REVIEW ARTICLE

Promising SINEs in the Treatment of Pancreatic Cancer

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ABSTRACT

Pancreatic cancer remains a deadly disease in urgent need of newer therapeutic modalities. Pancreatic tumors are very heterogeneous and carry alterations in many critical pathways (i.e. harboring a robust biological network) rendering the design of therapy against a single pathway unrealistic. The disease requires a broad form of therapy that can target the activation of multiple tumor suppressor proteins simultaneously. It has been consistently shown in pancreatic cancer biopsies that there is an over-expression of the nuclear exporter protein exportin 1 also known as Chromosome maintenance region 1. Nuclear exporter protein exportin 1 is recognized to export major nuclear residing tumor suppressor proteins causing their functional inactivation through their mislocalization to the cytoplasmic compartment. This makes exportin 1 an attractive therapeutic target in pancreatic cancer. Supporting this we have clearly demonstrated that targeted inhibition of exportin 1 by our specific inhibitors of nuclear export can restore the function of multiple tumor suppressor proteins leading the pancreatic cancer cell death and tumor inhibition in orthotopic models. Additionally, studies have demonstrated that specific inhibitors of nuclear export synergize with gemcitabine and nab-paclitaxel leading to enhanced pancreatic cancer growth inhibition, apoptosis, and disintegration of cancer stem cell mimicking spheroids. Collectively these studies have lead to a Phase Ib/II clinical evaluation of specific inhibitors of nuclear export with GEM and nab-paclitaxel in patients with metastatic pancreatic cancer (ClinicalTrials.gov Identifier: NCT02178436). In this mini-review we highlight the promising specific inhibitors of nuclear export in pancreatic cancer.

INTRODUCTION

Pancreatic cancer (PC) is an aggressive disease which kills approximately 40,000 Americans each year [1]. With a five-year survival rate of less than five percent, PC is considered the most lethal among all solid tumors. The major reasons for this dismal outcome include, lack of early detection markers [2], invasive behavior [3], and intrinsic resistance to therapeutic treatments [4]. Typically, pancreatic tumors are a heterogeneous population of cells that carry alterations in many critical pathways, rendering the use of targeted-therapy against a single pathway ineffective [5]. In addition, the disease is often diagnosed at a late stage, after the cancer has metastasized to distal sites. Therefore, focus on early detection methods, newer therapeutic targets and novel drug combinations is urgently needed to positively impact the survival of patients with PC.

Acquired resistance mechanisms and the tumor microenvironment make treating PC particularly challenging. Recently, several studies have identified a highly resistant subset of pancreatic cancer stem cells (PCSCs) with self-renewal capacity and a propensity to undergo epithelial to mesenchymal transition [6]. These cells express CD44, CD24, CD133 and epithelial specific antigen (ESA) at high levels, and are resistant to common standard-of-care agents, such as gemcitabine. Additionally, pancreatic tumors are often poorly vascularized due to highly desmoplastic stroma, which limits drug penetrance and efficacy [7]. Thus, further studies are needed to identify effective regimens against PCSCs and understand the key molecules that promote desmoplastic stroma. Together, this information will help identify biomarkers and potential targets to limit chemoresistance and disease recurrence.

Nuclear Protein Transport and Cancer

Nuclear transport is an evolutionarily conserved mechanism governed by a set of proteins termed karyopherins [8]. Exportin 1 (XPO1, CRM1) is a karyopherin, which binds over 200 cargo proteins with leucine-rich nuclear export signals (NES) and shuttles them through the nuclear pore complex into the cytoplasm [9]. This process is energy dependent and requires RanGTP binding in the nucleus and its hydrolysis to RanGDP in the cytoplasm. XPO1 is the sole exporter of key TSPs, such as p53, p21, FOXO3a, p27, Ikβ and Par-4 [10] (Figure 1). It is well recognized that nuclear localization of TSPs
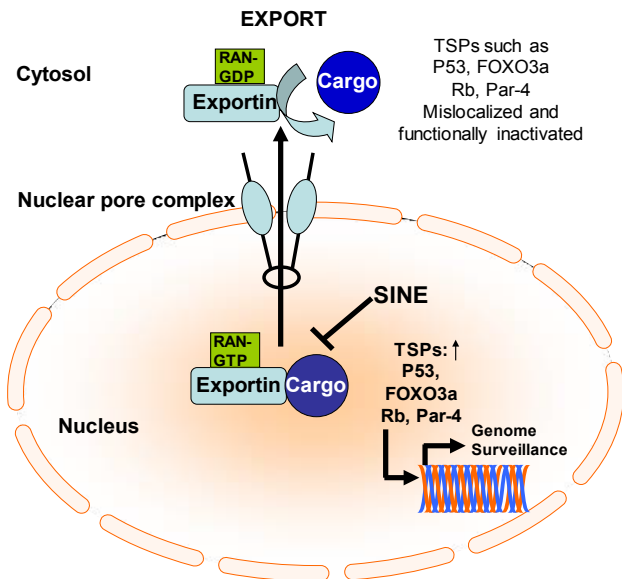
Figure 1. XPO1 regulates TSP function: Model of Ran-dependent nucleo-cytoplasmic transport of proteins. TSPs such as p53, FOXO3a, Rb and Par-4 are nuclear proteins that can be shuttled out of the nucleus by XPO1 in an RAN-GTP dependent manner. In cancer cells, over-expression of XPO1 hyper activates nuclear export of TSPs and other cell cycle regulators. Their mislocalization removes important cell cycle checkpoints and results in uncontrolled cell division."

