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RESEARCH ARTICLE

In Silico Analysis of Biomarker Potentials of miRNA-Mediated ceRNAs in Gastric Neoplasms

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Abstract

Objectives: The objective of this study is to define novel biomarkers for gastric neoplasm (GN) via *in silico* analysis that takes GN-specific miRNAs, finds their combinatorial target genes (potential ceRNAs), selects ones containing T-UCR among them and potentiates their relevance with GN. Based on this study we can plan new in vitro and in vivo studies.

Methods: Four miRNAs of which clinical relevances with GN were proved experimentally were exported via mirTarbase. Using the ComiR database, 1008 genes targeted by these 4 miRNAs simultaneously were identified. Genes containing T-UCR and showing potential ceRNA activity were extracted. Among GN-associated ceRNAs including T-UCR, we identified genes with significant expression differences between GN and normal stomach tissue using the GEPIA database. The statistical evaluation of the association of *NFAT5* and *CLK3* genes with GN was performed by Spearman correlation test in GEPIA database.

Results: GN-associated ceRNAs cross-matching with genes including T-UCR in their exonic regions were *NFAT5* and *CLK3*. We identified genes with significant expression differences between GN and normal stomach tissues among GN-associated ceRNAs including T-UCR. According to this analysis, only *NFAT5* gene was significantly higher expressed in GN than in normal stomach tissue while the other didn't show any significant differential expression pattern. *NFAT5* and *CLK3* genes were found to be significantly correlated with GN ($p<0.001$; $R=0.22$)

Conclusion: All in all, this is the study associating *NFAT5* gene with GN for the first time and giving it ongogenic potential for GN. Still, larger and more comprehensive studies are needed on this issue.

Key words: Gastric neoplasms; miRNA; ceRNA; T-UCR; *In silico* analysis

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Introduction

Gastric neoplasms may manifest in various different forms, depending on the cell of origin. The most common form is adenocarcinoma, while lymphoma, gastrointestinal stromal tumors (GISTs), carcinoids and other neoplasms are less frequently seen. Gastric adenocarcinoma is a particularly common cancer across the world, but particularly in the Far East. A lower incidence has been reported in the United Kingdom, but poor prognosis when the disease is in the late stage results in a significant impact on population health. Advanced disease is observed in the majority of patients at time of diagnosis (Schiller, 2017).

MicroRNAs (miRNAs) are small RNAs that are not encode a protein, but nevertheless potent coordinating capacities. They perform vital regulatory functions in a range of malign cancers, involving gastric cancer. Abnormal stated miRNAs are also involved in gastric carcinogenesis through modification of growth of cells, cell cycles, apoptosis, and migration of cells. Epigenetic and genetic alteration has been identified as one of the mechanisms responsible for miRNA dysregulation. MiRNA performs essential functions in the progression of gastric cancer by targeting oncogene or tumor suppressor gene expression. The first step in determining the roles of miRNAs in gastric cancer is to investigate differences in miRNA expression profiles between normal and tumor gastric tissues (Pan et al., 2013). Tumor suppressor miRNAs inhibit tumor formation by suppressing oncogenes. Relationship of microRNAs within cancer changes protein-encoding oncogene or tumor suppressor genes are known to cause cancer. Genetic cause of cancer with the recent demonstration of miRNAs in tumor formation.

Competing endogenous RNAs (ceRNAs) are transcripts capable of mutual regulation at the post-transcription level through competition for shared miRNAs. CeRNA networks link protein-coding mRNA functions with those of non-coding RNAs (ncRNAs), including microRNA, long ncRNA, pseudogenic RNA and circular RNA. Since any transcripts containing an miRNA response component are in theory capable of acting as ceRNAs, these may represent a widespread form of post-transcriptional gene expression regulation in physiological and pathological terms. A number of factors are known to be capable of affecting ceRNA activity, including the abundance and subcellular localization of ceRNA components, the binding affinity of miRNAs to their sponges, RNA editing, RNA secondary structures and RNA-binding proteins. Disturbance in these may lead to deregulation of ceRNA networks and thus to human diseases, including cancer (Qi et al., 2015).

In recent years, ncRNAs have generated considerable interest in terms of cell transformation. Ultraconserved regions (UCRs) were first discovered in 2004 following bioinformatic investigation of mouse, rat, and human genomes. UCRs consist of a minimum of 481 genomic sequences at least 200 bp in length (range 200-779 bp), and which are fully conserved (100% identity without any insertions or deletions) among the above three vertebrate species. A significant proportion of UCRs are transcribed (T-UCRs) in normal human tissues, and their expression levels have been observed to exhibit a ubiquitous and tissue-specific pattern. While the functions of T-UCRs are largely unclear, the high level of trans-species conservation they exhibit appears to suggest that they are of significant importance to ontogenesis/phylogenesis in mammals. Recent research into genome-wide expression has revealed that T-UCRs exhibit distinct profiles in different human cancers, representing further evidence of their role in carcinogenesis in humans (Fassan et al., 2014).

In recent years understanding their role in cancer, miRNAs have been hopeful in understanding the molecular pathology of cancer and developing molecular targeted therapies. Based on this feature of miRNAs, we aim to identify genes with potential oncogenic activity not previously identified *in silico* in gastric cancer. In line with our data, we aim to conduct further in vitro and in vivo studies on these miRNAs

Methods

Selection of miRNAs involved in the pathogenesis of gastric neoplasms

Four miRNAs clinically associated with gastric neoplasm and authenticated experimentally were exported over the MiRTarBase database. The miRTarBase database submits estimated and verified data concerning miRNA-target interaction. This enables researchers to confirm novel miRNA targets. The ‘Comfirmed Target module’ showed in this study by Chou et al. (2018).

