

## RESEARCH ARTICLE

# Mathematical Modeling of E6-p53 interactions in Cervical Cancer

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### Abstract

**Background:** Cervical cancer is the third most common cancer in women throughout the world. The human papillomavirus (HPV) E6 viral protein plays an essential role in proteasomal degradation of the cancer suppressant protein p53. As a result, p53 negative regulation and apoptosis relevant activities are abrogated, facilitating development of cervical cancer. **Methods:** A mathematical model of E6-p53 interactions was developed using mathematical laws. In-silico simulations were carried out on CellDesigner and as a test case the small molecule drug RITA was considered for its ability to rescue the functions of tumor suppressor p53 by inhibiting E6 mediated proteasomal degradation. **Results:** Using a computational model we scrutinized how p53 responds to RITA, and chemical reactions of this small molecule drug were incorporated to perceive the full effects. The evolved strategy allowed the p53 response and rescue of its tumor suppressor function to be delineated, RITA being found to block p53 interactions with E6 associated proteins. **Conclusion:** We could develop a model of E6-p53 interactions with incorporation of actions of the small molecule drug RITA. Suppression of E6 associated proteins by RITA induces accumulation of tumor suppressant p53. Using CellDesigner to encode the model ensured that it can be easily modified and extended as more data become available. This strategy should play an effective role in the development of therapies against cancer.

**Keywords:** Cervical cancer- E6- p53- small molecule drug- RITA- mathematical modeling

*Asian Pac J Cancer Prev*, **18** (4), 1057-1061

### Introduction

Throughout the world the second largest cancer in females is cervical cancer. Every year approximately 530,000 new cases of cervical cancer are diagnosed. In worldwide almost 275,000 women die due to this cancer. This ratio of deaths in women is 80% which mostly occur in developing countries (Beaudenon and Huibregtse, 2008). Cervical carcinoma in Pakistan is the fourth most common cancer in female. Double burden of diseases are faced by Pakistan like other developing countries. In Pakistan the cervical cancer is low as compared to the various western countries but the rate of mortality is higher (Badar et al., 2007).

Human papillomavirus (HPV) is the widespread sexually transmitted disease around the globe. In sexually active female it presents up to 75 % at early or late stages. While HPV infection is prevalent, a few people still be not aware of they are infected as the signs are not very clear. It is not yet understood that all cervical cancers (99.7%) are the consequence of earlier infection with one or more of the oncogenic types. It has been reported that the ratio of precancerous changes in cervical cancer tissue is 10% about up to 1 million females are infected. Among these,

about 8% will grow early cancer limited to the entire epithelial layer of the uterine cervix (Carcinoma in Situ; CIS), and only some will show invasive cancer until the precancerous lesions are identified and treat (Alam et al., 2008).

Numerous factors are implicated in cervical cancer risks, such as early marriage and pregnancy, sexual promiscuity, oral contraceptive, immune-compromised state, sexually-transmitted disease, human papillomavirus (HPV), and a number of oncogenes (Castellsagué and Muñoz, 2003). The viral genome of HPV encodes eight genes of which E6 and E7 have transforming properties. E6 and E7 have pleiotropic role such as, transmembrane signaling, cell cycle regulation, immortalization of primary cell line and regulation of chromosomal stability. The tumor suppressor's p53 and pRB are associated with high-risk HPV E6 and E7 oncoproteins, respectively, have been suggested as a mechanism by which these viral proteins induce tumors. The E6 oncoprotein degrade p53 and endorse cell proliferation through trimeric complex formation including E6, p53 and the E6-AP (E6-associated protein) (Kim et al., 2012).

The cell cycle is negatively regulates by p53 gene and thus lose the normal function and do not suppress

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the tumor; thus perturbing the control of cell cycle progression, which results to enhanced tumor development (Hollstein et al., 1991; Levine et al., 1991). The cell cycle checkpoints G1/S and G2/M phase no longer regulate by Rb and p53 and chromosomal duplications, centrosomal abnormalities occurred in infected cells (Michnov et al., 2012). Generally, it is accepted that the oncogenic behavior of E6 is independent to p53 and it contributes to the virus induced cellular transformation through targeting p53 for degradation.

