World Journal of Gastroenterology

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DOI: 10.3748/wjg.v20.i36.13172

World J Gastroenterol 2014 September 28; 20(36): 13172-13177
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CLINICAL TRIALS STUDY

Negative methylation status of *Vimentin* predicts improved prognosis in pancreatic carcinoma

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Supported by National Nature Science Foundation of China, No. 81001078
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Received: April 7, 2014 Revised: June 8, 2014
Accepted: June 26, 2014
Published online: September 28, 2014

Abstract

AIM: To determine the existence of a potential relationship between the methylation state of the *Vimentin* gene and its prognostic value in pancreatic cancer.

METHODS: Sixty-four primary tumor specimens and normal tissues were collected consecutively from pancreatic cancer patients during surgery at Hangzhou First People's Hospital and Affiliated Hospital of the Logistics University of the Chinese People's Armed Police Force. DNA was extracted from the samples and subsequently quantitative methylation-specific polymerase chain reaction was used to detect the *Vimentin* methylation status of the samples. All of the patients were followed up to December 2012. χ^2 test, Kaplan-Meier survival and Cox regression statistical models were used.

RESULTS: Out of 64 pancreatic cancer tissues, 21 were marked as *Vimentin* methylation-positive, and 43 were marked as *Vimentin* methylation-negative. The location of pancreatic carcinoma was related to the *Vimentin* methylation state. The pathological T staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the *Vimentin* methylation state ($P = 0.037$) were independent prognostic factors.

CONCLUSION: In our study, *Vimentin* methylation status can predict the prognosis of pancreatic cancer patients. However, additional experiments and clinical trials are needed to accurately validate this observation.

Key words: Vimentin; Methylation; Pancreatic carcinoma; Prognosis

Core tip: Vimentin is reported to be an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling. In our study, we found that pathological T staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the *Vimentin* gene methylation state ($P = 0.037$) were independent prognostic factors. However, additional experiments and clinical trials are needed to accurately validate this observation.

Zhou YF, Xu W, Wang X, Sun JS, Xiang JJ, Li ZS, Zhang XF. Negative methylation status of *Vimentin* predicts improved prognosis in pancreatic carcinoma. *World J Gastroenterol* 2014; 20(36): 13172-13177 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i36/13172.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i36.13172

INTRODUCTION

Pancreatic cancer is one of the most lethal malignan-

Table 1 Clinicopathological features of pancreatic carcinoma *n* (%)

Data	*Vimentin* methylated negative group (n = 43)[2]	*Vimentin* methylated positive group (n = 21)[3]	*P* value
Sex			0.582
Male	27 (62.8)	15 (71.4)	
Female	16 (37.2)	6 (28.6)	
Tumor position			0.007
Head	11 (25.6)	13 (61.9)	
Body and tail	32 (74.4)	8 (38.1)	
Preoperative CEA level			0.294
Normal	20 (46.5)	13 (61.9)	
Elevated	23 (53.5)	8 (38.1)	
Preoperative CA19-9 level			0.600
Normal	24 (55.8)	10 (47.6)	
Elevated	19 (44.2)	11 (52.4)	
Pathological N staging[1]			0.426
N0	21(48.8)	13 (61.9)	
N1	22 (51.2)	8 (38.1)	
Pathological T staging[1]			0.753
T1	14 (32.6)	5 (23.8)	
T2	19 (44.2)	11 (52.4)	
T3	10 (23.3)	5 (23.8)	
Adjuvant chemotherapy			0.791
No	20 (46.5)	11 (52.4)	
Yes	23 (53.5)	10 (47.6)	

[1]The pathological T and N staging was based on the UICC staging systems for pancreatic cancer; [2]Median age 54 years, range: 36-71 years; [3]Median age 53 years, range: 41-68 years.

cies in humans, wherein the 5-year survival rate is less than 5%[1,2]. Most patients are diagnosed at an advanced stage of disease because of the lack of easily observable symptoms[3]. As reported, only 20% of pancreatic cancer patients are determined as suitable candidates to be considered for surgical resection. Moreover, following surgery, the 5-year survival rate of pancreatic cancer patients is approximately only 15%-25% due to the high recurrence and metastatic rates[4,5]. In the advanced stage of disease, the prognosis for these patients remains poor, even after chemotherapy and radiotherapy. The median overall survival rate is 3-6 mo for patients with metastatic disease and 6-10 mo for patients with non-metastatic disease[6,7]. Thus, early detection of pancreatic cancer is clearly important in order to augment the potential benefits and rates of an operation, and the prognosis of the patients. Recently, CA19-9 has been commonly used as a biomarker of pancreatic cancer. However, the specificity and sensitivity of this biomarker have not been satisfactory[8-10].

