

The Principal Role of Several Members of HLA and IRF Genes in Prevention of Oral Mucositis After Chemoradiotherapy



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Received: May 14, 2021

Accepted: July 20, 2021

Published online October 20,
2021



Abstract

Introduction: One of the chemoradiotherapy (CRT) side effects is oral mucositis (OM). Since photobiomodulation therapy (PBMT) is a public method for the repair process, in the present study the mechanism of PBMT in the prevention of OM is investigated via the bioinformatics approach.

Methods: Six validated and significant differentially expressed genes (DEGs) associated with the prevention of OM in head and neck cancer (HNC) patients who had experienced CRT were extracted from the literature. After adding 50 neighbors from STRING, the network was constructed and analyzed. The results of the action map and network analysis were compared and discussed.

Results: HLA gene family were identified as central nodes of the analyzed network. Based on action map finding, activation is prominent action and IRF9 was the potent activator. The role of the IRF gene family was highlighted by action map analysis.

Conclusion: Regulation of the immune system by HLA and IRF genes family is a crucial factor in the prevention of OM in the studied patients.

Keywords: Laser; Gene expression; IRF; HLA; Oral mucositis.

Introduction

Radiotherapy is a public therapeutic method in the treatment of cancers, which usually plays a significant role in the treatment of cancers in combination with chemotherapy.¹ Reducing the side effects of radiotherapy that concern the life quality of the treated patients has attracted experts' attention in the treatment of patients.^{2,3} One of the side effects of radiotherapy on patients with head and neck cancer (HNC) is oral mucositis (OM).⁴ As it is pointed out, OM, besides the increment of treatment expense, causes significant morbidity and considerable delay in the treatment plan.⁵

Photobiomodulation therapy (PBMT) as a useful method in the treatment of various diseases is a public method in medicine.⁶ There are investigations about the molecular mechanism of PBMT that are concerned

with the molecular method by using the high throughput methods.⁷ Bioinformatics is a field that is tied to proteomics and genomics to solve complications which are associated with the interpretation of data about the molecular mechanism of various kinds of investigated biological systems.^{8,9} Network analysis as a bioinformatic technique is an interesting approach that is used to analyze the complex data about many studied diseases and interventions.^{10,11}

In the network analysis method, the set of genes, proteins, or metabolites are included in an interactome to form a network. Each element of the studied network has a unique characteristic feature relative to the other elements. Centrality parameters are different from the elements of the constructed network. In the scale-free networks, there are a few nodes that can be considered as

central nodes. The central nodes are the critical elements of the studied network.¹²⁻¹⁴ In the present study, DEGs which appeared in the mucosa of patients with HNC, who had received RCT due to laser irradiation (PBMT), were derived from the published data by Heliton et al¹⁵ and analyzed via network analysis to find the critical dysregulated genes.

Methods

The data is driven from the published article by Heliton et al.¹⁵ In the original study, 27 patients that were treated via chemoradiotherapy (CRT) for head and neck squamous cell carcinoma (HNSCC) in stages (III–IV) were included in the research. The patients were divided into two groups; laser group (LG) and placebo group (PG). The LG was treated by diode laser PhotonLase III[®] (DMC, São Carlos, São Paulo, Brazil) indium phosphide, gallium, and aluminum (InGaAlP), $\lambda 660$ nm, 100 mW, 4 J/cm², 1J with 0.24 cm² area, emitting continuous light with a Gaussian profile before CRT, and the PG was treated in the similar method without receiving a laser. Patients' oral mucosa was processed for RNA extraction. The cDNA microarray was performed for gene expression analysis. The finding was validated by the qPCR technique. The genes that were differentially expressed in one of the groups and were not DEGs in the other group were considered as the validated DEGs. More detail of the procedure is described in the published document by Heliton et al.¹⁵ The validated differentially expressed genes (DEGs) were included in the STRING "protein query". The relationship between the significant DEGs was identified by Cytoscape software.

Fifty first neighbors from the STRING database were added to the query DEGs and the protein-protein network was constructed by using Cytoscape. The network was analyzed and the centrality parameters of the node were determined. An action map was provided for the query DEGs and the 50 first neighbors by the CluePedia application of Cytoscape.

Results

The validated significant DEGs which are extracted from the article by Heliton et al are tabulated in Table 1. Three kinds of HLA genes including HLA-A, HLA-C, and HLA-DP and SPRR2E as the DEGs that were regulated in the laser group versus the PG and SPRR2P and SPRR2F as

the two DEGs which were dysregulated in the LG but were not differentially expressed in the PG are listed in Table 1. As it is shown in Figure 1, there are connections between the DEGs; however, SPRR2P is not recognized by STRING. The five queried DEGs including HLA-A, HLA-C, HLA-DPB1, SPRR2E, and SPRR2F plus 50 first neighbors from the STRING database were included in a PPI network including 55 nodes and 1083 edges. The layout network based on the degree value is represented in Figure 2.

The network was analyzed and results are presented in Table 2. Four centrality parameters including degree, betweenness centrality, closeness centrality, and stress were determined for the nodes of the studied network. The 55 elements of the network were studied via action map analysis. As it is shown in Figure 3, among the studied 55 genes, 20 individuals were included in the action map and the others remained as isolated nodes. Three actions including expression, activation, and inhibition were considered to illustrate the action map.

Discussion

Limited numbers of dysregulated genes (6 genes) were extracted from the original study which is related to the effect of laser irradiation. As it is shown in the result section, five genes among the six queried DEGs were recognized by the STRING database. Three kinds of human leukocyte antigen (HLA) genes and two small proline-rich protein (SPRR) individuals were introduced as the dysregulated genes. The HLA system is known as the regulator of immune response in humans and for the first time, it was identified in the leukocytes¹⁶. The SPRR gene family are the proteins that are strongly tempted through human epidermal keratinocytes differentiation.¹⁷

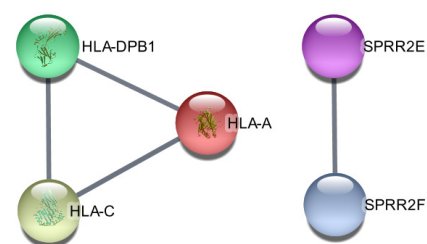


Figure 1. Connections Between the Recognized DEGs by STRING.

Table 1. Six Validated Significant DEGs Which Correspond to the Effect of Laser Irradiation Are Presented

Genes	<i>P</i> value < 0.05 in LG and <i>P</i> value > 0.05 in PG	<i>P</i> value < 0.05 in PG and <i>P</i> value > 0.05 in LG
HLA-A		*
HLA-C		*
HLA-DP		*
SPRR2E		*
SPRR2P	*	
SPRR2F	*	

Figure 2. PPI Network Including 5 Queried DEGs and 50 First Neighbors, Which Is a Layout Based on the Degree Value. Red to blue nodes and the bigger size of the nodes refer to the degree value increment.

R	Display Name	Degree	BC	CC	Stress
1	HLA-A	52	0.033	0.964	784
2	HLA-B	52	0.033	0.964	784
3	HLA-C	52	0.033	0.964	784
4	B2M	51	0.010	0.915	502
5	HLA-DPA1	51	0.010	0.915	502
6	HLA-DPB1	51	0.010	0.915	502
7	HLA-DQA1	51	0.010	0.915	502
8	HLA-DQA2	51	0.010	0.915	502
9	HLA-DQB1	51	0.010	0.915	502
10	HLA-DQB2	51	0.010	0.915	502
11	HLA-DRA	51	0.010	0.915	502
12	HLA-DRB1	51	0.010	0.915	502
13	HLA-DRB5	51	0.010	0.915	502
14	HLA-E	51	0.010	0.915	502
15	HLA-F	51	0.010	0.915	502
16	HLA-G	51	0.010	0.915	502
17	CIITA	47	0.004	0.857	246
18	CD44	44	0.001	0.818	90
19	FCGR1A	44	0.001	0.818	90
20	IFI30	44	0.001	0.818	86
21	GBP1	43	0.000	0.806	36
22	GBP2	43	0.000	0.806	36
23	ICAM1	43	0.000	0.806	36
24	IRF1	43	0.000	0.806	36
25	IRF2	43	0.000	0.806	36
26	IRF4	43	0.000	0.806	36
27	IRF9	43	0.000	0.806	36

Note: The queried DEGs are bolded. The highlighted rows refer to the genes that were presented in the action map (see Figure 3).

