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The Receptor for Advanced Glycation End Products Promotes Pancreatic Carcinogenesis and Accumulation of Myeloid-Derived Suppressor Cells

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Pancreatic ductal adenocarcinoma (PDA) has an aggressive natural history and is resistant to therapy. The receptor for advanced glycation end products (RAGE) is a pattern recognition receptor for many damage-associated molecular pattern molecules. RAGE is overexpressed in both human and murine models of PDA as well as most advanced epithelial neoplasms. The immunosuppressive nature of the PDA microenvironment is facilitated, in part, by the accumulation of regulatory immune cell infiltrates such as myeloid-derived suppressor cells (MDSCs). To study the role of RAGE expression in the setting of mutant Ras-promoted pancreatic carcinogenesis (KC), a triple-transgenic model of spontaneous murine PDA in a RAGE-null background (KCR) was generated. KCR mice had markedly delayed pancreatic carcinogenesis and a significant diminution of MDSCs compared with KC mice at comparable time points postweaning. Although RAGE was not required for the development or suppressor activity of MDSCs, its absence was associated with temporally limited pancreatic neoplasia and altered phenotype and function of the myeloid cells. In lieu of MDSCs, KCR animals at comparable time points exhibited mature CD11b+Gr1−F4/80+ cells that were not immunosuppressive in vitro. KCR mice also maintained a significantly less suppressive milieu evidenced by marked decreases in CCL22 in relation to CXCL10 and diminished serum levels of IL-6. The Journal of Immunology, 2013, 190: 1372–1379.

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Abbreviations used in this article: HMGB1, high-mobility group box 1; iNOS, inducible NO synthase; KC, Pdx1-Cre:KrasG12D/+;K C R , P d x 1 - C r e : K r a sG12D/+: Rage−/−; MDSC, myeloid-derived suppressor cell; NOS, NO synthase; PanIN, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma; RAGE, receptor for advanced glycation end products; Treg, regulatory T cell; WT, wild-type.

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The receptor for advanced glycation end products (RAGE) is an MHC class III–encoded protein, characterized as a damage-associated molecular pattern molecule receptor. It serves as the cognate receptor for the prototypical damage-associated molecular pattern, high-mobility group box 1 (HMGB1), and several S100 proteins including S100A8 and S100A9 (19, 20). RAGE-mediated signaling plays a role in the pathogenesis of epithelial derived cancers such as PDA by activating key survival pathways such as autophagy in cancer cells as well as propagating and sustaining promotor host inflammatory responses (20–23). Although normal pancreatic ductal epithelial cells do not routinely stain positively for RAGE, with the emergence and progression of pancreatic neoplasia, RAGE is markedly upregulated and overexpressed (22). The contribution of RAGE to intratumoral MDSC accumulation was first demonstrated in RAGE−/− mice in an inducible skin cancer model (24). In addition, mice deficient for the RAGE ligand, S100A9, exhibit a significant reduction in the incidence and burden of colitis-associated colorectal tumors and show demonstrable decreases in intratumoral and splenic MDSC frequency (18, 25, 26). To determine if RAGE plays a role in the accumulation of MDSCs during pancreatic carcinogenesis, we have backcrossed RAGE-null mice into the KC strain. The resultant Pdx1-Cre: KrasG12D/+; Rage−/− mice are termed KCR. We demonstrate in this study that the targeted ablation of RAGE in the emerging Kras-driven tumor microenvironment limits development of PanIN lesions and the associated accumulation of MDSCs.

Materials and Methods

Mouse strains

Wild-type (WT) C57BL/6 mice were purchased from Taconic Farms (Hudson, NY). RAGE knockout (Rage−/−; GFP) mice (SVEV129 × C57BL/6) were obtained from Dr. Angelika Bierhaus (27) as a kind gift. Pdx-1-Cre and KrasG12D/+ transgenic mice were obtained from the Mouse Models of Human Cancers Consortium/National Cancer Institute Mouse Repository. The genotypes Pdx1-Cre:KrasG12D/+ (termed KC) and Rage−/− were crossed to generate KCR mice. Genomic and recombination screens were done by PCR and analysis of GFP expression (data not shown).

