REVIEW

Promising SINEs for Embargoing Nuclear–Cytoplasmic Export as an Anticancer Strategy

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ABSTRACT In cancer cells, the nuclear–cytoplasmic transport machinery is frequently disrupted, resulting in mislocalization and loss of function for many key regulatory proteins. In this review, the mechanisms by which tumor cells co-opt the nuclear transport machinery to facilitate carcinogenesis, cell survival, drug resistance, and tumor progression will be elucidated, with a particular focus on the role of the nuclear–cytoplasmic export protein. The recent development of a new generation of selective inhibitors of nuclear export (XPO1 antagonists) and how these novel anticancer drugs may bring us closer to the implementation of this therapeutic strategy in the clinic will be discussed.

Significance: The nuclear transport mechanism is dysregulated in many malignancies and is associated with dysfunction of many regulatory proteins. Targeting this mechanism as an anticancer strategy has been compelling, and novel agents that selectively inhibit the nuclear export pathway have demonstrated preliminary evidence of clinical efficacy with an acceptable safety profile. *Cancer Discov; 4(5); 527–37. ©2014 AACR.*

INTRODUCTION

Normal cellular function is dependent upon the appropriate localization of proteins within specific intracellular compartments (1). There is growing evidence to suggest that the nuclear–cytoplasmic transport machinery is frequently disrupted by cancer cells, and that the subsequent mislocalization of key regulatory proteins facilitates carcinogenesis, cell survival, drug resistance, and tumor progression (2, 3). In this review, we will discuss the mechanisms of nuclear transport dysfunction in tumor cells, with a particular focus on the nuclear export apparatus as a therapeutic target, and how the recent emergence of a new generation of small-molecule selective inhibitors of nuclear export (SINE), specifically exportin 1 (XPO1) inhibitors, may provide a means to implement this therapeutic strategy in cancer treatment.

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NUCLEAR–CYTOPLASMIC TRANSPORT MECHANISMS

The Nuclear Pore Complex Is a Gateway between the Nucleus and Cytoplasm

In the eukaryotic cell, critical cellular processes, such as DNA synthesis, RNA transcription and translation, and protein processing, are carried out within distinct intracellular compartments (1). Small molecules (<40 kD) can passively diffuse across different compartments, but larger molecules, including most proteins and RNAs, require transport by signal-dependent and energy-dependent mechanisms (2). The site of active transport between the nucleus and the cytoplasm occurs at the nuclear pore complex (NPC), which is composed of basic building blocks called nucleoporin. NPC is a large macromolecular assembly that is embedded in the nuclear envelope and mediates the exchange of >40-kD molecules that pass between the nuclear and cytoplasmic compartments (3). Nuclear transport processes are highly regulated, and there is growing evidence to suggest that the ability to selectively modulate the geographic location of macromolecules within the cell is a key means to modulate cellular function (1, 3).

Nuclear–Cytoplasmic Transport Receptors and Signals

The majority of nuclear–cytoplasmic transport receptors belong to the karyopherin β family of proteins and act as carrier molecules that facilitate transit into (importins) and out

of (exportins) the nucleus via the NPC (4). Each karyopherin transport receptor recognizes a unique group of cargo proteins or RNA by the presence of either a nuclear localization signal (NLS) or a nuclear export signal (NES) in the amino acid sequence of the cargo protein, which directs them in and out of the nucleus, respectively (4). Three classes of nuclear–cytoplasmic transport signals have hitherto been identified: (i) the classical basic amino acid NLS sequences recognized by a heterodimer composed of importins α and β (5); (ii) a more complex NLS possessing N-terminal hydrophobic/basic motif and C-terminal RX2-5PY motifs found in karyopherin β cargo proteins, which directly binds to their specific carriers (6); and (iii) a hydrophobic leucine-rich NES recognized by the ubiquitous transport receptor chromosome maintenance protein 1 (CRM1, also known as XPO1; refs. 7, 8). Karyopherin β proteins also bind weakly to phenylalanine–glycine (FG) repeats in nucleoporins, thus targeting karyopherin β-cargo complexes to the NPC for translocation. At present, there are at least 19 karyopherin proteins identified in humans (9).

Current evidence suggests that nuclear–cytoplasmic export is mainly regulated by XPO1 in humans (3). XPO1 mediates the nuclear export of a small subset of RNAs, as well as over 200 eukaryotic proteins that possess a canonical hydrophobic leucine-rich NES (9). In addition, XPO1 also seems to regulate the nuclear export of some proteins [e.g., forkhead O transcription factor 3a (FOXO3a) and histone deacetylases] that form complexes with adapter molecules containing an NES, such as 14-3-3 proteins, a family of basic proteins with diverse physiologic functions that are also implicated in the nuclear cytoplasmic shuttling of proteins (3, 10, 11).

Nuclear Export Dynamics

The direction of transport across the NPC is determined by the concentration gradient of the small GTPase Ran. Levels of RanGTP in the nucleus are approximately 100-fold greater than those in the cytoplasm due to the location of the RanGTP exchange factor (RanGEF or RCC1) within the nucleus. Conversely, in the cytoplasm, the majority of Ran is in the GDP-bound form due to the cytoplasmic localization of (i) its GTPase-activating protein (RanGAP), (ii) the RanGTP-binding protein RanBP1, and (iii) homologous RanBP1 domains in cytoplasmic nucleoporin NUP358 (also known as RanBP2; refs. 3, 12). Once XPO1 is bound to its cargo, it forms a complex with RanGTP and exits the nucleus via the NPC (3). Upon leaving the nucleus, RanGTP undergoes hydrolysis by RanGAP and is converted to RanGDP, promoting dissociation of the XPO1 complex, and the cargo is subsequently released into the cytoplasm. Hence, interactions between transport receptors and the Ran GTPase control bidirectional transport, as translocation is dependent on the different nucleotide-bound states of Ran in the nucleus versus the cytoplasm (13, 14). A summary of the nuclear export pathway is depicted in Fig. 1.

XPO1-MEDIATED EXPORT AND CANCER

Dysregulation of growth-regulatory proteins (GRP) and functional inactivation of tumor-suppressor proteins are hallmarks of oncogenesis (15). Given that many tumor-suppressor proteins and GRPs perform their antineoplastic functions within the nucleus, any mechanism that could potentially enhance their nuclear export and cytoplasmic sequestration would effectively result in their functional inactivation, leading to a protumorigenic cellular state. Known examples of tumor-suppressor proteins and GRP sequestration within the cytoplasm of cancer cells include BRCA1 and FOXO proteins in breast cancer; RUNX3 in gastric cancer; p53 in breast, ovarian, and colorectal cancers, retinoblastoma, and neuroblastoma; adenomatosis polyposis coli (APC) or β-catenin in colon cancer; and BCR–ABL in chronic myelogenous leukemia (CML; refs. 2, 3, 16–21). The frequent observation of molecular localization signal alterations and the cytoplasmic mislocalization of these regulatory factors are consistent with functional linkage of nuclear export to cancer.

Overexpression and Alteration of XPO1-Mediated Export in Cancer

Increased expression of XPO1 has been observed and correlated with poor prognosis or resistance to chemotherapy in a number of solid (ovarian, pancreatic, brain, and cervical cancers, osteosarcoma, and melanoma) and hematologic malignancies [acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), multiple myeloma, and lymphoma; refs. 22–27]. Importantly, this increased expression of XPO1 seems to have functional significance in cancer cells. A study by van der Watt and colleagues (26) demonstrated that XPO1 is overexpressed in cervical cancer tissue and cell lines. In these tissue/cell lines, the inhibition of XPO1 significantly reduced cell proliferation and induced apoptosis, while sparing the noncancer cells. The exact mechanisms driving overexpression of XPO1 are unknown, but recent evidence suggests that the nuclear factor Y (NFY) and specificity protein 1 (Sp1) transcription factors may be responsible for XPO1 overexpression in cancer, and that *XPO1* transcription is inhibited when p53 interferes with NFY function following DNA damage (28). Given that many tumor-suppressor proteins and GRPs are exported by XPO1 in various cancers (Table 1), upregulation of XPO1-mediated export via XPO1 overexpression may be a common mechanism of enhancing nuclear export in cancer (2, 29).

