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Original Articles

African Americans with pancreatic ductal adenocarcinoma exhibit gender differences in Kaiso expression

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ABSTRACT

Kaiso, a bi-modal transcription factor, regulates gene expression, and is elevated in breast, prostate, and colon cancers. Depletion of Kaiso in other cancer types leads to a reduction in markers for the epithelial–mesenchymal transition (EMT) (Jones et al., 2014), however its clinical implications in pancreatic ductal adenocarcinoma (PDCA) have not been widely explored. PDCA is rarely detected at an early stage but is characterized by rapid progression and invasiveness. We now report the significance of the subcellular localization of Kaiso in PDCAs from African Americans. Kaiso expression is higher in the cytoplasm of invasive and metastatic pancreatic cancers. In males, cytoplasmic expression of Kaiso correlates with cancer grade and lymph node positivity. In male and female patients, cytoplasmic Kaiso expression correlates with invasiveness. Also, nuclear expression of Kaiso increases with increased invasiveness and lymph node positivity. Further, analysis of the largest PDCA dataset available on ONCOMINE shows that as Kaiso increases, there is an overall increase in Zeb1, which is the inverse for E-cadherin. Hence, these findings suggest a role for Kaiso in the progression of PDCAs, involving the EMT markers, E-cadherin and Zeb1.

Introduction

In the United States, pancreatic ductal adenocarcinoma (PDCA) is the fourth leading cause of cancer death. Worldwide, more than 200,000 people are diagnosed each year; only about 4% live for 5 years after diagnosis [1]. The incidence of PDCA in African Americans is 50%–90% higher than in other racial groups [2]. African American patients also have the poorest prognosis of any racial group. These devastating statistics are likely due to the fact that, at diagnosis, ~85% of patients have advanced, unresectable disease [3]. Although chemotherapeutics such as FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan) and Abraxane® (protein-bound paclitaxel) have been approved as mono- and multimodality therapies for late-stage disease, PDCA remains largely unresponsive to most chemotherapeutic agents [4,5]. This is particularly evident for African Americans, who demonstrate worse outcomes compared to Caucasians [2]. Thus, there is a need to understand, particularly for African Americans, the biological mechanisms that contribute to development and progression of PDCAs and to develop early indicators of aggressive disease.

Kaiso, a bimodal transcription factor, belonging to the BTB/POZ (Broad Complex, Tramtrak, Bric à brac/Pox virus and Zinc finger) subfamily of zinc-finger proteins, binds both methylated DNA and consensus Kaiso binding **s**ites (KBS) on DNA to regulate gene expression [6–8]. A function of Kaiso in tumorigenesis is methylation-dependent silencing of various tumor suppressor genes. For example, in prostate and breast cancers, Kaiso regulates genes that relate to de-differentiation, including those associated with the epithelial–mesenchymal transition (EMT), such as E-cadherin [8,9], Wnt 11 [10], and matrilysin [11]. Additionally, in MCF-7 breast cancer cells, Kaiso regulates the *cyclin D1* promoter via binding to methylated CpG-dinucleotides and KBS [12]. Progeny of KaisoTg/+ (Kaiso-overexpressing) mice crossed with ApcMin/+ mice (used for studies on colon carcinogenesis) demonstrates elevated expression of Wnt-related genes, increased inflammation, and increased tumorigenesis [13].

Based on its subcellular localization, elevated expression of Kaiso has shown promise as a prognostic biomarker for various tumor types [14,15]. In chronic leukemia and in non-small cell lung cancers, elevated levels of Kaiso are present predominantly in the cytoplasm [16,17]. However, in colorectal, prostate, and breast cancers, Kaiso is present in both the cytoplasm and the nucleus. In more aggressive tumors, Kaiso shows a trend of increased nuclear expression [12,18]. In prostate cancer cells, nuclear Kaiso silences E-cadherin and induces these cells to undergo an EMT [8,9]. Also, depletion of

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Kaiso in breast cancer cell lines causes a reduction in the EMT markers, N-cadherin and vimentin [8]. Nuclear Kaiso is elevated in prostate and breast tumors of African American patients, who typically have highly aggressive tumors of these types [8,9]. To date, however, there are no studies examining the expression of Kaiso in PDCA.

Herein we present findings that Kaiso expression is elevated in African American patients with PDCA. Tumors of males have a higher cytoplasmic expression that is associated with cancer grade and total lymph node positivity. Also, there is a correlation between cytoplasmic Kaiso expression and cancer aggressiveness in males and females. Nuclear Kaiso expression increases with increased invasiveness and total positive lymph nodes. There is also a correlation between the expression of Kaiso and Zeb1 with E-cadherin, an EMT marker. Furthermore, as determined with the largest PDAC patient data set available on ONCOMINE, there is elevated expression of Kaiso and ZEB1, which was inverse for expression of E-cadherin. Hence, these results show that Kaiso has an oncogenic role in the metastasis of PDCA.

