EJONS International Journal on Mathematic, Engineering and Natural Sciences ISSN 2602 - 4136

Article Arrival Date 18.05.2020 | Article Type Research Article | Article Published Date 12.06.2020

Doi Number: http://dx.doi.org/10.38063/ejons.251

A NOVEL ceRNA ANALYSIS FOR *LMNA* AND *ZMPSTE24* RELATED TO MECHANISMS OF PROGEROID LAMINOPATHIES

Nazlı Irmak GİRİTLİOĞLU

Molecular Biologist and Bioengineering Specialist, Istanbul/Turkey

ngiritlioglu@outlook.com, https://orcid.org/0000-0001-5034-0984

ABSTRACT

Progeroid syndromes are diseases that display old-age features such as skin aging (loss of elastin and skin thinning), cardiovascular problems, alopecia at young ages. One of the most known progeroid syndromes is Hutchinson-Gilford Progeria Syndrome (HGPS). HGPS is caused by *LMNA de novo* point mutation and as a result, a toxic protein that accumulates in the inner nuclear membrane called progerin is produced. Zinc Metallopeptidase STE24 (ZMPSTE24, also known as FACE-1 in humans) is an enzyme that has important functions in post-translational modifications. *ZMPSTE24* mutations also can cause progeroid syndromes. Competing endogenous RNA (ceRNA) is a relatively new hypothesis that claims RNAs communicate with each other and regulate each other's expressions via shared microRNA (miRNA)s. In this study, a ceRNA analysis was performed for mRNAs of *LMNA* and *ZMPSTE24*. Firstly, related miRNAs of *LMNA* and *ZMPSTE24* mRNAs were found in miRTarBase, and the results with both strong and less strong evidence were considered. Then, other genes related to these miRNAs that have only strong evidence in miRTarBase were chosen and hit scores of these genes were calculated. The Gene Ontology (GO) Resource GO Enrichment analysis algorithm was used for P values (<0.05) of molecular functions of these genes which have the highest hit scores. The highest hit score of transcripts associated with *LMNA* mRNAs is 3 (*BCL2*, *CCND1*, *CCND2*, *CDK6*, *EZH2*) and the highest hit score of transcripts associated with *ZMPSTE24* mRNAs is 2 (*CBL*, *CCND2*, *CLOCK*, *EZH2*, *MAPK14*, *SERP1*). The most important result in this study is EZH2 interaction with both *LMNA* and *ZMPSTE24* networks and its epigenetic involvement might be key for treatments of some progeroid syndromes. It was known that downregulated *EZH2* with loss of the H3K27me3 marks causes the weaker heterochromatin-lamina associations. The study provides a novel analysis for connected ceRNAs of *LMNA* and *ZMPSTE24* mRNAs.

Keywords: ceRNA, miRNA, Progeroid, *LMNA*, *ZMPSTE24*

1. INTRODUCTION

Nuclear lamins (type V intermediate filament proteins) are important, structural components of the proteinaceous meshwork called nuclear lamina (NL), and directly associated with the nuclear genome. NL provides a proper morphology to cells and has regulatory functions on epigenetic mechanisms (Hah & Kim, 2019). Lamins are capable of binding to chromatin, DNA, and histones (Shevelyov & Ulianov, 2019) so they control gene expressions. Vertebrates have two A type of lamins (lamins A and C) that are encoded by *LMNA*, also there are less abundant isoforms (lamin AΔ10 ve C2) (Dechat, Adam, Taimen, Shimi, & Goldman, 2010). A-type lamins are expressed in most somatic cell lines but not in undifferentiated pluripotent stem cells (Chojnowski, Ong, & Dreesen, 2015). Accelerated aging syndromes comprise of the many types of diseases which include classical/non-classical HGPS (both of them progerin producing type), non-progerin producing progeroid laminopathies (in consequence of heterozygous *LMNA* pathogenic variant or pathogenic variants in other genes such as *ZMPSTE24)*, non-progeroid laminopathies, and non-laminopathy progeroid

syndromes (Gordon, Brown, & Collins, 2003). Laminopathies are caused by the genes (*LMNA*, *LMNB1*, and *LMNB2*) which express the lamin proteins and *LMNA* mutations are the most involved type of these diseases such as muscular dystrophy, neuropathy, cardiomyopathy, metabolic disorders, and accelerated aging and their combinations (Ho & Hegele, 2019).

HGPS is one of the most known type of progeroid syndromes and caused by an aberrant process in post-translational modification of prelamin A. Cytosine (C) changes to thymine (T) in *LMNA* exon 11 (c.1824C>T, G608G). Prelamin A has CAAX (C=cysteine, A=aliphatic amino acid, and x=any amino acid) motif that goes through some processes at the C-terminus: 1) Farnesyltransferase adds a farnesyl group to Cysteine, 2) aaX aminoacids are cleavaged by zinc metalloproteinase (ZMPSTE24 or also known as FACE-1), 3) Prenylcysteine carboxy methyltransferase adds a carboxymethyl group to farnesylated cysteine, and 4) 15 terminal amino acid residues are cleavaged by ZMPSTE24. In HGPS, ZMPSTE24 does not recognize the second cleavage site because of the *de novo LMNA* mutation (then aberrant mRNA splicing) and a toxic protein is formed called progerin (Eriksson et al., 2003; Ramirez, Cadinanos, Varela, Freije, & Lopez-Otin, 2007). When the functional lamin A concentration is low, the cell would be more unstable, and external forces can easily affect it (Al-Saaidi & Bross, 2015). If the farnesylated prelamin A accumulates in the inner nuclear membrane, nuclear blebbing occurs (Yang et al., 2005).

