

5-1-2022

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Recommended Citation

Mehmood, Rashid; Jibiki, Kazuya; Alsafwani, Zakeya J.; Naseem, Muhammad; and Yasuhara, Noriko, "Systems genomics of nucleoporins provides prognostic insights into breast cancer" (2022). *All Works*. 5376.

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Full Length Research Article

Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received:
29/10/2021;
Date Revised:
28/12/2021;
Date Published Online:
25/05/2022;

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How to Cite:

Mehmood R, Jibiki K, Alsafwani ZJ, Naseem M, Yasuhara N (2022). Systems genomics of nucleoporins provides prognostic insights into breast cancer. Adv. Life Sci. 9(1): 98-110.

Keywords:

Nuclear Pore Complexes (NPCs); Nucleoporins; Breast cancer; Bioinformatics

Open Access



Systems genomics of nucleoporins provides prognostic insights into breast cancer

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Abstract

Background: Nucleoporins (Nups) constitute a large group of proteins that are structurally arranged at the nuclear envelope and facilitate the bidirectional movement of molecules across the nuclear membrane. In addition to regulating the shuttling of ribonucleoprotein complexes, RNAs and proteins, various Nups interact with chromatin either directly or indirectly, thus regulating gene expression. Any mutations or expression anomalies of Nups may lead to abnormal localization of critical molecules, or dysregulated expression of genes that they interact with. A comprehensive genomic study encompassing all Nup genes in relation to breast cancer is lacking.

Methods: We used genomic and transcriptomic datasets from Pan-Cancer TCGA (The Cancer Genome Atlas), Genotype-Tissue Expression (GTEx) and microarray platforms and conducted *in silico* analysis of all the genes encoding nucleoporins that are associated with the Nuclear Pore Complexes (NPCs). For mutation detection, we used cBioportal; for expression analysis, we used Xena and for patient survival plots, KMPlot was utilized.

Results: The genetic and molecular profile of nucleoporin genes identified multiple mutations and detected aberrant expression in breast cancer. Interestingly, *NUP133*, *AHCTF1*, *TPR*, *Nup121L* showed simultaneous gene amplification in nearly 10% of breast cancer patients. In addition, deregulated expression of some of the nucleoporins, namely, *NUP62*, *NUP 93*, *NUP98*, *NUP155*, *POM121L12*, *RAE1*, *SEC13*, *TPR* were correlated with patient prognosis.

Conclusion: The current study is the first one that unravels a comprehensive molecular and genetic profile of nucleoporins genes in breast cancer and underscores the critical roles of various nucleoporins in cancer progression. The identified molecules may advance our understanding of the etiology of the disease and serve as possible targets for novel therapeutic strategies in cancer.



Introduction

Nuclear pore complexes (NPCs), embedded in the nuclear envelope, provide a gateway for the movement of molecules in and out of the nucleus. The barrier function of the nuclear envelope is quintessential not only for maintaining the distinct composition of the nucleus but also for the unique functions therein. Two types of movements take place through the NPCs; passive diffusion that involves movements of small sized proteins, metabolites, and ions, and facilitative transport wherein large molecules including ribonucleoprotein complexes, RNAs and proteins cross the nuclear envelope. The facilitative transport requires particular transport receptors, called importins and exportins. Both of these types of transport are mediated by the NPCs [1]. NPCs are megadalton complexes (approximately 60-125MDa) and are composed of ~50 distinct proteins called nucleoporins (Nups), which provide binding sites for transport receptors for the transport of molecules in and out of the nucleus [2].

The NPCs are remarkably complicated structures and are composed of three structurally distinct regions. 1) the cytoplasmic ring from which filaments extend into the cytoplasm, 2) the nuclear basket that extends to the nucleoplasmic faces of the NPCs and, 3) the central framework which makes up the pore [3]. The correct positioning of various Nups in the NPC warrants their structural and functional roles, facilitating the transport of selective molecules [4]. The NPC assembly requires coordination between NUPs. For instance, Nup133, a component of the NPC scaffold, is required for Tpr and Nup153 assembly into the nuclear basket. For the transport of molecules into the nucleus, the karyopherins that bind NLS (Nuclear Localization Signal) containing cargoes interact with FG (Phe-Gly) repeats containing Nups of the NPCs and mediate the translocation of the transport complex [5]. Once into the nucleus, the association between Nups and importins is disengaged by the action of RanGTP by virtue of its very high binding affinity to importins [6]. Conversely, the nuclear export of a number of proteins is mediated by interaction of Exportins with RanGTP, while RanGTP independent export also exist, as in case of mRNAs [7].

In addition to their well-known role in the transport of molecules across the nuclear membrane, some of the Nups have been shown to regulate a variety of functions, including RNA processing, transcriptional activation, and chromatin modulation [8-10]. Their widespread functions are strengthened by the fact that some NUPs (i.e. NUP98, NUP62, NUP50, NUP153) are not only localized to the NPCs; instead, they exhibit dynamic features by moving off and on of the NPCs. Their shuttling in and out of the nucleus and interaction with

cytosolic and nuclear components point to their unorthodox functions. An interesting correlation was found between the expression levels of genes and their position in the vicinity of the NPCs [11]. The observation that the genomic regions nearby the NPCs are transcriptionally active led to the identification of their roles in transcriptional regulation [12, 13]. NUP53, for instance, was shown to not only bind DNA, but its binding pattern was co-enriched with other transcriptional activation markers, i.e., Histone H4K16 acetylation and RNA polymerase II [14]. Over the years, the roles of a battery of Nups were identified in regulating the chromatin landscape [15]. A subset of the Nups regulate expression of genes implicated in cellular identity thus regulating cellular differentiation and development [16, 17]. Moreover, recently, their roles in viral infection and innate immunity is also described [85, 86]. Therefore, it is not surprising that aberrant expression patterns of various Nups are associated with a wide range of medical conditions [18, 19].