Vimentin is reported as an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling[11,12]. Several investigators have previously shown that *Vimentin* is an important marker for the early detection of cancer, such as bladder cancer, hepatocellular carcinoma and colorectal cancer[13,14]. In addition, methylation of the *Vimentin* gene is described as a marker in several malignant tumors, including gastric carcinoma, colorectal carcinoma, cervical cancer and bladder cancer[13,15-17]. In our current study, we have attempted to identify the relationship between the methylation state of *Vimentin* and pancreatic cancer.

MATERIALS AND METHODS

Sample collection and DNA preparation

Sixty-four primary tumor specimens and normal tissues were collected consecutively from pancreatic cancer patients undergoing surgery at Hangzhou First People's Hospital and Affiliated Hospital of the Logistics University of the Chinese People's Armed Police Force. All specimens were confirmed by histopathology. Written informed consent was obtained from all patients. All the collected samples were stored at -80℃. DNA from the samples was extracted by QIAamp DNA Mini Kit (Catalog number: 51306, Qiagen, Hilden, Germany). The clinicopathological characteristics of the patients who were enrolled in our study are shown in Table 1.

Sodium bisulfite modification

Genomic tumor DNA (1 μg) and the corresponding normal pancreatic tissue specimens were subjected to bisulfite treatment using an Epitect Bisulfite Kit (Catalog No. 59104, Qiagen, Hilden, Germany)[18].

Quantitative methylation-specific polymerase chain reaction

The bisulfite-treated DNA was amplified using a quantitative methylation-specific polymerase chain reaction and a Thermal Cycler Dice® Real-time System TP800 (Takara Bio Inc., Otsu, Japan). Thermo-cycling was carried out in a final volume of 25 μL containing 1.0 μL of the DNA sample, 100 nmol/L each of the Vimentin or β-actin primers (forward and reverse sequences), and 12.5 μL of SYBR Premix Ex Taq Ⅱ (Takara Bio Inc., Otsu, Japan), which consists of Taq DNA polymerase,

Table 2 Univariate analysis of overall survival in pancreatic carcinoma

Variables	*n*	Median survival (mo)	*P* value
Sex			0.819
Male	42	13.45	
Female	22	10.84	
Preoperative CEA			0.260
Normal	33	15.15	
Elevated	31	13.61	
Preoperative CA19-9			0.947
Normal	40	14.12	
Elevated	24	15.09	
Tumor position			0.007
Body and tail	40	16.20	
Head	24	11.25	
Pathological T stage			< 0.001
T1	19	21.01	
T2	30	13.10	
T3	15	8.00	
Pathological N stage			0.311
N0	34	14.28	
N1	30	16.20	
Adjuvant chemotherapy			
Yes	33	16.12	0.015
No	31	13.07	
Vimentin methylation state			0.013
Positive	21	11.09	
Negative	43	16.03	

PCR reaction buffer and deoxynucleotide triphosphate mixture. The qPCR primer sequences for *Vimentin* were: *Vimentin* MS (sense), 5'-TCGTTTCGAGGTTTTC-GCGTTAGAGAC-3', and *Vimentin* MAS (antisense), 5'-CGACTAAAACTCGACCGACTCGCGA-3'. The PCR amplification consisted of 40 cycles (95℃ for 5 s and 55℃ for 30 s) after an initial denaturation step (95℃ for 10 s). To correct for differences between the samples in terms of both the quality and quantity of the purified DNA, β-actin was used as an internal control. The targets were obtained from the same bisulfite treated DNA.

Vimentin methylation scores

The relative levels of methylated *Vimentin* DNA in pancreatic cancer and the corresponding normal pancreatic tissues were calculated by normalizing to the internal control of β-actin. The *Vimentin* methylation scores were calculated by comparing the relative levels of *Vimentin* in pancreatic carcinoma with the corresponding levels in normal pancreatic tissue. The methylation status was recorded as positive when the *Vimentin* methylation score was greater than 1.0.

Follow-up

After treatment, the patients were monitored every month for the first year, every 3 mo for the second year, and then every 6 mo thereafter. Telephone calls and letters were used to identify patients who could not attend regular follow-up assessments. Complete data were collected for all 64 patients through December 31, 2012. The follow-up period ranged from 6 to 38 mo (with a median of 17 mo).