Mutations in *ZMPSTE24* also cause progeroid syndromes. Denecke et al. report a patient with homozygous *ZMPSTE24* null mutation and heterozygous *LMNA* mutation (Denecke et al., 2006). Shackleton et al. describe a 2 years old female patient that shows characteristics of HGPS, mandibuloacral dysplasia, and restrictive dermopathy with compound heterozygous *ZMPSTE24* mutations (Shackleton et al., 2005).

miRNAs are short (approximately 19-25 nucleotide), endogenous RNA molecules and basically, they have functions in gene silencing (Lu & Rothenberg, 2018). RNA-RNA interactions can comprise the regulations of RNA transcripts by miRNAs. In this context, ceRNA is a potent hypothesis that specifies the mRNAs of genes communicate between them and control each other's expressions with miRNAs (Tay, Rinn, & Pandolfi, 2014). There are some computational algorithms such as mirWalk (Dweep, Sticht, Pandey, & Gretz, 2011) or mirTaRBase (Huang et al., 2019) to show miRNAs that associate with mRNAs. Counts of shared miRNAs for mRNAs are indicated the connections between these mRNAs.

In this study, ceRNAs of *LMNA* and *ZMPSTE24* mRNAs were searched in bioinformatic tools and the potential ceRNAs were discussed.

2. MATERIALS AND METHODS

2.1. Detection of related miRNAs of *LMNA* and *ZMPSTE24* mRNAs based on strong and less strong evidence

Related miRNAs of *LMNA* and *ZMPSTE24* mRNAs were detected in miRTarBase which is a database that shows the validated miRNAs with strong and less strong experimental evidence (Huang et al., 2019) (Table 1 and Table 2).

Table 1. *LMNA* mRNAs related miRNAs with strong and less strong valuation methods in miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php)

miRTarbase ID	miRNA	Sequence	Target Site	Valuation Methods
MIRT019532	hsa-miR-340-5p	UUAUAAAGCAAUGAGACUGAUU	3' UTR	NGS
MIRT021388	hsa-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUGA	3' UTR	Microarray
MIRT022394	hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCC	3' UTR	Microarray and other
MIRT038445	hsa-miR-296-3p	GAGGGUUGGGUGGAGGCUCUCC	3' UTR	NGS
MIRT040169	hsa-miR-615-3p	UCCGAGCCUGGGUCUCCCUCUU	3' UTR	NGS
MIRT051592	hsa-let-7e-5p	UGAGGUAGGAGGUUGUAUAGUU	3' UTR	NGS
MIRT052409	hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU	3' UTR	NGS
MIRT731197	hsa-miR-205-5p	UCCUUCAUUCCACCGGAGUCUG	3' UTR	Reporter assay and microarray

Table 2. *ZMPSTE24* mRNAs related miRNAs with strong and less strong valuation methods in miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php)

miRTarbase ID	miRNA	Sequence	Target Site	Valuation Methods
MIRT004942	hsa-miR-98-5p	UGAGGUAGUAAGUUGUAUUGUU	3' UTR	qPCR and other
MIRT022576	hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCC	3' UTR	Microarray
MIRT438256	hsa-miR-141-3p	UAACACUGUCUGGUAAAGAUGG	3' UTR	Reporter assay

2.2. Determining the genes associated with *LMNA* and *ZMPSTE24* miRNAs and calculating the hit scores of the potential ceRNAs

All the miRNAs in Table 1 and Table 2 were searched in miRTarBase and the genes with only the strong evidence grade were selected (Table 3 and Table 4).

Hit score in this study means that the number of the genes according to their shared miRNAs. For example; *BCL* in Table 3 shares three miRNAs on the strong evidence grade with *LMNA*, so the hit score of *BCL* is 3.

2.3. GO molecular function analysis of the potential *LMNA* and *ZMPSTE24* ceRNAs

"Molecular function" term explains the functional activities of the gene products at the molecular level. The genes which have the highest hit scores were inserted into GO Enrichment Analysis (powered by PANTHER) algorithm (http://geneontology.org/) and "Molecular function" and "Homo sapiens" were selected. P values (<0.05) were ordered from the lowest to the highest (Table 7 and Table 8).

Table 3. The genes that their expressions are regulated by the same miRNAs with *LMNA*

miRNA	**Genes with strong evidence**
hsa-miR-340-5p	*CCND1, CCND2, CDK6, HNRNPA2B1, KRAS, MECP2, MET, PTBP1, PUM1, PUM2, RHOA, ROCK1, SKP2, SOX2*
hsa-miR-9-5p	*ACAT1, AP3B1, ATG5, BACE1, BCL2, BCL2L11, BCL6, CCND1, CCNG1, CD34, CDH1, CDX2, CHMP2B, CPEB4, CREB1, CUL4A, CXCR4, CYFIP2, DICER1, DRD2, ELAVL1, ENDOD1, ETS1, FOXO1, FOXO3, FOXP1, GRN, GSK3B, HBP1, HDAC4, ID2, IGF2BP1, IL6, JAK1, KLF17, KLF6, LHFPL2, MALAT1, MAP3K8, MIRLET7D, MMP13, MMP2, MMP9, MTHFD1, NF1, NFKB1, NR2E1, NRP1, NTRK3, ONECUT2, PDGFRB, POU2F2, PPARA, PRDM1, RAB34, RAB8B, REST, RHAG, RHOH, RYBP, SERPINB9, SIRT1, SOCS5, SOX7, SRF, STMN1, TAL1, TFRC, TGFBI, TGFBR2, TRAK2, VAMP5, VEGFA, VIM*
hsa-miR-124-3p	*ACAA2, ADIPOR2, AHR, AMOTL1, AR, ATP6V0E1, B4GALT1, BACE1, BDNF, CAMTA1, CAPNS1, CAV1, CBL, CCL2, CCND2, CD151, CD164, CD274, CDK2, CDK4, CDK6, CEBPA, CHMP2B, CLOCK, CTDSP1, CTGF, E2F6, EFNB1, EGR1, ELK3, ELOVL5, EZH2, FLOT1, FXN, GAS2L1, GRIA1, GRIA2, GRIA3, HMGA1, HNRNPA2B1, HOTAIR, IL6R, IQGAP1, ITGB1, ITGB3, JAG1, KLF6, LAMC1, MAGT1, MAPK14, MECP2, MTPN, NEAT1, NFATC1, NFKBIZ, NR3C1, NR3C2, PEA15, PIK3CA, PIM1, PPP1R13L, PRRX1, PTBP1, RAB38, RAC1, RAVER2, RDH10, RELA, REST, RGS4, RHOG, ROCK1, ROCK2, RRAS, SERP1, SIRT1, SLC16A1, SMYD3, SNAI2, SOS1, SPHK1, STAT3, SUCLG2, SURF4, SYCP1, TRIB3, TSC22D3, VAMP3, VIM, XRCC6*
hsa-miR-296-3p	*ICAM1, KCNH1*
hsa-miR-615-3p	*LCOR*
hsa-let-7e-5p	*AGO1, AURKB, CCND1, EIF3J, EZH2, FASLG, HMGA2, IGF1, IGF1R, LIN28A, MMP9, MPL, MYCN, SMC1A, TNFRSF10B, WNT1*
hsa-let-7a-5p	*AGO1, AMMECR1, APP, ARG2, AURKB, BCL2, CASP3, CASP8, CASP9, CCND2, CCR7, CDC34, CDK6, CDKN1A, DICER1, E2F1, E2F2, EGFR, EGR3, EIF2C4, EWSR1, EZH2, FOXA1, HAS2, HMGA1, HMGA2, HNRPDL, HRAS, IGF2, IGF2BP1, IL6, ITGB3, KRAS, LIN28A, MEIS1, MPL, MYC, NEFM, NF2, NFKB1, NKIRAS2, NR1I2, NRAS, PAK1, PRDM1, RAB40C, RAVER2, RRM2, SLC20A1, THBS1, TMED7, TNFRSF10B, TRIM71, TUSC2, UHRF1, UHRF2, VDR, ZFP36L1*
hsa-miR-205-5p	*ACSL1, ACSL4, AR, BCL2, BCL6, CTGF, CYR61, DDX5, E2F1, E2F5, EGLN2, ERBB2, ERBB3, ESRRG, EZR, HMGB1, HMGB3, IL24, IL32, INPPL1, ITGA5, KCNJ10, LAMC1, LRP1, LRRK2, MED1, PHLPP2, PRKCE, PTEN, PTPRM, RUNX2, SIGMAR1, SMAD1, SMAD4, SRC, TP73, VEGFA, YES, YY1, ZEB1, ZEB2*

Table 4. The genes that their expressions are regulated by the same miRNAs with *ZMPSTE24*

miRNA	**Genes with strong evidence**
hsa-miR-98-5p	*ACADM, AMMECR1, CASP3, CBL, CBX5, CCL5, CCND2, CCNJ, CHRNB1, CNOT4, CYP19A1, E2F1, E2F2, E2F5, EDN1, EZH2, HK2, HMGA2, HNRPDL, IGF1, IL13, IL6, KLF13, MEIS1, MPL, MYC, NCOA3, PBX3, PGRMC1, SERP1, SLC20A1, SNX6, SOCS4, THBS1, TUSC2, ZFP36L1*
hsa-miR-124-3p	*ACAA2, ADIPOR2, AHR, AMOTL1, AR, ATP6V0E1, B4GALT1, BACE1, BDNF, CAMTA1, CAPNS1, CAV1, CBL, CCL2, CCND2, CD151, CD164, CD274, CDK2, CDK4, CDK6, CEBPA, CHMP2B, CLOCK, CTDSP1, CTGF, E2F6, EFNB1, EGR1, ELK3, ELOVL5, EZH2, FLOT1, FXN, GAS2L1, GRIA1, GRIA2, GRIA3, HMGA1, HNRNPA2B1, HOTAIR, IL6R, IQGAP1, ITGB1, ITGB3, JAG1, KLF6, MAGT1, MAPK14, MECP2, MTPN, NEAT1, NFATC1, NFKBIZ, NR3C1, NR3C2, PEA15, PIK3CA, PIM1, PPP1R13L, PRRX1, PTBP1, RAB38, RAC1, RAVER2, RDH10, RELA, REST, RGS4, RHOG, ROCK1, ROCK2, RRAS, SERP1, SIRT1, SLC16A1, SMYD3, SNAI2, SOS1, SPHK1, STAT3, SUCLG2, SURF4, SYCP1, TRIB3, TSC22D3, VAMP3, VIM, XRCC6*
hsa-miR-141-3p	*ACVR2B, BAP1, BRD3, CDC25A, CDC25C, CDYL, CLOCK, CTBP2, DLX5, E2F3, EIF4E, ELAVL4, ELMO2, ERBB2IP, HDGF, HNRNPD, HOXB5, KEAP1, KLF11, KLF12, KLF5, KLHL20, MAP4K4, MAPK14, MAPK9, NR0B2, PHLPP1, PHLPP2, PPARA, PTEN, PTPRD, RASSF2, RIN2, SEPT7, SFPQ, SHC1, STAT4, STAT5A, STK3, TAZ, TCF7L1, TET1, TET3, TFDP2, TGFB2, TIAM1, TM4SF1, TRAPPC2P1, UBAP1, VAC14, WDR37, YAP1, YWHAG, ZEB1, ZEB2, ZFPM2*

Table 5. Hit scores of the genes related to *LMNA*

Gene	Hits	Gene	Hits	Gene	Hits	Gene	Hits	Gene	Hits
BCL2	3	*BDNF*	1	*FASLG*	1	*NFATC1*	1	*SPHK1*	1
CCND1	3	*CAMTA1*	1	*FLOT1*	1	*NFKBIZ*	1	*SRF*	1
CCND2	3	*CAPNS1*	1	*FOXA1*	1	*NKIRAS2*	1	*STAT3*	1
CDK6	3	*CASP3*	1	*FOXO1*	1	*NR1I2*	1	*STMN1*	1
EZH2	3	*CASP8*	1	*FOXO3*	1	*NR2E1*	1	*SUCLG2*	1
AGO1	2	*CASP9*	1	*FOXP1*	1	*NR3C1*	1	*SURF4*	1
AR	2	*CAV1*	1	*FXN*	1	*NR3C2*	1	*SYCP1*	1
AURKB	2	*CBL*	1	*GAS2L1*	1	*NRAS*	1	*TAL1*	1
BACE1	2	*CCL2*	1	*GRIA1*	1	*NRP1*	1	*THBS1-1*	1
BCL6	2	*CCNG1*	1	*GRIA2*	1	*NTRK3*	1	*TFRC*	1
CHMP2B	2	*CCR7*	1	*GRIA3*	1	*ONECUT2*	1	*TGFBI*	1
CTGF	2	*CD151*	1	*GRN*	1	*PAK1*	1	*TGFBR2*	1
DICER1	2	*CD164*	1	*GSK3B*	1	*PDGFRB*	1	*TMED7*	1
E2F1	2	*CD274*	1	*HAS2*	1	*PEA15*	1	*TRAK2*	1
HMGA1	2	*CD34*	1	*HBP1*	1	*PIK3CA*	1	*TRIB3*	1
HMGA2	2	*CDH1*	1	*HDAC4*	1	*PIM1*	1	*TRIM71*	1
HNRNPA2B1	2	*CDK2*	1	*HNRPDL*	1	*POU2F2*	1	*TSC22D3*	1
IGF2BP1	2	*CDK4*	1	*HRAS*	1	*PPARA*	1	*TUSC2*	1
IL6	2	*CDKN1A*	1	*HOTAIR*	1	*PPP1R13L*	1	*UHRF1*	1
ITGB3	2	*CDX2*	1	*ICAM1*	1	*PRRX1*	1	*UHRF2*	1
KLF6	2	*CEBPA*	1	*ID2*	1	*PUM1*	1	*VAMP3*	1
KRAS	2	*CLOCK*	1	*IGF1*	1	*PUM2*	1	*VAMP5*	1
LAMC1	2	*CPEB4*	1	*IGF1R*	1	*RAB34*	1	*VDR*	1
LIN28A	2	*CREB1*	1	*IGF2*	1	*RAB38*	1	*WNT1*	1
MECP2	2	*CTDSP1*	1	*IL6R*	1	*RAB40C*	1	*XRCC6*	1
MMP9	2	*CUL4A*	1	*IQGAP1*	1	*RAB8B*	1	*ZFP36L1*	1
MPL	2	*CXCR4*	1	*ITGB1*	1	*RAC1*	1		
NFKB1	2	*CYFIP2*	1	*JAG1*	1	*RDH10*	1		
PRDM1	2	*CYR61*	1	*JAK1*	1	*RELA*	1		
PTBP1	2	*DDX5*	1	*KCNH1*	1	*RGS4*	1		
RAVER2	2	*DRD2*	1	*KLF17*	1	*RHAG*	1		
REST	2	*E2F2*	1	*LCOR*	1	*RHOA*	1		
ROCK1	2	*E2F5*	1	*LHFPL2*	1	*RHOG*	1		
SIRT1	2	*E2F6*	1	*MALAT1*	1	*RHOH*	1		
TNFRSF10B	2	*EFNB1*	1	*MAGT1*	1	*ROCK2*	1		
VEGFA	2	*EGFR*	1	*MAP3K8*	1	*RRAS*	1		
VIM	2	*EGLN2*	1	*MAPK14*	1	*RRM2*	1		
ACAA2	1	*EGR1*	1	*MEIS1*	1	*RYBP*	1		
ACAT1	1	*EGR3*	1	*MET*	1	*SERP1*	1		
ACSL1	1	*EIF2C4*	1	*MIRLET7D*	1	*SERPINB9*	1		
ACSL4	1	*EIF3J*	1	*MMP13*	1	*SKP2*	1		
ADIPOR2	1	*ELAVL1*	1	*MMP2*	1	*SLC16A1*	1		
AMMECR1	1	*ELK3*	1	*MTHFD1*	1	*SLC20A1*	1		
AMOTL1	1	*ELOVL5*	1	*MTPN*	1	*SMC1A*	1		
AP3B1	1	*ENDOD1*	1	*MYC*	1	*SMYD3*	1		
APP	1	*ERBB2*	1	*MYCN*	1	*SNAI2*	1		
ARG2	1	*ERBB3*	1	*NEAT1*	1	*SOCS5*	1		
ATG5	1	*ESRRG*	1	*NEFM*	1	*SOS1*	1		
ATP6V0E1	1	*ETS1*	1	*NF1*	1	*SOX2*	1		
B4GALT1	1	*EWSR1*	1	*NF2*	1	*SOX7*	1		