Flow Cytometry

Flow cytometric analysis was performed on the C6 flow cytometer (Accuri Cytometers, Ann Arbor, MI) instrument provided by the University of Pittsburgh Cancer Institute Flow and Imaging Cytometry core facility and analyzed using FlowJo software (Tree Star, Ashland, OR). Murine spleens were homogenized through a 70-μm nylon filter (BD Biosciences, San Jose, CA) and washed with PBS. RBCs were lysed with RBC Lysis Buffer (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instructions. Single cell suspensions were then stained with corresponding isotype controls (all from BD Biosciences). The resulting single cell suspensions were then stained with the following fluorochromes: Abs: CD11b, CD11c, F4/80, Gr1, IL-4Rα, and corresponding isotype controls (all from BD Biosciences). In cases, the cells were then permeabilized with 0.2% Triton-X and stained with Abs to intracellular inducible NOS (iNOS)/NOS type II and Arginase-I (BD Biosciences). During analysis viable cells were identified via forward and side scatter and gated accordingly. Phenotyping of MDSC cell populations in KC and KCR mice (Fig. 3) were based on a CD11c−Gr1+ gate.

Histology

Harvested pancreatic tissue was formalin fixed (Sigma-Aldrich), stained with H&E, and mounted onto glass slides by the University of Pittsburgh Department of Pathology. Images were visualized and captured at a magnification of 10× by Nikon Eclipse E800 fluorescent microscope under bright field settings (Nikon, Melville, NY).

Coculture assays

A total of 4 × 10^5 bulk WT splenocytes were obtained as previously mentioned from C57/BL6 mice. To activate effector splenocytes, cells were incubated with T-activator CD3/CD28 Dynabeads (Dynal AS, Oslo, Norway) at a ratio of 1:1 according to the manufacturer’s instructions. For coculture conditions, splenocytes from KC and KCR mice were incubated with Gr1 or CD11b microbeads and magnetically separated using MACS LS separation columns (all from Miltenyi Biotec, Leiden, The Netherlands) according to the manufacturer’s instructions and purity assayed by flow cytometry. A total of 4 × 10^5 (termed low) or 8 × 10^5 (termed hi) Gr1+ or CD11b+ cells were incubated with bulk WT splenocytes for 96 h. The resulting supernatants were harvested for analysis.

In vitro differentiation of MDSCs from murine bone marrow

MDSCs were generated in vitro using the following method. Fresh bone marrow flushed from the femurs and tibias of mice was resuspended in media supplemented with G-CSF (100 ng/ml) and GM-CSF (250 U/ml) and cultured for 3 d. On day 4, 80 ng/ml of IL-13 was added to the culture for an additional 24 h (28). All cytokines were from eBioscience (San Diego, CA). Harvested cells routinely were 50% CD11b+Gr1+ and were magnetically sorted as previously mentioned for functional analysis.

ELISA analysis

Supernatants from coculture experiments or serum from murine peripheral blood (obtained via direct cardiac puncture) was assayed for detectable levels of IL-2, IFN-γ, and IL-6 by ELISA Ready-SET-Go! Kits (eBioscience) according to the manufacturer’s instructions.

Quantitative PCR

Pancreatic tissue from 35-wk-old mice was harvested and snap frozen in liquid nitrogen. mRNA was then isolated and analyzed for the relative quantity (RQ) of CCL22, CXCL10 mRNA and then normalized to a control housekeeping gene, HPRT1 and displayed as a ratio. Analysis was performed with StepOne software (Applied Biosystems, Foster City, CA).

Statistical analysis

Data are expressed as means ± SEM of at least two independent experiments performed in triplicate. Statistical analysis was performed using a two-tailed Student t test. The p values <0.05 were assigned statistical significance.

Results

Targeted ablation of RAGE attenuates development of high-grade PanIN lesions in KC mice

To determine if RAGE contributes to pancreatic carcinogenesis, 20- and 35-wk-old WT, RAGE-null, KC, and KCR mice were sacrificed and their pancreata harvested and stained with H&E for visualization of PanIN lesion formation. At 20 wk, both the KC and KCR strains show the occurrence of atypical ducts. However, glandular and islet cells remain largely intact, and both strains exhibit a paucity of fibrotic and inflammatory tissue (Fig. 1A). At 35 wk of age, KC mice exhibit extensive atypical ductal morphology within pancreatic tissue, the emergence of low- and high-grade PanIN lesions, and severe fibrogenesis indicative of emergent pancreatic carcinogenesis. In KC mice in which RAGE has been chromosomally ablated (KCR mice), the frequency of hyperplastic and dysplastic duct epithelium is substantially decreased and the integrity of glands and β cells in the islets is largely retained (Fig. 1B). Furthermore, we have previously reported that these mice have a significantly enhanced survival rate (22). These findings suggest that RAGE directly or indirectly contributes to the pathogenesis of early pancreatic carcinogenesis.

