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The role of nuclear pore complex in tumor microenvironment and metastasis

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Abstract

One of the main reasons for cancer mortality is caused by the highly invasive behavior of cancer cells, which often due to aggressive metastasis. Metastasis is mediated by various growth factors and cytokines, operating through numerous signaling pathways. Remarkably, all these metastatic signaling pathways must enter the nucleus through a single gatekeeper, the nuclear pore complex (NPC). NPCs are the only gateway between the cytoplasm and the nucleus. NPCs are among the largest proteinaceous assemblies in the cell and are composed of multiple copies of around 30 different proteins called nucleoporins (Nups). Here we review what is currently known about the NPC and its role in the mechanisms of tumor progression. We will also explore potential strategies to target metastatic pathways by manipulating the karyopherins (importins/exportins) of nucleocytoplasmic traffic through NPCs.

1 Introduction

Metastasis (from the Greek word for “change”) is the main cause of morbidity and death in most cancers [1]. Metastasis is cancer’s most deadly aspect; it is affected by several interconnected processes, including cell proliferation, angiogenesis, cell adhesion, migration, and invasion into the surrounding tissue [2]. Metastatic progression consists of tumor cell invasion from the primary tumor, intravasation, arrest, and extravasation of the circulatory system, subsequently angiogenesis and growth at a distant site [2-4]. Primary tumors comprise heterogeneous populations of cells with genetic modifications that allow them to overcome physical boundaries, disseminate, and colonize separate organs [5]. While several explanations account for treatment failure in cancer metastasis patients, the main obstacle to effective treatment is the heterogeneity of tumor cells, which comprise subpopulations of cells with diverse angiogenic, invasive, and metastatic properties. Even though metastases can have clonal origins, genetic instability results in immediate biological diversification and the regeneration of heterogeneous subpopulations of cells. Metastatic cells can be stimulated by the environment of a particular organ, and by systemic therapy as the metastases travel to lymph nodes and different organs [3]. The consequences of

metastasis are governed by numerous interactions between metastatic cells and homeostatic mechanisms that are unique to different organ microenvironments, each of which governs the extent of cancer cell proliferation, angiogenesis and invasion, as well as survival. Thus, treatment and prevention of metastasis must be targeted against cancer cells generally, with particular emphasis on factors associated with the growth and survival of metastatic cells [2].

2 Microenvironment

According to the Paget's "seed and soil" theory, a tumor cell's capability for metastatic colonization is determined by its unique characteristics ("seed") and by the host organ that tumor cells come across as they travel ("soil"), implying that metastasis progression and development are not random. Metastasis results only when the "seed and soil" are compatible and well suited [6,7]. To gain entrance into the microcirculation, tumor cells must degrade connective tissue, extracellular matrix, and basement membrane components that constitute barriers against invading tumor cells [8]. Epithelial to mesenchymal transition (EMT) was first conceived by Krug et al. in 1987 but not characterized until 1995 [9,10]; it is defined as the switch from non-motile,

polarized epithelial cells to motile, non-polarized mesenchymal cells, with the potential to migrate from primary tumor sites to distant organs, where they can seed and grow [9,10]. There are several well-known EMT markers, including E-cadherin, N-cadherin, Snail 1, Slug, and Twist [11]. A hallmark of EMT is a process named the “cadherin switch,” defined by a decrease in E-cadherin, the major component of adherent junctions, and a simultaneous increase of mesenchymal N-cadherin. This switch allows cells to lose adhesive affinity for other epithelial cells and become more migratory and invasive [11-13]. N-cadherin, which attaches to the cytoskeleton through interactions with both α- and β-catenin, signals through the GTPases Rac1 and Cdc42, and associates with the platelet-derived growth factor (PDGF) receptor, to initiate actin remodeling. By this means, it causes modifications in both cell adhesive properties and migratory status [11,14]. In addition to transcriptional repressors, another most potent inducer of EMT is the transforming growth factor-β (TGFβ), a known pluripotent growth factor, able to induce EMT in mammary, lung, pancreatic, colon, and many other cell types [11,14,15]. For metastasis to occur, all steps in the metastatic cascade must be take place. Therefore, the blockade of any single step in the metastatic cascade should slow down metastasis progression.

Numerous growth factors and cytokines differentially regulate signaling pathways that promote tumor progression and metastasis. Interestingly, to initiate transcriptional responses, all these metastatic signaling molecules must enter the nucleus through a gatekeeper: the nuclear pore complex (NPC). Here, we review what is current known about the NPC and its role in tumor progression mechanisms. We will also discuss potential strategies to target metastatic signaling cascades by manipulating nucleocytoplasmic traffic through NPCs.

3 Nuclear pore complex signatures in cancer

3.1 Nuclear pore complex

Intracellular communication between the nucleus and the cytoplasm is accomplished through the NPCs, which are thousands of cylindrical holes, at sites where inner and outer nuclear membranes join. The NPCs are made of ~30 different proteins named nucleoporins (Nups) (Fig. 1A) [16]. Nucleoporins are designated “Nup” followed by their predicted molecular weight; they are modular in their frequent use of the same structural motifs (coiled-coils, α solenoids, β propellers) [17]. Approximately a third of nucleoporins contain domains of phenylalanineglycine (FG) motifs interspersed with spacer sequences. These repeat domains are natively unstructured and serve as

interaction sites for transport receptors (karyopherins), which escort cargo through the pores. Some excellent reviews on NPC structure and function are available [17-24]. Here, we first focus on several Nups that are often abnormal in cancer patients.

3.2 Rae1

Ribonucleic acid export 1 (Rae1; also called GLE2 and mRNP41) was discovered in *Schizosaccharomyces pombe* in a genetic screen for RNA export proteins [25]. Because its mammalian homolog protein was found to be UV-cross-linked *in vivo* to poly(A) containing RNA, it was termed mRNP41 [26]. Moreover, its homolog in *Saccharomyces cerevisiae* was discovered to be synthetically lethal in combination with a null mutant of the glycine-leucine-phenylalanine-glycine (GLFG) nucleoporin Nup100, so Rae1 was also named GLFG lethal 2 (Gle2) [27]. Rae1 directly interacts with Nup116 through Gle2-binding sequence (GLEBS) [28]. An evolutionary highly conserved GLEBS motif was also found in the only GLFG nucleoporin of vertebrates, Nup98 [29,30].

