Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Expression of neuropeptide Y and its receptors Y1 and Y2 in pancreatic intraepithelial neoplasia and invasive pancreatic cancer in a transgenic mouse model and human samples of pancreatic cancer

*Jens Waldmann, MD, Volker Fendrich, MD, Martin Reichert, MD, Andreas Hecker, MD, Detlef K. Bartsch, MD, Winfried Padberg, MD, and Julia P.N. Holler, MD**

Department of Surgery, University Hospital Giessen and Marburg, Campus Giessen and Marburg, Giessen and Marburg, Germany

ARTICLE INFO

Article history:
Received 8 December 2016
Received in revised form
2 October 2017
Accepted 3 November 2017
Available online xxx

Keywords:
PanIN
Snail
Pancreatic injury
ADM
PDAC
NPY
Y1
Y2
IPMN

ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) is among the most dismal of human malignancies. Neuropeptides have shown to be implicated in angiogenesis, tumor growth, and formation of distant metastases in various solid tumors. In the present study, we used a genetically engineered mouse model of pancreatic cancer to evaluate the impact of neuropeptide Y (NPY) and its receptors 1 (Y1) and 2 (Y2) in preneoplastic lesions and pancreatic cancer as a potential target with antiproliferative properties. In addition, human PDAC tissue was analyzed.

Materials and methods: By interbreeding conditional *LsL-Trp53*^{R172H},*LsL-Kras*^{G12D} and *Pdx1-Cre* strains, we obtained *LsL-Kras*^{G12D};*LsL-Trp53*^{R172H};*Pdx1-Cre*(KPC), *LsL-Kras*^{G12D};*Pdx1-Cre*(KP) and control mice ($n = 8$ each). Mice were then followed in a longitudinal study for 3 to 6 mo. Pancreata were analyzed in regard to pancreatic intraepithelial neoplasia (PanIN) lesions and invasive carcinoma. Corresponding sections were then assessed by immunohistochemistry and quantitative polymerase chain reaction for NPY, Y1 and Y2 expression in murine and human samples.

Results: NPY and Y1 expressions were detected in human and murine pancreatic samples, but expression levels were similar in neoplastic and non-neoplastic tissue. Y2 revealed a significant increase of expression in the transgenic mouse model in PanIN lesions and pancreatic cancer compared to control. This holds also true for human samples of pancreatic cancer. Immunohistochemistry of Y2 in murine and human samples of PanINs and pancreatic carcinoma revealed an increased expression in PanIN lesions and pancreatic cancer.

Conclusions: Y2 is strongly overexpressed in pancreatic cancer and may modulate angiogenesis.

* *Corresponding author*. Department of Surgery, University Hospital Giessen and Marburg, Campus Giessen, D-35382 Giessen, Germany. Tel.: +49/641-985-44701; fax: +49/641-985-44709.
E-mail address: julia.p.holler@chiru.med.uni-giessen.de (J.P.N. Holler).
0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2017.11.010

Introduction

Pancreatic ductal adenocarcinoma (PDAC) of the pancreas kills >95% all of the 280,000 people affected by this disease annually worldwide. High mortality is a consequence of poor detection, distant metastases at initial diagnosis, and no effective therapy.[1,2]

Invasive PDAC is believed to descend from a spectrum of preneoplastic mucinous lesions with ductal features or briefly pancreatic intraepithelial neoplasias (PanINs), the most common precursor lesions observed in humans among other less common lesions as mucinous cystic neoplasias and intraductal papillary mucinous neoplasias.[3] Once initiated, PDAC present commonly as a fast-growing type of cancer. In a quantitative analysis focusing on timing of the genetic evolution of PDAC, Yachida *et al.*[4] found that at least a decade passes between the occurrence of the initiating mutation and the birth of the parental, nonmetastatic founder cell. Genetic mutations in preneoplastic lesions accumulate during tumor progression and lead to a stepwise atypia and ultimately invasive PDAC.[5] Activating mutations of the *Kras* gene mutations represent the earliest detectable mutations in preneoplastic lesions.[6] The utmost importance of *Kras* mutations for disease initiation has been shown in mice. The expression of a constitutively active $Kras^{G12D}$ allele induces PanINs and after a significant latency period also PDAC.[7] PanIN formation occurs simultaneously with acinar-to-ductal metaplasia, a common finding in pancreatitis and a highly significant risk factor for PDAC in humans.[8,9]

