Expression correlation and Copy Number Alteration analysis of *RNASEH2A* in cancer datasets confirms its association with cancer proliferation and specific cell cycle markers

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ABSTRACT

Ribonuclease H2A (RNASEH2A) is the catalytic subunit of RNASEH2, a key enzyme for removal of incorporated ribonucleotides in DNA and maintenance of genomic integrity for normal cellular growth and division. Gene Ontology analysis on genes showing high positive correlation with RNASEH2A expression in human Genotype -Tissue Expression (GTEx) dataset, suggested involvement of RNASEH2A in mitotic cell cycle regulation. Analysis of expression correlations was performed on a list of genes containing the RNASEH2 subunits RNASEH2A, RNASEH2B, and RNASEH2C, markers associated with cancer, and different cell cycle markers using The Cancer Genome Atlas(TCGA) Pan Cancer dataset, containing expression profiles of ~10,000 patient samples from 35 cancer types/subtypes. Clustering of expression correlation coefficient was performed to reorder genes according to their degree of association. The analysis revealed positive correlation of RNASEH2A with genes highly expressed in cancer and cancer proliferation markers as compared to RNASEH2B and RNASEH2C. The analysis further showed that RNASEH2A expression is highly associated with specific cell cycle markers, i.e., G2 and M cell cycle markers as opposed to G1 and S cell cycle markers. The observed correlations were successfully validated using the Broad institute's Cancer Cell Line Encyclopedia (CCLE) containing ~1000 cell lines. Copy Number Alteration information in TCGA Pan Cancer dataset showed high prevalence of RNASEH2A gene amplification in multiple cancer types/subtypes, further providing genomic evidence of elevated RNASEH2A expression in cancer. Our bioinformatic study, clearly shows increased co-expression of RNASEH2A with cancer proliferation and mitotic cell cycle markers, suggesting that RNASEH2A expression level could be a predictor of poor outcomes and a possible target for therapeutic interventions.

INTRODUCTION

Differential cell-cycle regulation of the RNASEH2A orthologous gene has been observed in yeast *S. Cerevisiae* and increase in levels of RNASEH2A has previously been observed with overexpression of several oncogenes in mesenchymal stem cells.

Study of co-expressed genes with RNASEH2A can reveal other genes involved with RNASEH2A either in common or interconnected biological processes.

Copy Number Alterations(CNAs) are somatic changes to chromosome structure that result in gain or loss in copies of sections of DNA

Classification of CNAs

- Deep deletion: deep loss/homozygous deletion;
- Shallow deletion: shallow loss/heterozygous deletion
- Diploid: homozygous genes
- Gain: a low-level gain/additional copies
- Amplification: high-level amplification

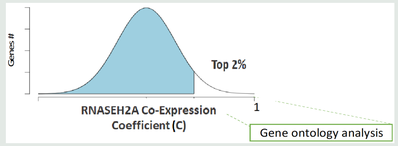
Deep deletions and **Amplification** causes highest burden, prognostic for cancer specific death.

OBJECTIVES

1. Find suggestive role of RNASEH2A in human tissues by using RNASEH2A co-expressed genes for gene ontology analysis
2. To elucidate the role of RNASEH2A in different cell cycle phases and in cancer via expression correlation analysis with marker genes
3. To validate RNASEH2A elevated expression in cancer by measuring the prevalence of CNAs of RNASEH2A in different cancer subtypes

MATERIAL (DATASETS)

1. RNA-seq expression data in Transcripts per million (TPM) from **Genotype-Tissue Expression (GTEx)** portal v7 is available for 53 different human tissues
2. RNA-Seq data expression from **Broad Institute Cancer Cell Line Encyclopedia (CCLE)** dataset in 1019 cancer cell lines from 26 different tissues of origin
3. Copy number alterations (CNAs) and RNA-Seq data from **The Cancer Genome Atlas (TCGA) Pan Cancer studies** involving 32 studies and 10,967 patients



