Pathology – Research and Practice 209 (2013) 635–639

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Influence of MRE11, RAD50 and NIBRIN protein expression on survival in pancreatic carcinoma after curative resection

Klemens Horst[a,*], Silke Ganzera[b], Wolfgang Kaisers[c], Johanna Munding[d], Berenike Flott-Rahmel[d], Andrea Tannapfel[d], Hubert Zirngibl[b]

[a] Department of Orthopedic Trauma Surgery, RWTH Aachen, Aachen, Germany
[b] Department for General and Visceral Surgery, HELIOS Klinikum Wuppertal, Wuppertal, Germany
[c] Bio-Medical-Research Center, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[d] Institute for Pathology, Ruhr-University-Bochum, Bochum, Germany

ARTICLE INFO

Article history:
Received 3 February 2013
Received in revised form 23 June 2013
Accepted 8 July 2013

Keywords:
MRE11
RAD50
NIBRIN
Survival
Pancreatic carcinoma

ABSTRACT

The MRE11/RAD50/NIBRIN complex, a protein complex that repairs DNA double-strand breaks, could serve as an early marker for new lesions in pancreatic cancer. We determined the expression of MRE11, RAD50 and NIBRIN, and their possible prognostic value regarding survival.

Forty-one patients with ductal adenocarcinoma of the pancreas were included. All underwent curative surgery. Immunohistochemistry was performed for MRE11, RAD50 and NIBRIN. Subsequent analyses were based on a modified immunoreactive score. Statistical analysis was conducted using the statistics program "R".

The mean follow-up period was 509 days. The mean age of the patients was 67 ± 8 years, male = 56%, female = 44%. Eighty-seven percent underwent a Kausch-Whipple procedure, whereas a left side resection was performed in 22% of patients. Positive lymph nodes were found in 80% of cases, and patients were staged UICC IIa (12%), IIb (56%) and IV (29%). Overall significant results were found for MRE11 ($p = 0.02$) and NIBRIN ($p = 0.01$) expression and postoperative survival.

We found a significant relation between the expression of MRE11, NIBRIN and the postoperative survival of patients with ductal adenocarcinoma. The link between the expression of the MRN complex, ATM and pancreatic cancer can be used to develop new treatment options for pancreatic carcinoma.

Introduction

Following colon and gastric cancer, pancreatic carcinoma is the most common malignant tumor of the gastrointestinal tract [14]. With 6300 male and 6600 female cases diagnosed annually in Germany, pancreatic cancer is responsible for about 3% of all oncologic diagnoses each year [4]. The five-year survival rate has risen from 2% to just 4.2% in the last 30 years [10]. Reasons for this low rate are delayed diagnosis and thus delayed curative resection, and rapid and aggressive cancer metastasis. Recovery from this disease can be achieved by resection.

The risk factors for this disease are known. At the molecular level, the most common changes are as follows: activation of oncogenes, inactivation of suppressor genes and defects in DNA repair genes [1].

Given the impact of this disease on death statistics and the small number of patients that can be offered curative resection, much attention has been given to achieving an early diagnosis. Unfortunately, however, there are no screening methods that help identify pancreatic cancer at an early stage.

Patients with ataxia telangiectasia (AT) have a higher risk of developing pancreatic carcinoma [21,25], as originally suggested by Swift et al. in 1976 [26]. Ataxia telangiectasia, also known as Louis–Bar Syndrome or Boder–Sedgwick Syndrome, is an inherited systemic disease characterized by an increased frequency of chromosome breakage, immune deficiency and oncologic diseases. In fact, patients with AT have a 100-fold higher risk of developing malignancy than healthy people [20,25]. Double-strand breaks in DNA and incorrect repair are central to the disease, and the protein kinase ataxia telangiectasia mutated (ATM) is important. The latter initiates the repair mechanism but its activation and functionality depends on how it is transported toward the DNA lesion [23]. ATM requires co-proteins that can be used for transport. The so-called MRN complex, a protein complex involving MRE11, RAD50 and NIBRIN, fulfills this role [29], detecting and transporting ATM to the damaged part of the DNA. Therefore, monitoring

* Corresponding author at: Department of Orthopaedic Trauma Surgery, University Hospital RWTH Aachen, Paulwelsstraße 30, 52074 Aachen, Germany.
Tel.: +49 0241 80 36785; fax: +49 0241 80 3388360.
E-mail address: khorst@ukaachen.de (K. Horst).

0344-0338/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.prp.2013.07.003

the MRN complex could be used to detect DNA double-strand breaks.

Hsu et al. showed that the MRN complex influences the development of breast carcinomas [16]. There is also evidence that mutations in double-strand repair mechanisms affect or even control the pathogenesis of breast cancer. Hsu et al. demonstrated that risk factors in specific genotypes significantly increase the risk of developing breast cancer compared to other genotypes [16]. In the early 1990s, Swift et al. showed that patients with AT have a higher risk of developing pancreatic cancer [25]. Wang et al. carried out a mutational analysis in 32 double-strand DNA break repair genes in breast and pancreatic carcinoma and suggested that inactivating mutations in RAD50 predispose to pancreatic cancer [30].

