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High Nuclear Hypoxia-Inducible Factor 1 Alpha Expression is a Predictor of Distant Recurrence in Patients with Resected Pancreatic Adenocarcinoma

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Abstract

Purpose—Nuclear Hypoxia-Inducible Factor 1 Alpha (HIF1a) is involved in tumor response to microenvironmental stress and may initiate development of micrometastases and chemoradiation resistance. The goal of this analysis was to evaluate HIF1a expression as a prognostic factor for distant recurrence (DR) and local recurrence (LR) following pancreatic adenocarcinoma (PAC) resection.

Materials/Methods—Tissue specimens were collected from 98 patients with PAC who underwent resection without neoadjuvant therapy between January 2000 and December 2011. LR was defined as radiographic or pathologic evidence of progressive disease in the pancreas, pancreatic bed, or associated nodal regions. DR was defined as radiographically or pathologically confirmed recurrent disease in other sites. IHC staining was performed and scored by an independent pathologist blinded to patient outcomes. High HIF1a overall expression score was defined as high percentage and intensity staining and score>1.33. Univariate analysis was

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performed for HIF1a score with LR alone and with DR. Multivariate logistic regression was used to determine predictors of LR and DR.

Results—Median follow-up time for all patients was 16.3 months. 8 (8%) patients demonstrated isolated LR, 26 (26.5%) patients had isolated DR, and 13 patients had both LR and DR. 53 (54%) patients had high HIF1a expression and 45 (46%) patients had low HIF1a expression. High HIF1a expression was significantly associated with DR (p=0.03) and low HIF1a expression was significantly associated with isolated LR (p=.03). On multivariate logistic regression analysis, high HIF1a was the only significant predictor of DR (OR=2.46 [95% CI 1.06–5.72]; p=.03). In patients with a known recurrence, a HIF1a score   2.5 demonstrated a specificity of 100% for DR.

Conclusions—High HIF1a expression is a significant predictor of distant failure versus isolated local failure in patients undergoing resection of pancreatic adenocarcinoma. HIF1a expression may have utility in determining candidates for adjuvant local radiation therapy and systemic chemotherapy.

Keywords

HIF-1α; biomarkers; pancreatic cancer; hypoxia

INTRODUCTION

Pancreatic adenocarcinoma (PAC) remains the fourth leading cause of cancer death in the United States today and carries an extremely poor prognosis, with 5-year survival rates remaining near 5%. Out of the estimated 44,000 newly diagnosed cases of pancreatic cancer in 2012, 37,400 deaths are expected.[1] Although incremental gains have been made in treatment strategies, complete resection remains the only hope for cure. Methods for stratifying patients and tailoring treatment for pancreatic adenocarcinoma rely mainly on descriptive clinicopathologic features such as TNM stage, margin status, perineural invasion and CA19-9, all of which have modest prognostic value. One accepted treatment algorithm after pancreatic resection is to administer adjuvant chemoradiation.[2,3] Even with curative resection, five-year survival rates remain around 20%. At least 40% of patients have metastatic disease at presentation, and another 30–40% have locally advanced disease not lending itself to curative intent resection. Several potential molecular biomarkers have been studied in pancreatic cancer with varying degrees of clinical utility.

Rapidly growing solid tumors appear to exhibit areas of hypoxia where the rate of growth outpaces blood vessel formation at the growing edge of the tumor. One of the pathways possibly linking hypoxia to angiogenesis is the Hypoxia-Inducible Factor 1 (HIF-1) pathway, which includes subunits HIF-1α and HIF-1β. Under physiologic conditions, HIF-1α is recognized by the von Hippel-Lindau tumor suppressor (pVHL) and tagged for degradation via the ubiquitin pathway. Under hypoxic conditions HIF-1α is *not* recognized by pVHL, leading to accumulation of HIF-1α and dimerization with HIF-1β. The dimer then translocates to the nucleus. Once inside the nucleus, HIF-1α transcribes several genes exhibiting multiple cellular signaling effects. While HIF-1β is a common subunit among many bHLH-PAS proteins and is constitutively expressed, HIF-1α is uniquely regulated in this way by available oxygen (O_2).[4] O_2 deprivation leads to increased signaling of nuclear

HIF-1α, which in turn causes tumor cells to experience an adaptive switch to angiogenic signaling, glycolytic metabolism, and subsequent micrometastases. This ability to respond to microenvironmental stress may lead in turn to tumor resistance to available thereapeutic approaches. High levels of HIF-1α activity likely increase tumor growth, vascularization and glucose metabolism and also reflect more clinically aggressive tumors due to an association with p53 mutations. HIF-1α expression has been suggested as a clinical marker of aggressive disease, poor prognosis and treatment resistance in other cancers, such as ovarian tumors[5], cervical cancer[6] and more recently in other gastrointestinal malignancies such as esophageal and colorectal cancer[7], potentially through regulation of hypoxia-based tumor-stromal interactions.[8] The aim of this study was to evaluate the role of HIF-1α expression in prognosticating survival and patterns of failure in patients with resectable PAC.