Genomic fusions involving Nups with other partners have been found in hematopoietic and non-hematopoietic malignancies [20, 21]. Additionally, the deregulated expression and mutations of some Nups are also linked with various cancers [22, 23]. Their roles in cancer progression may be due to specifically expediting the nuclear translocation of oncogenes, e.g., E2F1, MYC, thus enhancing their oncogenic activities [24] or through interacting with chromatin, thus regulating chromatin remodeling, and regulating the transcription of genes [25]. In breast cancer, only few studies have looked into the individual Nups for their roles in cellular transformation. NUP88 mRNA overexpression, for instance, has been shown to be associated with high aggressiveness of breast cancer [26]. Inverse correlation of NUP93 expression with the survival of triple-negative breast cancer patients by modulating cytoskeleton remodeling has been recently shown [27]. However, to our knowledge, a comprehensive study of all the nucleoporins cataloging their mutational profile, differential expression compared with the normal tissue and their correlation with breast cancer patient survival is lacking. In the current study, using genomic and transcriptomic datasets from The Cancer Genomic Atlas (TCGA) and microarray platforms, we analyzed all the known genes encoding components of NPCs in breast cancer. Our data suggest that majority of *NUPs* are mutated/aberrantly expressed in breast cancer, albeit with varying degrees, and point to the implications of *NUPS* in patients' prognosis.

Methods

Genetic alterations in Nup genes

In order to identify mutations in various Nups, we utilized TCGA (The Cancer Genome Atlas) data using cBioportal [28, 29]. At the cBioportal, breast cancer datasets are available from multiple sources. We selected the latest TCGA Breast Invasive Carcinoma (Firehouse legacy). In this category, there are 963 patients that had both mutation and CNA (Copy number alterations) data. Therefore, following genetic alterations were scanned in these 963 breast cancer patients' datasets; 1) Mutations, 2) CNA (copy number alterations). Ethical approval was not needed for the study as all datasets in this study were utilized from public databases (TCGA), and processing of data were according to the TCGA publication guidelines (<https://cancergenome.nih.gov/publications/guidelines>).

Nups and encoding genes nomenclature

Nucleoporins constitute all the proteins that make up the nuclear pore complexes. However, before their identification as a part of this huge nuclear pore complexes, they adapted different names based on interacting proteins and functions they were associated with. For the sake of consistency, we used Human Genome Organization (HUGO) gene nomenclature committee resource (<https://www.genenames.org/>). All the genes corresponding to nucleoporins were identified from this HUGO resource and published literature [30]. The list of Nups encoded by respective genes are listed in Table 1.

NUPs Interactome and network analysis

We mapped protein-protein interactions among NUPs specific proteins as high confidence interactome retrieved from STRING database (<https://string-db.org/>) [87]. The list of NUPs genes (table 1) was given as input identifiers to the multi-proteins search option of the STRING database. A stringent criterion for the selection of interactions as combined edge score greater than 0.9 (P-values for Bayesian confidence score) resulted in a densely connected network, composed of 35 nodes and 175 edges. For network analysis we used STRING inbuilt analysis function for degree and clustering co-efficient. We only selected experimental interactions and visualized the network in evidence mode as STRING output. The edges denote both functional and physical protein associations among the nodes as opted in the basic settings options.

Gene expression changes compared with the normal tissue

For expression comparison between normal tissue and breast cancer samples, we used Xena [67] which incorporates data from TCGA datasets and GTEx for comparison between the two [68].

The gene list in the table 1 was used to generate heat map. All the genes were incorporated in the UCSC Xena. After launching Xena, the first variable phenotype "main category" was selected. In the second Genomic variable, Gene expression was chosen, followed by incorporating all the genes encoding Nups. TCGA GTEx datasets was used to generate heatmap. Additionally, box plots for the individual genes were fetched from the "view chart" to make gene expression comparisons between GTEx and TCGA breast cancer patients' datasets. As Xena utilizes the same pipeline for TCGA and GTEx samples, therefore, both datasets are re-analyzed (using UCSC Toil RNA-seq recompute compendium) to eliminate batch effects [67].

Patient survival plots

Kaplan-Meier Plotter was used to generate Meier-Kaplan plots wherein multiple microarray expression datasets are incorporated from 7462 breast cancer patients [69]. In order to generate overall survival plots, the patients were split by median. For the best probe selection, "jetset" was selected for the shown time periods. The indicated p value does not include correction for multiple hypothesis testing by default [69].

Results

Mutational landscape of various Nups in breast cancer

In order to delineate the roles of nuclear transport process in breast cancer progression, we recently carried out bioinformatic analysis of TCGA datasets and showed that various genes encoding factors related to nuclear transport are mutated [70]. An equally important aspect of the nuclear transport process is the interaction of transport factors with the components of the NPCs and subsequent translocation to the nucleus. Therefore, mutations in the genes coding for Nups, the proteins that constitute the NPCs, may disrupt/expedite the nuclear transport process. Thus, it is imperative to identify various mutations in this category of genes. Therefore, using TCGA datasets, we characterized mutations in Nup genes and found that majority of the Nup genes were genetically altered in breast cancer patients. The alteration frequency differed markedly (ranging from 0.4% in *AAAS* to 15% in *AHCTF1*) (Figure 1) amongst various Nups. Interestingly, gene amplification appeared to be the most prevalent genetic anomaly. Additional mutations were also detected, including deep deletions, base substitutions and multiple alterations. A synergistic gene amplification in *AHCTF1*, *NUP133*, *NUP210L* and *TPR* was found in over 10% of patients. Figure 1B shows the frequency of mutations in the components of nuclear envelope based

on their location in the nuclear pore. To further expand on the possible consequences of genetic alterations on the nuclear pore architecture and to get functional insights into the NUPs genes, we mapped physical interactions among their encoded proteins. A densely connected network of NUPs proteins was derived from the publicly available STRING database [87]. As shown in the Figure 1C, there are 35 protein nodes in the network that constitute 175 edges with diverse functional implications. Interactions between Nup133, AHCTF1 and Nup210L, that are synergistically amplified, are clearly visible in the interactome. Interestingly, alteration frequency in all Nup genes was found to be in nearly 50% of the patients representing significance of this set of molecules (Supplementary figure 1A). Genetic alterations in Nups genes were detected in all breast cancer subtypes albeit with variable rates and types of genetic alterations. In order to validate our analysis, we used *PIK3CA*, *TP53*, *CDH1*, and *GATA3*. Consistent with literature, we found high prevalence of mutations in these control genes (Figure 1A).

After establishing that Nup genes are mutated in breast cancer, we stratified breast cancer patients into metaplastic breast cancer, breast invasive ductal carcinoma, breast mixed ductal and lobular carcinoma, breast invasive lobular carcinoma, and breast invasive mixed mucinous carcinoma and looked for mutations (Supplementary figure 1B). Additional analysis also identified co-occurrences and mutual exclusivities of these top mutated genes with several Nups (Table 2). The frequency of genetic alterations was not specific to breast cancer. We utilized TCGA PanCancer Atlas Studies that has datasets from a variety of cancers and identified similar trends in mutations in majority of the cancers. This shows that mutations in Nup genes span a spectrum of cancers (Figure 2).

Nups related genes undergo massive expression anomalies in breast cancer

Recent transcriptomic analysis showed massive transcriptomic deregulation is an important hallmark of cancer. A number of genes are not only aberrantly expressed in breast cancer, but may also serve as biomarkers. Therefore, we analyzed RNA levels of all Nup genes compared with the normal tissue. The cancer patient's data was from TCGA, and for comparison GTEx datasets were used. As can be seen in Figure 3A, several Nup genes showed deregulated expression in breast cancer. In majority of the genes analyzed, overexpression was detected to be major anomaly in cancer patients compared with the samples from GTEx. However, a few genes including *AAAS*, *POM121*, and *DDX19B* showed reduced expression in cancer patients

compared with the GTEx cohort. The high expression in TCGA datasets might be associated with high amplification rate, at least partially. Figure 3B shows comparisons of individual gene expression between TCGA and GTEx datasets. Collectively, majority of genes encoding Nups go through massive deregulation in expression in cancer patients.

Nups with correlation with patient overall survival.

Having established that genetic aberrations and expression changes in cancer patients prevail significantly, next was to determine if any of these changes in gene expression in Nups have a prognostic potential. Mining various microarray datasets using Meier-Kaplan (KM) Plotter [69], we calculated overall survival (OS) of breast cancer patients based on expression of Nup genes. Out of all the Nup genes tested, *NUP62*, *NUP93*, *RAE1*, *NUP155*, and *SEC13* overexpression showed poor overall survival, while *POM121L12*, *TPR*, and *NUP98* overexpression were correlated with better patient survival. *TOP2A*, a well-known marker for poor patient survival is also shown in the figure 4, which, consistent with its established role, showed poor patient OS. All other Nups that did not show any significant correlation with patient overall survival are shown in Supplementary figure 2. Overall, the analysis identifies several molecules that might have prognostic potential in breast cancer.

Discussion

In this study, we describe a comprehensive genetic and molecular landscape of nucleoporins genes in breast cancer. Our analysis shows that a number of Nup genes are not only mutated but also undergo drastic expression changes in breast cancer.

Among the Nup genes, we found that AHCTF1, NUP133, NUP210L and TPR were synergistically altered in around 10% of patients. Nup133 is located in the NPC scaffold forming the Y-complex together with Nup85, SEC13, Nup160, Nup107 [88]. It has been shown that Nup133 is instrumental in formation of the nuclear basket with its role in the Tpr and Nup153 recruitment to the NPC basket [89]. Moreover, Tpr is necessary for the nuclear export of mRNAs and proteins. Thus, if the gene products of NUP133 and TPR genes are synergistically altered, the assembly and the functions of NPC could be significantly altered. AHCTF1, also known as ELYS, is not only necessary for post-mitotic NPC assembly through recruitment of chromatin but also regulates the nuclear size [90]. Therefore, simultaneous changes in the quality and quantity of these NUPs could have a significant impact on the function of the nuclear pores, which may define the behavior of cancer.

