Plant Archives Volume 20 No. 1, 2020 pp. 456-460 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE *IN SILICO* GENE-TRANSCRIPTIONAL EFFECTS OF 2-((4-ACETOPHENYL)-2-CHLORO-N-METHYL) ETHYL AMMONIUM CHLORIDE ON THE ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINES, C7-14 (GLUCOCORTICOID SENSITIVE) AND C1-15 (GLUCOCORTICOID RESISTANCE)

Israa Najm Abdullah Al-Ibadi[1], Alyassari Abdulkareemsalman[3], neamah Ahmed jasim[1]*, Alaa Abdulaziz Abed[1A] and Miranl A Al-Rammahi[1]

[1*]Department of Zoonotic Disease research unit, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq.

[2]Department of pathology and poultry diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq.

[3]Department of microbiology, Al-Qasim green university, Iraq.

Abstract

Glucocorticoids (GCs) are well-known compounds for initiating apoptosis in acute lymphoblastic leukemia sensitive cell line, C7-14; however, studies have found that no GC-related apoptotic responses were recognized after prolong treatment using GCs suggesting the occurrence of genetic modification in the glucocorticoid receptor (GR) as observed by employing the GC-resistant cell line, C1-15. In attempts for finding new compounds that induce favorable effects even on the resistant cells, the current computational model was created to study the gene-transcriptional and translational effects of 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride (22EAC) on the C7-14 and C1-15 cell lines. The transcriptional and the translational model was built up and graphed using Cell Net Analyzer (CNA), a MATLAB toolbox. The 22EAC was introduced into the system using C7-14 and C1-15 cell lines as defined to the software by GR=1 or GR=0, respectively. The findings revealed major transcriptional and translational changes in both cell lines especially in the proinflammatory and cell death programing components. The outcomes demonstrated 33 and two upregulated and downregulated genes, respectively; however, the model recognized no alterations in the activities of 17 genes. The current model indicates successful effects of the 22EAC, as a replacing compound to the GCs in resistant cells, on the viability of the lymphoblastic leukemia cell lines which encourages future *in vitro* or *in vivo* studies for better understanding the molecular results of this compound.

Key words : 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride, glucocorticoid receptor, lymphoblastic leukemia.

Introduction

Cancer is considered as an uncontrolled-unusual cellular growth in which normal cell cycles and pathways are disrupted. The final product of such process is the creation of solid masses or leukemia that can be accompanied by malignancy-based metastases (Blackadar, 2016; Imran *et al.,* 2017; Terwilliger and Abdul-Hay, 2017; Zhang and Chen, 2018). The cases of cancer are increasingly occurred in the world; however, the average rate of livability with cancer is increased to exceed the known five-year period due to the fast-ongoing research and discovery of new and novel drugs and techniques for fighting this serious condition that takes the lives of several hundred-thousand patients every year, although the case mortality rate has been reduced due to those eradication and therapeutic protocols (Bray *et al.,* 2018; Siegel, Miller and Jemal, 2019).

Even though the presence of the old-fashioned and newly-identified cancer therapeutic agents, prolong use of these medicines has been documented to build up huge obstacles such as continuous development of resistance

***Author for correspondence :** E-mail: esraa.najm@qu.edu.iq

by cancerous cells against those agents due to the incidence of genetic mutations (Luzzatto, 2011; Vogelstein *et al.*, 2013; Iranzo, Martincorena and Koonin, 2018a). According to that, this continuousness in finding new cancer drugs is ascendingly in humongous progress (Iranzo, Martincorena and Koonin, 2018b; Maeda and Khatami, 2018; Sokolenko and Imyanitov, 2018; Wartman, 2018).

Glucocorticoid receptor (GR) is a well-known target that has successfully been used for decades in fighting cancers such as leukemia utilizing glucocorticoids (GCs) as GR acts as a non-oncogenic receptor in opposite to the androgen and estrogen receptors in the prostate or breast, respectively, that basically derive more cell growth (Pufall, 2015; Lin and Wang, 2016). Like other cancer therapeutic agents, cancer cells have developed new resistance against GCs by inducing modifications or a deletion in the GR after prolong use of GCs such as dexamethasone (DMS) leading to ineffectiveness of the GCs in eradicating the cancer cells (Conzen, 2017; Puhr *et al.*, 2018). Cell line based studies have identified those GR alterations in the glucocorticoid sensitive cell lines (C7-14) after treating them with DMS for long time transforming them into a glucocorticoid resistance cell line (C1-15) (Lynch *et al.*, 2010; Gu, Zhang and Zhang, 2019).

In attempts for finding new compounds that induce favorable effects even on the resistant cells, the current computational model was created to study the gene-transcriptional and translational effects of 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride (22EAC) on the C7-14 and C1-15 cell lines.

Materials and Methods

Software and cell lines

The transcriptional and the translational model was built up and graphed using CellNetAnalyzer (CNA, v2017.1c), a MATLAB toolbox. This tool enables building gene-regulatory models using interactional-site-based networking represented by the interactional nodes (for such interactions such as OR or AND functions) that reflect close-to-real-life interactions (Klamt, Saez-Rodriguez and Gilles, 2007). The 22EAC was introduced into the system using C7-14 and C1-15 cell lines as defined to the software by GR=1 or GR=0, respectively.

Connection of the model to the related databases

Connections of the model built here were initiated to the gene ontology database for out-putting the current model. Terms such as cell death and inflammation were introduced to the system for obtaining the findings of the model regarding the 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride (22EAC) therapy. For verifying the interactional results, a step of double-curation was performed for the second time.

LSSA result comparing scenarios

The LSSA result comparisons (node-based upregulation or downregulation) between both scenarios of GC-sensitive and GC-resistance were initiated relying on methods shown by (Tian *et al.*, 2013).

Correct prediction via *p*-value calculation

The Wolfram Alpha computational knowledge engine (http://www.wolframalpha.com/) was employed for better prediction of the interactions depending on the final calculated *p*-value recruiting the following searching term:

[a]Probability of [X] success in [Y] trials, chance of success is [Z][o]

Table 1: Dependency Matrix Comparison results.

Original	No Effect	Ambivalent	Weak Inhibitor	Weak Activator	Strong Inhibitor	Strong Activator
No Effect	N/A	665	30	30	0	0
Ambivalent	0	N/A	0	0	0	0
Weak Inhibitor	0	5	N/A	0	0	0
Weak Activator	0	21	0	N/A	0	0
Strong Inhibitor	0	1	0	0	N/A	0
Strong Activator	0	1	0	0	0	N/A

A) GR-knockout Comparison table.

No Effect	Ambivalent	Weak Inhibitor	Weak Activator	Strong Inhibitor	Strong Activator	Total
1602	960	5	30	1	3	2601

Table 1: B) Comparison between the two cell lines.

Original	No Effect	Ambivalent	Weak Inhibitor	Weak Activator	Strong Inhibitor	Strong Activator
No Effect	N/A	340	30	40	0	0
Ambivalent	0	N/A	0	0	0	0
Weak Inhibitor	0	9	N/A	0	0	0
Weak Activator	0	7	0	N/A	0	0
Strong Inhibitor	0	0	0	0	N/A	0
Strong Activator	0	0	0	0	0	N/A

Table 1: C) Full Matrix CIN052 Model.

Column1	Column2
No Effect	896
Ambivalent	1721
Weak Inhibitor	33
Weak Activator	52
Strong Inhibitor	0
Strong Activator	2
Total	2704

Table 2: LSSA Scenario Comparisons.
A) C7-14 (Full)

Column1	Column2	Column3
Upregulated	0	0.00
Unchanged	10	19.23
Downregulated	42	80.77
Total	52	100

Table 2: B) C1-15 (Full).

