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An animal model of preclinical diagnosis of pancreatic ductal adenocarcinomas

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDA) is a highly lethal disease, which is usually diagnosed in an advanced stage. Animal PDA models which reflect the human condition are clearly necessary to develop early diagnostic tools and explore new therapeutic approaches. We have established transgenic rats carrying a mutated H- or K-*ras* gene (Hras250 and Kras327) controlled by Cre/loxP activation. These animals develop PDA which are histopathologically similar to that in humans. We utilized this model to identify biomarkers to detect early PDA. We report here that serum levels of Erc/Mesothelin are significantly higher in rats bearing PDA than in controls. Importantly, the levels are significantly elevated in rats before grossly visible carcinomas develop. Even in rats with very small microscopic ductal carcinoma lesions, elevated serum Erc/Mesothelin can be detected. We believe this is the first report of a pancreas tumor animal model in which pre-symptomatic lesions can be diagnosed.

Introduction

Pancreatic ductal adenocarcinoma (PDA) carries the most dismal prognosis of all solid tumors. Preclinical detection of PDA is a necessary first step toward more successful treatment of this disease. Late manifestation of clinical symptoms, as well as the rapid and aggressive course of the disease contribute to its extremely high mortality. Most patients die within 1 year of diagnosis [1], and the 5 year survival rate is <5% [2]. Since the pancreas is located in a retroperitoneal cavity, detection of the tumor mass is possible only when it has reached a relatively large size. Furthermore, markers for the diagnosis of PDA have not yet been established. Consequently, diagnosis of pancreatic cancers when they are still treatable is extremely rare [3].

We have established transgenic rat lines carrying a human $H\textit{ras}^{G12V}$ (Hras250) [4] or a human $K\textit{ras}^{G12V}$ (Kras327) oncogene in which the expression of the transgene is regulated by the Cre/loxP system (termed *ras* Tg rats). Targeted activation of the transgene is accomplished by injection of a Cre recombinase-carrying adenovirus (AxCANCre) into the pancreatic ducts through the common bile duct. Neoplastic lesions in the *ras* Tg rats exhibit morphological similarities to those observed in human pancreas lesions. Early ductal lesions exhibit close similarity to intraepithelial neoplasias (PanIN category).

The rat *Erc* (expressed in renal cell carcinoma) gene was identified as a highly expressed gene in renal cell carcinoma of the Eker rat [5,6]. A human homolog of rat *Erc* is the *Mesothelin/megakaryocyte potentiating factor* (*MPF*) gene [7,8]. Mesothelin was identified as a cell surface antigen recognized by the monoclonal antibody K1 in human mesotheliomas and ovarian carcinomas [9–11]; MPF was independently identified in the culture supernatant of a human pancreatic carcinoma cell line, HPC-Y5 [12]. Human Mesothelin/MPF is derived from a common 71 kDa precursor [8,11]. The precursor protein is cleaved by a furin-like protease, and a 31 kDa NH_2-terminal peptide (MPF) is released into the extracellular

Abbreviations: PanIN, pancreatic intraepithelial neoplasias; PDA, pancreas ductal adenocarcinoma; AxCANCre, Cre recombinase-carrying adenovirus; TEF-1, transcription enhancer factor-1

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space, leaving a 40 kDa COOH-terminal peptide (Mesothelin) attached to the cell surface by a GPI-anchor [9]. To avoid confusion, we refer to the rat Erc protein and its human homolog as Erc/Mesothelin. Erc/Mesothelin is expressed in ovary and pancreas carcinoma tissue in humans [13–15] and can be used as a marker for PDA [15]. Recently, we developed a novel sandwich ELISA system for serum Erc/Mesothelin [16–19]. Using this serum assay system, the level of Erc/Mesothelin was found to be higher in samples from mesothelioma patients than in samples from subjects without pancreas lesions [16,17]. In the present study we report the use of Erc/Mesothelin as a reliable serum marker for pre-symptomatic, pre-malignant pancreas lesions in *ras* Tg rats.

Materials and methods

Animals. Male H*ras*^{G12V} transgenic (Hras250) rats were bred with female Sprague–Dawley rats by CLEA Japan Inc. (Tokyo, Japan) as previously reported [4]. Routine genotyping of Hras250 rats was performed using the primers 5′-TCGTGCTTTACGGTATCGCCGCTCCC GATT-3′ and 5′-GATCTGCTCCCTGTACTGGTGG-3′. For the generation of transgenic rats conditionally expressing human K*ras*^{G12V}, 3× hemagglutinin (HA) tagged K*ras*^{G12V} cDNA was subcloned into the SacI/KpnI site of pCALNL5 (DNA Bank, RIKEN BioResource Center, Ibaraki, Japan). The purified cassette was injected into the pronuclei of Sprague–Dawley rats (CLEA Japan, Tokyo, Japan) as previously reported [4,20]. Two lines were established (Kras301 and Kras327). In this study, we used the Kras327 line. Routine genotyping of Kras327 rats was performed using the primers 5′-TCTGGATCAAATCCGAAC GC-3′ and 5′-TGACCTGCTGTGTCGAGAAT-3′. Rats were maintained in plastic cages in an air-conditioned room with a 12-h light/12-h dark cycle. The experiments were conducted according to the 'Guidelines for Animal Experiments of the Nagoya City University Graduate School of Medical Sciences'.

