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NON-THEMATIC REVIEW

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. 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.

Keywords Nuclear pore complex · Tumor microenvironment · Metastasis · Nucleoporins · EGFR · TGF beta · Smad · Beta-catenin · NF kappa B · HIF-1 · microRNA

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

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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 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 currently 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].

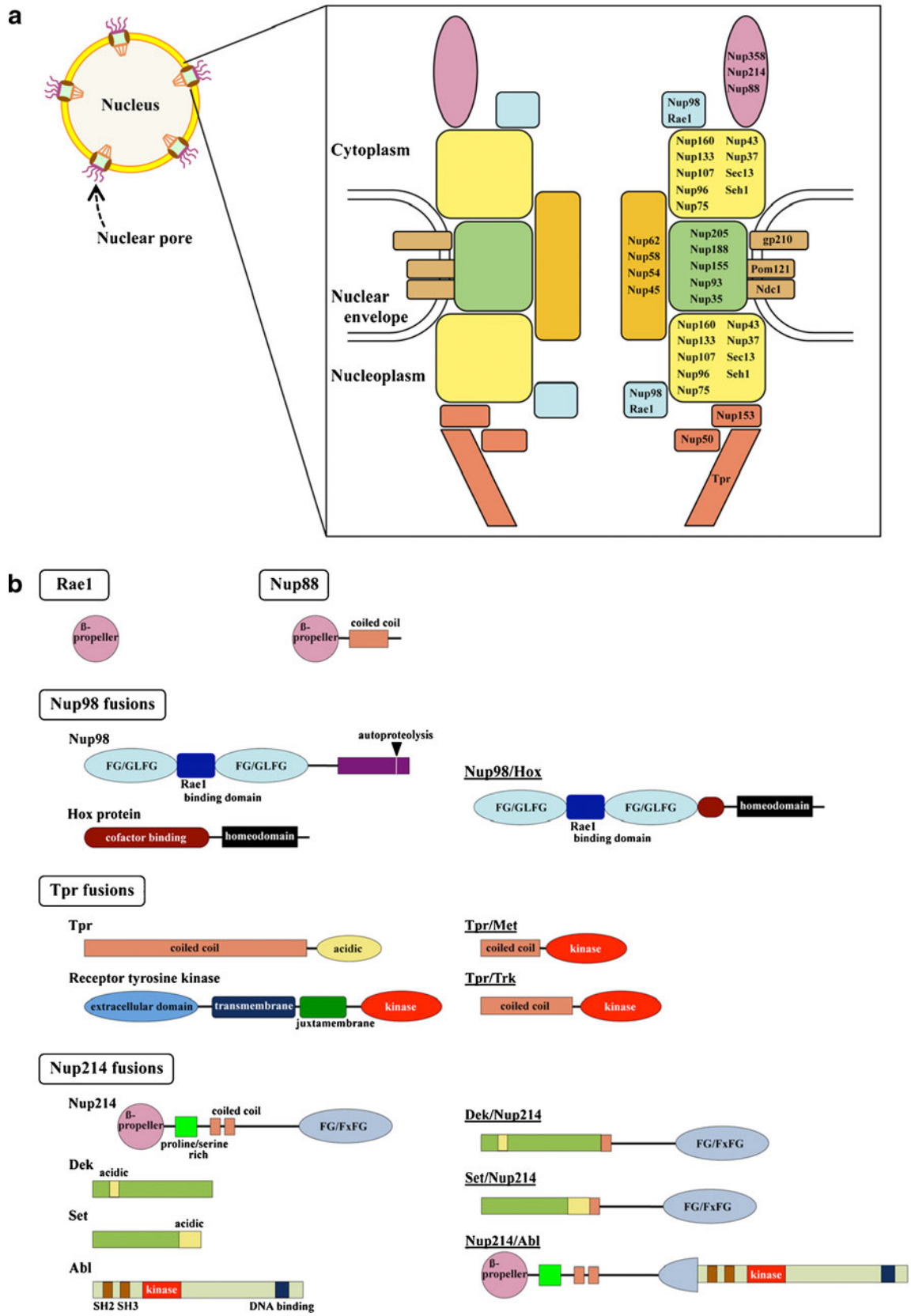

Fig. 1 Schematic representation of the nuclear pore complex (NPC) and nucleoporins. **a** Nuclear pores are large complexes embedded in the nuclear envelope. The NPC consists of ~30 different nuclear pore proteins (nucleoporins). Modified from [17, 21]. **b** Domain organization of nucleoporins and chimeric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

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 [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 [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

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 *Saccharomyces 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 (Figs. 3 and 4). 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