Rae1 contains seven WD40 repeats forming a seven-bladed β-propeller (Fig.1B). The β-propeller domain is a classic protein–protein interaction platform, capable of mediating associations with several proteins [31]. The interaction of Rae1 with the

Nup98 GLEBS motif appears to be critical to RNA export [30]. Moreover, Rae1 can interact with other proteins, not only in the nucleus but also in the cytoplasm, and reportedly has several functions in the formation of mitotic spindles [32-36]. In mitotic HeLa cells, Rae1 interacts and colocalizes with nuclear mitotic apparatus protein (NUMA) and cohesin subunit SMC1 to promote microtubule bundling at spindle poles [35,36]. Both depletion and overexpression of Rae1 lead to increased formation of multipolar spindles, an effect that can be counteracted by NUMA depletion or co-overexpression, respectively [35-37]. Imbalances in NUMA, SMC1 or Rae1 interactions cause formation of multipolar spindles and aneuploidy [33-35], increasing genomic instability and promoting tumorigenesis. Clinically, Rae1 is linked to breast cancer pathophysiology [38]. Remarkably, Rae1's binding partner, Nup98, is a proto-oncogene identified in numerous leukemogenic fusions with a variety of partner genes [39,40].

3.3 Nup98

Nup98 is expressed in two major forms, which vary because of differential splicing [41]. The first splice variant encodes a 920-amino acid protein (Fig. 1B). Nup98's N-terminal half contains FG and GLFG repeat motifs and is bisected by a small

coiled-coil domain (AA 181–224) that binds to the β-propeller nucleoporin Rae1; its C-terminus is a domain with a unique, β-sandwich structure that controls autoproteolytic activity [42] and is also important for directing Nup98 to the NPC [17,43,44]. Nup98 knockout mice die early in embryonic development [45]. The GLFG repeat region of Nup98 associates with nuclear transport receptors including importin-β, transportin, and the mRNA export receptor TAP [46]. The Nup98/Rae1 complex reportedly binds to the cdh1-form of anaphase-promoting complex/cyclosome (APC/C) [47,48]. In a clearly distinct mitotic role, Rae1 was found in a large complex of proteins (NuMA, cohesins) and RNA, required for mitotic spindle assembly, although Nup98 in this complex was not directly seen [17,49]. However, Nup98 is reported to regulate mitotic centromere-associated kinesin depolymerization activity independently during mitosis [50].

Two groups simultaneously reported Nup98 to be part of recurrent chromosomal translocation in acute myeloid leukemia (AML) patients [51,52]. This translocation (7p15:11p15) fuses the 5′ half of the *NUP98* gene with the 3′ portion of the *HOXA9* gene (Fig. 1B). The resulting 59 kDa fusion protein is expressed under control of the Nup98 promoter and contains nearly all the FG/GLFG repeats of Nup98 with the Rae1 binding site, and the DNA-binding homeodomain of HoxA9 [17]. Nup98/homeodomain

translocations are often found in AML patients, but are also related to chronic myeloid leukemia and pre-leukemic myelodysplastic syndrome (MDS) [39,40,53].

3.4 Translocated promoter region (Tpr)

Translocated promoter region (Tpr) is a 265 kDa nucleoporin found only at the nucleoplasmic face of the pore; it is a major component of the nuclear basket (Fig. 1A) [54]. While Tpr does not have nucleoporin FG repeats, it does comprise several heptad repeat or leucine zipper motifs [55]. Reportedly, Tpr forms filamentous structures, which originate from the nuclear basket and extend into the nuclear interior of amphibian oocytes [56], although the presence of such filaments in cultured somatic cells is debated [17,57,58]. Notably, Tpr may mediate the mitotic spindle checkpoint as binding partner and regulator of Mad1 and Mad2 [59-61].

Remarkably, Tpr was named for its initial isolation from a carcinogen-treated osteogenic sarcoma line as part of a chromosomal translocation (1q25:7q31) that fused N-terminal sequences of Tpr to the kinase domain of the proto-oncogene, Met [62]. Dimerization through the heptad repeats leads to constitutive activation of Met kinase activity, independent of ligand binding [63]. Tpr-Met was the first such activating fusion of a tyrosine kinase receptor to be identified, and served as the prototype for

understanding oncogenes producing translocated tyrosine kinase receptors that are activated by dimerization [17,64], of which more than 25 have been identified. Met is the cell-surface tyrosine kinase receptor for hepatocyte growth factor (HGF). Both Met and HGF have essential roles in cell migration during development, and in repair of adult tissues and in angiogenesis [17,64]. While Tpr translocations are uncommon in human tumors, Tpr-Met translocation is related to gastric carcinoma, in which it is thought to represent an early step in carcinogenesis [17].

3.5 Nup88: a potential marker for high grade tumors

Nup88 is a non-FG nucleoporin found mainly on the cytoplasm side of NPCs (Fig.1A). It is predicted to form two common NPC structural motifs: a β-propeller structure at the N-terminal domain and coiled-coils at the C-terminal domain (Fig. 1B). Nup88 interacts with the FG-repeat in nucleoporin Nup214 during the cell cycle [17] and in some systems, Nup62 [65].

Immunohistochemistry from Nup88 antibodies revealed overexpression of Nup88 in 75% of ovarian tumors [66]. Moreover, Nup88 was found to be overexpressed in a broad spectrum of sarcomas, lymphomas and mesotheliomas [67,68]. However, immunoblotting of several lung carcinoma samples showed that Nup88 overexpression

did not correlate with overexpression of the other nucleoporins tested, Nup214 and Nup153. Consistently, PCR quantitation of Nup88 confirmed that high Nup88 transcript levels correlated with a malignant phenotype [69]. Therefore, Nup 88 overexpression does not seem to indicate increased production of NPCs or upregulation of the Nup214/Nup88 subcomplex [17]. In tumors, Nup88 staining is prominent in the cytoplasm, often in granular dots [17], which agrees with earlier studies indicating that Nup88 accumulates in the cytoplasm when transiently overexpressed in cultured cells [70]. Notably, strong Nup88 staining was seen only in developing lung epithelia and intestinal crypts in normal fetal tissue samples [67]. In tumor tissues, intensity of Nup88 staining is correlated with tumor grade. Benign tumors and mild hyperplasias displayed little to no evidence of overexpression, whereas Nup88 overexpression was mainly found in more advanced tumors [17]. High Nup88 expression in breast, colorectal and hepatocellular carcinomas were often noted around the edges of tumors, suggesting a link to tumor aggressiveness [17,68,69]. Nup88 expression seems to increase during progression of carcinogenesis and to be most associated with poorly differentiated tumors. Therefore, Nup88 has been proposed as a marker of tumor state and a potential indicator of patient prognosis in breast, colorectal and hepatocellular carcinomas [17].

