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Upregulation of ABL1 Gene Expression Due to the Co-expression of PML-II and adenovirus Genome Transfection

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ABSTRACT

Several promyelocytic leukemia proteins (PML) isoforms are involved in a wide range of cellular functions, including innate immune responses, gene transcription, and apoptosis. Adenoviridae are non-enveloped viruses that can disrupt cellular systems that control cell cycle progression and apoptosis,. The study analyzed the gene expression of ABL-1(Tyrosine Kinase gene), into cells transfected with adenovirus and PML-II expression plasmids. A human breast cancer cell line (MCF-7) cells were plated in 24 well plates in 500 microliters of Dulbecco's Modified Eagle Medium (DMEM) for each well, and after 24 hours, the cells were harvested and used for gene expression analysis. So, the aim was evaluate the effect of the effect of PML-II on ABL1 gene expression.
The results showed that ABL1 gene expression was in transfected Ad5 and PML samples compared to the control.
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1. Introduction

Adenovirus causes a wide range of infections in respiratory tract, gastrointestinal tract conjunctiva, and rarely does it cause hemorrhagic cystitis or colitis, hepatitis, nephritis, pancreatitis and encephalitis [1]. The virus infects children and adults in closed places with more severe symptoms in young children (incomplete immune defaces) or people with an impaired immune response such as HIV- infected people or after transplantation. Severe adenovirus pneumonia can cause a fatality rate reaches 50%. More than 50 serotypes were identified with different tropisms to different tissues. The common serotypes vary among countries with a possibility of emerging of new strains [2].Adenovirus immediate region 1A (E1A) is a gene that produces a variety of E1A proteins during adenovirus replication [3, 4]. E1A gene encodes two main proteins (via alternative splicing) for adenovirus early replication; both affect the signaling pathway in mammalian cells [5]. The E1A-encoded proteins are expressed in the nucleus and influence the host cell's genetic control [6]. They promote the expression of other viral genes, also they can either boost or inhibit the expression of cellular genes depending on the cellular state and the need for expression of other viral genes [7]. The introduction of E1A fragment into cells may have adverse biological effects [8]. For example, promoting DNA synthesis and cell cycle progression [5]. As well as inhibiting differentiation (Webster and Kedes, 1988), and one of the causes of cellular transformation [9].

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Adenovirus E1B protein refers to one of two proteins produced by the adenovirus E1B gene: a 55kDa protein or a 19kDa protein. Adenovirus-infected cells require these two proteins to block apoptosis. E1B proteins help to block, apoptosis, which is caused by the E1A protein of the small adenovirus, which stabilizes the tumor suppressor p53 [10, 11].
PML gene was overexpressed to investigate its antagonistic effect against the ability of Ad5 to transform cells [12]. Ad5, PML, Ad5 and PML together were introduced into the cell and the gene expression of genes and their relationship to anti-tumor activity was analyzed [13].
Several cellular actions of the PML protein are linked to its anti-tumor effect. For example, PML activates the pro-apoptotic p53 function [14]. and recruits it into the PML-NBs, where PML binds to and suppresses the negative regulator of P53 to stabilize it to mediate its activity [15,16]. In response to IFN stimulation, the PML-II isoform regulates the expression of p53 and p53-mediated apoptosis.Overexpression of PML in gastric cancer cells causes a significant increase in cell apoptosis and a decrease in cell growth [17]. So the aim was to analyze the expression of ABL1 gene in case of overexpressing PML-II concomitant with Ad5 infection.

2. Materials and methods

2.1. Cell Culture

MCF7 cells were splitting them when they become confluent monolayer as the following: The media is removed and the flask is washed once with 2 ml of PBS (Phosphate Buffered Saline). The cells are treated with 2ml of trypsin – EDTA solution and incubated at 37 ° C for 5 minutes, with 5 ml from DMEM medium and pipetted a few times to disaggregate the clumps then at the appropriate concentration into a culture [18].

