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RAGE Gene Deletion Inhibits the Development and Progression of Ductal Neoplasia and Prolongs Survival in a Murine Model of Pancreatic Cancer

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Discussant

Dr. Emina H. Huang (Gainesville, FL): Congratulations, Dr. DiNorcia, for continued excellent work under the guidance of Drs. Ann Marie Schmidt and John Allendorf. Your presentation today is the culmination of a tremendous labor of breeding, genotyping, and pathologic dissection and you are commended for your efforts.

In your discussion, you refer to the issues that mechanism in your studies is only inferred. With the recent advances in combining molecular pathways and signatures from malignancies such as melanoma and non-small cell lung cancer in efforts to provide targeted treatment, I have three questions:

1. Are either soluble RAGE or a RAGE small molecule inhibitor, or cromolyn or other S100P antagonists potential treatments for pancreatic cancer?
2. Have you tried these interventions early on in the Tuveson mouse model?
3. Do you see a role for RAGE antagonism in those kindred with a familial pancreatic cancer or for those lacking metastatic disease, to prevent either metastases or recurrence?

Again, brilliant work and congratulations!

Closing Discussant

Dr. Joseph DiNorcia: Thank you, Dr. Huang, for your gracious comments and questions. RAGE inhibitors may be potential treatments for pancreatic cancer. It also is possible to envision inhibitors of downstream signaling as potential treatments once these post-receptor pathways are better defined. Given the complex role RAGE plays in differentially regulating cell survival and cell death, we have considerable more work to do prior to clinical application.

Targeting the ligand–RAGE axis also may be a strategy to improve the effectiveness of chemotherapy. For example, it has been shown that HMGB1 is released following tumor cell death and interacts with RAGE on neighboring tumor cells to promote survival. By inhibiting this ligand–RAGE interaction, we might be able to enhance a chemotherapeutic agent's ability to kill tumor cells.

We have treated a very small number of *Pdx1-Cre; Kras^{G12D/+}; p16^{Ink4a}–/–* mice with sRAGE. Although we noted no differences in the incidence of carcinoma, there was a significantly decreased stromal reaction in sRAGE-treated mice compared to controls, suggesting that stromal cells play roles in tumorigenesis via RAGE-dependent pathways.

Finally, perhaps the most practical clinical application of RAGE antagonism is in pancreatic cancer prevention. For example, RAGE inhibition in patients with IPMN may delay or arrest tumor progression to carcinoma. Or, in patients with localized pancreatic cancer, RAGE antagonism may prevent invasion and metastases by inhibiting tumor cell adhesion and motility. The ligand–RAGE axis is an attractive target for potential prophylaxis and treatment of pancreatic cancer and thus remains an exciting area for further study.

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Abstract

Background—The receptor for advanced glycation end-products (RAGE) is implicated in pancreatic tumorigenesis. Activating *Kras* mutations and *p16* inactivation are genetic abnormalities most commonly detected as pancreatic ductal epithelium progresses from intraepithelial neoplasia (PanIN) to adenocarcinoma (PDAC).

Objective—The aim of this study was to evaluate the effect of *RAGE* (or *AGER*) deletion on the development of PanIN and PDAC in conditional *Kras*^{G12D} mice.

Materials and Methods—*Pdx1-Cre*; *LSL-Kras*^{G12D/+} mice were crossed with *RAGE*^{-/-} mice to generate *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *RAGE*^{-/-} mice. *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-} mice

were crossed with *RAGE*^{-/-} mice to generate *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{-/-} mice. Pancreatic ducts were scored and compared to the relevant *RAGE*^{+/+} controls.

Results—At 16 weeks of age, *Pdx1-Cre; LSL-Kras*^{G12D/+}; *RAGE*^{-/-} mice had significantly fewer high-grade PanIN lesions than *Pdx1-Cre; LSL-Kras*^{G12D/+}; *RAGE*^{+/+} controls. At 12 weeks of age, none of the *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{-/-} mice had PDAC compared to a 45.5% incidence of PDAC in *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{+/+} controls. Finally, *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{-/-} mice also displayed markedly longer median survival.

