

Assessment of Post-Radiation Time Effect on Gene Expression Profiles of *Saccharomyces cerevisiae* Samples After Applying a UV Laser



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Abstract

Introduction: Widespread application of lasers in different fields of medicine implies more investigations into the molecular mechanism of laser effects on the human body. Network analysis of the dysregulated genes of *Saccharomyces cerevisiae* samples are irradiated by a UV laser and harvested 30 minutes after radiation compared with a 15-minute group is the aim of this research.

Methods: The significant dysregulated genes interacted via the STRING database, and the central nodes were determined by "Networkanalyzer" application of Cytoscape software. The critical genes and the related biological terms were identified via action map analysis and gene ontology assessment.

Results: The gene expression profiles of the samples with 30-minute post-radiation time were different from the samples with 15 minutes of post-radiation time. 9 potent central genes, 50% of which were similar to the nodes of the 15-minute group, were identified. The terms "positive regulation of telomere maintenance" were targeted in the two sample groups.

Conclusion: In spite of large alteration in the gene expression profiles of the samples, the results indicated that the main affected biological term for the 15-minute and 30-minute groups was similar.

Keywords: UV laser; Telomere; Network analysis; Post-radiation time; *Saccharomyces cerevisiae*.

Introduction

Widespread applications of lasers in medical fields have encouraged researchers to find the exact molecular mechanism of laser effects on the human body to identify the advantages and disadvantages of a laser as a therapeutic tool. On the other hand, lasers can be applied in other fields such as non-medical applications. Experiments indicate that most effects of lasers on the body can be considered beneficial, but they may be accompanied by side effects. The optimization of laser instruments and also the protocols of laser application are the prominent aims of researchers.¹⁻³

Gene expression profile analysis is a suitable method for the discovery of the molecular mechanism. The effects of different types of lasers on biological samples are investigated via network analysis, and valuable information is provided.⁴ In such analysis, gene expression alteration after intervention is assessed to find

targeted genes.⁵ Based on the applied method, it can lead to finding huge numbers of dysregulated genes. One of the best methods to analyze the data is network analysis.⁶ In this approach, the determined affected genes interact to form a network. Since the network is usually a scale-free network, there are few genes among all queried genes that play a critical role in constructing the network. These genes are known as central genes.⁷ Several centrality parameters are used to identify the central genes. Degree, betweenness centrality, closeness centrality, and stress are the four important centrality parameters that are considered to determine the central genes.^{8,9} The genes that are characterized by top values of more central parameters are the crucial genes which are highlighted as the basic elements of a network. Hub-bottleneck elements of the network are the individuals characterized by the values of degree and betweenness centrality. The central nodes of a network are the genes that are related to the

important dysregulated biological terms.¹⁰ It should be considered that the central genes which are categorized by high amounts of fold change play an important role in determining the dysregulated biological terms such as biological processes or biochemical pathways.^{11,12}

Action map analysis is the other valuable method for exploring a regulatory relationship between the queried genes. In this approach, some chemical properties of the assessed genes can be determined via directed links between the genes. Activation and inhibition are the two important actions that are accessible via action map analysis.¹³ In the present study, the effect of UV laser radiation on the gene expression profile of *Saccharomyces cerevisiae* samples was studied via network analysis to find the role of long post-radiation time (30 minutes versus 15 minutes).

Materials and Methods

Melinda Hauser et al published a research result about 30s UV laser radiation on *S. cerevisiae* samples. The irradiated samples were harvested 15, 30, and 60 minutes after radiation.¹⁴ In the present study, the data of the samples which were harvested 30 minutes after laser exposure was selected to analyze via network analysis. The gene expression profiles of the samples were compared with the controls, and the related Differentially expressed Genes (DEGs) were identified. Like the previous analysis (unpublished data submitted to journals), the significant DEGs were determined considering P value < 0.05 and ratio change > 2 . The results were compared with the data from the samples that were harvested 15 min after radiation to find the effect of the delay on harvesting. The significant DEGs were included in a network via the “protein query” of the STRING database and Cytoscape software. The constructed network was analyzed by “NetworkAnalyzer” application of Cytoscape.¹⁵ The hubs based on the degree value cut-off (mean+ 2SD) were introduced. The top 5% of the nodes based on betweenness centrality, closeness centrality, and stress were selected for more analysis.¹⁶ The hub-bottlenecks which were included in the other central nodes were identified as potent hub-bottleneck nodes. The finding was equated with the results of the samples in the 15-minute group. The potent hub-bottleneck nodes interacted by using CluePedia¹⁷ to find and explore the crucial individuals and regulatory properties of them. The results were discussed and interpreted considering the finding from the samples of the 15-minute group.

