Inhibition of nucleo-cytoplasmic shuttling through \textit{XPO1/CRM1}: a unique therapeutic approach for treatment of haematological and solid malignancies

Sneha S\textsuperscript{1}, Nagare R. P\textsuperscript{1}, Bindhya Sadhanandhan\textsuperscript{1}, Ramesh Shankar\textsuperscript{1}, Sidhanth Suresh, Manasa P, Krishna Priya, Trivadi S Ganesan\textsuperscript{1}, Manoj Garg\textsuperscript{1}

\textsuperscript{1}Department of Medical Oncology and Clinical Research, Cancer Institute (WIA), Chennai, Tamil Nadu, 600036, India

Correspondence: Manoj Garg
E-mail: manoj.garg@cancerinstitutewia.org or nuscsimg@gmail.com

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Cancer is one of the leading cause of morbidity and mortality worldwide. Regulated nucleo-cytoplasmic shuttling is very crucial for maintaining cellular homeostasis. Emerging evidence suggests that deregulation of the nucleo-cytoplasmic transport results in abnormal cell growth, cell cycle, apoptosis, tumor progression and drug resistance. Exportin-1 (also called as chromosome region maintenance 1) belongs to karyopherin-\(\beta\) superfamily and is the main mediator of nuclear export in several cell types. The \textit{XPO1}/\textit{CRM1} protein is overexpressed in liposarcoma, Ewing sarcoma, ovarian carcinoma, pancreatic cancer, hepatocellular carcinoma, lung carcinoma, osteosarcoma, gastric carcinoma, melanoma, glioma, acute myeloid leukemia, acute lymphocytic leukemia, chronic myeloid/lymphoid leukemia as well as multiple myeloma. Hot spot mutations were observed in many cancers. Higher levels of \textit{XPO1}/\textit{CRM1} are associated with poor prognosis, resistance to chemotherapy and recurrence in a large number of human malignancies. There are growing evidence that provided the foundation that inhibition of nuclear export by inhibiting nuclear export receptor (\textit{XPO1}) might be a potential targeted therapeutic approach for the treatment of human cancers in the clinic. In the present review, we will discuss the role of \textit{XPO1} in cancers and potential of selective inhibitors of nuclear export (\textit{XPO1} inhibitors) to restore the normal function of tumor suppressor and growth regulatory proteins by blocking their export. Selinexor (KPT-330) is an orally available, highly potent and is being tested in human phase-I/II clinical trials in both haematological and solid malignancies.

\textbf{Keywords:} Nucleo-cytoplasmic shuttling; exportin; SINE, selinexor; KPT-330; haematological malignancies; solid tumors; clinical trials; apoptosis; patient derived xenograft (PDX)

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Introduction

In eukaryotes, the nucleus is separated from the cytoplasm through nuclear envelope. Transport of specific proteins between the nucleus and cytoplasm is an important step for maintaining cell proliferation and apoptosis of normal and tumor tissues. Four important factors are involved in nuclear transport: (1) nucleoporins (2) RanGTPase (3) karyopherins (importins/exportins) (4) cargo proteins. Exportin 1 (XPO1; or chromosome region maintenance 1, CRM1) belongs to a member of the karyopherin-β superfamily. XPO1 is one of the well-known nuclear export receptor essential for trafficking of hundreds of cargo proteins and several RNA molecules from the nucleus to the cytoplasm [1-4]. XPO1 is required for recognizing and transporting cargo proteins through leucine-rich nuclear export signal (NES), which is dependent on the RanGTP/GDP axis [5,7]. RanGTP levels are very high in the nucleus due to the presence of RanGTP exchange factor (RanGEF, also known as RCC1) within the nucleus. RanGDP is residing in the cytoplasm due to the presence of GTPase-activating protein (RanGAP) within the cytoplasm (Figure 1A). Inside the nucleus, XPO1 bind to its cargo protein that contains leucine-rich NESs and forms a complex with RanGTP and leaves the nucleus through nuclear pore complex (NPC). In the cytoplasm, RanGTP undergoes hydrolysis by the RanGAP and is converted to RanGDP, which resulting segregation of the XPO1 complex to release the cargo into the cytoplasm [7-10] (Figure 1A). XPO1 cargo proteins include several tumor suppressors and growth-stimulatory proteins such as p53, p27kip1, p21cip1, p73, FOXO, STAT3, BRCA1, CDKN1A, RB1, IKB, APC, NPM1, topo2α and surviving [11-13] (Table 1).