Based on the current knowledge about the development, diagnostics, therapy and prognosis of pancreatic carcinoma, and the insights of Hsu et al. and Wang et al., the first step of our study was to demonstrate significant activity of the repair supporting genes MRE11, RAD50 and NIBRIN in pancreatic carcinoma.

All patients included in this study were treated by curative resection between 1996 and 2007. They either received a Whipple procedure or a left-sided resection. The results of immunohistochemistry were correlated with survival rates, including individual pathological and clinical parameters. The aim of this study was to determine the diagnostic and prognostic relevance of MRE11, RAD50 and NIBRIN in patients with pancreatic carcinomas that received curative resection.

Materials and methods

Between 1996 and 2007, 1192 patients with pancreatic carcinoma presented to the surgical department at the HELIOS University Hospital Wuppertal. Resection surgery was performed in 104 patients. Complete data were available for 85 patients. Histological material was available in 53 cases. After applying exclusion criteria to the latter cases, 41 patients were included in the study. The exclusion criteria were insufficient histological material ($n = 1$) and loss to follow-up ($n = 11$). Demographic, clinical (diagnostic, therapy, rehabilitation, progress) and detailed pathological information was recorded.

In order to document the timing of events and adjuvant therapy and the progression of disease or recovery, a questionnaire was created and sent to the patients' general physician.

Histological material was screened and immunohistochemistry was performed. The results were recorded as a modified Immunoreactive Score originally developed by Remmele und Stegner [22]. Information about immunohistochemistry in pancreatic tissue was obtained from the Swedish Human Proteome Resource (HPR). Investigations were carried out by two independent researchers. In the case of differing opinion, the results were discussed until a consensus was reached.

Statistical analysis was conducted using the statistics program "R" (Version 2.10.0) [28]. CRAN Packet "survival" (Version 2.35-7) and CRAN Packet "timereg" (Version 1.2-5) were used. Survival analysis was performed, and Log-rank tests were used for two-sided superiority tests. Estimated parameter values were calculated using semi parametric models. The multiplicative Cox-model was used. Graphics were generated using EXCEL 2010.

Table 1
UICC distribution of patients included in immunohistochemistry.

Patients	UICC						Total
	Ia	Ib	IIa	IIb	III	IV	
n	0	1	5	23	0	12	41
%	0	2	12	56	0	29	99

Results

Forty-one patients were included in this study. The mean follow-up lasted 509 days. The patients were 67 ± 8 years old (m = 66 ± 5.8 y; f = 69 ± 10 y), and 56% ($n = 23$) were male while 44% ($n = 18$) were female. The majority (80%, $n = 33$) had lymph node metastasis. Of these, 27% ($n = 9$) presented with stadium 1a and 73% ($n = 24$) with stadium 1b.

Seven patients (17%) were classified as ASA II while 12% ($n = 5$) were classified as ASA IV. The majority (68%, $n = 28$) were rated ASA III. Only 2% ($n = 1$) were graded ASA V. By the end of our observation period, only 4 patients were alive. Patients were classified by the UICC staging system (Table 1).

Data were analyzed using the Cox model. The parameters analyzed were age, sex, number of metastasized lymph nodes, and nuclear and cytoplasmic expression of p53, MRE11, RAD50 and NIBRIN.

Regression analysis was carried out, revealing that age, MRE11 and NIBRIN had a significant influence on survival (Table 2). A goodness of fit procedure did not reveal deviations from the model.

Older age ($p < 0.001$) and high levels of expression of MRE11 ($p = 0.02$) (Picture 1A) were associated with shorter survival. High levels of expression of NIBRIN ($p = 0.01$) (Picture 1B) had the opposite effect. High levels of expression of NIBRIN were associated with longer survival. RAD50 ($p = 0.33$) and nuclear p53 ($p = 0.943$) expression did not show a significant effect on survival. However, cytoplasmic expression of p53 was just below the level of significance ($p = 0.051$).

An extended Cox regression model did show that all variables were not time-dependent.

Discussion

The Robert-Koch Institute (Germany) estimates the incidence of pancreatic cancer in 2012 as 7800 cases in men and 7600 cases in women. Due to late occurring symptoms, only 15–20% of affected patients can be offered curative therapy.

Table 2
Cox model.

Parameter	Estimated values			Wald test	95% confidence interval	
	β	exp(β)	se(β)	*p*-Value	Lower limit	Upper limit
Gender	0.615	1.85	0.418	0.141	0.816	4.196
Age	0.141	1.151	0.033	0.00	1.08	1.227
Lymph node status	−0.043	0.958	0.569	0.94	0.314	2.921
MRE11 IRS	0.261	1.299	0.112	0.02	1.042	1.619
RAD50 IRS	0.086	1.089	0.088	0.33	0.917	1.294
NIBRIN IRS	−0.161	0.852	0.063	0.01	0.753	0.963

β = estimated value of regression coefficient in the proportional hazard model.
exp(β) = relative risk of death.
se(β) = standard deviation for estimated value.