

Therapeutic Targeting of Nuclear Export Inhibition in Lung Cancer

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ABSTRACT

Intracellular compartmentalization and trafficking of molecules plays a critical role in complex and essential cellular processes. In lung cancer and other malignancies, aberrant nucleocytoplasmic transport of tumor suppressor proteins and cell cycle regulators results in tumorigenesis and inactivation of apoptosis. Pharmacologic agents targeting this process, termed *selective inhibitors of nuclear export* (SINE), have demonstrated antitumor efficacy in preclinical models and human clinical trials. Exportin-1 (XPO1), which serves as the sole exporter of several tumor suppressor proteins and cell cycle regulators, including retinoblastoma, adenomatous polyposis coli, p53, p73, p21, p27, forkhead box O, signal transducer and activator of transcription 3, inhibitor of κ B, topoisomerase II, and protease activated receptor 4—is the principal focus of development of SINE. The most extensively studied of the SINE to date, the exportin-1 inhibitor selinexor (KPT-330 [Karyopharm Therapeutics, Inc., Newton Centre, MA]), has demonstrated single-agent anticancer activity and synergistic effects in combination regimens against multiple cancer types, with principal toxicities of low-grade cytopenias and gastrointestinal effects. SINE may have particular relevance in *KRAS*-driven tumors, for which this treatment strategy demonstrates significant synthetic lethality. A multicenter phase 1/2 clinical trial of selinexor in previously treated advanced *KRAS*-mutant NSCLC is under way.

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Intracellular compartmentalization and trafficking of molecules plays a critical role in complex and essential

cellular processes. Aberrant nucleocytoplasmic transport of tumor suppressor proteins and cell cycle regulators—mediated by importins and exportins—can result in tumorigenesis and inactivation of apoptosis. Several malignancies, including lung cancer, feature overexpression of these nuclear transport receptors. Pharmacologic targeting of this process has demonstrated antitumor efficacy. In this review article, we describe the mechanism, function, and therapeutic targeting of nuclear transport, with particular focus on application in lung cancer.

Nuclear Export Machinery

The nuclear envelope, comprising an inner and outer membrane, prevents the unrestricted diffusion of molecules larger than 40 kD between the nucleus and the cytoplasm. This regulated nuclear-cytoplasmic transport of proteins and other molecules plays a key role in cell functioning.¹ Within the nuclear envelope, nuclear pore complexes provide an aqueous channel for the active transport of molecules. The karyopherin-B protein family, which includes both importins and exportins, facilitates transport across these nuclear pores.² Cargo proteins destined for nuclear export have specific

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leucine-rich amino acid sequences known as nuclear export signals, which are recognized by exportin proteins.³ Nuclear-cytoplasmic transport is an active process requiring energy provided by RanGTP (Ran guanosine triphosphate). A complex between the cargo protein, the exportin molecule, and RanGTP is formed and transported across the nuclear pore complex to the cytoplasm.⁴ In the cytoplasm, Ran guanosine triphosphate causes hydrolysis of the RanGTP, releasing the cargo (which remains in the cytoplasm) and exportin protein (which is recycled back to the nucleus) (Fig. 1A).⁵ At least seven eukaryotic exportins have been identified (Table 1⁶⁻¹²). Although most of these are responsible for transport,¹³ exportin-1 (XPO1) (also known as chromosomal region maintenance 1) is a more ubiquitous receptor protein responsible for transporting approximately 220 proteins.^{6,14}

Role of Nuclear Export Functions in Normal Cell Physiology and Cancer

XPO1 is the sole exporter of several tumor suppressor proteins and cell cycle regulators, including retinoblastoma, adenomatous polyposis coli, p53, p73, p21, p27, forkhead box O, signal transducer and activator of transcription 3, inhibitor of κ B, topoisomerase