MATERIALS AND METHODS

Patient Selection

Ninety-eight patients were selected for this analysis, based on availability of pancreatic cancer resection specimens. These patients presented with early-stage PAC and underwent pancreaticoduodenectomy with curative intent between January of 2000 and January of 2011 at XXX Hospital. Patients who received neoadjuvant chemotherapy or radiation were excluded to avoid bias in biomarker interpretation. Approximately 30 of these patients were included in previous reports by the authors.[9–11] Overall survival and followup time were calculated using date of surgery as the initial contact. Chart review was conducted to obtain patient demographics, treatment characteristics and pathologic characteristics. Patients were followed for five years or until date of death. Local recurrence was defined as either recurrent disease in the pancreas, pancreatic bed or associated nodal regions. Distant recurrence was defined as radiologic or pathologically confirmed recurrent disease in other sites. Documented recurrence required either biopsy confirmed disease consistent with pancreatic primary or progressive disease on two consecutive post-treatment surveillance scans at any time point. Permission was obtained from the Institutional Review Board and patient confidentiality was maintained according to the Health Insurance and Patient Accessibility Act of 1996.

Immunohistochemical Analysis

This institution has a well defined tissue banking protocol in which representative fresh samples from the tumor are stored immediately after gross sectioning, often less than one hour after the specimen is resected, in order to minimize protein degradation. In order to prevent effects of intraoperative procedures, including electrocautery, representative tumor samples were chosen for the study away from tumor periphery. Representative sections of tumor were selected using formalin-fixed paraffin embedded slides reviewed by two expert pancreatic pathologists (NA, BS). Due to intratumoral heterogeneity, tissue microarray (TMA) blocks were created in quadruplicate using 5mm diameter punches from carefully selected cellular foci of tumor. The TMA cores were reviewed for homogeneity. TMA cores were stained for nuclear HIF-1α expression using anti-HIF-1α monoclonal mouse antibody (NB100-105, Novus Biologicals, Littleton, CO) under optimized staining conditions at a dilution of 1:400 using positive and negative control tissues according to manufacturer

recommendations. Due to heterogeneity of HIF-1α expression, all four TMA cores were reviewed for each sample. Nuclear expression levels of HIF-1α were determined by an experienced pancreatic pathologist (SB) blinded to patient outcomes and clinical data with an additional pancreatic pathologist available for review (NA). Immunohistochemical (IHC) expression scores were calculated based on a previously defined scoring system incorporating intensity and percent of cells staining.[10–13] Intensity of cells staining was recorded as follows: 0 = no staining, 1= weak staining, 2=moderate staining, 3=strong staining. Percentage of staining was recorded as follows: 0 = less than 1% of cells staining positive, 1= 1–10% of cells staining positive, 2 = 11–49% of cells staining positive, 3= 50–80% of cells staining positive, 4= greater than 80% of cells staining positive. A combined score was then calculated using the following equation: Score = ((1+ intensity)/3) X percent. This combined score equation allows for the percentage of cells staining to carry greater weight as it is thought to be more biologically relevant.

In addition to analysis of the calculated scores as a continuous variable, scores were divided into low and high expression levels. High expression level tumors were required to exhibit both high staining intensity (2–3) and high percentage of cells staining (>50%), and thus had a calculated score >1.33, while low expression level tumors exhibited low staining intensity (0–1) and low percentage of cells staining (<=50%) and thus had a calculated score   1.33.

Statistical Analysis

Descriptive characteristics were generated for all clinicopathologic covariates, patient demographics, clinical outcomes and biomarker intensity, percent and score. Median and interquartile ranges were generated for continuous variables and frequency statistics were generated for categorical variables. Descriptive characteristics were generated for the endpoints of recurrence pattern, death or loss to follow-up for all patients.