RAGE promotes splenic MDSC accumulation during Kras-mediated pancreatic carcinogenesis

MDSCs accumulate within the spleens of KC mice as they progress toward ductal adenocarcinoma (17). To determine if the integrity of the RAGE signaling pathway is an important factor in this observation, WT, RAGE-null, KC, and KCR mice were sacrificed at 35 wk of age at which point animals expressing mutant Kras display evidence of pancreatic carcinogenesis manifested by the presence of low and high grade PanIN lesions within the pancreatic ducts (Fig. 1B). Spleens from these mice were processed into single-cell suspensions for flow cytometric analysis of immuno-
mune cell frequencies. Both WT and RAGE-null animals exhibit comparable basal levels of CD11b<sup>+</sup>Gr1<sup>+</sup> MDSCs ranging from 3 to 4% (Fig. 2A) in the absence of apparent pathology. As has been previously documented, Kras-driven pancreatic carcinogenesis resulted in a significant increase in splenic MDSC frequency to 20–35% (17). Interestingly, KCR mice fail to accumulate MDSCs with a relative paucity of CD11b<sup>+</sup>Gr1<sup>+</sup> cells (Fig. 2A, 2B). This is significantly less than the KC strain (p = 0.0225) at comparable times. However other cells of myeloid-lineage such as myeloid dendritic cells (DCs) identified by the coexpression of CD11b and CD11c are elevated in both KC and KCR mice to similar extents, suggesting that the reduction in splenic MDSCs in the KCR strain is restricted to CD11b<sup>+</sup>Gr1<sup>+</sup> cells.

To observe how the kinetics of MDSC accumulation are affected by the presence or absence of RAGE, mice from all strains were sacrificed at 20, 24, 26, and 35 wk of age and their splenocytes assayed for MDSC frequency. As precursor lesions develop in the pancreas, the KC mice begin to accumulate high levels of MDSCs at 26 wk of age. This is significantly elevated when compared with KCR mice (p = 0.049) at the same time point. This becomes further accentuated by 35 wk of age (p = 0.023) (Fig. 2C). The absence of RAGE is associated with a significant lack of MDSC accumulation over time in these animals. These findings suggest that RAGE is critical for in vivo MDSC accumulation during pancreatic carcinogenesis. Of note, a study by Connolly et al. using the Kras-driven pancreatic neoplasia model with the additional p48 mutation has previously described. Cells from both strains were equally suppressive for levels of the proliferative T cell survival cytokine, IL-2 and the Th1-polarizing cytokine IFN-γ as indicators of relative T cell proliferation and activity. As expected, stimulation with anti-CD3/anti-CD28 activator beads alone or in a coculture with the isolated MDSCs from both the KC and KCR strains at a ratio of 1:1 (MDSC [low]) or 1:2 (MDSC [hi]). After 96 h of coculture, the resultant supernatants were harvested and analyzed by ELISA for levels of the proliferative T cell survival cytokine, IL-2 and the Th1-polarizing cytokine IFN-γ as indicators of relative T cell proliferation and activity. As expected, stimulation with anti-CD3/anti-CD28 activator beads induced significant secretion of both IL-2 (p < 0.01) and IFN-γ (p < 0.05) from WT splenocytes (Fig. 3C). When cocultured with MDSCs at either ratio isolated from KC or KCR mice, the secretion of these cytokines was inhibited and remained at non-stimulated levels. Importantly, there was no significant difference between MDSCs derived from either strain in terms of their suppressive capacity (Fig. 3C). To further validate the lack of a requirement for RAGE in MDSC hematopoiesis, MDSCs were generated in vitro from both non-tumor bearing WT and RAGE<sup>−/−</sup> mice. CD11b<sup>+</sup>Gr1<sup>+</sup> cells were generated to equal extents (50%) in marrow from both strains (Supplemental Fig. 1A). Furthermore, these cells were sorted and evaluated for suppressor function and previously described. Cells from both strains were equally suppressive (Supplemental Fig. 1B). These findings suggest that RAGE is not critical for the development of MDSCs in vivo or their ability to exert suppressor function in vitro. Moreover, the failure of KCR mice to accumulate splenic MDSCs during pancreatic carcinogenesis is not due to an impaired ability to generate functioning MDSCs.