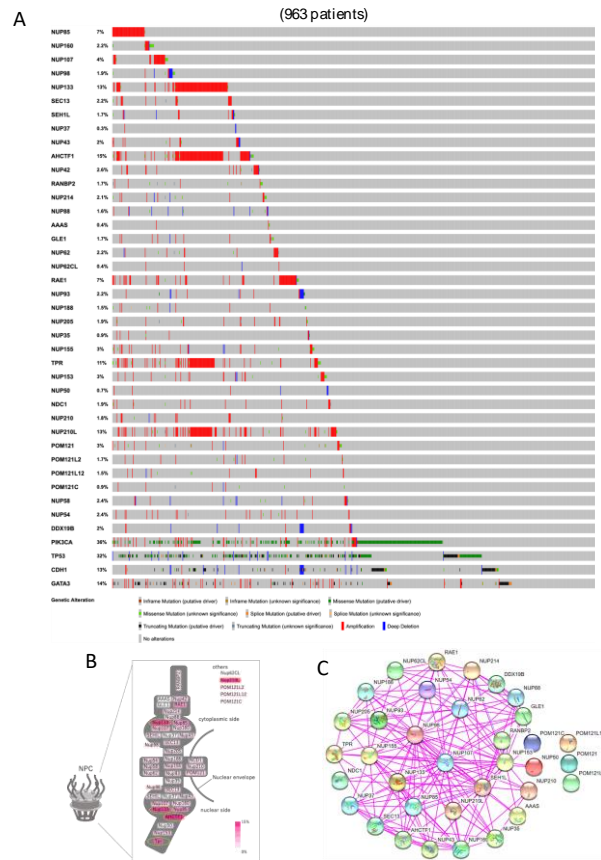


Figure 1: Genes encoding nucleoporins are genetically altered in breast cancer. (A) Various genetic alterations in nucleoporins are detected in breast cancer. Lines in the horizontal bars represent 963 breast cancer patients. Different color schemes in the figure represent amplification, deep deletion, in frame mutation, missense mutation and truncating mutations. The most prevalent genetic alteration detected among the nuclear transport family is the amplification. As positive controls, PIK3CA, TP53, CDH1 and GATA3 are used. (B) Mapping of NUP mutations in the NPC structure. The approximate positions of proteins and the mutation rate in colors are shown. (C) Mapping of NUPs specific interactome and network based topological insight. The round shapes are nodes with miniature NUPs protein structures in the spheres. The number of nodes of the network are 35, while straight lines are directionless (175) edges in the network. The average node degree of the network is 10, while the avg. local clustering coefficient is 0.699, the PPI enrichment p-value: < 1.0e-16. The network has significantly more interactions than random network.

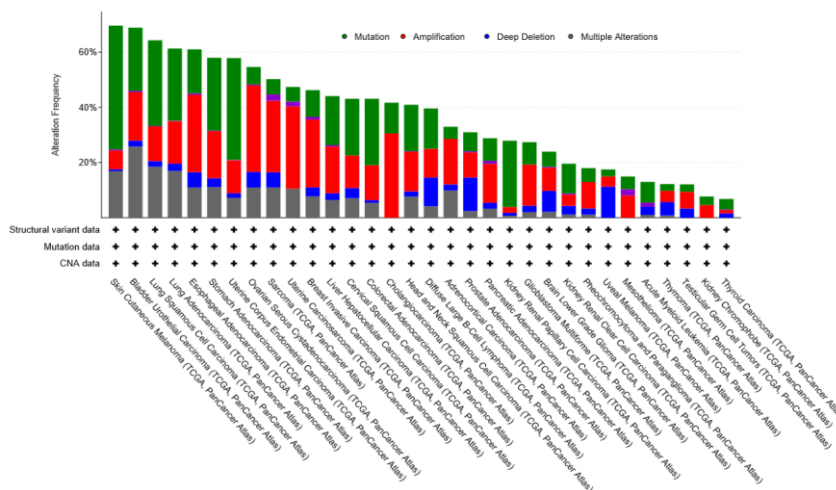


Figure 2: Genes encoding nucleoporins are mutated in a wide variety of cancers. TCGA PanCancer Atlas datasets were used to identify spectrum of mutations in different cancer types. Querying 10953 patients / 10967 samples in 32 studies. Queried Nup genes are altered in 4552 (42%) of queried samples.