Patient characteristics were summarized and compared between patients with high or low HIF-1α expression using ANOVA (analysis of variance) or Wilcoxon rank-sum test for numerical covariates and chi-square test or Fisher's exact test for categorical covariates where appropriate. Univariate association of HIF-1α expression with covariates was assessed using Wilcoxon rank-sum test or Kruskal-Wallis test for categorical covariates, where appropriate; and Spearman correlation for numerical covariates. Covariates included HIF-1α percentage, intensity and expression score, age, sex, ethnicity, receipt of adjuvant chemotherapy and/or radiation, tumor size, margin status, nodal status, grade, perineural invasion (PNI), and lymphovascular invasion (LVI). In order to eliminate death as a competing endpoint for recurrence and to directly compare isolated LR to DR, a subgroup analysis was performed including only patients with known recurrence before death or patients alive with disease. This excluded patients with no documented recurrence due to loss to follow-up or whose death was due to non-cancer causes. Univariate and Multivariable analysis of LR and DR were conducted using a logistic regression model. The best predictive model of LR and DR was identified using a backward variable selection method with an alpha level of removal set at 0.1. HIF-1α was forced in the multivariable model. Receiver operating characteristic (ROC) curves and predictive probability curves were generated for biomarker expression as a predictor of LR and DR. All analyses were done

using SAS 9.3 (SAS Institute, Inc., Cary, North Carolina) and significance level was set at 0.05.

RESULTS

Overall Characteristics

Median followup time was 16.3 months (range 0.85 – 61.6 months) and median survival time was 30.6 months (range 12.1–49.1 months). Median recurrence-free survival time was 9.05 months (range 0.85 – 61.6 months). Median age was 67.8 years (range 36–88 years). The patient population was 69.7% (n=62) Caucasian and 21.4% (n=27) African-American. At resection, median tumor maximum diameter was 3.5 cm (range 10–70 cm), 70% (n=71) patients were lymph node positive and 26.8% (n=26) patients had positive margins. Nuclear HIF-1α demonstrated differential expression among samples (figure 1). Median HIF-1α expression score was 2 (range 0–5.33) and mean score was 1.81 (SD ± 1.28). 85% (n=80) of patients received adjuvant chemotherapy and 20% (n=17) of patients received adjuvant radiotherapy. The remainder of the patient demographics and clinicopathologic characteristics are summarized in Table 1.

The only factors associated with high HIF-1α expression level on univariate analysis (table 2a, table 2b) were pattern of failure (p=.035) and age (p=.010).

Patterns of Failure

In all patients with recurrence, HIF-1α expression score was significantly associated with distant recurrence versus isolated local recurrence (p=.028, figure 2a). Both receiver operating characteristic curve (figure 2b) for HIF-1α score as a predictor of distant recurrence and predicted probability curve (figure 2c) demonstrate high specificity for distant recurrence with high HIF-1α score in all patients with recurrence. The percentage of each failure type by HIF-1α score (figure 2d) demonstrates the local recurrence group, which is composed primarily of low HIF-1α scores, the distant/local recurrence group, which is composed of a broad range of scores, and the distant recurrence group, which is composed of predominantly high scores.

On univariate analysis, low HIF-1α expression was significantly related to LR (OR 0.10 [95% CI 0.01–0.90]; p= .040, table 3) and high HIF-1α expression was significantly related to DR (OR 2.46 [95% CI 1.06 – 5.72]; p=.036, table 4). On multivariable analysis for LR, only low HIF-1α was a significant predictor of LR (OR 0.11 [95% CI .01–0.90]; p=.04). On multivariable analysis for DR, only high HIF-1α was a significant predictor of DR (OR 2.46 [95% CI 1.06–5.72]; p=.036).

DISCUSSION

In this study, high nuclear HIF-1α expression was highly specific as a predictive biomarker for distant recurrence of disease. Many biomarkers have been studied in pancreatic cancer, including other biomarkers involved in angiogenesis, tumor growth and invasion, and chemotherapy metabolism.[14,15] Few of these markers have been linked to specific patterns of failure in pancreatic cancer, as this study has demonstrated. The loss of one such

biomarker, SMAD4, was associated with increased pattern of distant failure in patients with advanced pancreatic cancer in a rapid autopsy series,[16] but this association did not stand in patients with earlier stage resectable pancreatic cancer,[17] potentially due to less aggressive tumor biology or earlier disease in these patients. This makes nuclear HIF-1α, to our knowledge, one of the first biomarkers to identify specific patterns of failure in early-stage pancreatic cancer patients.