Analysis of gastric neoplasm-specific miRNA-mediated ceRNAs

One thousand eight genes projected by these four miRNAs simultaneously were described using the ComiR database. ComiR is an online system employed for the purpose of combinatorial miRNA target estimation. It computes the potency of targeting by a group of miRNAs. When calculating the relay impact of one mRNA from a group of several miRNAs, the application employs utilizer-defined miRNA expression levels in a combinatorial manner based on appropriate machine learning techniques and thermodynamic modeling to elicit more accurate estimates. ComiR admits the opportunity of constituting a operational target for a group of miRNAs, based on relevant miRNA expression levels, for every gene (Coronnello and Benos, 2013).

We hope that the RNA transcripts of these genes will exhibit potential ceRNA activity for these miRNAs and that their arrangement is organized on the basis of miRNA-sponging mechanisms.

Matching of GN-associated ceRNA with genes including T-UCR

Bejerano et al. identified the UCRs in the human genome. Genes including these regions are graded as upstream, exonic or downstream in accordance, depending on the site of fixation within the gene (Bejerano et al., 2004). Genes with T-UCR in their exonic areas were also identified, and those exhibiting latent ceRNA activity were excerpted in our previous research.

Analysis of gastric neoplasm-related ceRNAs *including T-UCR in the sense of differential gene expression between gastric neoplasm and normal gastric tissues*

Genes exhibiting significant expression differences between gastric neoplasm and normal stomach tissue from GN-associated ceRNAs, including T-UCR were identified with the assistance of the GEPIA database. GEPIA (Gene Expression Profiling Interactive Analysis), a web-based tool to deliver fast and customizable functionalities based on The Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx) data. All plotting features in GEPIA are developed using R (version 3.3.2) and Perl (version 5.22.1) programs. (Tang et al., 2017).

Correl tests of NFAT5 and CLK3 genes in gastric neoplasm

Methods for analyzing gene expression are numerous and diverse. Expression-based clustering, for example, can be divided into supervised and unsupervised methods. Gene expression differential analysis is a classical supervised method, leading to the finding tumor-specific genes by comparing tumor to normal groups. Statistical analysis of the association between *NFAT5* and *CLK3* genes and gastric neoplasm was showed using the Spearman correlation test in the GEPIA database.

Results

A list of four miRNAs experimentally linked to gastric neoplasm using the miRTarbase database is shown in Table 1.

Table 1. List of miRNAs taking role in gastric neoplasms pathogenesis

1. hsa-miR-148a
2. hsa-miR-23a
3. hsa-miR-370
4. hsa-miR-429

A list of 1008 genes simultaneously targeted by these four miRNAs is shown supplementary 1. Wedeclared genes with T-UCR in their exonic regions from those listed by Bejerano et al., and these are shown in supplementary 2. Then, we extracted ones showing potential ceRNA activity in our previous analysis among them (Table 2). Genes exhibiting significant expression variation between gastric neoplasm and normal gastric tissues among gastric neoplasm-related ceRNAs with T-UCR were identified. In agreement with that analysis, expression of *NFAT5* was significantly higher in gastric neoplasm compared to normal stomach tissue, while no significant differential expression patterns were detected in the other genes (Table 3).

Table 2. List of gastric neoplasms-associated ceRNAs cross-matching with genes including T-UCR in their exonic regions

NFAT5
CLK3

Table 3. Expression values of GN-associated ceRNAs including T-UCR between gastric neoplasms and normal stomach tissues.

Gene ID	GN	Normal stomach
*NFAT5**	**8,94**	**4,03**
CLK3	30,1	36,53

*shows significantly differential expression pattern between GN and normal stomach tissues

Statistical analysis of the link between *NFAT5* and *CLK3* genes and gastric neoplasm was conducted through the GEPIA database. Spearman correlation analysis revealed that the *NFAT5* and CLK3 gene pair exhibited significant association with gastric neoplasm (Figure 1) (p=0.000; R=0.22).

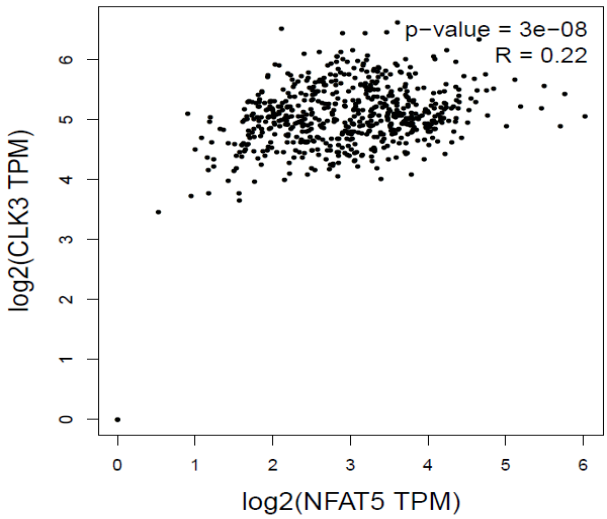

Figure 1: Spearman correlation analysis of *NFAT5* and *CLK3* genes with GN.