Statistical analysis

The χ^2 test was used to compare categorical variables between the palliatively operated group with the other groups. Student's *t*-tests were used to compare paired continuous variables. Univariate survival analysis was performed by Kaplan-Meier methods. Survival curves were compared using the log-rank test. Statistical analyses were performed with SPSS software version 20.0 for Windows (SPSS, Inc., Chicago, IL, United States). Statistical significance was defined as an alpha value of $P < 0.05$.

RESULTS

Vimentin methylation in pancreatic cancer and corresponding pancreatic tissues

We detected *Vimentin* methylation in pancreatic cancer and corresponding pancreatic tissues. Of the 64 pancreatic cancer tissues, 21 of them had a high-level methylation status and 45 of the corresponding pancreatic tissues had a high level of methylation. There were 9 pancreatic cancer tissues and 5 normal corresponding pancreatic tissues without methylation of the *Vimentin* gene. In addition, *Vimentin* methylation scores were recorded and informed that 43 of them were marked as *Vimentin* methylation-negative, and the remaining 21 were *Vimentin* methylation-positive.

Vimentin methylation state was related to the age and the diameter of the tumor

The clinicopathological factors seen between these two groups are summarized in Table 1. Moreover, we found that the location of the pancreatic carcinoma was associated with the status of *Vimentin* methylation. However, patient gender, preoperative serum tumor markers, lymph node metastasis and pathological T-stage were found not to be associated with the *Vimentin* methylation state.

Vimentin methylation state was an independent prognostic factor in pancreatic cancer

Univariate analysis showed that tumor position ($P = 0.002$), pathological T-staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.015$) and the *Vimentin* methylation state ($P = 0.013$) were prognostic factors in pancreatic carcinoma (Table 2 and Figure 1). Multivariate analysis showed that the pathological T-staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the *Vimentin* methylation state ($P = 0.037$) were independent prognostic factors (Table 3).

DISCUSSION

An estimated 44030 people were diagnosed with pancreatic cancer and approximately 37660 people died of pancreatic cancer in the United States in 2011[19]. Although the technology of radiotherapy and chemotherapy has been developed, the incidence and mortality rates have remained approximately the same over the past two decades. A mutation in the *CDKN2A* (*p16*) gene has been reported