Previous small studies have associated nuclear HIF-1α expression with lymph node metastases and decreased disease-free survival,[18] although data on overall survival has been inconsistent.[18–20] Nuclear HIF-1α has also been associated with both metastatic disease in other tumor types[5,6,21,22] and with aggressive tumor histology in cell line and tissue studies of pancreatic cancer.[4,19,23] Although cytoplasmic HIF-1α is expressed in both non-tumor and tumor pancreatic tissue, nuclear HIF-1α is expressed in approximately 88% of pancreatic ductal carcinomas versus only 16% of normal pancreas tissues, and is expressed by adjacent stroma to tumor in 43% cases, which suggests a role of HIF-1α in metastatic tumor behavior.[24–26] Nuclear HIF-1α appears to be more specific to tumor tissue[26] and the value of analyzing stromal expression remains under investigation.[8] Recent studies have suggested a potential link between tumor hypoxia and HIF-1α activated stromal proliferation that may promote metastatic behavior; this link deserves further evaluation.[8] It has been unclear whether nuclear HIF-1α expression levels are a result of treatment response by tumors or a marker of aggressive tumor behavior, thus this study analyzed only tissue samples from patients that had not been previously exposed to chemotherapy or radiation therapy. This inclusion criterion may contribute to the excellent median overall survival of this patient cohort, despite standard of care adjuvant treatment and good median follow up; this selection bias should be noted but does not preclude the useful interpretation of this data. The lower than expected recurrence rate may be attributed to strict requirements for documentation of recurrence in this retrospective analysis.

A pancreatic cancer biomarker that is predictive of failure patterns may be very timely as the role of radiation therapy versus isolated adjuvant chemotherapy in resected pancreatic cancer remains under debate. Several large studies have shown an advantage to adjuvant chemotherapy alone over adjuvant chemoradiation[27] yet 30% of PAC patients still die from locally aggressive disease rather than widespread metastatic disease.[16] Modern and advancing radiation therapy techniques, including intensity-modulated radiation therapy (IMRT) and stereotactic body radiation therapy (SBRT), allow the administration of larger radiation doses with sparing of surrounding structures and can provide excellent local control in selected patients.[28] Identifying those patients with resected PAC who are likely to develop isolated local or distant metastatic disease may have two-fold clinical benefit: prognostic information may help select patients for local radiation therapy and/or more aggressive systemic chemotherapy, and the development of targeted agents may prevent distant metastases and thus increase the effectiveness of local therapies and improve survival. With further investigation, it is also possible that this information could help stratify patients with resectable and borderline resectable tumors who would benefit from neoadjuvant therapy.

This data also justifies the further development of potential therapeutic targets, including HIF-1α inhibitors[29] and HSP90 inhibitors as both systemic agents and as radiosensitizing agents. When radiation is administered to a hypoxic tumor, the radiation dose must be nearly tripled in order to achieve comparable cell killing as compared to oxygenated tissue, which also increases normal tissue toxicity. Pancreatic adenocarcinoma is among the most hypoxic of all solid tumors, and HIF-1α inhibitors have been investigated pre-clinically as one method of increasing radiation response by decreasing resistance to radiation-induced apoptosis.[24,25] HIF-1α expression has also been associated with gemcitabine resistance in hypoxic environments, indicating HIF-1α may have a role in potentiating systemic therapeutic agents.[30]

Unfortunately, prognostic and predictive biomarker studies are fraught with issues of transparency and reproducibility. The Guidelines for the Reporting of Tumor MARKer Studies (REMARK) were developed and published in 2005 to provide a framework of study design and completeness of reporting for studies of prognostic biomarkers.[31–38] Although this retrospective study focuses on a predictive biomarker, the guidelines remain relevant and every effort was made to accurately report all 20 elements of the REMARK criteria. Similarly, the biological relevance of cut point determination in IHC studies, including HIF-1α, remains unclear, and thus biomarker studies remain prone to inaccurate conclusions due to arbitrary cut point delineation or determining cut points based on the produced data. Expression data was analyzed using continuous variables in addition to dichotomized expression levels, which minimizes this ambiguity; however, for the purposes of practical clinical utility, a pre-determined and standardized dichotomized system is necessary. The use of a uniform "scoring system" incorporating both intensity of staining and percentage of cells staining and standardized cut point determination allows better representation of overall expression and should be adopted and maintained in further prospective studies. The use of a quality controlled tissue banking protocol also will help minimize the interval between resection and fixation and susceptibility to fixation conditions of HIF-1α.