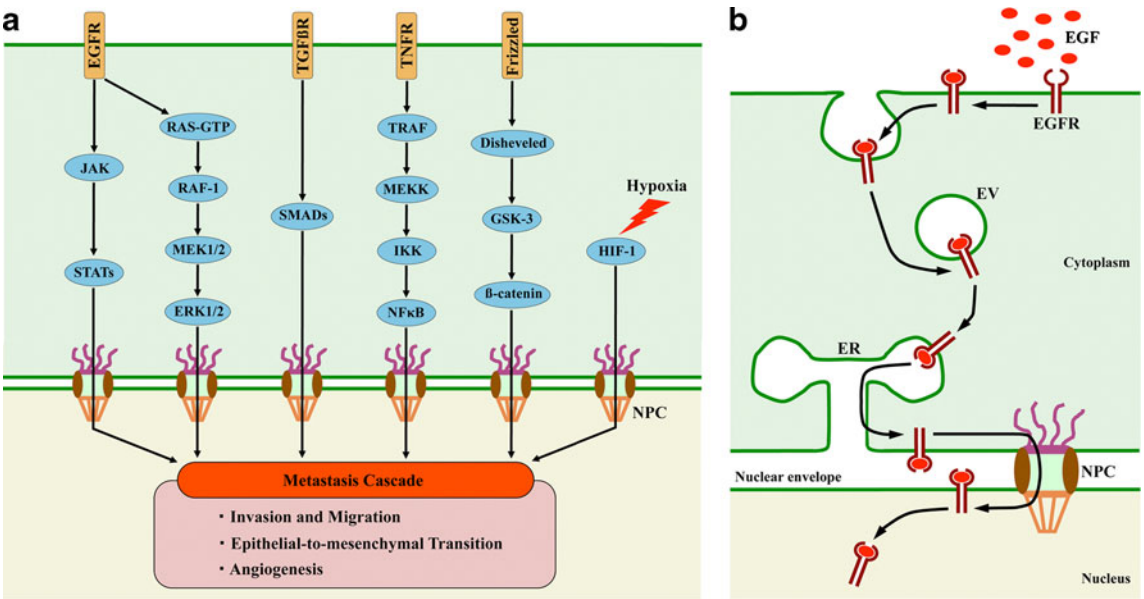

Fig. 2 **a** Diagram of signaling pathway and cascades of metastasis through NPC. **b** Model of epidermal growth factor receptor (EGFR) trafficking. After ligand-induced activation, EGFR is immediately internalized, mainly by clathrin-dependent endocytosis. Internalized EGFR embedded within early endosomes 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

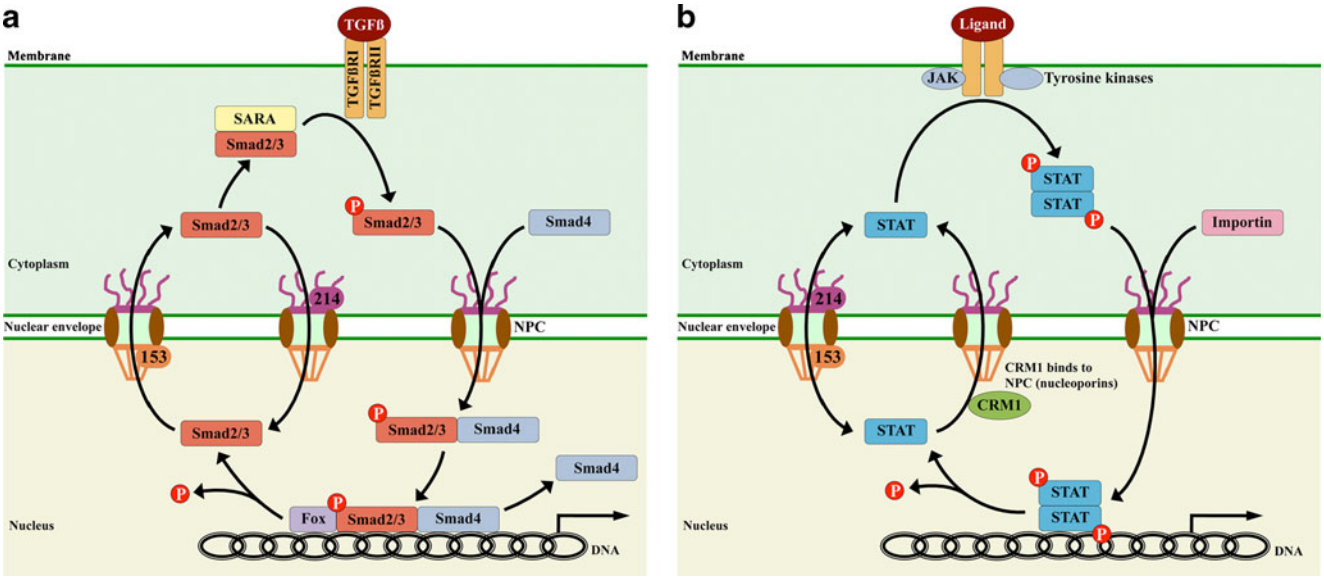

Fig. 3 Nuclear transport cycle of metastasis-associated transcription factors induced by cytokine through the NPC. **a** Model of SMAD nucleocytoplasmic shuttling. Unphosphorylated Smad2/3 shuttles between cytoplasm and nucleus via direct interactions with Nup153 during nuclear export and Nup214 during 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