3.6 Nup214/CAN

Nup214/CAN is an FG-repeat nucleoporin usually found in the cytoplasmic face of the NPC (Fig. 1A). Nup214/CAN contains repeats of both FG and FxFG types; its repeat domain is a high-affinity binding site for the nuclear protein export receptor, CRM-1/exportin-1. Other studies point to its role in mRNA export; indeed, the *S. cerevisae* Nup214/CAN homolog, Nup159, recruits RNA helicase Dbp5, a cofactor in mRNA export [71-74]. Like Tpr, the *Nup214/CAN* gene was first recognized as a component of a chromosomal translocation and, presumably because of its proximity to the *c-Abl* gene, was called *Cain* (*CAN*). This first chromosomal translocation (6p23:9q34) joined the *DEK* and *CAN* genes [75], and was followed shortly thereafter by identification of an intrachromosomal translocation (9q32:9q34) that fused the *SET* and *CAN* genes [76]. These translocations result in expression of fusion proteins that join virtually full Dek, Set or Abl proteins to the C-terminal two-thirds of Nup214, including a portion of the coiled coil domain and the FG-repeat domain (Fig. 1B) [17].

4 NPC as gatekeeper for metastasis activator traffic into nucleus

The transport of transcription factors or metastasis activators (Fig. 2A) and RNA across the nuclear envelope commonly requires transport factors

(karyopherins/importins) that bring cargo to the nuclear pore. Nuclear transport factors can move in and out of the nucleus due to their interaction with nucleoporins. This transportation process requires metabolic energy and is enhanced by a concentration gradient across the nuclear membrane of the GTP-bound form of the G-protein Ran, which is found mainly in the nucleus. The transport substrates are distinguished in their amino acid sequence by the presence of cis-acting nuclear localization signals (NLS) and/or nuclear export signals (NES). In particular, molecules that contain arginine/lysine-rich NLS are transported into the nucleus by the importin-α/β pathway. The classic NLS contains of either a single cluster of basic amino acids (monopartite NLS), or two such clusters separated by a nonspecific linker 10–12 amino acids long (bipartite NLS). The classic NLS binds directly to importin-α. Six importin-α isoforms have been identified in humans: importin-α1, importin-α3, importin-α4, importin-α5, importin-α6 and importin-α7 [77]. Based on their sequence similarity, importin-α molecules have been classified into three distinct subfamilies. All importin-α molecules have the ability to mediate nuclear import of NLS-containing substrates. However, importin-α molecules are functionally divergent. Importin-α associates with importin-β, which is responsible for docking importin–cargo complexes to the NPC cytoplasmic side, followed by translocation of the complex through the NPC [77-80]. Transcription

factors and metastasis-activating proteins import into nuclei, requiring association and dissociation between transport substrate, transport factors, and nucleoporins [17-24]. Here, we discuss (a) potential strategies to target metastatic pathways by manipulating NPC nucleocytoplasmic trafficking; (b) several oncologic pathways, such as those mediated by epidermal growth factor (EGFR), TGFβ, tumor necrosis factor receptor, β-catenin, hypoxia inducible factor (HIF), galectin-3 and NF-κB (Fig. 2A); and (c) metastasis regulation through NPC's orchestration of microRNA (miRNA) export (Fig. 5).

4.1 NPC regulates EGFR signal activator traffic into the nucleus

Epidermal growth factor signaling is initiated by binding EGF family members to the extracellular domain of erythroblastic leukemia viral oncogene homologue (ERBB) receptors. The ERBB receptor tyrosine kinase family consists of four members: epidermal growth factor receptor (EGFR; also called ERBB1 or HER1), ERBB2 (also called HER2, ERBB3 or HER3) and ERBB4 (also called HER4) [79-81]. Receptors and ligands of ERBB are frequently overexpressed by carcinoma cells [82], with EGFR and ERBB3 overexpressed in 50–70% of lung, colon and breast carcinoma, ERBB2 in 30%

of breast cancer patients, and ERBB4 in 50% of breast cancer patients and 22% of colon cancer patients [79].

Recent evidence shows that EGFR family receptors can be shuttled from the cell surface to the nucleus, where they transduce signals [80]. The NLS-containing proteins are transported into the nucleus by forming complexes with either importin-α/β or importin-β alone. Importin-β is responsible for nuclear translocation by directly associating with nucleoporins. Interestingly, various cell surface receptor tyrosine kinases (RTKs), including EGFR, ErbB-2 and FGFR1, translocate to the nucleus by importin-β–dependent mechanisms [80]. Moreover, the putative NLS of EGFR and ErbB-2 has been identified and importin-β has been shown to interact with it [83,84]. The tripartite NLS of EGFR, which comprises three clusters of basic amino acids (RRRHIVRKRTLRR; amino acids 645–657) is conserved among EGFR family members; it is located at the intracellular carboxyl terminus of EGFR. Interestingly, importin-β colocalizes with ErbB-2 in the endosomes, and importin-β, ErbB-2 and Nup358 (a nucleoporin located at the NPC cytoplasmic filaments), form a tri-complex and colocalize near the nuclear envelope (NE) [85]. These studies propose that the endocytic vesicles/endosomes may work as vehicles for EGFR/ErbB-2, using importin-β to convey cargo proteins through the NPC for nuclear translocation

[80,83,85]. Hung et al. proposed the term INTERNET, (integral trafficking from the ER [endoplasmic reticulum] to the NE transport), for the inner nuclear membrane targeting process of ER-to-NE transport of integral membrane proteins (Fig. 2B) [82]. This model provides a new direction for understanding how membrane-bound EGFR-family RTKs traffic from cell surface to nucleus [82].