Splenic myeloid cells derived from KCR mice exhibit a mature phenotype and do not exhibit suppressor function

A lack of MDSC accumulation during pancreatic carcinogenesis in KCR mice could be due to a failure in MDSC recruitment. Alternatively, the absence of RAGE could result in the induction of the differentiation of MDSCs from an immature (Gr1<sup>+</sup>) to a mature (Gr1<sup>−</sup>) phenotype or a failure to prevent such a differentiation
from occurring. To investigate the latter hypothesis, 35-wk-old WT, RAGE-null, KC, and KCR mice were sacked and their splenocytes analyzed for expression of CD11b and Gr1 and myeloid DC markers CD11b and CD11c from 35-wk-old WT (n = 2), RAGE-null (n = 2), KC (n = 8), and KCR (n = 5) mice. Note the boxes denoted MDSCs are distinct from the boxes denoting DC populations. (B) Scatter plots depicting the frequencies of splenic MDSCs identified by expression of CD11b, Gr1, and IL-4Rα and frequencies of splenic DCs of myeloid origin (CD11b+CD11c+) in mice from (A). (C) WT, RAGE-null, KC, and KCR mice were sacked at 20 (n = 2/strain), 24 (n = 2/strain), 26 (n = 2/strain), and 35 wk (n = 2, WT and RAGE-null; n = 8, KC; n = 5, KCR). The kinetics of splenic MDSC accumulation with the progression of pancreatic neoplasia (KC and KCR mice) is depicted.

**FIGURE 2.** RAGE ablation is associated with limited splenic MDSC accumulation during Ras-mediated pancreatic carcinogenesis. (A) Representative flow cytometry diagrams of splenocytes stained for cosurface expression of MDSC markers CD11b and Gr1 and myeloid DC markers CD11b and CD11c from 35-wk-old WT (n = 2), RAGE-null (n = 2), KC (n = 8), and KCR (n = 5) mice. Note the boxes denoted MDSCs are distinct from the boxes denoting DC populations. (B) Scatter plots depicting the frequencies of splenic MDSCs identified by expression of CD11b, Gr1, and IL-4Rα and frequencies of splenic DCs of myeloid origin (CD11b+CD11c+) in mice from (A). (C) WT, RAGE-null, KC, and KCR mice were sacked at 20 (n = 2/strain), 24 (n = 2/strain), 26 (n = 2/strain), and 35 wk (n = 2, WT and RAGE-null; n = 8, KC; n = 5, KCR). The kinetics of splenic MDSC accumulation with the progression of pancreatic neoplasia (KC and KCR mice) is depicted.
that lack suppressor activity and could contribute to both the paucity of classical MDSCs and the inhibited occurrence of malignant pancreatic lesions.

RAGE promotes a regulatory milieu within the emerging pancreatic tumor microenvironment

The presence or absence of host regulatory immune cell infiltrate in the pancreatic tumor microenvironment is in part regulated by factors present both within tumor tissue and circulating in the peripheral blood (33, 34). To investigate whether there were differences in the tumor microenvironments of both KC and KCR mice that might contribute to both the failure to accumulate MDSCs in the absence of RAGE and the influx of mature, nonsuppressive myeloid cells in KCR mice (Fig. 4), pancreatic tissue was harvested from 35-wk-old WT, RAGE-null, KC, and KCR mice and analyzed for mRNA levels of chemokines known to attract either immunoregulatory (CCL22) or immunostimulatory (CXCL10) immune cells by quantitative PCR (35, 36). To determine what the

**FIGURE 3.** RAGE is dispensable for the differentiation and suppressor activity of MDSC. (A) Representative flow cytometry diagrams of splenocytes from 20-wk-old KC (n = 2) and KCR (n = 2) mice stained for cosurface expression of MDSC markers CD11b and Gr1. (A and B) Flow diagrams of splenocytes gated for CD11b and Gr1 positivity and stained for surface expression of IL-4Rα and F4/80, and intracellular expression of iNOS/NOS type II and arginase-I from KC and KCR mice. (A) Graphical representation of the mean fluorescence intensity (MFI) of each marker (± SD). (B) MFI values illustrated on diagrams. Demarcations based on isotype controls (data not shown). (C) MDSCs from mice in (A) and (B) were magnetically separated and cocultured with bulk WT splenocytes stimulated with anti-CD3/anti-CD28 activator beads at ratios of 1:1 (MDSC [low]) and 2:1 (MDSC [hi]). Graphs depict IL-2 and IFN-γ levels detected in the resultant supernatants by ELISA (± SEM; *p < 0.05, **p < 0.01).