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 nucleo-

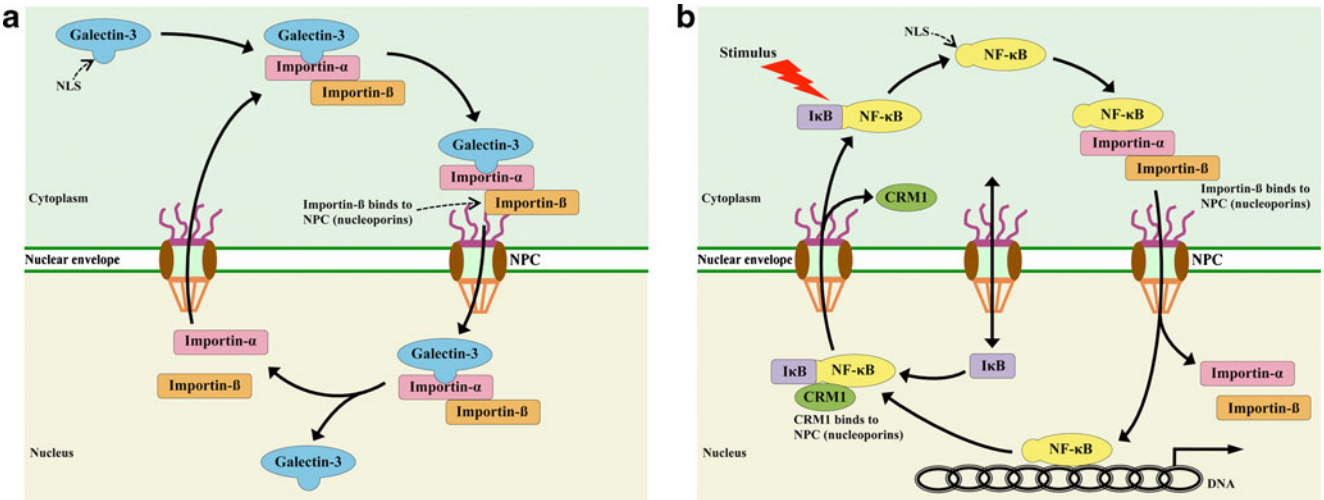

Fig. 4 Nuclear transport cycle of metastasis-associated factors through the NPC. **a** Model of galectin-3 nucleocytoplasmic shuttling. In the cytoplasm, galectin-3 NLS binds to importin-α, followed by binding to importin-ß. The galectin-3–importin-α/ß complex docks at nucleoporins and enters the nucleus.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

porins [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 transcription 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: EGFR (also called ERBB1 or HER1), ERBB2 (also called HER2), ERBB3 (also called 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, ERBB2, and FGFR1, translocate to the nucleus by importin-β-dependent mechanisms [80]. Moreover, the putative NLS of EGFR and ERBB2 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 ERBB2 in the endosomes, and importin-β, ERBB2, 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/ERBB2, 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 endoplasmic reticulum (ER) 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].

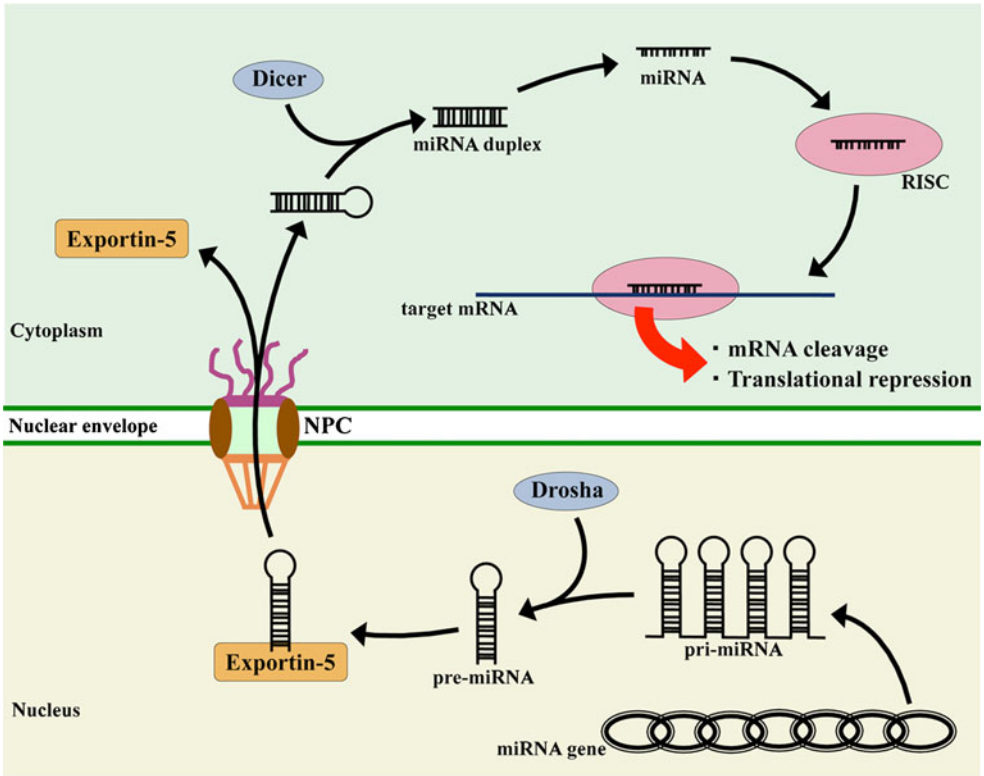

Fig. 5 Nuclear export of microRNA through NPC. MicroRNA genes are transcribed by RNA polymerase II in the nucleus. Primary miRNA (pri-miRNA) is depicted and cleaved by the Drosha RNase to form a miRNA precursor (pre-miRNA). The pre-miRNA is specifically recognized by exportin-5 and is transported to 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

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 [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 the TGFβ–SMAD pathway to the nucleus (Fig. 3a). Massagué and colleagues 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 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 carrier-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, 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 an 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 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 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 (Fig. 5) [111–113]. Which nucleoporins are responsible for the miRNA-exportin 5 complex or for mirtron exportation is currently unclear.