II, and protease-activated receptor 4.¹⁵ Under physiologic conditions, the regulated export of these molecules prevents their overactivity in the nucleus in the absence of oncogenic stimuli or DNA damage. In multiple cancer types, XPO1 overexpression leads to dysregulated export of these tumor suppressor proteins into the cytoplasm, where they are unable to exercise their effects, thereby resulting in aberrant growth signaling, inactivation of apoptosis, and tumor initiation and growth (Fig. 1B). XPO1 overexpression is also associated with drug resistance on account of export of drug targets such as topoisomerase II and galectin-3.^{16,17}

Nuclear Export Targeting

Given the critical role of nuclear export in cell cycle regulation and tumorigenesis, efforts to inhibit XPO1 pharmacologically have been undertaken. First-generation XPO1 inhibitors include natural products such as leptomycin B (see Table 1). Leptomycin B irreversibly alkylates an XPO1 cysteine residue (cysteine 528), preventing XPO1 binding to cargo protein nuclear export signals. This in turn leads to inhibition of export complex formation, as well as to nuclear retention of tumor suppressor proteins (Fig. 1C).¹⁸ Despite promising preclinical studies, strong dose-limiting

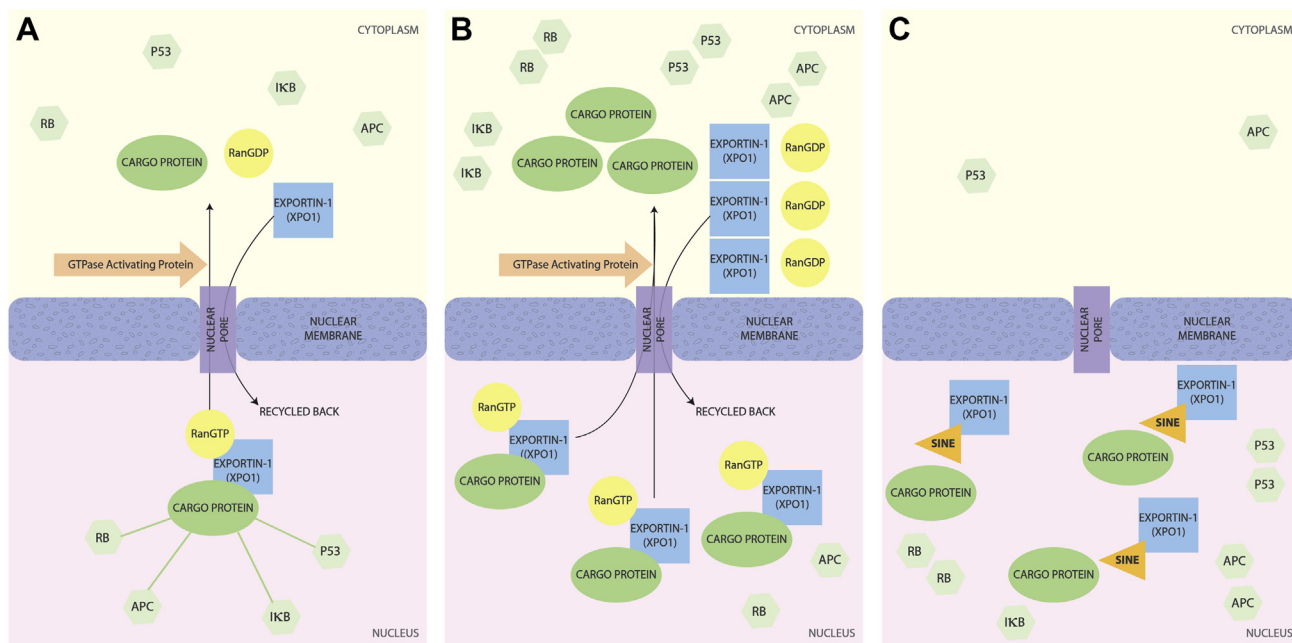


Figure 1. Export through the nuclear pore complex. (A) Physiologic state. Export complexes containing exportin-1 (XPO1), a cargo protein, and Ran guanosine triphosphate (RanGTP) are transported across the nuclear pore because of the RanGTP:Ran guanosine diphosphate gradient. Ran guanosine triphosphatase hydrolyzes RanGTP in the cytoplasm, leading to dissociation of the complex in the cytoplasm. (B) Cancer. Up-regulation of XPO1 results in dysregulated cytoplasmic transport of cell cycle regulators, leading to their accumulation in the cytoplasm and inability to exert their effects. In turn, this state leads to aberrant growth signaling, inactivation of apoptosis, and tumor initiation and growth. (C) Pharmacologic inhibition. Selective inhibitors of nuclear export (SINE) bind to XPO1 and prevent its interaction with cargo proteins, thereby inhibiting nuclear export. Cell cycle regulators are retained in the nucleus, leading to growth inhibition. RB, reginoblastoma; APC, adenomatous polyposis coli; GTPase, guanosine triphosphatase; I κ B, inhibitor of κ B.

Table 1. Characteristics of Exportin Molecules