1. Selection of gene pool of high positive correlation with RNAHSE2A to find RNASEH2A associated processes in Human Tissues

Pearson's Correlation coefficient was calculated for expressions values of each gene with expression values of RNASEH2A, termed as RNASEH2A Co-expression Coefficient(C). Top 2% of genes showing highest co-expression coefficient were used for Gene Ontology analysis

METHODOLOGY

Gene name (s)	Function/Role	References
CDKN2A, CDKN2B, CCND1, DHFR, CCNE1	G1 Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
	G1/S Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
AKT1-3, E2F4-5	S Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
CDKN2D, MDM2	G2 Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
CCNB2, TOPBP1	G2/M Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
APC, BUB1	M Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
E2F2, E2F3, CCNB1	M/G1 Cell Cycle Phase	A. Subramanian et al. (GSEA Database)
MYBL2, FOXM1, BUB1, AURKA, AURKB	Upregulated in cancer	M. Li et al
SCARA5, MYOM1	Downregulated in cancer	M. Li et al
PCNA, MKI67(Ki67), MCM2–MCM6, E2F1	Proliferative markers in cancer	M. L. Whitfield et al.
CCNE1, CCND1, CCNB1	Cell cycle markers associated with cancer (G1/S, G2 and M)	M. L. Whitfield et al.
RNASEH2A, RNASEH2B, RNASEH2C RNASEH1	Target genes of interest	This study

2.a Selected list of genes related to cancer proliferation and cell cycle phases

The above list contains 1. Markers involved/associated in different cell cycle phases, 2. Markers up/down regulated in cancer, 3. Cell cycle associated cancer markers, 4. Genes of our interest(RNASEH1, subunits of RNASEH2)

2.b Expression correlation analysis in TCGA and CCLE expression datasets

Pearson Correlation analysis was done between each pair 40 selected genes, followed by clustering to reorder genes with similar expression trends and high degree of association

3. Prevalence of Copy Number Alterations(CNAs) in TCGA Pan Cancer data

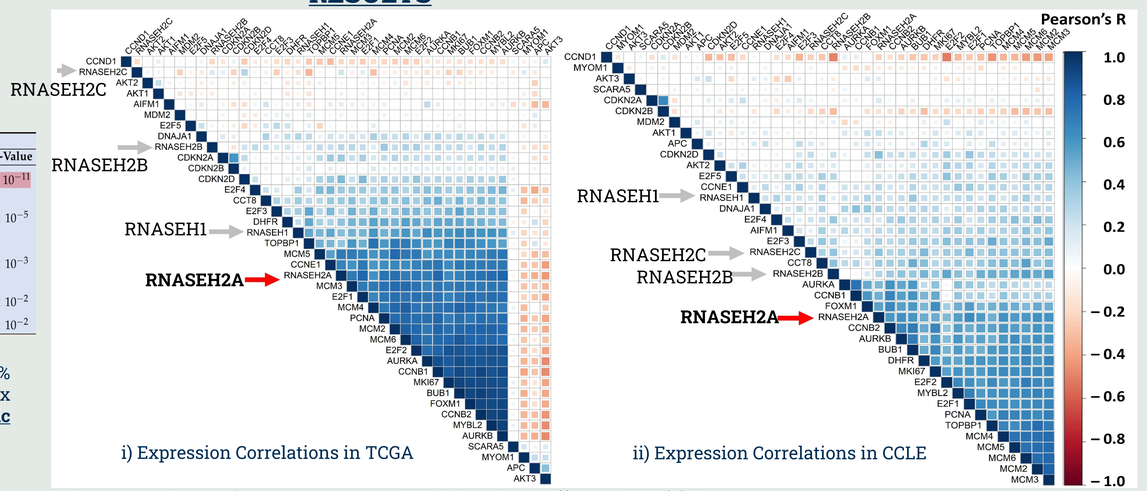
Prevalence for each CNA type was calculated in patients from each cancer subtype