2.2. Viability of Cells

Cells were grown in DMEM which contains 10% fetal bovine serum at 37 ° C and 100% relative humidity. When monolayer cells were detached by trypsin-EDTA to obtain a single cell suspension, the cell viability was counted using a hem cytometer [18].

2.3. Transfection

Cells were plated at a density of 0.5 x 106 cells per well in a 24-well plate contained 500 µl of DMEM culture for each well and then after 24 hours the 24-wells plate was divided into six groups: Each group (6- wells) row challenged with one of the following treatments: 1.2 µl of DNA fection reagent was mixed with 0.25 µg of Ad-5 plasmid, PML-II, Ad-5 and PML-II, DNA transfection reagent, only serum free media respectively prepared in 150 µl in serum free medium [19].

2.4. Gene expression

2.4.1 Isolation of total RNA from cell lines

RNA was isolated from cells using FavorPrep ™. Kit, Taiwan (Cat No.: FATRK001, 100) according to the manufacture instructions:
Cells were collected by spinning down at 14000 rpm for 1 min at 4C, the supernatant was discarded. The cellular pellet was lysed with 350 µl of FARB buffer containing 3.5 µl of β-Mercaptoethanol and vortexed vigorously for 1 min to re-suspend the cells completely.
The lysates were mixed with 1 volume of 70 % RNase – free ethanol and mixed well by vortex.

2.4.2. Converting RNA to cDNA

To convert RNA to cDNA Accupower RocketScript ^{RT} kit, Premix from (Bioneer, Korea), Cat No. : K-2101) was used.The extracted RNA templates were converted into complementary DNA (cDNA). The kit components were added to the reaction mixture and completed to a final volume of 20 ml according to Accupower Rocket Script ^{RT} Premix from (Bioneer) instruction.

2.4.3. Real time PCR reaction

The gene expression of ABL1 and GAPDH (which was used as endogenous calibrator gene) was analyzed using Bioneer Exicycler 96 and ROTOR GENE Q. The cDNA was utilized as a template for the qPCR. The Real Time PCR reaction was performed using RealMOD™Green SF 2X qPCR mix kit (iNtRON, Korea) according to the instruction enclosed with mentioned kit. and ABL1 specific primers Table 2 in Ad5, PML, Ad5 and PML, transfected and control treatments. Each sample was in 3 performed in triplicate and the averaged and standard deviation was extracted. The results were compared to the control samples. In order to qualify and quantify gene expression, the melting and amplification curves of the target genes were equitized and analyzed, all qPCR products were submitted to dissociation curve analysis to verify primer specificity.

Table 1. The type of cell lines.

No	Type	Location	Origin	Ref.
1-	MCF-7	Brest cancer (pleural effusion)	Human	[20].

Table 2. Sequences of ABL1 and GAPDH primers.

Gene	sequences		Tm	Ref.
ABL1	F.	5′-CGAAGGGAGGGTGTACCATTA-3′	79ºC	[21].
	R.	5′-CGAAGGGAGGGTGTACCATTA-3′		
GAPDH	F	5′- GGCCTCCAAGGAGTAAGAC-3′	83 ºC	[22].
	R	5′- CCCCTCTTCAAGGGGTCTAC-3′		

3. Relative gene expression

The relative gene expression analysis was performed using Livak and Schmittgen (2001) method (2−ΔΔCT) [23].

3.1. Statistical analysis

The PML gene overexpression were provided as the mean standard deviation (mean ± SD) in this research. We used independent sample t-tests to assess the Gene expression evaluation of a tumor suppressor gene.

3.2. Relative gene expression of ABL1

The results showed that ABL1 expression in Ad5 and PML transfected samples up.