Many peptide hormone receptors are overexpressed in human cancer allowing an *in vivo* targeting in a diagnostic or therapeutic background. NPY receptors are emerging candidates in this field, and Y1 and Y2 have been identified in many human cancers.[10] NPY receptors have been found to be expressed in tumor cells and blood vessels. With declining intensity, NPY receptors are found in the endocrine tumors (pheochromocytomas and paragangliomas), breast cancer, renal cell carcinomas, and ovarian cancer.[11-13] Tumor cells predominantly express Y1 or Y2, or both. Increasing evidence suggests that Y1 and Y2 may be selectively active and impair intracellular signaling.[14,15] Recent data from human cancer cell lines showed that there may be a role of NPY in tumor growth and angiogenesis.[13,16] In a transgenic null Y2 knockout mouse, Lee *et al.*[17] demonstrated that NPY-induced spontaneous and ischemic angiogenesis was primarily mediated by Y2.

We now show for the first time that NPY and Y2 is expressed in PanINs and pancreatic cancer in a genetically engineered transgenic mouse model of PDAC. Furthermore, a significant increase of Y2 expression in PanINs and pancreatic cancer was observed compared to control. These observations were confirmed in human specimen of chronic pancreatitis and pancreatic cancer suggesting a potential target with antiproliferative properties.

Materials and methods

Generation of LsL-$Kras^{G12D}$;LsL-$Trp53^{R172H}$;Pdx1-Cre(KPC), LsL-$Kras^{G12D}$;Pdx1-Cre(KP) mice

Conditional *LsL-*$Trp53^{R172H}$, *LsL-*$Kras^{G12D}$, and *Pdx1-Cre* strains were interbred to obtain double mutant (*LsL-*$Kras^{G12D}$*;Pdx1-Cre(KP)*) and triple (*LsL-*$Kras^{G12D}$*;LsL-*$Trp53^{R172H}$*;Pdx1-Cre(KPC*)) mutant mice on a mixed 129/SvJae/C57Bl/6 background as previously described.[18,19] All experiments were approved by the local committees for animal care (Regional Council Giessen, veterinary department) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publication No. 8023, revised 1978). Animals were maintained in a climate-controlled room kept at 22°C, exposed to a 12:12-h light-dark-cycle, and fed standard laboratory chow and water ad libitum as described by Plassmeier *et al.*[20]

Genotyping

For genotyping, genomic DNA was extracted from mice tail cuttings using the REDExtract-N-Amp Tissue PCR kit (Sigma−Aldrich, St. Louis, Missouri). Three PCR reactions were carried out for each animal, to test for the presence of the oncogenic *Kras* (using LoxP primers), *Snail1*, and *Pdx1-Cre* transgene constructs (using Cre-specific primers along with Gabra as positive control), respectively, as described by Plassmeier *et al.*[20] Primer sequences are available upon request.

Histologic evaluation

After longitudinal studies, mice were euthanized, and the pancreas was removed and inspected for grossly visible tumors and either preserved in 10% formalin solution (Sigma−Aldrich; Merck KGaA, Darmstadt, Germany) for histology or processed for RNA extraction (see below). Formalin-fixed, paraffin-embedded tissues were sectioned (4 μm) and stained with hematoxylin and eosin. Six sections (100 μm apart) of pancreatic tissues were histologically evaluated. PanIN lesions were classified according to histopathologic criteria as described by Plassmeier *et al.*[20]

To quantify the progression of PanIN lesions in KP, KPS, and KPS^{het} mice, the total number of ductal lesions and their grade were determined. About 100 to 130 pancreatic ducts of the entire fixed specimen (head, body, and tail of the pancreas) were analyzed for each animal. The relative proportion of each PanIN lesion to the overall number of analyzed ducts was recorded for each animal as described by Funahashi *et al.*[21]

Immunostaining

Organs were fixed with 4% buffered paraformaldehyde, embedded in paraffin, and cut into sections (6 μm). Slides were incubated with protease type XIV (0.5 mg/mL) (Sigma−Aldrich) in tris(hydroxymethyl)aminomethane hydrochloride (50 mM,