RESULTS

1.a. RNASEH2A gene expression is seen to be **highest in Testis (~50 TPM)**, which is more than 2X of RNASEH2A expression in any other human tissue in GTEx dataset

Process			
GO Term	Description	*p*-Value	FDR *q*-Value
GO 1903047	mitotic cell cycle process	2.80×10^{-14}	5.93×10^{-11}
GO 0070507	regulation of microtubule cytoskeleton organ	1.39×10^{-7}	8.40×10^{-5}
GO 0006974	cellular response to DNA damage stimulus	2.77×10^{-5}	5.86×10^{-3}
GO 0007059	chromosome segregation	9.00×10^{-5}	1.36×10^{-2}
GO 0006260	DNA replication	2.66×10^{-4}	3.12×10^{-2}

1.b. Gene Ontology(GO) term analysis of top 2% co-expressed genes with RNASEH2A in GTEx dataset reveals **possible involvement in Mitotic Cell Cycle Process**



i) Expression Correlations in TCGA ii) Expression Correlations in CCLE

2. Correlation Plots of selected genes in TCGA Pan Cancer(i) and CCLE(ii) Datasets with hierarchical clustering to reveal **close association of RNASEH2A (in red)** (as opposed to RNASEH2B, RNASEH2C and RNASEH1) **with cancer Proliferation and G2 and M Cell cycle markers**

Cancer Subtype(s)	Prevalence of Copy Number Alterations(CNAs) of RNASEH2A gene					Average RNASEH2A mRNA expression each CNA group, RSEM				
	Deep Deletion	Shallow Deletion	Diploid	Gain	Amplification	Deep Deletion	Shallow Deletion	Diploid	Gain	Amplification
Ovarian Epithelial Tumor	0.34%	29.15%	26.10%	36.27%	8.14%	649.61	730.15	1150.31	1444.92	2435.81
Endometrial Carcinoma		12.13%	69.67%	14.90%	3.29%		838.32	912.12	1721.50	2087.09
Adrenocortical Carcinoma		1.32%	34.21%	61.84%	2.63%		707.14	480.63	795.96	1114.81
Pleural Mesothelioma		8.05%	73.56%	16.09%	2.30%		631.88	534.82	975.79	1203.17
Esophageal Squamous Cell Carcinoma		34.04%	44.68%	19.15%	2.13%		775.80	697.66	974.64	2097.29
Cervical Squamous Cell Carcinoma	0.41%	26.23%	57.79%	13.52%	2.05%	1700.49	1289.19	1556.32	2079.49	7219.93
Diffuse Glioma	0.20%	3.33%	73.92%	20.78%	1.76%	205.72	413.52	351.50	461.54	404.56
Sarcoma		10.36%	50.20%	37.85%	1.59%		812.55	808.58	1356.59	1326.82
Invasive Breast Carcinoma	0.09%	21.25%	60.11%	17.23%	1.31%	384.40	618.31	615.99	940.40	1485.75
Ocular Melanoma		3.75%	92.50%	2.50%	1.25%		731.66	617.38	1234.06	798.60

3. Prevalence and average mRNA expression in patient samples with different Copy number variations for RNASEH2A gene in cancer subtypes. Average expression increases in patients found with amplified copy of RNASEH2A gene in comparison to average expression of patients with diploid RNASEH2A gene subtype. **Prevalence of Amplification of RNASEH2A gene copy is seen to be higher than prevalence of Deep Deletion in many cancer subtypes.**

CONCLUSION

1. GO analysis of RNASEH2A highly co-expressed genes in human tissues and correlation analysis in CCLE and TCGA concludes **RNASEH2A expression association with mitotic cell cycle regulation**
2. High positive correlation of RNASEH2A with markers unregulated in cancer and cancer proliferation markers suggests **RNASEH2A plays a role in cancer proliferation**
3. Higher prevalence of RNASEH2A amplification vs Deep Deletion in various cancer subtypes support and validate **high level of RNASEH2A and being associated with poor prognosis in multiple cancer types.**

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