

Nonlinear Modeling on Protein Kinase C(PKC)-Epsilon Inverse Regulation of Stress Fibers in Oncogenically Transformed Cells

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Abstract

Phorbol 12-myristate 13-acetate (PMA), a tumor promoter, provides effective means of reversibly converting tissue culture cells to a state which resembles that of transformed cells. PKC isozymes can be activated by PMA and cause reorganization of actin-containing structures. The purpose of the current research is to analyze the pattern in which PKC mediates dissolution of stress fibers (SFs) by a mathematical model. Several mathematical models were tried, including linear and nonlinear approximations, in order to establish the relationship between PKC- ϵ and SF accumulation in 1000W cells. Applying a non-linear approximation and using a bisection algorithm, an exponential function was derived to show how PKC- ϵ inversely controlled SFs. We use RNA interference (RNAi) technology to knock down the PKC- ϵ expression in order to validate this mathematical model. In the time course of SF formation following PMA exposure, a special feature of parallel arrays showed in cells with PKC- ϵ siRNA transfection.

1. Introduction

PKC is a family of serine, threonine kinases consisting of isozymes, α , β I, β II, γ , δ , ϵ , η , μ , θ , λ and ζ . With the exceptions of λ (t) and ζ PKCs conduct signaling downstream of both tyrosine kinase receptors and G-protein coupled receptors. Recent evidence [1] showed that PKC- ϵ was overexpressed in androgen-independent lines of prostatic epithelial cells. Other evidence [2] showed PKC- ϵ was linked to integrin β 1 through interaction with RACK, and associated with F-actin via its actin-binding motif, thereby mediating increased adhesion and mobility. The evidence from the Heckman's group indicated PKC- ϵ can decrease the SF formation in oncogenically

transformed cells. It is possible that the structure is affected by binding with actin in the association of adhesion sites [4]. The objective of this paper is to analyze the pattern in which PKC mediates dissolution of SFs by a mathematical model.

2. Methods

2.1. Mathematical modeling

The PKC- ϵ content in cells with PMA treatment and SF counts were published elsewhere [3]. Based on the assumption that the rate of PKC- ϵ decay is proportional to the PKC- ϵ level in the cell, we assumed a half-life of 24 hours in order to calculate PKC- ϵ contents in 1000W cells treated with anti-PKC- ϵ oligodeoxynucleotide (antisense) reagent. Matlab5.2 (MATLAB The language of technical computing, Math Works Inc., 1998) and Fathom1.1 (Dynamic statistics software, KLP Technologies, 2001) software were used to do the linear approximation. In nonlinear approximation, two C++ programs were developed by Li (unpublished data) to find the solution by the application of bisection algorithm.

2.2 siRNA transfection

The 1000W cell line, derived from the tracheal epithelium of a Fisher 344 rat, was used as an *in vitro* model for bronchogenic carcinoma. The cell population was nontumorigenic in initial tests in immune-suppressed, syngeneic animals, but positive after more than 40 passages *in vitro*. Cells were plated at a density of $3-4 \times 10^5$ per 60 mm dish in 3 ml of WIHC medium with FBS and antibiotics. Cells were incubated under normal growth conditions for 9 hours to allow attachment to dishes. The siRNA transfection follows Qiagen's RNAiFect transfection handbook. Following transfection, cells were treated with 2nM PMA and collected at various time intervals.

3. Results

The original data of PKC-ε and SF-forming cells seems to follow the curve of the following function:

$F(x) = F_0 * e^{-a(x_i - x_0)}$, where F is the percentage of SF-forming cells and x is the content of PKC-ε. By using the linear approximation software from Matlab and Fathom, we got two functions respectively:
 $F(x) = 0.3806 * e^{-0.3051(x-0.387)}$ and
 $F(x) = 0.6833 * e^{-0.727(x-0.387)}$.

Nonlinear approximation:

Applying least-square method, we want to get

$$\text{Min}_a \sum_{i=1}^n (y_i - f_0 e^{-a(x_i - x_0)})^2$$

So, we need to find the first derivative of the above equal to 0.

$$g(a) = \sum_{i=1}^n (y_i - f_0 e^{-a(x_i - x_0)}) e^{-a(x_i - x_0)} (x_i - x_0) = 0$$

Bisection algorithm is used to find a solution to the above function g(a) on the interval [0.84, 0.85], where g(0.84) and g(0.85) have opposite signs. The final approximation function is:

$$F(x) = 0.6833 * e^{-0.8412(x-0.387)}$$

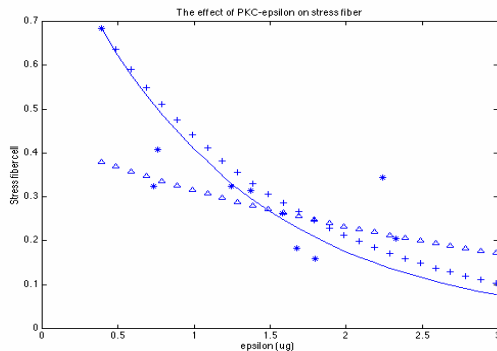


Fig.1. Mathematical model of the relationship between PKC-ε content and SF formation in 1000W cells. Compared to Matlab linear approximation (Δ) and Fathom linear approximation (+), nonlinear approximation (-) fits the original data (*) best.

Compared to scrambled sequence siRNA, cells with treatment of specific siRNA against PKC-ε showed SF formation of parallel arrays. The formation of parallel arrays appears correlated with a decline in the number of protrusions from the cell surface described elsewhere [5].

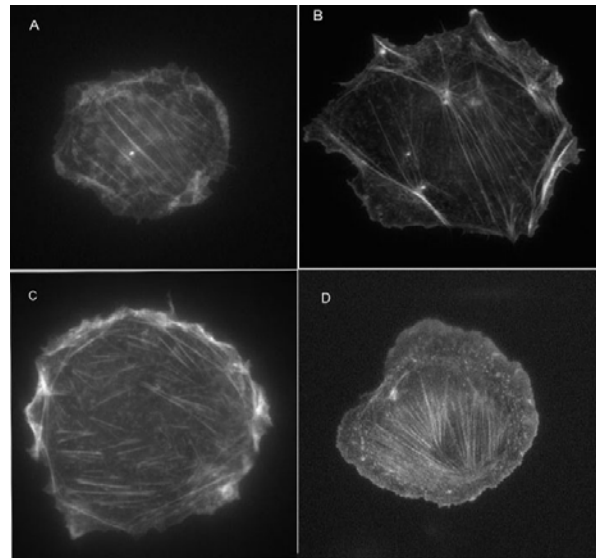


Fig.2. Characteristic appearance of 1000W cells after siRNA transfection. A) The appearance of cell exposed to random sequence siRNA is similar to that of unexposed cells at the peak of SF formation. In cells exposed to specific siRNA against PKC-ε (see B-D), PMA induces extensive SF formation, and often causes formation of parallel arrays. B) Treatment with PMA for 2 h, C) Treatment with PMA for 5 h, D) Treatment with PMA for 10 h. Magnifications: x1,100.

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