Anti-tumor effects of a ‘human & mouse cross-reactive’ anti-ADAM17 antibody in a pancreatic cancer model in vivo

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1. Introduction

Pancreas cancer is virtually fatal within a very short time after diagnosis. The most common type of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC), accounts for over 90% of all pancreatic malignancies. PDAC represents the fourth cause of death in cancer, with an overall 5-year survival rate of < 5% (Warshaw and Fernandez-del Castillo, 1992). Surgical resection with radiation therapy or chemotherapy remains the only potential curative treatment today (Hidalgo, 1992). Surgical resection with radiation therapy or chemotherapy remains the only potential curative treatment today (Hidalgo, 1992). However, only 5% to 25% of patients with resectable tumors and the median survival of these patients is < 2 years, predominantly due to the local and systemic recurrence after surgery. Therefore, better management strategies and more effective treatments for PDAC are urgently needed.

PDAC is preceded by the formation of non-invasive premalignant lesions; in particular pancreatic intraepithelial neoplasias (PanINs), the most common pancreatic precursor lesions. Based on histological analysis, PanINs can be classified into two pathological stages, low-grade PanINs (PanIN-1 and PanIN-2), and high-grade PanINs (PanIN-3) (Basturk et al., 2015; Cornish and Hruban, 2011; Koorstra et al., 2008). Overwhelming experimental evidence suggests that PanINs predominantly arise from acinar cells through a process referred as acinar-to-ductal metaplasia (ADM) which is characterized by the loss of acinar markers, carboxypeptidase A1 (CPA1) and amylase, and gain in expression of ductal markers, SOX9 and cytotkeratin-19 (CK19) (Kopp et al., 2012; Zhu et al., 2007). The transformation of pancreatic acini to dysplastic epithelium accompanies with a well-characterized sequence of genetic changes, including the activation of proto-oncogene KRAS and subsequent loss of various tumor suppressor genes P16^ink4a^, TRPS3, SMAD4 or BRCA2 (Hruban et al., 2000). Genetically engineered mouse models expressing oncogenic Kras together with conditional deletion of Trp53 have been shown to recapitulate the step-wise development of human pancreatic tumors (Bardeesy et al., 2015; Morton et al., 2008).

Upstream of KRAS, ErbB-mediated signaling cascades for example epidermal growth factor receptor (EGFR) signaling cascades play an
important role for pancreatic tumorigenesis. Without EGFR activation, mutant KRAS could not efficiently drive tumorigenesis of pancreas in vivo (Ardito et al., 2012; Navas et al., 2012). These pre-clinical studies have suggested that EGFR is a validated molecular target in pancreatic tumorigenesis. Combination of an EGFR tyrosine kinase inhibitor or have suggested that EGFR is a validated molecular target in pancreatic tumorigenesis. Without EGFR activation, J. Ye et al.

2.1. Antibodies and chemicals

Production of human anti-ADAM17 antibody A9(B8) has been described previously (Kwok et al., 2014). Briefly, A9(B8) IgG was expressed by transfection of HEK293 cells and the antibody was purified from conditioned medium by two Protein-A/G columns (GE Healthcare) and AKTA FPLC affinity chromatography (GE Healthcare), followed by dialysis in HEPES-buffered saline, pH 7.4 and filter-sterilized. Control human plasma IgG (R&D Systems 10-001-A) was used as a control in cell-based assays. N-TIMP-3 was prepared as described by Lee et al. (2001).

2.2. Cell culture

The PANC1 cell line was obtained from the American Tissue Type Collection (ATCC) and maintained in Dulbecco’s minimal essential medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco), 4 mM L-glutamine (Gibco), and 1% penicillin-streptomycin (Gibco) at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

2.3. Cell-surface shedding assays

To prepare for shedding assays carried out in PANC-1 cells, 1 × 10⁵ cells/well were plated on 48-well plates with 320 µl of medium for 18 h. Cells were then washed once with serum free media and incubated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) or PMA supplemented with 200 nM A9(B8) IgG or solvent control for 1 h. Soluble substrates were measured by sandwich Duoset kits (R & D systems) using the following kits: human TNF-α (TNFSF1A; cat. no. DY210), human soluble TNFR1-α (TNFRSF1A; cat. no. DY225), human TGF-α (cat. no. DY239), human AREG (cat. no. DY262), human IL-6-α (cat. no. DY227) and human HB-EGF (cat. no. DY259) according to manufacturer’s instructions. Absorbance was measured at 450 nm using Tecan Infinite-200. This process was performed three times for each cell line. Graphs were generated using GraphPad Prism 6 software (version 6.01).

2.4. IncuCyte cell proliferation analysis

PANC-1 cells were seeded at 5 × 10⁴ cells per well in 96-well, clear bottomed, tissue culture plates (Thermo Fisher Scientific) in 100 µl complete growth medium. After 8 h, cells were treated with 250 nM A9(B8) IgG or 250 nM normal human plasma IgG (R & D systems) for control. The plate was then inserted into the IncuCyte (Essen Bioscience) for real-time imaging, with three fields imaged per well under 10× magnification every 2 h for a total of 4 days. Data were analyzed using the IncuCyte Zoom software (version 2014A), which quantified cell surface area coverage as confluence values. All IncuCyte experiments were performed in triplicate.

2.5. Wound healing assay

Cells were assessed in wound healing scratch assays using the IncuCyte (Essen Bioscience). PANC-1 cells were seeded at 4 × 10⁴ cells on the 96-well ImageLock plates (Essen BioScience; cat. no. 4379) and incubated in complete media (DMEM with 10% FBS, and 4 mM L-glutamine) for 8 h. Wounds were made using the 96-pin WoundMaker (Essen BioScience) 1 h after the plate was washed twice with PBS, and incubated with DMEM containing 15% Charcoal/dextran-treated FBS (HyClone), 1% penicillin-streptomycin (Gibco), 4 mM L-glutamine (Gibco), 250 nM normal human plasma IgG (R & D systems) or 250 nM A9(B8) IgG. Cell migration was monitored in real time by IncuCyte, and wound width was measured by the IncuCyte software Zoom (version 2014A).

2.6. Mouse procedures

All mouse experiments described herein were approved by the University of Macau Animal Research Ethics Committees. A pharmacokinetic (PK) study was performed to assess the stability and half-life of A9(B8) antibody in serum. Six week old male and female C57BL/6 mice (three male; three female) were housed in a temperature and humidity-controlled room for the duration of the study. Blood samples pre-bleed were taken from each animal before treatment with A9(B8) IgG. Each animal was treated with 10 mg/kg A9(B8) IgG by intravenous tail (i.v.) injection. Blood samples were collected in heparinised tubes at selected time points post-antibody-injection (1, 4, 24, and 48 h and 7, 21, 28, 35, and 42 days). Samples were
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