**FIGURE 4.** Splenic myeloid cells derived from KCR mice exhibit a mature phenotype and do not exhibit suppressor function. (A) Representative flow cytometry diagrams of splenocytes from 35-wk-old KC (n = 6) and KCR (n = 5) mice stained for the cell-surface expression of the myeloid-lineage marker CD11b and the mature macrophage marker F4/80. (B) Scatter plot depicting the frequencies of splenic myeloid cells exhibiting immature phenotypes identified by the expression of CD11b and the lack of F4/80 expression in mice from (A). (C) CD11b+ splenocytes from mice in (A) and (B) were magnetically separated and cocultured with bulk WT splenocytes stimulated with anti-CD3/anti-CD28 activator beads at ratios of 1:1 (CD11b+ [low]) and 2:1 (CD11b+ [hi]). Graphs depict IL-2 and IFN-γ levels detected in the resultant supernatants by ELISA (± SEM, *p < 0.05).
relative levels of mRNA of these chemokines are in relation to each other, the ratio of CCL22 to CXCL10 was obtained. As expected, KC mice demonstrate a significant increase in CCL22: CXCL10, indicating a pronounced regulatory chemokine milieu in the pancreata of these mice. In the absence of RAGE, this ratio is significantly diminished ($p = 0.037$), suggested that the emerging tumor microenvironments of these animals is substantially less likely to recruit regulatory infiltrate (Fig. 5A).

We have recently reported that ablation of RAGE in the emerging tumor microenvironment attenuates STAT-3/IL-6 pathway activation in the KC model (22). IL-6, a cytokine often associated with epithelial-derived cancers and general tissue damage, has also been implicated in MDSC recruitment and persistence through a STAT3-dependent signaling pathway (37–39). To compare serum IL-6 levels between the KC and KCR strains, peripheral blood was obtained via direct cardiac puncture of 35 wk old mice and assayed for IL-6 concentration by ELISA. Although serum IL-6 levels were relatively low in WT and RAGE-null mice, KC mice exhibited a significant increase in the concentration of the cytokine in their peripheral blood. This was not the case for the KCR strain, which exhibited significantly diminished levels of IL-6 ($p = 0.038$), which were comparable with both WT and RAGE-null mice (Fig. 5B, left panel). Further illustrating the role of IL-6 in the recruitment and survival of MDSCs, mice were divided into two groups, regardless of strain: those with <100 pg/ml serum IL-6 and those with >100 pg/ml IL-6. The MDSC frequencies in the spleens from mice from these two groups were then assayed by flow cytometry. Mice with >100 pg/ml of serum IL-6 were significantly more likely to have greater splenic MDSC accumulation ($p = 0.022$) (Fig. 5B, right panel). Single-cell suspensions derived from the pancreata of 35 wk old mice from both strains were examined for MDSC frequencies as well as expression of F4/80 (Fig. 5C). A failure to accumulate MDSCs in response to endogenous carcinogenesis was observed in the KCR strain. This was in contrast with the KC strain, which showed a demonstrable expansion of these cells as has been previously reported (17). Interestingly, nearly uniform F4/80 expression was observed in CD11b+ cells in the KCR strain, but not in KC mice, where the vast majority of CD11b+ cells remained negative for F4/80. Taken together, these findings suggest that RAGE has a critical role in the promotion of an immunoregulatory milieu within the emergent tumor microenvironment during pancreatic carcinogenesis. The lack of the regulatory chemokine CCL22 in relation to the immunostimulatory chemokine CXCL10, coupled with the normal serum IL-6 levels in response to the diminished incidence and timing of Kras-mediated pancreatic neoplasia could contribute to inhibited MDSC accumulation in the absence of RAGE expression.