4.2 NPC regulates TGFβ signal activator traffic into the nucleus

TGFβ family cytokines, including TGFβ1, β2, and β3, are members of a large superfamily of pleiotropic growth factors, which include activins and bone morphogenetic proteins (BMPs) [86-89]. The TGFβ family of cytokines orchestrates complex physiological processes such as cell proliferation, differentiation, adhesion, matrix production, motility, and apoptosis. Initially, TGFβ was discovered as a component of a tumor cell secretion that produced a transformed phenotype in normal fibroblast [90]. With the identification of inactivating mutations within components of the TGFβ pathway in cancers, it became clear that TGFβ mediates a tumor suppressor pathway for many different types of cancer [91]. However, late-stage human carcinomas often become resistant to TGFβ growth inhibition, even while secreting elevated levels of TGFβ. Genetic manipulation of the TGFβ pathway in tumor cell lines and

experimental animal models validated the metastasis-promoting function of TGFβ in late-stage cancer progression [89,91]. Our current understanding of the TGFβ-SMAD pathway is discussed in great detail by several excellent recent reviews [87-93]. Clinical and experimental studies of metastasis have begun to shed light on the involvement of Smad proteins in the metastasis-enhancing function of TGFβ [91].

Recent studies have elucidated theTGFβ-SMAD pathway to the nucleus (Fig. 3A). Massagué et al. showed that SMAD nuclear import is mediated by direct interaction with Nup214/CAN [94], and found a direct interaction between SMAD and Nup153, a nucleoporin located at the nuclear side of the NPC [95]. By interacting with Nup214/CAN and Nup153, SMAD is capable of nuclear import as well as export (Fig. 3A). The Smad anchor for receptor activation (SARA) in the cytoplasm and SMAD DNA binding cofactor Fox in the nucleus compete with Nup214/CAN and Nup153 for recognition of overlapping hydrophobic patches on the MH2 domain of SMAD [94].

Similarly, direct binding of STAT to nucleoporins indicates that unphosphorylated STATs migrate into nuclei via specific molecular interactions with NPC components. Without cytokine stimulation, some unphosphorylated STAT proteins (STAT1, STAT3, and STAT5) can undergo rapid translocation through nuclear pores in a cytosol-unassisted manner, independent of carriers or metabolic energy. Thus, both

carrier-dependent and -independent translocation pathways determine intracellular distribution of STAT proteins (Fig. 3B) [96] .

4.3 NPC regulates galectin-3 signal activator traffic into the nucleus

Galectin-3, a member of an evolutionarily conserved family of β-galactoside-binding proteins, is widely expressed, and is involved in various biological functions. It can be found in cytoplasm, the nuclei, and even in extracellular spaces. It shuttles between cytoplasm and nucleus, where it undergoes post-translational modification such as phosphorylation of a serine residue by the protein kinase casein kinase 1 (CK1), which signals its export to cytoplasm and serves as a molecular switch for its sugar binding ability. Nuclear localization of galectin-3 is probably associated with normal cell proliferation because it is a required factor in the splicing of pre-mRNA [97-99]. In cancer, galectin-3 plays a significant role in tumor progression. It affects growth promotion and cell-cycle regulation through induction of cyclin D1 and c-Myc in nuclei of human breast epithelial cells. *In vivo*, the cytoplasmic versus nuclear expression of galectin-3 is associated with tumor invasion and metastasis. Loss of nuclear galectin-3 expression is reported in colon and prostate carcinomas. Similarly, nuclear galectin-3 levels are markedly decreased during progression from normal to

cancerous states in tongue carcinomas. In lung carcinoma, nuclear galectin-3 is a predictor of recurrence and/or poor prognosis. In patients with esophageal squamous cell carcinoma, elevated nuclear galectin-3 expression may be an important pathological parameter, related to histological differentiation and vascular invasion [97-99].

Human galectin-3 has a NLS-like sequence, 223HRVKKL228, in the C-terminal region that is similar to p53 and c-Myc NLSs. Proteins containing NLS, such as p53, are transported into the nucleus by the importin-α/β (karyopherin) complex. Importin-α is the receptor subunit that recognizes NLS, a cluster of basic amino acid residues that complex with importin-β to pass through the nuclear pore. Nakahara et al. showed that deletion of the C-terminal region of galectin-3 protein without the NLS-like motif (1–222) results in complete impairment of nuclear accumulation, whereas a C-terminal deletion protein that includes this motif (1–229) can be accumulated in the nucleus [97-99]. In addition, substitution of Arg to Ala at position 224 (R224A) of human galectin-3 efficiently abolishes nuclear localization. Nakahara et al. revealed that galectin-3 directly binds to importin-α proteins and complexes with importin-α/β complex *in vivo* (Fig. 4A). Moreover, failure to translocate from the cytoplasm to the nucleus resulted in a rapid degradation of galectin-3 [97-99].

4.4 NPC regulates β-catenin signaling activator traffic into the nucleus

Adenomatous polyposis coli (APC) and β-catenin, two key interacting proteins implicated in development and cancer, travel in and out of the nucleus in response to internal and external signals [100]. The Wnt/β-catenin signaling cascade is a core signal transduction pathway driving tissue morphogenesis during both development and tumor initiation and progression in human cancers. Wnt's mechanism for regulating the nuclear signaling form of β-catenin is a major research focus [101]. Although Wnt causes β-catenin to enter the nucleus, it is unclear whether other cell-surface changes (such as changes in the adhesion complex) also induce nuclear translocation of β-catenin. The physiological conditions under which membrane-associated β-catenin is released are also poorly understood (Fig. 2A) [100].

4.5 NPC regulates hypoxia signal activator traffic into the nucleus

Multicellular organisms have adaptive mechanisms to maintain cellular and tissue function upon reduction of O_2 tension. The important mediator of these mechanisms is the hypoxia-inducible transcription factor-1 (HIF-1). HIF-1 consists of two subunits: HIF-1α and ARNT (HIF-1β) [102]. In order to function, HIF-1α must enter the nucleus. Entry of proteins into the nucleus is mediated by nuclear import receptors, which

typically belong to the importin-β family. Fifteen members of this family act as monomers in mammalian cells, while 11 receptors are heterodimers of importin-β, with an adaptor protein such as importin-α. All these receptors interact with their transport cargoes by associating with nuclear import signals [103]. Mylonis et al. found that HIF-1α is a shuttling protein that can be exported from the nucleus to the cytoplasm by CRM1 via an atypical C-terminal hydrophobic NES located at amino acids 632–639 [104]. Nuclear export of HIF-1α is inhibited by MAPK-dependent phosphorylation of two serine residues (Ser641 and Ser643) that lie in close proximity to the NES [105]. It is thus possible that nuclear accumulation of HIF-1α and its regulation may comprise both nuclear import and nuclear export routes (Fig. 2A) [102].

