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p38 MAPK in Pancreatic Cancer: Finding a Protective Needle in the Haystack

Murray Korc

Departments of Medicine, Biochemistry and Molecular Biology, Indiana University School of Medicine, the Melvin and Bren Simon Cancer Center and the Pancreatic Cancer Signature Center, Indianapolis, Indiana, United States of America

Summary

Activated p38 MAPK alpha (pp38α) is a good prognostic marker in pancreatic ductal adenocarcinoma (PDAC) that could be used to personalize therapy. pp38α suppresses JNK-mediated proliferation, both *in vitro* and *in vivo*. The current findings support testing of combination therapies that include JNK targeting and/or suppressing negative regulators of pp38α.

In this issue of *Clinical Cancer Research*, Zhong and colleagues (1) performed biomarker profiling to identify survival-predicting pathways in resectable pancreatic ductal adenocarcinoma (PDAC). Using tissues obtained from 36 patients through a rapid autopsy program, they prepared a discovery set consisting of a tissue microarray (TMA) of primary and metastatic lesions (Fig. 1A). Markers were grouped into three clusters: 1) biomarkers with abnormal immunostaining patterns when compared to normal epithelial cells within the same sections (cluster 1); 2) biomarkers with expression heterogeneity (cluster 2); and 3) biomarkers with similar patterns of expression intensity or cellular localization to normal epithelial cells. The authors focused on the 12 proteins in cluster 2, all of which have been implicated in PDAC pathobiology: β-catenin, cyclin D1, EGFR, mesothelin, MUC5AC, p53, p63, phospho-p38 MAPK (pp38), phospho-JNK (pJNK), phospho-AKT, S100A2, and YAP. They next prepared five TMAs from 83 resected PDACs (Fig. 1A) and determined that cytoplasmic pp38 overexpression in the cancer cells correlated with significantly prolonged survival (24.4 months) vs. the low expression group (14.7 months), and with decreased lymph node involvement.

Interestingly, 41 of the 83 resected patients completed a course of adjuvant therapy, consisting of 1-2 cycles of gemcitabine followed by 5-FU and radiation therapy, followed by another 2-4 months of gemcitabine. Patients whose cancers exhibited high pp38 scores had a longer overall survival (28.4 months) vs. the low expression group (15.0 months). In another group of 26 patients treated according to a similar protocol (RTOG 97-04), high pp38 expression was an independent predictor of improved patient survival. Together, these observations support the conclusion that pp38 is a prognostic marker in PDAC, and

Corresponding author: Murray Korc, M.D., Indiana University School of Medicine, 980 W. Walnut Street, Indianapolis, IN 46202, Phone: 317-278-6410, Fax: 317-274-8046, mkorc@iu.edu.

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There are no potential conflicts of interest.

underscore the usefulness of TMA analysis using well-annotated patient samples obtained through a rapid autopsy program.

PDAC is a deadly cancer that is remarkably recalcitrant to chemotherapy and radiation therapy (2). Multiple features contribute to therapeutic resistance. These include the overexpression of growth factors and their respective high-affinity receptors, excessive production of cytokines, a desmoplastic microenvironment with areas of hypoxia and attenuated/leaky vascular perfusion, a plethora of molecular alterations that include mutated Kras in 95% of PDAC patients, aberrant non-coding RNA expression, and suppression of cancer-directed immune mechanisms (3-5). Consequently, PDAC exhibits complex signaling cascades and loss of negative growth constraints. For example, there are four p38 mitogen activated protein kinases (MAPKs) encoded by four genes (6). *MAPK11* encodes p38 β , *MAPK12* encodes p38 γ , and *MAPK13* encodes p38 δ , whereas *MAPK14* encodes p38 α , which was the focus of the current study. Other members of this family include the ERK1 and ERK2 MAPKs that are activated by mitogenic growth factors and three JNKs (7). JNK1 is encoded by *MAPK8*, whereas JNK2 and JNK3 are encoded by *MAPK9* and *MAPK10*, respectively. JNK1 and JNK2 are ubiquitous, but JNK3 is mostly expressed in the brain. These pathways are often depicted in a modular and highly structured manner in which a MAPK kinase kinase (MAP3K) such as Raf activates a MAPK kinase (MAP2K) such as MEK, which in turn activates a MAPK such as ERK1 (Fig. 1 B). However, the context-dependence of the actions of different MAPKs, the multiplicity of MAP3Ks and MAP2Ks, their ability to cross-talk, and the fact that these pathways are modulated by multiple cytokines, growth factors, and negative signals such as phosphatases has stymied previous efforts to assess the exact role of pp38 in PDAC.

