Protein-Protein Interaction Network Analysis and Identification of Key Players in nor-NOHA and NOHA Mediated Pathways for Treatment of Cancer through Arginase Inhibition: Insights from Systems Biology

Ishtiaque Ahammad[1]*

1. Department of Biochemistry and Microbiology, North South University, Dhaka, Bangladesh.
** Corresponding author. Email:* ishtiaque.ahammad@northsouth.edu

Abstract

L-arginine is involved in a number of biological processes in our bodies. Metabolism of L-arginine by the enzyme arginase has been found to be associated with cancer cell proliferation. Arginase inhibition has been proposed as a potential therapeutic means to inhibit this process. N-hydroxy-nor-L-Arg (nor-NOHA) and N (omega)-hydroxy-L-arginine (NOHA) has shown promise in inhibiting cancer progression through arginase inhibition. In this study, nor-NOHA and NOHA-associated genes and proteins were analyzed with several Bioinformatics and Systems Biology tools to identify the associated pathways and the key players involved so that a more comprehensive view of the molecular mechanisms including the regulatory mechanisms can be achieved and more potential targets for treatment of cancer can be discovered. Based on the analyses carried out, 3 significant modules have been identified from the PPI network. Five pathways/processes have been found to be significantly associated with nor-NOHA and NOHA associated genes. Out of the 1996 proteins in the PPI network, 4 have been identified as hub proteins- SOD, SOD1, AMD1, and NOS2. These 4 proteins have been implicated in cancer by other studies. Thus, this study provided further validation into the claim of these 4 proteins being potential targets for cancer treatment.

Keywords

Arginase; nor-NOHA; NOHA; Cancer

Introduction

L-arginine, a basic amino acid, has a major role in a number of systems in our bodies including the immune system, particularly on the proliferation of T lymphocytes [1]. The enzyme arginase metabolizes L-arginine to L-ornithine which is important in the biochemical pathways involved in cell proliferation [2]. Two isoforms of arginase exists: arginase I, and arginase II. Arginase I is

a cytosolic enzyme which is primarily found in hepatocytes, erythrocytes, and granulocytes [3], [4] and Arginase II is found in the mitochondria of various tissues, including kidney, brain, and prostate [5], [6]. Arginase II activity has been shown to be increased in breast, colon, and prostate cancer [7], [8]

Arginase inhibition has been found to suppress proliferation of breast cancer cells. Therefore it is being considered as a new therapeutic target for suppressing breast cancer cell growth. In tumor cells with elevated arginase activity, arginase inhibitors can be a potential therapeutic option for treating cancer [9].

N(omega)-hydroxy-L-arginine (NOHA) has been found to selectively inhibit cell proliferation and induce apoptosis in high arginase expressing MDA-MB-468 cells [7]. NOHA is also involved in the modulation of T cell receptor CD3zeta which undergoes down-regulation in cancer [7].

Injection of N-hydroxy-nor-L-Arg (nor-NOHA) blocked growth of lung cancer cells in mice [10]. In another study, nor-NOHA significantly ($P = 0.01$) reduced arginase II activity and suppressed growth of cells with high arginase activity in renal carcinoma [9]

The purpose of this study was to identify crucial genes and proteins involved in the nor-NOHA and NOHA mediated pathways in order to shed more light on their mechanism of action and the key players involved. For this, nor-NOHA and NOHA-associated genes from several databases was identified. And multiple Bioinformatics and Systems Biology tools have been used to identify more nor-NOHA and NOHA-associated key genes. They included pathway enrichment analysis, protein-protein interaction (PPI) network, module analysis and transcriptional regulatory network analysis.

Methods

Nor-NOHA and NOHA-Associated Genes

“nor-NOHA” and “NOHA” were used as keywords to search in three databases for genes associated with these compounds and the results were combined to build a set of Nor-NOHA and NOHA-associated genes. The three databases used were-

- GeneCards (version 3.0), a searchable, integrative database which provides comprehensive information on all annotated and predicted human genes [11].
- Search Tool for Interacting Chemicals (STICH, version 5.0), a database of known and predicted interactions between chemicals and proteins [12]
- Comparative Toxicogenomics Database (CTD), which provides manually curated information concerning chemical–gene/protein interactions, chemical–disease as well as gene–disease relationships was used [13].

PPI Network

Two databases were combined to predict the Protein-Protein Interaction (PPI) pairs among the nor-NOHA and NOHA-associated genes. The databases used were-

- Biological General Repository for Interaction Datasets (BioGRID, version 3.4, https://wiki.thebiogrid.org/) [14]
- The Molecular Interaction Database (MINT, 2012 update, https://mint.bio.uniroma2.it/) [15]

Using the software Cytoscape (http://www.cytoscape.org) [16], a PPI network was visualized for Nor-NOHA and NOHA-associated genes.

Using the CytoNCA plug-in [17] (version 2.1.6, http://apps.cytoscape.org/apps/cytonca) in Cytoscape, degree centrality (DC), betweenness centrality (BC), and closeness centrality of the nodes of the PPI network were subjected to analysis to identify the hub proteins [18]. "Without weight." was set as the parameter.

Module Analysis

For module analysis of the PPI network, MCODE plug-in [19] (version 1.4.2; http://apps.cytoscape.org/apps/mcode; parameters set as degree cut-off = 2, maximum depth = 100, node score cut-off = 0.2, and -core = 2) in Cytoscape was used.

Pathway Enrichment Analysis

KEGG pathway enrichment analysis for the nodes of top modules was carried out with JEPETTO plug-in [20] in Cytoscape.

Transcriptional Regulatory Network Construction

Transcription factors (TFs) among nor-NOHA and NOHA-associated genes were searched and then their targets were identified using the transcriptional regulatory relationships unravelled by a sentence-based text-mining (TRRUST, http://www.grnpedia.org/trrust/) [21] database.

Finally, a transcriptional regulatory network of the hub proteins was constructed using Cytoscape [16].