In our research study, we develop mathematical model and simulate the chemical reactions of p53 pathway which are infected by E6 viral protein in cervical cancer. We also examine the E6-p53 interaction by using a computational model how p53 responds to drug RITA, chemical reaction of small molecule drug RITA is incorporated to perceive the full effect. Consequently, there are other important cellular targets for HPV E6 proteins, in the pathogenesis of HPV the E6-p53 interaction having fundamental significance and its association represent a significant therapeutic target for the control and eradication of a number of important human cancers (Lee and Tameru, 2012). In drug designing the pharmacophore model seems to be a very helpful tool serving in the designing and development of new lead compounds, there is a need to design and develop new drugs with minimal side effects and improved efficacy for cancer (Haseeb and Hussain, 2015).

## Materials and Methods

It was found out that integrative model of E6, E6AP and p53 for cervical cancer was not reported. Therefore, the combinatory model was generated from different literatures to investigate the role of E6-p53 interactions and their associated proteins in cervical cancer, as shown in (Figure 1) (Thomas et al., 1999). The chemical reactions were formulated from integrative model by using mathematical laws such as, Mass action and Michaelis–Menten kinetics (Michaelis and Menten, 1913).

It is shown that small molecule drugs playing a key role in the inhibition of oncogenic proteins. In our previous study we used small molecule drug Nutlin to suppress the interaction of Mdm2 and p53 to revive the function of tumor suppressant p53 (Haseeb et al., 2017). Almost, similar strategy was applied in this study and small molecule drug RITA is used for the inhibition of E6-p53 interaction. The chemical reaction for drug RITA was generated and applied on integrative model.

In Silico simulation are carried out on CellDesigner (Funahashi et al., 2003). CellDesigner is a very powerful tool for biological network generation and simulation. Implementation of chemical reactions are performed in CellDesigner by applying different parameters and investigate the behavior of E6-p53 interaction.

### Chemical Reactions

1.  $E1 + Ub + ATP \rightarrow E1\_Ub + AMP$  (Proctor et al., 2007).

2.  $E2 + E1\_Ub \rightarrow E2\_Ub + E1$  (Proctor et al., 2007).

3.  $E6 + E6AP \rightarrow E6\_E6AP$

(4a).  $E6\_E6AP + P53 \rightarrow E6\_p53 + E6AP$  (Inactivation of p53 in low HPV types)

(4b).  $P53 + E6\_E6AP + E2\_Ub \rightarrow [P53\_Ub (m) \_E6\_E6AP] + E2$  (Degradation in high HPV types)

5.  $[P53\_Ub (m) \_E6\_E6AP] + E2\_Ub \rightarrow P53\_Ub (p) + E6\_E6AP + E2$

6.  $P53\_Ub (p) + Proteasome \rightarrow P53\_Ub (p) \_proteasome$

7.  $P53\_Ub (p) \_Proteasome + ATP \rightarrow P53degraded + Ub (p) + proteasome + ADP$

8.  $E6\_E6AP\_P53 + Rk1 p53\_R + E6 + E6AP$

Small molecule drug RITA is used to rescue the p53 function in cervical cancer. RITA block the activity of E6 mediated degradation and reactivate the p53 activity. In reaction the small molecule RITA binds with p53 by a constant rate k1. It inhibits the E6-p53 interaction and the concentration of p53 starts increasing and does not degrade.