Overexpression of XPO1 leads to mislocalization of key regulatory proteins and is associated with resistance to chemotherapy, poor prognosis and short survival in many human malignancies [14-16]. XPO1 hot mutations (E571) were found in approximately 4% of primary chronic lymphocytic leukemia (CLL) patients [17,18]. Interestingly, high frequency of XPO1 was observed in relapse CLL patient samples compared to untreated [19]. A high rate of XPO1 mutation (E571K) was also seen in primary mediastinal B-cell lymphoma (PMBL) [20]. XPO1 missense mutation has also been identified in esophageal squamous cell carcinoma [21] and thyroid cancer [22]. Functional significance of these mutations remains unknown. Also, we have analysed TCGA data and observed that XPO1 is either overexpressed or mutated in a number of haematological and solid tumors (Figure-2). We do not know the actual mechanisms responsible for overexpression of XPO1. Recently, there is an evidence suggests that the nuclear factor Y (NFY) and specificity protein 1 (Sp1) transcription factors may be responsible for XPO1 overexpression in cancer. Transcription of XPO1 is suppressed when nuclear p53 interferes with NFY function [23]. However, increased expression of XPO1 caused mislocalization of p53 in cancers.

Previously, small molecule inhibitors were used against XPO1 and many of these include Valtrate, Leptomycin B (LMB), goniotohalamin, anguinomycin, ratjadone, N-azolylacrylates, and CBS9106 [24-28] (Figure-3). These inhibitors formed irreversible binding with the cysteine residue (Cys528) in the NES-binding groove of XPO1 [26]. LMB is the first natural XPO1 inhibitor which restores functions of the several tumor suppressor proteins. LMB has been shown to suppress the cellular proliferation of human cancer cell lines in vitro as well as in murine xenograft models [26]. However, this drug had modest efficacy and severe toxicity (e.g. malaise, anorexia, vomiting, and nausea) in preclinical animal models, as well as in patients with refractory solid tumors in phase-I human clinical trial [29]. Later, CBS9106 was identified as an oral and reversible XPO1 inhibitor [23]. CBS9106 was effective in preclinical cancer models but have not entered into the clinical trials [25, 30]. Recently, a new class of orally bioavailable small molecules known as Selective Inhibitors of Nuclear Export (SINE) has been developed (Karyopharm Therapeutics Inc.). SINE selectively and reversibly binds to Cys528 residue located in the cargo-binding groove of XPO1. This inhibits XPO1 binding to its target cargo proteins (Figure-1B). SINE compound includes KPT-8602, selinexor (KPT-330), verdinexor (KPT-335), KPT-276, KPT-251, KPT-249, KPT-185 and KPT-176 (Figure-3). Among those, selinexor is the most advanced SINE and patients with hematologic and solid are treated with selinexor in phase-I/II clinical trials (http://www.clinicaltrials.gov). In the present review, we will discuss selective inhibitors of nuclear export (SINE) as a novel class of potential anti-tumor agents in different kind cancers and how these novel drugs may guide us for the treatment of cancer patient in the clinic.

Role of selective inhibitors of nuclear export (XPO1 inhibitors) in haematological malignancies

XPO1 inhibitors have been extensively studied as a new anticancer strategy for haematologic malignancies. Increased expression of XPO1 was correlated with poor survival in patients with AML [15]. KPT-185 decreased cell growth, induced G1 arrest and apoptosis as well as myeloid differentiation through p53-CEBA pathway in both primary AML samples and cell lines in liquid culture [31]. This study found that c-Kit and FLT3 were downregulated after KPT-185 treatment of AML cell line and primary samples [31]. We showed that KPT-330 in combination with standardAML induction therapy synergistically inhibited cell
growth of FLT3-ITD positive AML cell lines [32]. Nucleophosmin 1 (NPM1) is a nucleolar tumor suppressor phosphoprotein which moves between the nucleolus and cytoplasm through Ran-GTP complex and mediates p53 dependent apoptosis [33]. NPM1 frameshift mutations were observed in approximately 30% of patients with AML [32, 34]. NPM1 mutations disrupt tryptophans at C-terminus leading to XPO1 dependent transport of NPM1 in the cytoplasm [35].