Supplementary 1. List of genes targeted bu these 4GN-associated miRNAs simultaneously

AVPR1A	0.9048
AAK1	0.9202
ABCC3	0.9049
ABI2	0.9239
ACADSB	0.9122
ACER3	0.905
ACP6	0.9129
ACSS3	0.9052
ACVR1C	0.9173
ACVR2B	0.9216
ADAM10	0.9201
ADAM12	0.9212
ADAM22	0.9054
ADAMTS4	0.9054
ADAMTS5	0.9197
ADAMTS6	0.9213
ADARB1	0.9051
ADCY1	0.9202
ADCYAP1R1	0.9128
ADRBK2	0.9055
AFF1	0.9197
AFF2	0.9133
AFF3	0.9051
AGAP1	0.923
AGFG1	0.9172
AGO1	0.9217
AGO2	0.9116
AGO3	0.9231
AJAP1	0.9201

AK4	0.9053
AKAP5	0.9172
AKAP6	0.9054
AKT2	0.9114
AMER2	0.9131
ANK2	0.9172
ANKFY1	0.9125
ANKRD11	0.9201
ANKRD9	0.9049
ANO5	0.9111
ANTXR2	0.9131
AP1M1	0.9178
AP5M1	0.9201
APC	0.9052
APOL3	0.9104
APOL6	0.9055
ARHGAP19	0.9051
ARHGAP20	0.9174
ARHGAP31	0.9049
ARHGAP32	0.9052
ARIH1	0.9227
ARL10	0.9134
ARL5A	0.9167
ARL8B	0.9146
ARNT2	0.9213
ARPIN	0.9174
ARSD	0.9117
ASAP1	0.9165
ASRGL1	0.909

ATF7	0.9127
ATG9A	0.9127
ATP10A	0.9051
ATP11A	0.9198
ATP11B	0.9117
ATP2A2	0.9051
ATP8A1	0.9198
ATP8A2	0.9054
ATRX	0.923
ATXN1	0.9216
ATXN1L	0.9054
ATXN3	0.9202
ATXN7L3B	0.9175
B3GALT5	0.9201
B4GALT4	0.905
B4GALT5	0.9163
BACH2	0.913
BAG2	0.9125
BCAR1	0.9128
BCAS4	0.9054
BCL2L11	0.9209
BICD1	0.9197
BICD2	0.905
BMF	0.9049
BMP1	0.912
BMP2K	0.9166
BMPR1A	0.923
BNC2	0.9216
BNIP2	0.917

BRCA1	0.9171	CDKL5	0.9177	CUL3	0.9049
BRWD1	0.9173	CDS2	0.9133	CUX1	0.9216
C12orf49	0.9176	CDYL2	0.9174	CXCL12	0.9162
C14orf37	0.9125	CECR2	0.9173	CXorf23	0.9196
C16orf52	0.9168	CELF1	0.9225	CYB561D1	0.905
C17orf51	0.92	CELF2	0.9132	CYB5R4	0.9132
C17orf70	0.9048	CENPO	0.9117	CYTH3	0.9119
C18orf25	0.9116	CENPP	0.9176	DAPK2	0.9175
C18orf32	0.9195	CEP192	0.9132	DBNL	0.9226
C1orf95	0.9056	CEP250	0.9133	DBT	0.9133
C2orf71	0.9162	CEP78	0.913	DCAF7	0.9052
C4orf32	0.9132	CEP85L	0.9207	DCN	0.917
C5orf56	0.9116	CERS6	0.9125	DCP2	0.9233
C9orf114	0.9208	CFL2	0.9232	DCUN1D3	0.905
CA12	0.9049	CFLAR	0.9057	DCX	0.9053
CA5B	0.9051	CHML	0.9123	DDI2	0.9177
CAB39L	0.9113	CHRM3	0.905	DDX53	0.9165
CACNA1E	0.9176	CHRNA7	0.9167	DGKE	0.9131
CACNG8	0.9177	CHST11	0.9127	DGKH	0.9227
CACUL1	0.923	CHST9	0.9056	DGKI	0.9176
CADM1	0.9132	CIITA	0.9133	DIRAS2	0.9113
CADM2	0.9056	CLCN3	0.9104	DIS3	0.9215
CAMK4	0.9201	CLCN6	0.912	DISC1	0.9052
CAMSAP2	0.911	CLEC16A	0.905	DLG5	0.9174
CAPRIN2	0.913	CLEC2D	0.9049	DLGAP2	0.9055
CARD8	0.905	**CLK3**	**0.9177**	DNAJC10	0.9231
CASK	0.913	CLMN	0.9134	DNAJC15	0.9131
CASP10	0.9166	CLOCK	0.9201	DNAJC18	0.9167
CBFA2T2	0.9052	CLVS2	0.9237	DNAJC5	0.9196
CBX5	0.9177	CNKSR3	0.9238	DNASE1	0.9177
CCDC127	0.9201	CNNM2	0.9227	DNM3	0.9127
CCDC144A	0.9115	CNOT4	0.9103	DNMT3A	0.9176
CCDC50	0.9175	CNOT6L	0.9228	DOK6	0.9176
CCDC85C	0.9217	CNTNAP2	0.9049	DR1	0.9216
CCDC93	0.9128	CNTNAP3B	0.9119	DRP2	0.9122
CCNT2	0.9127	CNTROB	0.9121	DSC2	0.9215
CCSAP	0.9125	COL20A1	0.9195	DTNA	0.917
CD226	0.9055	CPD	0.9125	DTWD1	0.9227
CD84	0.9053	CPEB3	0.9053	DYRK2	0.9174
CDCP1	0.9117	CPM	0.9127	EEF2K	0.9051
CDH23	0.9173	CPSF6	0.9127	EGFR	0.9176
CDH7	0.9214	CRTAP	0.9198	EIF2AK2	0.9133
CDH8	0.9232	CSRNP3	0.9134	EIF4E3	0.9216
CDHR1	0.9211	CTNNA3	0.9211	EIF4G1	0.905