To delineate the mechanisms whereby pp38 exerts its protective actions, Zhong and colleagues studied seven recently derived human pancreatic cancer cell lines. Three of these cell lines (Panc5.04, A10.7 and A38.44) expressed pp38 and its downstream target phospho-ATF2 (pATF2), suggesting that pp38 was active in these cells. They next used three distinct p38 inhibitors (SB202190, SB203580 and SB239063) and determined that all three pyridinyl imidazoles enhanced cell proliferation and decreased pATF2 levels, confirming the presence of active pp38 in these cells. However, these inhibitors do not exert completely superimposable effects, and they exhibit off-target actions at high concentrations. Nonetheless, subsequent experiments with 10 μ M SB202190 revealed that inhibition of pp38 led to enhanced proliferation under both normoxic and hypoxic conditions, indicating that pp38 suppressed pancreatic cancer cell proliferation even in circumstances that mimic the pancreatic tumor microenvironment.

SB202190 also increased pJNK levels in Panc5.04, A10.7 and A38.44 cells, whereas pJNK inhibition by SP600125 blocked the growth-stimulatory effects of p38 inhibition. Classically, JNKs and p38 MAPKs are activated by MKK4/7 and MKK3/6, respectively (Fig. 1B). However, MKK4 can also activate p38 α , which in turn can inhibit MKK4/7 through a complex negative feedback loop. Zhong and colleagues determined that MKK7 phosphorylation, and hence activation, increased in all three cell lines as a consequence of p38 inhibition by SB202190. They next demonstrated that siRNA-mediated suppression of MKK7 markedly attenuated the proliferative actions of SB202190. Thus, both SP600125-

mediated JNK inhibition and si-RNA-mediated knockdown of MKK7 prevented SB202190-induced proliferation, confirming that pp38-mediated growth-inhibitory effects in PDAC are due to its ability to suppress pJNK proliferative actions.

Zhong and colleagues next tested the effects of SB202190 and SP600125 in a subcutaneous xenograft model, using Panc5.04 and A10.7 cells expressing pp38, and A2.1 and A6L cells that express very low levels of either pp38 or pJNK. In tumors derived from Panc5.04 and A10.7 cells, inhibition of pp38 resulted in enhanced growth, whereas pJNK inhibition with SP600125 attenuated growth. By contrast, tumors derived from A2.1 and A6L cells were not affected by either inhibitor. Although the studies were not performed in an orthotopic model that could have provided information on metastases, these observations indicate that pp38 signaling is not only a predictive marker of outcome in resected PDAC, but is also a protective tumor suppressor that antagonizes the deleterious actions of JNK.

This study is important for several reasons. First, the finding that pp38 is a favorable prognostic marker in PDAC is a major discovery, given the pivotal role of MAPKs in PDAC pathobiology and the complexity of these signaling pathways. Second, the study suggests that pp38 may be a biomarker delineating when adjuvant radio-chemotherapy would be beneficial in resected PDAC patients, which would be useful clinically given previous negative results with this therapeutic approach (8). Third, p38 activation has been previously shown to attenuate cell proliferation by such diverse mechanisms as suppressive effects at both the G1/S and G2M transitions, inhibition of EGF receptor signaling and ERK1/2 activation, antagonizing pJNK actions and upregulating p53 activity (9). However, this is the first demonstration that pp38 acts as a tumor suppressor in PDAC by inhibiting JNK actions. Fourth, it is now possible to consider additional pre-clinical studies followed by personalized therapy trials based on targeting JNK, which is activated downstream of oncogenic Kras in PDAC and which promotes the self-renewal of cancer stem cell like cells thereby sustaining their role in tumor initiation (10).