Exportin protein	Alternative Name	Chromosome	Cargo	Role in Cancer	Reference
XPO1	CRM1 protein	2p15	>200 macromolecules, including protein and RNA (including several tumor suppressor genes and cell cycle regulators, such as p53, p21, Rb, APC, and FOXO)	Up-regulated in multiple cancer types, associated with tumorigenesis and drug resistance	Stade et al. (1997) ⁶
Exportin-2	CAS protein, chromosome segregation 1-like protein	20q13.13	Importin- α	Overexpressed in thyroid cancer	Holzer et al., (2016) ⁷
Exportin-3, (Exportin-T)	Karyopherin- β , tRNA exportin	12q14.2	tRNA	–	Leisegag et al. (2012) ⁸
Exportin-4	KIAA1721, FLJ13046,	13q12.11	Broad substrate specificity, including SMAD3 and eIF5A	–	Kurisasi et al. (2006) ⁹
Exportin-5	Ran-binding protein 21, KIAA1291	6p21.1	Proteins bearing a double-stranded RNA binding domain and double-stranded RNAs, micro-RNA precursors, tRNA, and eIF1A	Inactivating mutations associated with microsatellite instability	Melo et al. (2010) ¹⁰
Exportin-6	Ran-binding protein 20, KIAA0370, FLJ22519	16p11.2	Actin	–	Stuven et al. (2003) ¹¹
Exportin-7	Ran-binding protein 16, KIAA0745	8p21.3	eIF4A1, ARHGAP1, VPS26A, VPS29, VPS35 and SFN, and p50RhoGAP	–	Mingot et al. (2004) ¹²

XPO1, exportin-1; CRM1, chromosome region maintenance 1; Rb, retinoblastoma; APC, adenomatous polyposis coli; FOXO, forkhead box O; CAS, cellular apoptosis susceptibility; tRNA, transfer RNA; SMAD3, mothers against decapentaplegic homolog 3; eIF, eukaryotic translation initiation factor; ARHGAP1, rho GTPase activating protein 1; VPS, vacuolar protein sorting-associated protein; SFN, stratifin.

toxicities (anorexia and nausea) and minimal clinical benefit in early studies limited development of leptomycin B.¹⁹ Newer pharmacologic agents, termed *selective inhibitors of nuclear export* (SINE), reversibly bind the XPO1 cysteine 528 residue. To date, the most extensively studied of the SINE is selinexor (KPT-330 [Karyopharm Therapeutics, Inc., Newton Centre, MA]). In multiple in vitro and in vivo models, selinexor has demonstrated single-agent anticancer activity and synergistic effects in combination regimens (Table 2). Globally, selinexor has been administered to more than 2100 patients. Common adverse events include low-grade nausea (62%), fatigue (60%), anorexia (51%), thrombocytopenia (42%), and vomiting (37%), which have generally been readily managed with standard supportive care measures.

