Selective Inhibitors of Nuclear Export Block Pancreatic Cancer Cell Proliferation and Reduce Tumor Growth in Mice

ASFAR S. AZMI,[1] AMRO ABOUKAMEEL,[2] BIN BAO,[1] FAZLUL H. SARKAR,[1] PHILIP A. PHILIP,[2] MICHAEL KAUFFMAN,[3] SHARON SHACHAM,[3] and RAMZI M. MOHAMMAD[2]

[1]*Department of Pathology, Wayne State University, Detroit, Michigan;* [2]*Karyopharm Therapeutics, Natick, Massachusetts;* [3]*Department of Oncology, Karmanos Cancer Institute, Detroit, Michigan*

See Covering the Cover synopsis on page 257.

BACKGROUND & AIMS: Tumor-suppressor proteins are inactivated by many different mechanisms, including nuclear exclusion by chromosome region maintenance (CRM)-1. Increased tumor levels of CRM-1 have been correlated with poor prognosis of patients with pancreatic cancer, making it a therapeutic target. Selective inhibitors of nuclear export (SINEs) bind to CRM-1 to irreversibly inhibit its ability to export proteins; we investigated a new class of SINEs in pancreatic cancer cells. **METHODS:** We studied the effects of SINE analogs in a panel of pancreatic cancer cell lines and nontransformed human pancreatic ductal epithelial cells using proliferation, apoptosis, immunoblot, co-immunoprecipitation, small inhibitor RNA, and fluorescence microscopy analyses. The effects of the SINEs also were investigated in mice with subcutaneous and orthotopic tumors. **RESULTS:** SINEs (KPT-185, KPT-127, KPT-205, and KPT-227) inhibited proliferation and promoted apoptosis of pancreatic cancer cells, but did not affect human pancreatic ductal epithelial cells. The nuclei of cells incubated with KPT-185 accumulated tumor-suppressor proteins (p27, FOXO, p73, and prostate apoptosis response-4 [PAR-4]) and inhibited interactions between CRM-1 and these proteins. Mutations in the region of CRM-1 that bind to SINEs (Cys-528), or small inhibitor RNA knockdown of PAR-4, prevented the ability of KPT-185 to block proliferation and induce apoptosis of pancreatic cancer cells. Oral administration of KPT-330 to mice reduced growth of subcutaneous and orthotopic xenograft tumors without major toxicity. Analysis of tumor remnants showed that KPT-330 disrupted the interaction between CRM-1 and PAR-4, activated PAR-4 signaling, and reduced proliferation of tumor cells. **CONCLUSIONS: We identified SINEs that inhibit CRM-1 and promote nuclear accumulation of tumor-suppressor proteins in pancreatic cancer cells. Oral administration of the drug to mice reduces growth of xenograft tumors.**

Keywords: Exportin; Translocation; Nucleus; Small Molecule Inhibitor; CRM1.

Treatment of pancreatic cancer is an unmet clinical problem with annual deaths exceeding breast and prostate cancers, largely owing to the lack of effective drug therapies.[1] Such dismal statistics indicate that newer targets and drugs urgently need to be identified. Chromosome region maintenance 1 (CRM-1; also referred to as *exportin1* or *Xpo1*) is one of the major, nonredundant receptors for the export of proteins out of the nucleus. It is a member of the importin β superfamily of nuclear transport receptors, recognizing proteins bearing a leucine-rich nuclear export sequence (NES).[2] Among the various targets of CRM-1 are tumor-suppressor proteins (TSPs) such as p53, p27, p21, FOXO, and prostate apoptosis response-4 (PAR-4).[3] Nuclear exclusion of these and other TSPs by CRM-1 renders cancer cells resistant to apoptosis.[4] A significant mechanism of action of the commonly used chemotherapeutic drugs such as gemcitabine, 5-fluorouracil, and platinum-based compounds is by activation of various TSPs through their nuclear retention. However, increased CRM-1 expression levels in cancer cells result in mislocalization of important TSPs via a constant nuclear export, resulting in the attenuation of their tumor-suppressor function, and contributing to treatment failure. In support of its role in cancer maintenance, increased CRM-1 expression has been found to be correlated with poor overall survival in multiple tumors including pancreatic cancer.[5] Therefore, targeted inhibition of CRM-1 is an attractive therapeutic strategy for forcing the TSPs to be retained in the nucleus to allow them to function properly and induce cancer-specific apoptosis.

Earlier approaches to target CRM-1 led to the development of the natural product CRM-1 inhibitor leptomycin B (LMB).[6,7] However, a single phase I trial of parenterally administered LMB showed marked gastrointestinal toxicity and constitutional intolerance, thereby limiting its clinical use.[8] Semisynthetic modification of LMB markedly improved pharmacokinetic (PK) properties, and improved the therapeutic window of these parenterally administered LMB derivatives in animals.[9] Nevertheless,

Abbreviations used in this paper: CRM-1, chromosome region maintenance 1; FITC, fluorescein isothiocyanate; HPDE, human pancreatic ductal epithelial; IC_{50}, median inhibitory concentration; LMB, leptomycin B; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NES, nuclear export sequence; PAR-4, prostate apoptosis response-4; PARP, poly (ADP-ribose) polymerase; PK, pharmacokinetic; PKA, protein kinase A; SINE, selective inhibitors of nuclear export; siRNA, small inhibitor RNA; TSP, tumor-suppressor proteins.

© 2013 by the AGA Institute
0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2012.10.036

their clinical potential has not been explored. Together, these data suggest that newer CRM-1 inhibitors with high specificity, cancer cell selectivity, and low toxicity are needed.