Tumor induction and pathological examination. AxCANCre was amplified in HEK293 cells and then purified using Vivapure Adenopack (Vivascience, Hannover, Germany). The titer of the adenovirus

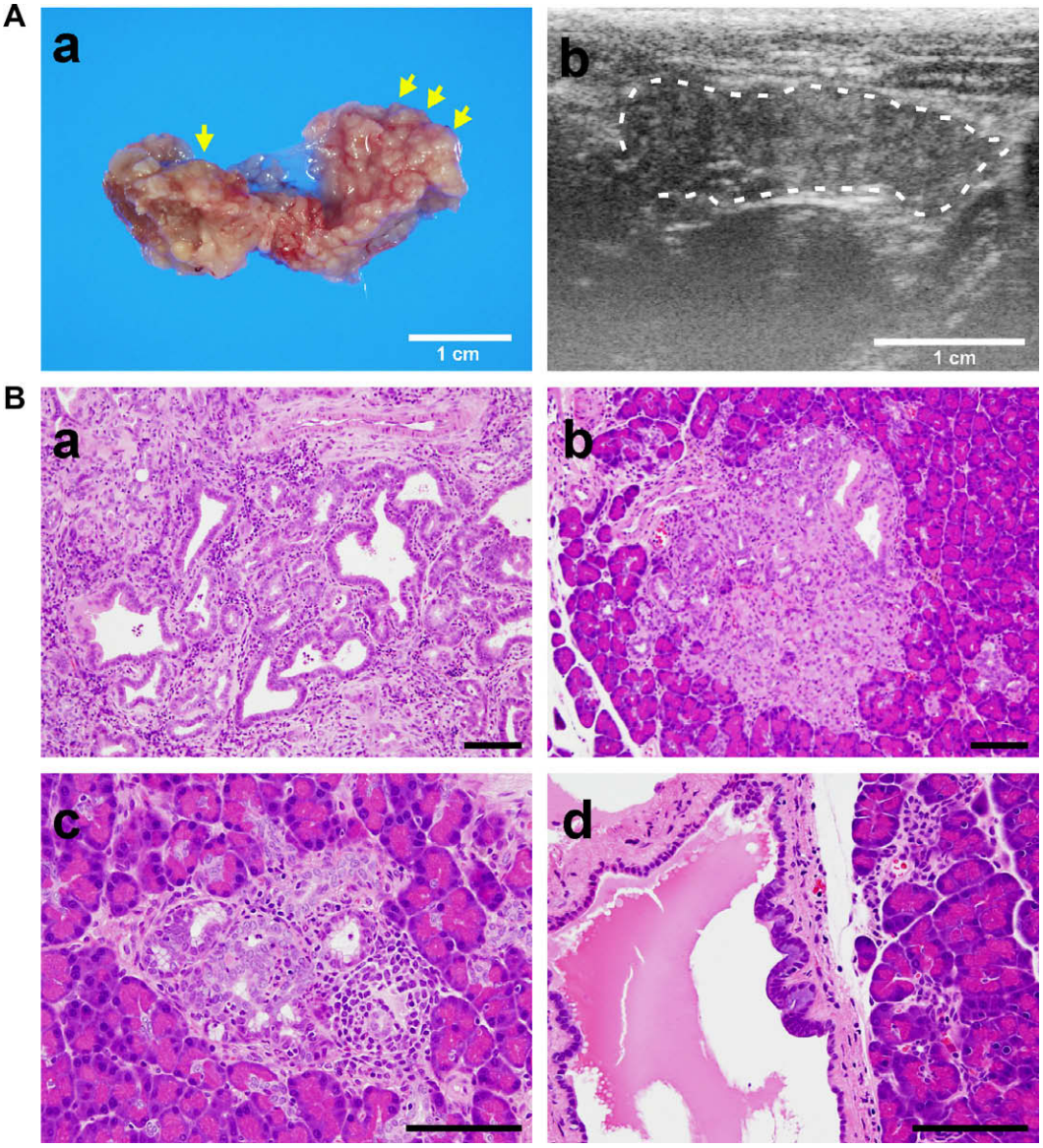

Fig. 1. Pancreas tumors developed in *ras* Tg rats. Animals were killed 3–4 weeks after injection of recombinant AxCANCre into the pancreas of adult *ras* Tg rats. (A) Macroscopic appearance of the pancreas with advanced multiple tumors (arrows) in an Hras250 rat (a). At this stage, multiple tumor nodules can be visualized by ultrasound image analysis (inside broken line) (b). (B) Histological appearance of pancreatic lesions. Large carcinoma (a), small carcinomas (b,c) and a PanIN-1a like lesion with slightly atypical duct epithelium (d). (a,b) are in Hras250 rats and (c) and (d) are grossly invisible small lesions in Kras327 rats. Bars = 100 μm.