4.6 NPC regulates NF-κB signal activator traffic into the nucleus

NF-κB transcription factors are dimers of polypeptides belonging to the Rel family of proteins. Under basal conditions, members of the inhibitor of kappa B (IκB) family retain NF-κB in the cytoplasm in an inactive state, thereby inhibiting transcription [106]. In mammals, the NF-κB family of transcription factors has five members: p50, p52, p65 (RelA), c-Rel and RelB [77,106]. The heterodimer p50/p65 is the most common form of

NF-κB in most cell types, but all members of the family can associate to form homo- or heterodimers, except for RelB, which can only form heterodimers *in vivo* [77].

Activation of NF-κB is controlled mainly via nuclear translocation. In addition, in response to initiating stimuli, IκB members dissociate from NF-κB, which unmasks NLSs present in the NF-κB subunits [107]. This allows translocation of NF-κB to the nucleus and subsequent transcriptional activation of target genes. Regulation of NF-κB is achieved through a myriad of proteins, and can occur via different mechanisms [107]. One of these mechanisms is regulated export of NF-κB subunits out of the nucleus [108]. CRM1 is a nuclear export receptor located on the nuclear envelope; it recognizes leucine-rich NESs in NF-κB subunits and thereby transports NES-containing proteins into the cytoplasm. Inhibition of CRM1-dependent nuclear export under basal conditions results in accumulation of NF-κB within the nucleus, indicating that NF-κB constitutively shuttles between the cytoplasm and the nucleus [109]. Besides IκB-α and NF-κB, other regulatory proteins of the NF-κB pathway undergo regulated nucleocytoplasmic shuttling to control transcription of NF-κB responsible genes (Fig. 4B) [107,110].

5 NPCs orchestrate export of miRNAs serving as metastasis suppressors

MicroRNAs are functional RNA molecules that are transcribed from DNA sequences of RNA genes, but not translated into protein [111]; MiRNAs are small noncoding, double-stranded RNA molecules that mediate expression of target genes with complementary sequences [112-114]; they bind to their target mRNAs based on sequence complementarity, and inhibit protein translation by degrading mRNA. In cancer, miRNAs function as regulatory molecules, acting as oncogenes or tumor suppressors [111]. Recently, Ma et al. found that miR-10b is more highly expressed in breast cancer metastatic cells than it is in nonmetastatic cells [115], although their results are debated by Gee et al. [116].

With the exception of a minor class of miRNAs called mirtrons [114], which are processed by the RNA splicing machinery, most miRNAs are processed from primary transcripts into precursor miRNA (pre-miRNAs) within the nucleus by a complex containing the RNase III enzyme Drosha and its partner DGCR8 [114]. Pre-miRNAs are transported to the cytoplasm by the nuclear karyopherin exportin-5 in a Ran-GTP-dependent manner, and processed a second time by the RNase III family member Dicer to generate a ~20–25-nt duplex, one strand of which is incorporated into

the RNA-induced silencing complex (RISC) (Fig. 5) [111-113]. Which nucleoporins are responsible for miRNA-exportin 5 complex, or for mirtron exportation, is currently unclear.

Perspectives

Each Nup contributing to carcinogenesis seems to do so in a unique manner, and each Nup's contribution to tumor progression is largely unknown. Therefore, although Nups offer potential targets for cancer therapies, strategies using different metastatic pathways through manipulation of NPC karyopherins (importins/exportins) have not been thoroughly investigated.

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Figure legends

Fig. 1 Schematic representation of the nuclear pore complex (NPC) and nucleoporins. **A)** Nuclear pores are large complexes embedded in the nuclear envelope. The NPC consists of ~30 different nuclear pore proteins (nucleoporins). Modified from [17, 21]. **B)** Domain organization of nucleoporins and chimeric fusion proteins following chromosomal translocations. Modified from [17].

Fig. 2 A) Diagram of signaling pathway and cascades of metastasis through NPC. **B)** Model of epidermal growth factor receptor (EGFR) trafficking. After ligand-induced activation, EGFR is immediately internalized, mainly by clathrin-dependent endocytosis. Internalized EGFR embedded within early endosomes is transported to the nucleus. Several potential mechanisms may be involved in nuclear trafficking of EGFR; a newly proposed pathway is shown here [82]. EGFR localized in the ER is transported to the nucleus through the NPC via outer nuclear membrane and inner nuclear membrane. EV: endocytic vesicle; ER: endoplasmic reticulum.