Cancerous inhibitor of PP2A (CIP2A) is an oncoprotein that inhibits protein serine/threonine phosphatase 2A (PP2A), a protein distributed within the nucleus and cytoplasm that controls the phosphorylation of numerous proteins involved in cell signaling and regulates cell growth and apoptosis, and has recently been identified as a substrate of the XPO1 export pathway (30). CIP2A also binds directly to c-Myc and inhibits PP2A activity toward the c-Myc serine (Ser) 62 residue, thus preventing proteolytic degradation of c-Myc (31). CIP2A has also been shown to transform human cells when overexpressed and to promote anchorage-independent cell growth and *in vivo* tumor formation (31). CIP2A overexpression has been observed in bladder, tongue, hepatocellular, and colon cancers, non–small cell lung carcinoma, head and neck squamous cell carcinoma, and CML (31, 32). Cytoplasmic overexpression of CIP2A has also been associated with higher-grade, advanced-stage, and poorer outcomes in serous ovarian cancer (33).

In addition to nuclear transport, XPO1 is also involved in the regulation of mitotic checkpoints, spindle assembly, and postmitotic nuclear envelope reassembly (34). XPO1 has been shown to localize to kinetochores, where it forms a complex that is essential for RanGTP-dependent recruitment

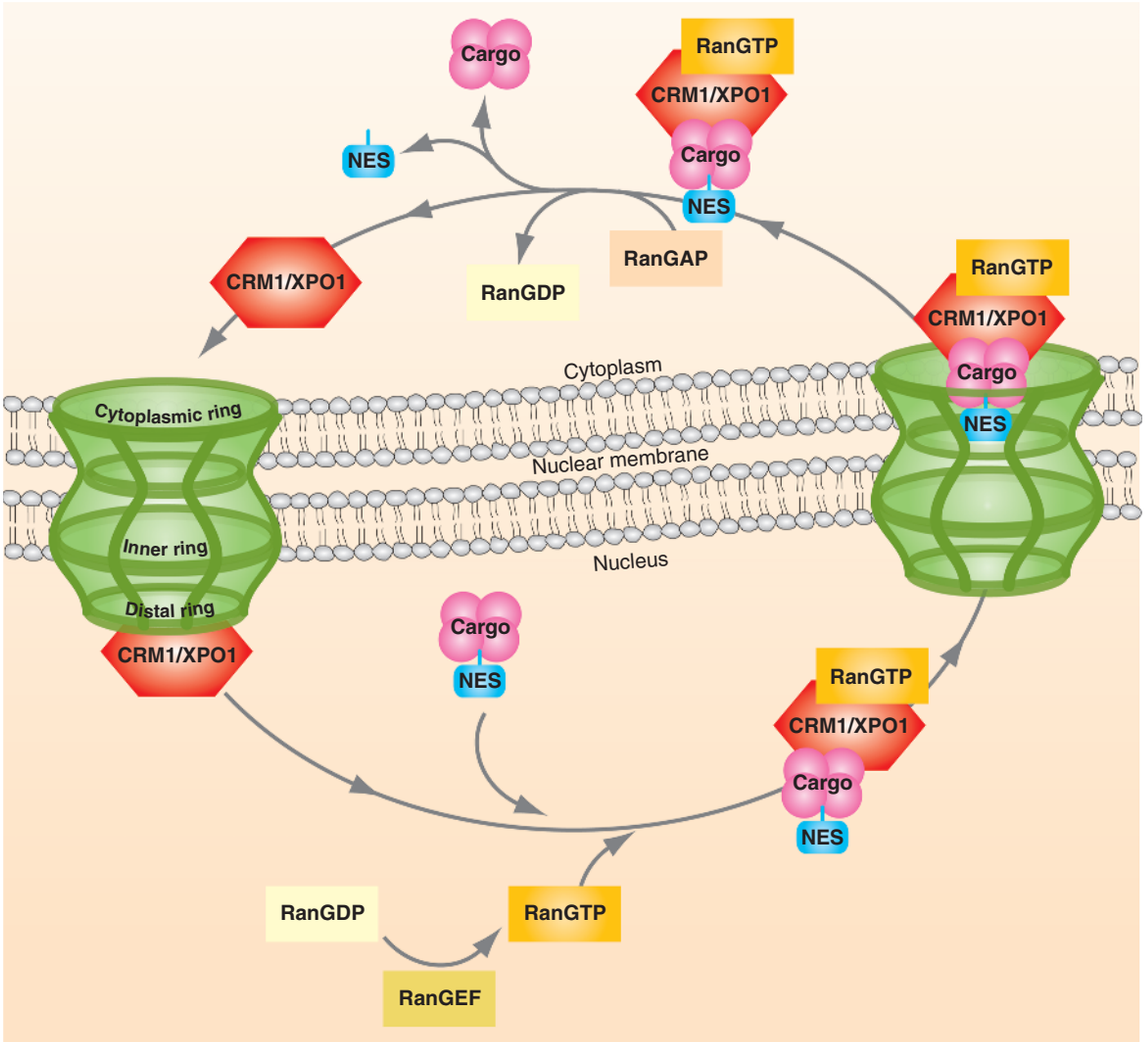

Figure 1. Situated in the nuclear membrane, the NPC (green) is composed of multiple rings held together in a basket configuration that measures 200 nm in length. The complex is open-ended at the nucleus and the cytoplasm and traffics proteins between them. Cargo proteins containing an NES bind to CRM1 and RanGTP in the nucleus, whereupon they are shuttled to the cytoplasm through the NPC (right). Hydrolysis of RanGTP to RanGDP by RanGAP in the cytoplasm dissociates the complex and XPO1 (CRM1) is free to be transported back through an NPC into the nucleus where the process resumes.

of RanGAP and RanBP2 to kinetochores (34). Furthermore, inhibition of XPO1 by the XPO1 inhibitor Leptomycin-B has been shown to disrupt mitotic progression and chromosome segregation at anaphase (34). Hence, XPO1 plays a critical role in mitotic spindle assembly and function and may, therefore, be upregulated to facilitate a dual role of enhancing cell proliferation while exporting tumor-suppressor proteins that have growth-inhibitory and apoptotic functions in tumors. The p53 tumor-suppressor protein regulates multiple signaling pathways to induce cell-cycle arrest for DNA damage repair to maintain genomic integrity and, if necessary, to induce apoptosis (35). It is estimated that about 50% of malignant tumors harbor mutations or deletions in the *TP53* gene (36). In addition, p53 function is also crucially dependent on its subcellular nuclear localization, which is primarily regulated by the XPO1-mediated nuclear export pathway (37, 38). Both p53 and XPO1 seem to be involved in a reciprocal regulatory loop where, as described earlier, nuclear p53 can repress XPO1 transcription by interfering with NFY function (28), while increased activity of XPO1 can cause p53 mislocalization and dysfunction in cancers (39). Cytoplasmic sequestration of wild-type p53 has been reported in a variety of tumors, including breast, colorectal, and hepatocellular carcinomas (40).

Finally, mutations in a highly conserved region of *XPO1* have been observed in CLL, although the functional significance of this remains unknown (41).

