Human Adenovirus Type 5 Infection and Tumor Suppressor Factors

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Abstract

Several promyelocytic leukemia proteins (PML) isoforms are involved in a wide range of cellular functions, including innate immunological responses, gene transcription, and apoptosis. Adenoviridae are non-enveloped viruses that can disrupt cellular systems that control cell cycle progression and apoptosis, as well as those that affect the synthesis of mRNA viruses that cause cancer. The study analyzed the gene expression of IFN (A fused gene formed when pieces of chromosomes 9 and 22 break off and trade places), into cells transfected with adenovirus and PML 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 in this study. The 24-well plate was split into six rows, which were as follows: Each 6-well row was challenged with 1.2 mL of Ad-5 plasmid, PML-II, Ad-5, and PML-II, DNA transfection reagent, only serum-free medium.

The results the IFN gene expression assay revealed the threefold increase in IFN expression in transfected Ad5 and PML samples compared to the control. The study has recommended the expression of other genes that are implicated in other anti-tumor pathways the gene expression at the protein level, investigating the effect of other PML isoforms.

Keywords: Adenovirus type 5, PML gene, Transform cells, apoptosis, Tumor suppressor gene, Oncogenic protein, Transfection. IFN

1. Introduction

Adenovirus causes a wide range of infections in respiratory tract, gastrointestinal tract conjunctiva, and rarely it causes 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 [8], and one of the causes of cellular transformation [9].

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 [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 [3]. 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 [14, 15]. 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 [16].

2. Aim of the study

Suppress the role of adenovirus in transforming cells through PML gene overexpression.

3. Materials and Methods

Cell Culture

Type of cell line used in the study MCF7, PC3. In vitro maintenance of cell lines. The cell lines are maintained by 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

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from DMEM medium and pipetted a few times to disaggregate the clumps then at the appropriate concentration into a culture.

Viability of Cells

Cell lines are grown in DMEM which contains 10% fetal bovine serum at 37 ° C and 100% relative humidity. When monolayer cells are detached by trypsin-EDTA to obtain a single cell suspension, the cell viability is measured using a hem cytometer.

Transfection

The gene IFN using DNA transfection. Cells were plated at a density of 0.5 x 10^6 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 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.

Evaluate gene expression

Isolation of total RNA from cell lines

The isolation of RNA from cell line was achieved using FavorPrep ™. Taiwan (Cat No.: FATRK001, 100) according to the manufacture instructions:

A range of 1 – 5 million cells were collected by spinning down at 14000 rpm for 1 min at 4 °C, 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 vortexing.

The FARB Mini columns were moved to a collection tube and transfer the ethanol containing sample mixtures to the FARB Mini column then centrifuged at 14,000 rpm for 1 min, the flow- through was discarded and the FARB Mini columns were moved back to the collection tube.

RNA-containing columns were washed with 500 µl of washing buffer 1, the FARB Mini column and then spun down for 1 min at 14,000 rpm. The flow-through was discarded and the FARB Mini columns were transferred back to the collection tube.

The columns were washed twice with 750 µl of wash buffer 2 and were centrifuged at 14,000 rpm for 1 min, the flow through was removed and then the columns moved back to the collection tube. Step 8 was repeated for one more wash. The columns were dried at 14,000 rpm for an additional 3 min. The pure RNA was eluted using 50 µl of elution buffer. The columns were left for 1 min at room temperature. The columns were left for 1 min at room temperature then spun down at 14,000 rpm for 1 min to collect the RNA and stored at -70° C.

Converting RNA to cDNA

To convert RNA to cDNA Accupower RocketScript ^{RT} Premix from (Bioneer, Korea), Cat No. : K-2101) was used relying on reverse transcriptase enzymes.

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.

Gene expression

Real time PCR reaction

The type of cell lines used in the study were obtained from cell culture Table (1). In order to assess the gene expression of the BCR-ABL1 and GAPDH and GAPDH (which was used as endogenous calibrator gene). 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 BCR-ABL specific primers (Table 2) in Ad5, PML, Ad5 and PML, transfected and control treatments. Each sample was in 3 performed in triplicat 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	[17].
2-	PC3	Prostate cancer	Human	[18].

Table (2): Sequences of BCR-ABL and GAPDH primers.