Cytoplasmic localization of NPM1 has been shown to restore nuclear localization after treatment with XPO1 inhibitors (KPT-185). Nuclear localization of mutant NPM1 was associated with profound antileukemic activity in primary blasts obtained from patients with AML as well as in established AML cell lines. Interestingly, leukemic cells with wild-type NPM1 were also sensitive to XPO1

<table>
<thead>
<tr>
<th>Cargo proteins</th>
<th>Biological relevance of cargo protein</th>
<th>Function</th>
<th>Implicated cancer</th>
</tr>
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<tbody>
<tr>
<td>CDK1A (p21WAF1)</td>
<td>Involved in cell cycle arrest in G1 phase and plays a regulatory role in S phase DNA replication and DNA damage repair by inhibiting cyclin-CDK2.</td>
<td>Cell-cycle inhibitor</td>
<td>CML, breast and ovarian carcinoma</td>
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<tr>
<td>CDK1B (p27KIP1)</td>
<td>Blocks cell cycle in the G0/G1 phase upon differentiation signals or cellular stress.</td>
<td>Cell-cycle inhibitor</td>
<td>Breast, thyroid colon, esophageal, ovarian, carcinomas</td>
</tr>
<tr>
<td>P53</td>
<td>Acts as a tumor suppressor and trigger cell cycle arrest, apoptosis, senescence, DNA repair, DNA damage and change the metabolism depending on physiological conditions. Also, known as Guardian of the genome.</td>
<td>Tumor Suppressor</td>
<td>Neuroblastoma, retinoblastoma, liposarcoma, breast, colorectal, and ovarian carcinoma</td>
</tr>
<tr>
<td>FOXO1, FOXO3a, FOXO4, FOXO6</td>
<td>Regulate expression of target genes which triggers cellular proliferative, cell cycle, differentiation, and cell death.</td>
<td>Tumor Suppressor</td>
<td>Melanoma, glioblastoma, breast, prostate, and thyroid carcinoma</td>
</tr>
<tr>
<td>APC</td>
<td>Negative regulator of WNT signaling by rapid degradation of β-catenin.</td>
<td>Tumor Suppressor</td>
<td>Colorectal carcinoma</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>Accumulation of cells in the G1 phase of cell cycle.</td>
<td>Cell–cycle</td>
<td>Melanoma, CLL, and myelomas</td>
</tr>
<tr>
<td>NPM1</td>
<td>Nuclear protein implicated in ribosome biogenesis, centrosome duplication, protein chaperoning, histone assembly, cell proliferation, and regulation of tumor suppressor’s p53/TP53 and ARF.</td>
<td>Tumor Suppressor</td>
<td>AML, CLL, and breast carcinoma</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11), resulting in a fusion gene known as BCR-ABL1 and results in constitutive activation of tyrosine kinase activity.</td>
<td>Tyrosine Kinase</td>
<td>CML</td>
</tr>
<tr>
<td>RASSF2</td>
<td>Interacts with KRAS and promotes cell-cycle arrest and apoptosis.</td>
<td>Tumor Suppressor</td>
<td>Thyroid and nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>STAT3</td>
<td>Acts as an activator in response to cytokine and growth factors. Plays a key role in cellular processes such as cell growth, cell differentiation, and cell death.</td>
<td>Transcription factor</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Survivin</td>
<td>Negative regulator of an X-linked inhibitor of apoptosis (XIAP) that prevent apoptotic cell death. Important for chromosomal segregation, alignment during mitosis and cytokinesis.</td>
<td>Inhibitors of apoptosis</td>
<td>Breast, cervix, oral and oropharyngeal carcinoma</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Regulate transcription, DNA repair of double-stranded breaks, and recombination to maintain genomic stability.</td>
<td>Tumor Suppressor</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>iκB</td>
<td>Activate NF-κB signaling and induce apoptosis of cancer cells.</td>
<td>Tumor Suppressor</td>
<td>Hodgkin’s disease, ALL, thyroid, pancreas, breast and ovarian carcinoma</td>
</tr>
<tr>
<td>RB1</td>
<td>Inhibits G1 to S phase of the cell cycle by targeting transcription factors E2F.</td>
<td>Tumor Suppressor</td>
<td>Retinoblastoma, NSCLC, AIDS-related Burkitt’s Lymphoma</td>
</tr>
<tr>
<td>RUNX3</td>
<td>Inhibits colon carcinoma by attenuating WNT signaling and enhancing TGF-β.</td>
<td>Tumor Suppressor</td>
<td>Breast, prostate, Colon, pancreatic, gastric, laryngeal carcinoma</td>
</tr>
<tr>
<td>Topoisomerase 1</td>
<td>Enzyme that catalyzes the relaxation of super-coiled DNA during replication, transcription, recombination, and chromosome condensation.</td>
<td>Relaxation of chromatin</td>
<td>Anaplastic astrocytoma and neuroblastoma</td>
</tr>
<tr>
<td>CIP2A</td>
<td>Inhibits tumor suppressor activity of PP2A and stabilizes the oncogenic activity of c-Myc by preventing proteolytic degradation.</td>
<td>Oncogenic protein</td>
<td>CML, breast, and ovarian tumors</td>
</tr>
<tr>
<td>WEE1</td>
<td>Involved in mitotic arrest (G2-M) by inhibiting CDK1 activity.</td>
<td>Tumor Suppressor</td>
<td>Breast, prostate, lung and colon carcinoma</td>
</tr>
<tr>
<td>Vitamin D3 upregulated protein 1 (VDUP1)</td>
<td>Involved in nuclear export of hypoxia-inducible factor-1α and degrade HEF-1α in the cytoplasm through the proteasomal pathway.</td>
<td>Tumor Suppressor</td>
<td>Breast, cervical, lung, colon, and kidney carcinoma</td>
</tr>
<tr>
<td>Transducer ofErbB-2 (TOB)</td>
<td>Induces arrest at G1 –S phase, up-regulates cyclin-dependent kinase inhibitor p27, and down-regulates the anti-apoptotic proteins Bcl-2 and Bcl-XL.</td>
<td>Tumor Suppressor</td>
<td>Breast carcinoma</td>
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Table 1. Cargo proteins exported from nucleus to cytoplasm through XPO1
inhibitors. These studies clearly proved that p53 was responsible for the antileukemic effect of XPO1 inhibitors which was independent of NPM1 mutation status. MDM2 is overexpressed in AML and is involved in p53 degradation. Nutlin-3a is a specific and potent inhibitor of MDM2 and found to restore expression of p53 in nucleus and cytoplasm. KPT-185 and Nutlin-3a combination showed increased apoptosis of AML cells by increasing nuclear localization of p53. On the contrary, Etchin et al have reported that KPT-185 exhibits antileukemic activity independent p53 in AML cells [36]. The patients with AML achieve remission with standard chemotherapy, but the majority of patients frequently relapse due to the incomplete targeting of leukemia-initiating cells (LICs). Most recently, KPT-8602 (a second generation oral XPO1 inhibitor) was synthesized and showed better tolerability than KPT-330. KPT-8602 has been shown to be very effective in eliminating leukemic blasts and LICs in AML patient-derived xenograft (PDX) models [37]. Most importantly, XPO1 inhibitors had a negligible cytotoxic effect on normal human haematopoietic stem and progenitor cells.