Activation of Oncogenic Pathways and XPO1-Mediated Nuclear Export

Cytoplasmic mislocalization of tumor-suppressor proteins and GRPs can also be induced by activation of oncogenic pathways within cells. The PI3K–AKT oncogenic signaling pathway is activated in many human epithelial cancers,

Table 1. Tumor-suppressor proteins, cell-cycle inhibitors, transcription factors, and drug targets that undergo XPO1-mediated nuclear export

Protein	Role	Brief description of function	Tumors in which XPO-mediated nuclear export may be functionally relevant
p53	Tumor suppressor	Guardian of the genome: induces cell-cycle arrest, activates DNA repair proteins to maintain genomic integrity and, if necessary, initiates apoptosis following DNA damage.	Colorectal, breast, and ovarian cancer, retinoblastomas, and neuroblastoma
APC	Tumor suppressor	Inhibits WNT signaling by targeting β-catenin for proteasome-mediated degradation in the cytoplasm.	Colorectal cancer
Activating transcription factor 2 (ATF2)	Transcription factors	Member of the leucine zipper family of transcription factors that regulates transcription of genes involved in antiapoptosis, cell growth, and DNA damage response.	Melanoma, prostate cancer
BCR–ABL	Tyrosine kinase	Constitutively active BCR–ABL tyrosine kinase. Caused by reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11), resulting in the formation of the *BCR–ABL* fusion gene.	CML
Bcl2-related ovarian killer protein (BOK)	Proapoptotic protein	Exerts proapoptotic function through the mitochondrial pathway.	Breast and cervical cancer cell lines
BRCA1	Tumor suppressor	Regulates the DNA damage response and repair functions in the nucleus and at centrosomes to inhibit centrosome duplication after DNA damage.	Breast cancer
CIP2A	Oncogenic protein	Inhibits protein PP2A and stabilizes the oncogenic transcription factor c-Myc.	Head and neck and colon cancer
CDK inhibitor 1 (p21, CIP1)	Cell-cycle inhibitor	Regulates cell-cycle progression from G_1-phase by inhibiting the activity of cyclin-CDK2 or cyclin-CDK1 complexes.	Ovarian and breast cancer and CML
CDK inhibitor 1B (p27, KIP1)	Cell-cycle inhibitor	Inhibits cyclin E/CDK2, thus blocking G_1–S phase transition.	Ovarian, esophageal, thyroid, colon, and breast carcinomas
ERK	Oncogenic protein	Cytoplasmic localization of activated ERK1/2 is required for its antiapoptotic activity.	Melanoma
ER-α	Prevents S-phase entry	Nuclear export of ER-α into the cytoplasm is required for estradiol-dependent activation of DNA synthesis and entry into S-phase of the cell cycle.	Breast cancer
FOXO transcription factors (FOXO1, FOXO3a, FOXO4 and FOXO6)	Tumor suppressor	Negative regulators of cell proliferation, cell survival, and cell-cycle progression.	Breast, prostate, and thyroid cancer, glioblastoma, and melanoma
Galectin-3	Regulator of proliferation/apoptosis	Multifunctional protein that regulates cell proliferation and can induce apoptosis in cancer cells.	Thyroid, prostate, and breast cancer
HSP90	Molecular chaperone	HSP90 chaperone machinery is required to prevent mutated and overexpressed oncoproteins from misfolding and degradation in cancer cells.	Breast cancer
Inl1/hSNF5	Tumor suppressor	Component of the chromatin remodeling SWI/SNF complex whose tumor-suppressor functions include inducing G_1 arrest and controlling the mitotic spindle checkpoint. Deleted in malignant rhabdoid tumors.	Malignant rhabdoid tumors

(*continued*)

Table 1. Tumor-suppressor proteins, cell-cycle inhibitors, transcription factors, and drug targets that undergo XPO1-mediated nuclear export (*Continued*)

Protein	Role	Brief description of function	Tumors in which XPO-mediated nuclear export may be functionally relevant
Moesin–ezrin–radixin-like protein (Merlin/NF2)	Tumor suppressor	Neurofibromatosis type 2 (*NF2*) gene encodes the tumor-suppressor protein Merlin, which is an ERM (ezrin, radixin, and moesin) protein family member that regulates proliferation, apoptosis, survival, motility, adhesion, and invasion.	Inherited schwannomas, meningiomas, and ependymomas
Nucleophosmin (NPM)	Tumor suppressor	Centrosome-associated protein with aberrant cytoplasmic expression observed in several hematologic malignancies.	AML and breast cancer
Neuronal Wiskott–Aldrich syndrome protein (N-WASP)/focal adhesion kinase (FAK)	Regulators of actin cytoskeleton	Phosphorylation of FAK leads to phosphorylation of N-WASP, which promotes cell motility and invasion.	Neural Wiskott–Aldrich syndrome, breast, esophageal, and ovarian cancer
Ras association domain-containing protein 2 (RASSF2)	Tumor suppressor	Binds KRAS with the characteristics of an effector and promotes apoptosis and cell-cycle arrest.	Thyroid cancer, nasopharyngeal carcinoma
Retinoblastoma (RB1)	Tumor suppressor	Inhibits G_1–S phase transition of the cell cycle in response to DNA damage.	Retinoblastoma
RUNX3	Tumor suppressor	Associated with transforming growth factor-β signaling and functions as a tumor suppressor in gastric carcinogenesis. Suppresses the development of colon cancer by forming a complex with β-catenin-TCF4 and attenuating WNT-mediated signaling activity.	Breast, colon, and gastric cancer
Survivin	Inhibitor of apoptosis	Member of inhibitor-of-apoptosis protein family (IAP) that exerts its cytoprotective effects when located in the cytoplasm by inhibiting apoptotic pathways.	Breast cancer, oral, and oropharyngeal squamous cell carcinoma
Transducer of ErbB-2 (TOB)	Tumor suppressor	Cell-cycle inhibitor that induces arrest at G_1–S phase, upregulates cyclin-dependent kinase inhibitor p27, and downregulates the antiapoptotic proteins Bcl-2 and Bcl-XL	Breast cancer
Topoisomerase I	Relaxation of supercoiled DNA	Enzyme that catalyzes the relaxation of supercoiled DNA during replication, transcription, recombination, and chromosome condensation. Cellular target of camptothecin.	Anaplastic astrocytoma and neuroblastomas
Topoisomerase IIα	Relaxation of supercoiled DNA	Enzyme that catalyzes the relaxation of supercoiled DNA during replication, transcription, recombination, and chromosome condensation. Cellular target of doxorubicn and etoposide.	Multiple myeloma
Vitamin D3 upregulated protein 1 (VDUP1)	Tumor suppressor	Mediates nuclear export of the hypoxia-inducible factor-1α (HIF-1α), a transcriptional activator of angiogenesis, anaerobic metabolism, and survival pathways, leading to proteasomal degradation of HIF-1α in the cytoplasm.	Cervical and lung cancer cell lines
WEE1	Tumor suppressor	Inhibitor of CDK1 and key molecule in maintaining G_2 cell-cycle checkpoint arrest for premitotic DNA repair.	Currently unknown—but reduced expression of WEE1 has been observed in non–small cell lung, colon, and prostate cancer

resulting in the upregulation of several downstream target genes that inhibit apoptosis and promote cell proliferation (42). Several downstream PI3K–AKT signaling substrates that negatively regulate cell proliferation, cell survival, and cell-cycle progression have been shown to be functionally inactivated because of cytoplasmic sequestration following PI3K–AKT pathway activation, including the FOXO transcription factors and the cell-cycle inhibitor p27 (11, 43, 44). The lipid phosphatase PTEN negatively regulates the PI3K–AKT pathway, and mutations in the *PTEN* gene have frequently been found in a variety of cancers, including glioblastoma multiforme, melanoma, renal cell carcinoma, and prostate, ovarian, and endometrial cancers (45, 46). In *PTEN*-null cancer cells, constitutive activation of AKT leads to phosphorylation of FOXO transcription factors at multiple sites, thus preventing FOXO DNA binding and transcriptional activity (47). Hyperphosphorylation of the FOXO proteins also facilitates binding with 14-3-3, which then promotes XPO1-mediated nuclear export of FOXO (11). Colon cancer and renal cancer cell lines that do not express PTEN show constitutive mislocalization and loss of function for FOXO proteins, which in turn result in loss of regulation for metabolism for many cell types (47).