**Discussion**

In this study, we demonstrate that RAGE plays a critical role in promoting pancreatic neoplasia and the resultant immunosuppres-
sive tumor microenvironment. When RAGE is ablated in mice that spontaneously form PanIN lesions due to mutant Kras expression, the emergence and progression of these malignant lesions are substantially attenuated. Concurrently, the accumulation of splenic MDSCs that occurs over time in KC mice is limited in the absence of RAGE. Our findings suggest that the integrity of RAGE is critical for pancreatic carcinogenesis and responding MDSC accumulation. Importantly, RAGE is not required for the development of MDSCs from myelopoietic progenitor cells or their specific inhibitory activity, as MDSCs are found in both RAGE-null and KCR mouse strains and are phenotypically and functionally intact. Interestingly, during pancreatic neoplasia in the absence of RAGE, a substantial majority of myeloid cells (CD11b+) exhibit a more mature phenotype manifested by the expression of the mature macrophage marker F4/80 and a loss of Gr1 expression (18, 32). When isolated and evaluated in an in vitro suppression assay, these cells had notably lost their inhibitory activity. This suggested that RAGE is a factor in either myeloid cell plasticity or determining which myeloid subsets respond to emergent pancreatic carcinogenesis. However, alternative explanations for the maturation of myeloid cells toward a more mature F4/80+ phenotype exist. Inability to recruit more suppressive cells types or apoptosis of other myeloid subsets could explain this observation. Additionally, the chemokine milieu in the pancreatic tumor microenvironment was markedly less regulatory in KCR mice illustrated by the relative abundance of CXCL10 in relation to CCL22. The levels of IL-6, which have been implicated in the STAT3-dependent recruitment and retention of MDSCs (38), were also significantly decreased in the KCR strain, and these levels directly correlated with measured MDSC frequency within the emergent pancreatic tumor microenvironment. The proinflammatory proteins S100A8/A9 induce MDSCs by interacting with RAGE and other glycoproteins on the surface of MDSCs and promote their migration via NF-κB–dependent signaling (18). In addition, RAGE overexpressed within the tumor and stromal compartments is concurrently ligated by S100A8/A9, which induces a regulatory chemokine tumor gene profile and serves as a positive-feedback loop for the further recruitment of MDSCs (18, 25). Our findings suggest that RAGE ablation in the emerging tumor microenvironment leads to diminished signaling through the IL-6/STAT3 pathway. It is likely that the failure to accumulate MDSCs in KCR mice is due to a combined effect of RAGE deletion in both the tumor and bone marrow compartments in the global RAGE knockout strain. Future studies will examine targeted ablation in each exclusive compartment to further elucidate these mechanisms. Other cells of myeloid lineage (particularly dendritic cells and macrophages) have been shown by our group and others to secrete another RAGE ligand, HMGBl, in response to various maturational stimuli, therefore HMGBl secretion by MDSCs represents an additional mechanism for MDSC recruitment to be explored (40, 41).

Our observations suggest an important mechanism driving an immunosuppressive environment during early pancreatic carcinogenesis. However, several questions remain concerning the relationship between cancer-related inflammation and the accrual of MDSCs. First, it is not clear if the pancreatic carcinogenesis that promotes the accumulation of MDSCs is an epiphenomenon or if the recruitment of MDSCs contributes to carcinogenesis by limiting effective immune editing. Secondly, the failure of various immunotherapies, particularly within the context of pancreatic cancer, is attributed to the presence of host regulatory immune cells like MDSCs and Tregs (6). It is not clear if targeting MDSC function in these settings may render these therapies more effective. Our results demonstrating a critical role for RAGE in MDSC accumulation in the context of pancreatic carcinogenesis identifies a novel pathway that can be explored to address these questions possibly by assessing established strategies such as IL-2 administration in this model (42). Lastly, given the role of RAGE in promoting autophagy within tumor cells and evidence of immune cell-mediated autophagy (particularly T and NK cells), it would be interesting to explore whether interactions with MDSCs modulate these observations in KCR mice (21).

Acknowledgments

We dedicate this manuscript to the memory of Angelika Bierhaus, Ph.D., our friend and benefactor. She was a pioneering RAGE biologist, generous and kind in her dealings with colleagues, who provided us the mice with which we completed this work and who sadly died on April 15, 2012 after a long and courageous battle with cancer. Angelika had a great love of life, and she was generous, kind, and warm hearted. She was always full of plans for scientific endeavors, even when her disease began to take its toll. She remained steadfast that she would not be defeated, but despite recently celebrating her 50th birthday, she was aware her remaining time was short. She dedicated herself to research, but despite her incredible strength, she was not able to overcome the disease that tragically took her life. We also thank the University of Pittsburgh Cancer Institute Flow and Imaging Cytometry core facility as well as the University of Pittsburgh Cancer Institute Animal Facility.

Disclosures

The authors have no financial conflicts of interest.

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