6 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 [117]. 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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References

1. Murphy, P. M. (2001). Chemokines and the molecular basis of cancer metastasis. *The New England Journal of Medicine, 345*, 833–835.
2. Khan, N., & Mukhtar, H. (2010). Cancer and metastasis: Prevention and treatment by green tea. *Cancer and Metastasis Reviews, 29*, 435–445.
3. Steeg, P. S. (2006). Tumor metastasis: Mechanistic insights and clinical challenges. *Natural Medicines, 12*, 895–904.
4. Chambers, A. F., Groom, A. C., & MacDonald, I. C. (2002). Dissemination and growth of cancer cells in metastatic sites. *Nature Reviews. Cancer, 2*, 563–572.
5. Chiang, A. C., & Massague, J. (2008). Molecular basis of metastasis. *The New England Journal of Medicine, 359*, 2814–2823.
6. Paget, S. (1889). The distribution of secondary growths in cancer of the breast. *Lancet, 1*, 571–573.
7. Paget, S. (1889). The distribution of secondary growths in cancer of the breast. *Cancer and Metastasis Reviews, 8*, 98–101.
8. Funasaka, T., & Raz, A. (2007). The role of autocrine motility factor in tumor and tumor microenvironment. *Cancer and Metastasis Reviews, 26*, 725–735.
9. Krug, E. L., Mjaatvedt, C. H., & Markwald, R. R. (1987). Extracellular matrix from embryonic myocardium elicits an early morphogenetic event in cardiac endothelial differentiation. *Developmental Biology, 120*, 348–355.
10. Hay, E. D. (1995). An overview of epithelio-mesenchymal transformation. *Acta Anat Basel, 154*, 8–20.
11. Finger, E. C., & Giaccia, A. J. (2010). Hypoxia, inflammation, and the tumor microenvironment in metastatic disease. *Cancer and Metastasis Reviews, 29*, 285–293.
12. Gravdal, K., Halvorsen, O. J., Haukaas, S. A., & Akslen, L. A. (2007). A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clinical Cancer Research, 13*, 7003–7011.
13. Margulis, A., Zhang, W., Alt-Holland, A., Crawford, H. C., Fusenig, N. E., & Garlick, J. A. (2005). E-cadherin suppression accelerates squamous cell carcinoma progression in three-dimensional, human tissue constructs. *Cancer Research, 65*, 1783–1791.
14. Yilmaz, M., & Christofori, G. (2009). EMT, the cytoskeleton, and cancer cell invasion. *Cancer and Metastasis Reviews, 28*, 15–33.
15. Dumont, N., Bakin, A. V., & Arteaga, C. L. (2003). Autocrine transforming growth factor-beta signaling mediates Smad-independent motility in human cancer cells. *The Journal of Biological Chemistry, 278*, 3275–3285.
16. Strambio-De-Castillia, C., Niepel, M., & Rout, M. P. (2010). The nuclear pore complex: Bridging nuclear transport and gene regulation. *Nature Reviews. Molecular Cell Biology, 11*, 490–501.
17. Xu, S., & Powers, M. A. (2009). Nuclear pore proteins and cancer. *Seminars in Cell & Developmental Biology, 20*, 620–630.
18. Tran, E. J., & Wente, S. R. (2006). Dynamic nuclear pore complexes: Life on the edge. *Cell, 125*, 1041–1053.
19. Hetzer, M. W. (2010). The nuclear envelope. *Cold Spring Harb Perspect Biol, 2*, a000539.
20. Hetzer, M. W., & Wente, S. R. (2009). Border control at the nucleus: Biogenesis and organization of the nuclear membrane and pore complexes. *Developmental Cell, 17*, 606–616.
21. Lim, R. Y., Aebi, U., & Fahrenkrog, B. (2008). Towards reconciling structure and function in the nuclear pore complex. *Histochemistry and Cell Biology, 129*, 105–116.
22. Schwartz, T. U. (2005). Modularity within the architecture of the nuclear pore complex. *Current Opinion in Structural Biology, 15*, 221–226.
23. Rout, M. P., Aitchison, J. D., Suprapto, A., Hjertaas, K., Zhao, Y., & Chait, B. T. (2000). The yeast nuclear pore complex: Composition, architecture, and transport mechanism. *The Journal of Cell Biology, 148*, 635–651.
24. Blobel, G. (2010) Three-dimensional organization of chromatids by nuclear envelope-associated structures. *Cold Spring Harb Symp Quant Biol*.
25. Brown, J. A., Bharathi, A., Ghosh, A., Whalen, W., Fitzgerald, E., & Dhar, R. (1995). A mutation in the *Schizosaccharomyces pombe* rae1 gene causes defects in poly(A) + RNA export and in the cytoskeleton. *The Journal of Biological Chemistry, 270*, 7411–7419.
26. Kraemer, D., & Blobel, G. (1997). mRNA binding protein mrnp 41 localizes to both nucleus and cytoplasm. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 9119–9124.
27. Murphy, R., Watkins, J. L., & Wente, S. R. (1996). GLE2, a *Saccharomyces cerevisiae* homologue of the *Schizosaccharomyces pombe* export factor RAE1, is required for nuclear pore complex structure and function. *Molecular Biology of the Cell, 7*, 1921–1937.
28. Bailer, S. M., Siniossoglou, S., Podtelejnikov, A., Hellwig, A., Mann, M., & Hurt, E. (1998). Nup116p and nup100p are interchangeable through a conserved motif which constitutes a docking site for the mRNA transport factor gle2p. *The EMBO Journal, 17*, 1107–1119.
29. Pritchard, C. E., Fornerod, M., Kasper, L. H., & van Deursen, J. M. (1999). RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. *The Journal of Cell Biology, 145*, 237–254.
30. Ren, Y., Seo, H. S., Blobel, G., & Hoelz, A. (2010). Structural and functional analysis of the interaction between the nucleoporin Nup98 and the mRNA export factor Rae1. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 10406–10411.
31. Paoli, M. (2001). Protein folds propelled by diversity. *Progress in Biophysics and Molecular Biology, 76*, 103–130.
32. Blower, M. D., Nachury, M., Heald, R., & Weis, K. (2005). A Rae1-containing ribonucleoprotein complex is required for mitotic spindle assembly. *Cell, 121*, 223–234.
33. Wong, R. W. (2010). Interaction between Rae1 and cohesin subunit SMC1 is required for proper spindle formation. *Cell Cycle, 9*, 198–200.