Results and Discussion

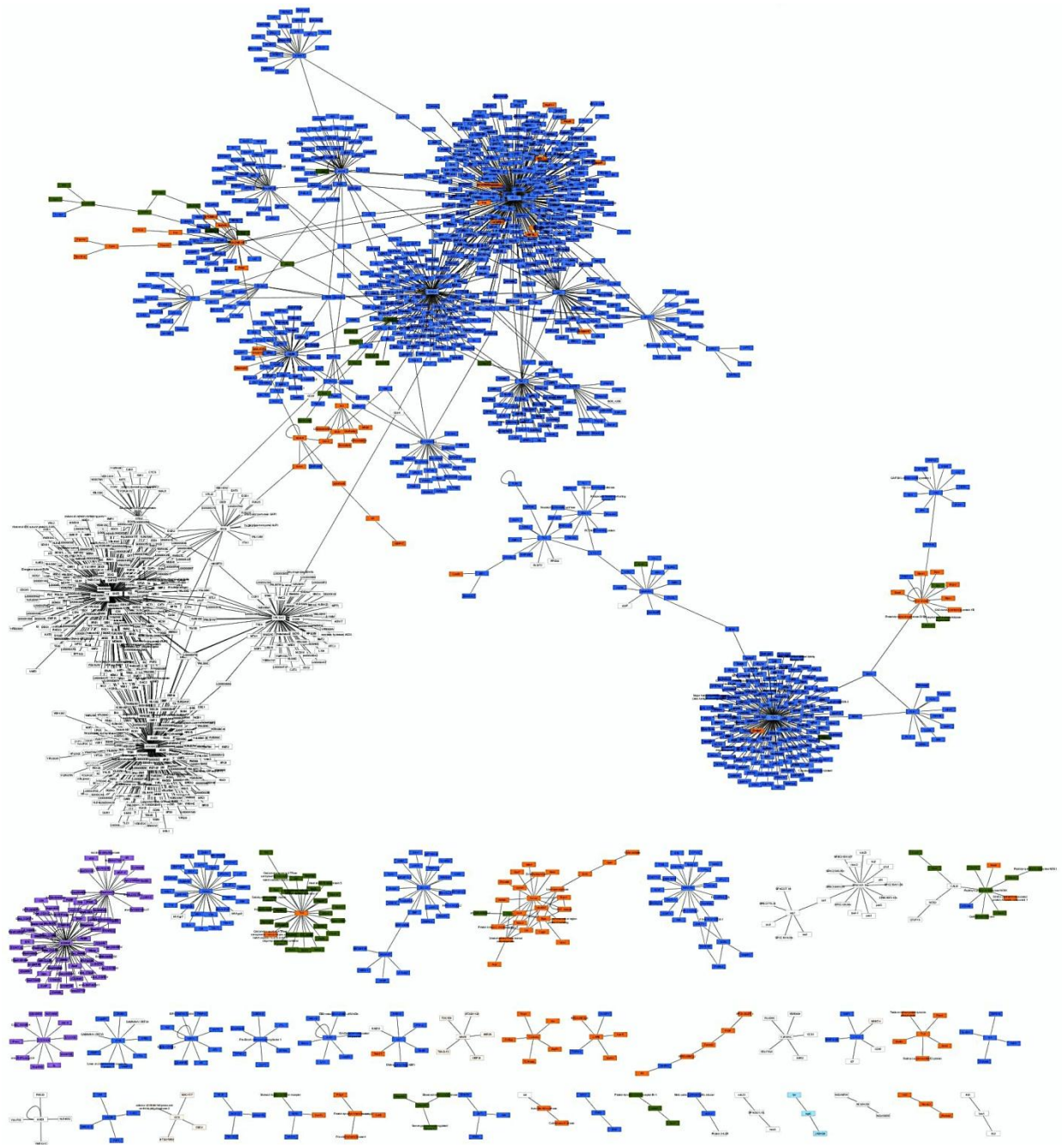

Figure 1: Protein-protein interaction network of nor-NOHA and NOHA associated genes. It contains 1996 nodes (proteins) and 2132 edges (interactions).

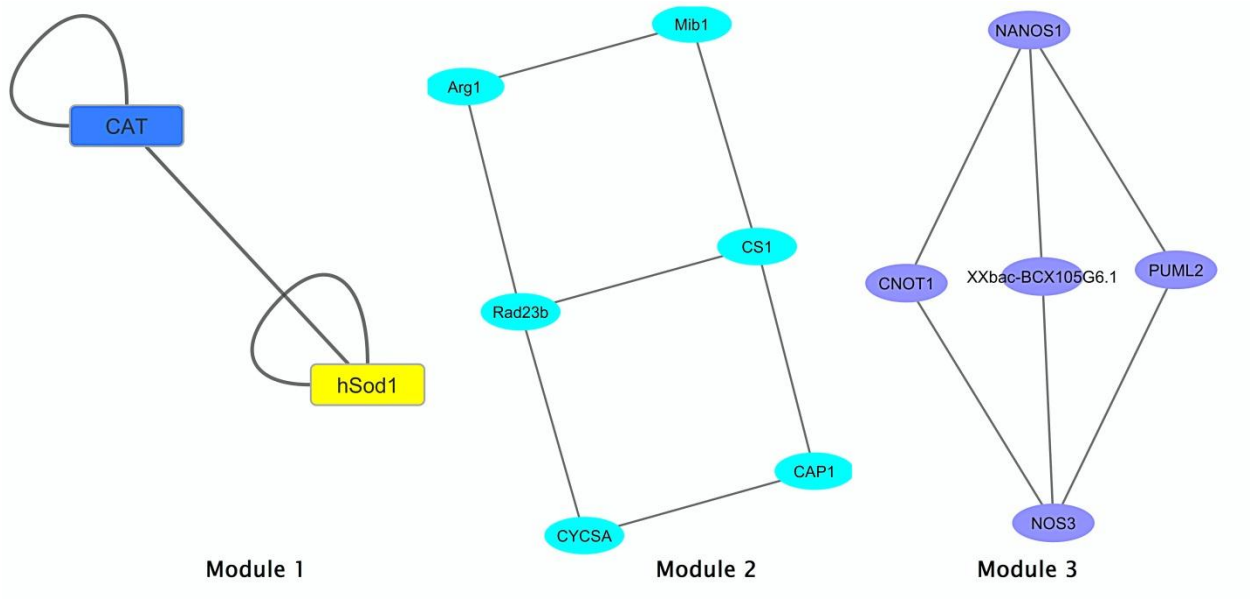

Figure 2: Module 1, 2 and 3 derived using MCODE plug-in in Cytoscape with parameters set as degree cut-off = 2, maximum depth = 100, node score cut-off = 0.2, and -core = 2