As monotherapy, selinexor has induced responses in hematologic malignancies and yielded disease control in solid tumors.^{20–22} In one study, 31% of evaluable patients had an objective response with use of selinexor across a spectrum of non-Hodgkin's lymphoma subtypes, with a median duration of response exceeding 10 months.²² In another study, among 157 evaluable patients with advanced or metastatic solid tumors, single-agent selinexor resulted in an objective

response rate of 4% and a stable disease rate of 43%.²³

Preclinical Studies and Clinical Trials in Lung Cancer

XPO1 is overexpressed in lung cancer cells, particularly those arising in the setting of exposure to nicotine-derived nitrosamine ketone (a tobacco carcinogen).²⁴ Preclinical studies have demonstrated antitumor activity of SINE in NSCLC cell lines and xenografts.^{25,26} SINE have shown efficacy against EGFR inhibitor-resistant NSCLC cell lines in a time- and dose-dependent manner.²⁶ Synergism with chemotherapy and radiation therapy has been demonstrated in the presence of diverse molecular alterations, including *EGFR*, *p53*, *RAS*, and phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha gene (*PIK3CA*) mutations.^{25,27}

Efficacy against *KRAS*-mutant lung adenocarcinoma, a disease setting lacking specific targeted therapies to date, appears particularly promising. In a multigenomic screen of 4700 biological processes in more than 100 human NSCLC cell lines, nuclear transport machinery emerged as the sole process exhibiting synthetic-lethal

Table 2. Inhibitors of Nuclear Export Tested in Humans

Drug	Parent Company	Molecular Target	Trial Phase	Indication for Human Use	ClinicalTrials.gov Identifier(s)
First-generation nuclear export inhibitor					
Leptomycin (also called elactocin)	Warner-Lambert/Pfizer, New York, New York	Exportin-1	1	Advanced refractory solid cancers	
Second-generation nuclear export inhibitors					
SL-801	Stemline Therapeutics, Inc., New York, New York	Exportin-1	1	Advanced solid tumors	NCT02667873
Selinexor (KPT-330)	Karyopharm Therapeutics, Inc., Newton Centre, MA	Exportin-1	2/ 3	Acute myelogenous leukemia, myelodysplastic syndrome, T-cell lymphoma, B-cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, glioblastoma, gynecological cancers, lung cancer, head and neck cancer, sarcoma, melanoma, breast cancer, prostate cancer, colon cancer, pancreatic cancer, gastric cancer, esophageal cancer, and salivary gland tumors	(>50 trials in total). Lung cancer trials: NCT02536495, NCT02351505, NCT03095612, NCT02250885, NCT02213133
Verdinexor (KPT-335)	Karyopharm Therapeutics, Inc., Newton Centre, MA	Exportin-1	1	Antiviral agent (approved for canine lymphoma)	NCT02431364
KPT-8602	Karyopharm Therapeutics, Inc., Newton Centre, MA	Exportin-1	1/ 2	Relapsed refractory multiple myeloma	NCT02649790

interactions in *KRAS*-driven cancers.²⁸ In this study, the primary mechanism of cell kill was intolerance to nuclear accumulation of I κ B with consequent inhibition of nuclear factor κ B transcription activity. Rare cases of intrinsic resistance (<20%) were associated with follistatin-like 5 gene (*FSTL5*) mutations and attributed to yes-associated protein 1 (YAP1) activation. With few exceptions, nuclear export inhibition had limited efficacy against *KRAS* wild-type cell lines.

In summary, the broad genomic landscape in lung cancer makes it an attractive clinical setting for SINE. To date, selinexor trials in advanced squamous cell lung cancer (NCT02536495) and relapsed SCLC (NCT02351505) have been initiated.²³ A phase 1/2 trial in previously treated advanced *KRAS*-mutant NSCLC is under way (NCT03095612).

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