Conclusion—Loss of RAGE function inhibited the development of PanIN and progression to PDAC and significantly prolonged survival in these mouse models. Further work is needed to target the ligand–RAGE axis for possible early intervention and prophylaxis in patients at risk for developing pancreatic cancer.

Keywords

RAGE; AGER; PanIN; Pancreatic adenocarcinoma; Pancreatic cancer; *Pdx1-Cre*; *Kras*; *p16*; *RAGE*^{-/-} mice

Introduction

Pancreatic cancer is one of the most lethal gastrointestinal malignancies and the fourth leading cause of cancer-related deaths in the USA.¹ The overall incidence and mortality have changed little in the past three decades with estimates of 43,140 new cases and 36,800 deaths from the disease in 2010. Estimated 5-year survival is approximately 5% in part because the disease is diagnosed at late stages when it is no longer amenable to curative resection. Thus, there is a need to identify novel targets for early intervention in pancreatic cancer.

Pancreatic ductal adenocarcinoma (PDAC) is thought to follow an adenoma–carcinoma sequence, arising from noninvasive precursor lesions called pancreatic intraepithelial neoplasia (PanIN) and progressing through increasingly severe stages of cellular atypia to invasive PDAC.² On the molecular level, these lesions show multi-step changes that include early activating mutations in the proto-oncogene, *KRAS*, and subsequent inactivating mutations in tumor-suppressor genes such as *p16*^{INK4A}.³ The central role of these genetic alterations is evidenced by the presence of mutations in *KRAS* and inactivating mutations in *p16*^{INK4A} in over 90% of cases of human PDAC.^{4,5}

In this context, the preclinical study of PanIN progression to PDAC has been made possible by genetically modified mouse models that recapitulate human pancreatic neoplasia on both genetic and histomorphologic levels. The conditional *Kras*^{G12D} model employs Cre-Lox recombination to create murine pancreatic ductal lesions that closely resemble human PanINs. In brief, the mouse genomic *Kras* is engineered to contain a G to A transition in codon 12 that results in a glycine to aspartic acid substitution in the expressed protein and subsequent constitutive downstream signaling of Ras effector pathways.⁴ A *Lox-STOP-Lox* (*LSL*) construct upstream from the modified *Kras* locus silences the mutation. When

interbred with mice that express Cre recombinase under the pancreas-specific promoter, *Pdx1*, the silencing cassette is excised, resulting in expression of the mutant *Kras* allele and development of early PanIN lesions. The additional knockout of *p16^{Ink4a}* causes more rapid development of PanIN and PDAC and allows for the study of progression from noninvasive to invasive pancreatic ductal disease⁵⁻⁷ (Qiu et al.; manuscript in revision). The conditional *Kras^{G12D}* model thus is a valuable tool to explore possible novel targets for early intervention in pancreatic cancer.

One such target is the receptor for advanced glycation end-products (RAGE). RAGE is a multi-ligand, transmembrane cell surface receptor of the immunoglobulin superfamily whose ligands include S100 proteins and high-mobility group box-1 (HMGB1).⁸ Ligand–RAGE interactions lead to initiation of downstream signaling pathways that perpetuate inflammation, promote cell survival, and inhibit cell death.⁹ The ligand–RAGE axis thus has been linked to carcinogenesis.¹⁰⁻¹⁷ Recent work with RAGE and pancreatic cancer has identified the ligand–RAGE axis as a promising target for intervention in this disease. RAGE and its ligands have been shown to be over-expressed in human PDAC and to correlate with tumor proliferation and invasiveness.^{13,18-20} Expression of S100 proteins, for example, increases as ductal lesions progress from PanIN to PDAC.²¹ Furthermore, release of HMGB1 from pancreatic tumor cells has been shown to enhance tumor cell survival via a RAGE-dependent pathway.⁹

Together, these data suggest that the ligand–RAGE axis plays an important role in the development and progression of pancreatic cancer. We previously reported that genetic deletion of *RAGE* significantly inhibited tumorigenesis and progression to invasive disease in a mouse model of colorectal carcinoma.²² In the present study, we describe the potential benefit of loss of RAGE function in inhibiting PanIN development and progression to PDAC in a conditional *Kras^{G12D}* mouse model.