34. Wong, R. W. (2010). An update on cohesin function as a 'molecular glue' on chromosomes and spindles. *Cell Cycle, 9*, 1754–1758.
35. Wong, R. W., & Blobel, G. (2008). Cohesin subunit SMC1 associates with mitotic microtubules at the spindle pole. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 15441–15445.
36. Wong, R. W., Blobel, G., & Coutavas, E. (2006). Rae1 interaction with NuMA is required for bipolar spindle formation. *Proceedings of the National Academy of Sciences of the United States of America, 103, 19783–19787.*
37. Guttinger, S., Laurell, E., & Kutay, U. (2009). Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nature Reviews. Molecular Cell Biology, 10*, 178–191.
38. Chin, K., DeVries, S., Fridlyand, J., Spellman, P. T., Roydasgupta, R., Kuo, W. L., et al. (2006). Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell, 10*, 529–541.
39. Moore, M. A. (2010). A cancer fate in the hands of a samurai. *Natural Medicines, 16*, 963–965.
40. Moore, M. A., Chung, K. Y., Plasilova, M., Schuringa, J. J., Shieh, J. H., Zhou, P., et al. (2007). NUP98 dysregulation in myeloid leukemogenesis. *Annals of the New York Academy of Sciences, 1106*, 114–142.
41. Radu, A., Moore, M. S., & Blobel, G. (1995). The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell, 81*, 215–222.
42. Fontoura, B. M., Blobel, G., & Matunis, M. J. (1999). A conserved biogenesis pathway for nucleoporins: Proteolytic processing of a 186-kilodalton precursor generates Nup98 and the novel nucleoporin, Nup96. *The Journal of Cell Biology, 144*, 1097–1112.
43. Vasu, S., Shah, S., Orjalo, A., Park, M., Fischer, W. H., & Forbes, D. J. (2001). Novel vertebrate nucleoporins Nup133 and Nup160 play a role in mRNA export. *The Journal of Cell Biology, 155*, 339–354.
44. Griffis, E. R., Altan, N., Lippincott-Schwartz, J., & Powers, M. A. (2002). Nup98 is a mobile nucleoporin with transcription-dependent dynamics. *Molecular Biology of the Cell, 13*, 1282–1297.
45. Wu, X., Kasper, L. H., Mantcheva, R. T., Mantchev, G. T., Springett, M. J., & van Deursen, J. M. (2001). Disruption of the FG nucleoporin NUP98 causes selective changes in nuclear pore complex stoichiometry and function. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 3191–3196.
46. Blevins, M. B., Smith, A. M., Phillips, E. M., & Powers, M. A. (2003). Complex formation among the RNA export proteins Nup98, Rae1/Gle2, and TAP. *The Journal of Biological Chemistry, 278*, 20979–20988.
47. Jeganathan, K. B., Baker, D. J., & van Deursen, J. M. (2006). Securin associates with APCCdh1 in prometaphase but its destruction is delayed by Rae1 and Nup98 until the metaphase/anaphase transition. *Cell Cycle, 5*, 366–370.
48. Jeganathan, K. B., Malureanu, L., & van Deursen, J. M. (2005). The Rae1-Nup98 complex prevents aneuploidy by inhibiting securin degradation. *Nature, 438*, 1036–1039.
49. Wozniak, R., Burke, B., & Doye, V. (2010). Nuclear transport and the mitotic apparatus: An evolving relationship. *Cellular and Molecular Life Sciences, 67*, 2215–2230.
50. Cross, M.K., & Powers, M.A. (2011) Nup98 regulates bipolar spindle assembly through association with microtubules and opposition of MCAK. *Mol Biol Cell*. in press.
51. Nakamura, T., Largaespada, D. A., Lee, M. P., Johnson, L. A., Ohyashiki, K., Toyama, K., et al. (1996). Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. *Nature Genetics, 12*, 154–158.
52. Borrow, J., Shearman, A. M., Stanton, V. P., Jr., Becher, R., Collins, T., Williams, A. J., et al. (1996). The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. *Nature Genetics, 12*, 159–167.
53. Moore, M. A. (2005). Converging pathways in leukemogenesis and stem cell self-renewal. *Experimental Hematology, 33*, 719–737.
54. Krull, S., Thyberg, J., Bjorkroth, B., Rackwitz, H. R., & Cordes, V. C. (2004). Nucleoporins as components of the nuclear pore complex core structure and Tpr as the architectural element of the nuclear basket. *Molecular Biology of the Cell, 15*, 4261–4277.
55. Byrd, D. A., Sweet, D. J., Pante, N., Konstantinov, K. N., Guan, T., Saphire, A. C., et al. (1994). Tpr, a large coiled coil protein whose amino terminus is involved in activation of oncogenic kinases, is localized to the cytoplasmic surface of the nuclear pore complex. *The Journal of Cell Biology, 127*, 1515–1526.
56. Cordes, V. C., Reidenbach, S., Rackwitz, H. R., & Franke, W. W. (1997). Identification of protein p270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments. *The Journal of Cell Biology, 136*, 515–529.
57. Fontoura, B. M., Dales, S., Blobel, G., & Zhong, H. (2001). The nucleoporin Nup98 associates with the intranuclear filamentous protein network of TPR. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 3208–3213.
58. Frosst, P., Guan, T., Subauste, C., Hahn, K., & Gerace, L. (2002). Tpr is localized within the nuclear basket of the pore complex and has a role in nuclear protein export. *The Journal of Cell Biology, 156*, 617–630.
59. Lee, S. H., Sterling, H., Burlingame, A., & McCormick, F. (2008). Tpr directly binds to Mad1 and Mad2 and is important for the Mad1-Mad2-mediated mitotic spindle checkpoint. *Genes & Development, 22*, 2926–2931.
60. Nakano, H., Funasaka, T., Hashizume, C., & Wong, R. W. (2010). Nucleoporin translocated promoter region (Tpr) associates with dynein complex, preventing chromosome lagging formation during mitosis. *The Journal of Biological Chemistry, 285*, 10841–10849.
61. Strambio-de-Castillia, C., Blobel, G., & Rout, M. P. (1999). Proteins connecting the nuclear pore complex with the nuclear interior. *The Journal of Cell Biology, 144*, 839–855.
62. Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. M., et al. (1984). Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature, 311*, 29–33.
63. Park, M., Dean, M., Cooper, C. S., Schmidt, M., O'Brien, S. J., Blair, D. G., et al. (1986). Mechanism of met oncogene activation. *Cell, 45*, 895–904.
64. Peschard, P., & Park, M. (2007). From Tpr-Met to Met, tumorigenesis and tubes. *Oncogene, 26*, 1276–1285.
65. Macaulay, C., Meier, E., & Forbes, D. J. (1995). Differential mitotic phosphorylation of proteins of the nuclear pore complex. *The Journal of Biological Chemistry, 270*, 254–262.
66. Martinez, N., Alonso, A., Moragues, M. D., Ponton, J., & Schneider, J. (1999). The nuclear pore complex protein Nup88 is overexpressed in tumor cells. *Cancer Research, 59*, 5408–5411.
67. Gould, V. E., Martinez, N., Orucevic, A., Schneider, J., & Alonso, A. (2000). A novel, nuclear pore-associated, widely distributed molecule overexpressed in oncogenesis and development. *The American Journal of Pathology, 157*, 1605–1613.
68. Gould, V. E., Orucevic, A., Zentgraf, H., Gattuso, P., Martinez, N., & Alonso, A. (2002). Nup88 (karyoporin) in human malignant neoplasms and dysplasias: Correlations of immunostaining of tissue sections, cytologic smears, and immunoblot analysis. *Human Pathology, 33*, 536–544.
69. Agudo, D., Gomez-Esquer, F., Martinez-Arribas, F., Nunez-Villar, M. J., Pollan, M., & Schneider, J. (2004). Nup88 mRNA

