Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com

Biotic / Abiotic Stress Influences on Human Epidermal Keratinocyte Cells

Seyed Mohammad Motevalli [a], Fateme Mirzajani [b,*]

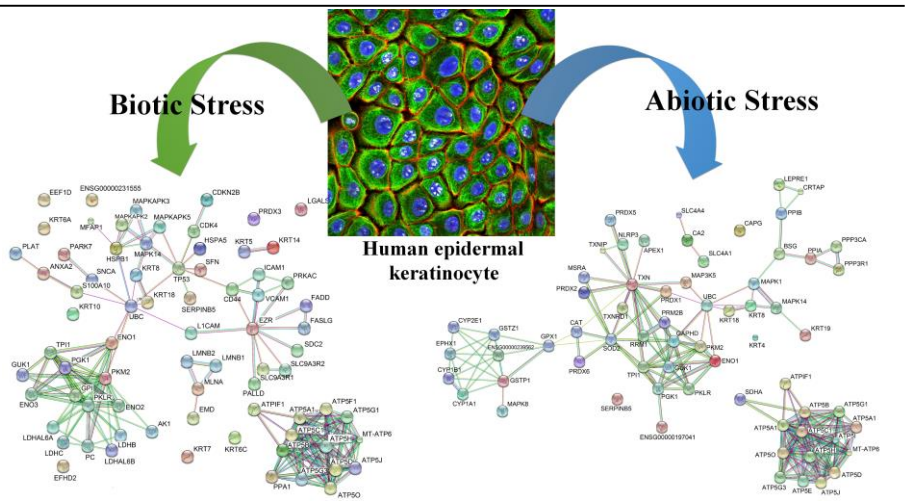
[a] *National Center for Nanoscience and Technology, Chinese Academy of Sciences, Beijing, China*

[b] *Departmrnt of Nanobiotechnology, Protein Research Center, Shahid Beheshti University, G.C., Tehran, Iran*

ARTICLE INFO

Article history:
Received: 04 May 2019
Accepted: 22 May 2019
Available online: 31 July 2019
Manuscript ID: PCBR-1905-1035

GRAPHICAL ABSTRACT



KEYWORDS

Abiotic stress
Biotic stress
Epidermal cell
Silicon dioxide
Protein networks

ABSTRACT

Human epidermal keratinocyte cells are the first defence blocks against the aggressive agents such as pathogens and xenobiotic materials. Synthesis process of the cellular proteins can be affected by abiotic stresses (aBS like: SiO_2 nanoparticles) and biotic stresses (BS like: Virus), leading to the alteration of consumed energy profiles of proteins. The consumed energy profile of each protein was calculated based on their consumed ATP during the amino acids synthesizing procedure. Cells consumed more ATP, more energy, to synthesize more proteins under BS conditions compare to aBS conditions. Our results suggested that, the cells infected by pathogens are tend to survive longer than the treated cells by xenobiotic materials. Our data analysis revealed that the most energy reduction took place under aBS conditions. So, aBS could have severe effect on energy production pathways and decrease the energy source of cells. Moreover, the results demonstrated that the complexity of cellular protein networks under aBS conditions were more than BS conditions. It seems the cellular energy reduction under aBS conditions is one of the important factors in cell death. In addition, the position of proteins in the protein network was another important factor that should be carefully considered.

* Corresponding author: Tel: +989128023246
E-mail: f_mirzajani@sbu.ac.ir
†Electronic Supplementary Information (ESI) available

1. Introduction

Mammalian skin is composed of at least three parts including epidermis, hair follicle, and associated glands. The basal layer of the human epidermis is constructed from a heterogeneous population of proliferative and differentiating cells [1]. Keratinocyte is the outermost layer of skin which forms a natural barrier against pathogens, ultra violet (UV) radiation, heat and water loss. Viruses are one of the most important Biotic Stresses (BS) can severely alter the human cell natural physiology and lead them to be cancerous. Human papillomaviruses (HPVs) are small, double strand, non-enveloped DNA viruses that belong to papillomaviridae family [2]. They typically infect the basal layer of skin and mucosal epithelium of the genital tract, mouth, anus or respiratory organs. In this regard, the HPV types could be categorized in two groups which are high and low risk groups [3].