EIF5	0.9048	FGF7	0.9121	GOLGB1	0.9133
ELFN2	0.9129	FGFR1OP	0.9217	GOLT1B	0.9106
ELK4	0.9174	FIGN	0.9215	GPATCH2L	0.9238
ELP2	0.9052	FILIP1	0.9123	GPR107	0.913
EMC10	0.9056	FKBP15	0.905	GPR161	0.9049
ENAH	0.9176	FKTN	0.913	GPR180	0.9197
ENTPD1	0.9056	FLNA	0.9056	GPRC5B	0.9125
EPHA5	0.9123	FLRT2	0.9241	GPRIN3	0.9134
EPHA8	0.9106	FMN1	0.9177	GRAMD1B	0.9049
EPHB6	0.9122	FNTA	0.9049	GREM1	0.9227
EPN1	0.9217	FOSL2	0.9128	GRIK3	0.9229
EPT1	0.9053	FOXK1	0.9177	GRIN2A	0.9132
ERBB2	0.9049	FOXP2	0.913	GRIN2B	0.9241
ERBB2IP	0.916	FREM2	0.9052	GTDC1	0.9176
ERBB4	0.9175	FRK	0.9231	GTF2H5	0.9132
ERI1	0.911	FRY	0.9192	GTF3C4	0.9123
ESRRG	0.9162	FSD1L	0.9209	GTPBP10	0.9213
ETNK1	0.913	FTO	0.9056	GUCY1A2	0.9134
ETV5	0.9109	FUT4	0.9224	GXYLT1	0.9197
EXOC5	0.9052	FUT9	0.9233	HDAC2	0.9053
EXOSC9	0.9111	FXR1	0.9175	HDAC9	0.9198
EXT1	0.9171	FZD3	0.9134	HECW2	0.9174
FAM126A	0.9133	GAB1	0.9051	HEG1	0.9194
FAM126B	0.9225	GABRA4	0.9054	HELB	0.9176
FAM168B	0.917	GABRG3	0.9176	HELZ	0.9174
FAM179A	0.9176	GALR1	0.9133	HEMK1	0.9231
FAM193B	0.9049	GAN	0.9217	HFE	0.9116
FAM204A	0.9202	GAS2L3	0.9189	HHIP	0.9051
FAM217B	0.9103	GCC2	0.9054	HIF1AN	0.9227
FAM26E	0.9201	GDAP2	0.9199	HIPK2	0.9234
FAM63B	0.92	GDF11	0.9056	HIPK3	0.9123
FAM83F	0.9178	GFOD1	0.9176	HLA-A	0.908
FAM9C	0.9209	GFPT1	0.9053	HLA-A	0.9194
FARP1	0.9175	GJA3	0.9122	HMGA2	0.9053
FARP2	0.9126	GLRA3	0.9173	HMHA1	0.9049
FAT3	0.9198	GMFB	0.9214	HNRNPA3	0.9049
FBXL4	0.9172	GMPPB	0.9051	HOOK3	0.9056
FBXO22	0.923	GMPS	0.905	HS2ST1	0.9171
FBXO25	0.9177	GNAI3	0.9238	HS6ST3	0.9131
FBXO30	0.9174	GNAO1	0.9168	HSBP1	0.9132
FBXO32	0.9129	GNB1L	0.9054	HSD17B2	0.9055
FEM1A	0.9055	GNB5	0.9132	HSPA12A	0.9122
FER	0.9216	GNPDA2	0.9118	HTT	0.9194
FGF14	0.9236	GOLGA6L2	0.9228	ICA1L	0.9173