Materials and Methods

Animals

Pdx1-Cre,⁴ *LSL-Kras^{G12D/+}*,²³ and *p16^{Ink4a}–/–*²⁴ mouse strains were obtained from the National Cancer Institute Mouse Repository (Frederick, MD). *RAGE* knock-out (*RAGE^{–/–}*) mice were generated in the C57BL/6 background as described previously.^{25,26} Animals were maintained in a specific pathogen-free facility of Columbia University (New York, NY). Mice were genotyped using DNA extracted from tail biopsies (Qiagen, Valencia, CA) for allele-specific polymerase chain reaction. All animal experiments were approved by the Institutional Animal Care and Use Committee of Columbia University and conformed to the guidelines outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

PanIN Mouse Model

The *Pdx1-Cre*; *LSL-Kras^{G12D/+}* mouse model of PanIN has been described previously.⁴ In brief, the pancreas-specific promoter, *Pdx1*, directs Cre recombinase-mediated excision of a floxed STOP cassette in pancreas progenitor cells, leading to the expression of the *Kras^{G12D}*

mutation in mature pancreatic cell lineages.²⁷ The mice develop early PanINs that progress to advanced PanINs and eventually to PDAC. To evaluate the impact of *RAGE* deletion on the development of PanIN, three lines of mice, *Pdx1-Cre*, *LSL-Kras*^{G12D/+}, and *RAGE*^{-/-}, were interbred to generate *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *RAGE*^{-/-} mice and *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *RAGE*^{+/+} controls. Specifically, the *RAGE* null locus was bred into separate *Pdx1-Cre* and *LSL-Kras*^{G12D/+} mouse lines to generate *Pdx1-Cre*; *RAGE*^{+/-} and *LSL-Kras*^{G12D/+}; *RAGE*^{+/-} mice. These mice were crossed as the final step to generate the experimental and control mice. Care was taken to cross-breed in a manner that ensured offspring were heterozygous for *Pdx1-Cre* and *Kras*^{G12D}.

PDAC Mouse Model

The *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-} mouse model of PDAC has been described previously.^{5,6} The homozygous deletion of *p16*^{Ink4a} promotes more rapid progression of PanINs to invasive PDAC. The model thus recapitulates the full spectrum of human PanINs and PDAC in a shorter period of time than the model described by Hingorani et al.⁴ (Qiu et al.; manuscript in revision). To evaluate the impact of *RAGE* deletion on the development of PDAC, four lines of mice, *Pdx1-Cre*, *LSL-Kras*^{G12D/+}, *p16*^{Ink4a-/-}, and *RAGE*^{-/-}, were interbred to generate *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{-/-} mice and *Pdx1-Cre*; *LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{+/+} controls. Specifically, the *p16* null locus and the *RAGE* null locus were bred into separate *Pdx1-Cre* and *LSL-Kras*^{G12D/+} mouse lines to generate *Pdx1-Cre*; *p16*^{Ink4a+/-}; *RAGE*^{+/-} and *LSL-Kras*^{G12D/+}; *p16*^{Ink4a+/-}; *RAGE*^{+/-} mice. These mice were crossed as the final step to generate the experimental and control mice. Care was taken to crossbreed in a manner that ensured offspring were heterozygous for *Pdx1-Cre* and *Kras*^{G12D}.