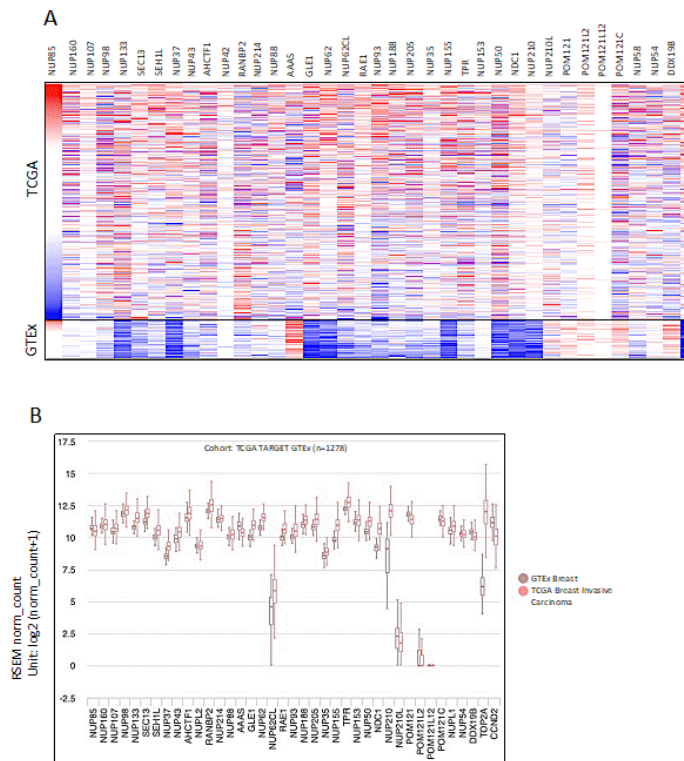


Figure 3: Comparison of expression of genes that encode nucleoporins. (A) Over/under expression of all the genes is represented by red/blue bars respectively. The data from GTEx is used to compare the expression of normal breast tissue with breast cancer samples from TCGA. TOP2A and CCND2 are used as controls. (B) Comparisons of individual Nup genes expression in normal breast samples from TCGA and breast cancer patient samples. The p values for individual genes are calculated using Welch's t-test. NUP85-p = 2.391e-13 (t = 7.535), NUP160-p = 0.00004021 (t = -4.647), NUP107-p = 3.156e-20 (t = -9.694), NUP98-p = 1.599e-13 (t = -7.674), NUP133-p = 6.677e-127 (t = -30.64), SEC13-p = 7.633e-80 (t = -24.77), SEH11-p = 4.966e-46 (t = -16.36), NUP37-p = 1.090e-112 (t = -32.86), NUP43-p = 1.143e-34 (t = -14.02), AHCTF1-p = 1.012e-15 (t = -8.435), NUPL2-p = 0.02267 (t = -2.287), RANBP2-p = 3.152e-47 (t = -15.70), NUP214-p = 0.001859 (t = -3.141), NUP88-p = 4.548e-20 (t = -9.647), AAAS-p = 0.000 (t = 15.51), GLE1-p = 2.849e-131 (t = -38.31), NUP62-p = 1.600e-121 (t = -35.62), NUP62CL-p = 1.344e-25 (t = -11.73), RAE1-p = 8.397e-108 (t = -27.93), NUP93-p = 5.991e-63 (t = -18.83), NUP188-p = 1.730e-30 (t = -12.67), NUP205-p = 8.034e-82 (t = -21.80), NUP35-p = 2.813e-17 (t = -9.110), NUP155-p = 1.007e-155 (t = -37.19), TPR-p = 1.705e-51 (t = -16.93), NUP153-p = 0.00005305 (t = -4.073), NUP50-p = 5.891e-100 (t = -28.78), NDC1-p = 1.897e-168 (t = -45.57), NUP210-p = 3.468e-71 (t = -28.35), NUP210L-p = 0.0001713 (t = 3.825), POM121-p = 0.000 (t = 16.28), POM121L2-p = 0.06428 (t = 1.859), POM121L12-p = 0.005488 (t = 2.810), POM121C-p = 2.887e-15 (t = 8.203), NUPL1-p = 2.792e-18 (t = -9.235), NUP54-p = 0.7115 (t = 0.3700), DDX19B-p = 0.000 (t = 12.45).

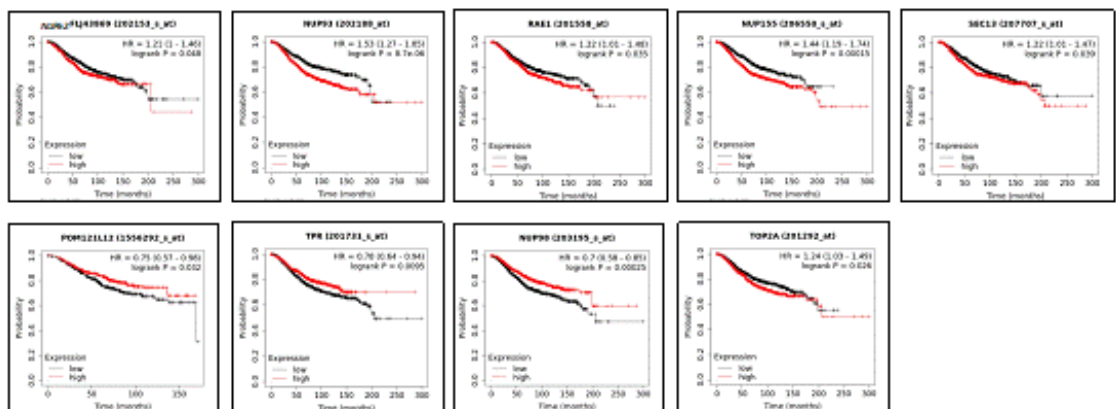


Figure 4: Correlation of Nup genes with breast cancer patients over all survival. Expression of Nup genes correlate with over all patient samples. Overall patient survival shown by Meier-Kaplan plots. X axis shows Overall survival percentage, and Y axis shows Months after the diagnosis. High expression group is indicated by red, while the low expression group is shown by the black color. The patients (n=1402) were split by median. p-values were determined using the Log-Rank test.

Nucleoporin Protein Name	Gene symbol	Reference
NUCLEOPORIN 85	NUP85	[31]
NUCLEOPORIN 160	NUP160	[32]
NUCLEOPORIN 107	NUP107	[33]
NUCLEOPORIN 98/96	NUP98	[34, 35]
NUCLEOPORIN 135	NUP135	[32]
SEC13	SEC13	[36]
SEH1 LIKE NUCLEOPORIN	SEH1L	[37]
NUCLEOPORIN 37	NUP37	[38]
NUCLEOPORIN 43	NUP43	[38]
ELYS	AHCTF1	[39]
NUCLEOPORIN 42	NUPL2/NUP42	[40]
RAN BINDING PROTEIN 2	RANBP2	[41]
NUCLEOPORIN 214	NUP214	[42]
NUCLEOPORIN 88	NUP88	[43]
ALADIN WD REPEAT NUCLEOPORIN	AAAS	[44]
GLE1	GLE1	[45]
NUCLEOPORIN 62	NUP62	[46]
NUCLEOPORIN 62 C-TERMINAL LIKE	NUP62CL	[47]
RAE1	RAE1	[48]
NUCLEOPORIN 93	NUP93	[49]
NUCLEOPORIN 188	NUP188	[50]
NUCLEOPORIN 205	NUP205	[51]
NUCLEOPORIN 35	NUP35	[52]
NUCLEOPORIN 155	NUP155	[53]
TPR	TPR	[54]
NUCLEOPORIN 153	NUP153	[55]
NUCLEOPORIN 50	NUP50	[56]
NDC1	NDC1	[57]
NUCLEOPORIN 210	NUP210	[58]
NUCLEOPORIN 210L	NUP210L	[59]
POM121	POM121	[60]
POM121 transmembrane nucleoporin like 2	POM121L2	[61]
POM121 transmembrane nucleoporin like 12	POM121L12	[62]
POM121 transmembrane nucleoporin C	POM121C	[63]
NUCLEOPORIN 58/45	NUPL1/NUP58	[64]
NUCLEOPORIN 54	NUP54	[65]
DDX19	DDX19B	[66]