This study suggests that nuclear HIF-1α expression level at time of resection for early-stage PAC is a predictor of eventual distant failure versus local failure. While this analysis provides important hypothesis-generating data supported by previous studies of HIF-1α expression in PAC, future studies are necessary to validate these findings and to prospectively evaluate the role of HIF-1α expression in a larger, more homogeneous patient cohort, such as evaluation as a secondary endpoint in a future cooperative group trial.

Acknowledgments

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Summary

This analysis examined high hypoxia-inducible factor 1α as a predictor of isolated local recurrence versus distant recurrence in patients with resected pancreatic adenocarcinoma. High HIF1a expression is a significant predictor of distant failure versus isolated local failure in patients undergoing resection of pancreatic adenocarcinoma. HIF1a expression may have utility as a clinical decision-making tool in determining candidates for adjuvant local radiation therapy and systemic chemotherapy.
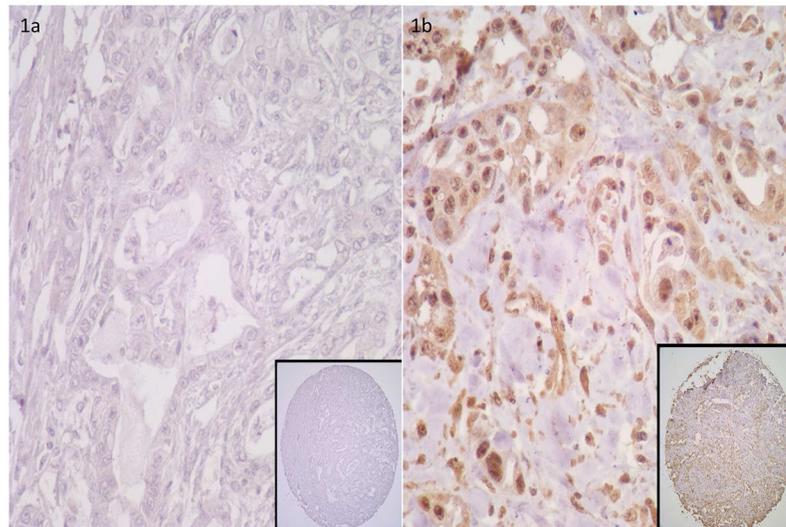
Figure 1.
HIF1a is differentially expressed via immunohistochemistical analysis. Low intensity of staining and low percentage of staining (score 0) are demonstrated in (1a) and high intensity of staining ad high percentage of staining (score 5.3) are demonstrated in (1b). Inset images are TMA core at ×100 magnification and large images are at ×400 magnification. Figure (1b) demonstrates nuclear positivity of HIF1a.
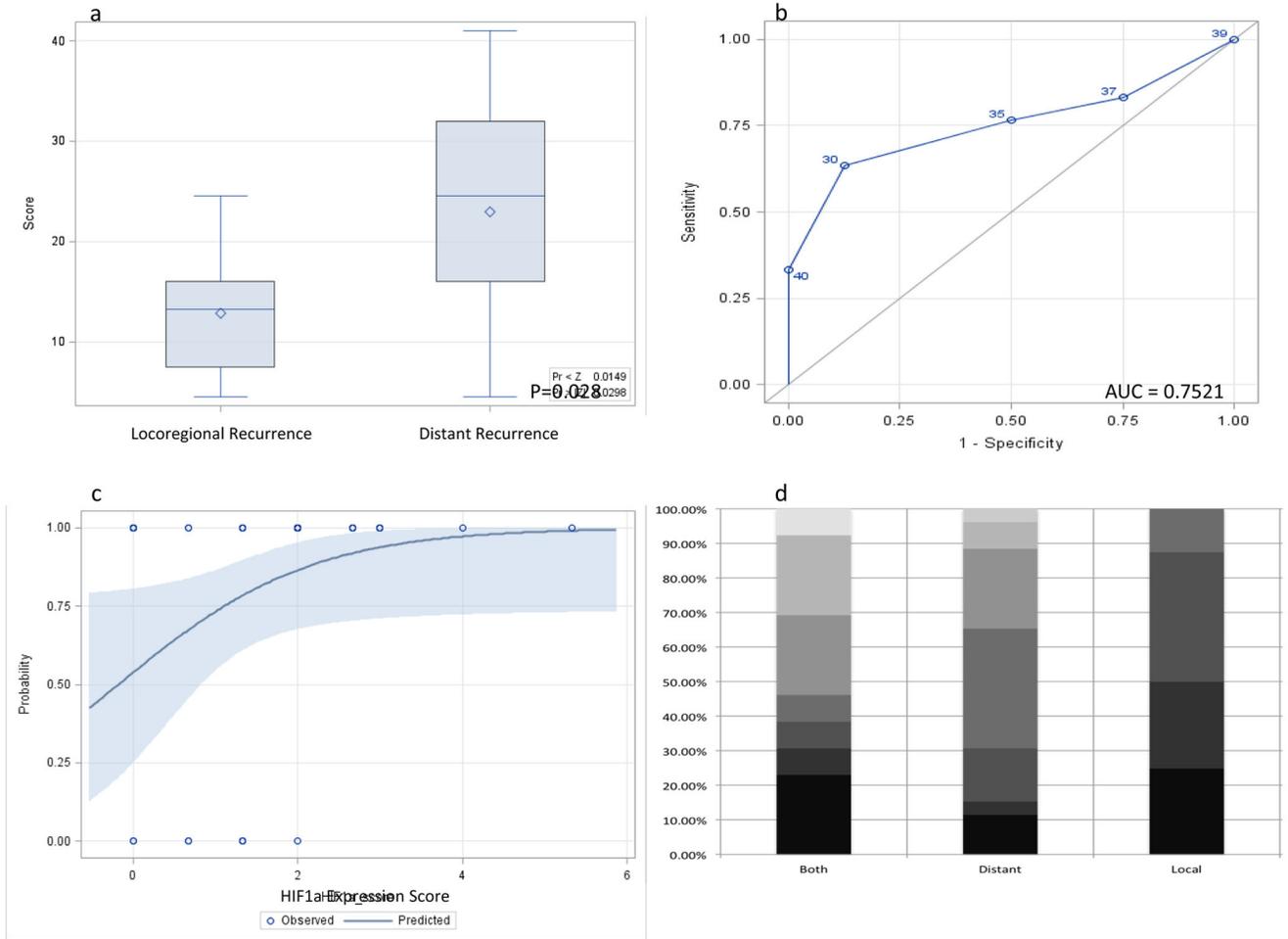
Figure 2.
Distribution of Wilcoxon scores for HIF1a score by site of recurrence (2a) demonstrates statistically significant difference between mean HIF1a score for isolated locoregional versus distant recurrence. Both Receiver operating characteristic curve (2b) for HIF1a score as a predictor of distant recurrence and predicted probability curve (2c) demonstrate high specificity for distant recurrence with high HIF1a score in all patients with recurrence. The percentage of each failure type by HIF1a score (2d), with darkest shading representing lowest scores and lightest shading representing higher scores, demonstrates the local recurrence group composed primarily of low HIF1a scores, distant/local recurrence group composed of a broad range of scores and the distant recurrence group composed of predominantly high scores. Small numbers above figure 2b represent predicted probabilities.