overexpression is associated with high aggressiveness of breast cancer. *International Journal of Cancer, 109*, 717–720.
70. Bastos, R., Ribas de Pouplana, L., Enarson, M., Bodoor, K., & Burke, B. (1997). Nup84, a novel nucleoporin that is associated with CAN/Nup214 on the cytoplasmic face of the nuclear pore complex. *The Journal of Cell Biology, 137*, 989–1000.
71. van Deursen, J., Boer, J., Kasper, L., & Grosveld, G. (1996). G2 arrest and impaired nucleocytoplasmic transport in mouse embryos lacking the proto-oncogene CAN/Nup214. *The EMBO Journal, 15*, 5574–5583.
72. Walther, T. C., Pickersgill, H. S., Cordes, V. C., Goldberg, M. W., Allen, T. D., Mattaj, I. W., et al. (2002). The cytoplasmic filaments of the nuclear pore complex are dispensable for selective nuclear protein import. *The Journal of Cell Biology, 158*, 63–77.
73. von Moeller, H., Basquin, C., & Conti, E. (2009). The mRNA export protein DBP5 binds RNA and the cytoplasmic nucleoporin NUP214 in a mutually exclusive manner. *Nature Structural & Molecular Biology, 16*, 247–254.
74. Napetschnig, J., Kassube, S. A., Debler, E. W., Wong, R. W., Blobel, G., & Hoelz, A. (2009). Structural and functional analysis of the interaction between the nucleoporin Nup214 and the DEAD-box helicase Ddx19. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 3089–3094.
75. von Lindern, M., Fornerod, M., van Baal, S., Jaegle, M., de Wit, T., Buijs, A., et al. (1992). The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. *Molecular and Cellular Biology, 12*, 1687–1697.
76. von Lindern, M., van Baal, S., Wiegant, J., Raap, A., Hagemeijer, A., & Grosveld, G. (1992). Can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3′ half to different genes: Characterization of the set gene. *Molecular and Cellular Biology, 12*, 3346–3355.
77. Fagerlund, R., Melen, K., Cao, X., & Julkunen, I. (2008). NF-kappaB p52, RelB and c-Rel are transported into the nucleus via a subset of importin alpha molecules. *Cellular Signalling, 20*, 1442–1451.
78. Weis, K. (2003). Regulating access to the genome: Nucleocytoplasmic transport throughout the cell cycle. *Cell, 112*, 441–451.
79. Lu, X., & Kang, Y. (2010). Epidermal growth factor signalling and bone metastasis. *British Journal of Cancer, 102*, 457–461.
80. Wang, Y. N., Yamaguchi, H., Hsu, J. M., & Hung, M. C. (2010). Nuclear trafficking of the epidermal growth factor receptor family membrane proteins. *Oncogene, 29*, 3997–4006.
81. Hynes, N. E., & MacDonald, G. (2009). ErbB receptors and signaling pathways in cancer. *Current Opinion in Cell Biology, 21*, 177–184.
82. Citri, A., & Yarden, Y. (2006). EGF-ERBB signalling: Towards the systems level. *Nature Reviews. Molecular Cell Biology, 7*, 505–516.
83. Lo, H. W., Ali-Seyed, M., Wu, Y., Bartholomeusz, G., Hsu, S. C., & Hung, M. C. (2006). Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. *Journal of Cellular Biochemistry, 98*, 1570–1583.
84. Vrailas-Mortimer, A. D., Majumdar, N., Middleton, G., Cooke, E. M., & Marenda, D. R. (2007). Delta and Egfr expression are regulated by Importin-7/Moleskin in Drosophila wing development. *Developmental Biology, 308*, 534–546.
85. Giri, D. K., Ali-Seyed, M., Li, L. Y., Lee, D. F., Ling, P., Bartholomeusz, G., et al. (2005). Endosomal transport of ErbB-2: Mechanism for nuclear entry of the cell surface receptor. *Molecular and Cellular Biology, 25*, 11005–11018.
86. Nguyen, D. X., Bos, P. D., & Massague, J. (2009). Metastasis: From dissemination to organ-specific colonization. *Nature Reviews. Cancer, 9*, 274–284.
