cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Mitogen-activated Protein Kinase Kinase Activity Maintains Acinar-to-Ductal Metaplasia and Is Required for Organ Regeneration in Pancreatitis

Christopher J. Halbrook,[1,2] Hui-Ju Wen,[1,2] Jeanine M. Ruggeri,[1,2] Kenneth K. Takeuchi,[1,2] Yaqing Zhang,[3] Marina Pasca di Magliano,[3] and Howard C. Crawford[1,2]

[1]Department of Molecular and Integrative Physiology, [2]Department of Internal Medicine, and [3]Department of Surgery, University of Michigan, Ann Arbor, Michigan

SUMMARY

Mitogen-activated protein kinase kinase signaling is required for initiation and maintenance of pancreatitis. Inhibition of this signaling pathway attenuates inflammation and fibrosis, but also limits organ regeneration.

BACKGROUND & AIMS: Mitogen-activated protein kinase (MAPK) signaling in the exocrine pancreas has been extensively studied in the context of pancreatic cancer, where its potential as a therapeutic target is limited by acquired drug resistance. However, its role in pancreatitis is less understood. We investigated the role of mitogen-activated protein kinase kinase (MEK)-initiated MAPK signaling in pancreatitis to determine the potential for MEK inhibition in treating pancreatitis patients.

METHODS: To examine the role of MEK signaling in pancreatitis, we used both genetic and pharmacologic approaches to inhibit the MAPK signaling pathway in a murine model of cerulein-induced pancreatitis. We generated mice harboring inducible short hairpins targeting the MEK isoforms *Map2k1* and/or *Map2k2* specifically in the pancreatic epithelium. We also used the MEK inhibitor trametinib to determine the efficacy of systemic inhibition in mice with pancreatitis.

RESULTS: We demonstrated an essential role for MEK signaling in the initiation of pancreatitis. We showed that both systemic and parenchyma-specific MEK inhibition in established pancreatitis induces epithelial differentiation and stromal remodeling. However, systemic MEK inhibition also leads to a loss of the proliferative capacity of the pancreas, preventing the restoration of organ mass.

CONCLUSIONS: MEK activity is required for the initiation and maintenance of pancreatitis. MEK inhibition may be useful in the treatment of chronic pancreatitis to interrupt the vicious cycle of destruction and repair but at the expense of organ regeneration. *(Cell Mol Gastroenterol Hepatol 2017;3:99–118; http://dx.doi.org/10.1016/j.jcmgh.2016.09.009)*

Keywords: Inflammation; Wound Healing; Tissue Regeneration; ADM.

Pancreatitis is the most frequent cause for hospitalization for a gastrointestinal disease.[1] Repeated bouts of acute pancreatitis cause a necrosis-fibrosis sequence leading to chronic pancreatitis (CP), which is characterized by progressive and potentially irreversible damage to the pancreas.[2] Although some acinar cells are lost during pancreatitis through necrosis,[3] other acinar cells undergo acinar-to-ductal metaplasia (ADM).[4] ADM are proliferative duct-like structures theoretically capable of regenerating acinar cells lost in pancreatitis.[4–6] ADM induction has been linked to several mechanisms including ductal ectasia,[7] activation of nuclear factor kappa B (NF-κB),[8,9] Notch receptors,[10,11] and epidermal growth factor receptor (EGFR). Activation of EGFR by ectopic ligands has been demonstrated to drive ADM in ex vivo cultures[10,12] and in vivo.[12,13]

High levels of RAS activity, established through transgenic overexpression of oncogenic *Kras,* are sufficient to drive CP and ADM.[14] This effect is mediated through RAS activation of NF-κB signaling, which propagates a feed-forward signaling loop promoting chronic inflammation.[15] We and others have demonstrated that endogenously expressed mutant *Kras* requires EGFR to achieve sufficient RAS activity to induce ADM and tumorigenesis.[16,17] We also observed that pharmacologic inhibition of mitogen-activated protein kinase kinase (MEK) is sufficient to block KRAS-driven ADM and subsequent tumor formation,[17] whereas MEK inhibition of established pancreatic intraepithelial neoplasia induces acinar cell redifferentiation.[18] Taken together, these data strongly support a key role for *KRAS-MEK* signaling in the formation and maintenance of pancreatic preneoplasia.

Abbreviations used in this paper: **ADM, acinar-to-ductal metaplasia; BrdU, bromodeoxyuridine; CP, chronic pancreatitis; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; NF-κB, nuclear factor kappa B; pERK, phosphorylated extracellular signal–regulated kinase; qRT-PCR, quantitative reverse transcriptase–polymerase chain reaction; sh, short hairpin; WT, wild-type.**