4. The results

- Gene expression
- Specificity and amplification curves of gene specific primers

In order to assess the gene expression of the ABL1 and GAPDH. The cDNA was prepared by reverse transcription from RNA and utilized as a template for the first strand of DNA qPCR. In order to identify each gene, the melting and amplification curves of the target genes were evaluated, and all qPCR products were submitted to dissociation curve analysis to verify primer specificity.

GAPDH was used as an internal calibrator gene in order to correct the amplification and conducting the ΔΔ CT analysis. Each sample was prepared in 3 replicates and were averaged and standard deviation was extracted. The results were compared to the control samples, master mix and SYBR green was used as intercalating dye to achieve the quantification regulated to 2.5 to 3 -fold compared to the control sample.

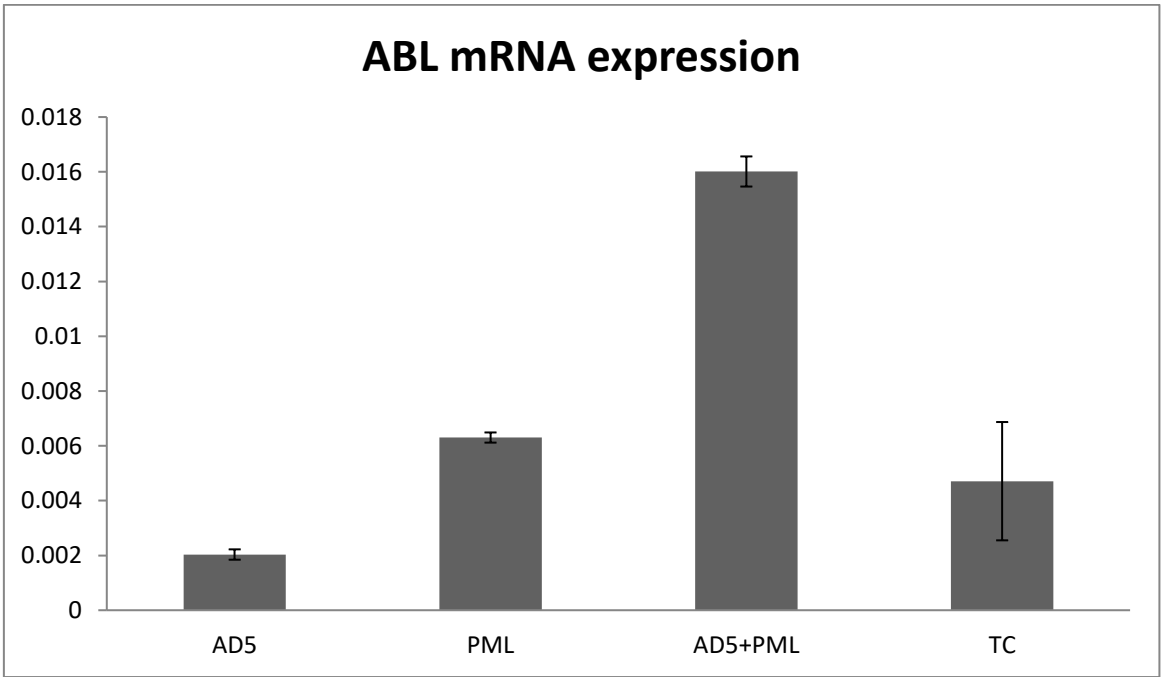

Fig. 1. ABL relative expression level with or without the overexpression of Ad5 or PML or Ad5 and JAK plasmids in MCF7.

Equivalent cultures of MCF7 were transfected with Ad5, PML, Ad5 and PML, Lipofectamine or left without any treatment. Total RNA was extracted at 24 hours post transfection, reverse transcribed and the synthesized DNA were used as a template for qPCR relative expression assay using SYBR green master mix. Data were analyzed by ΔΔ CTs and normalized to (CAPDH) house-keeping gene.