Results

A total of 329 DEGs were determined as significant and characterized individuals and were imported to the “protein query” of the STRING database via Cytoscape software. The distribution of gene expression fold changes as a function of the number of the significant DEGs for the two groups of samples (15-min and 30-min groups) are presented in Figure 1. However, parts of the

two curves overlap, and gross differential parts appear. Among the 329 queried DEGs, 326 genes were recognized by the STRING database. The network includes 28 isolated nodes and a main connected component which was formed from 298 nodes and 2379 edges. Topological analysis revealed that the degree value cut-off was 44 for determining the hub nodes. 15 hubs were identified. More analysis led to the introduction of 9 hub-bottleneck nodes. Centrality analysis showed that all hub-bottleneck nodes were central nodes based on closeness centrality and stress. The 9 key dysregulated genes and their centrality properties are shown in Table 1. Two actions including activation and inhibition were determined for the 9 potent central DEGs. As it is depicted in Figure 2, RPN11 and HSP82 activate UBI4 respectively. The other

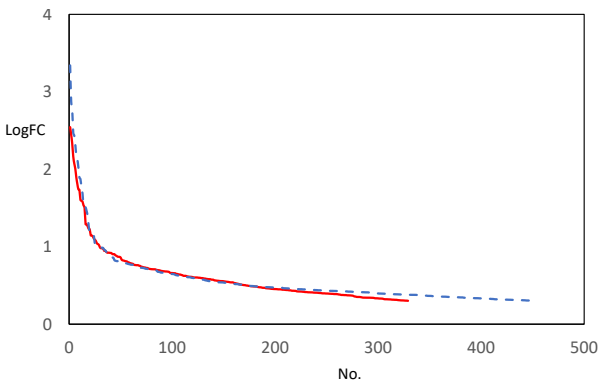


Figure 1. The Distribution of Fold Changes for the Significant DEGs. The dashed line and the solid line refer to 15-minute and 30-minute groups respectively.

Table 1. The 9 Key Central DEGs of the Network or the 30-Minute Group of the Samples

No.	Display Name	Degree	BC	CC	Stress
1	TFS1	65	0.246	0.48	37702
2	RPN11	63	0.754	0.46	46994
3	PRX1	61	0.350	0.48	42662
4	NQM1	59	0.175	0.47	33670
5	GAD1	58	0.088	0.45	26652
6	UBI4	53	0.368	0.45	38550
7	GLK1	48	0.000	0.46	21836
8	SOD1	46	1.000	0.48	74490
9	HSP82	46	0.158	0.45	33452

BC, betweenness centrality; CC, closeness centrality.

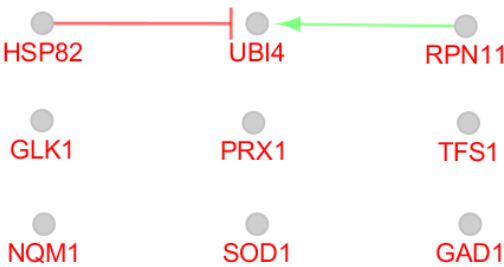


Figure 2. Activation and Inhibition Relationships Between the 9 Potent Central Nodes. The green and red arrows refer to activation and inhibition actions respectively.

central nodes remain isolated.

Discussion

Producing large amounts of data is a property of high throughput methods.¹⁸ In such experiments, interpretation of findings is an important feature of analysis. In the present study, among a large number of significant DEGs, 9 individuals were highlighted as the critical targets of the laser. Our previous analysis (unpublished data) revealed that network analysis of gene expression profiles of samples, which were harvested 15 minutes after irradiation, led to the introduction of 11 central DEGs. As it is depicted in Figure 1, the long duration of post-radiation time affects the number of significant DEGs and also the range of fold change. 329 significant DEGs were determined for analysis of 30-minute samples, while 452 significant DEGs were identified for the 15-minute group. Increasing 15-minute for post-radiation resting led to the reduction of 123 significant DEGs. The maximum amount of fold change for 15-minute and 30-minute groups were 2193 and 350 respectively. A reduction in significant DEGs and maximum fold change of the 30-minute group relative to the 15-minute group can be interpreted as the activation of the repair system in the exposed cells. Corresponding to the reduction of DEGs and the maximum value of fold change for the 30-minute group relative to the 15-minute group, a decrease in the central nodes from 11 to 9 is a logical process.

More analysis showed that among 9 central nodes of the 30-minute group network, 56% of nodes, including RPN11, PRX1, UBI4, GLK1, and HSP82, were common with the 11 central nodes of the 15-minute group network. It can be concluded that the long duration of post-radiation time not only changes the number of significant DEGs and the maximum value of fold change but also alters the number and combination of the central nodes of the related networks.

RPN11 is a 26S proteasome regulatory subunit¹⁹; this metalloisopeptidase hydrolyzes the ubiquitin molecule which is transported to the proteasome for degradation.²⁰ Investigations have revealed that UBI4 deletion leads to stopped accumulation of ubiquitin, cell tolerance reduction to some stresses such as oxidative, osmotic, cell wall perturbing and heat-shock stresses. Other dysregulated processes such as decreased transcriptional levels of antioxidant genes expression is also reported.²¹

The regulatory relationship between the central nodes showed that RPN11, UBI4, HSC82, and HSP82 were the core of the targeted genes in the 15min group. Enriched analysis revealed that “positive regulation of telomere maintenance” was related to these four elements of the described core. Considering alteration of gene expression profiles and also topological properties of the analyzed networks for the two 15-minute and 30-minute groups, regulatory pattern between the critical genes and also the

related biological terms may change for the two groups. As it is shown in Figure 2, RPN11, UBI4, and HSP82 are the elements of the core of regulatory DEGs related to the 30-minute group. It is an amazing finding that like the core of the regulatory subnetwork of the 15-minute group, the core is repeated for the 30-minute group; however, HSC82 is missed. The common biological term related to the two cores of 15-minute and 30-minute groups is “positive regulation of telomere maintenance”. The role of HSP82 in the inhibition of the assembly of the unextendable telomere structure has been reported by DeZwaan et al.²²

In the 30-minute group, UBI4 was activated by RPN11 and inhibited by HSP82. It seems that like the 15-minute group, the critically affected gene in the 30-minute group is UBI4. UBI4 was up-regulated in the 15-minute group 3.5 times. It was expected that the rate of UBI4 up-regulation would be reduced relative to the amount of its fold change in the 15min group, but the fold changes of UBI4 in 15-minute and 30-minute groups were 3.9 and 8.1 respectively. The fold changes of up-regulated RPN11 which activated UBI4 in the two groups were 2.24 and 2.75 for 15-minute and 30-minute groups respectively. HSP82 that inhibited UBI4 was up-regulated in the two groups about 7 times. It seems that the hyper-activation of UBI4 is almost similar in the two groups of samples. It is reported that the overexpression of UBI4 may assist as a pointer for stress which implies enhanced activity of the ubiquitin-proteasome system.²³

Conclusion

It can be concluded that post-radiation times of 15 min and 30 min have different gene expression profiles; however, the critical target genes are similar. It seems that “positive regulation of telomere maintenance” is supported by a cellular response to UV laser radiation. This conclusion may be real that laser application may slow the aging process for a long period of time. However, more pieces of evidence are required. In addition, it should be considered that some effects of laser radiation undergo the repair process and may be deleted from biological media.

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Conflicts of Interest

The authors declare they have no conflicts of interest.

Ethical Considerations

Not applicable.

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