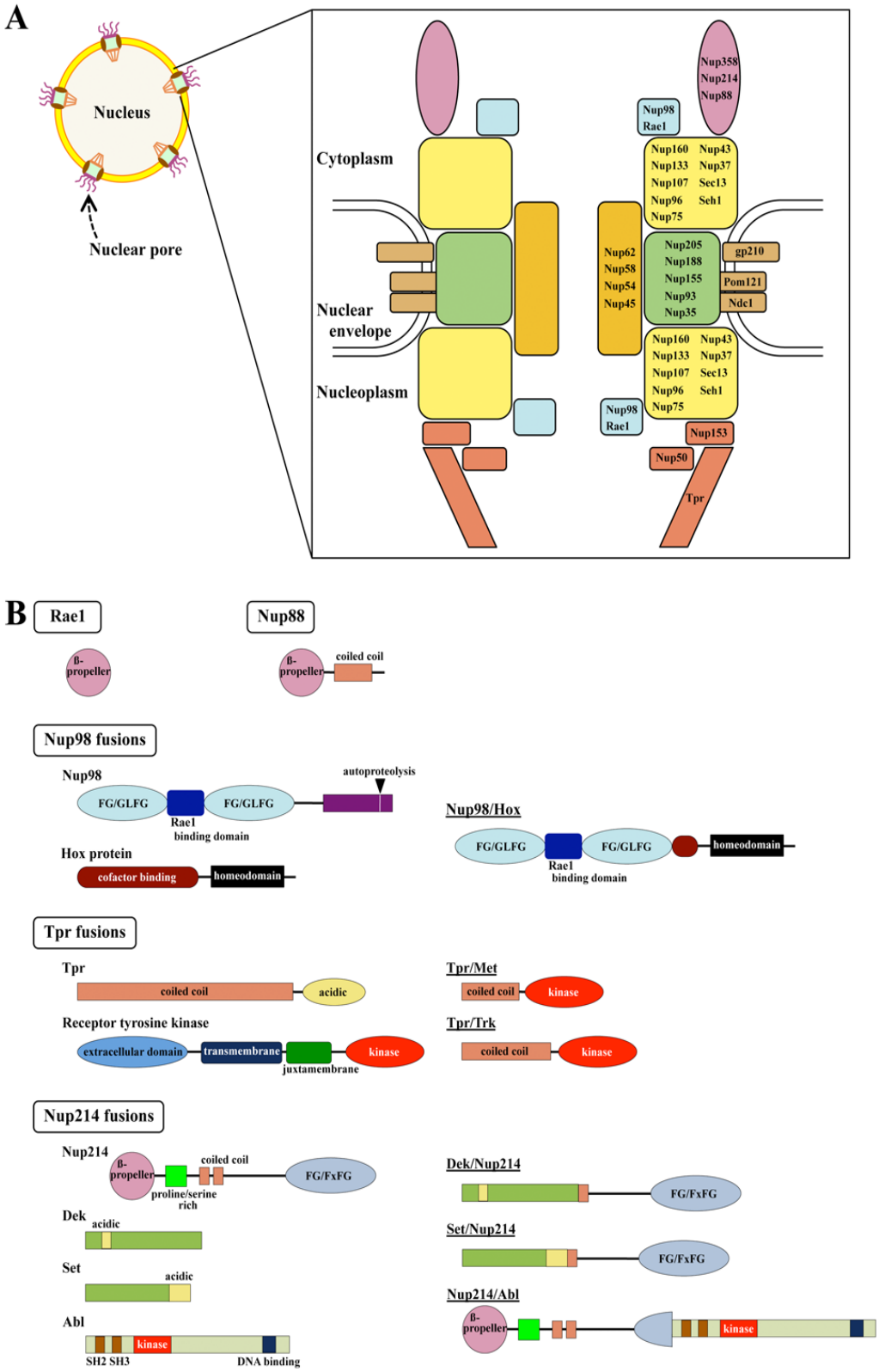
Fig. 3 Nuclear transport cycle of metastasis-associated transcription factors induced by cytokine through the NPC. **A)** Model of SMAD nucleocytoplasmic shuttling.

Unphosphorylated SMAD2/3 shuttles between cytoplasm and nucleus via direct interactions with Nup153 during nuclear export, and Nup214 during nuclear import. Phosphorylated SMAD2/3 forms heterodimeric complexes with SMAD4; these complexes translocate to the nucleus through the NPC, where they can interact with transcriptional co-factors such as Fox, and bind to target genes to initiate transcription. **B)** Model of STAT nucleocytoplasmic shuttling. Unphosphorylated STAT constitutively shuttles between cytoplasm and nucleus via direct interactions with Nup153 and Nup214. NES-mediated transport of unphosphorylated STAT via CRM1 enhances the export rate and causes cytoplasmic accumulation. After cytokine-induced receptor activation, STAT is tyrosine-phosphorylated, dimerizes, and is translocated into the nucleus by binding to importin, which interact with NPC components. Nuclear STAT can bind target genes to initiate transcription.

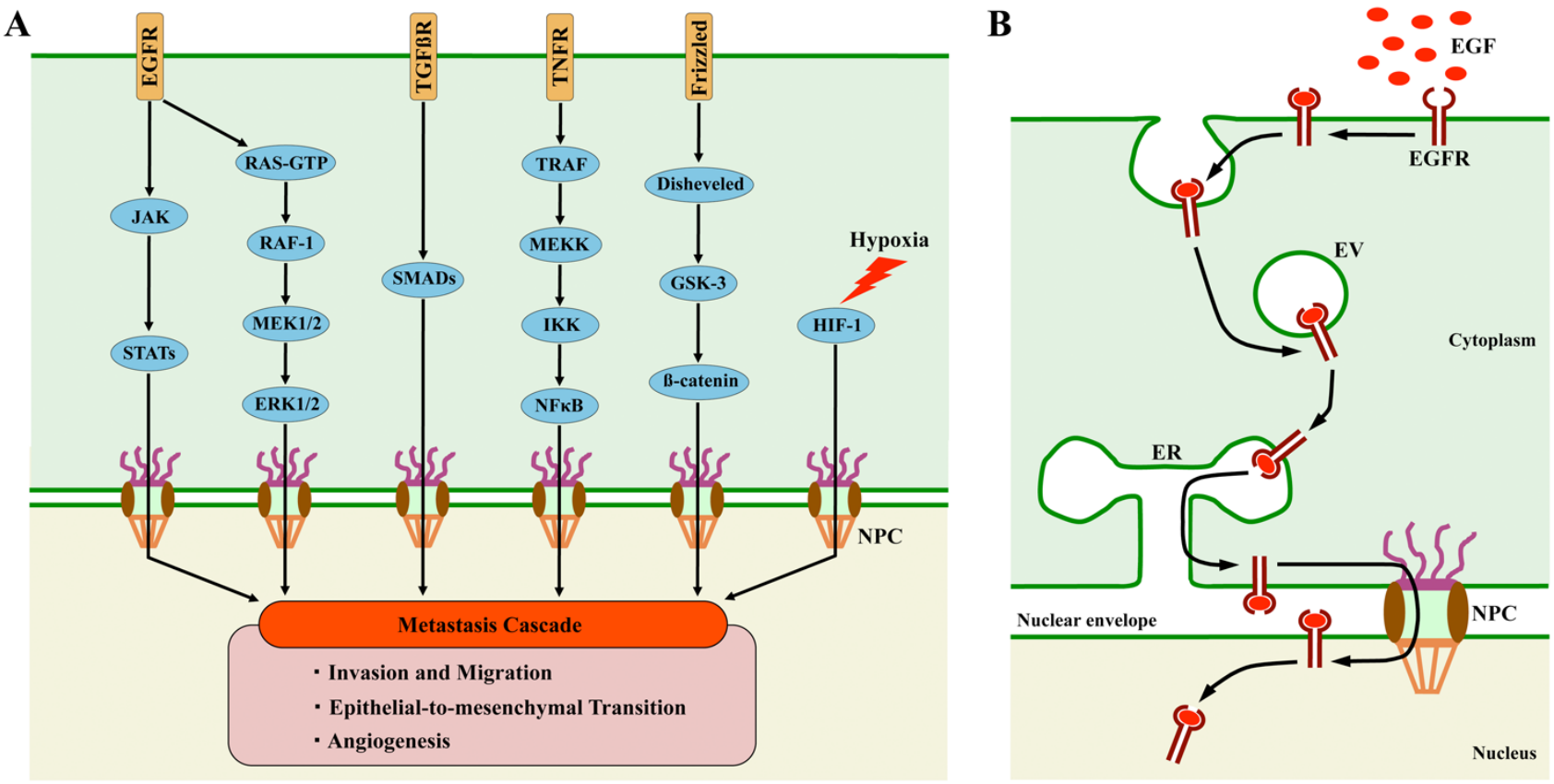
Fig. 4 Nuclear transport cycle of metastasis-associated factors through the NPC. **A)** Model of galectin-3 nucleocytoplasmic shuttling. In the cytoplasm, galectin-3 NLS binds to importin-α, followed by binding to importin-ß. The galectin-3–importin-α/ß complex docks at nucleoporins and enters the nucleus. The complex dissociates in the nucleus, releasing the galectin-3; the importin-α and -ß are then exported through the