According to the linear representation of the HPV genome and their ORFs, there are six gene expression patterns. Along with this representation, there are three main oncogenes groups called E5, E6, and E7 [4]. In this research, we have focused on E6 and E7 groups. They are involved in high-risk HPV types that play an important role in carcinogenesis. These viruses interfere with various cellular proteins and lead to the cell transformation and immortalization.

Nanoparticles have been used as effective biomaterials. They have unique and impressive features, including mechanical, optical, chemical, electrical, and biological applications [5]. Silicon dioxide (SiO_2) nanoparticles, due to their stability, low toxicity, and ability to be functionalized with a range of different molecules and polymers have been widely used for biomedical purposes such as energy engineering [6], bio imaging, drug delivery, cancer treatment and semiconductor manufacturing. It has been shown that amorphous SiO_2 nanoparticles can change the MRC-5 cells expression and induce oxidative stress; while they have lower toxicity on A549 and HeLa cells than bare nanoparticles (iron oxide without coating) [7].

On the other hand, ZnO, TiO_2, Al_2O_3 nanoparticles exhibited adverse effects on cell viability and cell proliferation of A549 carcinoma cells [8]. In addition, SiO_2 nanoparticles increased the DNA damage and apoptosis in IL-6 cells [9]. The SiO_2 nanoparticles have effect on the cell phenotype through the protein expression alteration. These changes could be applied genetically or epigenetically. Generally, cells can tolerate any stress by the modulating of gene and protein expression in different levels, such as the transcription, processing and translation. Whereas translation is a high-demanding process, this is the main cell target for control and management of stress [10].

2. Materials and Methods

Protein Sequence Extraction

All protein sequences, based on their accession numbers, were extracted from NCBI database [11]. In the following, the numbers of each protein's amino acid were calculated based on CLC Main Workbench V. 6.6.2 [12].

Consumed energy profile calculation

Protein fold changes in aBS (SiO_2 nanoparticle) and BS (papillomavirus) were reported by Yang et al. [11] and Merkley *et al*. [12]. For aBS (equation 1) and BS (equation 2), the ATP change of each protein was calculated based on equations 1 and 2:

ATP change= ((1/[x])-1) ×protein value Equation 1

ATP change=(x-1) ×protein value Equation 2

Where the "x" is the fold change number that observed for each protein. "Protein value" is the multiplication of each amino acid numbers in its amino acid cost [13].

X= (Numbers of increased ATP used in amino acids synthesizing process)/ (Total numbers of decreased and increased ATP used in amino acids synthesizing process) ×100

Where "X" is the total positive consumed energy profile of all groups of proteins under aBS and BS conditions.

X= (Numbers of free individual proteins in the protein network)/ (Total numbers of protein in the protein network) ×100

Where "X" is the percentage of free proteins in the protein network (not involved in a cluster).

Protein's network drawing

Cellular protein networks under aBS and BS conditions were plotted based on [14] at the highest confidence (required confidence=0.9) and maximum number (interactors shown=50) [15]. The whole procedure is shown in a flowchart (Figure 1).

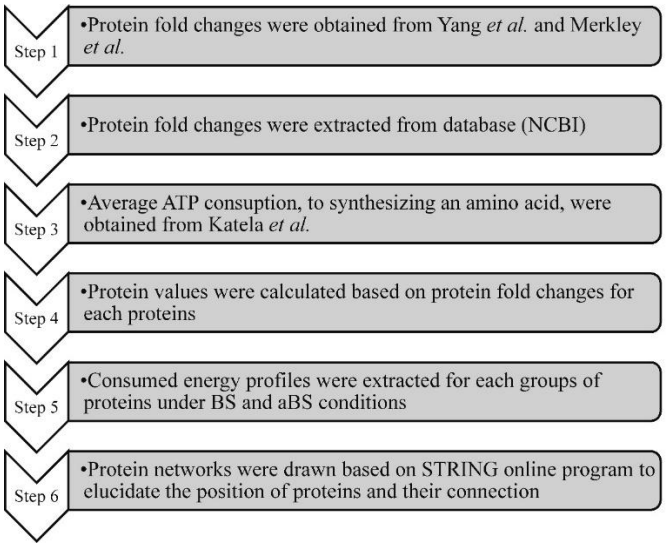


Fig 1. the flowchart shows the process of the calculation of consumed energy profiles under BS and aBS conditions and their interaction in a protein network.