Table 6. Hit scores of the genes related to *ZMPSTE24*

Gene	Hits	Gene	Hits	Gene	Hits	Gene	Hits	Gene	Hits
CBL	2	CDK4	1	HNRNPA2B1	1	PBX3	1	SOCS4	1
CCND2	2	CDK6	1	HNRNPD	1	PEA15	1	SOS1	1
CLOCK	2	CEBPA	1	HNRNPDL	1	PGRMC1	1	SPHK1	1
EZH2	2	CHMP2B	1	HOTAIR	1	PHLPP1	1	STAT3	1
MAPK14	2	CHRNB1	1	HOXB5	1	PHLPP2	1	STAT4	1
SERP1	2	CNOT4	1	IGF1	1	PIK3CA	1	STAT5A	1
ACAA2	1	CTBP2	1	IL13	1	PIM1	1	STK3	1
ACADM	1	CTDSP1	1	IL6	1	PPARA	1	SUCLG2	1
ACVR2B	1	CTGF	1	IL6R	1	PPP1R13L	1	SURF4	1
ADIPOR2	1	CYP19A1	1	IQGAP1	1	PRRX1	1	SYCP1	1
AHR	1	DLX5	1	ITGB1	1	PTBP1	1	TAZ	1
AMMECR1	1	E2F1	1	ITGB3	1	PTEN	1	TCF7L1	1
AMOTL1	1	E2F2	1	JAG1	1	PTPRD	1	TET1	1
AR	1	E2F3	1	KEAP1	1	RAB38	1	TET3	1
ATP6V0E1	1	E2F5	1	KLF11	1	RAC1	1	TFDP2	1
B4GALT1	1	E2F6	1	KLF12	1	RASSF2	1	TGFB2	1
BACE1	1	EDN1	1	KLF13	1	RAVER2	1	THBS1	1
BAP1	1	EFNB1	1	KLF5	1	RDH10	1	TIAM1	1
BDNF	1	EGR1	1	KLF6	1	RELA	1	TM4SF1	1
BRD3	1	EIF4E	1	KLHL20	1	REST	1	TRAPPC2P1	1
CAMTA1	1	ELAVL4	1	MAGT1	1	RGS4	1	TRIB3	1
CAPNS1	1	ELK3	1	MAP4K4	1	RHOG	1	TSC22D3	1
CASP3	1	ELMO2	1	MAPK9	1	RIN2	1	TUSC2	1
CAV1	1	ELOVL5	1	MECP2	1	ROCK1	1	UBAP1	1
CBX5	1	ERBB2IP	1	MEIS1	1	ROCK2	1	VAMP3	1
CCL2	1	FLOT1	1	MPL	1	RRAS	1	VIM	1
CCL5	1	FXN	1	MTPN	1	SEPT7	1	VAC14	1
CCNJ	1	GAS2L1	1	MYC	1	SFPQ	1	WDR37	1
CD151	1	GRIA1	1	NCOA3	1	SHC1	1	XRCC6	1
CD164	1	GRIA2	1	NEAT1	1	SIRT1	1	YAP1	1
CD274	1	GRIA3	1	NFATC1	1	SLC16A1	1	YWHAG	1
CDC25A	1	HDGF	1	NFKBIZ	1	SLC20A1	1	ZEB1	1
CDC25C	1	HK2	1	NR0B2	1	SMYD3	1	ZEB2	1
CDYL	1	HMGA1	1	NR3C1	1	SNAI2	1	ZFPM2	1
CDK2	1	HMGA2	1	NR3C2	1	SNX6	1		

3. RESULTS AND DISCUSSION

The highest hit scores belong to *BCL2*, *CCND1*, *CCND2*, *CDK6*, and *EZH2* for *LMNA* mRNAs that are seen in Table 5. A study about the potential *LMNA* ceRNAs for HGPS indicated these genes (Arancio, Giordano, & Pizzolanti, 2013); but in this study, the genes only have strong evidence and all *LMNA* mRNAs were considered for ceRNA detection, based on the current data. BCL2 protein family members have roles in apoptosis. Products of *BCL2* are integral outer mitochondrial membrane proteins and show anti-apoptotic effects ("GeneCards", 2020). Also, studies proved that BCL2 is related to aging because of its interaction with Beclin-1 (encoded by *BECN1*) which has a function in the regulation of autophagy (Kang, Zeh, Lotze, & Tang, 2011; Xu & Qin, 2019). *CCND1* and *CCND2* were not surprising and the kinase activity functions have the lowest P values (Table 7). Basically, cyclins regulate CDKs. *CCND1* malfunction is related to some human cancers (Dai et al., 2016; Mohammedi, Doula, Mesli, & Senhadji, 2019; NCBI, 2020a). A study in 2017 showed that the amounts of BCL2 and CCND1 decrease with age (Kim et al., 2017). Cyclin D2 is also related to various human cancers (NCBI, 2020b; Parry & Engh, 2012). Tschen et al. found that Cyclin D2