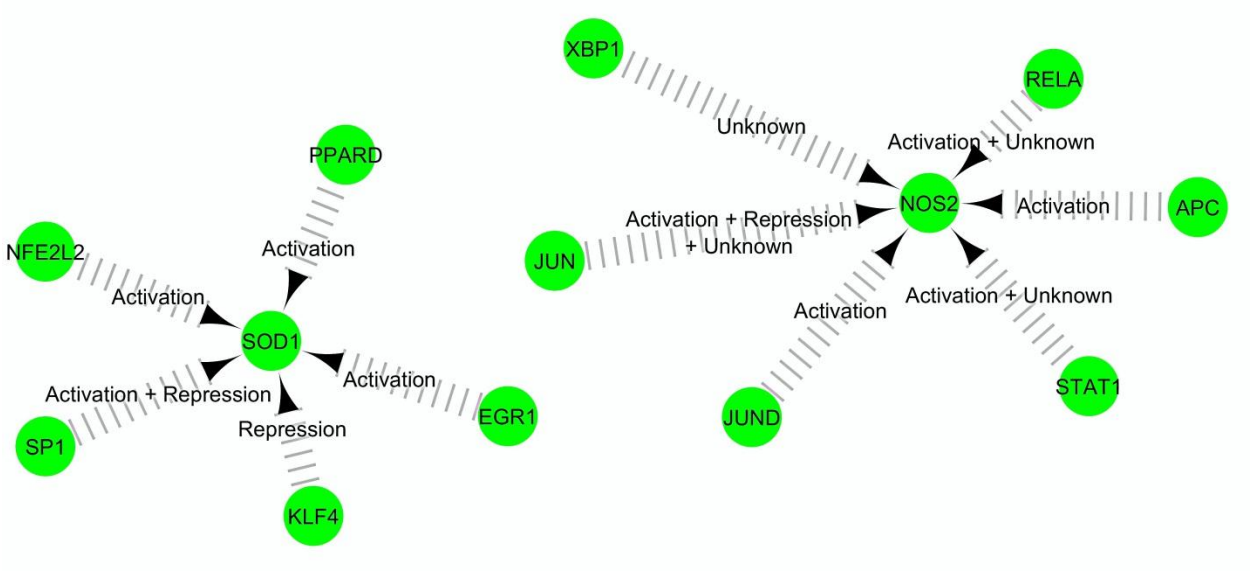

Figure 3: Transcriptional regulatory network of hub proteins SOD1 and NOS2

Table 1: Nor-NOHA and NOHA associated genes

Nor-NOHA associated genes	NOHA associated genes
NOS3	NOS1
ARG2	MARC2
NOS2	NOS2
CD247	MARC1
NOS1	NOS3
SOD1	ARG1
DDAH1	CYCS
IL6	ODC1
AGMAT	SOD1
	AMD1
	CAT
	ARG2
	STX8
	UROD
	AGMAT

Table 2: Hub proteins in the PPI network according to Degree Centrality, Betweenness Centrality and Closeness Centrality using CytoNCA

Degree Centrality		Betweenness Centrality		Closeness Centrality	
Gene	Score	Gene	Score	Gene	Score
SOD	336.0	SOD	1271250.132142436	SOD	0.0038243429647416014
SOD1	239.0	SOD1	774804.0421345556	CCS1	0.0038173221921919734
AMD1	208.0	CCS1	597881.8676198863	SOD1	0.003820160825854615
NOS2	146.0	AMD1	436818.27082915726	GSH1	0.0038169885126128162
ODC1	68.0	NOS2	308769.3082637827	UBC	0.003815663639055696
CuZnSOD	62.0	ODC1	195981.77444007777	CAT	0.0038166363585598754

CYCS	49.0	GSH1	142337.01101673584	HSPABP2	0.003815154317167466
CAT	49.0	HUR	132458.55204446075	HSPH2	0.003815154317167466
CARB	46.0	CARB	111604.5321192979	KARP1	0.003814432239231872
NOS3	46.0	NOS3	110840.35731586967	NDPKB	0.003814432239231872

Table 3: KEGG pathway enrichment analysis result for the nodes of top modules

Pathway or Process	**XD-score**	**q-value**	**Overlap/Size**
Arginine and proline metabolism	0.21151	0.09869	2/37
Tryptophan metabolism	0.14914	1.00000	1/26
Nucleotide excision repair	0.09053	1.00000	1/42
Amyotrophic lateral sclerosis (ALS)	0.08040	1.00000	1/47
Peroxisome	0.06672	1.00000	1/56
VEGF signaling pathway	0.05981	1.00000	1/62
Amoebiasis	0.03611	1.00000	1/98
Protein processing in endoplasmic reticulum	0.02407	1.00000	1/139
Calcium signaling pathway	0.02161	1.00000	1/152
Metabolic pathways	0.01404	1.00000	3/640

Table 4: Genes involved in the top 10 enriched KEGG pathways

Pathway or Process	**Genes Involved**
Arginine and proline metabolism	ABP1,DAO,PUM2,NANOS1,GOT2,GLUD1,CKM,CKB,NOS1,ARG1,SAT1, SAT2,ARG2,ODC1,SRM,NOS2,RAD23B,CPS1,CAP1,OTC,ASS1,ALDH18A 1,ASL,OAT,NOS3,SMS,P4HA2,ALDH2,GLS2,PYCR2,GLS,GLUL,MIB1,PY CRL,AL,DH7A1,CKMT2,GAMT,GLUD2,unknown,GOT1,CAT,P4HA3,P4H A1,EPRS,RARS,SIRT7
Tryptophan metabolism	PUM2,NANOS1,ABP1,RAD23B,ECHS1,IL4I1,CAP1,NOS3,OGDH,ACAT2, TPH1, AANAT,ALDH2,ACAT1,WARS,ARG1,HADHA,IDO1,KYNU,MIB1,GCDH, ALDH7A1,CYP1A1,EHHADH,WARS2,CAT,CYP1A2,HAAO,DDC,CYP1B1

	,HADH,TDO2, ACMSD
Nucleotide excision repair	ERCC5,GTF2H1,ERCC2,RPA3,RPA2,RPA1,ERCC3,ERCC4,CDK7,PCNA,ERCC8, DDB1,MNAT1,ERCC1,DDB2,RAD23A,XPC,GTF2H3,GTF2H2,RAD23B,XPA, CCNH,GTF2H5,POLD2,RFC1,RFC2,RFC5,POLE2,POLE,LIG1,POLD1,RPA4,RFC3,POLD3,POLD4,POLE3,POLE4,RFC4,PUM2,NANOS1,RBX1,CUL4A,CUL4B,CETN2,CAP1,NOS3,ARG1,MIB1,CAT
Amyotrophic lateral sclerosis (ALS)	BCL2L1,TP53,CYCS,BAD,BCL2,BID,CASP9,CASP1,BAX,APAF1,DAXX,PPP3CA, CASP3,SOD1,PPP3CB,MAPK14,MAPK11,RAC1,MAP3K5,PUM2,NANOS1, MAP2K3,MAP2K6,MAPK12,MAPK13,GRIN2B,GRIN1,GRIN2A,CCS,NOS1,ARG1,GRIN2D,PRPH2,PRPH,NEFH,NEFM,ALS2,TNFRSF1A,TNFRSF1B, RAD23B, RAB5A,CAP1,GRIA1,unknown,NOS3,GRIA2,TOMM40,GRIN2C,PPP3CC, MIB1,SLC1A2,CAT,DERL1
Peroxisome	PHYH,PEX7,ABCD3,ACSL6,PUM2,NANOS1,GSTK1,FAR1,GNPAT,AGPS, PEX19,PEX3,PEX10,PEX2,SLC25A17,PEX13,PXMP4,ABCD1,ABCD2,PEX14,PEX12,PEX16,PEX11B,PEX11A,ACAA1,PEX5,AGXT,HAO1,DDO,SCP2,EHHADH,ACOX1, PEX1,PEX6,DAO,NOS2,RAD23B,PECR,CRAT,PMVK,SOD1,CAP1,IDH1,SOD2, NOS3,ACSL4,HSD17B4,ECH1,PRDX1,ARG1,ACOX3,MIB1,PRDX5,MVK, PGAM5,HMGCL,ABCD4,EPHX2,CAT,PIPOX,HACL1,BAAT,IDH2
VEGF signaling pathway	PTK2,SRC,PLCG1,PXN,PIK3R1,RAF1,PRKCA,AKT1,KDR,HRAS,PLCG2,SH2D2A, BAD,MAPK3,MAPK1,MAP2K1,KRAS,MAP2K2,RAC1,CDC42,NFATC1,PRKCG, VEGFA,PPP3CA,PPP3CB,CASP9,MAPKAPK2,NOS3,AKT2,PUM2,NANOS1,NFATC4,MAPK14,NFATC2,HSPB1,RAC2,PIK3CA,PIK3CD,PIK3CB,PLA2G4A,MAPKAPK3,MAPK13,PIK3R3,PIK3R2,SHC2,PIK3CG,PLA2G2A,PLA2G1B,MAPK11,PLA2G5,PIK3R5,PRKCB,RAD23B,MAPK12,CAP1,NFAT5,NFATC3,unknown,AKT3,PLA2G10,PTGS2,ARG1,PPP3CC,MIB1,RAC3,CAT,SPHK1,JMJD7-PLA2G4B, TBC1D3F,PLEKHG2,OPHN1,CDC42BPG,CDC42SE1,TCL1A,PDE3B,TCL1B,ICMT,PLCE1,SHOC2,RASSF2,NRP1,NRAS,KSR1,ALS2CR12,EPX,FOXP3,DUSP9,DUSP10,MAPKSP1,DUSP5,PTPRR,PTPN5,NRGN,DUSP2,ZNHIT1,NOXA1,C11orf17,PLA2R1,MIR1538,SPHK2
Amoebiasis	PTK2,ACTN1,VCL,PIK3R1,ITGB2,TGFB1,COL2A1,TGFB2,LAMB1,LAMA5,LAMC1,PRKCA,GNA15,PLCB1,ACTN4,LAMA3,NOS2,RELA,PRKACA, NFKB1,RAB7A,RAB5A,ITGAM,CD14,PRKACB,PRKACG,PRKCB,PUM2, NANOS1,LAMB3,FN1,COL1A2,COL4A4,COL1A1,COL4A2,COL4A1,COL4A6,COL5A3,GNAQ,PIK3CA,PLCB2,PIK3CD,PIK3CB,IL1R1,TLR2,PIK3R3,TLR4,PIK3R2,COL5A1,C8B,C8A,C9,C8G,RAB5C,LAMB2,LAMC2,TGFB3,PIK3CG,PIK3R5,IL1B,ARG1,ARG2,IL12B,IL12A,IL1R2,CTSG,SERPINB1

	3,ACTN2,PRKCG,CASP3,GNA11,SERPINB2,RAD23B, COL3A1,CD1D,GNAS,SERPINB1,IFNG,CAP1,CSF2,SERPINB3,PLCB3,COL11A1, NOS3,RAB5B,SERPINB9,ADCY1,LAMA1,IL6,HSPB1,PLCB4,CXCL1,IL8, LAMA4,MIB1,SERPINB4,PRKX,IL10,SERPINB6,MUC2,CAT,COL11A2,GNA14,LAMA2, COL5A2
Protein processing in endoplasmic reticulum	UBQLN1,UBQLN4,P4HB,SSR4,SSR3,SEC63,SEC62,HSPA5,SEC61B,VCP, ATXN3,HSPA8,HSP90B1,UFD1L,NPLOC4,NSFL1C,PLAA,UBE4B,AMFR, DERL1, RAD23A,RAD23B, TRAF2,MAP3K5,ERN1,MAPK9,HSPA1A,HSPBP1,BCL2, DNAJA2,BAG2,PPP1R15A,EIF2AK2,DNAJC3,STUB1,DNAJB11,DNAJB1, HSPA2,UBQLN2,RPN1,SYVN1,ERP29,DNAJA1,CANX,PRKCSH,BCAP31, PDIA3,EDEM1,MBTPS1,MAPK8,MAP2K7,BAK1,BAX,PUM2,NANOS1,HSP90AB1,SKP1, HSPH1,SAR1B,EIF2AK3,NFE2L2,ATF4,RBX1,CUL1,FBXO2,CALR,HSP90AA1,NOS3,EIF2AK1,DNAJC10,SEC23B,SIL1,DNAJC1,SEC24B,SEC13,STT3A,RPN2,DAD1,TUSC3,STT3B,UBE2D3,RNF5,NGLY1,UBE2E1,UBE2G1,SEC24C,SEC23A, ERO1LB,ERO1L,TRAM1,GANAB,EIF2S1,SEC24D,SEC31A,CRYAB,CRYAA,PARK2,UBE2D2,UBE2D1,DDIT3,UBE2E3,UBE2G2,UBE2J2,HERPUD1,SAR1A,DNAJC5,MBTPS2,HYOU1,CAPN1,UBE2E2,CAP1,SEC61A1,MAPK10,SEC61A2,ATF6, RRBP1,ERLEC1,UBE2D4,SEL1L,SEC24A,DERL2,LMAN1,CAPN2,ARG1,PDIA4,EDEM2,MOGS,XBP1,DERL3,UGGT1,CKAP4,OS9,MIB1,UGGT2,SSR1,EIF2AK4,PREB,MAN1A2,WFS1,MAN1C1,CAT,DNAJB2,HSPA6,PDIA6,SSR2,HSPA1L
Calcium signaling pathway	PLCG1,EGFR,ERBB2,PLCG2,PDGFRB,GRIN2A,PDGFRA,AGTR1,PTGFR, PTGER1,PRKCA,GRM5,NOS1,ITPKA,GNA15,ATP2B2,MYLK,ATP2B1,ITPKB,ADRA1B, PLCB1,AVPR1A,GRM1,PRKCG,GRIN1,GRIN2D,PRKACA,PRKACB,PRKACG,ITPR1,RYR1,PHKA1,ITPR2,CACNA1C,PLN,RYR2,PPP3CA,ATP2B4, PPP3CB,CAMK4,CALM1,PLCD1,CAMK2G,PHKA2,CAMK2A,PHKB,PHKG2,PLCB3,PHKG1,ADCY8,PDE1A,PRKCB,PUM2,SLC25A5,NANOS1,SLC25A6,SLC25A4,ADRB1,PTK2B,ERBB4,ERBB3,TBXA2R,GNA11,GNAQ,HTR2A,ITPR3,ADRB2,CHRM2,TRPC1,EDNRB,EDNRA,HTR2B,BDKRB2,PLCB2,HTR6,CHRM3,BDKRB1,HTR2C,ADCY3,CAMK2B,PPID,F2R,GNAS, ADCY2,LHCGR,ADRB3,CAMK2D,ATP2A2,PTGER3,CACNA1S,ARG1,GNA14,NOS3,DRD1,ATP2A1,RYR3,ADCY1,ADRA1A,SLC8A3,SLC8A2,SLC8A1,ADRA1D,P2RX2,P2RX1,P2RX3,CCKAR,CCKBR,CACNA1G,CD38,TACR2,TACR1,TACR3,NOS2,PLCE1,RAD23B,VDAC3,VDAC2,CAP1,unknown, ADORA2B,CACNA1D,CACNA1A,ATP2A3,P2RX7,CACNA1B,GRIN2C,P2RX5,PLCD4,CACNA1E,P2RX6,PPP3CC,MYLK2,PLCB4,NTSR1,OXTR,AVPR1B,TNNC2,

	MIB1,TNNC1,PTAFR,CALML5,BST1,DRD5,PRKX,HRH2,CHRM1,ADCY7,CAT,GRPR,SPHK1,CALML3,P2RX4,PLCD3,TRHR,CHRNA7,HTR7,CACNA1H,CHRM5,CALM2,CAMKK2,BCAR3,DAB1,TNS3,CACNB3,RNF41,NRG2,HTR4,BANK1,VDAC1P1,PPIF,CABP2,RIC8B,TAC4,CAMK2N2,AVP,SLN,ADORA2A,CHRFAM7A,CACNA1F,SPHK2,CACNA1I
Metabolic pathways	NSDHL,SC4MOL,HSD17B7,PHPT1,DNMT3L,ENO1,GAPDH,POLR2B,DLAT,POLR2C,PKM2,GPI,PGK1,TPI1,PGAM2,PLCG1,PLCG2,PLD2,DGKZ,ATP5C1,ATP5B,POLA2,ATP5A1,ATP5D,ATP5F1,ATP5E,ATP5J,ATP5L,ATP5H,ATP5I,NDUFB3,ATP5G1,MTATP6,IDH3A,IDH3B,POLR1C,PMM2,ATP6V1B2,PFKM,IDH3G,ME1,ACO2,DLST,DLD,NDUFB8,NDUFAB1,NDUFA7,NDUFS1,NDUFA6,NDUFS6,NDUFB9,NDUFV2,NDUFS2,NDUFB10,NDUFA5,NDUFS7,MTND1,NDUFA4,NDUFA1,NDUFB1,NDUFA9,NDUFV3,NDUFV1,MTND4,NDUFB6,NDUFB2,NDUFA3,NDUFA8,NDUFB5,NDUFB4,NDUFB7,NDUFA10,NDUFC2,NDUFS3,NDUFS5,NDUFS8,NDUFC1,NDUFS4,NDUFA2,NME3,NME2,PTGDS,HK2,HK3,PAFAH1B3,POLR3H,IMPDH1,TK1,BPGM,DPM1,DPM2,DPM3,PIGC,PIGA,ATP6V1A,ATP6V1G1,MTHFD1,ATP6V0D1,ATP6V1D,ATP6V1H,ATP6V0A1,ATP6V1F,ATP6V1E1,ATP6V1C1,MCCC1,GCLM,GCLC,SRM,QARS,CBS,MAT1A,POLR2A,POLR2J,POLR2E,POLR2F,POLR2I,POLR2D,POLR2K,POLR2H,POLR2G,POLR2L,PFKP,RAD23B,INPP5K,PIP5K1A,PLD1,PIP5KL1,POLD2,POLE2,POLE,POLA1,PRIM2,PRIM1,POLD1,POLD3,POLD4,POLE3,POLE4,AK2,PAFAH1B2,ISYNA1,RPN2,DUT,ACLY,ATIC,CBR1,RRM1,RRM2,FECH,PRPS2,POLR3C,PDHA1,PDHX,PDHB,GMPPB,DGKE,PUM2,NANOS1,POLR3B,POLR3F,POLR3A,POLR3D,POLR1E,POLR1D,POLR3K,POLR3G,HMGCR,POLR1A,POLR1B,GART,PGD,PC,NME7,NT5C2,PAFAH1B1,PIGH,PIGQ,PIGY,B4GALT1,LALBA,STT3A,RPN1,DAD1,UQCRC1,PON2,UQCRC2,UQCRH,GANAB,PFAS,HADHAHMGCS1,ACACA,GLUD2,OGDH,QDPR,PDHA2,CYC1,PIP5K1B,HK1,ENO2,ALDOA,ALDOC,SQLE,LTA4H,PFKL,ADH1B,TUSC3,STT3B,RRM2B,ALG5,PLA2G2A,ALOX12,PLA2G1B,ACACB,FH,MDH2,CAD,PAICS,GOT2,APRT,ASL,COQ6,ECHS1,AUH,DPYD,GMPPA,IMPDH2,HIBCH,GLUD1,CS,PGM1,PRPS1,UMPS,PLA2G4A,PLA2G5,HPGDS,ALDOB,ATP6V0E1,DNMT3A,DNMT1,DNMT3B,CKM,CKB,MAT2A,MAT2B,ST3GAL2,NOS1,ARG1,PCYT2,GBE1,ATP6V0A4,ALDH2,PIK3C2B,PIK3C2A,GPAA1,GLB1,GALNS,SORD,ADH1A,LIPT1,EPRS,AK5,UGDH,SAT1,SEPHS1,SAT2,B4GALT3,SDHB,SDHA,SDHD,SDHC,ENO3,SPTLC2,SPTLC1,UQCRFS1,DGKH,DGKD,TYRP1,TYR,MCCC2,PIK3C3,ARG2,ACADL,HADHB,COX5B,COX5A,EXT2,EXT1,GALNT5,COX4I1,MTCO1,PIGU,PIGT,PIGK,PIGS,SUCLG2,SUCLG1,ME3,ASAH1,SMPD1,GAD1,GAD2,HAAO,SUCLA2,HSD11B1,PON3,SCP2,EHHADH,ACOX1,ACAA2,MVK,GALE,ALPP,UCKL1,UCK2,CYP11A1,CYP11B2,ALAS2,CYP11B1,ZNRD1,ODC1,AGPAT6,ADPGK,ALG13,AHCY,GCH1,DAO,MTMR7,CD38,NDST2,ACSS2,NADSYN1,PON1,ALPL,PLCD1,AGK,UPP2,NOS2,MTAP,NADK,PLCE1,REV3L,AKR1B1,RPIA,HMBS,PIP5K1C,CDS1,ST6GAL1,CEL,ALOX5,INPP4A,GPT,MDH1,AGPS,CPS1,ALAS1,IL4I1,CPOX,ALDH6A1,ALAD,CDA,PCYT1A,SHMT1,PNP,APIP,UPP1,MAN2A2,PMVK,KHK,RDH8,FDPS,ADH7,PLCB2,TKT,HPRT1,CAP1,PKLR,ADSS,PSAT1,OTC,PI4K2B,MVD,MTHFD2,

	TRIT1,LCT,IDH1,CTPS2,UQCRQ,ASS1,HEXB,PPAP2B,INPPL1,IMPA2,ACADVL,ALG10,ALG10B,AK1,ITPKA,PLCB3,ALDH18A1,ACAA1,BCAT1,AGXT,OAT,DGKA,NOS3,OCRL,PPAP2A,DGKQ,HYAL2,COASY,SMS,ACSL4,INPP5A,ATP6V0B,ACAT2,LDHA,PLA2G10,PIGB,ASNS,COMT,GLYCTK,ATP6AP1,SI,TPH1,AANAT,ITPKB,GUSB,TH,FBP1,DCTD,HSD17B10,P4HA2,LIAS,PANK2,MGLL,ACAT1,ATP6V0C,HSD17B4,NT5E,GDA,GLS2,CRLS1,SYNJ1,SYNJ2,PYCR2,HSD3B2,FDFT1,NDUFA4L2,BCKDHB,GLS,SLC27A5,PHGDH,LIPC,GCK,PLCD4,FDXACB1,ACOX3,MTMR2,PGS1,SHMT2,MTR,GLUL,ATP6V1B1,MOGS,TRDMT1,IDO1,PLCB1,PTGS1,NANS,KYNU,FLAD1,PSPH,CTPS,GGT1,GAL3ST1,PPAT,PMM1,ITPK1,PLCB4,MECR,COX6B1,COX6C,ADK,MPI,HAO1,MIB1,NAT2,NAT1,UGT8,PCYT1B,COX17,PPCDC,QPRT,DBT,NNT,COQ7,IMPA1,PYCRL,MTMR6,ADI1,INPP1,GCDH,SMPD2,BST1,POLG,PI4KB,ACSL6,ALDH7A1,PAH,MAN1A2,CKMT2,PAPSS1,PPAP2C,ST3GAL4,MPST,GAMT,CHKA,LDHB,PDXK,GMPS,CMAS,CBR3,PGLS,AASDHPPT,NME5,Unknown,MTCO2,GNPDA1,ADA,GOT1,GALNT6,SPR,UXS1,TPO,DHCR24,AMPD2,PRDX6,AMPD1,CTH,ACADM,HMGCL,AKR1A1,MAN1C1,GALNT12,GALNT14,GALNT10,ST3GAL3,ALG2,HYI,CHPF,MGAT1,EPHX2,CAT,PIPOX,PTGES2,MUT,UGP2,ST6GALNAC6,SGMS1,PTGES,G6PD,HYAL3,SPHK1,IDUA,CYP2E1,CYP2C9,CYP1A2,CYP17A1,CYP2C19,MTHFR,TALDO1,JMJD7PLA2G4B,CDO1,FBP2,COX4I2,GGT7,GALNTL2,AOC3,G6PC,PPOX,DDC,CNDP1,UGCG,DTYMK,PAPSS2,B3GNT4,MTHFS,PLCD3,SMPD4,BAAT,PGAP1,GRHPR,CYP19A1,TYMS,PTS,HADH,ETNK2,GALNT1,TKTL2,BPNT1,GALNT2,DEGS1,GALNT13,PGAM1,GALNT11,MTCO3,PLA2G7,UQCRB,DPYS,MGAT4B,INPP5E,P4HA3,DHCR7,GALK2,AHCYL1,TDO2,ACMSD,GFPT1,ATP5G3,ALPI,PANK4,AHCYL2,FUT4,ALOX12B,UROS,CMPK1,SGPL1,GCNT1,ANPEP,FASN,DCXR,ADH5,ENPP1,RPE,AKR1B10,GLDC,GBA,IDH2,NT5C1B,MT-ND2,INPP5B,MT-ND3,ACAD8,P4HA1

Table 5: Transcription factors targeting nor-NOHA and NOHA associated genes

#	Key TF	Description	Target genes	Mode of action
1	SP1	Sp1 transcription factor	CAT	Unknown
			IL6	Activation
			NOS1	Unknown
			NOS3	Activation
			NOS3	Unknown
			ODC1	Unknown
			SOD1	Activation

			SOD1	Repression
2	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	IL6	Unknown
			ODC1	Activation
			ODC1	Unknown
3	DDIT3	DNA-damage-inducible transcript 3	IL6	Activation
			NOS3	Repression
4	XBP1	X-box binding protein 1	IL6	Unknown
			NOS2	Unknown
5	JUN	jun proto-oncogene	IL6	Activation
			IL6	Unknown
			NOS2	Activation
			NOS2	Repression
			NOS2	Unknown
			NOS3	Unknown
6	AR	androgen receptor	ARG1	Activation
			ARG2	Activation
7	JUND	jun D proto-oncogene	IL6	Activation
			NOS2	Activation
8	KLF4	Kruppel-like factor 4 (gut)	IL6	Activation
			ODC1	Repression
			SOD1	Repression
9	STAT1	signal transducer and activator of transcription 1, 91kDa	IL6	Repression
			NOS2	Activation
			NOS2	Unknown
10	EGR1	early growth response 1	IL6	Repression
			SOD1	Activation

11	PPARD	peroxisome proliferator-activated receptor delta	CAT	Activation
			SOD1	Activation
12	APC	adenomatous polyposis coli	NOS2	Activation
			ODC1	Repression
13	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	CAT	Activation
			SOD1	Activation
14	XBP1	X-box binding protein 1	IL6	Unknown
			NOS2	Unknown
15	RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	IL6	Activation
			IL6	Repression
			IL6	Unknown
			NOS1	Activation
			NOS2	Activation
			NOS2	Unknown
			NOS3	Activation
16	CREB1	cAMP responsive element binding protein 1	IL6	Activation
			ODC1	Unknown

In this study, a total of 19 nor-NOHA and NOHA-associated genes were identified from GeneCards, STICH and CTD databases (Table 1) and their protein-protein interaction network was constructed (Figure 1). Based on Betweenness Centrality, Closeness Centrality, and Degree Centrality scores SOD, SOD1, AMD1, and NOS2 were established as hub nodes in the Protein-Protein Interaction network of these genes and their interactions (Table 2). Three distinct modules (modules 1, 2 and 3) of the PPI network were identified (Figure 2). Arginine and proline metabolism, tryptophan metabolism, nucleotide excision repair, amyotrophic lateral sclerosis (ALS) and peroxisome were the top 5 pathways/processes the proteins in these modules were found to be significantly involved in (Table 3). Only 2 out of the 4 hub proteins, namely SOD1 and NOS2 were present in the TRRUST database of transcription factors (Table 5). So a transcriptional regulatory network of them was constructed (Figure 3). SOD1 has been found to be the target of transcription factors SP1, KLF4, EGR1, PPARD and NFE2L2 while NOS2 was found to be the target of transcription factors XBP1, JUN, JUND, STAT1, APC, XBP1, and RELA.

Superoxide dismutase has long been considered a target for the selective killing of cancer cells [22]. It has also been labeled as body's natural cancer fighter [23]. SOD1 however has been only recently been proposed as a novel target for cancer therapy [24] and its role in cancer is also being revealed [25]. More recently being elucidated is the role of AMD1 in cancer which has been found to be upregulated in human prostate cancer [26]. NOS2 is another one which has been recently being seen as a emerging target for cancer treatment [27][28].

Conclusion

To conclude, a total of 19 nor-NOHA and NOHA-associated genes have been identified. SOD, SOD1, AMD1, and NOS2 have been revealed as key players in nor-NOHA and NOHA mediated pathways. Interestingly, these proteins have been shown to be associated with cancer in other studies as well. Therefore, this study has further validated the arguments for targeting them for treating cancer.

References

[1] J. B. Ochoa, J. Strange, P. Kearney, G. Gellin, E. Endean, and E. Fitzpatrick, "Effects of L-Arginine on the Proliferation of T Lymphocyte Subpopulations," *J. Parenter. Enter. Nutr.*, vol. 25, no. 1, pp. 23–29, Jan. 2001.

[2] G. Wu and S. M. Morris, "Arginine metabolism: nitric oxide and beyond.," *Biochem. J.*, vol. 336 (Pt 1), pp. 1–17, Nov. 1998.

[3] K. Miyanaka *et al.*, "Immunohistochemical localization of arginase II and other enzymes of arginine metabolism in rat kidney and liver.," *Histochem. J.*, vol. 30, no. 10, pp. 741–51, Oct. 1998.

[4] M. Munder *et al.*, "Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity," *Blood*, vol. 105, no. 6, pp. 2549–2556, Mar. 2005.

[5] T. Gotoh, M. Araki, and M. Mori, "Chromosomal Localization of the Human Arginase II Gene and Tissue Distribution of Its mRNA," *Biochem. Biophys. Res. Commun.*, vol. 233, no. 2, pp. 487–491, Apr. 1997.

[6] O. Braissant, T. Gotoh, M. Loup, M. Mori, and C. Bachmann, "L-arginine uptake, the citrulline-NO cycle and arginase II in the rat brain: an in situ hybridization study.," *Brain Res. Mol. Brain Res.*, vol. 70, no. 2, pp. 231–41, Jul. 1999.

[7] R. Singh, S. Pervin, A. Karimi, S. Cederbaum, and G. Chaudhuri, "Arginase activity in human breast cancer cell lines: N(omega)-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells.," *Cancer Res.*, vol. 60, no. 12, pp. 3305–12, Jun. 2000.

[8] S. M. Mumenthaler *et al.*, "Expression of arginase II in prostate cancer.," *Int. J. Oncol.*,

vol. 32, no. 2, pp. 357–65, Feb. 2008.

[9] F. Wu and H. Gehring, “Structural requirements for novel coenzyme-substrate derivatives to inhibit intracellular ornithine decarboxylase and cell proliferation,” *FASEB J.*, vol. 23, no. 2, pp. 565–574, Feb. 2009.

[10] P. C. Rodriguez *et al.*, “Arginase I Production in the Tumor Microenvironment by Mature Myeloid Cells Inhibits T-Cell Receptor Expression and Antigen-Specific T-Cell Responses,” *Cancer Res.*, vol. 64, no. 16, pp. 5839–5849, Aug. 2004.

[11] M. Safran *et al.*, “GeneCards Version 3: the human gene integrator,” *Database*, vol. 2010, no. 0, p. baq020-baq020, Aug. 2010.

[12] D. Szklarczyk, A. Santos, C. von Mering, L. J. Jensen, P. Bork, and M. Kuhn, “STITCH 5: augmenting protein–chemical interaction networks with tissue and affinity data,” *Nucleic Acids Res.*, vol. 44, no. D1, pp. D380–D384, Jan. 2016.

[13] A. P. Davis *et al.*, “The Comparative Toxicogenomics Database: update 2017,” *Nucleic Acids Res.*, vol. 45, no. D1, pp. D972–D978, Jan. 2017.

[14] A. Chatr-aryamontri *et al.*, “The BioGRID interaction database: 2015 update,” *Nucleic Acids Res.*, vol. 43, no. D1, pp. D470–D478, Jan. 2015.

[15] L. Licata *et al.*, “MINT, the molecular interaction database: 2012 update,” *Nucleic Acids Res.*, vol. 40, no. D1, pp. D857–D861, Jan. 2012.

[16] R. Saito *et al.*, “A travel guide to Cytoscape plugins,” *Nat. Methods*, vol. 9, no. 11, pp. 1069–1076, Nov. 2012.

[17] M. Li, Y. Lu, Z. Niu, and F.-X. Wu, “United Complex Centrality for Identification of Essential Proteins from PPI Networks,” *IEEE/ACM Trans. Comput. Biol. Bioinforma.*, vol. 14, no. 2, pp. 370–380, Mar. 2017.

[18] X. He and J. Zhang, “Why Do Hubs Tend to Be Essential in Protein Networks?”

[19] G. D. Bader and C. W. Hogue, “An automated method for finding molecular complexes in large protein interaction networks,” *BMC Bioinformatics*, vol. 4, no. 1, p. 2, Jan. 2003.

[20] C. Winterhalter, P. Widera, and N. Krasnogor, “JEPETTO: a Cytoscape plugin for gene set enrichment and topological analysis based on interaction networks,” *Bioinformatics*, vol. 30, no. 7, pp. 1029–1030, Apr. 2014.

[21] H. Han *et al.*, “TRRUST: a reference database of human transcriptional regulatory interactions,” *Sci. Rep.*, vol. 5, no. 1, p. 11432, Sep. 2015.

[22] P. Huang, L. Feng, E. A. Oldham, M. J. Keating, and W. Plunkett, “Superoxide dismutase as a target for the selective killing of cancer cells.,” *Nature*, vol. 407, no. 6802, pp. 390–395, Sep. 2000.

[23] T. M. Brasky *et al.*, “Serum Phospholipid Fatty Acids and Prostate Cancer Risk: Results From the Prostate Cancer Prevention Trial,” *Am. J. Epidemiol.*, vol. 173, no. 12, pp. 1429–1439, Jun. 2011.

[24] L. Papa, G. Manfredi, and D. Germain, “SOD1, an unexpected novel target for cancer therapy.,” *Genes Cancer*, vol. 5, no. 1–2, p. 15, Apr. 2014.

[25] S. Zhang *et al.*, “The superoxide dismutase 1 3′UTR maintains high expression of the SOD1 gene in cancer cells: The involvement of the RNA-binding protein AUF-1,” *Free Radic. Biol. Med.*, vol. 85, pp. 33–44, Aug. 2015.

[26] A. Zabala-Letona *et al.*, “mTORC1-dependent AMD1 regulation sustains polyamine metabolism in prostate cancer,” *Nature*, vol. 547, no. 7661, pp. 109–113, Jun. 2017.

[27] D. D. Thomas and D. A. Wink, “NOS2 as an Emergent Player in Progression of Cancer,” *Antioxid. Redox Signal.*, vol. 26, no. 17, pp. 963–965, Jun. 2017.

[28] D. Basudhar *et al.*, “Coexpression of NOS2 and COX2 accelerates tumor growth and reduces survival in estrogen receptor-negative breast cancer.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 114, no. 49, pp. 13030–13035, Dec. 2017.