The cell-cycle inhibitor p27 can also be sequestered in the cytoplasm or exported from the nucleus by XPO1 via AKT phosphorylation (48). Under normal conditions, p27 is localized in the nucleus, where it acts as a cell-cycle inhibitor by binding to and inhibiting the activity of cyclin-dependent kinase 2 (CDK2), a serine/threonine protein kinase that promotes G_1–S phase cell-cycle progression (3). Phosphorylation of the p27 NLS by AKT has been shown to impair nuclear import with subsequent cytoplasmic sequestration of p27, leaving unbound CDK2 within the nucleus free to facilitate cell-cycle progression and tumorigenesis (48, 49). In addition, RAS activation may lead to XPO1-mediated nuclear export of p27 via phosphorylation of p27 at Ser10 by the PI3K–AKT and RAF–MEK–ERK pathways (50, 51). Cytoplasmic localization of p27 has been observed in esophageal, thyroid, colon, and breast cancers (3). Wang and colleagues (52) recently demonstrated that increased XPO1 and phosphorylated Ser10 p27 (pSer10p27) expression levels were associated with advanced-stage and high-grade epithelial ovarian cancer and poorer overall survival. Knockdown of XPO1 and pSer10p27 expression levels also leads to cell-cycle arrest and inhibition of cell proliferation in SKOV3 cells *in vitro* and *in vivo* (52).

The homologous recombination DNA repair proteins BRCA1 and RAD51 have also been shown to be functionally inactivated because of cytoplasmic sequestration following PI3K–AKT pathway activation (20). Cytoplasmic localization of BRCA1 has been observed in 60% of primary invasive ductal breast cancers, with a high correlation between cytoplasmic BRCA1 localization and AKT1 activity (based on increased levels of phosphorylated AKT1) in the tumors (20). The exact mechanism of AKT1-induced cytoplasmic sequestration of BRCA1 is currently unknown, but nuclear export of BRCA1 via the XPO1 pathway has previously been described following DNA damage caused by ionizing radiation (53), and XPO1 has also been implicated in the regulation of BRCA1 centrosomal localization, thus suggesting that AKT-induced nuclear export of BRCA1 may be XPO1 mediated (54). The relationship between AKT1 activity and homologous recombination–related tumor-suppressor protein mislocalization and function has recently been further highlighted by data showing that BRCA1 deficiency in cells may itself lead to activation of AKT1 and impaired CHK1 nuclear localization (55). Impaired CHK1 nuclear import disrupts the interaction between CHK1 and RAD51 in the nucleus, resulting in homologous recombination repair defects (55).

Recent data suggest that the nuclear export of the estrogen receptor α (ERα) into the cytoplasm is required for estrogen-dependent activation of DNA synthesis and S-phase entry, resulting in increased proliferative activity of breast cancer cells (56, 57). Following estrogen stimulation, PI3K–AKT phosphorylates FOXO1, also known as forkhead in rhabdomyosarcoma (FKHR; ref. 57). In addition, estrogen also activates the nonreceptor protooncogene SRC, resulting in tyrosine phosphorylation of ERα, which in turn leads to assembly of an ERα–FKHR complex within the nucleus and its subsequent nuclear export via XPO1. The cytoplasmic localization of the ER-α–FKHR leads to downregulation of FKHR transcriptional activity and upregulation of cyclin D1 transcription, resulting in DNA synthesis and S-phase transition (56).

Other examples of intracellular mislocalization of tumor-suppressor proteins and GRPs mediated by activation of oncogenic pathways include the functional inactivation of the RUNX3 tumor-suppressor protein by phosphorylation by SRC kinase activity and subsequent cytoplasmic mislocalization via XPO1-mediated nuclear export in gastric and breast cancers, and aberrant activation of the WNT pathway in more than 90% of colorectal cancers (17, 19). The p53 tumor-suppressor protein is also mislocalized. Hence, the development and progression of cancer mediated by upregulation of oncogenic signaling pathways (e.g., PI3K–AKT, SRC, and WNT) and inactivation of tumor-suppressor proteins may be partly underpinned by nuclear–cytoplasmic mislocalization of key regulatory proteins.

XPO1-MEDIATED NUCLEAR EXPORT AND DRUG RESISTANCE IN CANCER

There is also accumulating evidence to suggest that XPO1 may mediate drug resistance in a variety of tumor types by facilitating the nuclear export of various drug targets. These include topoisomerase I α (topo1α) and topo2α, galectin-3, and BCR–ABL (2).

The nuclear protein topo2α functions as a homodimer that disentangles DNA and relieves the torsional stress in supercoiled DNA caused by the process of DNA replication (58). Chemotherapeutic agents, such as doxorubicin and etoposide, inhibit topo2α during DNA replication and produce cleavable complexes, resulting in double-stranded DNA breaks and cell death (59). It has been demonstrated that for DNA damage to occur in this manner, topo2α must be localized within the nucleus and in physical contact with DNA (21). In myeloma cells, nuclear export of topo2α to the cytoplasm via XPO1 has been associated with resistance to topo2α inhibitors, and reversal of the topo2α inhibitor-resistant phenotype can be induced by the XPO1 inhibitors ratjadone C or Leptomycin-B or by siRNA-mediated

knockdown of XPO1 protein expression (21). Depletion of XPO1 by siRNA or ratjadone C has been shown to result in synergistic sensitivity of myeloma cells, obtained from bone marrow aspirates of patients with multiple myeloma and from human multiple myeloma cell lines, to doxorubicin and etoposide (21).

Nuclear galectin-3 is a multifunctional protein that regulates cell proliferation and can induce apoptosis in cancer cells. The subcellular location of galectin-3 seems to be tightly regulated by specific selective mechanisms via the NPC depending on cell type, growth conditions, or neoplastic transformation (60). Nuclear-to-cytoplasmic translocation of galectin-3 has been observed in prostate and breast cancer cells following treatment with cytotoxic agents, with subsequent inhibition of apoptosis in these cells (61, 62). Treatment of the breast cancer cells by both cisplatin and the nuclear export inhibitor Leptomycin-B has been shown to induce nuclear retention of galectin-3 and apoptosis, thus reversing cisplatin resistance (61).

The development of the BCR–ABL kinase inhibitor imatinib mesylate (Gleevec; Novartis) has revolutionized the treatment of CML and has become its standard first-line treatment (2, 16). Development of drug resistance is, however, a common event when the disease progresses to its accelerated or blast phase despite imatinib treatment. The main mechanisms underlying the development of imatinib resistance are point mutations in the kinase domain, *BCR–ABL* amplification and clonal evolution [i.e., the development of additional nonrandom chromosomal abnormalities besides the balanced translocation of t(9:22)(q34:q11.2) that results in the Philadelphia chromosome; ref. 16]. The ABL protein contains three putative NLSs and a single NES. In normal cells, the ABL kinase functions in the cell nucleus and is activated by DNA damage to induce the activation of the p73 tumor-suppressor protein (63). In fact, the BCR–ABL protein has been found to retain the apoptotic functions of the wild-type ABL protein, and induces apoptosis in leukemic cells when maintained in the nucleus using the export inhibitor Leptomycin-B (64). Furthermore, inactivation of BCR–ABL by imatinib partially restores nuclear localization of the protein (2). A strategy of combined treatment with imatinib and XPO1 inhibition by Leptomycin-B to effectively trap BCR–ABL in the cell nucleus has been shown to induce cell death in imatinib-resistant cells that displayed *BCR–ABL* amplification or signs of clonal evolution (16).

NUCLEAR EXPORT INHIBITORS AS ANTICANCER THERAPEUTICS

In view of the aforementioned evidence supporting the role of nuclear transport dysregulation in maintaining the neoplastic phenotype and drug resistance, targeting the nuclear transport mechanism as a therapeutic strategy has gained interest within the oncology community in recent years. The main focus of this strategy has been the targeted inhibition of XPO1, with the hypothesis that inhibition of nuclear export will result in forced nuclear retention and thereby activation of several key tumor-suppressor proteins and GRPs, for example, p53 pathway proteins, with consequent induction of cell-cycle arrest and apoptosis in tumor cells while sparing normal cells.