sufficient for regulation of β cell self-renewal (Tschen et al., 2017). Yamak et al. indicated that Cyclin D2 positively regulates transcription factor GATA Binding Protein 4 (GATA4) (Yamak, Latinkic, Dali, Temsah, & Nemer, 2014) that is required for embryogenesis, cardiomyocyte growth and differentiation, and normal testicular development ("GeneCards", 2020; Yamak et al., 2014). Filipkowski et al. showed that Cyclin D2 also have key roles in adult brain neurogenesis (Filipkowski & Kaczmarek, 2018). *CDK6* and *LMNA* association is not much studied according to current literature, but it is known that they are indirectly related to each other. A study in 2007 showed that Cyclin D3 has a direct interaction as binding to Lamin A/C (Mariappan, Gurung, Thanumalayan, & Parnaik, 2007) and also is a regulatory subunit of CDK6 (Dechat et al., 2008).

Table 7. GO molecular function (Top 20) analysis of ceRNAs of *LMNA* mRNAs ("The Gene Ontology Resource: 20 years and still GOing strong," 2019)

GO molecular function	Raw P value
cyclin-dependent protein serine/threonine kinase regulator activity	5.84E-05
protein kinase activity	2.51E-04
phosphotransferase activity, alcohol group as acceptor	4.00E-04
kinase activity	5.44E-04
catalytic activity, acting on a protein	6.05E-04
FBXO family protein binding	7.19E-04
transferase activity	7.53E-04
protein kinase regulator activity	7.86E-04
transferase activity, transferring phosphorus-containing groups	9.86E-04
kinase regulator activity	1.05E-03
transcription corepressor activity	1.41E-03
histone methyltransferase activity (H3-K27 specific)	1.44E-03
BH3 domain binding	1.68E-03
primary miRNA binding	2.16E-03
BH domain binding	2.87E-03
death domain binding	2.87E-03
proline-rich region binding	5.02E-03
RNA polymerase II core promoter sequence-specific DNA binding	5.74E-03
molecular function regulator	6.21E-03
cyclin-dependent protein serine/threonine kinase activity	7.17E-03

CBL, *CCND2*, *CLOCK*, *EZH2*, *MAPK14*, and *SERP1* has 2 hits for *ZMPSTE24* mRNAs that are seen in Table 6. CBL is a proto-oncogene and encodes a RING finger E3 ubiquitin ligase which has a function in substrate degradation by the proteasome. Products of the CLOCK has key functions in the regulation of circadian rhythms. *MAPK14* expression product is a functionary in the wide range of biological processes such as cell proliferation/differentiation, gene transcription/regulation, and development. *SERP1* has roles in the protection of unfolded target proteins and avoids their degradation during endoplasmic reticulum stress ("GeneCards", 2020).

Table 8. GO molecular function (Top 20) analysis of ceRNAs of *ZMPSTE24* mRNAs ("The Gene Ontology Resource: 20 years and still GOing strong," 2019)

GO molecular function	Raw P value
transferase activity	1.02E-04
chromatin DNA binding	4.15E-04
protein kinase binding	6.14E-04
kinase binding	8.81E-04
catalytic activity, acting on a protein	1.66E-03
histone methyltransferase activity (H3-K27 specific)	1.73E-03
NFAT protein binding	1.73E-03
mitogen-activated protein kinase p38 binding	1.73E-03
primary miRNA binding	2.59E-03
phosphatidylinositol 3-kinase regulatory subunit binding	3.16E-03
MAP kinase activity	4.31E-03
MAP kinase kinase activity	5.45E-03
RNA polymerase II core promoter sequence-specific DNA binding	6.89E-03
mitogen-activated protein kinase binding	7.74E-03
catalytic activity	7.87E-03
ephrin receptor binding	8.60E-03
phosphatidylinositol 3-kinase binding	8.89E-03
core promoter sequence-specific DNA binding	1.00E-02
epidermal growth factor receptor binding	1.00E-02
chromatin binding	1.03E-02

EZH2 mRNAs have a relation with both *LMNA* and *ZMSPTE24* mRNAs. *EZH2* encodes a member of the Polycomb-group proteins that keep the silenced state of genes at the transcriptional level; this is heritable and also this member is responsible for methylation of Lys-9 (H3K9me) and Lys-27 (H3K27me) of histone H3 ("GeneCards", 2020). Long-term silencing depends on some mechanisms which are DNA methylation, histone modifications, and using the Polycomb-group proteins. Chromatin and lamina binding occurs at the lamin associate domains (LADs) which are characterized by the H3K9me2, H3K9me3, and H3K27me3 marks. There are two types of LADs and one of these types is called constitutive LAD which is directly related to the NL. Epigenetic alterations can cause irreversible changes in cell fate. If H3K27me3 occurs on the gene promotors, this condition directly affects the gene expressions. Gene density is the key point of H3K27me3 loss or gain. Gene-poor regions are willing to lose H3K27me3 whereas gene-rich regions are the opposite. H3K9me3 is constitutive heterochromatin and downregulated in HGPS cells, so the association of pericentric regions and the NL gets weaker. One of the key HGPS characteristics is the loss of heterochromatin. McCord and coworkers found that progerin accumulation in the NL causes the alteration of H3K27me3 marks with the downregulated *EZH2* and as a result, heterochromatin-lamina interactions are damaged. The inactive X chromosome ($X_{i)}$, associated with the NL, is also involved in the epigenetic landscape. X_i is facultative heterochromatin and inactivation already occurs in the embryonic state for humans (Balaton et. al, 2018; Crasto & Di Pasquale, 2018; McCord et al., 2013;

Arancio et. al, 2014; Shumaker et al., 2006). A study in 2006 showed that H3K27me3 was lost on X_i in the early passaged cells from a female HGPS patient and *EZH2* was 9 to 10 fold downregulated in these cells (Shumaker et al., 2006).