ICE2	0.9175	KCNQ5	0.9119	LRRK2	0.9173
ICOSLG	0.913	KCTD15	0.9121	LSAMP	0.9176
IDS	0.9127	KCTD16	0.9226	LYNX1	0.9123
IFITM10	0.9164	KDM3B	0.9199	LYRM2	0.9126
IGF2BP1	0.9129	KDM5A	0.9051	MACC1	0.9171
IGSF10	0.9119	KDM7A	0.9213	MAP3K2	0.9201
IKZF1	0.9125	KIAA0930	0.9198	MAP3K9	0.9133
IL17RD	0.9054	KIAA1045	0.9124	MAPK1	0.9201
IL6R	0.9124	KIAA1244	0.9216	MAPK13	0.9052
IL6ST	0.913	KIAA1456	0.9053	MAS1	0.9056
ILDR2	0.9201	KIAA1462	0.9171	MBNL3	0.9176
IMPG1	0.9125	KIAA1549	0.9054	MBOAT2	0.9054
INO80D	0.9201	KIAA1614	0.9175	MBP	0.9177
INPP4A	0.9176	KIAA1958	0.9175	MCC	0.905
INTS6	0.9217	KIAA2018	0.9129	MCFD2	0.9205
INTU	0.9133	KIDINS220	0.9199	MCTP2	0.9169
IPCEF1	0.9172	KIF1B	0.9128	MDGA1	0.9055
IPMK	0.9125	KIF26B	0.9054	MDM2	0.9131
IPO9	0.9216	KIF6	0.9215	MDM4	0.9133
IRAK3	0.9051	KLC1	0.9234	MECP2	0.9056
IRGQ	0.9053	KLF12	0.9216	MED12L	0.9123
ITGA11	0.9172	KLHL21	0.9051	MED13L	0.9201
ITGA9	0.9212	KLHL28	0.9116	MEGF9	0.919
ITM2B	0.9132	KLHL42	0.905	MEIS1	0.9123
ITSN1	0.9178	KLHL6	0.9126	MESDC2	0.9122
IYD	0.9213	KMT2C	0.9236	METTL8	0.9175
JAKMIP2	0.9168	KPNA4	0.913	MEX3C	0.9121
JMY	0.905	KRR1	0.9226	MGAT4A	0.9131
KALRN	0.9053	KRT222	0.9105	MGAT4C	0.9238
KAT7	0.9132	KSR1	0.9212	MGAT5	0.9173
KATNAL1	0.913	KSR2	0.9134	MGLL	0.9124
KCNA1	0.9052	KYNU	0.9236	MIEF1	0.9165
KCNB1	0.9132	LANCL3	0.9056	MITF	0.9114
KCNC1	0.9052	LCOR	0.9054	MKLN1	0.923
KCNC4	0.9238	LCORL	0.9048	MLEC	0.9128
KCND3	0.9113	LDLRAD4	0.9053	MLXIP	0.9126
KCNH5	0.92	LGALS8	0.9127	MLYCD	0.9134
KCNJ15	0.9131	LPGAT1	0.9214	MMP16	0.9057
KCNJ6	0.924	LPHN3	0.9131	MOB1B	0.913
KCNK5	0.9103	LPP	0.9231	MON2	0.9131
KCNMA1	0.9055	LRIG2	0.9199	MOSPD2	0.9103
KCNN3	0.9134	LRRC58	0.9197	MPP6	0.9128
KCNQ3	0.9215	LRRC8B	0.9051	MPRIP	0.9176
KCNQ4	0.9099	LRRK1	0.9133	MR1	0.9131

MRE11A	0.9167	NIPA1	0.9197	PCNXL4	0.9236
MROH5	0.9124	NKD1	0.9174	PCYT1B	0.9122
MRPL35	0.9114	NKTR	0.9131	PDE4B	0.9098
MRPL42	0.9217	NLGN4X	0.9116	PDE4DIP	0.9049
MRPS25	0.9126	NOL4L	0.9049	PDE5A	0.919
MTF1	0.9198	NOVA1	0.9214	PDE7A	0.915
MTMR10	0.9211	NOVA2	0.9198	PDIK1L	0.9101
MTMR9	0.9197	NOX5	0.9174	PDK1	0.9231
MTR	0.9054	NQO2	0.9216	PDPR	0.9174
MTUS1	0.9118	NR6A1	0.905	PDXK	0.9131
MXD1	0.9125	NRDE2	0.9227	PDZD8	0.9166
MYLK	0.9199	NRXN3	0.9175	PEAK1	0.9231
MYO18A	0.92	NT5DC1	0.905	PELP1	0.9159
MYO18B	0.9225	NT5DC3	0.9053	PEX11A	0.9152
MYO5C	0.9172	NTNG2	0.9157	PEX26	0.9234
MYO9A	0.916	NTPCR	0.905	PGBD5	0.9056
N4BP2	0.9176	NTRK3	0.9238	PHACTR1	0.9167
N4BP2L2	0.92	NUCKS1	0.9127	PHACTR2	0.92
NA	0.9049	NUDCD2	0.9051	PHC3	0.9176
NA	0.9053	NUDT3	0.92	PHEX	0.9121
NA	0.9055	NUDT4	0.9132	PHF3	0.9053
NA	0.9125	NUFIP2	0.9198	PHKG2	0.9126
NA	0.913	NUPL1	0.9171	PIGP	0.9131
NA	0.9216	ODF2L	0.9196	PIK3C3	0.9173
NA	0.9239	OGFRL1	0.9176	PIK3CA	0.9223
NABP1	0.9201	ONECUT2	0.9134	PITPNM3	0.9048
NACC2	0.9051	ORAI2	0.9134	PLCXD3	0.9048
NAP1L1	0.9215	ORC4	0.9049	PLEKHA1	0.9057
NCKAP1	0.924	OSBPL8	0.9122	PLEKHA3	0.9057
NCOA2	0.9122	OTUD4	0.9115	PLEKHA8	0.9174
NDST1	0.9171	OTUD7A	0.9216	PLEKHG4B	0.9233
NDUFA5	0.9127	OTULIN	0.9175	PLEKHM1	0.9128
NDUFA9	0.9055	PAG1	0.9216	PLLP	0.9052
NDUFS1	0.9133	PAK3	0.9132	PLXNA4	0.9199
NEDD4	0.9198	PANK3	0.9133	PNRC2	0.9146
NEGR1	0.9226	PAPD5	0.9051	POLE	0.9226
NF1	0.9176	PARD3B	0.9168	POLR1A	0.9175
NFASC	0.9215	PAX5	0.9226	POLR3D	0.9048
NFAT5	0.9236	PAXIP1	0.9122	POU2F1	0.9178
NFIA	0.9233	PBX1	0.9198	PPARA	0.9175
NFIB	0.9175	PCDH10	0.9194	PPIP5K2	0.9202
NFIC	0.9053	PCDH19	0.912	PPM1A	0.9175
NHLRC2	0.9055	PCDH9	0.9239	PPM1F	0.9169
NIN	0.9127	PCDHA4	0.9052	PPP1CB	0.9166