Column1	Column2	Column3
Upregulated	10	19.23
Unchanged	42	80.77
Downregulated	0	0.00
Total	52	100

Table 2: C) C7-14 (Full) vs GR-knockout.

Column1	Column2	Column3
Upregulated	33	63.46
Unchanged	17	32.69
Downregulated	2	3.85
Total	52	100

[X]: Correct prediction number

[Y]: Prediction total number

[Z]: Success chance (one to three out of three possible outputs).

Results

The findings revealed major transcriptional and translational changes in both cell lines especially in the proinflammatory and cell death programing components. The outcomes demonstrated 33 and two upregulated and downregulated genes, respectively; however, the model recognized no alterations in the activities of 17 genes, Figs. 1 and 2. (Table 1, 2) show the matrixes and comparisons with their resulted gene and interactional regulations in details.

Discussion

GR plays important roles in fighting cancerous cells

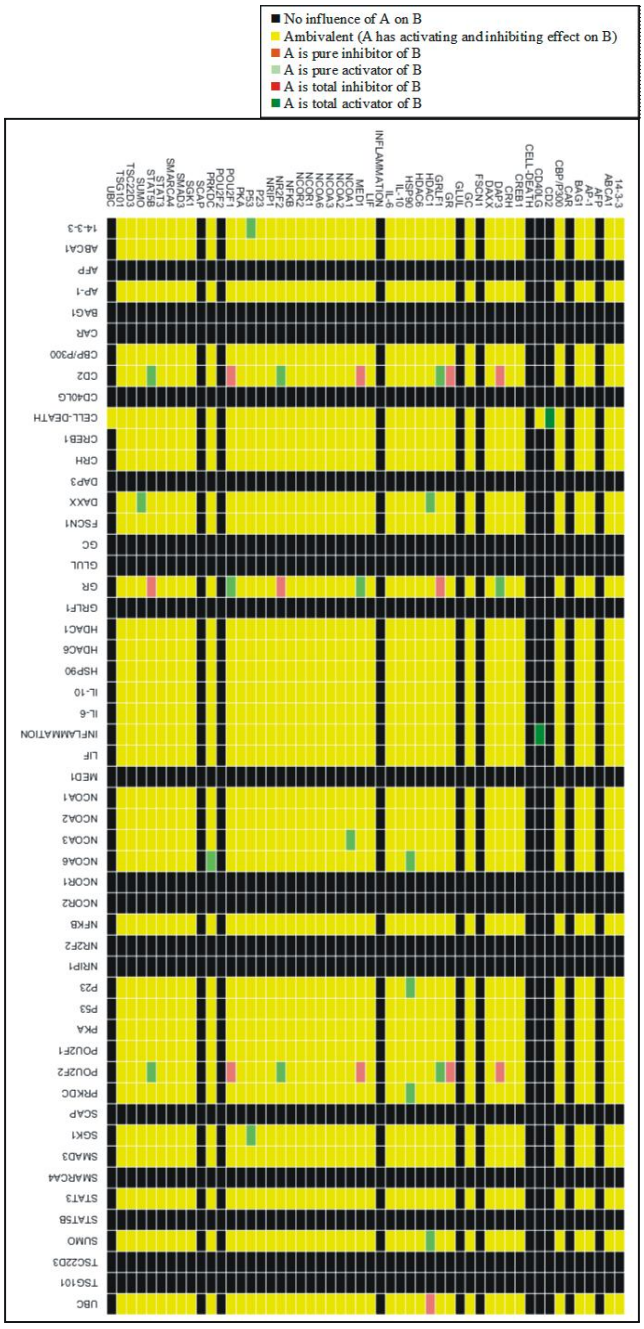

Fig. 1: Dependency Matrix for 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride (22EAC) project CIN052 (GR activation deleted).

due to the effects of GCs in leading the cell into apoptosis (Pufall, 2015; Lin and Wang, 2016). However, those cancer therapeutic agents face resistance initiated by the GR alteration (deactivation) after prolong use of GCs leading to unsuccessfulness of the GC treatment (Conzen, 2017; Puhr *et al.*, 2018). Those alterations in the GR have been seen in the C7-14 after treating them with DMS for long time transforming them into C1-15 (Lynch *et al.*, 2010; Gu, Zhang and Zhang, 2019). In attempts for finding new compounds that induce favorable

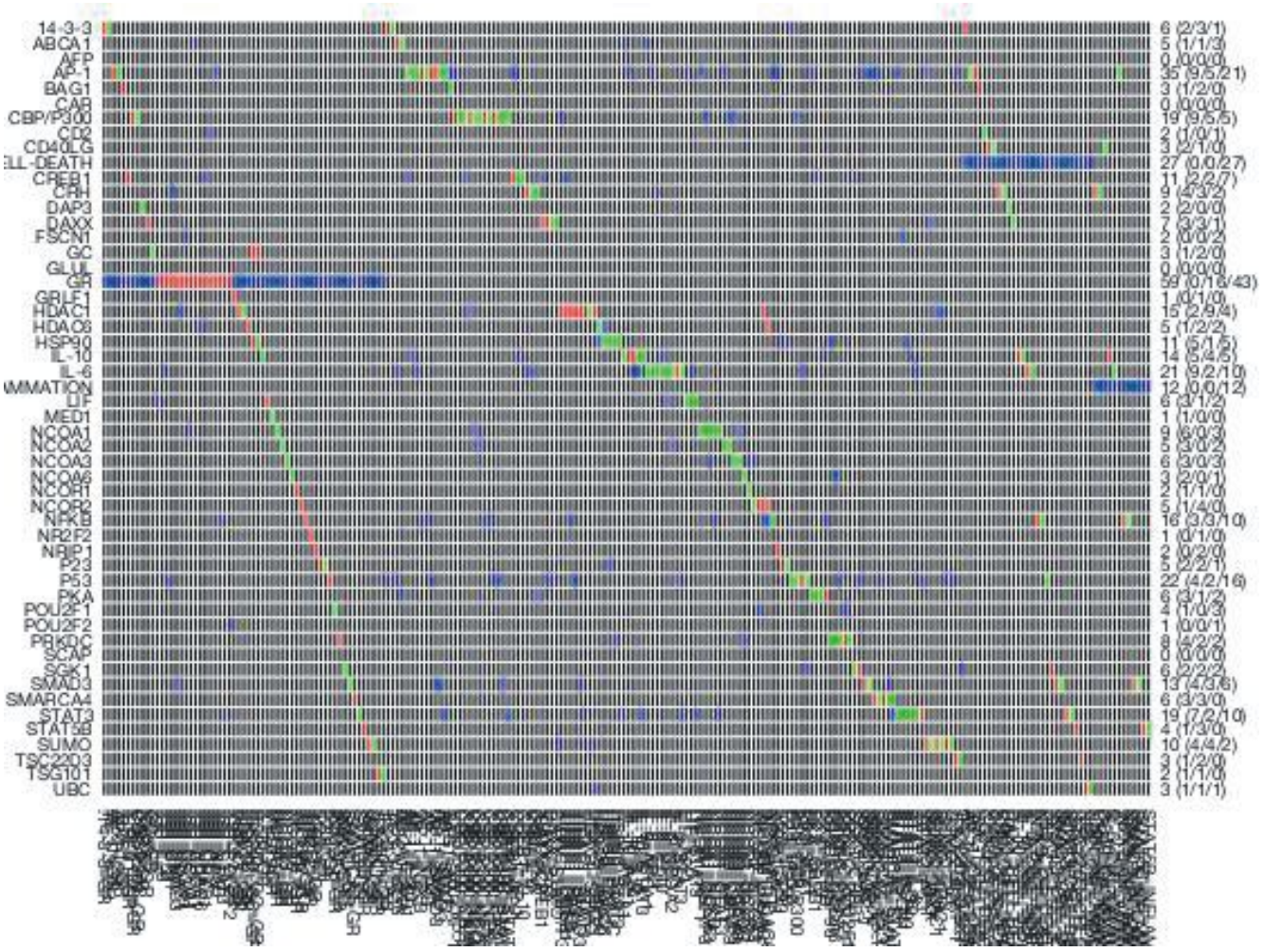

Fig. 2: Interaction Matrix for 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride (22EAC) project CIN052.

effects even on the resistant cells, the current computational model was created to study the gene-transcriptional and translational effects of 22EAC on the C7-14 and C1-15 cell lines.