## Results

We have built a mathematical model of the cervical cancer pathway. The model was simulated and its initial amounts and parameters were chosen. In the cervical cancer pathway, the model reactions leading to the first ubiquitination step, E1 is an activating enzyme which activates the Ub protein and makes a dimer. The initial amount of E1 is 100 molecule and ubiquitin molecule is 4,000 the energy required for this reaction is ATP 10,000 molecule for making the complex of E1Ub. The E1Ub consumed energy of ATP molecules about 6,000 which declines in output simulation graph and the rest of the energy AMP which is released in reaction is shown in graph is increased 4,000 molecules (Figure 2). This reaction takes  $2.0 \times 10^{-4}$  time and the unit is 1/molecule $\times$ sec. The rate of this reaction is  $2.0 \times 10^{-4}$  and used Michaelis–Menten kinetics. Then E1\_Ub complex transfer Ub protein to E2 which is conjugating enzyme. The initial amount of E1\_Ub is 0 and E2 conjugating enzyme is 100 which is taken from the model, which takes  $1.0 \times 10^{-3}$  molecule-1sec-1 and the dimerization of E2\_Ub is increased (Proctor et al., 2007). (Figure 3) indicates the concentration of E6 and E6AP is enhanced. The concentration of E6AP is 100 molecule and law of mass action is used for this reaction. The initial amount of E6 is 50 molecules, we assume for this model and check the degraded p53 with respect to time. The 45% of E6AP is consumed and the E6 viral protein is used and decline from 50 to 0. The product of E6 and E6AP increases from 0 to 45 (Figure 4).

This means that E6 and E6Ap have strong relationship with each other. The inactivation of p53 in low risk HPV types, E6 and E6AP target the p53 and make a complex. The initial concentration of p53 is 5 molecules, the

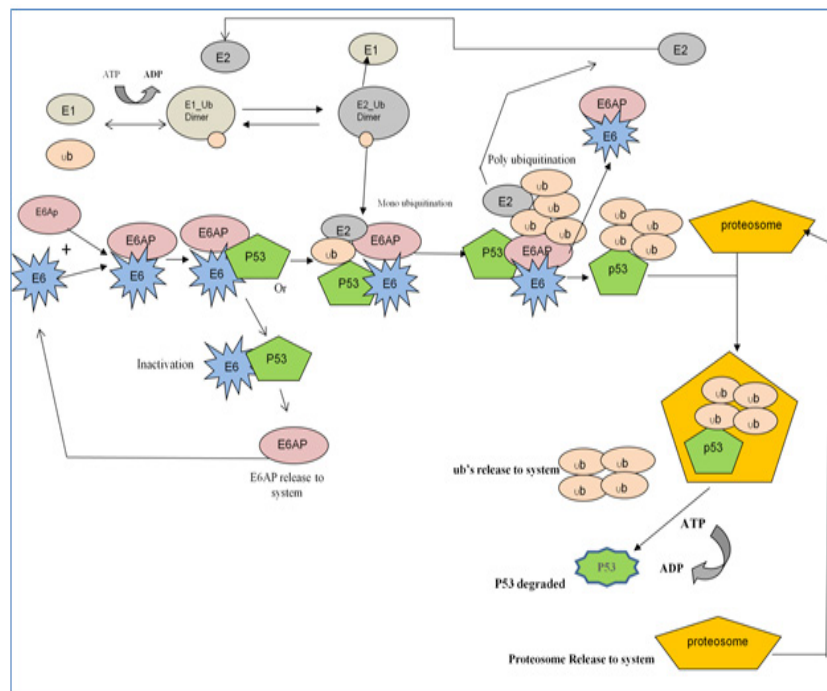


Figure 1. This Figure Shows the IntegrativeModel of E6, p53, E6AP and Their Role in Proteasomal Degradation of p53 and Causing Cervical Cancer. (Thomas et al., 1999; Tommasino et al., 2003; White et al., 2014).

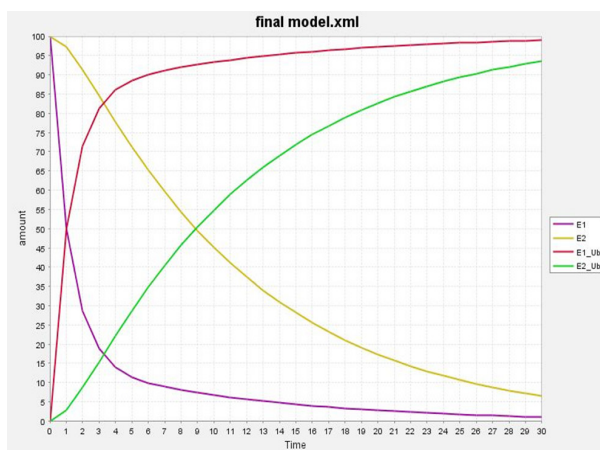


Figure 2. The Concentration of E1\_Ub and E2\_Ub is Increased in Time  $K_{bin} * E1_{Ub}$  and  $\bar{K}_{bin} * E2_{Ub}$