T cell acute lymphoblastic leukemia (T-ALL) is a lethal disease in 50-70% of adult [38]. KPT-185 and KPT-330 showed a dramatic increase in the apoptosis in a panel of 14 T-ALL cell lines having the different genomic abnormality in a dose-dependent fashion. NSG mice were injected with MOLT4 cells expressing luciferase for the development of leukemia [39]. KPT-251 and KPT-330 treatment showed striking anti-leukemic activity in these mice while sparing normal haematopoietic cells. Walker and colleague found that KPT-185 and KPT-330 strongly triggered apoptosis and impaired the clonogenic potential in Philadelphia-positive ALL (Ph+ ALL) and chronic myeloid leukemia blast crisis (CML-BC) [40]. This study provided evidence that combination of KPT-330 with imatinib led to synergistic effects and might be used in the patients with TKI resistance [40]. The most likely mechanism of KPT-330 in Ph+ ALL was the nuclear accumulation of SET and CIP2A and restoration of various tumor suppressors such as PP2A, p53, p21, and FOXO3a [40]. KPT-8602 showed potent anti-leukemic activity against T-ALL mouse model as well as in two PDX ALL models [41]. Phase-I clinical trials were initiated using selinexor in combination with fludarabine and cytarabine for the pediatric patients with relapsed/refractory leukemia. In this study 7 of 15 patients (47%) achieved a complete response or complete response with incomplete count recovery [42].

