Shc scores into low-risk (·····) and high-risk (—) groups (P = 0.0004) with cutoff points as in (*A*). *C,* Kaplan-Meier graphical analysis of RFS in patients from the combined cohorts partitioned on p66 Shc scores by Xtile into low-risk (p66 Shc score, ≤1.7; ·····), intermediate-risk (p66 Shc score, 1.8-2.5; - – - –), and high-risk (p66 Shc score, ›2.5; —) groups (P = 0.01). *D,* Kaplan-Meier graphical analysis of DSS in patients from the combined cohorts partitioned on p66 Shc scores into low-risk (·····), intermediate-risk (- – - –), and high-risk (—) groups (P = 0.009) with cutoff points as in (*C*).

Table 3. Multivariate analysis for RFS and DSS in the combined cohorts

Covariates	RFS*		DSS*	
	HR (95% CI)	*P* †	HR (95% CI)	*P*
Base model				
Sex	1.9 (0.9-3.7)	0.07	2.1 (0.8-5.5)	0.11
Age	1.0 (1.0-1.0)	0.12	1.0 (1.0-1.0)	0.59
Tumor grade	2.0 (1.0-4.2)	0.06	1.6 (0.6-4.2)	0.31
LVI	2.7 (1.3-5.7)	**0.008**	3.2 (1.2-8.9)	**0.025**
With p66 Shc (continuous)				
Sex	1.9 (1.0-3.8)	0.06	2.3 (0.9-6.0)	0.08
Age	1.0 (1.0-1.0)	0.07	1.0 (1.0-1.0)	0.38
Tumor grade	2.3 (1.1-4.7)	**0.031**	1.6 (0.6-4.2)	0.31
LVI	2.6 (1.2-5.5)	**0.015**	4.1 (1.4-12)	**0.010**
p66 Shc (full range) ‡	13.0 (1.7-97)	**0.012**	36.6 (3.5-411)	**0.004**
With p66 Shc (binary categorical)§				
Sex	1.8 (0.9-3.6)	0.09	1.9 (0.8-4.9)	0.16
Age	1.0 (0.95-1.0)	**0.050**	1.0 (1.0-1.0)	0.34
Tumor grade	2.2 (1.0-4.5)	**0.041**	1.5 (0.6-3.9)	0.39
LVI	2.6 (1.2-5.7)	**0.013**	4.9 (1.6-15)	**0.006**
p66 Shc category 2	2.7 (1.4-5.6)	**0.005**	5.0 (2.0-12)	**0.0002**
With p66 Shc (ternary categorical)§				
Sex	1.9 (1.0-3.9)	0.06	2.0 (0.8-5.2)	0.14
Age	1.0 (1.0-1.0)	**0.043**	1.0 (1.0-1.0)	0.32
Tumor grade	2.4 (1.1-5.2)	**0.023**	1.6 (0.6-4.3)	0.33
LVI	2.5 (1.2-5.4)	**0.020**	4.7 (1.5-15)	**0.008**
p66 Shc category 2	1.7 (0.7-3.8)	0.21	2.0 (0.6-6.8)	0.26
p66 Shc category 3	3.7 (1.5-9.0)	**0.003**	7.5 (2.6-25)	**0.001**

*RFS and DSS from multivariate Cox proportional hazards model are given based on time from initial diagnosis to first relapse of disease or death from disease.
† *P* is given for multivariate analysis of patients. Statistically significant values ($P < 0.05$) are in boldface.
‡ The full-range HR reflects the relative hazard comparing the highest observed score to the lowest observed score for p66 Shc. In this case, there are 5 units of change from highest to lowest score, so the HR for a 1-unit change was raised to the 5th power.
§ The reference category for each analysis is defined as category 1 or the lowest scores of p66 Shc.

pathologic measures of tumor aggressiveness, such as grade or LVI. Taken together, the results of both the univariate and multivariate analyses firmly support our hypothesis that p66 Shc can be used to prognose the risk of disease recurrence and disease-specific death in stage IIA colon cancer. PY-Shc was found not to be statistically significant in the separate cohorts; however, with a larger sample size and longer follow-up, the value of using PY-Shc, in addition to p66 Shc, may be revealed.

In this report, we have shown that p66 Shc is a strong hazard in stage IIA colon cancer for both RFS and DSS. In contrast to this finding, we have previously reported that p66 Shc is a strong protector in early-stage invasive breast cancer for RFS and DSS (12, 13). A possible explanation for these diametrically opposed observations can be derived from the known cellular functions of p66 Shc. In normal cells, p66 Shc down-regulates tyrosine kinase signaling through Ras to the mitogen-activated protein kinase pathway (for review, see ref. 10) and also drives p53-dependent apoptosis resulting from oxidative stress (11). Aggressive breast cancer cells often rely on dysregulated signaling from growth factor receptor tyrosine kinases to drive signaling through Shc to Ras and the mitogen-activated protein kinase cascade (14). This signaling requirement may engender selective pressure to down-regulate expression of p66 Shc.

A second selective pressure to down-regulate p66 Shc is p66 Shc–mediated apoptosis in response to oxidative stress. Oxidative stress, often experienced by aggressive tumors, can be the result of growth factor signaling, mitochondrial activity, invasion of the solid tumor by the host inflammatory cells, and vascular reperfusion of hypoxic tissue in response to successful tumor angiogenesis (15, 16). In this scenario, independent dysregulation of the Ras pathway and/or dysregulation of p53 or other apoptotic regulators could lessen the selective pressures to down-regulate p66 Shc.

Whereas early-stage invasive breast cancers have a low incidence of Ras mutations and a modest (12-40%) incidence of dysregulated p53 and other apoptotic regulators (17, 18), colon cancers have a 38% incidence of mutated K-Ras (19) and a 40% to 60% incidence of mutated p53 (20–22). Additionally, mutations in the apoptotic regulators, phosphatidylinositol 3-kinase and phosphatase and tensin homologue, have been reported in 30% and 18% of colorectal cancers, respectively (23, 24). Thus, most aggressive early-stage breast cancers, in contrast to colon cancers, would be expected to have strong selective pressure to down-regulate p66 Shc. Consistent with this notion, we have noticed that the prognostic ability of p53 in breast cancer is primarily associated with patients whose tumors have not down-regulated p66 Shc.[12]

A number of other biomarkers have been reported to have a significant ability to prognose disease outcome for early-stage colon cancer. For many, such as loss of heterozygosity at

[12] A.R. Frackelton, Jr., unpublished data.

chromosome 18q, thymidylate synthase, p53, or dihydropyrimidine dehydrogenase (4, 9), either there is only minimal clinical significance in comparison with routine clinical information or the tests are impractical, imprecise, or costly to implement on a large scale. Recently, however, several biomarkers including microsatellite instability, Claudin-1, epidermal growth factor receptor, and TUCAN have joined thymidylate synthase in retaining significance in full multivariate analyses (4, 8, 25–27). To the extent that p66 Shc and these other markers are independent, a prognostic test combining p66 Shc and these markers could offer additional increments in the ability of clinicians to specifically quantify the risk of recurrent disease faced by their individual patients with stage IIA colon cancer. Additional studies of p66 Shc in colon cancer are currently proposed to examine this hypothesis.

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