3. Results and Discussion

The biotic stress (BS), in the following discussion, means the stress caused by viruses; while the abiotic stress (aBS) means the stress caused by nanoparticles. Our results suggested that the energy profile of HaCAT cells were highly depleted in aBS. While, it showed higher ATP consumption under BS compare to normal conditions. The average consumed ATP under BS and aBS conditions were 3967.5 and -969.5, respectively. Also with respect to expressed proteins type in both stress conditions, the proteins production cost (it means how many ATP was used in each protein pathway synthesizing) under BS (14008.15 ATP) was more than aBS (10815.2 ATP). Also, all protein's categories of cells under BS showed a higher protein production cost compare to the cells under aBS. According to this classification, aBS had not direct effect on the cell cycle and signaling pathway-associated proteins. The maximum decrease and increase in the energy profile of cells was occurred in metabolism-associated proteins and cytoskeleton-associated proteins, respectively, in cells under aBS (Table 1). While, the maximum decrease and increase was observed in apoptosis and tumor-associated proteins and molecular chaperones, respectively, under BS conditions. Among all groups of proteins under aBS, the cytoskeleton-associated proteins were the only group that illustrated a positive consumed energy profile. It worth to mention that, a positive consumed energy profile means cells under aBS had higher protein expression. While this group of proteins showed a negative consumed energy profile and lower protein expression. Among all groups of proteins with a negative consumed energy profile, oxidative stress-associated proteins showed the lowest value which means they illustrated a higher protein expression. (Table 1). Molecular chaperons showed a positive and negative consumed energy profile under BS and aBS conditions, respectively. It means, HaCAT cells preferred to produce more molecular chaperons under BS compare to aBS. Table 1 demonstrate the consumed energy profile alteration of each protein category under BS and aBS conditions. Our results illustrated that the total positive consumed energy profile of all groups of proteins was higher in cells under BS conditions (48.7%) compare to cells were under aBS conditions (7.1%).

Figure 1 and 2 revealed that cells under BS conditions had higher number and variation of proteins compare to cells under aBS conditions. Therefore, the cellular protein' network under BS conditions is more complex than aBS conditions. In addition, proteins are not involved in a cluster (free individual proteins) have higher frequency under BS conditions (36%) compare to aBS conditions (19%).

Decreasing of protein synthesis is usually observed in response to cellular damaging which is usually called as stress-induced damage minimization [16]. The regulation of translation procedure help to reveal cells' behavior which lead to understand cells immediate and selective responses. Our data are in agreement with previous reports and suggest that, the protein expression under aBS conditions (such as SiO_2 nanoparticles) is reduced; while it increases under the BS conditions (like papillomavirus). Consequently, the energy consumption (the number of used ATP in amino acids and proteins synthesis process) under the aBS and BS conditions were decreased and increased, respectively. Several reports have shown that, the total protein expression has been inhibited/decreased under aBS conditions. However, compare to aBS conditions, cells show a broad range of responses to different types of aggressive agents under BS conditions [18]. In other words, it seems the complicated and versatile interactions between aggressive agents and cells, affect the protein expression in different levels. However, cells generally take a similar way to respond to xenobiotic materials by decreasing the consumed energy profile.

The results suggested that the consumed energy profile of metabolism-associated proteins under BS (like virus) conditions, based on virus type and their synergistic interactions, had different effects on cells. But in aBS conditions (like SiO_2 nanoparticles) they showed a same response to all sizes of nanoparticles. It has been observed that metabolism-associated proteins demonstrated a higher negative consumed energy profile under aBS conditions compare to BS conditions. It means in a virus

infection, cells survives for a longer time and they keep their growth in abnormal conditions. Generally, it has been shown that in cells under BS conditions (virus types and their interaction), has versatile consumed energy profile; while cells under aBS conditions (all sizes of SiO_2 nanoparticles), showed same behaviors (Figure 2 and Figure 3).

Table 1. Average ATP change (consumed energy profile) for protein groups having biotic stress and abiotic stress

Protein categories	SiO_2 nanoparticle			papillomavirus		
	micro	30	15	E6	E7	E6 & E7
Cytoskeleton-associated proteins	1364.45	12642.58	11036.72	-5462.67	159.94	-4297.12
Oxidative stress-associated proteins	-1379.85	-2972.12	-3286.78	5883.1	4813.45	1083.8
Apoptosis and tumour-associated proteins	-3607.8	-5111.05	-5612.13	-4076.24	-7473.11	-6657.86
Cell cycle associated proteins	-	-	-	-6116.01	3909.47	3027.03
Molecular chaperones	-2400.39	-3663.76	-3598.46	30470.49	3556	22462.03
Signalling pathway associated proteins	-	-	-	2675.54	4298.62	4865.34
Metabolism-associated proteins	-3060.44	-6356.32	-6541.77	11343.26	-1528.28	-854.59

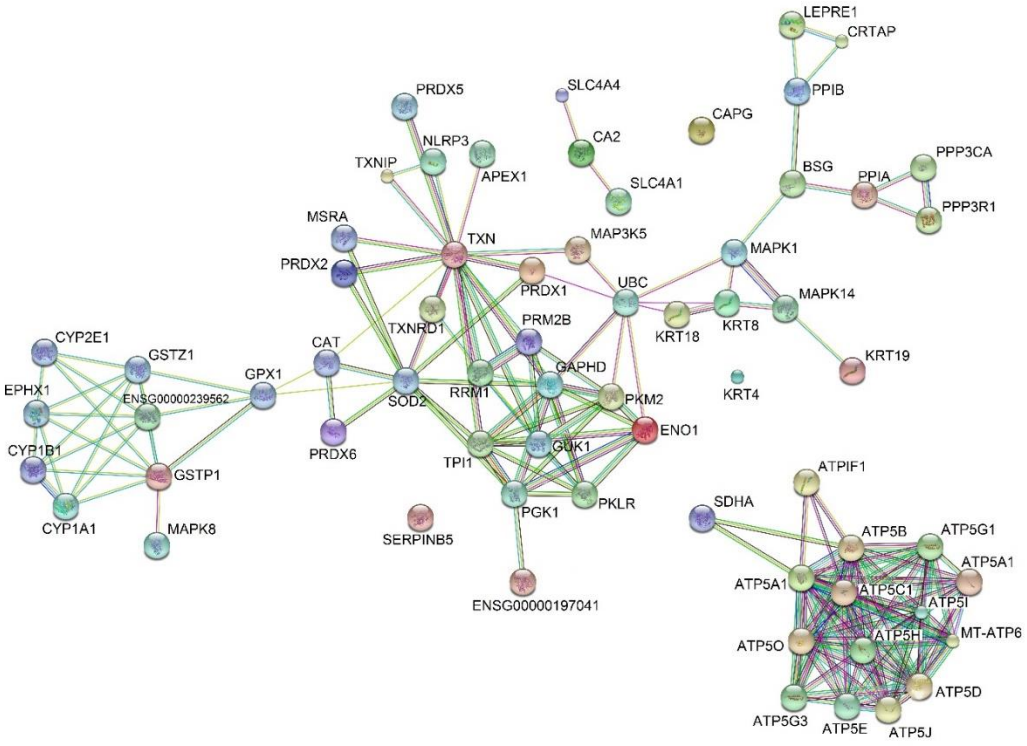


Fig. 2. The protein network of HaCaT cells under aBS conditions (SiO_2 nanoparticle) with high confidence degree and maximum numbers. It showed lower complexity with lower numbers of proteins. Most of proteins were involved in distinct clusters.

According to previous reports, cells exposed to SiO_2 nanoparticles and papillomaviruses encountered with oxidative stress [17, 18]. Our results depicted that the C under BS and aBS were increased and decreased, respectively. It means cells under BS conditions tend to synthesis proteins that confront with reactive oxygen species (ROS). While synthesizing the defensive proteins (SiO_2 nanoparticles) is overlooked in cells under aBS conditions It seems cells under BS conditions can reduce the cellular damage by synthesizing defensive proteins which result to increase of cell viability [19][20]. Cytoskeleton-associated proteins were the only group of proteins that were increased under aBS conditions [21, 22].