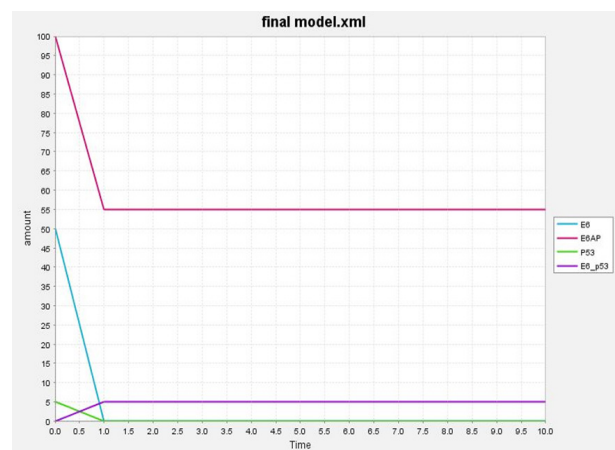


Figure 4. The Degradation Rate of Inactive p53 is Increases (Purple Line) due to the Binding of E6 Viral Protein and the Actual Amount of Active p53 is Decline from 5, Shown in (Green Line).



Figure 3. The Concentration of E6\_E6AP Complex Increased (Yellow Line) with Respect to Term  $k3a * 1$

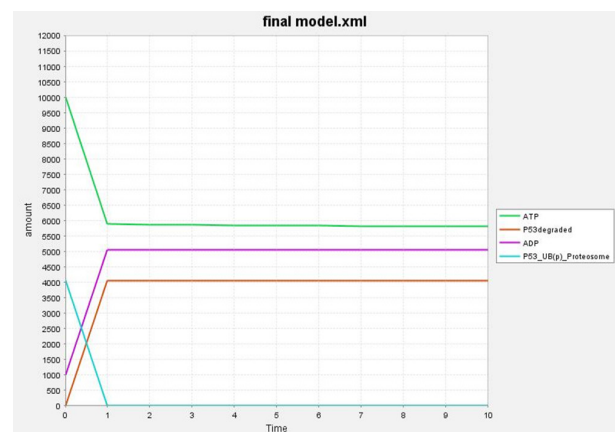


Figure 5. The Degradation Rate of p53 in Cervical Cancer in Presence of Viral Oncogenic Protein E6

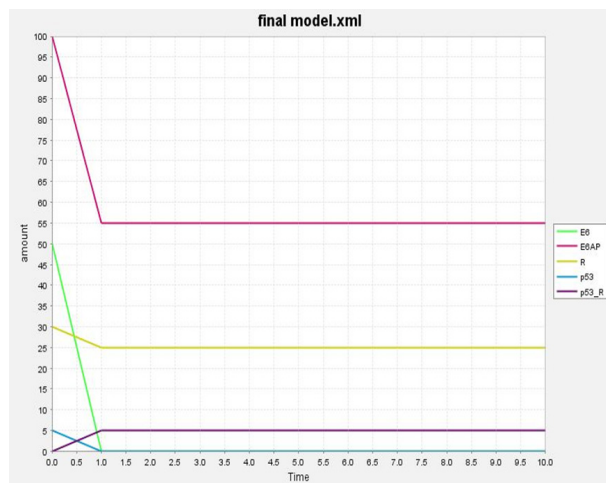


Figure 6. P53 and RITA Complex (Purple) Reached to Its Original Value in Time k1 and the Concentration of E6 and E6AP is Decreased

Table 1. The Initial Amount and Units Which are Given as Follows

Species	Initial amount	Units	References
E1	100	Molecule	
Ub	4,000	Molecule	(Proctor et al., 2007; Proctor and Gray, 2010)
ATP	10,000	Molecule	
E1_Ub	0	Molecule	
AMP	1,000	Molecule	
E2	100	Molecule	Assume
E2_Ub	0	Molecule	
E6	50	Molecule	
E6AP	100	Molecule	(Proctor et al., 2007)
E6_E6AP	0	Molecule	
E6_P53	0	Molecule	
P53	5	Molecule	
P53_Ub(M)_E6_E6AP	0	Molecule	
P53_Ub(P)	0	Molecule	
Proteasome	500	Molecule	
P53_Ub(p)_proteasome	0	Molecule	
Degraded P53	0	Molecule	
ADP	1,000	Molecule	
Ub (P)	0	Molecule	

concentration of p53 is consumed due to the viral E6 and E6AP dimerization. The rate of p53 inactivation is increased due to the binding of E6 viral protein to p53 and free E6AP release to the system and decline as shown in (Figure 4).