Table 1: Various Nucleoporins (Nups) and genes encoding them.

A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
NUP133	AHCTF1	801	17	35	110	>3	<0.001	<0.001	Co-occurrence
TPR	NUP210L	805	37	48	73	>3	<0.001	<0.001	Co-occurrence
NUP133	TPR	798	55	38	72	>3	<0.001	<0.001	Co-occurrence
AHCTF1	TPR	783	70	35	75	>3	<0.001	<0.001	Co-occurrence
AHCTF1	NUP210L	773	69	45	76	>3	<0.001	<0.001	Co-occurrence
NUP133	NUP210L	785	57	51	70	>3	<0.001	<0.001	Co-occurrence
SEC13	NUP210	937	9	5	12	>3	<0.001	<0.001	Co-occurrence
NUP93	DDX19B	934	10	8	11	>3	<0.001	<0.001	Co-occurrence
DDX19B	CDH1	831	3	113	16	>3	<0.001	<0.001	Co-occurrence
GLE1	NUP188	940	9	7	7	>3	<0.001	<0.001	Co-occurrence
TP53	CDH1	540	294	116	13	-2.280	<0.001	<0.001	Mutual exclusivity
NUP214	NUP188	936	13	7	7	>3	<0.001	<0.001	Co-occurrence
NUP153	POM121L2	924	23	8	8	>3	<0.001	<0.001	Co-occurrence
POM121	POM121C	931	23	3	6	>3	<0.001	<0.001	Co-occurrence
NUP93	CDH1	826	8	116	13	>3	<0.001	<0.001	Co-occurrence
NUP42	POM121L12	950	19	8	6	>3	<0.001	<0.001	Co-occurrence
NUP214	GLE1	932	15	11	5	>3	<0.001	<0.001	Co-occurrence
PIK3CA	GATA3	509	319	108	27	-1.326	<0.001	<0.001	Mutual exclusivity
NUP153	TP53	646	10	286	21	2.246	<0.001	0.001	Co-occurrence
SEC13	NUP188	932	17	10	4	>3	<0.001	0.006	Co-occurrence
TPR	POM121	835	99	18	11	2.366	<0.001	0.006	Co-occurrence
NUP133	POM121C	833	121	3	6	>3	<0.001	0.011	Co-occurrence
NUP98	NUP214	929	14	16	4	>3	<0.001	0.012	Co-occurrence
POM121L2	TP53	652	4	295	12	2.729	<0.001	0.015	Co-occurrence
NUP85	NUP155	876	57	22	8	2.482	<0.001	0.016	Co-occurrence
NUP42	NUP205	924	21	14	4	>3	<0.001	0.027	Co-occurrence
POM121	GATA3	810	18	124	11	1.997	0.001	0.031	Co-occurrence
NUP42	PIK3CA	594	23	344	2	-2.736	0.001	0.042	Mutual exclusivity
NUP188	NUP210	955	11	14	3	>3	0.001	0.042	Co-occurrence
NUP85	RAE1	840	53	58	12	1.713	0.002	0.042	Co-occurrence
NUP153	POM121	908	26	24	5	2.863	0.002	0.045	Co-occurrence
NUP85	SEC13	883	59	15	6	2.582	0.002	0.045	Co-occurrence
SEC13	AHCTF1	806	12	136	9	2.152	0.002	0.045	Co-occurrence
RAE1	TP53	620	36	273	34	1.101	0.002	0.045	Co-occurrence

Table 2: Mutual exclusivity cooccurrence

We recently catalogued genetic and molecular attributes of all the genes encoding nuclear transport factors in breast cancer [70]. A crucial part of the nuclear transport process is the interaction of Nups with the actual transport receptors. Nups constitute a group of proteins that constitute the nuclear pore complexes. Their critical role in regulating transport of molecules across the nuclear membrane is already established [71]. One of the categories of cargo that traverse through the nuclear membrane is the proteins related to regulation of cell cycle. Therefore, the functions of Nups in cell cycle regulation become pivotal. Mislocalization of cell cycle regulators has been linked to various cancers [24]. Our knowledge of individual Nups is rudimentary with limited evidence of *NUP43*, *NUP88* and *NUP98* to be drivers of breast cancer [72, 73]. However, an overarching molecular profiling of Nup genes in cancer was lacking. Our study fills this gap by providing comprehensive analysis of all Nup genes in breast cancer.