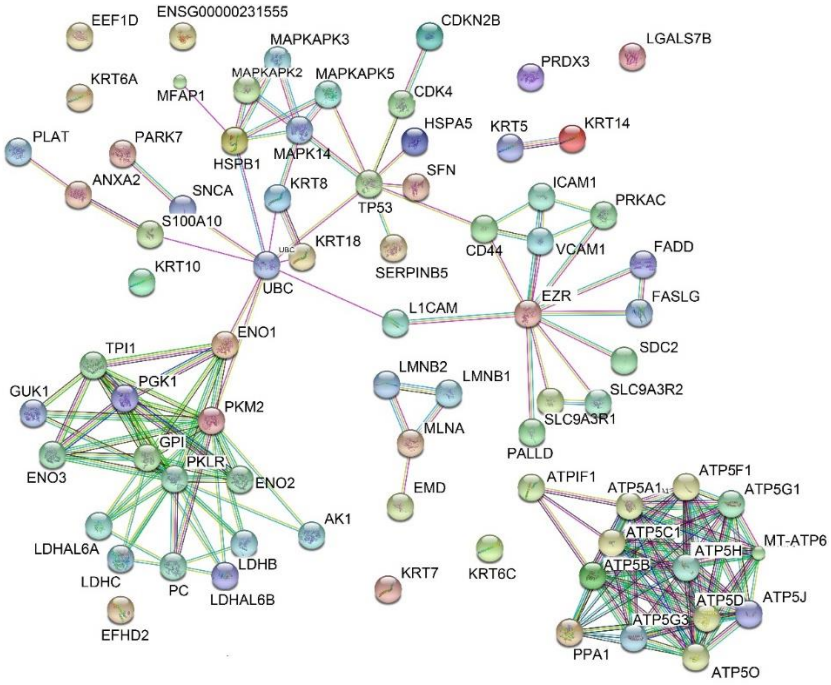


Fig. 2. The protein network of HaCaT cells under BS conditions (papillomavirus) with high confidence degree and maximum numbers. It showed higher complexity with more numbers of proteins. Also, numbers of free individual proteins were more than the protein network under aBS conditions.

Figures 1 and 2 demonstrates that cells have more complicated protein networks under BS conditions than aBS conditions. Additionally, according to the protein networks, it can be concluded that the alteration of consumed energy profiles are not directly related to the applied treatment features. In other words, it seems the protein location is as important as protein function in the protein network.

PRDX1, PRDX2 and TXN are connected in the network; therefore, the decreasing of consumed energy profile of these proteins may not be directly related to the BS and aBS conditions. In other words, they could have crosstalk to each other and the increasing and decreasing of each protein expression is not a direct influence of treatment conditions [23].

4. Conclusion

Cells under BS conditions showed mainly positive consumed energy profiles compare to cells under aBS conditions which revealed mainly negative consumed energy profiles [24]. Also, the cells under BS conditions could tolerate pathogens stress and survive longer than the cells treated by xenobiotic materials. Additionally, numbers and variation of proteins in cells under BS conditions were more than cells under aBS conditions. Therefore, cells responses to environmental stresses are more complicated and versatile under BS conditions compared with the aBS conditions. Moreover, the position of proteins in the network is as important as their function. Consequently, cellular responses to different stimuli are not always the direct influence of stresses, however the proteins interactions are another important factor which must be considered thoughtfully.

Conflict of interest

The authors declare that they have no competing interests.

References

[1] N. O. Fortunel, M. T. Martin, *Int. J. Radiat. Biol.*, 2012, **88**: 677

[2] R. Faridi, A. Zahra, K. Khan, M. Idrees, *Virol. J.,* 2011, **8**: 269

[3] P. G. Rossi, F. Chini, S. Bisanzi, E. Burroni, G. Carillo, A. Lattanzi, P. Capparucci, *Infect. Agent. Cancer*, 2011, **6**: 2

[4] S. Boulenouar, C. Weyn, M. Van Noppen, M. Moussa Ali, M. Favre, P. O. Delvenne, V. Fontaine, *Carcinogenesis*, 2010, **31**:473