Table 1

Patient Demographics, Tumor Characteristics and Treatment Characteristics

Variable	Level	N (%)
Local Recurrence Alone	No	89 (91.75)
	Yes	8 (8.25)
	Missing	1
Distant Recurrence	No	58 (59.79)
	Yes	39 (40.21)
	Missing	1
Failure Patterns	No failure	50 (51.55)
	Distant Recurrence	26 (26.8)
	Local Recurrence	8 (8.25)
	Distant and Local Recurrence	13 (13.4)
	Missing	1
Race	Non-White	27 (30.34)
	White	62 (69.66)
	Missing	9
Margin Status at Resection	Positive margins	26 (26.8)
	Negative margins	71 (73.2)
	Missing	1
Grade of differentiation of tumor	Low Grade, well-differentiated	4 (4.08)
	Moderate Grade, moderately differentiated	77 (78.57)
	High Grade, poorly differentiated	17 (17.35)
Nodal Status at Resection	Positive	70 (71.43)
	Negative	28 (28.57)
Perineural invasion at resection	Positive	83 (84.69)
	Negative	15 (15.31)
Lymphovascular invasion at resection	Positive	55 (56.12)
	Negative	43 (43.88)
Adjuvant chemotherapy or radiation therapy after resection	Yes	80 (85.11)
	No	14 (14.89)
	Missing	4
Radiation therapy after resection	Yes	17 (20)
	No	68 (80)
	Missing	13
Age	Mean (± SD)	66.22 (± 11.6)
	Median (Range)	68 (36 – 88)
	Missing	4
Tumor size (cm)	Mean (± SD)	3.43 (± 1.27)
	Median (Range)	3.5 (1 – 7)
	Missing	3
HIF-1 alpha Score	Mean (± SD)	1.81 (± 1.28)

Variable	Level	N (%)
	Median (Range)	2 (0 – 5.33)
	Missing	0

Total N=98. Data are presented as number of patients (%), mean (± SD) or median (range).