and their intact DNA binding domains are essential for proper activity [11]. However, over-expression of XPO1 in cancer cells results in TSPs efflux and their inability to regulate cell cycle activity in the nucleus [12]. Supporting this, over-expression of XPO1 has been associated with chemoresistance and poor survival in solid tumors [13].

Drugging the Aberrant Nuclear Transport in PC

Emerging evidence suggests that XPO1 plays a critical role in PC. A 2009 study by Huang and colleagues evaluated tissue specimens from 69 PC and 10 normal patients [14]. Increased XPO1 expression was observed in PC tissue (P=0.007), and was associated with larger tumor size (P=0.01), lymph node metastases (P=0.004) and liver metastases (P=0.003). In addition, the expression of XPO1 was an independent prognostic determinant for poor progression-free and overall survival (95% CI, 1.27-5.39). Recently, Mahipal and colleagues evaluated the expression of XPO1 in 70 nonmalignant and 91 pancreatic carcinoma samples (unpublished work J Clin Oncol 31, 2013 (suppl; abstr e15115). The median IHC score was 6 (range: 0-9) and 3 (range: 1-9) in malignant and nonmalignant pancreatic tissue samples, respectively (P<0.0001). High XPO1 expression was found in 11% (8/70) of normal tissue samples and 69% (63/91) of tumor samples (P<0.0001). There were 48 samples of PC tissue and normal tissue obtained from same patient, and among these patients, 33 (69%) had higher XPO1 expression, 7 (15%) had similar expression and 8 (17%) had lower expression in malignant tissue samples compared to adjacent normal tissues. From this analysis, it was concluded that XPO1 expression is elevated in PC compared to nonmalignant pancreatic tissue, reinforcing its putative oncogenic activity and potential as a therapeutic target.

Collectively, these patient based studies demonstrate that targeted inhibition of XPO1 using specific small molecule inhibitors (SIMs) has potential [15] for the treatment of PC. The first agent to be developed as a potent XPO1 inhibitor, Leptomycin B (LMB), was derived from yeast saccromyces as an anti-fungal compound [16]. LMB covalently binds to and modifies a critical amino acid, Cys528, on XPO1 rendering it ineffective as a nuclear protein exporter [17]. However, LMB displayed considerable toxicity, leading to its withdrawal after a Phase I clinical study and restricting its use to only an *in vitro* tool compound [18]. Since this failed trial, a number of newer derivatives of LMB have been developed as XPO1 inhibitors [19]. However, none of these newer agents have been clinically evaluated. Recently, Karyopharm Therapeutics has developed Selective Inhibitor of Nuclear Export (SINE) compounds, which are a novel class of XPO1 inhibitors being tested in human clinical trials [20-24]. These compounds bind covalently in a slow reversible fashion to cys528 in the XPO1 NES binding pocket. This binding inactivates XPO1 export activity [25]. Unlike LMB, the SINE compound currently in early stage clinical trials is orally bioavailable and well tolerated in patients (J Clin Oncol 32, 2014 (suppl 3; abstr 482)). Several studies have demonstrated that functional inhibition of XPO1 with SINE compounds locks TSPs in the nucleus allowing the re-activation of cell cycle checkpoints. Re-introduction of TSP activity in the nucleus results in cell cycle arrest and apoptosis of cancer cells and only reversible cell cycle arrest in normal cells. SINE compounds have broad activity against different tumor types with IC_{50}s typically in the low nanomolar range [26]. Recent proof of concept studies showed that SINE compounds can suppress PC cell proliferation and tumor growth in mice [27, 28] and induce minimal apoptosis in immortalized human pancreatic ductal epithelial cells (HPDE). Further, we demonstrated that SINE compounds can re-align additional novel tumor suppressors such as F-BOX family member FBW7 and prostate apoptosis response-4 (Par-4) [28, 27]. Based on these studies, SINE compounds were rapidly introduced into 34 different Phase I and Phase II trials for solid tumors and hematological malignancies (ClinicalTrials.Gov). These studies clearly show the superiority of SINE compounds compared to LMB and warrant further clinical investigation in patients with PC.