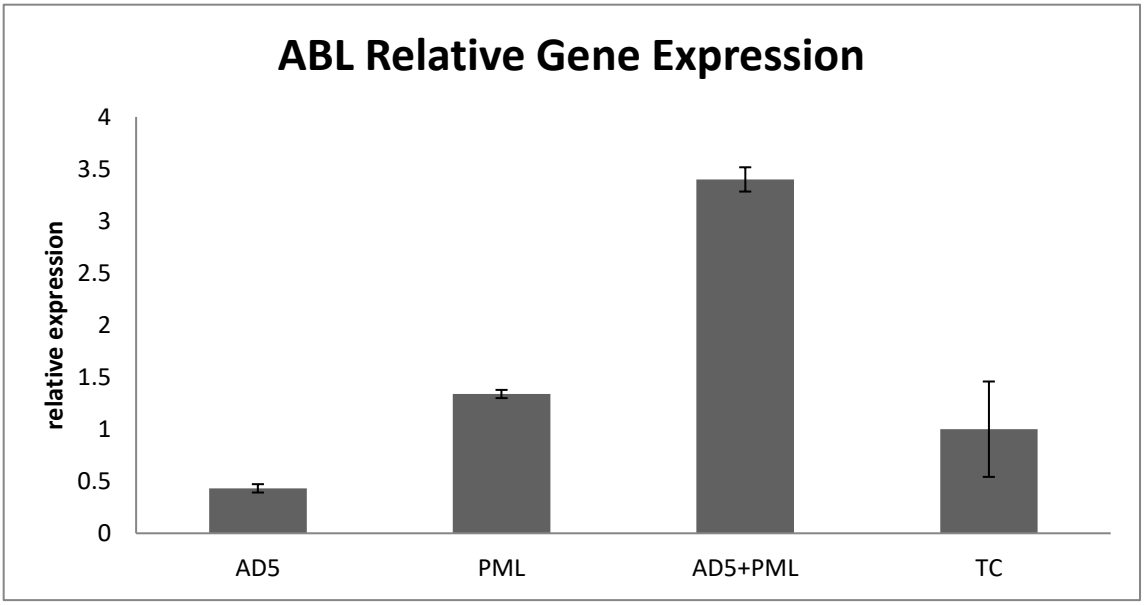

Fig. 2. fold change analysis with or without the overexpression of Ad5 or PML or Ad5 and PML plasmids in MCF7.

Equivalent cultures of MCF7 were transfected with Ad5, PML, Ad5 and PML, Lipofectamine or left without any treatment. Total RNA was extracted at 24 hours post transfection, reverse transcribed and the synthesized DNA were used as a template for qPCR relative expression assay using SYBR green master mix. Data were analyzed by ΔΔ CTs and normalized to (CAPDH) house-keeping gene. (t test P =0.003219).

5. Discussion

Since the ABL1 protein is implicated in upregulating the DNA damage -induced apoptosis, it was necessary to evaluate its overexpression [24]. PML-II gene was overexpressed to investigate its antagonistic effect against the ability of Ad5 to transform cells. Ad5, PML, Ad5 and PML together were introduced into the cell and the gene expression of genes and their relationship to anti-tumor activity was analysed [25]. Tyrosine-protein kinase gene expression was performed in the transfected samples, Ad5, PML, Ad5 and PML and control samples. The results showed that the gene expression of ABL1 in the Ad5 and PML transfected samples was 3-fold high compared to the control sample. Tyrosine kinase plays an important role in cell growth and survival activities such as cytoskeleton remodeling in response to extracellular stimuli, cell motility and adhesion, cellular endocytosis, autophagy, DNA damage response, apoptosis, as well as many other factors [26]. ABL is implicated in different multiple cellular functions including apoptosis; ABL controls PUMA, p53-regulatory modulator of apoptosis and to cause intrinsic apoptotic events downstream. ABL regulates DNA damage response suggesting that ABL enhances the apoptosis by increasing the levels of apoptosis protein. ABL regulates expression of the cell cycle inhibitor p21 after DNA damage in the absence of p53. Suggests that ABL1 which is encoded by the ABL1 gene (previous symbol ABL) located on chromosome 9 [27]. ABL is transported to in a response to DNA damage resulting in ABL buildup [28]. PUMA, a pro-apoptotic p53-dependent gene is regulated by PML-II isoform, and hence is involved in cell death [29]. Similar to the previous article, we speculate that the ABL1 gene on chromosome 9 encodes ABL1. ABL is transferred in response to DNA damage. Nuclear ABL then activates the transcription factors p53 and p73, which promotes apoptosis. PUMA, a p53-dependent pro-apoptotic gene, is implicated in cell death. Another evidence about the role of PML in apoptosis, PML enhances cell apoptosis, which in turn reduced cell proliferation. Apoptosis implicated genes such as Bcl-2, Bak, and caspase-9, are modulated by p73 in response to PML effects [30].