4. CONCLUSIONS

There are not many studies about mRNAs of *LMNA* and *ZMPSTE24* ceRNAs in the current literature. Farnesyltransferase inhibitors, statins, and biphosphonates are using in clinical trials of Progeria. New therapy approaches are always a necessity for these syndromes and unraveling the unknown mechanisms is very important. Not just the standard pathway regulatory drugs, also the drugs that generate the reverse effect on *EZH2* downregulation should be considered for the treatments at as young as possible ages. *EZH2*-related treatments might be a good start. There are also minor conclusions to be noted in this study. Current literature is weak for cyclins, Cbl proto-oncogene, clock genes, and *ZMPSTE24* relations, also Cyclin dependent kinase 6 for *LMNA*. This study contains genes that have only strong experimental evidence. There are also different ceRNAs for *LMNA* and *ZMPSTE24* mRNAs which do not mention in this analysis.

REFERENCES

Al-Saaidi, R., & Bross, P. (2015). Do lamin A and lamin C have unique roles? *Chromosoma*, *124*(1), 1-12. doi:10.1007/s00412-014-0484-7

Arancio, W., Giordano, C., & Pizzolanti, G. (2013). A ceRNA analysis on LMNA gene focusing on the Hutchinson-Gilford progeria syndrome. *J Clin Bioinforma, 3*(1), 2. doi:10.1186/2043-9113-3-2

Arancio W, Pizzolanti G, Genovese SI, Pitrone M, Giordano C. Epigenetic involvement in Hutchinson-Gilford progeria syndrome: a mini-review. *Gerontology*. 2014;60(3):197-203. doi:10.1159/000357206

Balaton BP, Dixon-McDougall T, Peeters SB, Brown CJ. The eXceptional nature of the X chromosome. *Hum Mol Genet*. 2018;27(R2):R242-R249. doi:10.1093/hmg/ddy148

Chojnowski, A., Ong, P. F., & Dreesen, O. (2015). Nuclear lamina remodelling and its implications for human disease. *Cell Tissue Res, 360*(3), 621-631. doi:10.1007/s00441-014-2069-4

Crasto, S., & Di Pasquale, E. (2018). Induced Pluripotent Stem Cells to Study Mechanisms of Laminopathies: Focus on Epigenetics. *Frontiers in cell and developmental biology*, *6*, 172. https://doi.org/10.3389/fcell.2018.00172

Dai, J., Wei, R. J., Li, R., Feng, J. B., Yu, Y. L., & Liu, P. S. (2016). A study of CCND1 with epithelial ovarian cancer cell proliferation and apoptosis. *Eur Rev Med Pharmacol Sci*, *20*(20), 4230-4235.

Dechat, T., Adam, S. A., Taimen, P., Shimi, T., & Goldman, R. D. (2010). Nuclear lamins. *Cold Spring Harbor perspectives in biology*, *2*(11), a000547-a000547. Retrieved from https://pubmed.ncbi.nlm.nih.gov/20826548

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2964183/. doi:10.1101/cshperspect.a000547

Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D. K., Solimando, L., & Goldman, R. D. (2008). Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes & development*, *22*(7), 832-853. Retrieved from https://pubmed.ncbi.nlm.nih.gov/18381888

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2732390/. doi:10.1101/gad.1652708

Denecke, J., Brune, T., Feldhaus, T., Robenek, H., Kranz, C., Auchus, R. J., . . . Marquardt, T. (2006). A homozygous ZMPSTE24 null mutation in combination with a heterozygous mutation in the LMNA gene causes Hutchinson-Gilford progeria syndrome (HGPS): insights into the

pathophysiology of HGPS. *Human Mutation, 27*(6), 524-531. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1002/humu.20315. doi:10.1002/humu.20315

Dweep, H., Sticht, C., Pandey, P., & Gretz, N. (2011). miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. *J Biomed Inform, 44*(5), 839-847. doi:10.1016/j.jbi.2011.05.002

Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., . . . Collins, F. S. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature, 423*(6937), 293-298. doi:10.1038/nature01629

Filipkowski, R. K., & Kaczmarek, L. (2018). Severely impaired adult brain neurogenesis in cyclin D2 knock-out mice produces very limited phenotypic changes. *Prog Neuropsychopharmacol Biol Psychiatry, 80*(Pt A), 63-67. doi:10.1016/j.pnpbp.2017.03.028

The Gene Ontology Resource: 20 years and still GOing strong. (2019). *Nucleic Acids Res, 47*(D1), D330-d338. doi:10.1093/nar/gky1055

GeneCards. (2020). *https://www.genecards.org/*.

Gordon, L. B., Brown, W. T., & Collins, F. S. (2003). *Hutchinson-Gilford Progeria Syndrome*. Seattle (WA): University of Washington: GeneReviews® [Internet].