The findings revealed major transcriptional and translational changes in both cell lines especially in the proinflammatory and cell death programing components. The outcomes demonstrated 33 and two upregulated and downregulated genes, respectively. As it was noticed from the current work results, apoptotic related pathway upregulations were the major influential changes. Apoptosis or programmed cell death is an ATP-mediated process which organized and performed by certain enzymes (proteases for cytoskeleton and endonucleases for DNA or RNA). This step is urgently needed when a particular cell reaches to a level of threat to the body (Akhtar and Bokhari, 2019). Sometimes, apoptosis may occur in response to various cellular physiological cycles. The dying cell undergoes a morphological phenomenon in which condensation of chromatin and pyknosis (nuclear fragmentation), blebbing of plasma membrane, and shrinkage of the cell. Finally, the cell is sheared into two small fragments covered a layer of membrane which is called (apoptotic bodies) that phagocytic-removed without inflammatory processes (Reed, 2000). Proinflammatory responses are important in worsening the inflammatory responses which is important fighting the cancerous cells that might help in initiating the programmed cell death (Landskron *et al.*, 2014; Chen *et al.*, 2018).

Conclusion

The current model indicates successful effects of the 22EAC, as a replacing compound to the GCs in resistant cells, on the viability of the lymphoblastic leukemia cell lines which encourages future *in vitro* or *in vivo* studies for better understanding the molecular results of this compound.

References

Akhtar, F. and S.R.A. Bokhari (2019) *Apoptosis*, *StatPearls*. StatPearls Publishing. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29762996 (Accessed: 2

August 2019).

Blackadar, C.B. (2016). 'Historical review of the causes of cancer'. *World Journal of Clinical Oncology*, **7(1):** 54. doi: 10.5306/wjco.v7.i1.54.

Bray, F. *et al.* (2018) 'Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries'. *CA: A Cancer Journal for Clinicians*, **68(6):** 394–424. doi: 10.3322/caac.21492.

Chen, L. *et al.* (2018). 'Inflammatory responses and inflammation-associated diseases in organs.', *Oncotarget*. Impact Journals, LLC, **9(6):** 7204–7218. doi: 10.18632/oncotarget.23208.

Conzen, P.S.D. (2017). 'Recent Advances in Understanding Glucocorticoid Receptor Function in Cancer', *Clinical advances in hematology & oncology/ : H&O*. NIH Public Access, **15(5):** 338. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5996375/ (Accessed: 2 August 2019).

Gu, L., G. Zhang and Y. Zhang (2019). 'A novel method to establish glucocorticoid resistant acute lymphoblastic leukemia cell lines'. *Journal of Experimental & Clinical Cancer Research Bio. Med. Central*, **38(1):** 269. doi: 10.1186/s13046-019-1280-2.

Imran, A. *et al.* (2017). 'Role of Molecular Biology in Cancer Treatment: A Review Article'. *Iranian journal of public health*, **46(11):** 1475–1485. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29167765 (Accessed: 2 August 2019).

Iranzo, J., I. Martincorena and E.V. Koonin (2018a). 'Cancer-mutation network and the number and specificity of driver mutations'. *Proceedings of the National Academy of Sciences*, **115(26):** E6010–E6019. doi: 10.1073/pnas.1803155115.