Naturally Derived XPO1 Inhibitors

Inhibition of XPO1 as a therapeutic strategy has been studied for almost two decades. The bacterial toxin Leptomycin-B was the first selective, irreversible XPO1 inhibitor that was discovered and subsequently tested in a clinical trial (65). Leptomycin-B has potent antitumor activity *in vitro*, but is also toxic to normal cells (66). Despite marked toxicities in animals, Leptomycin-B was administered intravenously to patients with refractory solid tumors in a phase I study by Newlands and colleagues (65). Toxicities included profound gastrointestinal side effects, including nausea, vomiting, watery diarrhea along with marked anorexia, fatigue/asthenia, and malaise, whereas typical side effects of cytotoxic agents such as neutropenia, mucositis, and alopecia were not observed. Transient tumor marker responses in patients with ovarian carcinoma and trophoblastic tumor and stable disease in a patient with refractory sarcoma were reported, but the associated severe toxicities led to its development being abandoned (65).

Leptomycin-B exerts its inhibitory effect on XPO1 by irreversibly alkylating the reactive site cysteine residue (Cys528; ref. 29). Recently, it was demonstrated that upon binding to XPO1, Leptomycin-B undergoes hydrolysis of the lactone ring, which in turn enables persistent binding of the drug to XPO1 (67). Alkylation of Cys528 prevents XPO1 from binding to the leucine-rich nuclear export sequence of the cargo protein substrate, hence inhibiting the formation of the XPO1–cargo–RanGTP export complex and effectively blocking nuclear export (3, 29). There are now data suggesting that the severe toxicities observed following treatment with Leptomycin-B involve additional off-target effects caused by binding to cysteine proteases as well as the irreversible blockade of all XPO1 functions *per se* (68). Therefore, a selective and reversible inhibitor might possess potent anticancer activity with an improved tolerability profile. Most of the currently available XPO1 inhibitors function by permanently modifying the reactive site Cys528 and preventing XPO1 binding to the NES of cargo proteins (2). Since the discovery of Leptomycin-B, other naturally derived inhibitors have been identified, including the ratjadone analogs (A, B, C, and D) isolated from *Sorangium cellulosum*, which have a similar chemical structure to Leptomycin-B and use an identical molecular mechanism to modify XPO1, but these compounds have not been tested *in vivo* (2).

Synthetic XPO1 Inhibitors

Kosan Biosciences Inc., before its acquisition by Bristol-Myers Squibb, created derivatives of Leptomycin-B, including KOS-2464, with better pharmacologic properties that showed a much improved therapeutic index across multiple tumor xenografts (69). Significantly better tolerability and less weight loss than with Leptomycin-B were observed in animals, although transient anorexia remained (69). More recently, a small-molecule reversible inhibitor of XPO1, CBS9106, which induced cell-cycle arrest and apoptosis as a single agent in 60 different human cancer cell lines, including bladder, breast, colon, and lung cancer, at submicromolar concentrations has been described (70). Unlike the Leptomycin-B analogs, CBS9106 binds to the Cys528 residue by forming a reversible

covalent bond with it and was found to inhibit tumor growth in xenograft models without significant weight loss or mortality (70). Both of these compounds have not progressed into the clinic but are at least indicative that XPO1 can be inhibited with an adequate therapeutic window.

Perhaps the compounds that herald the most promise for the clinical utility of XPO1 inhibition are the new small-molecule inhibitors of XPO1, also known as selective inhibitors of nuclear export (SINE). These compounds share a similar structure with the N-azolylacrylate analog PKF050-638, a highly specific and reversible small-molecule XPO1 inhibitor that was originally developed to prevent nuclear export of the HIV-1 Rev protein, an essential regulator of the HIV-1 mRNA expression (71). Karyopharm Therapeutics has developed a series of SINE compounds [KPT-115, KPT-127, KPT-185, KPT-251, KPT-276, KPT-330 (selinexor), and KPT-335 (verdinexor)] that have been shown to inhibit XPO1-mediated export of p53, p73, RB1, FOXO proteins, APC, IκB, NPM, topo2α, and survivin. Preclinical studies of these compounds result in dose-dependent cytotoxicity for a number of hematologic and solid tumors [i.e., CLL, AML, CML, multiple myeloma, non–Hodgkin lymphoma (NHL), melanoma, and renal, breast, pancreatic, prostate, and colorectal cancer cells; refs. 2, 21, 68, 72–78]. These highly specific inhibitors of XPO1 are water soluble, and they bind to the reactive site Cys528 residue by forming a slowly reversible covalent bond (79). SINEs have been shown to be selectively cytotoxic for neoplastic cells with a half maximal effective concentration (EC_{50}) of 10 to 1,000 nmol/L versus >5 to 20 μmol/L in nonneoplastic cell lines, and their cytotoxic effects seem to be independent of known genetic alterations such as $BRAF^{V600E}$ mutations in melanoma or *TP53*, *PI3K*, *AKT*, and *BRCA1/2* mutation status in breast cancer cell lines (24, 75). Synergistic activity using KPT-185 in combination with SN38 and oxaliplatin in colorectal cancer cell lines and KPT-251 with chemotherapy in prostate cancer cell lines has also been observed (72, 73). In murine and monkey pharmacokinetic studies, KPT-185 showed limited bioavailability and systemic exposure, whereas KPT-276 and selinexor showed >50% bioavailability reaching C_{max} >5 μmol/L following 10 mg/kg oral administration of drug (24).

Preclinical *in vitro* and *in vivo* data have shown that both hematologic and solid tumor cells are susceptible to single-agent cytotoxicity caused by selinexor, consistent with its restoration of multiple tumor-suppressor and growth-regulatory pathways, leading to the death of cancer cells (24, 75, 79). Interestingly, SINE treatment also results in reduction in expression of multiple oncoproteins, including cMyc in multiple myeloma, cKIT and FLT3 in AML, and cyclin D1 in mantle cell lymphoma, as well as proteasome-mediated degradation of the XPO1 protein itself. The major adverse event across all tested species to date (mice, rats, dogs, and monkeys) is reversible weight reduction accompanied by reduced food intake without significant vomiting or diarrhea (79). The improved toxicity profile of these newer XPO1 inhibitors relative to previous XPO1 inhibitors, such as Leptomycin-B, is thought to be related to their exquisite specificity for XPO1 with no detectable binding to other proteins, including the cysteine proteases, believed to be the cause of poor tolerance to Leptomycin-B (68).

The SINE XPO1 inhibitors have also been investigated in a comparative oncology setting, using dogs with newly diagnosed and chemotherapy-refractory cancers (80). In one study, the SINE compound verdinexor was administered in 17 dogs with NHL, mast cell tumor, or osteosarcoma. Common toxicities included anorexia, vomiting, and diarrhea. Dose-limiting toxicities were anorexia and vomiting, which occurred at 2 mg/kg twice weekly. Among 16 evaluable dogs, 2 achieved partial responses (both with NHL) and 12 others achieved stable disease, indicating activity of XPO1 inhibitors in large-animal models (80). Verdinexor is currently in phase II studies in dogs with newly diagnosed or recurring lymphoma.