Most popular and known association of Nups with various malignancies including leukemic and non leukemic are through juxtaposition of nup genes with a wide range of other genes [20, 21]. *Nup98*, for instance, is frequently identified to be translocated with a number of factors and mediate transcription changes depending on its fusion partner [74]. However, there is little knowledge regarding the role of individual Nups in cancer progression. Their molecular analysis of individual role of Nups is critical as they perform diverse aspects of cell cycle either by regulating the nucleocytoplasmic shuttling of various molecules implicated in cancer or by direct or indirect interaction with chromatin., thus regulating expression of genes with possible implications with cancer. An evidence in support of the first aspect was recently reported in prostate cancer wherein overexpression of *POM121* led to enhanced nuclear import of *E2F1*, *MYC*, and *AR*, eventually affecting tumorigenesis, proliferation, and survival in lethal prostate cancer [24]. We identified that, in breast cancer, several other Nups were either mutated or had deregulated expression. Interestingly, some of them are implicated in directing nuclear transport by interacting with transport receptors [75], implicating possible nuclear transport related functions.

In addition to above mentioned functions of Nups, evidence regarding their un-expected role in coordinating cell cycle regulation is being established [91]. A recent report highlighted the role of *Nup155* in regulating translation of *P21* which, a key downstream effector of *P53* response in liver cancer, thus regulating *P53* network [76]. *Nup155* also emerged as an important modulator of chromatin remodeling by interaction with *HDAC4* [77]. More recently, *Nup155* has been shown to

regulate non-coding RNA network mouse embryonic stem cells (mESCs). Partial loss of *Nup155* led to reduction in abundance of *miR290–295* cluster with concomitant loss of pluripotency factors in mES cells thus affecting pluripotency [78]. The involvement of a single nucleoporin in a wide variety of signaling networks including pluripotency underscores widespread functions. Our data show that *NUP155* is not only significantly upregulated in breast cancer but also correlated with poor patient survival. It remains to be seen if and how individual pathways regulated by *Nup155* converge onto the pathogenic phenotype in breast cancer.

Another novel molecule that was identified through our in silico analysis is *SEC13*. Although *SEC13* is generally associated with *COPII* vesicle for the anterograde movement of molecules from ER to Golgi, its location in the nuclear pore is well established where it is a part of *Nup107-160* subcomplex [37]. Additionally, its nucleocytoplasmic shuttling of *SEC13* is also reported [79]. In the nucleus, it is also shown to bind kinetochores [37]. Its multi-compartmental distribution indicates a variety of possible functions. Our analysis indicates that it is not only significantly upregulated, but also correlates with poor patient survival, thus warranting deeper exploration.

It is pertinent to note that majority of Nups were upregulated in breast cancer patients (Figure 3). While expression of most of them is correlated with poor patient survival, thus pointing to their roles as oncogenes, overexpression of some Nups correlated with better overall patient survival. The Nups in this category included *Nup98*, *TPR* and *POM121L12*. The correlation between their overexpression and better patient survival might provide a hint for their possible tumor suppressor functions. Further studies are required to realize their role in cell survival and proliferation by using loss of function and gain of function assays in cellular models.

As nucleoporins feed multiple arms of the cellular physiology, it is challenging to identify what particular altered functions might lead to tumorigenesis. Finding the downstream effects is important as it provides an opportunity to target the specific pathway for therapeutic purpose. e.g. Importazole was used to reduce tumorigenic potential in *POM121* overexpressed cells as it was mediated through interaction with importin β [80]. However, individual Nups might have role in the disease progression that is independent of nuclear transport functions, e.g the role of *Nup62* in regulating gastric cancer metastasis by regulating *Wnt/ β -Catenin* and *TGF- β* signaling pathways [81]. In such circumstances, individual Nups and/or the

downstream pathways that are altered might have to be unraveled for eventual targeting.

Although inhibitors for nuclear transport receptors are relatively well studied in cancer, specific inhibitors for Nups are equally promising in various malignancies considering their massive deregulation, as exemplified by the current study in breast cancer. The mutations in Nup genes were represented in 50% of the breast cancer patients in addition to deregulated expression representing an important vulnerable point. The repertoire of small molecules perturbing the nuclear pore permeability by disrupting FG interactions is limited to only trans1-2 cyclohexanediol (CHD) and Pitstop2 [82, 83]. Despite their ability to compromise the NPC permeability barrier effectively, their systemic effects on the NPCs may hinder their use in therapeutics. Design and synthesis of small molecules targeting specific components of NPCs remains an arduous task, albeit with potential to pave way for novel therapeutic strategies in breast cancer.

Competing Interest

The authors declare no competing interests.

Authors' Contribution

R.M. and N.Y. conceived and designed the study, interpreted data, and finalized the manuscript; R.M., M. N., and Z. A. conducted Bioinformatics analysis; R.M., K.J., M.N., and N.Y. were involved in manuscript writing; All authors read and approved the manuscript.

Acknowledgement

A grant from Alfaisal University (IRG 20410) to RM is thankfully acknowledged.