87. Padua, D., & Massague, J. (2009). Roles of TGFbeta in metastasis. *Cell Research, 19*, 89–102.
88. Massague, J. (2008). TGFbeta in Cancer. *Cell, 134*, 215–230.
89. Miyazono, K. (2009). Transforming growth factor-beta signaling in epithelial-mesenchymal transition and progression of cancer. *Proceedings of the Japan Academy. Series B: Physical and Biological Sciences, 85*, 314–323.
90. Roberts, A. B., Flanders, K. C., Heine, U. I., Jakowlew, S., Kondaiah, P., Kim, S. J., et al. (1990). Transforming growth factor-beta: Multifunctional regulator of differentiation and development. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 327*, 145–154.
91. Kang, Y. (2006). Pro-metastasis function of TGFbeta mediated by the Smad pathway. *Journal of Cellular Biochemistry, 98*, 1380–1390.
92. Korpal, M., & Kang, Y. (2010). Targeting the transforming growth factor-beta signalling pathway in metastatic cancer. *European Journal of Cancer, 46*, 1232–1240.
93. Kang, J. S., Liu, C., & Derynck, R. (2009). New regulatory mechanisms of TGF-beta receptor function. *Trends in Cell Biology, 19*, 385–394.
94. Xu, L., Kang, Y., Col, S., & Massague, J. (2002). Smad2 nucleocytoplasmic shuttling by nucleoporins CAN/Nup214 and Nup153 feeds TGFbeta signaling complexes in the cytoplasm and nucleus. *Molecular Cell, 10*, 271–282.
95. Sukegawa, J., & Blobel, G. (1993). A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. *Cell, 72*, 29–38.
96. Marg, A., Shan, Y., Meyer, T., Meissner, T., Brandenburg, M., & Vinkemeier, U. (2004). Nucleocytoplasmic shuttling by nucleoporins Nup153 and Nup214 and CRM1-dependent nuclear export control the subcellular distribution of latent Stat1. *The Journal of Cell Biology, 165*, 823–833.
97. Nakahara, S., Hogan, V., Inohara, H., & Raz, A. (2006). Importin-mediated nuclear translocation of galectin-3. *The Journal of Biological Chemistry, 281*, 39649–39659.
98. Nakahara, S., Oka, N., Wang, Y., Hogan, V., Inohara, H., & Raz, A. (2006). Characterization of the nuclear import pathways of galectin-3. *Cancer Research, 66*, 9995–10006.
99. Nakahara, S., & Raz, A. (2007). Regulation of cancer-related gene expression by galectin-3 and the molecular mechanism of its nuclear import pathway. *Cancer and Metastasis Reviews, 26*, 605–610.
100. Henderson, B. R., & Fagotto, F. (2002). The ins and outs of APC and beta-catenin nuclear transport. *EMBO Reports, 3*, 834–839.
101. Thorne, M. E., & Gottardi, C. J. (2005). Terminating Wnt signals: A novel nuclear export mechanism targets activated (beta)-catenin. *The Journal of Cell Biology, 171*, 761–763.
102. Chachami, G., Paraskeva, E., Mingot, J. M., Braliou, G. G., Gorlich, D., & Simos, G. (2009). Transport of hypoxia-inducible factor HIF-1alpha into the nucleus involves importins 4 and 7. *Biochemical and Biophysical Research Communications, 390*, 235–240.
103. Pemberton, L. F., & Paschal, B. M. (2005). Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic, 6*, 187–198.
104. Mylonis, I., Chachami, G., Paraskeva, E., & Simos, G. (2008). Atypical CRM1-dependent nuclear export signal mediates regulation of hypoxia-inducible factor-1alpha by MAPK. *The Journal of Biological Chemistry, 283*, 27620–27627.
105. Mylonis, I., Chachami, G., Samiotaki, M., Panayotou, G., Paraskeva, E., Kalousi, A., et al. (2006). Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. *The Journal of Biological Chemistry, 281*, 33095–33106.