PPP1R12B	0.9177
PPP1R13B	0.9128
PPP2R1B	0.9188
PPP2R5E	0.921
PRDM11	0.9132
PRDM15	0.9226
PRDM16	0.9052
PRKAA2	0.9054
PRKCA	0.9132
PRKCB	0.9052
PRLR	0.9176
PRPF38A	0.9122
PRRC2B	0.9048
PRRG3	0.9049
PRTG	0.9233
PSD3	0.92
PSMG4	0.9049
PTAR1	0.9215
PTBP2	0.9133
PTBP3	0.905
PTCH1	0.9057
PTCHD1	0.923
PTEN	0.9172
PTGER3	0.9198
PTK2	0.9171
PTPN11	0.9125
PTPN14	0.923
PTPN23	0.9048
PTPRT	0.9133
PURA	0.9201
PURB	0.9054
PVRL1	0.9048
PYGO1	0.9174
QKI	0.9226
RAB11FIP2	0.9165
RAB11FIP4	0.92
RAB15	0.9106
RAB21	0.9217
RAB3C	0.9201
RAB3IP	0.9132
RAB6B	0.9173
RAD51D	0.9131
RALY	0.9052
RAP1A	0.9206
RAP1B	0.9227
RAPGEF1	0.9162
RASAL2	0.9133
RASGEF1B	0.9049
RASSF5	0.9128
RASSF8	0.9166
RBBP4	0.9129
RBM25	0.9124
RBM28	0.9227
RBMS2	0.9129
RBMS3	0.905
RC3H2	0.9212
REL	0.9055
REPS1	0.9125
REPS2	0.913
RET	0.9116
REV1	0.9127
REV3L	0.9216
RFX7	0.92
RGMA	0.9057
RICTOR	0.9053
RIF1	0.9199
RILPL2	0.9051
RIMKLA	0.9198
RIMS2	0.9168
RNF115	0.9055
RNF150	0.9054
RNF152	0.9132
RNF165	0.9055
RNF217	0.92
RNF24	0.9175
RORA	0.9133
RORB	0.9175
RPAP2	0.9217
RPS6KA5	0.9241
RPS6KB1	0.9118
RRP15	0.9199
RTEL1-TNFRSF6B	0.9118
RTKN2	0.9049
RUNX1T1	0.9126
S100A7A	0.9049
SAMD12	0.9131
SAR1A	0.9052
SARM1	0.9201
SCAI	0.9176
SCN3B	0.9048
SCN8A	0.9209
SCO1	0.9056
SCOC	0.9119
SCUBE1	0.9132
SDHC	0.9057
SDK2	0.905
SDR42E1	0.9216
SEC22C	0.9049
SEMA3A	0.9051
SEMA5A	0.9133
SEMA6D	0.9169
SERINC3	0.9116
SERINC5	0.9052
SESN2	0.9096
SESN3	0.9225
SF3B3	0.9053
SGCD	0.9199
SH3BP2	0.9056
SH3PXD2A	0.9215
SH3TC2	0.9132
SHE	0.913
SHPRH	0.9216
SHROOM4	0.9198
SIK2	0.9175
SIK3	0.9209
SIM1	0.9129
SIX4	0.9122
SKP1	0.9216
SLC16A7	0.9133
SLC1A2	0.9201
SLC24A4	0.9214
SLC30A4	0.9052
SLC30A9	0.9126
SLC35B4	0.9194
SLC35C2	0.9129
SLC35E3	0.9217
SLC39A9	0.9122
SLC43A2	0.9214
SLC44A1	0.9132
SLC4A4	0.912
SLC4A7	0.9104
SLC4A8	0.9056

SLC5A3	0.9177	STRN	0.9049	TMEM184A	0.9052
SLC7A11	0.9133	STX7	0.9217	TMEM192	0.9176
SLC7A14	0.9172	STXBP4	0.9134	TMEM200C	0.9052
SLC7A2	0.9048	STXBP6	0.9155	TMOD1	0.9101
SLC7A6	0.905	SUGT1	0.9202	TMOD2	0.9132
SLC8A1	0.9236	SULT1B1	0.905	TMOD3	0.92
SLCO5A1	0.905	SV2B	0.9228	TNFAIP8	0.92
SLITRK5	0.9238	SV2C	0.9176	TNKS1BP1	0.921
SMAD2	0.9241	SYK	0.9121	TNPO1	0.9053
SMAD5	0.9225	SYNE3	0.9216	TNRC6A	0.9226
SMC1A	0.9198	SYNJ1	0.9119	TNRC6B	0.9134
SMG9	0.9121	SYT14	0.9134	TOM1L2	0.9194
SMURF2	0.9131	SYT16	0.9134	TPCN1	0.9116
SNAP91	0.9125	TAB3	0.9052	TPPP	0.9052
SNTB2	0.9199	TACR3	0.9166	TREM1	0.909
SNX1	0.9133	TBC1D15	0.9119	TRHDE	0.9234
SNX27	0.9173	TBC1D16	0.9201	TRIL	0.9102
SNX30	0.9054	TBC1D32	0.9173	TRIM33	0.9168
SNX33	0.913	TBC1D5	0.9121	TRIM44	0.9227
SNX8	0.9191	TBX18	0.9169	TRIOBP	0.9237
SOD2	0.9134	TCF4	0.913	TRMT5	0.9122
SOGA3 KIAA0408	0.9055	TET2	0.9198	TROVE2	0.9199
SORT1	0.9128	TET3	0.913	TRPM3	0.9129
SOS1	0.9194	TEX14	0.905	TRPS1	0.9127
SOX5	0.9122	TFDP2	0.9104	TSC1	0.9053
SP3	0.9115	TFEC	0.9195	TSC2	0.9051
SPATA2	0.911	TG	0.9195	TSC22D2	0.9177
SPEF2	0.9097	TGFBR3	0.913	TSPAN14	0.9231
SPRY3	0.9131	THBS1	0.9124	TSPAN3	0.9129
SREK1IP1	0.9131	THRB	0.9051	TTBK2	0.9173
SRGAP1	0.9237	THSD7A	0.9168	TTC39B	0.9176
SRRM4	0.9172	THUMPD3	0.9101	TTC7B	0.924
SSBP2	0.9132	TMED3	0.924	TTL	0.9057
SSH2	0.9124	TMED5	0.9171	TTPAL	0.9128
SSTR2	0.9052	TMED7	0.9181	TXLNG	0.9108
ST6GALNAC3	0.9124	TMEM120B	0.9053	TXNDC15	0.9115
ST8SIA1	0.9132	TMEM127	0.913	TXNL1	0.9128
ST8SIA3	0.9176	TMEM132B	0.9174	UBA6	0.9051
ST8SIA5	0.9057	TMEM154	0.9235	UBN2	0.9238
STAM2	0.9116	TMEM164	0.9196	UBXN10	0.9048
STARD8	0.9197	TMEM168	0.9049	UBXN7	0.9132
STK24	0.9216	TMEM170A	0.921	UFM1	0.9151
STK35	0.9212	TMEM170B	0.9133	UHMK1	0.9131
STOX2	0.913	TMEM178B	0.9177	UNC119B	0.9124