Oral selinexor is currently being developed and evaluated for tolerability and single-agent antitumor activity in phase I clinical trials of patients with solid tumors (trial number NCT01607892; http://clinicaltrials.gov, last accessed on February 14, 2014), and the data from these studies have hitherto been presented only in meeting abstracts. In these studies, selinexor is administered orally at either 8 doses or 10 doses in each 28-day cycle. The preliminary results of the phase I solid-tumor study with the 10 doses per cycle were presented recently. At the time of presentation, 2 of 3 patients had experienced dose-limiting toxicities at 40 mg/m^2, with grade 3 anorexia with dehydration and fatigue in 1 patient, and grade 3 fatigue in another. Commonly observed nonhematologic toxicities included fatigue, nausea, and anorexia that were mostly low grade (grade 1–2), whereas the most common hematologic toxicity was thrombocytopenia. Toxicities were all reversible upon stopping the drug. Among the 39 patients treated, at least 7 patients had disease stabilization of more than 6 months, including patients who were treated at low dose levels. Selinexor has a dose-proportional pharmacokinetic profile and a half-life of 5 to 7 hours, and paired biopsies, done in 12 patients, showed increased nuclear retention of a number of tumor-suppressor proteins, including FOXO1, IκB, and p53, following treatment (81). The alternative dosing regimen in which the drug is administered twice weekly (i.e., 8 doses every 28 days) is currently being investigated in patients with advanced solid tumors, and the maximum-tolerated dose (MTD) has not been reached to date.

In the parallel phase I study in advanced, progressive hematologic malignancies (NCT01607892), patients with relapsed/refractory NHL, CLL, multiple myeloma, or AML were dosed with oral selinexor (8–10 doses every 28 days; refs. 82–84). The adverse-event profile was similar to that observed in the solid-tumor study, and MTD on the eight doses per cycle schedule has not been reached to date. Durable responses and disease stabilization with single-agent oral selinexor across all disease subtypes have been observed, with some patients remaining on study for over 1 year. In CML, recently published data have demonstrated that treatment with selinexor results in apoptosis and impaired the clonogenic potential of leukemic, but not normal, CD34(+) progenitors (85). As a proof of mechanism, following exposure to selinexor, nuclear accumulation of CIP2A, SET (an oncoprotein that inhibits PP2A activity), IκBα (an inhibitor of the NF-κB transcription factor), FOXO3a, p53, and p21, with subsequent reactivation of PP2A within the cytoplasm and reduction in BCR–ABL levels, was noted (85). Antileukemic activity in a CML blast crisis mouse model was observed following treatment with selinexor, and treatment of a single

patient in accelerated-phase CML with Selinexor also led to a reduction in bone pain, splenomegaly, and immature myeloid blasts in the peripheral blood (85).

CONCLUSIONS

There is preclinical evidence to suggest that inhibition of XPO1-mediated nuclear export leading to nuclear retention and functional reactivation of tumor-suppressor proteins and GRPs may be a viable therapeutic anticancer strategy. Until recently, the development of this pharmacologic class of compounds has been hampered by the narrow therapeutic window of XPO1 inhibition (2, 65). New-generation XPO1 inhibitors with improved preclinical efficacy, pharmacokinetics, oral bioavailability, and reduced toxicities in animal models have generated renewed interest in this approach and are now being tested in early-phase clinical trials (79).

Notably, despite the encouraging early signals, and apart from the obvious lack of published clinical toxicity and efficacy data, there are crucial questions that remain unresolved in the context of nuclear export inhibition as a therapeutic strategy. These include the challenges of identifying potential predictive biomarkers of efficacy to optimize patient selection for these drugs, and the possible cumulative toxicities of long-term nuclear export inhibition. Despite these reservations, the emerging data from early-phase clinical studies seem encouraging. In addition, given the noted synergistic effects on cytotoxicity in neoplastic cells, the potential for combining XPO1 inhibition with conventional chemotherapy, such as topoisomerase inhibitors, is open for exploration in the future (21, 73, 86). Moreover, combination strategies with other targeted therapeutics may be another option for consideration, and recent preclinical data showing that combined XPO1 and BRAF inhibition leads to synergistic tumor regression in *BRAF*-mutant melanoma suggest that this may be a viable therapeutic approach as well (87).

Ultimately, it may be overly optimistic to expect that the inhibition of nuclear–cytoplasmic transport will be an effective target in all cancer types, or that the therapeutic efficacy of this approach will not eventually be hampered by intrinsic and acquired resistance mechanisms and/or toxicities engendered by sustained physiologic inhibition of such transport in normal cells. In the context of drug resistance, it is worth noting that the Cys528 residue, which seems to be the main target of XPO1 inhibitors, is not part of an active site of XPO1 and also seems to be nonessential for XPO1/CRM1 function. Mutant forms of XPO1/CRM1 that carry a Cys528–Ser mutation can substitute for wild-type CRM1 in mammalian cells, and *Saccharomyces cerevisiae* express a form of CRM1 that contains a threonine at this position, instead of a cysteine. Human cells that express CRM1 with the Cys528–Ser mutation have been shown to be resistant to the effects of Leptomycin-B (88).

Nonetheless, the strategy of imposing a molecular embargo on nuclear transport as a backbone for new avenues of cancer therapy may be on the verge of becoming a clinical reality.

Disclosure of Potential Conflicts of Interest

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REFERENCES

1. Zaidi SK, Young DW, Javed A, Pratap J, Montecino M, van Wijnen A, et al. Nuclear microenvironments in biological control and cancer. Nat Rev Cancer 2007;7:454–63.
2. Turner JG, Dawson J, Sullivan DM. Nuclear export of proteins and drug resistance in cancer. Biochem Pharmacol 2012;83:1021–32.
3. Kau TR, Way JC, Silver PA. Nuclear transport and cancer: from mechanism to intervention. Nat Rev Cancer 2004;4:106–17.
4. Cook A, Bono F, Jinek M, Conti E. Structural biology of nucleocytoplasmic transport. Annu Rev Biochem 2007;76:647–71.
5. Lange A, Mills RE, Lange CJ, Stewart M, Devine SE, Corbett AH. Classical nuclear localization signals: definition, function, and interaction with importin alpha. J Biol Chem 2007;282:5101–5.
6. Lee BJ, Cansizoglu AE, Suel KE, Louis TH, Zhang Z, Chook YM. Rules for nuclear localization sequence recognition by karyopherin beta 2. Cell 2006;126:543–58.
7. Dong X, Biswas A, Suel KE, Jackson LK, Martinez R, Gu H, et al. Structural basis for leucine-rich nuclear export signal recognition by CRM1. Nature 2009;458:1136–41.
8. Kutay U, Guttinger S. Leucine-rich nuclear-export signals: born to be weak. Trends Cell Biol 2005;15:121–4.
9. Xu D, Farmer A, Chook YM. Recognition of nuclear targeting signals by Karyopherin-beta proteins. Curr Opin Struct Biol 2010;20:782–90.
10. Kao HY. Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. J Biol Chem 2001;276:47496–507.
11. Yang JY, Hung MC. A new fork for clinical application: targeting forkhead transcription factors in cancer. Clin Cancer Res 2009;15:752–7.
12. Bischoff FR, Klebe C, Kretschmer J, Wittinghofer A, Ponstingl H. RanGAP1 induces GTPase activity of nuclear Ras-related Ran. Proc Natl Acad Sci U S A 1994;91:2587–91.
13. Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Gorlich D. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. EMBO J 1997;16:6535–47.
14. Marelli M, Dilworth DJ, Wozniak RW, Aitchison JD. The dynamics of karyopherin-mediated nuclear transport. Biochem Cell Biol 2001;79:603–12.
15. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
16. Kancha RK, von Bubnoff N, Miething C, Peschel C, Gotze KS, Duyster J. Imatinib and leptomycin B are effective in overcoming imatinib-resistance due to Bcr-Abl amplification and clonal evolution but not due to Bcr-Abl kinase domain mutation. Haematologica 2008;93:1718–22.
17. Goh YM, Cinghu S, Hong ET, Lee YS, Kim JH, Jang JW, et al. Src kinase phosphorylates RUNX3 at tyrosine residues and localizes the protein in the cytoplasm. J Biol Chem 2010;285:10122–9.
18. Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY, et al. IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 2004;117:225–37.
19. Ito K, Liu Q, Salto-Tellez M, Yano T, Tada K, Ida H, et al. RUNX3, a novel tumor suppressor, is frequently inactivated in gastric cancer by protein mislocalization. Cancer Res 2005;65:7743–50.
20. Plo I, Laulier C, Gauthier L, Lebrun F, Calvo F, Lopez BS. AKT1 inhibits homologous recombination by inducing cytoplasmic retention of BRCA1 and RAD51. Cancer Res 2008;68:9404–12.
21. Turner JG, Marchion DC, Dawson JL, Emmons MF, Hazlehurst LA, Washausen P, et al. Human multiple myeloma cells are sensitized to topoisomerase II inhibitors by CRM1 inhibition. Cancer Res 2009;69:6899–905.
22. Noske A, Weichert W, Niesporek S, Röske A, Buckendahl A-C, Koch I, et al. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. Cancer 2008;112:1733–43.
23. Huang WY, Yue L, Qiu WS, Wang LW, Zhou XH, Sun YJ. Prognostic value of CRM1 in pancreas cancer. Clin Invest Med 2009;32:E315.