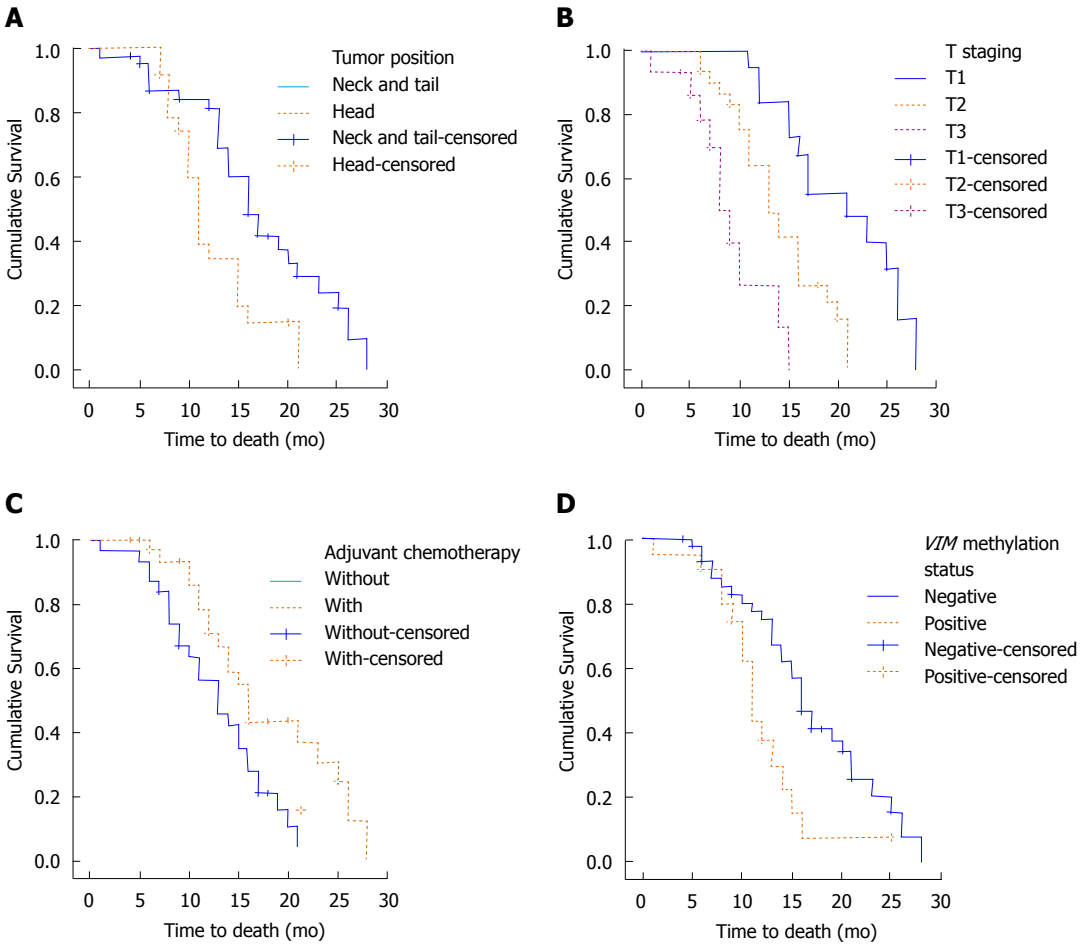

Figure 1 Univariate analysis: Tumor position, pathological T staging, adjuvant chemotherapy and *vimentin* methylation state were prognostic factors for the pancreatic carcinoma patients. A: Tumor position; B: T staging; C: Adjuvant chemotherapy; D: *VIM* methylation status.

Table 3 Multivariate analyses of overall survival in pancreatic carcinoma (Cox's regression model)

Variable	HR	95%CI	*P* value
OS in all pancreatic cancer patients			
Tumor position	1.789	0.873-3.666	0.112
Pathological T staging	4.026	2.283-7.101	<0.001
Adjuvant chemotherapy	0.356	0.181-0.698	0.003
Vimentin methylation state	2.250	1.052-4.813	0.037

OS: Overall survival; HR: Hazard ratio.

in families with pancreatic cancer and melanoma[20,21]. An excess of pancreatic cancer is also seen in families harboring breast cancer susceptibility gene-2 mutations, and particular mutations in the *PALB2* and *MSH2* genes have recently been identified as possibly increasing pancreatic cancer susceptibility[22-25]. Vimentin is a key molecular marker of epithelial-mesenchymal transition, but it is not yet associated with either the occurrence or degree of malignancy of pancreatic carcinoma.

CpG methylation is a major epigenetic modification of genome DNA that is involved in the regulation of cell-specific gene expression[26]. It has been proposed that this modification may cause transcriptional repression by directly modulating transcription factor function or by triggering the formation of inactive chromatin[27,28]. We found that of 64 pancreatic cancer tissues, 21 of them displayed a high level of methylation status and 45 of the corresponding pancreatic tissues displayed a high level of methylation. Moreover, survival analysis showed that the *Vimentin* methylation status was an independent prognostic factor as well as a prognostic marker in T-staging and adjuvant chemotherapy. We are also aware that a low methylation status is always associated with high *Vimentin* expression levels. Additionally, *Vimentin* has been shown to be associated with several pathways, including cell adhesion, cytoplasmic microtubule assembly, and cytoskeleton remodeling. Higher *Vimentin* expression in pancreatic cancer cells may imply a higher state of malignancy of these cells, with an associated higher metastatic ability. The detailed mechanism of *Vimentin* and its gene methylation status requires further study.

Our observations only covered 64 pancreatic cancer patients, which is a small population sample. Although our results showed that the *Vimentin* methylation status could be used to predict prognosis in pancreatic cancer, more studies and clinical trials are needed to validate this result. In summary, our study showed that pancreatic cancer patients exhibiting a negative *Vimentin* methylation

status displayed a poorer prognosis as compared with those with a positive status. The role of *Vimentin* methylation in pancreatic cancer warrants further empirical exploration.

COMMENTS

Background

Vimentin is reported as an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling. In their current study, authors have attempted to identify the relationship between the methylation state of the *Vimentin* gene and pancreatic cancer.

Research frontiers

Several investigators have previously shown that *Vimentin* is an important marker for the early detection of cancer, such as bladder cancer, hepatocellular carcinoma and colorectal cancer. In addition, methylation of *Vimentin* is described as a marker in several malignant tumors, including gastric carcinoma, colorectal carcinoma, cervical cancer and bladder cancer.

Innovations and breakthroughs

The location of pancreatic carcinoma was related to the *Vimentin* methylation state. The pathological T staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the *Vimentin* methylation state ($P = 0.037$) were independent prognostic factors.