Table 2A

Univariate association of HIF-1 alpha Score (binary) with covariates

Covariate	Level	HIF-1 alpha Score (High > 1.33)		P-value[*]
		High (N=53)	Low (N=45)	
Local Recurrence Alone	No	51 (98.08)	38 (84.44)	**0.023**
	Yes	1 (1.92)	7 (15.56)	
Distant Recurrence	No	26 (50)	32 (71.11)	**0.034**
	Yes	26 (50)	13 (28.89)	
Failure Patterns	No failure	25 (48.08)	25 (55.56)	**0.035**
	Distant Recurrence	18 (34.62)	8 (17.78)	
	Local Recurrence	1 (1.92)	7 (15.56)	
	Distant and Local Recurrence	8 (15.38)	5 (11.11)	
Race	Non-White	15 (30.61)	12 (30)	0.950
	White	34 (69.39)	28 (70)	
Margin Status at Resection	Positive margins	14 (26.42)	12 (27.27)	0.924
	Negative margins	39 (73.58)	32 (72.73)	
Grade of differentiation of tumor	High Grade	12 (22.64)	5 (11.11)	0.133
	Low Grade or Moderate Grade	41 (77.36)	40 (88.89)	
Nodal Status at Resection	Positive	34 (64.15)	36 (80)	0.083
	Negative	19 (35.85)	9 (20)	
Perineural invasion at resection	Positive	44 (83.02)	39 (86.67)	0.617
	Negative	9 (16.98)	6 (13.33)	
Lymphovascular invasion at resection	Positive	31 (58.49)	24 (53.33)	0.608
	Negative	22 (41.51)	21 (46.67)	
Adjuvant chemotherapy or radiation therapy after resection	Yes	43 (84.31)	37 (86.05)	0.814
	No	8 (15.69)	6 (13.95)	
Radiation therapy after resection	Yes	10 (22.22)	7 (17.5)	0.587
	No	35 (77.78)	33 (82.5)	
Age	Mean (± SD)	63.36 (± 12.79)	69.48 (± 9.16)	**0.010**
Tumor size (cm)	Mean (± SD)	3.49 (± 1.41)	3.36 (± 1.11)	0.619

Data are presented as number of patients (%), mean (± SD) or median (range). Categories with N<98 reflect missing data; see Table 1 for missing data.

[*] The p-value is calculated by ANOVA or Wilcoxon rank-sum test for numerical covariates; and chi-square test or Fisher's exact test for categorical covariates, where appropriate.

Table 2B

Univariate association of HIF-1 alpha Score (continuous) with covariates

Variable	Level	N	Mean (± SD)	Median (Range)	P-value
				HIF-1 alpha Score	
Local Recurrence Alone	No	89	1.89 (± 1.3)	2 (0 – 5.33)	**0.030**
	Yes	8	0.92 (± 0.71)	1 (0 – 2)	
Distant Recurrence	No	58	1.71 (± 1.35)	1.33 (0 – 5.33)	0.250
	Yes	39	1.96 (± 1.19)	2 (0 – 5.33)	
Failure Patterns	No failure	50	1.83 (± 1.39)	1.67 (0 – 5.33)	0.161
	Distant Recurrence	26	1.92 (± 0.98)	2 (0 – 4)	
	Local Recurrence	8	0.92 (± 0.71)	1 (0 – 2)	
	Distant and Local Recurrence	13	2.03 (± 1.57)	2.67 (0 – 5.33)	
Race:binary	Non-White	27	1.89 (± 1.31)	2 (0 – 5.33)	0.751
	White	62	1.79 (± 1.29)	2 (0 – 5.33)	
Margin Status at Resection	Positive margins	26	1.86 (± 1.52)	2 (0 – 5.33)	0.947
	Negative margins	71	1.8 (± 1.21)	2 (0 – 5.33)	
Grade of differentiation of tumor	High Grade	17	2 (± 1.07)	2 (0 – 4)	0.374
	Low Grade or Moderate	81	1.77 (± 1.33)	2 (0 – 5.33)	
Nodal Status at Resection	Positive	70	1.77 (± 1.29)	1.33 (0 – 5.33)	0.572
	Negative	28	1.92 (± 1.29)	2 (0 – 4)	
Perineural invasion at resection	Positive	83	1.82 (± 1.31)	2 (0 – 5.33)	0.992
	Negative	15	1.78 (± 1.17)	2 (0 – 4)	
Lymphovascular invasion at resection	Positive	55	1.93 (± 1.33)	2 (0 – 5.33)	0.283
	Negative	43	1.66 (± 1.23)	2 (0 – 5.33)	
Adjuvant chemotherapy or radiation therapy after resection	Yes	80	1.8 (± 1.23)	2 (0 – 5.33)	0.690
	No	14	1.76 (± 1.6)	2 (0 – 5.33)	
Radiation therapy after resection	Yes	17	1.8 (± 1.65)	2 (0 – 5.33)	0.798
	No	68	1.8 (± 1.21)	2 (0 – 5.33)	