Histopathologic Evaluation

Formalin-fixed, paraffin-embedded pancreata were sectioned (5 µm thick) and stained with H&E. Sections (100 µm apart) of the entire pancreas were evaluated by a gastrointestinal pathologist (H. Remotti) blinded to the experimental groups. PanIN lesions were classified according to criteria recommended in the literature.^{28,29} To quantify the progression of PanIN lesions, ductal lesions were evaluated by a comprehensive scoring system that involved microscopic examination of all terminal ducts in the lobular units of the pancreas. A median of 38 lobular units were analyzed for each animal. Lobular units containing multiple ductal profiles were scored as normal or with respect to the highest degree of PanIN identified. The relative proportion of each PanIN lesion to the total number of ducts analyzed was recorded for each animal. Invasive carcinomas were evaluated by gross tumor dimensions with histologic confirmation.

Immunohistochemistry

Tissue sections were de-paraffinized in xylene and rehydrated in a graded ethanol series. Sections to be stained with the antibody to RAGE or HMGB1 were pretreated with trypsin for 20 min. Sections to be stained with the antibody to S100 were heated by boiling in 10 mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. All sections were further treated with 0.3% H₂O₂ for 30 min before immunostaining. After

blocking with 10% normal goat serum (Vector Laboratories, Burlingame, CA), serial sections were stained with the rabbit polyclonal antibodies to RAGE (1:100; house),³⁰ HMGB1 (1:50; ProteinTech Group Inc., Chicago, IL), and S100 (1:100; Abcam, Cambridge, MA), and were incubated overnight at 4°C in a humidified chamber. After washing with phosphate-buffered saline (PBS), sections were incubated with biotinylated goat anti-rabbit immunoglobulin (1:200; Vector Laboratories Inc., Burlingame, CA) followed by peroxidase-conjugated avidin (Sigma, St. Louis, MO). Location of peroxidase-conjugates was revealed using 3-amino-9-ethylcarbazole as the chromogen.

Statistics

Categorical variables were compared using Fisher's exact test and reported as number and percentage (%). To calculate the rate of duct dysplasia, loglinear Poisson regression was used to model the number of ducts displaying PanIN-2 lesions or greater as a function of the experimental group, with the offset equal to the natural log of the total number of ducts counted. When data were overdispersed, a negative binomial regression model was fit in the same fashion. Survival probabilities were estimated using Kaplan–Meier methods, and strata were compared using the log-rank test. A *p* value of less than 0.05 was considered statistically significant. Data analyses were performed with SAS software, Version 9.2 of the SAS System for Microsoft Windows (Copyright © 2009 SAS Institute Inc., Cary, NC, USA).

Results

RAGE and RAGE Ligand Expression

Histologic examination of H&E-stained sections of pancreata from *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}* mice showed a spectrum of ductal neoplasia, ranging from PanIN-1 to invasive PDAC (Fig. 1). Representative sections of normal, dysplastic, and cancerous ducts revealed expression of RAGE, S100, and HMGB1 (Fig. 2).

RAGE Deletion Inhibits PanIN Development in *Pdx1-Cre; LSL-Kras^{G12D/+}* Mice

At 16 weeks of age, *Pdx1-Cre; LSL-Kras^{G12D/+}; RAGE^{-/-}* mice (*n*=6) had a 67% lower rate of high-grade PanINs compared to *RAGE^{+/+}* controls (*n*=6; *p*=0.0005). The *RAGE^{-/-}* mice also had a significantly lower incidence of high-grade PanINs compared to *RAGE^{+/+}* controls (5.1% vs. 15.3%, *p*=0.0003; Fig. 3)

RAGE Deletion Inhibits PanIN Development and Progression to PDAC in *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}* Mice

At 12 weeks of age, *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}; RAGE^{-/-}* mice (*n*=11) had a 36.7% lower rate of high-grade PanINs compared to *RAGE^{+/+}* controls (*n*=11; *p*=0.2069; Fig. 4a). The *RAGE^{-/-}* mice also had a significantly lower incidence of high-grade PanINs compared to *RAGE^{+/+}* controls (11.3% vs. 19.9%, *p*=0.0075; Fig. 4b). Strikingly, the *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}; RAGE^{-/-}* mice had more benign histopathology with no mouse harboring PDAC compared to 45.5% PDAC in *RAGE^{+/+}* controls (*p*=0.0351; Fig. 4c).