Applications

This result showed that the *Vimentin* methylation status could be used to predict prognosis in pancreatic cancer.

Peer review

The manuscript is very interesting. The authors try to determine the existence of a potential relationship between the methylation state of *Vimentin* and its prognostic value in pancreatic cancer. In total, 64 primary tumor specimens and normal tissues were collected in this study. The authors found that *Vimentin* methylation status can predict the prognosis of pancreatic cancer patients.

REFERENCES

1 **Michalski CW**, Weitz J, Büchler MW. Surgery insight: surgical management of pancreatic cancer. *Nat Clin Pract Oncol* 2007; **4**: 526-535 [PMID: 17728711 DOI: 10.1038/ncponc0925]

2 **Hirata K**, Egawa S, Kimura Y, Nobuoka T, Oshima H, Katsuramaki T, Mizuguchi T, Furuhata T. Current status of surgery for pancreatic cancer. *Dig Surg* 2007; **24**: 137-147 [PMID: 17476103 DOI: 10.1159/000102067]

3 **Cardenes HR**, Chiorean EG, Dewitt J, Schmidt M, Loehrer P. Locally advanced pancreatic cancer: current therapeutic approach. *Oncologist* 2006; **11**: 612-623 [PMID: 16794240 DOI: 10.1634/theoncologist.11-6-612]

4 **Carpelan-Holmström M**, Nordling S, Pukkala E, Sankila R, Lüttges J, Klöppel G, Haglund C. Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. *Gut* 2005; **54**: 385-387 [PMID: 15710987 DOI: 10.1136/gut.2004.047191]

5 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

6 **Sultana A**, Smith CT, Cunningham D, Starling N, Neoptolemos JP, Ghaneh P. Meta-analyses of chemotherapy for locally advanced and metastatic pancreatic cancer. *J Clin Oncol* 2007; **25**: 2607-2615 [PMID: 17577041 DOI: 10.1200/JCO.2006.09.2551]

7 **Hawes RH**, Xiong Q, Waxman I, Chang KJ, Evans DB, Abbruzzese JL. A multispecialty approach to the diagnosis and management of pancreatic cancer. *Am J Gastroenterol* 2000; **95**: 17-31 [PMID: 10638554 DOI: 10.1111/j.1572-0241.2000.01699.x]

8 **Wu X**, Lu XH, Xu T, Qian JM, Zhao P, Guo XZ, Yang XO, Jiang WJ. Evaluation of the diagnostic value of serum tumor markers, and fecal k-ras and p53 gene mutations for pancreatic cancer. *Chin J Dig Dis* 2006; **7**: 170-174 [PMID: 16808798]

9 **Leung TK**, Lee CM, Wang FC, Chen HC, Wang HJ. Difficulty with diagnosis of malignant pancreatic neoplasms coexisting with chronic pancreatitis. *World J Gastroenterol* 2005; **11**: 5075-5078 [PMID: 16124071]

10 **Zhou G**, Chiu D, Qin D, Niu L, Cai J, He L, Huang W, Xu K. The efficacy evaluation of cryosurgery in pancreatic cancer patients with the expression of CD44v6, integrin-β1, CA199, and CEA. *Mol Biotechnol* 2012; **52**: 59-67 [PMID: 22382453 DOI: 10.1007/s12033]

11 **Lee JM**, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006; **172**: 973-981 [PMID: 16567498 DOI: 10.1083/jcb.200601018]

12 **Ivaska J**, Pallari HM, Nevo J, Eriksson JE. Novel functions of vimentin in cell adhesion, migration, and signaling. *Exp Cell Res* 2007; **313**: 2050-2062 [PMID: 17512929 DOI: 10.1016/j.yexcr.2007.03.040]

13 **Costa VL**, Henrique R, Danielsen SA, Duarte-Pereira S, Eknaes M, Skotheim RI, Rodrigues A, Magalhães JS, Oliveira J, Lothe RA, Teixeira MR, Jerónimo C, Lind GE. Three epigenetic biomarkers, GDF15, TMEFF2, and VIM, accurately predict bladder cancer from DNA-based analyses of urine samples. *Clin Cancer Res* 2010; **16**: 5842-5851 [PMID: 20975101 DOI: 10.1158/1078-0432.CCR-10-1312]

14 **Wong KF**, Luk JM. Discovery of lamin B1 and vimentin as circulating biomarkers for early hepatocellular carcinoma. *Methods Mol Biol* 2012; **909**: 295-310 [PMID: 22903723]