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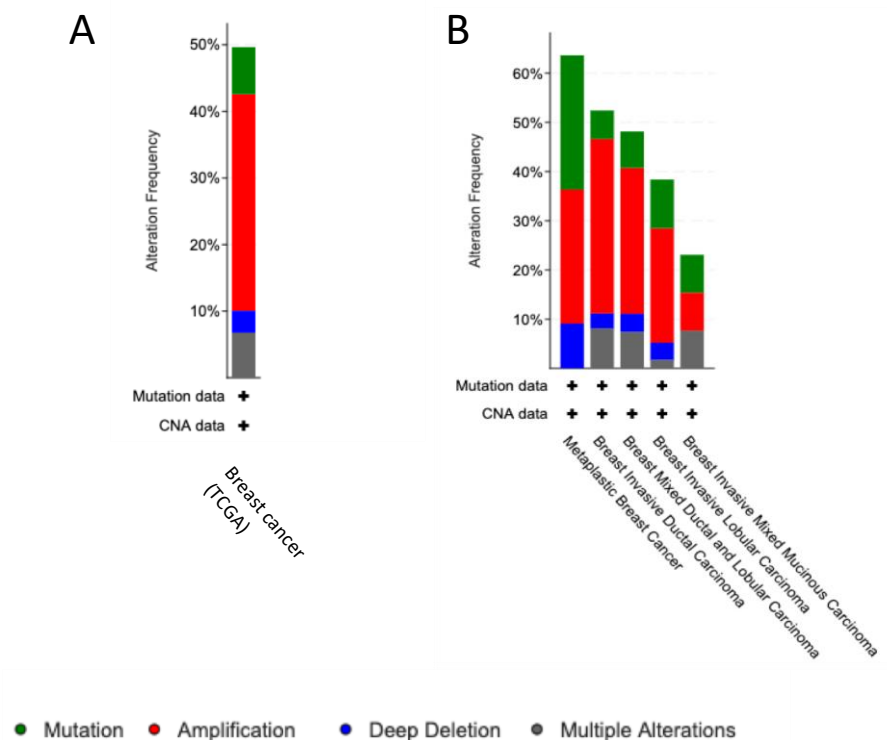
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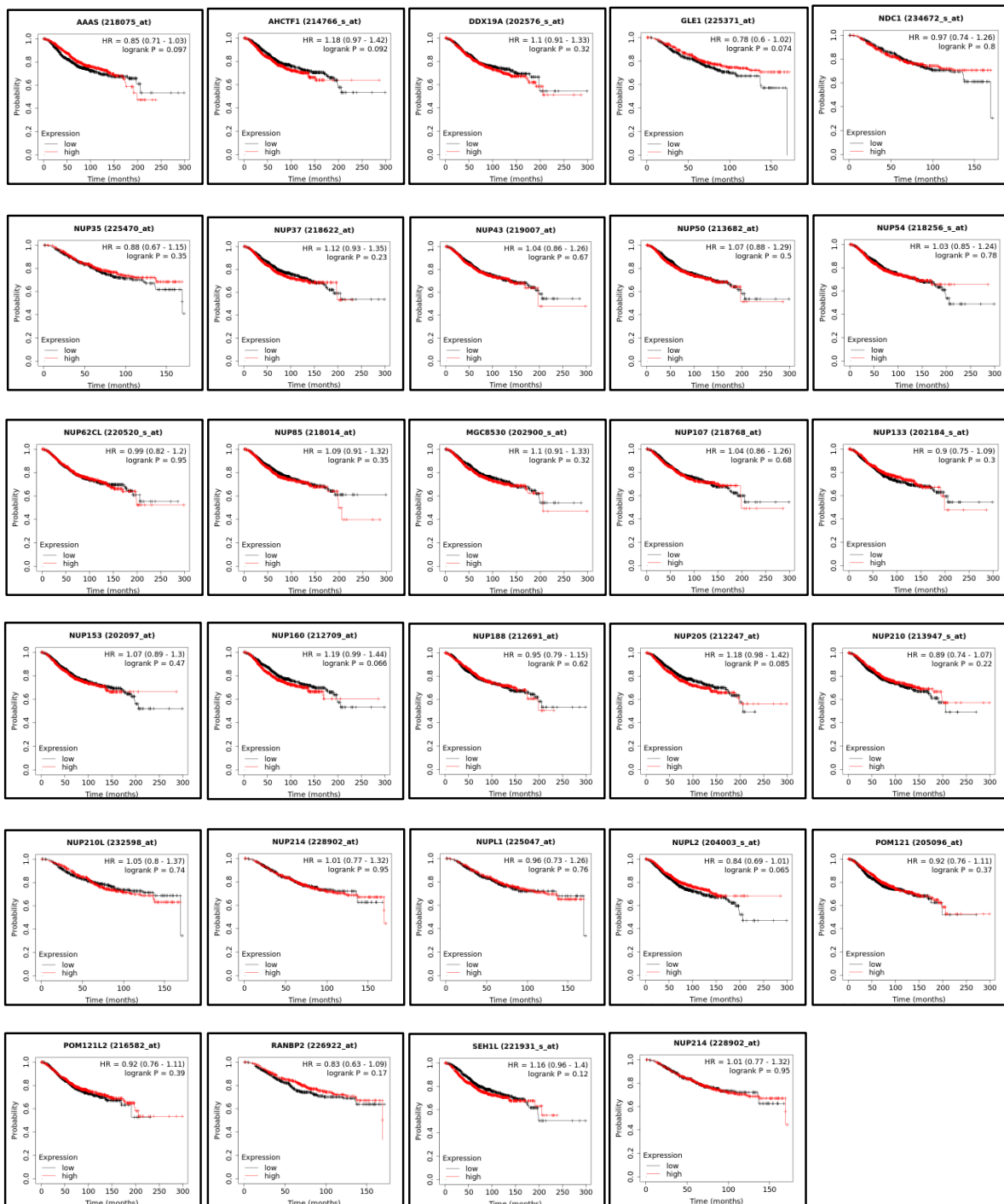
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Supplementary Figure 1



Supplementary Figure 1: A) Overall mutation frequency in the nucleoporins group in breast cancer. (B) Representation of mutations in nucleoporin genes in different breast cancer subtypes.

Supplementary Figure 2



Supplementary Figure 2: Nup genes without a significant correlation with patient survival. Overall patient survival shown by Meier-Kaplan plots. X axis shows Overall survival percentage, and Y axis shows Months after the diagnosis. High expression group is indicated by red, while the low expression group is shown by the black color. The patients (n=1402) were split by median. p-values were determined using the Log-Rank test.