Gene		sequences	Tm	Reference
IFN	F.	5′-ATTGCCTCAAGGACAGGATG3′	82 ºC	[19].
	R.	5′-GGCCTTCAGGTAATGCAGAA-3′		
GAPDH	F	5′- GGCCTCCAAGGAGTAAGAC-3′	83 ºC	[20].
	R	5′-CCCCTCTTCAAGGGGTCTAC- 3′		

Relative gene expression

The relative gene expression analysis was performed using Livak et al. [21] method (2−ΔΔCT).

4. Statistical Analysis

The PML gene overexpression were provided as the mean standard deviation (mean ± SD) in this research. We used independent sample t-tests and ANOVA to assess the Gene expression evaluation of a tumor suppressor gene We used univariate and multivariate analysis to Increases its antagonistic ability against adenovirus type 5. We used receiver operating characteristic analysis to assess the diagnostic performance of the researched parameters. Using Microsoft Excel, we edited, sorted, coded, classified, and tabulated data. For all statistical studies,

we used IBM SPSS (version 26.0). At a p-value of less than 0.05, we regarded statistical differences or relationships to be significant.

5. The results

3.1 Gene expression

3.1.1 Specificity and amplification curves of gene specific primers

In order to assess the gene expression of the IFN 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.

Relative gene expression of IFN

The results showed that IFN expression in Ad5 and PML transfected samples up-regulated to 3 fold compared to the control sample.

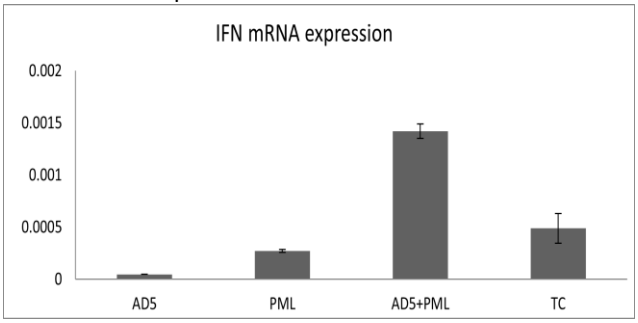

*Figure (1): IFN relative expression level with or without the overexpression of Ad5 or PML or Ad5 and PML plasmids in MCF7. Equivalent cultures of MCF7 were transfected with Ad5, PML, Ad5 and PML, Lipofectamine or left without any treatment. Total RNA was extracted at 24 hours post transfection, reverse 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.*

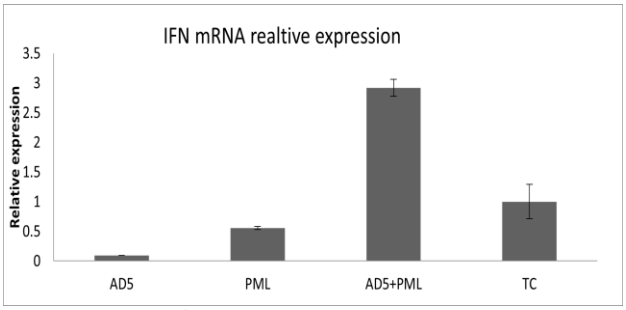

*Figure (4): IFN fold change analysis with or without the overexpression of Ad5 or PML or Ad5 and PML plasmids in MCF7. Equivalent cultures of MCF7 were transfected with Ad5, PML, Ad5 and PML, Lipofectamine or left without any treatment. Total RNA was extracted at 24 hours post transfection, reverse 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 =0.023662).*

6. Discussion

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].

PML functions in tumor suppression

Several cellular actions of the PML protein are linked to its anti-tumor effect. For example, PML activates the pro-apoptotic p53 function 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 [14, 15]. 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 [16].

IFN

Transfected, Ad5, PML, Ad5 and PML, and control samples were tested for BCR-ABL gene expression. In comparison to the control sample, IFN gene expression was shown to be 3-fold increase in Ad5 and PML transfected samples.

MYC which is a proto-oncogene component of works as a transcription factor controls apoptosis [22]. And the cell cycle in Chronic myeloid leukemia CML cells and is associated with the development of leukemia [23]. likely that apoptosis activation is increased in MCF-7 cells after treatment with the PML pathway, as long as IFN 3 fold-regulation relative to our control sample is suppressed.

Cell death decreases after IFN-γ stimulation when PML-II is absent. Induction of apoptosis by IFNs is more effective when PML is present [24], and also suggests that PML-II is one of the important isoforms involved in IFNα-mediated apoptosis. In addition, PML depletion decreases TRAIL expression in hepatocellular carcinoma cells, which accordingly, PML-II knockdown reduces gene expression [23, 25].

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