CLL is a lymphoid malignancy and is characterized by immunophenotype of CD19+CD5−CD23−sIgdim expressing clonal mature B cells [43]. Next generation sequencing of untreated and relapsed/refractory CLL showed a high incidence of concurrent mutations that mostly affect the TP53, ATM, SP3B1 and XPO1 genes [19]. Mutations in these genes have a poor prognosis [84-91]. Lapalombella et al. evaluated the efficacy of SINE (KPT-185, KPT-251) in CLL. In their study, they observed that KPT-185 induced
significant cell death in human CLL cells comparing to normal B cells from healthy donors. Eμ-TCL1-SCID mice develop the disease, which is very similar to that of CLL patients. Interestingly, KPT-251 treatment of these transgenic mice reported strikingly longer disease-free survival than mice receiving fludarabine. The effect of KPT-185 and KPT-251 was due to a restoration of nuclear localization of the TSPs such as FOXO, IκB, and p53 both in vitro and in vivo [44]. In another study, they found that selinexor was effective in ibrutinib resistant CLL cells and also works synergistically in combination with ibrutinib in CLL [43]. Most recently, next generation XPO1 inhibitor (KPT-8602) showed greater anticancer effect due to the wider therapeutic window, more efficacy, and negligible off-target effect and in CLL [45].

Mantle cell lymphoma (MCL) has grave prognosis due to chemo-resistance. Recent studies showed that XPO1 highly expressed in MCL patients samples than normal B-cell controls [46, 47]. KPT-185 and KPT-276 were found to be associated with significant increase in the apoptosis in MCL cell lines and mouse models in a p53 dependent as well as p53 independent manner. Also, it was observed that these drugs downregulated c-myc and NFκB [46, 47]. Sequencing of PMBL samples showed recurrent hot-mutation (E571K) of XPO1. PMBL cell lines are sensitive to the SINE compound [20]. Verdinexor (KPT-335) has been approved by Food and Drug Administration for the treatment of non-Hodgkin lymphoma (NHL), osteosarcoma, mast cell tumor and melanoma in canines [48]. This study used oral doses of verdinexor (1.75 mg/kg two times a week) and showed significant response with the manageable toxicity [48]. Currently, verdinexor is in phase II trials in dogs either with primary or recurring lymphoma. Thirty-two pre-treated patients with refractory lymphoma were treated with selinexor in phase-I clinical trials and showed that drug was effective in all these patients.

XPO1 expression was found to be very higher in bortezomib-resistant multiple myeloma (MM). KPT-185,
KPT-330 have shown to induce cytotoxicity by inducing the nuclear accumulation of p53, IκB, FOXO3a, p21, PP2A and impair osteoclastogenesis by blocking RANKL mediated activation of NFκB, and NKFAT1c. The combination of KPT-185 with bortezomib showed synergistic effect on growth arrest in MM cells. However, no synergy was observed with KPT-276 when combined with other chemotherapeutic agents such as bortezomib, dexamethasone and melphalan in a different studies \[49\]. Interestingly, this study found that SINEs suppressed the expression of cell division cycle 25 homolog A, bromodomain-containing protein 4 (BRD4) and c-Myc \[49\]. Moreover, combined treatment with selinexor and carfilzomib caused significant apoptosis of myeloma cells and primary plasma cells derived from relapsing/refractory myeloma patients \[50\]. The combination of selinexor and doxorubicin was highly effective against acquired drug resistance in \textit{ex vivo} samples obtained from patients with either relapsed or refractory myeloma as well as \textit{in vivo} xenograft models of myeloma. Their study has shown that combination preventing nuclear export of \textit{TOP2A} and promoted DNA damage which resulted in apoptosis of resistant cells. Phase-I clinical trials have been initiated with selinexor and doxorubicin (ClinicalTrials.gov NCT02186834, NCT02336815, NCT02343042).