RAGE Deletion Prolongs Survival in *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}* Mice

To test the effect of genetic deletion of *RAGE* on survival, 12 *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}; RAGE^{-/-}* mice and 19 *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}; RAGE^{+/+}* controls were aged and followed. The *RAGE^{-/-}* mice lived significantly longer than their *RAGE^{+/+}* controls with median survivals of 21.9 and 13.0 weeks, respectively ($p=0.0051$; Fig. 5). Necropsy and pathologic analysis revealed that tumors were ductal adenocarcinomas with varied histologic patterns of high cytologic grade: glandular, solid spindle (sarcomatoid), or solid epithelioid (anaplastic). Often the greatest degree of cytologic atypia and pleomorphism was demonstrated in the epithelioid areas. PDAC in the *RAGE^{-/-}* mice tended to exhibit a greater percentage of glandular differentiation whereas PDAC in the *RAGE^{+/+}* controls tended to exhibit a greater percentage of more poorly differentiated components, characterized by diffuse infiltrate of spindle or epithelioid tumor cells (Fig. 6).

Discussion

Genetically engineered mouse models that recapitulate human disease are useful in the preclinical study of pancreatic carcinogenesis.³¹⁻³⁴ Conditional *KRAS* transgenic models slowly develop a full range of PanINs with progression to PDAC after a prolonged latency.³⁵ They are excellent models for the study of PanIN, but require lengthy time periods for the study of PDAC. When a second genetic alteration is added to the model, invasive disease develops more rapidly. Aguirre et al.⁶ reported that deletion of *p16^{Ink4a}* cooperated with the activation of *Kras^{G12D}* to promote the malignant conversion of PanINs to PDAC. Given these data, we chose *Pdx1-Cre; LSL-Kras^{G12D/+}* mice to model the development of PanIN and *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a-/-}* mice to model the progression to PDAC.

Several reports in the literature have investigated possible targets for intervention in pancreatic cancer by using variations on the conditional *Kras^{G12D}* transgenic mouse model. Mohammed et al.³⁶ used *p48^{Cre/+}; LSL-Kras^{G12D/+}* mice to study the efficacy of gefitinib, an epidermal growth factor receptor (EGFR) inhibitor, on the development of PanIN. They treated the mice with gefitinib and found significant suppression of PanIN development and progression to PDAC, highlighting EGFR as a potential target for chemoprevention of pancreatic cancer. Funahashi et al.²⁷ used *Pdx1-Cre; LSL-Kras^{G12D/+}* mice to study the effects of nimesulide, a selective COX-2 inhibitor, on the development of PanIN. They reported that pancreatic ducts in treated mice displayed 70% PanIN-1, 10% PanIN-2, and 0% PanIN-3 compared to 50%, 30%, and 10%, respectively, in controls. COX-2 inhibition thus delayed the progression of PanIN, suggesting a role for anti-inflammatory agents in pancreatic cancer chemoprevention.

Another potential target for chemoprevention of pancreatic cancer is RAGE. Found on both inflammatory and epithelial tumor cells, RAGE has been implicated in cellular and molecular pathways that promote inflammation and carcinogenesis.¹⁰⁻¹⁷ It is known that pancreatic cancer cells express RAGE and its ligands, and there is mounting evidence that the ligand-RAGE axis plays an important and complex role in the biology of pancreatic cancer. Using pancreatic cancer cell lines, Arumugam et al.³⁷ demonstrated that, via interaction with RAGE, S100P promotes pancreatic cancer cell proliferation, survival, and

invasion. Whiteman et al.²¹ suggested that invasion of pancreatic cancer cells occurs via S100P-mediated cytoskeletal changes. In further studies, Arumugam et al.³⁸ demonstrated that inhibition of RAGE interaction with S100P led to enhanced anti-tumor activity of gemcitabine in a xenograft model of pancreatic cancer. Blockade of S100P interaction with RAGE by a RAGE antagonist peptide inhibited these biological effects, suggesting that interference with the ligand–RAGE axis may be a novel approach for pancreatic cancer treatment.