106. Ghosh, S., May, M. J., & Kopp, E. B. (1998). NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annual Review of Immunology, 16*, 225–260.
107. Muller, P. A., van de Sluis, B., Groot, A. J., Verbeek, D., Vonk, W. I., Maine, G. N., et al. (2009). Nuclear-cytosolic transport of COMMD1 regulates NF-kappaB and HIF-1 activity. *Traffic, 10*, 514–527.
108. Johnson, C., Van Antwerp, D., & Hope, T. J. (1999). An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of IkappaBalpha. *The EMBO Journal, 18*, 6682–6693.
109. Carlotti, F., Dower, S. K., & Qwarnstrom, E. E. (2000). Dynamic shuttling of nuclear factor kappa B between the nucleus and cytoplasm as a consequence of inhibitor dissociation. *The Journal of Biological Chemistry, 275*, 41028–41034.
110. Birbach, A., Gold, P., Binder, B. R., Hofer, E., de Martin, R., & Schmid, J. A. (2002). Signaling molecules of the NF-kappa B pathway shuttle constitutively between cytoplasm and nucleus. *The Journal of Biological Chemistry, 277*, 10842–10851.
111. Shenouda, S. K., & Alahari, S. K. (2009). MicroRNA function in cancer: Oncogene or a tumor suppressor? *Cancer and Metastasis Reviews, 28*, 369–378.
112. Castanotto, D., Lingeman, R., Riggs, A. D., & Rossi, J. J. (2009). CRM1 mediates nuclear-cytoplasmic shuttling of mature microRNAs. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 21655–21659.
113. Lee, S.J., Jiko, C., Yamashita, E., & Tsukihara, T. (2011) Selective nuclear export mechanism of small RNAs. *Curr Opin Struct Biol*. in press.
114. Okamura, K., Hagen, J. W., Duan, H., Tyler, D. M., & Lai, E. C. (2007). The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. *Cell, 130*, 89–100.
115. Ma, L., Teruya-Feldstein, J., & Weinberg, R. A. (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature, 449*, 682–688.
116. Gee, H.E., Camps, C., Buffa, F. M., Colella, S., Sheldon, H., Gleadle, J.M., Ragoussis, J., Harris, A.L. (2008) MicroRNA-10b and breast cancer metastasis. *Nature*, 455, E8-9; author reply E9.
117. Nakano, H., Wang, W., Hashizume, C., Funasaka, T., Sato, H., & Wong, R. W. (2011). Unexpected role of nucleoporins in coordination of cell progression. *Cell Cycle* 10, 425–433