The p53 ubiquitination occur through ubiquitin pathway in high HPV risk types. The mono-ubiquitination occurs when the dimer of E6 and E6AP binds to p53 and the conjugating enzyme transfer Ub's protein to the

Table 2. List of Reactions (Reactant and Product), Parameter Values and the Rate Law for the Model

Chemical reaction	Reactant	Product	Rate law	Parameter	Values	Units
E1_Ub binding	E1 + Ub	E1_Ub	Michaelis-Menten kinetics	kbinE1Ub	2.00E-04	1/(molecule*second)
E2_Ub binding	E2 + Ub	E2_Ub	Michaelis-Menten kinetics	KbinE2Ub	0.001	1/(molecule*second)
E6_E6AP dimer	E6+ E6AP	E6_E6AP	Law of mass action	K3a	1	1/(molecule*second)
P53 inactivation	E6_E6AP+P53	E6_p53+E6AP	Law of mass action	K4a	1	1/(molecule*second)
Monoubiquitination	P53+ E6_E6AP+E2_Ub	[P53_Ub(m)_E6_E6AP] + E2	Law of mass action	K4b	1	1/(molecule*second)
Polubiquitination	P53_Ub (m) _E6_E6AP]+ E2_Ub	P53_Ub(p)+	Law of mass action	K5a	1	1/(molecule*second)
Proteasome binding	P53_Ub(p)+ Proteasome	P53_Ub(p)_proteasome	Law of mass action	K6a	1	1/(molecule*second)
Proteasome Activity	P53_Ub(p)_Proteasome+ ATP	P53degraded+ Ub(p)+proteasome + ADP	Law of mass action	K7a	1	1/(molecule*second)



Table 3. List of Initial Conditions and Parameter for Small Molecule Drug RITA

Drug	Parameter	Dose	Reactant	Product	Species	Initial amount
Rita (Zhao et al., 2010)	K1	30	R+E6_E6AP_p53	P53_R+E6+E6AP	P53	5
					E6	50
					E6AP	100
					Rita	30

complex. Numbers of Ub's protein are attached to p53 and then this protein is targeted to the proteasomal degradation.

The ATP energy is required for the p53 degradation when it target proteasome. The amount of ATP energy is 10,000 molecules and this energy is consumed from 10,000 to 6,000. It means that this reaction required 4000 ATP molecule for the degradation of p53 (Figure 5).

The initial concentration is taken 5 molecules for p53 and drug RITA concentration is 30 mg. By targeting the complex p53\_E6\_E6AP on small molecule drug the original concentration of p53 is achieved as shown in (Figure 6).

## Discussion

Our research highlighted the cellular system in cervical cancer. There are many other viral proteins of cervical cancer, which affect different pathways that we have not considered here. For example, E7 is viral oncogenic protein of cervical cancer which also affects the (Rb) tumor suppressor protein and leads to abnormal cell growth and cause tumor formation. This study gives important information about the molecular mechanisms of a system; by applying control system we can inhibit the activity of one component and enhance the other to control such type of diseases like cervical cancer.

In this research the integrative model was proposed and chemical reactions are designed from the proposed model. The chemical reactions were implemented in CellDesigner and the in silico simulation reveal the interaction of E6 and p53. By targeting the complex of viral E6, E6AP and p53 on drug (Rita) the function of tumor suppressor p53 is rescued. The physiological based pharmacokinetics (PBPK) model is needed to define the exact dosage of Rita drug. Therefore, modulating the exact dosage of Rita drug may be a useful therapeutic strategy.

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