6. Conclusions

The increase in ABL1 up to 3 fold compared to the control might be a good inducer to the apoptosis pathways.Increase in the gene expression of ABL1 up regulates the apoptosis, immunity and inflammatory response and even antiviral response.ABL regulates the expression of the cell cycle inhibitor p21 after DNA damage in the absence of p53. and activates the proactive pathways, therefore ABL1 induction may be beneficial to the cell.

References

[1] H. T. The wainy, H. Hasony, The Medical Journal of Basrah University **37**(1), 37 (2019).
[2] J. P. Lynch, A. E. Kajon, InSeminars in respiratory and critical care medicine **37**, 04, 586 (2016).
[3] Z. W. Atwan, Basrah Journal of Science **37**(1), 73 (2019).
[4] Y. Zheng, Stamminger T. PLoS pathogens **12**(1), 73 (2016).
[5] D. P. Molloy, K. J. Smith, A. E. Milner, P. H. Gallimore, R. J. Grand , Journal of Biological Chemistry **274**(6), 3503 (1999).
[6] L. Crisostomo, A. M. Soriano, J. R. Frost, O. Olanubi, M. Mendez, P. Pelka, Viruses **9**(12), 387 (2017).
[7] S. M. Frisch, J. S. Mymryk, Nature reviews Molecular cell biology **3**(6), 441 (2002).
[8] P. A. Boulanger, G. E. Blair, Biochemical journal **275**(Pt 2), 281 (1991).
[9] J. M. Routes, S. Ryan, A. Clase, T. Miura, A. Kuhl, T. A. Potter, J. L. Cook, The Journal of Immunology **165**(8), 4522 (2000).
[10] E. White, R. Cipriani, Molecular and cellular biology **10**(1), 120 (1990).
[11] S. W. Lowe, H. E. Ruley, Genes & development **7**(4), 535 (1993).
[12] A. Chikhalya, M. Dittmann, Y. Zheng, S. Sohn, C. Rice, P. Hearing, Mbio **12**(6), 21 (2021).
[13] E. Shirbhate, R. Veerasamy, S. H. Boddu, A. Tiwari, H. Rajak, Drug Discovery Today **2**, 9 (2022).
[14] H. Al-Salman, E. Ali, M. Jabir, G. Sulaiman, European Food Research and Technology **246**(8), 020 (2020).
[15] R. Bernardi, P. Pandolfi, Nature reviews Molecular cell biology **8**(12), 1006 (2007).
[16] K. Takahashi, N. Yoshida, N. Murakami, K. Kawata, H. Ishizaki, M. Tanaka, Molecular biology of the cell **18**(5), 1701 (2007).
[17] Z. Xu, J. Chen, L. Shao, W. Ma, Tumor Biology **36**(10), 8047 (2015).
[18] R. I. Freshney, International journal of cancer **126**(1), 302 (2010).
[19] S. Rodin, L. Antonsson, O. Hovatta, K. Tryggvason, Nature protocols **9**(10), 2354 (2014).
[20] N. Zare-Feizabadi, Z. Amiri-Tehranizadeh, P. Sharifi-Rad, P. Mokaberi, DNA and Cell Biology **40**(8), 1039 (2021).
[21] Z. Yan, Q. Sun, H. Zhang, Y. Han, J. Qiao, M. Niu, S. Zhu, K. Zhao, Q. Wu, Oncology Letters **18**(5), 5139 (2019).
[22] K. McLoughlin, E. Pedrini, M. MacMahon, J. Guduric, Frontiers in medicine **11**, 10 (2019).
[23] K. Livak, T. Schmittgen, methods **25**(4), 402 (2001).
[24] Y. Dasgupta, M. Koptyra, G. Hoser, K. Kantekure, D. Roy, Blood **127**(17), 2131 (2016).
[25] Z. Du, C. M. Lovly, Mol Cancer **17**(58), 18 (2018).
[26] K. Yoshida, K. Yamaguchi, T. Natsume, Nat Cell Biol **7**, 278 (2005).
[27] J. Gong, A. Costanzo, H. Q. Yang, Nature **399**, 806 (2010).
[28] Y. Shaul, M. Ben-Yehoyada , Cell Res **15**(1), 33 (2005).