Materials and methods

Patient population

PDCA tissue microarrays (TMAs) were obtained from Emory University (Atlanta, GA). All specimens were originally collected utilizing Institutional Review Board-approved protocols. Additionally, the Institutional Review Boards of Tuskegee University and Troy University approved the use of these tissues for this study. For each patient, the following clinicopathological characteristics were assessed: gender, histological grade, pre-invasive tumor size, size and extent of the invasive tumor, lymph node positive tissues, and extent of regional lymph node metastases.

Immunohistochemistry (IHC)

IHC was performed with the anti-Kaiso clone 6F (Upstate Biotechnology, MA), and TMAs were stained for evaluation by IHC [19]. Briefly, cells were scored blindly, and individual specimens stained for Kaiso were scored separately for cytoplasmic and nuclear staining. Immunostaining was evaluated by determining the percentage of malignant cells in four random fields that demonstrated staining on a scale of 0–3. Scores of 0 (no staining), 1 (10–30% staining), 2 (40–70% staining), and 3 (71–100% staining) were assigned as previously described [8,9,20].

Statistical analyses

For all experiments, statistical calculations were performed with Microsoft Excel or GraphPad prism software; version 6. Independent Student's t-tests were utilized to determine statistical differences between means of experimental and control values. Tissue correlations were made using chi-square and Fisher's exact test. Any tissues that were damaged or lacked sufficient information were removed from the overall cohort. All p values were considered statistically significant at <0.05.

Results

Kaiso expression in human PDCA tissue

Previously, we reported that Kaiso expression is higher in aggressive breast and prostate cancers and in tumors of African Americans [21]. However, there have been few reports of Kaiso expression in PDCAs. To evaluate Kaiso expression and localization in PDCA progression, IHC was used to evaluate samples of tumors from 31 African American patients, 17 females and 14 males (Table 1). For low-grade malignant tissues, there was high expression of Kaiso in the cytoplasm and low levels in the nuclei (Fig. 1A and C, Table 2). In high-grade tumors, there was staining for Kaiso in both cytoplasm and nuclei (Fig. 1B and D, Table 2). As determined by immunofluorescence staining for Kaiso, there was a similar pattern of expression in low-grade and high-grade tumors, with low to absent expression of Kaiso in normal and adjacent normal tissue (Supplemental Fig. S1). Thus, Kaiso is robustly expressed in PDCAs.

Table 1
Clinical and pathological characteristics of patient cohort.

	Number of patients	
Characteristics	31*	
Gender		
Female	17	54.80%
Male	14	45.16%
Race		
AA	31	100%
Grade**		
High	10	32.26%
Low	20	64.52%
Tumor size**		
≥2	6	19.35%
≤2	24	77.42%
LN positive**		
≥2	20	64.52%
≤2	10	32.26%
Stage T**		
≥2 (T1-T2)	7	22.58%
≤2 (T3-T4)	21	67.74%
Stage N**		
N0	10	32.26%
N1-N3	18	58.06%

NOTE: *Patients missing due to the lack of clinical characteristics or tissue damage was excluded from the analysis.
** Data are missing for few patients.

Cytoplasmic and nuclear Kaiso expression is associated with higher tumor grade and size

To determine the clinical relevance of Kaiso in PDCA, the association of Kaiso expression with the clinical grade of tumors, which is typically used as a prognostic factor, was analyzed. The tissues were analyzed and scored (0–3) blindly and subsequently analyzed quantitatively. In the overall patient population, expression of cytoplasmic Kaiso was higher in grade 2 tumors compared to grade 1 tumors ($P = 0.0435$) (Fig. 2A). For nuclear expression, there was no significant difference between tumor grades (Fig. 2A). When the population was separated according to gender, there were also no differences between nuclear expression and cytoplasmic expression in relation to tumor grade (Fig. 2B). Females, however, showed elevated expression of cytoplasmic Kaiso in both grade 1 and grade 2 tumors, compared

Table 2
Correlation of sub-cellular Kaiso expression with patient and tumor characteristics.

Characteristics	Patients	Cytoplasmic expression		p*	Nuclear expression		p*
		<1.5	≥1.5		<1.5	≥1.5	
Gender							
Male	42	9	21	0.9186	18	12	0.1524
Female	51	14	31		34	11	
Stage T (male)							
Non-invasive	6	4	1	**0.0101**	5	0	0.0608
Invasive	30	4	16		11	9	
Stage T (female)							
Non-invasive	15	6	5	**0.0418**	7	4	0.3421
Invasive	33	7	25		25	7	
LN positive (male)							
No	27	9	11	**0.0112**	15	5	**0.0177**
Yes	15	0	10		3	7	
LN positive (female)							
No	33	10	19	0.3824	22	7	0.7549
Yes	15	3	11		10	4	
Grade (male)							
Low	21	7	7	0.0701	10	4	0.232
High	18	3	13		8	8	
Grade (female)							
Low	39	12	24	0.5196	26	10	0.2981
High	12	2	7		8	1	

* p value considered significant at ≤0.05.
Chi square test was employed for determining correlation.

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