**Role of XPO1 and selective inhibitors of nuclear export (SINE) in sarcomas and solid tumors**

Liposarcoma is the most common type of soft tissue sarcoma with a high recurrence rate and less responsive to available therapies \[51, 52\]. To date, complete surgical is the best therapeutic option. Most of the patients have tumors recurrence and metastasis after surgery, which is associated with a high mortality rate \[51, 53\]. Recently, we showed that \textit{XPO1} protein was overexpressed in different histological subtypes of liposarcoma in patient samples than benign lipoma as well as normal fat \[52\]. Selinexor treatment showed decreased cellular growth as well as induced cell cycle arrest and apoptosis of liposarcoma cells \textit{in vitro}, which was independent of \textit{p53} expression or mutational status \[52, 54\]. Further, oral administration of selinexor led to significant decrease in the tumor growth and increased in apoptosis of liposarcoma cells in a xenograft murine model which was associated with decreased staining of \textit{Ki}-67, CD31 and increased staining of TUNEL \[52, 54\]. Interestingly, selinexor
treatment increased the expression of insulin-like growth factor binding protein 5 (IGFBP5) in liposarcoma cells and suppressed the phosphorylation of both IGF-1R and AKT in an IGF-1 dependent manner \cite{52}. Growth inhibitory effect of selinexor treatment was partially rescued by silencing of IGFBP5 in liposarcoma cells and concluded that IGFBP5 act as tumor suppressor in liposarcoma. Selinexor decreased the expression oncogenic aurora kinase A and B in liposarcoma cells. Currently, phase-I/II study using selinexor in patients with sarcoma including well-differentiated/dedifferentiated liposarcoma, myxoid liposarcoma, leiomyosarcoma and synovial sarcoma reported strong anti-tumor activity at a well-tolerated dose of 35 mg/m\textsuperscript{2} (approximately 60 mg flat dose) \cite{55}. In this report, selinexor was found to be very effective in 78% of the patients with liposarcoma \cite{55}. Altogether, preclinical and phase-IIB clinical trials strongly suggest that inhibition of XPO1 using SINE compounds might be an attractive approach for treatment of liposarcoma, a disease for which current treatment options are very limited.

Ewing sarcoma (EWS) is an aggressive bone malignancy is characterized by chromosomal translocations involving EWS gene and ETS transcription factor \cite{56, 57}. EWS-FLI1 binds to the promoter of insulin-like growth factor binding protein 3 (IGFBP3) and transcriptionally suppresses the expression of IGFBP3 and resulted in an increased IGF-1 signaling \cite{58}. In the recent study from our group, it was shown that XPO1 is highly expressed in EWS \cite{59}. Interestingly, EWS-FLI1 was found to be another target of XPO1 in EWS cells and selinexor treatment dramatically markedly suppressed the expression of the EWS-FLI1 fusion protein and increased the expression of the IGFBP3, which in turn inhibits IGF-1 signaling. The combination of selinexor and linsitinib (IGF-IR inhibitor), displayed synergistic anti-proliferation/antitumor activity on EWS cells both in vitro and in vivo \cite{59}.

HCC is the third most common cause of cancer-related deaths worldwide \cite{60}. Sorafenib is an FDA approved drug for treatment of patients with HCC but most patients either did not respond to sorafenib therapy or develop resistance\cite{61}. Meta-analysis confirmed overexpression XPO1 in HCC compared to normal liver tissue. XPO1 inhibitors (LMB, KPT-276, and KPT-330) have been shown to inhibit the viability and induced apoptosis of HCC cells in liquid culture as well in HCC xenograft model \cite{62}. KPT-330 treatment was shown to be associated with the increased expression of the tumor suppressor proteins p53 and p27 and reduced expression of HCC promoting proteins, c-Myc and c-Met \cite{62}.