UNC13A	0.905
USP15	0.9215
USP31	0.9174
USP35	0.9163
USP38	0.9052
USP42	0.9049
USP45	0.905
USP46	0.913
USP49	0.9054
USP6	0.9053
USP6NL	0.9227
USP8	0.9231
UVSSA	0.9126
VAMP4	0.9049
VANGL1	0.9176
VAPA	0.9199
VASH2	0.9181
VCPIP1	0.9173
VGLL3	0.9175
VKORC1L1	0.9126
VLDLR	0.9129
VPS35	0.9123
VTA1	0.9131
VTI1A	0.9164
VWC2	0.9132
WASF3	0.9161
WDFY2	0.9133
WDR11	0.9161
WDR62	0.9197
WDR7	0.9216
WDR82	0.916
WHSC1L1	0.9126
WNK3	0.9196
WNT2B	0.9233
WSCD1	0.9118
WTIP	0.9177
XIAP	0.9174
XKR4	0.9234
XPO1	0.917
XPO4	0.9225
XYLT1	0.9131
YIPF4	0.9217
YIPF6	0.9051
YOD1	0.9198
YY1	0.913
ZADH2	0.9131
ZBED3	0.9126
ZBTB25	0.9215
ZBTB34	0.9195
ZBTB37	0.9239
ZBTB44	0.9053
ZBTB8A	0.9049
ZBTB8B	0.9202
ZC3H12C	0.9172
ZC3H14	0.9239
ZC3H6	0.9215
ZC3H8	0.917
ZDHHC17	0.9183
ZDHHC18	0.9121
ZDHHC2	0.9127
ZDHHC21	0.9215
ZEB1	0.9232
ZFHX4	0.9114
ZFP90	0.9127
ZFYVE20	0.9167
ZFYVE26	0.9054
ZHX3	0.9175
ZNF107	0.9164
ZNF117	0.9238
ZNF138	0.9226
ZNF142	0.913
ZNF189	0.9114
ZNF207	0.923
ZNF223	0.9106
ZNF226	0.9231
ZNF230	0.9125
ZNF233	0.9182
ZNF257	0.9232
ZNF26	0.9234
ZNF268	0.9057
ZNF273	0.9227
ZNF286A	0.9128
ZNF286B	0.9127
ZNF292	0.9111
ZNF37A	0.9131
ZNF431	0.9177
ZNF445	0.9176

Supplementary 2: List of genes containing T-UCR in their exonic regions according to the study of Bejerano et al.

uc.143	218	AB014560
uc.203	203	AB067798
uc.135	201	AK096400
uc.339	252	ATP5G2
uc.413	272	BC060758
uc.49	207	BC060860
uc.61	326	BCL11A
uc.324	225	C11orf8
uc.285	232	CARP-1
uc.233	266	CENTG3
uc.393	**275**	**CLK3**
uc.185	411	CLK4
uc.184	230	CPEB4
uc.471	239	DDX3X
uc.331	218	DLG2
uc.13	237	EIF2C1
uc.194	201	EPHA7
uc.183	236	FBXW1B
uc.333	270	FLJ25530
uc.478	252	GRIA3

uc.479	302	GRIA3
uc.282	207	GRIN1
uc.97	442	HAT1
uc.144	205	HNRPDL
uc.186	305	HNRPH1
uc.263	207	HNRPK
uc.264	267	HNRPK
uc.443	239	HNRPM
uc.45	203	HNRPU
uc.46	217	HNRPU
uc.409	244	L32833
uc.174	260	MATR3
uc.129	212	MBNL1
uc.356	251	MBNL2
uc.375	300	MIPOL1
uc.292	217	MLR2
uc.406	**211**	**NFAT5**
uc.473	222	NLGN3
uc.378	251	NRXN3
uc.475	397	OGT
uc.280	220	PBX3
uc.338	223	PCBP2
uc.376	290	PRPF39
uc.377	217	PRPF39
uc.33	312	PTBP2
uc.102	338	PTD004
uc.48	298	PUM2
uc.477	209	RAB9B
uc.395	249	RBBP6
uc.330	207	RBM14
uc.455	245	RNPC2
uc.419	289	SFRS1
uc.138	419	SFRS10
uc.28	355	SFRS11
uc.189	573	SFRS3
uc.456	320	SFRS6
uc.50	222	SFRS7
uc.454	208	SLC23A1
uc.193	319	SYNCRIP
uc.436	210	TCF4
uc.414	246	THRA
uc.313	231	TIAL1
uc.208	218	TRA2A
uc.209	250	TRA2A
uc.77	296	ZFHX1B
uc.151	214	ZFR
uc.474	210	ZNF261