Future studies should delineate why and how p38 α becomes activated in PDAC, whether it is possible to activate p38 α in those cancers where it is not active, and how various p38 and JNK isoforms differ in their actions in PDAC. It will also be important to determine whether it is possible to target JNK more efficiently and to suppress the actions of negative regulators of pp38 such as DUSP1/ MKP1 which inactivates pp38 and enhances tumorigenicity and chemoresistance in PDAC (11-12).

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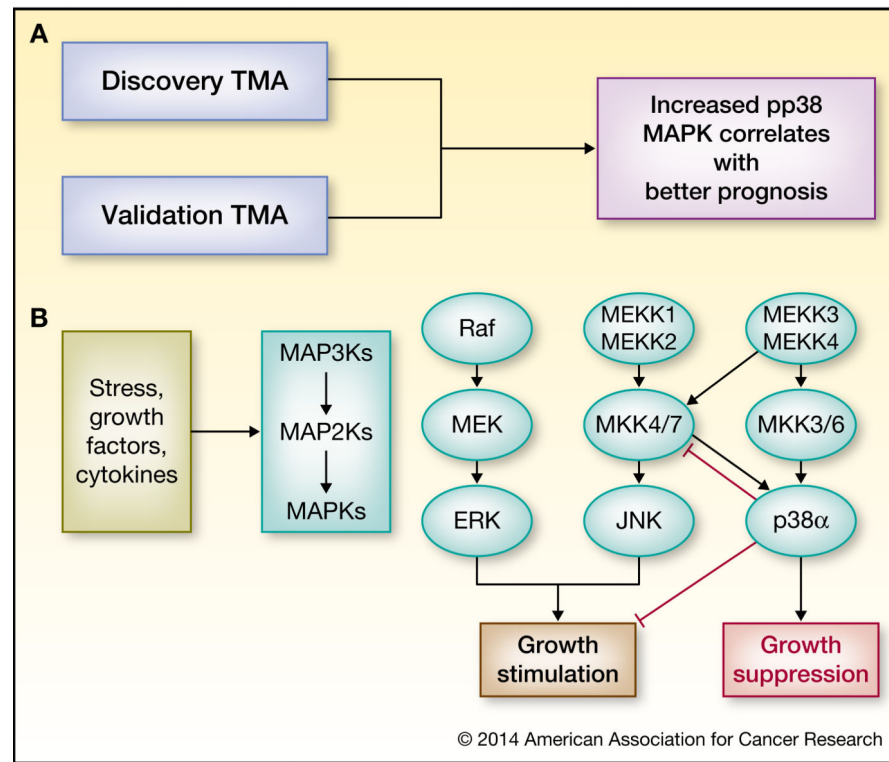


Figure 1.

Uncovering phospho-p38 MAPK as a good prognostic marker that suppresses JNK-induced mitogenesis in pancreatic cancer. **A.** A discovery tissue microarray (TMA) and a validation TMA were used to identify increased phospho-p38 MAPK (pp38) as a good prognostic marker in PDAC. **B.** Schematic representation of MAPK signaling pathways. Stress pathways, growth factors, and cytokines can activate MAPK signaling. The indicated MAPK kinase kinases (MAP3Ks) activate MAPK kinases (MAP2Ks), which activate MAPKs, as explained in the text. In general MKK4/7 activate JNKs, whereas MKK3/6 activate p38 MAPKs. In addition, MKK4 can activate p38 MAPK alpha (p38 α). Activated p38 α (pp38 α) can in turn inhibit MKK4/7, thereby blocking JNK activation and abrogating its growth-stimulatory actions. In addition, activated p38 α can suppress mitogenic signaling through other mechanisms as discussed in the text.