NPC. **B)** Model of NF-κB nucleocytoplasmic shuttling. In the cytoplasm, the NF-κB is inactivated by IκB, which masks the NLS. Following stimulation, IκB is phosphorylated by IκB kinase, leading to proteasome-mediated degradation of IκB. The released NF-κB binds to importin-α and is thus translocated into the nucleus, where it can bind to target genes to initiate transcription. NF-κB then forms a complex with IκB; this complex is exported through a CRM1-dependent pathway. The NF-κB-IκB complex will enter a new round of nuclear import, whereas the IκB may transport to the nucleus for another export cycle.

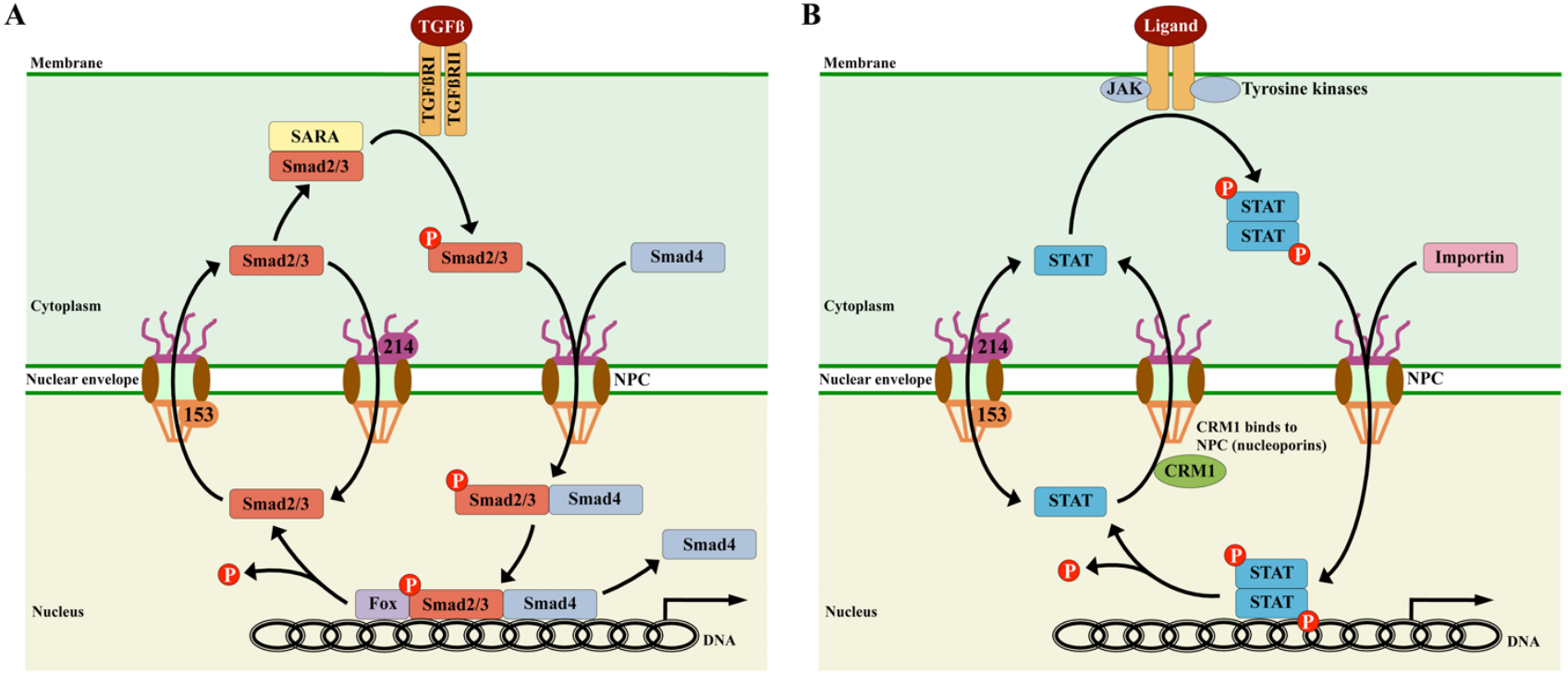
Fig. 5 Nuclear export of microRNA through NPC. MicroRNA genes are transcribed by RNA polymerase II in the nucleus. Primary miRNA (pri-miRNA) is depicted and cleaved by the Drosha RNase to form a miRNA precursor (pre-miRNA). The pre-miRNA is specifically recognized by exportin-5, and is transported to the cytoplasm through the NPC. Following release from exportin-5, the endoribonuclease Dicer further cleaves the pre-miRNA into a miRNA duplex; a single-stranded miRNA is then formed by unwinding the duplex, which assembles into the RNA-induced silencing complex (RISC). The miRNA-RISC complex can bind to mRNA to cause degradation of mRNA and/or inhibition of protein translation.