[29] X. Meng, University of Warwick **3**, 65 (2018).
[30] Z. X. Xu, A. Timanova-Atanasova, R. X. Zhao, Mol Cell Biol 23(12), 4247 (2003).

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ارتفاع مستوى التعبير الجيني لجين ABL1 نتيجة لرفع التعبير الجيني الخارجة لجين PML-II

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معلومات البحث

الاستلام ١٥ آذار ٢٠٢٢
القبول ٢٩ اذار ٢٠٢٢
النشر ٣١ تموز ٢٠٢٢

الكلمات المفتاحية

فيروس الورم الحليمي, جين الـ PML تحول الخلايا, الموت المبرمج , الجينات الكابحة للورم البروتينات المسببة للاورام..

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الملخص

تشارك العديد من النظائر البروتينبه لبروتينات ابيضاض الدم النخاعي (PML)في مجموعة واسعة من الوظائف الخلوية ، بما في ذلك الاستجابات المناعية الفطرية ، والنسخ الجيني ، والاستماتة. Adenoviridae هي فيروسات غير مغلفة يمكنها أن تعطل الأنظمة الخلوية التي تتحكم في تقدم دورة الخلية وموت الخلايا المبرمج حللت الدراسة التعبير الجيني لـ ABL1 في الخلايا المصابة بالفيروس الغدي والبلازميدات التعبير عن PML. تم استزراع خلايا من خط خلايا سرطان الثدي البشري (MCF-7) في ٢٤ لوحة جيدة في ٥٠٠ ميكرولتر من Dulbecco's Modified Eagle Medium (DMEM) لكل حفرة ، وبعد ٢٤ ساعة ، تم حصاد الخلايا واستخدامها في الدراسة. تم تقسيم الطبق المكون من ٢٤ حفره إلى ستة صفوف ، والتي كانت على النحو التالي: تم اضافة ١,٢ مل من العامل المساعد لادخال البلازمد DNA fection و ٠,٢٥ مايكروغرام من البلازميد Ad-5 ، و PML-II ، و Ad-5 ، و PML-II ، وكاشف تعداء الحمض النووي ، فقط وسط خال من المصل. كشفت نتائج اختبار التعبير الجيني BCR-ABL عن زيادة بمقدار ثلاثة أضعاف في تعبير -ABL في عينات Ad5 و PML المنقولة مقارنةً بمجموعة التحكم. أوصت الدراسة بالتعبير عن الجينات الأخرى التي لها دور في مسارات أخرى مضادة للورم في التعبير الجيني على مستوى البروتين ، والتحقيق في تأثير النظائر البروتينية لـ PML الأخرى..

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