Targeted nuclear factor-kappaB suppression enhances gemcitabine response in human pancreatic tumor cell line murine xenografts

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Background. *Pancreatic ductal adenocarcinoma (PDAC) is an almost uniformly fatal malignancy characterized by resistance to chemotherapy. Currently, gemcitabine is the agent used most commonly but demonstrates only a partial response. The transcription factor nuclear factor-kappaB (NF-κB), known to be involved in the inflammatory response, is constitutively activated in PDAC and further activated by gemcitabine. Our aim was to examine the effects of targeted NF-κB suppression on gemcitabine resistance using an in vivo tumor growth model.*

Methods. *To suppress the NF-κB pathway, the mutant IκBα super-repressor protein was stably expressed in PaCa-2 human PDAC cells. Athymic mice were injected subcutaneously with IκBα-super-repressor (SR) or vector-expressing PaCa-2 cells and randomized to receive phosphate-buffered saline (PBS) or 100 mg/kg gemcitabine(gem) for 4 weeks.*

Results. *The mean increase in tumor volume was 47 mm^3 (89%) and 196 mm^3 (326%) in gem/SR and gem/vector groups, respectively (P = .03). The PBS-treated groups demonstrated greater tumor growth, ~340 mm^3 (850%) increase, in both PBS/vector and PBS/SR groups. Intratumoral NF-κB activity was decreased in gem/SR compared with the gem/vector group (P = .04). Decreased Ki-67 positivity was noted in gem/SR (49%) versus gem/vector tumors (73%) (P = .04), with no difference in apoptosis (apoptag, P = .3) or angiogenesis (CD31+, P = .9).*

Conclusion. *Stable IκBα-SR expression in vivo potentiated the antitumor effects of gemcitabine, resulting in decreased tumor growth in association with decreased cell proliferation. Molecular suppression of the NF-κB pathway decreases successfully gemcitabine resistance in a relatively chemoresistant PDAC line. Thus, NF-κB–targeted agents may complement gemcitabine-based therapies and decrease chemoresistance in patients with PDAC. (Surgery 2015;158:881-9.)*

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Advances in early detection as well as surgical and chemotherapeutic treatments have resulted in improved outcomes for many solid-organ malignancies during the past 2 decades. Adenocarcinoma of the pancreas, the most common pancreatic malignancy, has remained largely refractory to such advances. In the United States, approximately 46,420 people would have been diagnosed with pancreatic cancer in 2014, with 39,590 succumbing to the disease.[1] The poor survival rate can be explained in part by its insidious nature; pancreatic cancer often remains largely asymptomatic until it reaches a locally advanced or metastatic stage. In addition, even when detected in its earliest form and resected promptly, survival remains dismal, with a median survival of approximately 2 years.[2,3]

Most patients diagnosed with pancreas cancer present with distant metastatic disease. These patients derive no benefit from operative resection and rely solely on chemotherapeutic agents

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for improvements in longevity. The workhorses of current chemotherapy for pancreatic ductal adenocarcinoma (PDAC) are 5-flourouracil and gemcitabine (2′,2′-difluorodeoxycytidine), a nucleoside analog that inhibits deoxyribonucleic acid (DNA) replication by intercalating in the penultimate nucleoside position in place of cytidine, thereby arresting cell growth.[4] The efficacy of gemcitabine in the primary and adjuvant setting is limited. In patients with advanced unresectable disease, only 7–14% of patients demonstrate any response to gemcitabine chemotherapy, which translates to a 1- to 4-month improvement in progression-free interval.[5] When used in the adjuvant setting after operative resection, this agent demonstrates a 3- to 4-month increase in median survival compared with resection alone.[6] Numerous mechanisms have been proposed for gemcitabine resistance in PDAC, which include alterations in molecule transport and uptake, rapid metabolism of the drug, alterations in the drug target, and antiapoptotic factors expressed within the tumor cells.[7]

Nuclear factor-kappaB (NF-κB) is a ubiquitous transcription factor within animal cells. Inactive NF-κB resides in the cell cytoplasm bound to the inhibitor proteins IκB-α or IκB-β. Phosphorylation by IκB kinase targets the inhibitor proteins for degradation by the 26S proteosome, allowing NF-κB to cross the nuclear membrane and activate its downstream targets.[8] Dysregulation of NF-κB activity has been implicated in numerous inflammatory and neoplastic conditions.[9] NF-κB has also been shown to be constitutively activated in human PDAC and PDAC cell lines.[10-12] In addition, human PDAC cells exposed to gemcitabine exhibited increased NF-κB activity in vitro, an undesired response which may contribute to chemoresistance.[10,13-15]

We reported recently that pharmacologic disruption of the NF-κB pathway in vitro when using dimethylaminoparthenolde, an orally bioavailable analogue of the sesquiterpine lactone parthenolide, improves gemcitabine efficacy.[16] Furthermore, in genetically engineered mouse PDAC models, dimethylaminoparthenolde in combination with gemcitabine prevented the progression of premalignant pancreatic lesions and increased survival.[17,18] The limitation of pharmacologic inhibition of NF-κB lies in its potential lack of target specificity. To more directly evaluate the role of NF-κB inhibition in chemoresistance, a molecular means of blocking NF-κB activation is needed.

In the present study, we investigated the effects of NF-κB suppression on gemcitabine resistance in an in vivo model of tumor growth. Specifically, the IκBα super-repressor (SR) protein mutated at phosphorylation sites S32A and S36A was stably expressed in the PaCa-2 human PDAC cell line. The mutant IκBα-SR is unable to be phosphorylated and therefore, remains bound to NF-κB and preventing its activation. We hypothesize that NF-κB suppression will counter gemcitabine resistance in the IκBα-SR expressing PaCa-2 tumors.

MATERIALS AND METHODS

Cell lines and compounds. PaCa-2 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained as recommended. Gemcitabine (GEMZAR; Eli Lilly, Indianapolis, IN) was dissolved in sterile water and stored at 4°C. Recombinant human tumor necrosis factor-alpha (TNF-α) (R&D Systems, Minneapolis, MN) was dissolved in phosphate buffered saline containing 0.1% BSA (10 μg/ml) and stored at −20°C.

Lentiviral construct and transduction. The pCL6IEGwo/IκBαSR lentiviral construct was made by inserting the IκBαSR element from the pcDNA3/IκBαSR plasmid (provided kindly by Harikrishna Nakshatri, Indiana University School of Medicine, Indianapolis, IN)[19] into the *Eco*R1/*Bam*H1 sites of the pCL6IEGwo lentiviral vector (a generous gift from Helmut Hanenberg, MD, Herman B. Wells Center for Pediatric Research, Indianapolis, IN). The empty pCL6IEGwo lentiviral vector was used as the control. PaCa-2 cells were transduced with the 2 constructs and sorted for green fluorescence protein to generate stable PaCa-vector and PaCa-SR cell lines.

Cell-proliferation assay. Cell proliferation was determined using a colorimetric assay, the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega, Madison, WI). Cells were plated in triplicate in 96-well plates. Twenty-four hours later, gemcitabine or vehicle control (water) was administered. After treatment for the indicated time period, cells were incubated with 20 μL of CellTiter 96 AQ_{ueous} One Solution Reagent, and absorbance was recorded at 490 nm. Percent cell growth was determined from a ratio of average absorbances of the treatment wells to the control wells.

Western blotting. Cells were lysed in radioimmunoprecipitation assay buffer (phosphate-buffered saline [PBS], 1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate,