Effect of UV Laser Radiation on “Positive Regulation of Telomere Maintenance” in *Saccharomyces cerevisiae*

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Received: October 17, 2021

Accepted: December 21, 2021

Published online: December 28, 2021

Abstract

Introduction: Excessive exposure to ultraviolet (UV) radiation may cause a variety of skin cancers and damage to the eye lens. The assessment of different aspects of UV damage has attracted researchers' interest. UV radiation to simple biological models such as *Saccharomyces cerevisiae* of yeast family could help to find out different molecular changes resulting from radiation. The assessment and network analysis of gene expression data about yeast cells radiated by the UV laser was the aim of this study.

Methods: The gene expression profiles of *S. cerevisiae* samples in the presence of the UV laser at 30 seconds radiation and 15 minutes' post-radiation time are compared with the control profiles. The significant expressed genes interacted and the central nodes and related biological terms were identified.

Results: The main connected component of the network including 427 nodes was analyzed and 11 central differentially expressed genes (DEGs) were determined. RPN11, UBI4, HSP82, and HSC82 as critical DEGs and “positive regulation of telomere maintenance” as a related biological term were introduced.

Conclusion: The finding has provided a new perspective on laser application in the rejuvenation process. It seems that the laser can be used as a suitable agent against the aging process which is a limiting factor in human life.

Keywords: UV laser; Telomere; *Saccharomyces cerevisiae*; Gene; Data analysis.

Introduction

Ultraviolet (UV) ray molecular damage to skin cells may lead to various complications. UV can be divided into three types: A, B and C. The third type with a wavelength of 100 to 280 nm is absorbed by the Earth's atmosphere ozone. But type A with a wavelength of 430 to 400 nm and 10% of type B with a wavelength of 280 to 320 nm could reach the earth's surface. In environmental abundance, UV rays can cause various complications in the human body, such as sunburn¹ and various skin cancers.² Damage to the cornea of the eye is also one of these complications.³ The direct absorption of UV energy by cells leads to damage to DNA and proteins and indirectly to the

formation of free radicals.⁴ UV radiation with a short wavelength is carcinogenic (280-315 nm) and has been the focus of most researches. However, it should be noted that long-wavelength UV radiation can also cause cell damage. Short-wavelength UV causes damage to DNA, while long-wavelength radiation acts indirectly, and the produced free oxygen radicals from the reactions could be absorbed by other cell molecules.⁵ Molecular functions of UV lead to various skin carcinomas and are still unclear as it is a mutagen and has the properties of a tumor initiator and a tumor promoter.¹ However, the benefits of UV by mediating the natural synthesis of vitamin D and endorphins in skin are inevitable.⁶ Atrophy, pigmentation

changes, wrinkling and malignancy are among the profound health risks carried by excessive exposure to UV.⁷ The three most common types of skin cancer; basal cell carcinoma, squamous cell carcinoma, and malignant melanoma are epidemiologically linked to UV radiation and molecular mechanism of these disorders is not clear properly.⁸ Such genetic factors as polymorphism of the Melanocortin 1 receptor (MCR1) gene could influence the risk of UV mediated skin disease. This gene is involved in UV sensitivity and skin cancer.⁹ MCR1 found on the surface of melanocytes could bind to α -melanocyte stimulating hormone (MSH) and transmit differentiation into the cell through the activation of adenylyl cyclase and cAMP production.^{10, 11} The risk of skin cancer is influenced by UV exposure and skin pigmentation.¹² Malignant melanoma of skin arising from epidermal melanocytes is a metastatic-prone malignancy.¹³ Two major non-melanomatous skin cancers are basal cell carcinomas and squamous cell carcinomas, both derived from keratinocytes. UV mutations in cancer-relevant genes such as p53 tumor suppressor in squamous cell carcinoma are well characterized.¹⁴ The exome analysis of melanomas has revealed that UV radiation has a direct mutagenic role in the pathogenesis of melanoma.¹⁵ UV-induced DNA mutations are causative factors of melanoma.¹⁶ Mutations caused by UV lead to ROS generation, such as superoxide anion, hydrogen peroxide and the hydroxyl radical.¹⁷ Nucleotides are susceptible to free radical injury, and the oxidation of nucleotide bases causes mutagenesis.¹⁸ UV radiation induces abnormality in transcription, DNA replication, and base pairing.¹⁹ UV-induced photoproducts and other bulky DNA lesions could be repaired by nucleotide excision repair as an evolutionary mechanism.²⁰

Saccharomyces cerevisiae is one of the simple biological models for studying the genome and how UV rays affect biological molecules. Facile genetic manipulation and the large degree of homology between genes in yeast and humans make it useful,²¹ and about 30% of known genes involved in human disease have yeast orthologues.²² Many yeast genes involved in damage response checkpoints and DNA repair by UV irradiation have human orthologues and their mutation induces cancer sensitivity.²³

Network analysis is a useful method for screening the dysregulated genes based on properties of the studied genes in interaction with the other elements of the analyzed network.²⁴ A network contains nodes connected to each other via edges and there are few nodes playing a critical role in network construction named central nodes.²⁵ Analyzing the network interactions between proteins and genes modified by UV in yeast has made us develop the relationship between proteins and genes important in this regard.

In the present study, the gene expression profiles of *S. cerevisiae* cells which are treated by the UV laser were

compared with the controls. Significantly expressed genes were determined to construct a network, and critical elements of the network such as hubs and bottlenecks were introduced to analyze interactions between key genes to find the prominent effects of UV on *S. cerevisiae* genes.

Materials and Methods

Original data were extracted from the reported document by Hauser et al.²⁶ As it is published by this research group, the gene expression profiles of *S. cerevisiae* samples in the presence of the UV laser at 30 seconds and 15 minutes after irradiation harvesting were compared with control profiles. The full details of the methods are described in the published data by the authors. The data were screened based on *P* value <0.05 and ratio change >2. The characterized genes were included in an interactome by the “protein query” of the STRING database via Cytoscape software. The network was analyzed by “NetworkAnalyzer” application of Cytoscape. Mean + 2SD was considered as the cut-off value of the degree to identify hub nodes. Five percent of top nodes based on betweenness centrality were determined as bottlenecks. Common hubs and bottlenecks were selected as hub-bottleneck nodes. The other group of nodes (top 5% nodes based on closeness centrality and stress) was identified. Common genes between the two central groups and the hub-bottleneck nodes were determined as critical DEGs.

The connection between the critical DEGs was formed via STRING, and a sub-network was constructed. The critical nodes were screened via action map analysis. The nodes interacted by directional connections which were corresponded to activation, inhibition, and expression actions via CluePedia application of Cytoscape. The key critical DEGs were enriched for biological terms by using ClueGO plugin of Cytoscape.

Results

A total of 452 differentially expressed genes (DEGs) were screened among the reported genes. Since 4 proteins were not identified in data banks, 448 individuals were a candidate for more investigations. The network including 19 isolated genes, 1 paired node, and 427 interacted individuals by 3617 edges was constructed via undirected connections. Degree value 47 was determined as the cut-off value to determine the hub nodes. Based on degree value 47, 19 top nodes were identified as hubs (see Table 1). Analysis revealed that 11 DEGs among the determined hubs were hub-bottleneck nodes. More investigation indicated that all hub-bottlenecks were presented in the top nodes which were characterized based on closeness centrality and stress values. The binding property of the critical DEGs was investigated via network analysis. Connections between the hub-bottleneck nodes are illustrated in Figure 1. As it is depicted in Figure 1, HSP104

is connected to the 10 first neighbors, while ZWF1 and GLK1 are linked to 5 critical DEGs.

The action map including activation, inhibition, and expression is shown in Figure 2. As shown in Figure 2, there is no expression relationship between the critical DEGs and 4 DEGs including RPN11, UBI4, HSP82, and HSC82 (as the key critical DEGs) that are connected by inhibition and activation links.

One class (positive regulation of telomere maintenance)

Table 1. Hub Nodes of the Network and the Related Centrality Parameters Including Degree, Betweenness Centrality (BC), Closeness Centrality (CC), and Stress

No.	Display name	Degree	BC	CC	Stress
1	HSP104	79	0.0238	0.4754	86408
2	HSP82	76	0.0335	0.4671	93394
3	HSP42	75	0.0376	0.4723	86366
4	RPN11	73	0.0437	0.4566	97224
5	HSC82	72	0.0300	0.4620	85568
6	CDC48	63	0.0352	0.4465	76188
7	UBI4	61	0.0197	0.4401	46348
8	PRX1	60	0.0285	0.4595	71656
9	GLK1	60	0.0312	0.4532	68380
10	HSP78	57	0.0122	0.4503	42096
11	HSP26	55	0.0122	0.4522	39472
12	TPS1	55	0.0141	0.4508	41198
13	PNC1	53	0.0134	0.4470	31464
14	DCS2	53	0.0088	0.4320	26994
15	RTC3	51	0.0117	0.4286	28452
16	ZWF1	49	0.0248	0.4465	58312
17	ATG8	48	0.0280	0.4378	59254
18	RPN10	48	0.0081	0.4247	25578
19	GLC3	47	0.0111	0.4316	24950

Note: The nodes of No. 1-9 and 16-17 are hub-bottleneck nodes.

Figure 1. A Sub-network Including the Hub-Bottleneck Nodes. The bigger size and blue color refer to higher value of degree.

including 4 biological terms (“positive regulation of telomere maintenance”, “positive regulation of telomere maintenance via telomerase”, “positive regulation of telomere maintenance via telomere lengthening”, and “positive regulation of DNA biosynthetic process” are related to the key critical DEGs (see Figure 3).

Discussion

The centrality analysis of PPIs usually leads to the introduction of a few key genes which play critical roles in network construction and also managing the altered biological processes under the studied condition. In the present study, 11 critical DEGs were identified in *S. cerevisiae* samples which were irradiated 30s and harvested after 15 min. of laser radiation. Action map analysis results highlighted RPN11, UBI4, HSC82, and HSP82 as the crucial targets of laser radiation; however, UBI4 was determined as prominent DEGs. Based on the reported data, UBI4 was upregulated with a fold change of 3.9. As it is shown in Figure 2, RPN11 activated UBI4, while HSC82 and HSP82 inhibited UBI4. RPN11 was upregulated 2.24

Figure 2. Action Map for the 11 Critical DEGs. Green and red arrows refer to activation and inhibition respectively.

Figure 3. Biological terms Related to the Key Critical DEGs. The label of the term by which the class is characterized is colored.

times versus HSC82 that was downregulated 2.3 times. HSP82, the inhibitor of UBI4, was upregulated about 7 times. It can be concluded that the overall upregulation and downregulation of activators and inhibitors lead to hyper-activation of UBI4 which is upregulated. This calculation is an estimation of the occurred processes. This finding indicates that upregulation and hyper-activation of UBI4 is the prominent advantage of laser radiation in the applied condition. Since “positive regulation of telomere maintenance” is the main biological term that is related to these 4 DEGs, it seems that telomere is an important target of the laser in the irradiated samples.

Telomeres are nucleoprotein structures on the chromosome end. Telomeres provide protection chromosome ends from enzymatic degradation and maintain chromosomal and genomic stability. Loss of telomere function may cause genetic instability and cancer progression. Chromosomal stability is the character of normal cells, while cancer cells have frequent chromosomal changes.²⁷ Loss of a telomere results in chromosomal end-to-end fusion, chromosomal rearrangements and instability.²⁸ The use of yeast as a simple model for the study of ultraviolet light on the structure of telomeres in this study showed a change in the expression of some genes in the genomic profile of yeast. Our results demonstrated that UBI4 expression was affected by RPN11 & HSC82 and HSP82. UBI4 or polyubiquitin gene of *S. cerevisiae* is a modulator of life span, and UBI4 is induced in response to DNA damaging agents and heat shock. UBI4 is also expressed in higher levels in cells growing by respiration.²⁹ UBI4 gene deficiency leads to early induction of apoptosis and shortened replicative lifespan in yeast.³⁰ Zhao et al believe that the overexpression of UBI4 in yeast will increase resistance to toxin and the overexpression of UBI4 is associated with increased ubiquitin and ubiquitinated proteins.²⁹ RPN11 could upregulate UBI4 according to our results. RPN11 is served as the essential deubiquitinase of the proteasome. On the other hand, HSC82 and HSP82 genes inhibit UBI4 based on our data. However, the overexpression of HSC82 and HSP82 resulted in telomere shortening in wild-type cells of yeast.³¹ Figure 3 demonstrates the relationship between our key genes and telomere maintenance with positive regulation according to ClueGO results. Our data suggest the connections between telomere survival and the effect of the UV laser on behalf of 4 key genes, namely RPN11, UBI4, HSC82, and HSP82, targeted as regulators.

Conclusion

The finding indicates that “positive regulation of telomere maintenance” is related to RPN11, UBI4, HSC82, and HSP82. This term is affected by 30s UV-laser radiation on the *S. cerevisiae* samples which are harvested 15 min. after exposure. This result offers a new perspective on laser application in the rejuvenation process. It seems that laser

potential in the promotion of life quality is higher than that of the usual aspects of its application in the present time of human life. It may be a suitable candidate against the aging process which is a limiting factor in human life. However, the possible side effects of laser application in human life should be investigated continuously.

Acknowledgment

Shahid Beheshti University of Medical Sciences supported this research.

Conflicts of Interests

The authors declare they have no conflicts of interest.

Ethical Considerations

Not applicable.

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