Combination Studies with SINE and Towards Clinical Application

With the recent FDA approval, a major fraction of patients with PC now receive GEM-nab-paclitaxel based therapies [29]. GEM is a pro-drug that enters cells by facilitated transport [30]. Once in the cytoplasm, it is phosphorylated by deoxycytidine kinase (dCK) to an active form [31]. Both GEM diphosphate (dFdCTP) and GEM triphosphate (dFdCTP) inhibit the processes required for DNA synthesis. On the other hand, nab-paclitaxel primarily works through targeting tubulin thereby abrogating mitotic spindle formation and integrity of the cells cytoskeleton [32]. Importantly, the gene encoding XPO1 is required for the higher order

maintenance of chromosome structure, spindle assembly and centrosome replication [33]. Building on this front, our gemcitabine and nab-paclitaxel resistant PCSC and their corresponding spheroid models, MiaPaCa-2 Gemcitabine-nab-paclitaxel resistant (MiaPaCa-2 GPR) and AsPC-1 Gemcitabine-nab-paclitaxel resistant (AsPC-1 GPR), demonstrate enhanced expression of XPO1 (unpublished work abstract in pancreatology 2013, 13, 2, e15). Interestingly, unlike parent cells that are responsive to SINE compounds ($IC_{50}\sim150$ nM), the GPR models are more resistant (two fold higher IC_{50}; unpublished work). During the course of our studies we observed that SINE compounds synergize with GEM [34] and GEM-nab-paclitaxel (unpublished work). Most importantly, GEM-nab-paclitaxel-SINE combination was effective in eliminating the GEM resistant spheroid models of PC that carry stem cell like (CSCs) features. Primary indicators point to the enhanced nuclear retention of important TSPs as the major mechanism of GEM-nab-paclitaxel-KPT enhanced anti-tumor activity. Nuclear retention of TSPs was associated with suppression of drug resistance and "stemness" markers. SINE compounds inhibit DNA damage repair and were shown in vitro and in vivo to synergize with chemotherapy and radiation treatment to induce DNA damage. Therefore, we predict that the standard of care agents in combination with SINE compounds will be synergistic and increase PC cell death. In view of the pre-clinical efficacy, the combination of GEM-nab-paclitaxel-SINE is now under evaluation in a Phase Ib/II clinical study for the treatment of metastatic PC (ClinicalTrials.gov Identifier: NCT02178436).

Conclusions

XPO1 is over-expressed in cancer and has been recognized as a druggable therapeutic target. However, the protein carries essential roles for the normal function of non-cancerous cells as well. Therefore, the clinical feasibility of any XPO1 targeted strategy has a number of hurdles. While molecular mechanism(s) of action of the first generation XPO1 inhibitor LMB were well defined, the drug proved highly toxic in preclinical models and was discontinued in the clinic, the primary reason being the incomplete understanding and validation of entire sets of pathways modulated by this master exporter. This is coupled with a lack of complete evaluation of the effects of XPO1 inhibition. As recently demonstrated by us and independent groups, in addition to its role in the regulation of cell cycle division, XPO1 inhibition interferes with important and complex processes such as miRNA processing [35], RNA translation and epithelial-to-mesenchymal transition [36, 37]. These findings indicate that additional pre-clinical studies in a panel of disease models is required to optimize novel XPO1 inhibitors for applications in cancer and other non oncology diseases. Major unanswered questions remain as to whether there are differences in cellular responses between cell types (cancer with aberrant genome vs. normal cells with normal genome versus precancerous lesions). Performing such studies in different cell and animal models will greatly facilitate the optimization of

XPO1 inhibitor therapies in the clinic, as well as in the design of novel strategies targeting nuclear transport [38]. These approaches will greatly enhance the clinical utilizations of SINE and related compounds to overcome therapy resistance in patients with PC.

Conflicting Interest

The authors had no conflicts of interest

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