Given these data from pancreatic cell lines, we endeavored to investigate the role of RAGE in pancreatic cancer tumorigenesis in an orthotopic mouse model. First, to confirm that RAGE and its ligands were relevant to the model, we stained representative sections of pancreata from the conditional *Kras*^{G12D} model and demonstrated the presence of RAGE and its ligands S100 and HMGB1 in the neoplastic pancreatic ductal epithelium. Next, we used *Pdx1-Cre; LSL-Kras*^{G12D/+} and *RAGE*^{-/-} mice to investigate the role of RAGE on PanIN development. By breeding the *RAGE* null locus into the model, we were able to demonstrate a significant decrease in incidence and rate of PanIN-2 and PanIN-3 development in 16-week-old mice. We chose 16 weeks of age to allow the mice sufficient time to develop a spectrum of precursor lesions, but not so much time as to develop invasive disease. To investigate the role of RAGE on PanIN progression to PDAC, we used *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-} and *RAGE*^{-/-} mice. We bred the *RAGE* null locus into the model and demonstrated a significant decrease in incidence of PanIN-2 and PanIN-3 development with no progression to PDAC in 12-week-old mice. We chose 12 weeks of age to allow adequate time for progression to PDAC. Importantly, this difference in disease progression correlated with improved mouse survival. To our knowledge, this study represents the first use of genetic *RAGE* deletion in a conditional *Kras*^{G12D} transgenic mouse model.

Our study demonstrates a clear, RAGE-mediated phenomenon. In light of recent literature, we can hypothesize that RAGE and its ligands facilitate the development of an inflammatory tumor microenvironment that promotes carcinogenesis in the pancreatic ducts. The inflammatory cytokine, HMGB1, for example, is released upon pancreatic tumor cell death and, depending on its redox status, promotes tumor cell survival and resistance to chemotherapeutic agents.^{39,40} Building on this work, Kang et al.⁹ established a direct link between inflammatory mediators in the tumor microenvironment and resistance to apoptosis in pancreatic cancer cells. They showed that targeted knockdown of RAGE significantly increased tumor cell death via a p53-dependent pathway and that forced over-expression of RAGE promoted tumor cell survival via decreased phosphorylation of the mammalian target of rapamycin. They thus suggested a new paradigm in tumor biology whereby pro-inflammatory factors aggregate in the tumor microenvironment and interact with RAGE to increase programmed cell survival and decrease programmed cell death. It is plausible that lack of RAGE-mediated inflammatory signaling within the pancreatic ductal epithelium in our *Pdx1-Cre; LSL-Kras*^{G12D/+}; *RAGE*^{-/-} and *Pdx1-Cre; LSL-Kras*^{G12D/+}; *p16*^{Ink4a-/-}; *RAGE*^{-/-} mice contributes to less aggressive pathology and prolonged survival. Additional investigation into differences in RAGE signaling within pancreatic ductal epithelial cells

versus inflammatory cells within the tumor microenvironment may help further elucidate the role of RAGE signaling in the pathogenesis of pancreatic cancer.

Conclusion

This study provides evidence that RAGE signaling plays an important role in pancreatic tumorigenesis. Using established murine models of PanIN and PDAC, we showed that loss of function via genetic deletion of *RAGE* inhibited PanIN development in *Pdx1-Cre; LSL-Kras^{G12D/+}; RAGE^{-/-}* mice and progression to PDAC in *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}; RAGE^{-/-}* mice. Importantly, the inhibitory effects significantly prolonged mouse survival. Taken together, these data link RAGE to the development and progression of premalignant pancreatic ductal neoplasia. Further cellular and molecular work is needed to target the ligand–RAGE axis for possible early intervention and prophylaxis in patients at risk for developing pancreatic cancer.

