Repression of E-Cadherin by the Polycomb Group Protein EZH2 in Pancreatic Cancer

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BACKGROUND

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer deaths in the United States. Single-agent gemcitabine remains the standard treatment for advanced PDA, which has shown improvement in disease-related symptoms and a modest benefit in survival. A recently discovered histone methyltransferase termed enhancer of zeste homologue 2 (EZH2) was found to be overexpressed in a variety of carcinomas including PDA. Silencing of E-cadherin was proposed as a mechanism by which EZH2 mediates tumor aggressiveness. Furthermore, in-vitro studies showed EZH2 depletion sensitizes pancreatic cancer cells to gemcitabine. In this study we correlated EZH2 with E-cadherin expression in PDA, and evaluated response to gemcitabine in relation to EZH2 expression.

DESIGN

43 PDAs, 14 intraductal papillary mucinous neoplasms (IPMNs), and 5 chronic pancreatitis (CP) cases were stained with EZH2 (BD Bioscience; 1:25) and E-cadherin (Zymed; 1:1,000). Cases with diffuse weak staining, or strong staining in less than 30% of tumor nuclei were considered to have low EZH2 expression. High EZH2 expression was defined as strong nuclear staining in >30% of tumor cells. E-cadherin expression was scored on membrane positivity as follows: 0 (0-10%); 1 (10-25%); 2 (25-75%), and 3 (>75%). E-cadherin scores were considered normal at 3, reduced at 2, and negative at 1 or 0. Statistical analysis was performed using Fisher's exact and Kruskal-Wallis tests, depending on the discrete or continuous nature of the other factors. A Kaplan-Meier curve was stratified by EZH2 expression to assess survival.

RESULT

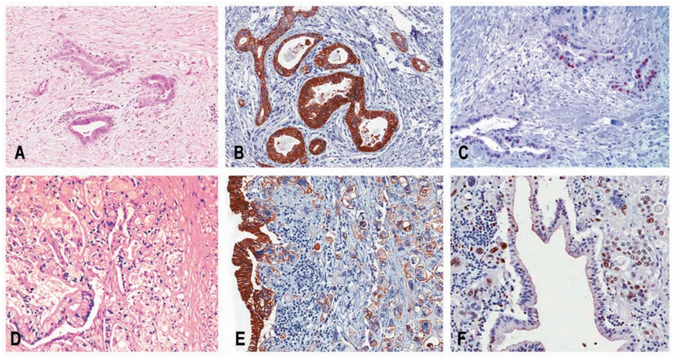


Figure 1 (A-F)

EZH2 and E-cadherin expression in well-differentiated PDA (1A-Hematoxylin-eosin, original magnification x 200). Strong membranous E-cadherin expression in PDA (1B- Immunostain, original magnification x 200). Focal nuclear expression of EZH2 in PDA (1C- Immunostain, original magnification x 200).

EZH2 and E-cadherin expression in poorly-differentiated PDA (1D-Hematoxylin-eosin, original magnification x 200). Strong membranous E-cadherin expression in a benign duct with reduced staining in PDA (1E-Immunostain, original magnification x 200). Diffuse nuclear expression of EZH2 in PDA with no staining in a benign duct (1F- Immunostain, original magnification x 200).

High EZH2 expression in PDA was significantly associated with decreased E-cadherin expression (70% vs. 35%), node-positivity (82% vs. 40%), and larger tumor size (4 cm vs. 2.4 cm). There was a trend for longer survival (35 vs. 15 months) in gemcitabine treated patients with low vs. high EZH2 expression. High EZH2 expression was detected in IMPN with moderate-severe dysplasia, however not in CP.

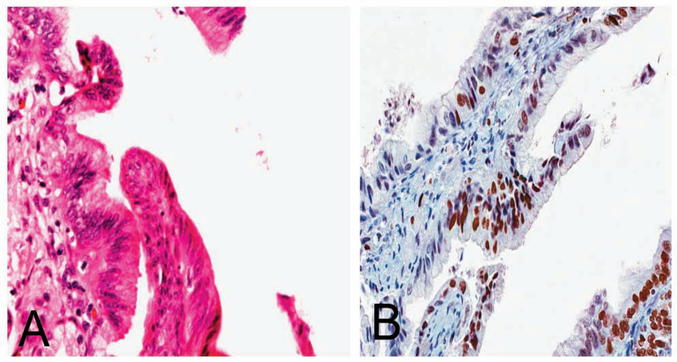


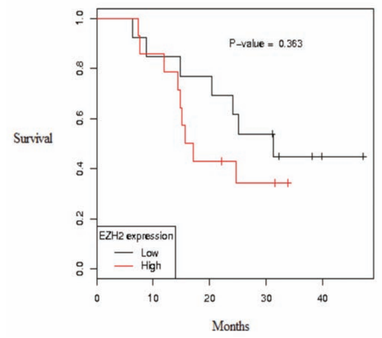
Figure 2 (A-B)

Diffuse EZH2 expression in IPMN with severe dysplasia. (3A-Hematoxylin-eosin, original magnification x 200, 3B-Immunostain, original magnification x 200). Cases with mild dysplasia were negative.

Descriptive Statistics by EZH2 expression

	Low N=20	High N=23
E-Cadherin expression		
Low	35%	70%
High	65%	30%
T stage		
T1	20%	0%
T2	20%	23%
T3	60%	77%
N stage		
N0	60%	18%
N1	40%	82%
Age (Yrs)	62.5	67.00
Gender		
Female	55%	23%
Male	45%	77%
Grade		
1	20%	10%
2	70%	62%
3	10%	29%
Tumor size (mm)	2.4	4.0
Extrapancreatic extension		
No	35%	23%
Yes	65%	77%

Kaplan-Meier survival curve of PDA stratified by EZH2 expression



CONCLUSION

Our study suggests that E-cadherin downregulation may lead to EZH2-mediated invasion and metastasis. While strong diffuse EZH2 expression is seen in PDA, overexpression may be present in IPMN.