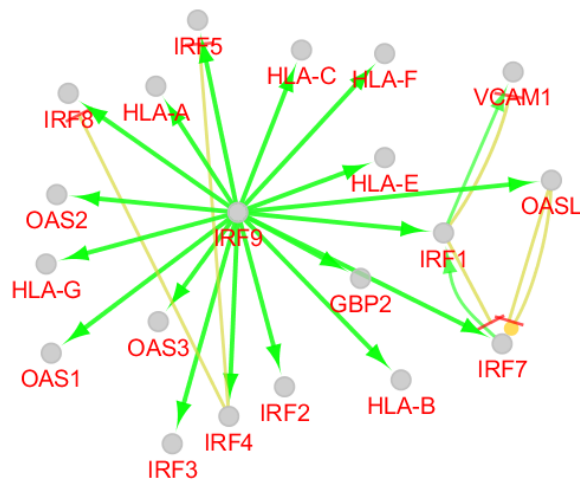


Figure 3. Action Map of 5 Queried DEGs and 50 First Neighbors. Among 55 genes, 20 individuals were included in the action map by ClueGO and the other ones (35 genes) were isolated nodes. Green and yellow refer to activation and expression actions respectively.

Investigations indicate that SPRR genes react to UV irradiation.¹⁸

Fifty first neighbors were added to the 5 recognized queried DEGs to construct a scale-free network. It is possible that a few nodes of the network play a critical role in the network. As it is shown in Table 1, the 16 top central nodes except B2M belong to the HLA Family. Beta-2-microglobulin (B2M) is involved in the modulation of the immune system, the stimulation of melanocytes, the function of the kidney, and inflammatory diseases.¹⁹

Action map analysis revealed that among the 55 studied genes, 20 individuals participated in the network. Three kinds of actions including expression, activation, and inhibition were investigated. As it is represented in Figure 3, there is no inhibition action between the 20 interacted genes. Activation is prominent action between the studied genes. Expression relationship is mostly between the interferon regulatory factor (IRF) gene family. The IRF family are a set of genes that play a role in the regulation of innate antiviral response (mainly the regulation of interferons and interferon-stimulated genes expression in vertebrates).²⁰ As it is depicted in Figure 3, IRF9 activates all elements of the network except VCAM1. However, IRF9 activates IRF1 and IRF7, and IRF7 activates IRF1, and finally, IRF1 is linked to VCAM1 by activation connection. It can be concluded that IRF9 is the central activator in the action map. Investigation indicates that IRF9 is involved in antiviral and antibacterial immunity.²¹ As shown in Table 2, the elements of the action map are colored. IRF9 is located in the row of 27 which indicates that it can be considered as a middle element of the table. It should be noted that the upper part of the table is mainly occupied by HLA genes. The content of Table 2 and also Figure 3 indicates that SPRR2E and SPRR2F (the

two queried DEGs) did not appear as central or regulatory genes in network analysis.

Our findings show that HLA and IRF genes are the two important genes that were regulated by laser irradiation. It seems that OM after CRT is prevented by the regulation of the immune system which is mainly administrated by the regulation of HLA and IRF gene families. Xiao et al published data about the decreased activity of IRFs in HNC patients²². Marcussen et al pointed to HLAs as the associated factors in OM.²³ As it is shown in Table 2, SPRR2E and SPRR2F appear as nodes that have minimal interaction with the other elements of the network and based on this finding they have not been discussed as critical factors in response to laser irradiation.

Conclusion

The findings indicate that HLA and IRF genes are the main regulated genes by PBMT in the prevention of OM in the HNC patients after CRT. On the other hand, immune system regulation by PBMT is the principal action in the prevention of OM. This finding is important as it improves laser quality and reduces possible side effects of laser applications in the clinical field.

Ethical Considerations

Not applicable.

Conflicts of Interest

There is no conflict of interest.

Acknowledgment

This project is supported by Shahid Beheshti University of Medical Sciences.

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