Hah, J., & Kim, D. H. (2019). Deciphering Nuclear Mechanobiology in Laminopathy. *Cells, 8*(3). doi:10.3390/cells8030231

Ho, R., & Hegele, R. A. (2019). Complex effects of laminopathy mutations on nuclear structure and function. *Clin Genet, 95*(2), 199-209. doi:10.1111/cge.13455

Huang, H.-Y., Lin, Y.-C.-D., Li, J., Huang, K.-Y., Shrestha, S., Hong, H.-C., . . . Huang, H.-D. (2019). miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database. *Nucleic Acids Research, 48*(D1), D148-D154. Retrieved from https://doi.org/10.1093/nar/gkz896. doi:10.1093/nar/gkz896

Kang, R., Zeh, H. J., Lotze, M. T., & Tang, D. (2011). The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ, 18*(4), 571-580. doi:10.1038/cdd.2010.191

Kim, J.-H., Lee, B.-R., Choi, E.-S., Lee, K.-M., Choi, S.-K., Cho, J. H., . . . Kim, E. (2017). Reverse Expression of Aging-Associated Molecules through Transfection of miRNAs to Aged Mice. *Molecular therapy. Nucleic acids, 6*, 106-115. Retrieved from https://pubmed.ncbi.nlm.nih.gov/28325277

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5363412/. doi:10.1016/j.omtn.2016.11.005

Lu, T. X., & Rothenberg, M. E. (2018). MicroRNA. *The Journal of allergy and clinical immunology, 141*(4), 1202-1207. Retrieved from https://pubmed.ncbi.nlm.nih.gov/29074454

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5889965/. doi:10.1016/j.jaci.2017.08.034

Mariappan, I., Gurung, R., Thanumalayan, S., & Parnaik, V. K. (2007). Identification of cyclin D3 as a new interaction partner of lamin A/C. *Biochemical and Biophysical Research Communications, 355*(4), 981-985. Retrieved from http://www.sciencedirect.com/science/article/pii/S0006291X07003415. doi:https://doi.org/10.1016/j.bbrc.2007.02.060

McCord, R. P., Nazario-Toole, A., Zhang, H., Chines, P. S., Zhan, Y., Erdos, M. R., . . . Cao, K. (2013). Correlated alterations in genome organization, histone methylation, and DNA-lamin A/C interactions in Hutchinson-Gilford progeria syndrome. *Genome Res, 23*(2), 260-269. doi:10.1101/gr.138032.112

Mohammedi, L., Doula, F. D., Mesli, F., & Senhadji, R. (2019). Cyclin D1 overexpression in Algerian breast cancer women: correlation with CCND1 amplification and clinicopathological parameters. *Afr Health Sci, 19*(2), 2140-2146. doi:10.4314/ahs.v19i2.38

NCBI. (2020a). CCND1 cyclin D1 [Homo sapiens (human)]. Retrieved from https://www.ncbi.nlm.nih.gov/gene/595#top.

NCBI. (2020b). CCND2 cyclin D2 [Homo sapiens (human)]. Retrieved from https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=894.

Parry, P. V., & Engh, J. A. (2012). The role of cyclin-d2 in the tumorgenesis of glioblastoma. *Neurosurgery, 71*(6), N22-23. doi:10.1227/01.neu.0000423047.43054.58

Ramirez, C. L., Cadinanos, J., Varela, I., Freije, J. M., & Lopez-Otin, C. (2007). Human progeroid syndromes, aging and cancer: new genetic and epigenetic insights into old questions. *Cell Mol Life Sci, 64*(2), 155-170. doi:10.1007/s00018-006-6349-3

Shackleton, S., Smallwood, D. T., Clayton, P., Wilson, L. C., Agarwal, A. K., Garg, A., & Trembath, R. C. (2005). Compound heterozygous ZMPSTE24 mutations reduce prelamin A processing and result in a severe progeroid phenotype. *J Med Genet, 42*(6), e36. doi:10.1136/jmg.2004.029751

Shevelyov, Y. Y., & Ulianov, S. V. (2019). The Nuclear Lamina as an Organizer of Chromosome Architecture. *Cells, 8*(2). doi:10.3390/cells8020136

Shumaker, D. K., Dechat, T., Kohlmaier, A., Adam, S. A., Bozovsky, M. R., Erdos, M. R., . . . Goldman, R. D. (2006). Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci U S A, 103*(23), 8703-8708. doi:10.1073/pnas.0602569103

Tay, Y., Rinn, J., & Pandolfi, P. P. (2014). The multilayered complexity of ceRNA crosstalk and competition. *Nature, 505*(7483), 344-352. doi:10.1038/nature12986

Tschen, S. I., Zeng, C., Field, L., Dhawan, S., Bhushan, A., & Georgia, S. (2017). Cyclin D2 is sufficient to drive beta cell self-renewal and regeneration. *Cell Cycle, 16*(22), 2183-2191. doi:10.1080/15384101.2017.1319999

Xu, H. D., & Qin, Z. H. (2019). Beclin 1, Bcl-2 and Autophagy. *Adv Exp Med Biol, 1206*, 109-126. doi:10.1007/978-981-15-0602-4_5

Yamak, A., Latinkic, B. V., Dali, R., Temsah, R., & Nemer, M. (2014). Cyclin D2 is a GATA4 cofactor in cardiogenesis. *Proc Natl Acad Sci U S A, 111*(4), 1415-1420. doi:10.1073/pnas.1312993111

Yang, S. H., Bergo, M. O., Toth, J. I., Qiao, X., Hu, Y., Sandoval, S., . . . Fong, L. G. (2005). Blocking protein farnesyltransferase improves nuclear blebbing in mouse fibroblasts with a targeted Hutchinson-Gilford progeria syndrome mutation. *Proc Natl Acad Sci U S A, 102*(29), 10291-10296. doi:10.1073/pnas.0504641102