24. Lesinski GB, Yang J, Bill MA, Landesman Y, Shacham S, Kauffman M, et al. Effect of small inhibitors of nuclear export (SINE) on growth inhibition and apoptosis of human melanoma cells. J Clin Oncol 30, 2012 (suppl; abstr e13549).
25. Shen A, Wang Y, Zhao Y, Zou L, Sun L, Cheng C. Expression of CRM1 in human gliomas and its significance in p27 expression and clinical prognosis. Neurosurgery 2009;65:153–9.
26. van der Watt PJ, Maske CP, Hendricks DT, Parker MI, Denny L, Govender D, et al. The Karyopherin proteins, Crm1 and Karyopherin beta1, are overexpressed in cervical cancer and are critical for cancer cell survival and proliferation. Int J Cancer 2009;124:1829–40.
27. Yao Y, Dong Y, Lin F, Zhao H, Shen Z, Chen P, et al. The expression of CRM1 is associated with prognosis in human osteosarcoma. Oncol Rep 2009;21:229–35.
28. van der Watt PJ, Leaner VD. The nuclear exporter, Crm1, is regulated by NFY and Sp1 in cancer cells and repressed by p53 in response to DNA damage. Biochim Biophys Acta 2011;1809:316–26.
29. Nguyen KT, Holloway MP, Altura RA. The CRM1 nuclear export protein in normal development and disease. Int J Biochem Mol Biol 2012;3:137–51.
30. Thakar K, Karaca S, Port SA, Urlaub H, Kehlenbach RH. Identification of CRM1-dependent nuclear export cargos using quantitative mass spectrometry. Mol Cell Proteomics 2013;12:664–78.
31. Junttila MR, Puustinen P, Niemela M, Ahola R, Arnold H, Bottzauw T, et al. CIP2A inhibits PP2A in human malignancies. Cell 2007;130: 51–62.
32. Khanna A, Pimanda JE, Westermarck J. Cancerous inhibitor of protein phosphatase 2A, an emerging human oncoprotein and a potential cancer therapy target. Cancer Res 2013;73:6548–53.
33. Bockelman C, Lassus H, Hemmes A, Leminen A, Westermarck J, Haglund C, et al. Prognostic role of CIP2A expression in serous ovarian cancer. Br J Cancer 2011;105:989–95.
34. Arnaoutov A, Azuma Y, Ribbeck K, Joseph J, Boyarchuk Y, Karpova T, et al. Crm1 is a mitotic effector of Ran-GTP in somatic cells. Nat Cell Biol 2005;7:626–32.
35. Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol 2007;8:275–83.
36. Soussi T. TP53 mutations in human cancer: database reassessment and prospects for the next decade. Adv Cancer Res 2011;110:107–39.
37. Cai X, Liu X. Inhibition of Thr-55 phosphorylation restores p53 nuclear localization and sensitizes cancer cells to DNA damage. Proc Natl Acad Sci U S A 2008;105:16958–63.
38. Freedman DA, Levine AJ. Nuclear export is required for degradation of endogenous p53 by MDM2 and human papillomavirus E6. Mol Cell Biol 1998;18:7288–93.
39. Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ, Wahl GM. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. EMBO J 1999;18:1660–72.
40. O'Brate A, Giannakakou P. The importance of p53 location: nuclear or cytoplasmic zip code?Drug Resist Updat 2003;6:313–22.
41. Puente XS, Pinyol M, Quesada V, Conde L, Ordonez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011;475:101–5.
42. Yap TA, Garrett MD, Walton MI, Raynaud F, de Bono JS, Workman P. Targeting the PI3K–AKT–mTOR pathway: progress, pitfalls, and promises. Curr Opin Pharmacol 2008;8:393–412.
43. Blain SW, Massague J. Breast cancer banishes p27 from nucleus. Nat Med 2002;8:1076–8.
44. Vogt PK, Jiang H, Aoki M. Triple layer control: phosphorylation, acetylation and ubiquitination of FOXO proteins. Cell Cycle 2005;4: 908–13.
45. Zhang S, Yu D. PI(3)king apart PTEN's role in cancer. Clin Cancer Res 2010;16:4325–30.
46. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med 2011;365:2473–83.
47. Nakamura N, Ramaswamy S, Vazquez F, Signoretti S, Loda M, Sellers WR. Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol Cell Biol 2000;20:8969–82.
48. Shin I, Yakes FM, Rojo F, Shin NY, Bakin AV, Baselga J, et al. PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. Nat Med 2002;8:1145–52.
49. Chu IM, Hengst L, Slingerland JM. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer 2008;8:253–67.
50. Besson A, Gurian-West M, Chen X, Kelly-Spratt KS, Kemp CJ, Roberts JM. A pathway in quiescent cells that controls p27Kip1 stability, subcellular localization, and tumor suppression. Genes Dev 2006;20: 47–64.
51. Fujita N, Sato S, Katayama K, Tsuruo T. Akt-dependent phosphorylation of p27Kip1 promotes binding to 14–3-3 and cytoplasmic localization. J Biol Chem 2002;277:28706–13.
52. Wang Y, Xiang J, Ji F, Deng Y, Tang C, Yang S, et al. Knockdown of CRM1 inhibits the nuclear export of p27(Kip1) phosphorylated at serine 10 and plays a role in the pathogenesis of epithelial ovarian cancer. Cancer Lett 2014;343:6–13.
53. Feng Z, Kachnic L, Zhang J, Powell SN, Xia F. DNA damage induces p53-dependent BRCA1 nuclear export. J Biol Chem 2004;279: 28574–84.
54. Brodie KM, Henderson BR. Characterization of BRCA1 protein targeting, dynamics, and function at the centrosome: a role for the nuclear export signal, CRM1, and Aurora A kinase. J Biol Chem 2012; 287:7701–16.
55. Jia Y, Song W, Zhang F, Yan J, Yang Q. Akt1 inhibits homologous recombination in Brca1-deficient cells by blocking the Chk1-Rad51 pathway. Oncogene 2013;32:1943–9.
56. Castoria G, Giovannelli P, Lombardi M, De Rosa C, Giraldi T, de Falco A, et al. Tyrosine phosphorylation of estradiol receptor by Src regulates its hormone-dependent nuclear export and cell cycle progression in breast cancer cells. Oncogene 2012;31:4868–77.
57. Lombardi M, Castoria G, Migliaccio A, Barone MV, Di Stasio R, Ciociola A, et al. Hormone-dependent nuclear export of estradiol receptor and DNA synthesis in breast cancer cells. J Cell Biol 2008;182:327–40.
58. Robinson MJ, Martin BA, Gootz TD, McGuirk PR, Moynihan M, Sutcliffe JA, et al. Effects of quinolone derivatives on eukaryotic topoisomerase II. A novel mechanism for enhancement of enzyme-mediated DNA cleavage. J Biol Chem 1991;266:14585–92.
59. Robinson MJ, Osheroff N. Effects of antineoplastic drugs on the post-strand-passage DNA cleavage/religation equilibrium of topoisomerase II. Biochemistry 1991;30:1807–13.
60. Fukumori T, Kanayama H, Raz A. The role of galectin-3 in cancer drug resistance. Drug Resist Updat 2007;10:101–8.
61. Takenaka Y, Fukumori T, Yoshii T, Oka N, Inohara H, Kim HR, et al. Nuclear export of phosphorylated galectin-3 regulates its antiapoptotic activity in response to chemotherapeutic drugs. Mol Cell Biol 2004;24:4395–406.
62. Fukumori T, Oka N, Takenaka Y, Nangia-Makker P, Elsamman E, Kasai T, et al. Galectin-3 regulates mitochondrial stability and antiapoptotic function in response to anticancer drug in prostate cancer. Cancer Res 2006;66:3114–9.
63. Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG Jr, Levrero M, et al. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 1999;399:806–9.
64. Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med 2001;7:228–34.
65. Newlands ES, Rustin GJ, Brampton MH. Phase I trial of elactocin. Br J Cancer 1996;74:648–9.
66. Kau TR, Schroeder F, Ramaswamy S, Wojciechowski CL, Zhao JJ, Roberts TM, et al. A chemical genetic screen identifies inhibitors of regulated nuclear export of a forkhead transcription factor in PTEN-deficient tumor cells. Cancer Cell 2003;4:463–76.
67. Sun Q, Carrasco YP, Hu Y, Guo X, Mirzaei H, Macmillan J, et al. Nuclear export inhibition through covalent conjugation and

