ARE-mediated regulatory system in glioma

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Introduction:

Recently among the mechanisms involved into tumor biology a role of posttranscriptional regulation through ARE-mediated mRNA decay has been recognized [Schoenberg, Maquat, 2012]. ARE stands for Adenine Uridine Rich Elements and designates a type of a nucleotide sequence present in 3' UTRs of some genes. Approximately 8% of all mammalian mRNAs contain ARE [Gruber et al., 2011], noteworthy, those transcripts encode proteins of particular functional groups that are tightly involved into tumorigenesis: growth factors and cell cycle controlling proteins, mediators of inflammation and metabolism [Schoenberg, Maquat, 2012]. ARE sequence is used by ARE-binding proteins that either protect mRNAs from decay (stabilize mRNA) or just the other way round enhance the degradation of the molecules. Several decay-controlling factors have been identified by far: HUR, TTP, KSRP, AUF1, BRF1 and BRF2 [Schoenberg, Maquat, 2012]. A number of studies demonstrate that those factors might be of high relevance to cancer development and represent promising targets for design of anti-cancer drugs with systemic effect.

In spite of intensive studies, glioma remains among malignant tumors with the poorest prognosis: the most abundant form of the pathology – glioblastoma is characterized with a median survival of only 0.5 year [Gravendeel et al., 2009]. A few studies have demonstrated that ARE-mediated post-transcriptional control might play a significant role in glioma biology [Hitti et al., 2016; Fillipova et al., 2012]. However ARE-mediated control system has not been investigated on the whole transcriptome level by far. In the present study, we use a large publicly available dataset with whole transcriptome expression levels to study the behavior of ARE-binding factors and ARE-containing genes in glioma. Our results reveal complex regulatory interactions among ARE-binding factors in glioma and indicate that at the level of transcriptome ARE-containing genes form clusters with distinguished biological functions relevant to tumor biology. Overall, our data point to importance of ARE-mediated post-transcriptional regulation in glioma.

Materials and methods

Dataset retrieval and processing

The microarray dataset GSE16011 was downloaded from GEO database (www.geo.com). The dataset contained data on the expression values for the majority of genes in 278 samples of gliomas of different histological types and 8 control healthy brain tissue samples [Gravendeel et al., 2009]. The selection criteria for this dataset included 1) dataset size; 2) presence of data on healthy controls; 3) in vitro support of the assessments previously made by meta-analysis of the microarray data [Ivliev et al., 2011; Ivliev et al., 2012].

Lists of ARE-binding factors and ARE-containing mRNAs

The list of six ARE-binding proteins regulating mRNA turnover was retrieved from the publications [Schoenberg, Macquat, 2012; Anderson, 2009] and included: HUR, TTP, KSRP, AUF1, BRF1 and BRF2. List of ARE-containing mRNAs was built using database ARED (http://brp.kfshrc.edu.sa/AredOrg/index.jsp) and included 2984 genes.

Analyses of differential gene expression and correlation

Differential expression was performed using the Mann–Whitney U test. For correlation analysis Spearman's rank correlation coefficient was used.

Coexpression analysis (WGCNA)

Weighted correlation network analysis (WGCNA) was performed using instructions and recommendations from: (labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/). List of ARE-containing mRNAs was used for network construction.

Functional annotation of the modules

Functional annotation of the modules was performed on the basis of analysis of their gene composition. DAVID (https://david.ncifcrf.gov/) was used to test each module for enrichment in genes with particular GO, SwissProt, and InterPro terms compared with the background list of all genes on the array.

Results:

Glioma transcriptome is enriched with ARE-containing differentially expressed genes

Analysis of differential expression revealed that approximately a half (1485) out of 2984 ARE-containing genes were significantly altered in gliomas (were DEGs) (p-value < 2.2e-16). This indicates that glioma transcriptome is significantly enriched in ARE-containing genes.

TTP but not HuR is upregulated in gliomas

Previous data revealed that HUR overexpression at the expense of TTP activity in glioma might be responsible for extensive stabilization of genes that promote tumor development

[Fillipova et al., 2011]. Thus, we addressed the question of whether or not HUR upregulation and TTP downregulation might be traced on the transcriptome level. Surprisingly, our data revealed that HUR is not altered, while TTP is significantly upregulated in tumor tissues. This result indicates that TTP might be tightly connected to glioma transcriptome alteration.

ARE-binding factors antagonistically control similar sets of genes

In order to further investigate possible involvement of ARE-binding factors into glioma transcriptome regulation we analyzed genes that have the expression profiles that significantly correlate to the profiles revealed for HUR, TTP, KSRP, AUF1, BRF1 and BRF2. Functional annotation revealed that gene groups correlating to each of the ARE-binding factors were enriched with genes, involved into innate immune response and RNA-binding. These data was clearly relevant to multiple published results [reviewed in: Anderson, 2009] and indicate that the factors act a single system. Noteworthy, there was a surprising phenomenon about the observed correlation: genes that were positively correlated to TTP (TTP+ genes) were negatively correlated with the rest of ARE-binding factors and vice versa. Moreover, TTP+ genes were enriched with immune response related genes, while TTP- genes contained RNA-binding factors. Together these data reveal antagonistic manner of regulatory system that involves TTP and the rest ARE-binding factors and point to a possible connection between TTP overexpression and dysregulation of immune response within brain tumors.

WGCNA identifies modules with biological functions relevant to tumor biology

Overall 13 modules were retrieved upon WGCNA analysis. Module annotation using DAVID revealed that they were characterized with transparent biological functions: transcription, cell junction formation and immune response. This data indicate that ARE-containing genes form clusters with distinguished biological functions relevant to tumor biology in glioma.

Discussion

In the present study we have performed the analysis of ARE-containing gene expression alteration and functional clustering and analysed behaviour of ARE-binding genes. Our data revealed that ARE-containing genes form substantial portion of glioma transcriptome and are significantly altered in tumour tissues comparing to healthy brain tissues. Moreover, weighted correlation network analysis demonstrated that ARE-containing genes constructed a network that comprised gene modules with transparent biological functions. The processes that are regulated involving ARE-containing genes include transcription, cell junction formation and innate immune response.

Another direction of our analysis related to ARE-binding factors. On contrary to literature data we failed to observe HUR upregulation, but observed TTP overexpression. Analysis of

correlations between ARE-binding factors and other genes demonstrated an interesting pattern of antagonistic interplay between TTP and other ARE-binding factors. Interestingly, a recent publication [Hitti et al., 2016] has also indications on TTP/HUR antagonistic relations revealed on the level of transcription.

Altogether, our data demonstrate that ARE-mediated post-transcriptional regulatory system is involved into glioma biology and ARE-binding factors might represent promising targets for development of novel effective anti-cancer therapeutics.

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