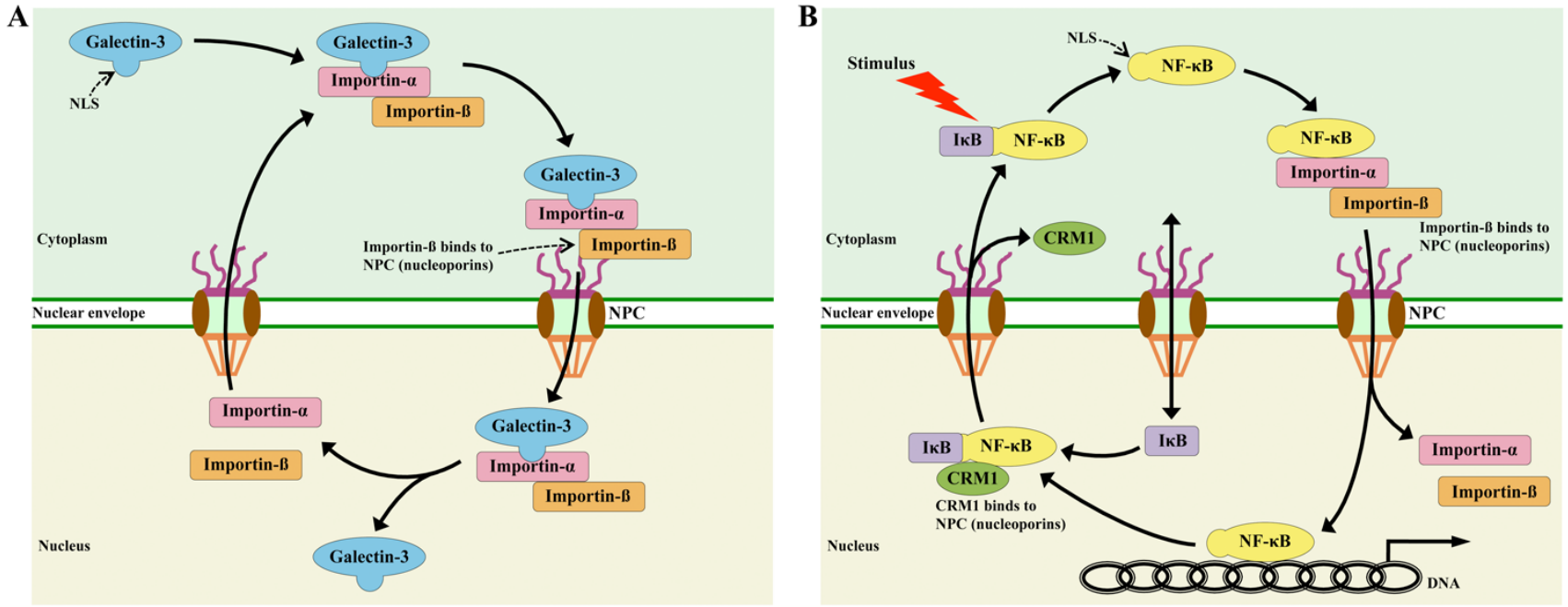
Funasaka & Wong_Figure 1



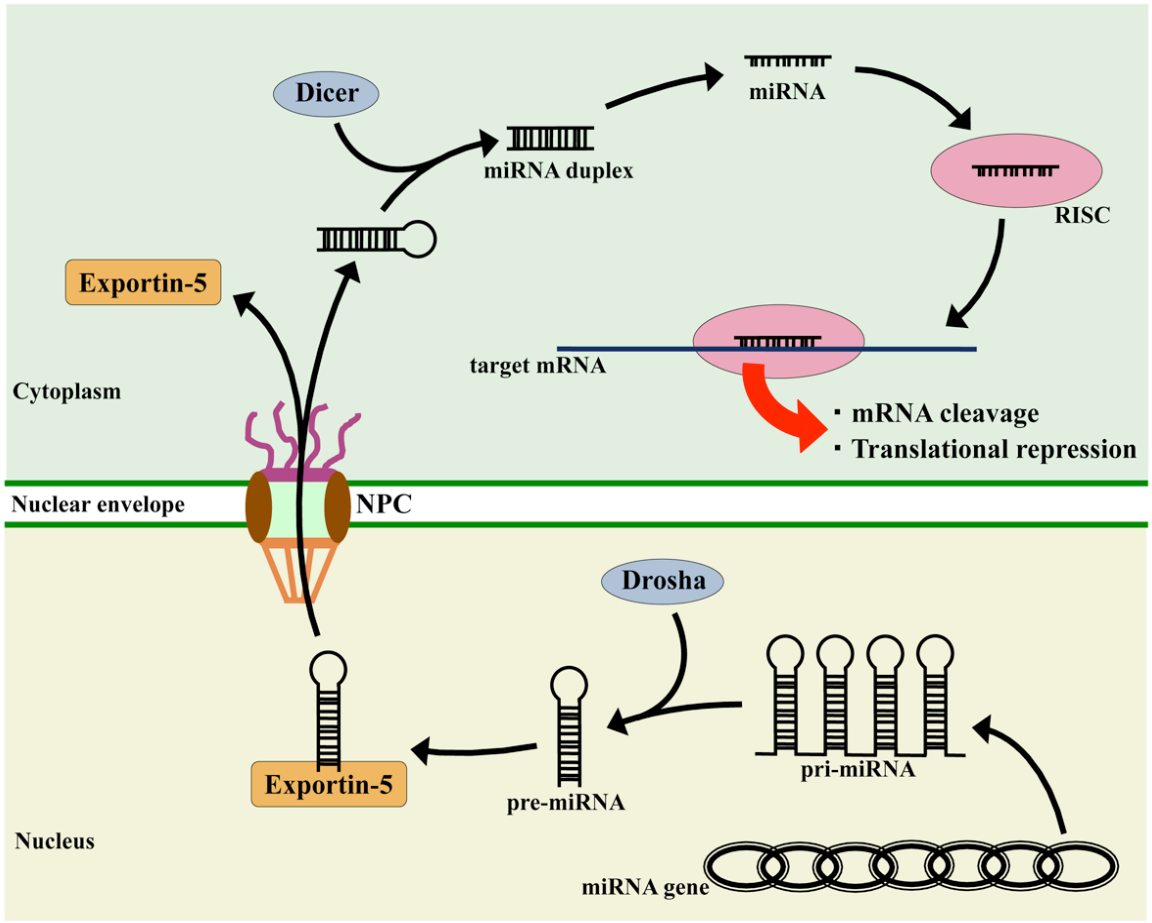
Funasaka & Wong_Figure 2



Funasaka & Wong_Figure 3



Funasaka & Wong_Figure 4



Funasaka & Wong_Figure 5