15 **Shirahata A**, Sakata M, Sakuraba K, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. Vimentin methylation as a marker for advanced colorectal carcinoma. *Anticancer Res* 2009; **29**: 279-281 [PMID: 19331162]

16 **Shirahata A**, Sakuraba K, Kitamura Y, Yokomizo K, Gotou T, Saitou M, Kigawa G, Nemoto H, Sanada Y, Hibi K. Detection of vimentin methylation in the serum of patients with gastric cancer. *Anticancer Res* 2012; **32**: 791-794 [PMID: 22399595]

17 **Jung S**, Yi L, Kim J, Jeong D, Oh T, Kim CH, Kim CJ, Shin J, An S, Lee MS. The role of vimentin as a methylation biomarker for early diagnosis of cervical cancer. *Mol Cells* 2011; **31**: 405-411 [PMID: 21491170 DOI: 10.1007/s10059-011-0229-x]

18 **Hibi K**, Sakata M, Sakuraba K, Shirahata A, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y. Aberrant methylation of the HACE1 gene is frequently detected in advanced colorectal cancer. *Anticancer Res* 2008; **28**: 1581-1584 [PMID: 18630515]

19 **Siegel R**, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; **61**: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]

20 **Ghiorzo P**, Gargiulo S, Nasti S, Pastorino L, Battistuzzi L, Bruno W, Bonelli L, Taveggia P, Pugliese V, Borgonovo G, Mastracci L, Fornarini G, Romagnoli P, Iiritano E, Savarino V, Bianchi-Scarrà G. Predicting the risk of pancreatic cancer: on CDKN2A mutations in the melanoma-pancreatic cancer syndrome in Italy. *J Clin Oncol* 2007; **25**: 5336-537; author reply 5336-537; [PMID: 18024887 DOI: 10.1200/JCO.2007.13.5624]

21 **Whelan AJ**, Bartsch D, Goodfellow PJ. Brief report: a familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor gene. *N Engl J Med* 1995; **333**: 975-977 [PMID: 7666917 DOI: 10.1056/NEJM199510123331505]

22 **Ferrone CR**, Levine DA, Tang LH, Allen PJ, Jarnagin W, Brennan MF, Offit K, Robson ME. BRCA germline mutations in Jewish patients with pancreatic adenocarcinoma. *J Clin Oncol* 2009; **27**: 433-438 [PMID: 19064968 DOI: 10.1200/JCO.2008.18.5546]

23 **Hahn SA**, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, Gerdes B, Kress R, Ziegler A, Raeburn JA, Campra D, Grützmann R, Rehder H, Rothmund M, Schmiegel W, Neoptol-

emos JP, Bartsch DK. BRCA2 germline mutations in familial pancreatic carcinoma. *J Natl Cancer Inst* 2003; **95**: 214-221 [PMID: 12569143 DOI: 10.1093/jnci/95.3.214]

24 **Slater EP**, Langer P, Niemczyk E, Strauch K, Butler J, Habbe N, Neoptolemos JP, Greenhalf W, Bartsch DK. PALB2 mutations in European familial pancreatic cancer families. *Clin Genet* 2010; **78**: 490-494 [PMID: 20412113 DOI: 10.1111/j.1399-0004.2010.01425.x]

25 **Lindor NM**, Petersen GM, Spurdle AB, Thompson B, Goldgar DE, Thibodeau SN. Pancreatic cancer and a novel MSH2 germline alteration. *Pancreas* 2011; **40**: 1138-1140 [PMID: 21926548 DOI: 10.1097/MPA.0b013e318220c217]

26 **Bird A**. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; **16**: 6-21 [PMID: 11782440 DOI: 10.1101/gad.947102]

27 **Shin TH**, Paterson AJ, Grant JH, Meluch AA, Kudlow JE. 5-Azacytidine treatment of HA-A melanoma cells induces Sp1 activity and concomitant transforming growth factor alpha expression. *Mol Cell Biol* 1992; **12**: 3998-4006 [PMID: 1380648]

28 **Ammanamanchi S**, Kim SJ, Sun LZ, Brattain MG. Induction of transforming growth factor-beta receptor type II expression in estrogen receptor-positive breast cancer cells through SP1 activation by 5-aza-2′-deoxycytidine. *J Biol Chem* 1998; **273**: 16527-16534 [PMID: 9632722 DOI: 10.1074/jbc.273.26.16527]

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ISSN 1007-9327