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Fig. 1.

H&E-stained sections of the pancreas from a *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}* mouse at 12 weeks of age demonstrate a spectrum of ductal neoplasia. **a** $\times 10$ magnification shows an entire pancreas. **b** $\times 100$ magnification of the indicated section demonstrates pancreatic ducts with varying degrees of dysplasia. **c** $\times 200$ magnification of the indicated section demonstrates a PanIN-1 lesion. **d** $\times 200$ magnification of the indicated section demonstrates ducts with PanIN-2 and PanIN-3 lesions. **e** $\times 200$ magnification of the indicated section demonstrates PDAC

Fig. 2.

RAGE and its ligands, S100 and HMGB1, are expressed in the pancreatic ductal epithelium of a *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}* mouse at 12 weeks of age. H&E-stained sections demonstrate normal and dysplastic ductal architecture. Immunohistochemical staining of serial sections demonstrates expression of RAGE, S100, and HMGB1. **a** normal pancreatic ducts. **b** PanIN2 and PanIN3. **c** PDAC

Fig. 3.

RAGE deletion inhibits PanIN development in *Pdx1-Cre; LSL-Kras^{G12D/+}* mice. *Pdx1-Cre; LSL-Kras^{G12D/+}; RAGE^{-/-}* mice ($n=6$) had a significantly lower rate and incidence of high-grade PanINs compared to *RAGE^{+/+}* controls ($n=6$) at 16 weeks of age. **a** Proportion of PanIN-2 and PanIN-3 ducts compared to normal and PanIN-1 ducts for each mouse are shown. **b** Proportion of PanIN-2 and PanIN-3 ducts compared to normal and PanIN-1 ducts for all mice in each group are shown. $*p < 0.01$

Fig. 4.

RAGE deletion inhibits development and progression of ductal neoplasia in *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}* mice. *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}; RAGE^{-/-}* mice ($n=11$) had a decreased rate and a significantly lower incidence of high-grade PanINs compared to *RAGE^{+/+}* controls ($n=11$) at 12 weeks of age. **a** Proportion of PanIN-2 and PanIN-3 ducts compared to normal and PanIN-1 ducts for each mouse are shown. One mouse in the *RAGE^{+/+}* group had no normal ducts or PanIN lesions because of diffuse PDAC and therefore is not represented. **b** Proportion of PanIN-2 and PanIN-3 ducts compared to normal and PanIN-1 ducts for all mice in each group are shown. **c** *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}; RAGE^{-/-}* mice had more benign histopathology with no mouse harboring PDAC compared to 45.5% PDAC in *RAGE^{+/+}* controls. Mice are classified according to the highest grade lesion seen on histopathologic examination. NS=not significant, * $p<0.01$

Fig. 5.

RAGE deletion prolongs survival in *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}* mice. Twelve *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}; RAGE^{-/-}* mice and 19 *Pdx1-Cre; LSL-Kras^{G12D/+}; p16^{Ink4a}^{-/-}; RAGE^{+/+}* controls were aged and followed. The *RAGE*^{-/-} mice lived significantly longer than their *RAGE*^{+/+} controls with median survivals of 21.9 weeks and 13.0 weeks, respectively ($p=0.0051$)

Fig. 6. H&E-stained sections from pancreata obtained at necropsy from *Pdx1-Cre; LSL-Kras^{G12D/+}; 16Ink4a^{-/-}; RAGE^{-/-}* mice and *Pdx1-Cre; LSL-Kras^{G12D/+}; p16Ink4a^{-/-}; RAGE^{+/+}* controls. Tumors were ductal adenocarcinomas with varied histologic patterns. **a** glandular; **b** solid spindle (sarcomatoid); **c** solid epithelioid (anaplastic)