Overexpression of epidermal growth factor receptor (EGFR) is observed in approximately 60% of the patients with metastatic NSCLC and usually treated with EGFR-tyrosine kinase inhibitors (TKI, gefitinib or erlotinib) \cite{63, 64}. NSCLC patients acquired resistance to EGFR-TKI therapy due to mutations in EGFR (exon 20), KRAS and PIK3CA \cite{65}. Sun et al. reported that EGFR-TKI resistant NSCLC cells were highly sensitive to selinexor treatment in vitro as well as in murine xenograft model. The anti-proliferative effect of selinexor against p53 wild type NSCLC cells was mainly due restoration p53 expression inside the nucleus. Interestingly, selinexor displayed promising antitumor activity against several NSCLC with or without their mutational status of p53, RAS, EGFR, PI3K, phosphatase, and tensin homologue \cite{66}. The effectiveness of selinexor in NSCLC was mainly due to the restoration of tumor suppressive function of p73 because p73-knockdown cells were resistant to growth and apoptosis after treatment with selinexor \cite{66}. Selinexor displayed synergistic anti-proliferative activity in combination with cisplatin. Another report also confirmed that XPO1 inhibitors (KPT-185 and KPT-276) have an anti-cancer effect in NSCLC preclinical models \cite{67}. Most recently, nuclear export receptor XPO1 has been identified as a druggable target in KRAS-mutant NSCLC using short interfering RNA screening \cite{68}. Silencing of XPO1 showed a marked decrease in the viability of KRAS-mutant cells. Additionally, XPO1 inhibitors (KPT-185 and KPT-330) verified that IC50 value for KRAS-mutant cell lines was significantly lower than KRAS-wild type cells. Further, XPO1 inhibitor selinexor (KPT-330) confirmed greater selectivity and anti-tumor activity towards KRAS-mutant in xenograft murine models (KRAS-wild-type and KRAS-mutant NSCLC cells), patient-derived xenograft model with KRASG12D and genetically engineered mouse (KrasLSL-G12D, p53fl/fl) \cite{68}. Selinexor treatment showed stable disease in patients with KRAS-mutation (44%) versus the patients with wild-type KRAS in phase-I clinical trials in colon cancer \cite{69}. This should be further evaluated in phase-I/II clinical trial for the lung carcinoma.

Robust cytoplasmic expression of survivin has been demonstrated as an independent risk factor correlated with worse prognosis in breast carcinoma. However, nuclear expression of survivin has been validated as a marker of favorable outcome. Cytoplasmic survivin plays an important role in tumor progression by stabilizing X-linked inhibitor of apoptosis (XIAP). Transport of survivin from the nucleus to the cytoplasm is mediated through the XPO1–Ran-GTP complex. XPO1 inhibitors (KPT-251, KPT-276, and KPT-330) displayed a high rate of apoptosis in breast cancer cell line \cite{70}. Also, XPO1 inhibitors have been shown to decrease in cytosolic survivin protein with an increase in nuclear survivin protein in these cells. In this study, KPT-276
suppresses the transactivation of STAT3 which in turn inhibit transcription of survivin as one of the mechanisms to deplete survivin protein [70]. Selinexor (KPT-330) displayed a significant reduction in the growth of breast tumor in xenograft. This has been shown to be correlated with increased nuclear localization of survivin protein and decreased nuclear location of STAT3.

Pancreatic carcinoma has a grave prognosis with a high mortality rate. KPT-185 was shown to export prostate apoptosis response-4 (PAR-4) to nucleus to get phosphorylated. This activated PAR-4 caused apoptosis of pancreatic cell lines [71]. Selinexor and gemcitabine increased p27, decreased survivin, inhibited accumulation of DNA repair proteins and promoted apoptosis.

In renal cell carcinoma (RCC), KPT-251 was shown to have potent antitumor activity compared to sorafenib in murine xenografts. Anti-tumor activity of KPT-185 was associated with nuclear localization of p53 and p21 to in RCC [72].

Conclusion

Nucleo-cytoplasmic shuttling is a highly-regulated process and has been observed to play an important role in the pathogenesis of several diseases including cancers. This process is required for maintaining the balance between cell survival and death inside the cell. XPO1/CRM1 is one of the most important proteins involved in nuclear export of hundreds of cargoes. XPO1 is highly overexpressed or mutated in human malignancies. This has led to the development of new therapeutic strategies to target this phenomenon. In last decade, a large number of targeted small molecule inhibitors of nuclear export were developed and synthesized (also termed as SINE) and checked for their anti-tumor activity in a variety of cancers both in vitro and in vivo. Now, these drugs are entering in clinical trials for their efficacy in different types of haematological and solid malignancies. So far, selinexor is the most advanced oral, reversible, unique class of targeted agents for several types of haematological and solid malignancies. In future, development of more advanced inhibitors with low cytotoxicity will pave the way.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations


Author Contributions

M.G. conceived the design of review and wrote the manuscript. S.S., M.G. generated the figures, R.N., B.S., R.S., M.P., K.P., S.S., M.G. prepared table, perform literature search, collect data from TCGA and arranged the references. T.S.G., critically proof-read, provide scientific suggestions. All authors read and approved the final manuscript.

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