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Review

Clinical translation of nuclear export inhibitors in cancer

William T. Senapedis*, Erkan Baloglu, Yosef Landesman

Karyopharm Therapeutics, Inc., 2 Mercer Road, Natick, MA 01760, United States

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ABSTRACT

Clinical targeting of multi-dimensional proteins such as the proteasome has been efficacious in recent years. Inhibitors such as bortezomib and carfilzomib have been used successfully to treat multiple myeloma despite early skepticism surrounding unsubstantiated toxic side effects. Another target of this magnitude is ready to emerge as a clinically viable option for targeting various neoplasias. This target, XPO1 (exportin-1 also known as Chromosome Region Maintenance 1 (CRM1)), is the transport protein responsible for nuclear export of many of the major tumor suppressor proteins and cell growth regulators. Up-regulation of XPO1 protein, a common occurrence in a variety of cancers, can lead to aberrant cytoplasmic localization and degradation of tumor suppressors such as p53 and FOXO. Therefore, inhibition of XPO1 using specific small molecules collectively called Selective Inhibitors of Nuclear Export (SINE) could potentially restore normal tumor suppressor function and have universal application for the treatment of cancer. This review will discuss the current pre-clinical data on SINE compounds in both hematological and solid malignancies. Cancer treatment through direct inhibition of the proteasome and the nuclear export machinery should instill optimism for further targeting of critical cellular pathways.

1. Introduction

Cellular reactions are highly specialized processes in which temporal and spatial control are critical for maintaining proper growth and proliferation. Precisely controlled compartmentalization within the cell is critical to maintaining complex processes and sustaining life at every level. If the delicate balance is disrupted in this ecosystem, it can lead to cancer cell development. Eukaryotic cells are divided into two major compartments, the cytoplasm and the nucleus, separated by a physical barrier (nuclear membrane) which controls intracellular signaling. DNA synthesis, RNA transcription/transport, protein translation/maturation, and cell division are only a few of the critical cellular functions that depend upon nuclear transport [1–4]. Delicate control of nuclear import and export is provided by the karyopherin-β family of proteins and the nuclear pore complex (NPC) [5–9]. The NPC is made up of many nucleoporins which allows molecules less than 40 kDa to passively move through the nuclear envelope. Active transport for larger macromolecules requires karyopherins. These transporters interact with a chaperone protein (Ran) and the NPC embedded in the nuclear envelope to actively shuttle >40 kDa proteins into and out of the nucleus [10]. Karyopherins can be hijacked by viruses to promote infections and viral replication [11–14]. DNA amplification/mutation of karyopherins can also cause improper localization of cellular components and can lead to neuronal diseases, chronic inflammation, and cancer [4,8,10,15–20].

Targeting of critical cellular mechanisms, such as the proteasome pathway, for treatment of cancer have been initially viewed with skepticism, though eventually open new avenues for the treatment of multiple myeloma and other cancers. For example the introduction of bortezomib which specifically targets the proteasome was regarded as potentially lethal to normal cells. The prevailing concern was that inhibiting such an essential cellular process necessary for all cells would be severely toxic to healthy cells and was destined for failure before being tested in human trials. Despite the early angst in targeting the proteasome, bortezomib and now carfilzomib are FDA-approved proteasome inhibitor drugs used for the treatment of multiple myeloma with the potential to treat other forms of neoplasia [21,22]. Similar concerns are being articulated about targeting nuclear export because of the failure of Leptomycin B (elactocin) in a single Phase I human trial completed, due to highly toxic side effects with no therapeutic index. However, almost 20 years since the first clinical trial of nuclear export inhibition, Selective Inhibitors of Nuclear Export (SINE) designed through a combination of traditional structure–activity relationship (SAR) and computational modeling show promise in pre-clinical applications across a diverse array of cancer cells with

* Corresponding author. Tel.: +1 781 350 0868.
E-mail addresses: william@karyopharm.com, william.senapedis@gmail.com (W.T. Senapedis).

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improved tolerability [15,23]. Additionally, outcomes of several ongoing Phase I/II clinical trials (KPT-330/selinexor; clinicaltrials.gov) suggest that oral SINE has clear anti-cancer activity with acceptable tolerability across multiple solid and hematological malignancies and may help build continued trust in future targeting of principal cellular pathways. This review will cover pre-clinical development of nuclear transport inhibitors for the treatment of neoplasia.

2. Nuclear transport machinery

The eukaryotic cell is divided roughly into two milieus: the cytosol, which is separated from the surrounding environment by the plasma membrane, and the nucleoplasm, which is enveloped by the nuclear membrane. DNA/RNA synthesis, protein translation, cell growth, proliferation, apoptosis, etc. are all dependent on tight control of biochemical processes that occur in the cytoplasm and the nucleus as well as crosstalk and transport between the two compartments [1–9].

Tyrosine kinase receptors (TKRs) and ion channels direct most of the signals at the plasma membrane (PM) [24–26]. At the nuclear surface molecules less than 40 kDa passively move through a large mega-Dalton multi-meric protein called the nuclear pore complex (NPC). For proteins greater than 40 kDa, a family of ~15 transporter proteins called karyopherin-βs chaperone shuttling of cargoes into and out of the nucleus through the NPC (Fig. 1) [7,9]. The NPC is made up of ~30 different types of nucleoporins (Nups) and physically resembles a basketball hoop with a proteinaceous net localized to the nucleus with cilia-like fibrils protruding into the cytoplasm [3]. The pore itself is lined with hydrophobic sequences of phenylalanine-glycine residues, or FG-repeats, that facilitate directed transport of importin-bound cargoes [3]. Most karyopherins have very specialized nuclear import or export function with three transporters (Karyopherin-α/β and exportin-1) being the best studied to date [7,9]. Nuclear import is most notably regulated by importin-α (karyopherin-α) and importin-β1 (karyopherin-β1) [7,9]. Importin-α recognizes and binds to cargo proteins containing a short canonical sequence of basic amino acids referred to as a nuclear localization signal (NLS) [7] (Fig. 1). Once in the nucleus, release of imported cargoes is prompted by the charged form of Ran (Ran-GTP, a member of the Ras family of small GTPases) [4,7,9,27]. Although importin-α/β1 cargo transport is typical, it is important to note that there are proteins imported by importin-β1 without the need of the accessory transporter importin-α [7].