*
The p-value is calculated by Wilcoxon rank-sum test or Kruskal-Wallis test for categorical covariates, where appropriate; and Spearman correlation for numerical covariates. Categories with N<98 reflect missing data; see Table 1 for missing data.

Table 3

Univariate analysis of Isolated Local Recurrence

Covariate	Level	N	Odds Ratio	Local Recurrence Alone=Yes		
				95%CI Low	95%CI Up	OR P-value
HIF-1 alpha Score (High > 1.33)	High	52	0.106	0.013	0.902	**0.040**
	Low	45	1 (Ref)	-	-	-
Race:binary	Non-White	27	0.896	0.163	4.935	0.900
	White	61	1 (Ref)	-	-	-
Margin Status at Resection	Positive margins	26	1.696	0.375	7.663	0.493
	Negative margins	70	1 (Ref)	-	-	-
Grade of differentiation of tumor	High Grade	16	0.000 *	0.000	NA	-
	Low Grade or Moderate Grade	81	1 (Ref)	-	-	-
Nodal Status at Resection	Positive	70	0.615	0.136	2.774	0.527
	Negative	27	1 (Ref)	-	-	-
Perineural invasion at resection	Positive	82	0.513	0.093	2.823	0.443
	Negative	15	1 (Ref)	-	-	-
Lymphovascular invasion at resection	Positive	55	0.745	0.175	3.170	0.690
	Negative	42	1 (Ref)	-	-	-
Adjuvant chemotherapy or radiation therapy after resection	Yes	79	1.264	0.143	11.144	0.833
	No	14	1 (Ref)	-	-	-
Radiation therapy after resection	Yes	17	1.356	0.248	7.399	0.725
	No	67	1 (Ref)	-	-	-
HIF-1 alpha Score		97	0.488	0.238	0.998	**0.049**
Age		93	0.985	0.927	1.046	0.615
Tumor size (cm)		94	0.945	0.531	1.679	0.846

*
Due to no event in patients with high grade. All patients without missing data were included in fitted model; see Table 1 for missing data.

Table 4

Univariate analysis of Distant Recurrence (with or without Local Recurrence)

Covariate	Level	N	Odds Ratio	95%CI Low	95%CI Up	OR P-value
HIF-1 alpha Score (High > 1.33)	High	52	2.461	1.059	5.719	**0.036**
	Low	45	1 (Ref)	-	-	-
Race:binary	Non-White	27	1.660	0.666	4.137	0.277
	White	61	1 (Ref)	-	-	-
Margin Status at Resection	Positive margins	26	0.938	0.372	2.361	0.891
	Negative margins	70	1 (Ref)	-	-	-
Grade of differentiation of tumor	High Grade	16	0.873	0.289	2.635	0.809
	Low Grade or Moderate Grade	81	1 (Ref)	-	-	-
Nodal Status at Resection	Positive	70	0.970	0.392	2.396	0.947
	Negative	27	1 (Ref)	-	-	-
Perineural invasion at resection	Positive	82	1.010	0.329	3.106	0.986
	Negative	15	1 (Ref)	-	-	-
Lymphovascular invasion at resection	Positive	55	0.980	0.432	2.224	0.962
	Negative	42	1 (Ref)	-	-	-
Adjuvant chemotherapy or radiation therapy after resection	Yes	79	1.291	0.396	4.207	0.671
	No	14	1 (Ref)	-	-	-
Radiation therapy after resection	Yes	17	2.016	0.688	5.908	0.201
	No	67	1 (Ref)	-	-	-
HIF-1 alpha Score		97	1.165	0.847	1.601	0.347
Age		93	0.964	0.928	1.000	0.051
Tumor size (cm)		94	1.109	0.802	1.534	0.532

F1 All patients without missing data were included in fitted model; see Table 1 for missing data.