[5] a) M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli, and S. Laurent, *Chem. Rev.,* 2011, **111**: 5610 b) M. Soleiman-Beigi, Z. Arzehgar, *Synlett*, 2018, **29**:986 c) S. Sajjadifar, Z. Arzehgar, S. Khoshpoori, *J. Inorg. Organomet. Polym. Mater.,* 2018, **28**:837 d) Z. Arzehgar, S. Sajjadifar, H. Arandiyan, *Asian J. Green Chem*., 2019, **3**:43 e) M. Soleiman-Beigi, Z. Arzehgar, *J. Sulfur Chem.,* 2015, **36**:395 f) M. Soleiman-Beigi, Z. Arzehgar, *Monatsh Chem*., 2016, **147**:1759 f) M. Soleiman-Beigi Z. Arzehgar *Heteroatom Chem*., 2016, **26**:355 g) M. Soleiman-Beigi, Z. Arzehgar, B. Movassagh, *Synthesis*, 2010, 392

[6] M. M. Byranvand, A. Dabirian, A. N. Kharat, and N. Taghavinia, *RSC Adv.,* 2015, **5**: 33098

[7] M. A. Malvindi, V. De Matteis, A. Galeone, V. Brunetti, G. C. Anyfantis, A. Athanassiou, P. P. Pompa, *PLoS One*, 2014, **9**: e85835

[8] X. Q. Zhang, L. H. Yin, M. Tang, Y. P. Pu, *Biomed. Environ. Sci.,* 2011, **24**: 661

[9] J. Duan, Y. Yu, Y. Li, Y. Yu, Y. Li, X. Zhou, Z. Sun, *PLoS One*, 2013, **8**: e62087

[10] S. Echevarría-Zomeño, E. Yángüez, N. Fernández-Bautista, A. Castro-Sanz, A. Ferrando, M. Castellano, *Int. J. Mol. Sci*., 2013, **14**: 4670

[11] http://www.ncbi.nlm.nih.gov/

[12] https://www.qiagenbioinformatics.com/products/clc-main-workbench/

[13] X. Yang, J. Liu, H. He, L. Zhou, C. Gong, X. Wang, B. Zhang, *Part. Fibre Toxicol*., 2010, **7**: 1

[14] X. Q. Zhang, L. H. Yin, M. Tang, Y. P. Pu, *Biomed. Environ. Sci.,* 2011, **24**: 661

[15] M. A. Merkley, E. Hildebrandt, R. H.Podolsky, H. Arnouk, D. G. Ferris, W. S. Dynan, H. 2009, Stöppler, *Proteome Sci.,* , **7**: 29

[16] C. Kaleta, S. Schäuble, U. Rinas, S. Schuster, *Biotechnol. J.,* 2013, **8**: 1105

[17] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Kuhn, *Nucleic Acids Res*., 2015, **43**: D447

[18] H. Kim, K. Strange, *Am. J. Physiol. Cell Physiol*., 2013, **305**: C1257

[19] A. M. Pickering, R. A. Linder, H. Zhang, H. J. Forman, K. J. A. Davies, *J. Biol. Chem*., 2012, **287**: 10021

[20] C. Goldring, N. Kitteringham, R. Jenkins, I. Copple, J.-F. Jeannin, B. K. Park, *J. Exp. Biol*., 2006, **209**: 2337

[21] I. Passagne, M. Morille, M. Rousset, I. Pujalté, B. L'azou, *Toxicology*., 2012, **299**:112

[22] K.D. Quint, R. E. Genders, M. N.C de Koning, C. Borgogna, M. Gariglio, J. N. Bouwes Bavinck, J. Doorbar, M.C. Feltkamp, 2015, *J. Pathology,* **235**: 342

[23] L. Cicchini, R.Z Blumhagen, J.A Westrich, M.E Myers, C.J Warren, C. Siska, D. Raben, K.J Kechris, D. Pyeon, 2017, *Scientific reports* ,**7**: 3633

[24] M.M Sthijns, W. Thongkam, C. Albrecht, B. Hellack, A. Bast, G. Haenen, R.P.F. Schins. *Toxicology in Vitro*, 2017, **40**: 223