Discussion

Gastric neoplasm is the leading cause of cancer-related deaths. According to research conducted in 2008, gastric neoplasm is the fourth most common cancer in the world and ranks second among cancers that cause death. The death rate from this cancer is higher than that from malignant tumors such as colon, breast and prostate cancers. The development of this cancer is complex, involving a number of genetic and epigenetic alterations of oncogenes, tumor suppressor genes, deoxyribonucleic acid (DNA) repair genes, cell cycle regulators, and signaling molecules. Oncogenes are activated at different stages of the course of gastric neoplasm, and some tumor suppressor genes are inactivated. Numerous studies have shown that miRNAs can be effective in carcinogenesis. Changes in expression levels of miRNAs in different types of cancer have been investigated, and miRNAs have been observed to differ between normal and pathological tissues (Sevignani et al., 2006; Zhou et al., 2010). Various miRNAs have been shown to play a specific role in tumor progression and metastasis in the differentiation of cancer cells (Kim et al., 2011; Calin et al., 2002; Michael et al., 2003; Metzler et al., 2004; Chan et al., 2005; He et al., 2005; Sevli et al., 2010; Lamy et al., 2006; Iorio et al., 2005) The purpose of this study was to describe novel biomarkers for GN through *in silico* analysis involving gastric neoplasm-specific miRNAs, by determining their combinatorial target genes (potential ceRNAs), selecting those with T-UCR and potentiating their association with gastric neoplasm using statistical correlation techniques.

Four miRNAs experimentally related to gastric neoplasm were identified through the miRTarbase database (Table I). Genes with equal ComiR abundance were listed through 1008 genes targeted concurrently by these four miRNAs. Genes with T-UCR in their exonic regions were described from the genes containing T-UCR listed by Bejerano et al. (Bejerano et al., 2004). We then considered those exhibiting probable ceRNA activity in our earlier analysis (Table II). Next, we choosed genes with significant differences in expression between gastric neoplasm and normal gastric tissues from GN-related ceRNAs involving T-UCR. This test revealed significantly higher NFAT5 expression in gastric neoplasm than in normal stomach tissue,

while the other exhibited no significantly different expression pattern. In addition, the NFAT5 and CLK3 gene pair were substantively associated with gastric neoplasm based on the Spearman correlation analysis findings.

These NFAT5 genes have not previously been experimentally linked to gastric neoplasm. Ours is the first study to associate these two genes with gastric neoplasm. NFAT family contains five different proteins one of them is NFAT5 protein. But, NFAT1 to 4 proteins are regulated by calcineurin, NFAT5 is controlled by osmotic pressure at the nuclear localization, transcriptional and expression levels. When stimulated, NFAT5 triggers target gene transcription by binding to tonicity enhancer elements) in various coordinator domains which are all responsible for supplying cells in order to facilitate their survival under hypertonic conditions (Cheung and Ko, 2017). NFAT5 gene shows its oncogenic role via different pathways in such diseases as renal cell carcinoma, breast cancer, lung adenocarcinoma and colon cancer. NFAT5-related expression of S100A4 projects the migration and proliferation of renal carcinoma cells (Küper et al., 2014). Additionally, NFAT5/STAT3 interaction soften synergism of high salt with IL-17 towards induction of VEGF-A expression in breast cancer cells (Amara S et al., 2016). NFAT5 also stimulates the migration and proliferation of pulmonary adenocarcinoma cells, in part by modulating AQP5 expression (Guo and Jin, 2015). The Src kinase pathway is also involved in NFAT5-mediated S100A4 induction through hyperosmotic stress in colon cancer cells (Chen et al., 2011). NFAT5 is also a tumor suppressor that functions by suppressing invasion and triggering apoptosis in hepatocellular carcinoma.

Conclusion

The NFAT5 gene was correlated with gastric neoplasm in our study, and *in silico* analysis results predict that they may potentially play an oncogenic role in gastric neoplasm. The inconsistent results concerning their roles in varying forms of cancer suggests that our study findings will be preliminary for subsequent in vitro and in vivo studies performed to determine the roles of the NFAT5 gene in gastric neoplasm progression. Fatal one among urological cancers. RCC is causedby the accumulation of many genetic and

Ethics Committee Approval:
Since it is a in silico study, there is no need for an ethics committee approval
Peer-review: Externally peer-reviewed.
Author Contributions: Externally peer-reviewed. Author Contributions: Concept- D.U.A.; Design D.U.A., S.E.; Supervision-D.U.A., S.E.; Materials D.U.A., S.E.; Data Collection and/or Processing D.U.A., S.E.; Analysis and/or Interpretation-D.U.A; Literature Review-D.U.A.; Writing-D.U.A.; Critical Review- S.E
Conflict of Interest: No conflict of interest was declared by the author.
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