Most current article

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2016.09.009

In contrast to tumorigenesis, the role of mitogen-activated protein kinase (MAPK) signaling in the induction and persistence of pancreatitis in the absence of oncogenic *Kras* is less well-elucidated. Pancreatitis is marked by an influx of macrophages that can release cytokines such as tumor necrosis factor-α and RANTES driving ADM by activation of NF-κB.[8] In addition, alternatively activated macrophages promote the activation of pancreatic stellate cells, further enhancing the fibroinflammatory response.[19] It has been postulated that stromally derived cytokines and growth factors are primarily responsible for driving acinar cell damage and ADM.[8] However, expression of EGFR ligands and EGFR activation is commonly observed in human CP, and in mice, parenchymal ablation of either EGFR or ADAM17, the primary EGFR ligand sheddase, prevents ADM and the stromal response in a cerulein model of pancreatitis.[17] These data collectively suggest that MEK signaling in epithelial cells, downstream of EGFR activation, is required for initiation of pancreatitis, including ADM and the fibroinflammatory response. The possibility that MEK activity is important for maintaining ADM suggests that MEK inhibitors may offer a treatment strategy for CP in human patients, for which there currently are no effective alternatives.[2]

Here we have determined that inhibition of MAPK signaling in cerulein-induced pancreatitis by treatment with the MEK inhibitor trametinib blocked CP development. Furthermore, short-term trametinib treatment of established pancreatitis restored exocrine tissue and dramatically reduced inflammation and fibrosis. However, inhibition of MEK interfered with the restorative capacity of the organ by blocking cell proliferation. With longer-term trametinib treatment, loss of organ regeneration was even more pronounced. By using short hairpin (sh) RNA mouse models individually targeting both major MEK isoforms, we found that parenchyma-specific knockdown of MEK blocked pancreatitis-induced ADM and the associated inflammation and fibrosis. Together, these data show that MEK signaling is a potent driver of the overall pancreatitis phenotype and is required for limited organ regeneration.

Results

Blockade of Mitogen-activated Protein Kinase Signaling Prevents Chronic Pancreatitis

Previously we showed that parenchymal EGFR and its activation by ADAM17 are required for pancreatic tumorigenesis,[17] attributing this effect to a reduction in downstream MEK activation. We also found that parenchymal ablation of EGFR or ADAM17 blunted all aspects of experimental pancreatitis.[17] However, in each of these models, EGFR signaling was chronically inhibited before disease onset, preventing us from examining acute effects. Here we set out to explore the feasibility of acute MEK inhibition as a potential treatment for CP. First, we performed immunohistochemistry for phosphorylated extracellular signal–regulated kinase (pERK) on a human pancreas tissue microarray that included normal and CP samples (Figure 1*A*) to determine whether MEK activity is potentially relevant to human CP. Ten of 12 CP samples showed pERK positivity in the epithelia and 12 of 12 in stromal cells. In contrast, 2 of 56 normal pancreas samples showed pERK positivity in the epithelia and 4 of 56 in the stroma. This ERK activity may be a result of being normal tissue in close proximity to cancer.

Pancreatitis can be induced in mice by administration of supramaximal doses of cerulein, a cholecystokinin ortholog, with the extent of damage determined by the amount and duration of treatment. Mild treatment regimens induce symptoms of acute pancreatitis, marked by acinar cell necrosis and an innate immune response. A more severe treatment protocol results in a phenotype more reminiscent of human CP, marked by ADM, fibrosis, and innate and adaptive immune responses.[17] However, unlike human CP, damage induced by chronic cerulein treatment resolves over time after cerulein withdrawal.

To investigate whether systemic inhibition of MEK blocked cerulein-induced pancreatitis in a manner similar to EGFR gene ablation, we pretreated mice with either the MEK inhibitor trametinib (T-CP) or vehicle (V-CP) and then continued this treatment with a cerulein treatment regimen that elicits a CP-like phenotype (Figure 1*B*) or saline as a vehicle control. The efficacy of trametinib treatment was verified by immunoblotting tissue lysates harvested from V-CP and T-CP groups, where ~60% lower pERK levels were observed (Figure 1*C*).

Histologic examination of pancreas tissue demonstrated that cerulein treatment strongly induced a dropout of acinar tissue and a gain of fibrotic stroma (Figure 1*D*). V-CP mice had ~70% loss of acinar cell area, defined by area positive for amylase by immunohistochemistry (Figure 1*E*), compared with saline controls. Loss of acinar cells was accompanied by gain of a picrosirius-positive fibrotic stroma (Figure 1*F*), rich in inflammatory cells. Pancreata from T-CP animals had dramatically more amylase-positive acinar tissue compared with vehicle control, as well as significantly less fibrosis. The fibroinflammatory response and acinar dropout correlated to organ atrophy associated with CP, with V-CP pancreata losing 60% of their relative pancreatic mass compared with saline control (Figure 1*G*). In contrast, T-CP mice lost only 35% of relative pancreas mass compared with saline control. There were no apparent differences in the tissue of mice treated with trametinib or vehicle in the absence of cerulein (Figure 1*H*).

To support the histologic findings, RNA was harvested from the tissue of V-CP and T-CP mice. Quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) was performed to assess the acinar vs ductal composition of the pancreas (Figure 1*I*). As expected, levels of amylase (Amy2b) were dramatically higher and levels of the ductal marker cytokeratin 19 (Krt19) were significantly lower in T-CP pancreata vs V-CP pancreata. Transcripts for the acinar-specific transcription factors Ptf1a and Mist1 confirmed higher levels of acinar differentiation in T-CP mice compared with V-CP mice.

Consistent with the epithelial response, examination of immune cell infiltration in the cerulein-treated tissue