By using a high-throughput, in silico, structure-based design, we have developed novel, small-molecule, highly selective inhibitors of nuclear export (SINEs) that covalently bind to the CRM-1 cargo-binding groove and irreversibly inhibit the protein's export function. SINEs have broad specificity against different tumor types with median inhibitory concentrations (IC_{50}s) in the submicromolar range, sparing normal cells (Supplementary Figure 1), and have recently entered phase I clinical trials for both solid tumors (NCT01607905) and advanced hematologic malignancies (NCT01607892).

As documented earlier, prostate apoptosis response-4 (Par-4) is a cancer cell–selective, pro-apoptotic protein that functions intracellularly in the cytoplasmic and nuclear compartments as a TSP.[10] Under external stress conditions in most cancer cells, ectopic PAR-4 readily translocates to the nucleus to induce apoptosis.[11] In contrast, in normal cells, ectopic PAR-4 is localized predominantly to the cytoplasm and does not induce apoptosis unless a second apoptotic insult occurs. Consistent with these observations, PAR-4 has been found to be down-regulated in many cancers, such as renal cell carcinoma,[12] neuroblastoma,[13] endometrial cancer,[14] breast cancer,[15] and pancreatic cancer.[16] We showed that activation and nuclear localization of PAR-4 can lead to significant apoptosis in pancreatic cancer cells.[17,18] Based on these investigations, we proposed PAR-4 activation as a potential therapeutic strategy in pancreatic cancer.[19] Because the TSP PAR-4 contains a canonical leucine-rich NES, it may be a cargo for CRM-1–mediated nuclear export, and therefore an ideal candidate to investigate using our newly developed SINEs.

In this report, we investigated the anticancer potential of SINEs against pancreatic cancer cell lines and in 3 xenograft models (1 subcutaneous and 2 orthotopic) and evaluated the role of different TSPs in general, and, more specifically, the role of PAR-4 in these effects. The data presented here show that SINE activity is mediated through nuclear localization of multiple tumor-suppressor proteins, particularly PAR-4. Our findings support the development of SINEs for the clinical treatment of pancreatic cancer.

Materials and Methods

Cell Lines and Culture Condition

AsPC-1, BxPC-3, and HPAC cells were obtained from American Type Culture Collection. Colo-357 and human pancreatic ductal epithelial (HPDE) cells were a gift from Dr Paul Chiao (MD Anderson Cancer Center, Houston, TX). Pancreatic cancer cells were grown in Dulbecco's modified Eagle medium with 5% penicillin and streptomycin supplemented with fetal bovine serum (Sigma) and 5% glutamine (Invitrogen). HPDE was cultured in keratinocyte serum-free medium supplemented with epidermal growth factor and bovine pituitary extract (Life Technologies). These cell lines have been tested and authenticated in our Core facility.

Cell Growth Inhibition 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay

Pancreatic cancer cells and normal HPDE cells were seeded at a density of 3×10^3 cells per well in 96-well microtiter culture plates. After overnight incubation, medium was removed and replaced with fresh medium containing KPT SINEs at indicated concentrations (0–200 nmol/L). In another set of experiments, pancreatic cancer cells were first exposed to calyculin (0–15 nmol/L) for 5–30 minutes followed by KPT-SINEs (0–150 nmol/L for 24–72 h). Upon completion of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to published methods [17]

Quantification of Apoptosis by Histone DNA Enzyme-Linked Immunosorbent Assay and Annexin V Fluorescein Isothiocyanate Assay

Cell apoptosis was detected by using Annexin V fluorescein isothiocyanate (FITC) (Biovision, Danvers, MA) and histone DNA enzyme-linked immunosorbent assay Detection Kit (Roche, Life Sciences) according to the manufacturer's protocol.[18]

Western Blot Analysis

Preparation of cellular lysates, protein concentration determination, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis has been described previously.[20]

Immunofluorescence and PAR-4 Cellular Staining

For protein localization experiments, cells were grown on glass chamber slides and exposed to KPT-185 in the absence or presence of different small inhibitor (si)RNAs (control siRNA and PAR-4–specific siRNA) at indicated concentrations for 24 hours. In another set of experiments, cells on cultured slides were exposed to short-term treatment with calyculin A (0–15 nmol/L) for 5–30 minutes followed by KPT-185 treatment for 24 hours. At the end of the treatment, immunofluorescence was performed according to our previously published methods.[20]

siRNA and Transfections

Cells were transfected with either control siRNA or PAR-4 siRNA using Lipofectamine 2000 (Invitrogen) according to published methods.[21] After the siRNA treatment period, cells were treated further with KPT-185 in 96-well plates for MTT assay, and 6-well plates for Annexin V FITC assay, respectively. Transfection efficiency was evaluated by fluorescence microscopy as published previously.[20]

Site-Directed Mutations in CRM-1

CRM-1 cys528 mutants (that carry aberrant KPT-SINE–recognizable NES-recognizing sequence) were developed by transiently transfecting AsPC-1 and BxPC-3 cells with wild-type and mutant Ser-528 CRM-1 according to published methods.[22]

Development of Subcutaneous and Orthotopic Animal Xenografts and Preclinical Efficacy Trial

Four-week-old female ICR-SCID mice (Taconic Laboratory) were adapted to animal housing and xenografts were de-

Download English Version:

https://daneshyari.com/en/article/3293276

Download Persian Version:

https://daneshyari.com/article/3293276

Daneshyari.com