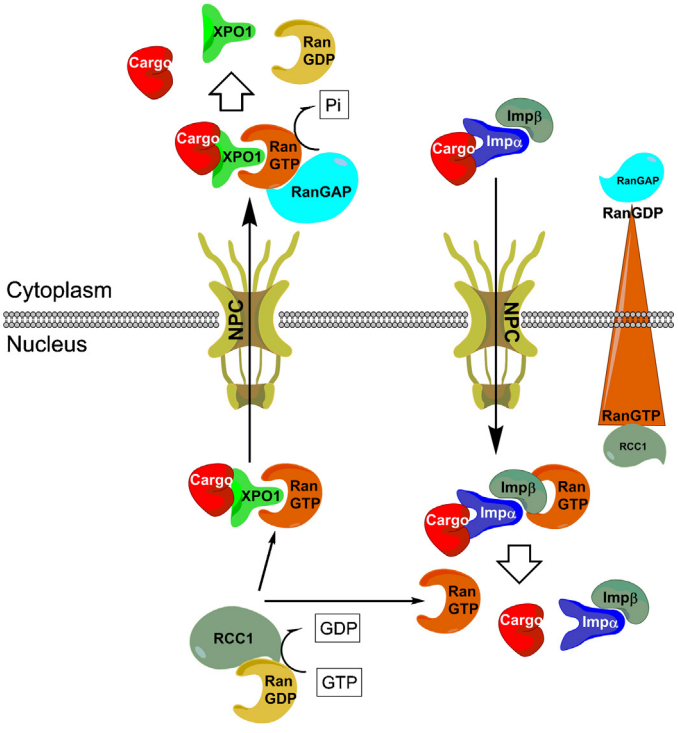

Fig. 1. Nuclear import occurs when importin-α (Impα) recognizes and binds the nuclear localization signal (NLS) on cargo proteins. Import is facilitated through subsequent binding of importin-β (Impβ) in the cytoplasm and the multi-meric complex is transported through the nuclear pore complex (NPC). Once in the nucleus RanGTP binding disrupts the import complex releasing cargo proteins. Nuclear export occurs when exportin-1 (XPO1) recognizes and binds the nuclear export signal (NLS) on cargo proteins. Export is facilitated through subsequent binding of RanGTP and transported through the NPC. Once in the cytoplasm RanGTP is hydrolyzed to RanGDP by Ran GTPase activating protein (RanGAP) and releasing XPO1 and cargo proteins. These two proteins (RCC1 and RanGAP1) ensure tight control of import and export through the Ran GDP:GTP gradient.

Nuclear export is controlled by similar basic principles and some of the same proteins but in the opposite direction (Fig. 1). Chromosome Region Maintenance 1, or exportin-1 (CRM1/XPO1), binds short, canonical leucine-rich sequences called nuclear export signals (NES) in cargo proteins marked for nuclear export in the presence of GTP-loaded Ran [5,16,28–39]. This complex passes through the nuclear pore to the cytoplasm where hydrolysis of Ran-GTP to Ran-GDP releases XPO1 cargoes. The GTP exchange factor, regulator of chromosome condensation 1 (RCC1) is exclusively sequestered in the nucleus, leading to increase concentration of Ran-GTP in the nucleus. On the other hand, GTPase activating protein, RanGAP1, is localized to the cytosol, leading to accumulation of Ran GDP. These two proteins (RCC1 and RanGAP1) ensure tight control of import and export through the Ran GDP:GTP gradient. Additionally, many cargo proteins undergo post-translational modification and adopt conformational changes to ensure spatial and temporal control of NLS/NES exposure leading to proper compartmentalization [40–44].

3. Nuclear export and inhibitors

Correct compartmentalization is necessary for proper cellular growth and apoptosis control. There are numerous studies showing that protein up-regulation, or RNA/DNA amplification of importin α, β1 and XPO1, correlates with neoplasia and poor cancer prognosis. For example, increased mRNA and/or protein expression of karyopherins has been observed in ovarian, pancreatic, and cervical cancer cells as well as in glioma, osteosarcoma, renal cell carcinoma, metastatic melanoma, leukemias, multiple myeloma, and mantle cell lymphoma cell lines [45–57]. The same elevation of mRNA or protein has been shown in large cohorts of patient samples [45–57]. These observations support a hypothesis that perturbation of the delicate balance of nuclear transport can change the spatial localization of certain RNAs and proteins in the cell and therefore may lead to cancer and various other maladies.

XPO1 controls the nucleo-cytoplasmic localization of more than 200 NES-containing proteins, many of which are major tumor suppressor proteins (TSPs), cell cycle and immune response regulators [33,35]. A majority of these proteins are linked to cancer, including p53, p21, p27, adenomatous polyposis coli (APC), retinoblastoma (pRb), forkhead box protein O (FOXO), IκB, and topoisomerase II (topo IIa) [15,16,50,58–62]. Cytoplasmic localization of these proteins away from DNA promoter sequences and other target proteins (kinases, oncogenes, etc.) can lead to aberrant growth signals and inactivation of apoptosis, which are hallmarks of cancer development and progression. Cancer cells can co-opt the nuclear transport machinery to prevent proper cell cycle and apoptosis signals from