Iranzo, J., I. Martincorena and E.V. Koonin (2018b). 'Cancer-mutation network and the number and specificity of driver mutations'. *Proceedings of the National Academy of Sciences*, **115(26):** E6010–E6019. doi: 10.1073/pnas.1803155115.

Klamt, S., J. Saez-Rodriguez and E.D. Gilles (2007). 'Structural and functional analysis of cellular networks with CellNetAnalyzer'. *BMC Systems Biology*. *Bio. Med. Central*, **1(1):** 2. doi: 10.1186/1752-0509-1-2.

Landskron, G. *et al.* (2014). 'Chronic inflammation and cytokines in the tumor microenvironment'. *Journal of immunology research*, Hindawi Limited, 149185. doi: 10.1155/2014/149185.

Lin, K.T. and L.H. Wang (2016). 'New dimension of glucocorticoids in cancer treatment'. *Steroids*, **111:** 84–88. doi: 10.1016/j.steroids.2016.02.019.

Luzzatto, L. (2011). 'Somatic mutations in cancer development.', *Environmental health/ : a global access science source*. BioMed. Central, **10 Suppl 1(Suppl 1):** S12. doi: 10.1186/1476-069X-10-S1-S12.

Lynch, J.T. *et al.* (2010). 'The role of glucocorticoid receptor phosphorylation in Mcl-1 and NOXA gene expression.', *Molecular cancer*. BioMed. Central, **9:** 38. doi: 10.1186/1476-4598-9-38.

Maeda, H. and M. Khatami (2018). 'Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs.', *Clinical and translational medicine*. Springer, **7(1):** 11. doi: 10.1186/s40169-018-0185-6.

Pufall, M.A. (2015). 'Glucocorticoids and Cancer', in *Advances in experimental medicine and biology*, 315–333. doi: 10.1007/978-1-4939-2895-8_14.

Puhr, M. *et al.* (2018). 'The Glucocorticoid Receptor Is a Key Player for Prostate Cancer Cell Survival and a Target for Improved Antiandrogen Therapy'. *Clinical Cancer Research*, **24(4):** 927–938. doi: 10.1158/1078-0432.CCR-17-0989.

Reed, J.C. (2000). 'Mechanisms of apoptosis.', *The American journal of pathology*. *American Society for Investigative Pathology*, **157(5):** 1415–30. doi: 10.1016/S0002-9440(10)64779-7.

Siegel, R.L., K.D. Miller and A. Jemal (2019). 'Cancer statistics, 2019'. *CA: A Cancer Journal for Clinicians*, **69(1):** 7–34. doi: 10.3322/caac.21551.

Sokolenko, A.P. and E.N. Imyanitov (2018). 'Molecular Diagnostics in Clinical Oncology'. *Frontiers in Molecular Biosciences*. Frontiers Media SA, **5:** 76. doi: 10.3389/FMOLB.2018.00076.

Terwilliger, T. and M. Abdul-Hay (2017). 'Acute lymphoblastic leukemia: a comprehensive review and 2017 update.', *Blood cancer journal*. Nature Publishing Group, **7(6):** e577. doi: 10.1038/bcj.2017.53.

Tian, K. *et al.* (2013). 'Dynamics of DNA Damage Induced Pathways to Cancer', *PLoS ONE*. Edited by P. Csermely. *Public Library of Science*, **8(9):** e72303. doi: 10.1371/journal.pone.0072303.

Vogelstein, B. *et al.* (2013). 'Cancer genome landscapes.', *Science (New York, N.Y.)*. NIH Public Access, **339(6127):** 1546–58. doi: 10.1126/science.1235122.

Wartman, L.D. (2018). 'The future of cancer treatment using precision oncogenomics.', *Cold Spring Harbor molecular case studies*. Cold Spring Harbor Laboratory Press, **4(2):** doi: 10.1101/mcs.a002824.

Zhang, H. and J. Chen (2018). 'Current status and future directions of cancer immunotherapy.', *Journal of Cancer*. Ivyspring International Publisher, **9(10):** 1773–1781. doi: 10.7150/jca.24577.