hydrolysis of Leptomycin B by CRM1. Proc Natl Acad Sci U S A 2013;110: 1303–8.
68. Lapalombella R, Sun Q, Williams K, Tangeman L, Jha S, Zhong Y, et al. Selective inhibitors of nuclear export (SINE) show that CRM1/XPO1 is a target in chronic lymphocytic leukemia. Blood 2012;120:4621–34.
69. Mutka SC, Yang WQ, Dong SD, Ward SL, Craig DA, Timmermans PB, et al. Identification of nuclear export inhibitors with potent anticancer activity *in vivo*. Cancer Res 2009;69:510–7.
70. Sakakibara K, Saito N, Sato T, Suzuki A, Hasegawa Y, Friedman JM, et al. CBS9106 is a novel reversible oral CRM1 inhibitor with CRM1 degrading activity. Blood 2011;118:3922–31.
71. Daelemans D, Afonina E, Nilsson J, Werner G, Kjems J, De Clercq E, et al. A synthetic HIV-1 Rev inhibitor interfering with the CRM1-mediated nuclear export. Proc Natl Acad Sci U S A 2002;99: 14440–5.
72. Azmi AS, Kauffman M, McCauley D, Shacham S, Mohammad RM. Novel small-molecule CRM-1 inhibitor for GI cancer therapy. J Clin Oncol 30, 2012 (suppl 4; abstr 245).
73. Chung HW, Salas Fragomeni RA, Shacham S, Kauffman M, Cusack JC. Effect of inhibition of nuclear protein export using a novel CRM1 inhibitor on the apoptotic response to SN38 in preclinical colon cancer models. J Clin Oncol 30, 2012 (suppl 4; abstr 609).
74. Inoue H, Kauffman M, Shacham S, Landesman Y, Weiss RH. Evaluation of selective inhibitors of nuclear export (SINE) CRM1 inhibitors for the treatment of renal cell carcinoma (RCC). J Clin Oncol 30, 2012 (suppl; abstr 4634).
75. McCauley D, Landesman Y, Senapedis W, Kashyap T, Saint-Martin J-R, Plamondon L, et al. Preclinical evaluation of selective inhibitors of nuclear export (SINE) in basal-like breast cancer (BLBC). J Clin Oncol 30, 2012 (suppl; abstr 1055).
76. Shacham S, Barnard S, Kisseberth W, Ito D, Jensen K, Borgotti A, et al. Results of a phase I dose escalation study of the novel, oral CRM1 selective inhibitor of nuclear export (SINE) KPT-335 in dogs with spontaneous non-Hodgkin's lymphomas (NHL) [abstract]. In: Proceedings of the 54th ASH Annual Meeting and Exposition; 2012 Dec 8–11; Atlanta, GA. Abstract nr 161.
77. Shacham S, Gravina GL, Ricevuto E, Mancini A, Chin L, Shechter S, et al. Preclinical evaluation of selective inhibitors of nuclear export (SINE) CRM1 (XPO1) inhibitors in prostate cancer (PrCa). J Clin Oncol 30, 2012 (suppl; abstr e15200).
78. Shacham S, Kauffman M, Sandanayaka V, Draetta G, Shechter S, Williams J, et al. Preclinical development of small-molecule CRM1 inhibitors as novel therapy for the treatment of colorectal cancer (CRC). J Clin Oncol 29, 2011 (suppl 4; abstr 430).
79. Etchin J, Sun Q, Kentsis A, Farmer A, Zhang ZC, Sanda T, et al. Antileukemic activity of nuclear export inhibitors that spare normal hematopoietic cells. Leukemia 2013;27:66–74.
80. London CA, Bernabe LF, Barnard S, Kisseberth WC, Borgatti A, Henson M, et al. Preclinical evaluation of the novel, orally bioavailable Selective Inhibitor of Nuclear Export (SINE) KPT-335 in spontaneous canine cancer: results of a phase I study. PLoS ONE 2014;9:e87585.
81. Razak A, Sorensen M, Mahipal M, Shacham S. First-in-class, first-in-human phase I trial of KPT-330, a selective inhibitor of nuclear export (SINE) in patients with advanced solid tumors. J Clin Oncol 31, 2013 (suppl; abstr 2505).
82. Chen C, Gutierrez M, de Nully Brown P, Gabrail N, Baz R, Reece DE, et al. A first-in-class oral selective inhibitor of nuclear export (SINE) XPO1/CRM1 antagonist in patients (pts) with relapsed/refractory multiple myeloma (MM) or Waldenstrom's macroglobulinemia (WM). Blood 2013;122.
83. Kuruvilla J, Gutierrez M, Shah B, Gabrail NY, de Nully Brown P, Stone RM, et al. Preliminary evidence of anti tumor activity of Selinexor (KPT-330) in a phase i trial of a first-in-class oral selective inhibitor of nuclear export (SINE) in patients (pts) with relapsed/refractory non Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Blood 2013;122:90.
84. Savona M, Garzon R, de Nully Brown P, Yee K, Lance JE, Gutierrez M, et al. Phase I trial of Selinexor (KPT-330), a first-in-class oral selective inhibitor of nuclear export (SINE) in patients (pts) with advanced acute myelogenous leukemia (AML). Blood 2013;122:1440.
85. Walker CJ, Oaks JJ, Santhanam R, Neviani P, Harb JG, Ferenchak G, et al. Preclinical and clinical efficacy of XPO1/CRM1 inhibition by the karyopherin inhibitor KPT-330 in Ph+ leukemias. Blood 2013;122:3034–44.
86. Abdel-Naim AB, Lu C, Shao C, Cobos E, Singh KP, Gao W. Chemotherapeutic sensitization of leptomycin B resistant lung cancer cells by pretreatment with doxorubicin. PLoS ONE 2012;7:e32895.
87. Salas Fragomeni RA, Chung HW, Landesman Y, Senapedis W, Saint-Martin JR, Tsao H, et al. CRM1 and BRAF inhibition synergize and induce tumor regression in BRAF-mutant melanoma. Mol Cancer Ther 2013;12:1171–9.
88. Akakura S, Yoshida M, Yoneda Y, Horinouchi S. A role for Hsc70 in regulating nucleocytoplasmic transport of a temperature-sensitive p53 (p53Val-135). J Biol Chem 2001;276:14649–57.