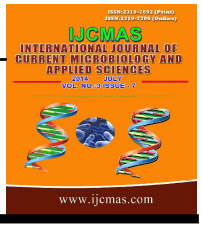
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9(2014) pp. 1045-1058**
http://www.ijcmas.com



Original Research Article

In silico analysis of Costunolide Dependent Responses of ErbB Signaling Pathway

Ramakrishnan Ganesan[1]*, S.Venkatesan[2] and Krishnaswamy Muthuchelian[3]

[1]Department of Microbiology, PK.N. College, Thirumangalam, Madurai, India
[2]Department of Environmental Science, Periyar University, Salem, India
[3]School of Energy Science, Madurai Kamaraj University, Madurai, India
**Corresponding author*

Keywords

ERbB signaling, Erk, Akt, Costunolide, modeling.

A B S T R A C T

During the past decade, our knowledge of molecular mechanisms involved in growth factor signaling has proliferated almost explosively. However, the kinetics and control of information transfer through signaling networks remain poorly understood. Deregulation of ErbB signaling plays a key role in the progression of multiple human cancers. To understand ErbB signaling quantitatively, in this work we combine traditional experiments with computational modeling, building a model that describes how stimulation of ErbB receptor leads to activation of two critical downstream proteins, extracellular-signal-regulated kinase (ERK) and Akt, thus in turn, leads to the cell survival and proliferation respectively. As the time course kinetics of Akt and ERK activities seemed to be transient and complex, we constructed a mathematical simulation model for ErbB induced signaling to explain the dynamics of the regulation mechanism in this signal transduction cascade. We also modeled the impact of the phytochemical compound Costunolide isolated from *Costus speciosus* on this pathway. Results showed that Costunolide was able to inhibit the survival of Akt, but not Erk. Hence, further studies are needed for the costunolide to block both Akt and Erk simultaneously.

Introduction

Molecularly targeted therapies are transforming the treatment of cancer at various levels (1). Small molecule inhibitors that target the cancer dependent enzymes raise the possibility of rational approaches to cancer therapy. The wealth of molecular information from the recent genomics technologies offers a remarkable opportunity for new target discovery (2, 3). Systems level view of perturbed networks or pathways can provide promising therapeutic

ErbB receptor tyrosine kinases play essential roles in cellular proliferation and differentiation, and their deregulated expression or mutation highly correlates with the incidence of certain types of human cancer (4, 5, 6). A complex network of interactions between the activated receptors, recruited proteins, and plasma membrane molecules eventually culminates in the

activation of multiple downstream effectors, including extracellular-signal regulated kinase (ERK) and protein kinase B/Akt, which are implicated in the control of proliferation and survival. Abnormalities within the ErbB signaling network correlate with the development of several cancer types, and multiple drugs that target these defects have been used to treat cancer successfully (7). Knowledge of some specific ErbB signaling network defects associated with tumorigenesis has led to the development of successful cancer treatments. Furthermore, there are instances where potentially drug-sensitive cancers either do not respond and/or eventually become resistant to treatment (8). Improving the efficacy of these targeted treatments requires a more detailed understanding of the mechanisms by which cancer-correlated network properties cause deregulation of the entire ErbB signaling network.

Different ErbB ligands can stimulate different network activation dynamics, and that there is a connection between ligand-dependent activation kinetics and cell fate, to understand how the ErbB signaling network controls cell fate, we must first elucidate the mechanisms that control ligand-dependent activation kinetics. Similarly, understanding ligand-dependent signaling mechanisms is a key step in understanding how the ErbB network's deregulation contributes to tumorogenesis. Because the ErbB signaling system is a highly interconnected, dynamic network containing multiple feedback loops, it is difficult to predict the response of the network solely by qualitative means. It is becoming increasingly clear that quantitative methods are required to understand the mechanisms by which signaling networks function.

There are two major oncogenic pathways such as PI3K pathway and Erk pathway. The ErbB receptors are stimulated upon the binding of their cognate growth factors, which results in the stimulation of multiple downstream signaling cascades. These signaling pathways are important for a wide range of cellular functions including protein synthesis, transcription, angiogenesis, and regulation of the cell cycle, cell proliferation and survival. These two pathways are modeled and simulated using the parameters obtained from the literature. This study, thus, predicts the systems behavior by integrating several levels of useful information.

Materials and methods

Kegg (Kyoto Encyclopedia of Genes and Genomes)

Kegg is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals. It was initiated by the Japanese human genome programme in 1995. The KEGG databases have disease information computerized in 2 forms. Pathway maps and gene/molecule lists. In the KEGG pathway database, there are pathway maps for the molecular systems in both normal and perturbed states. The molecular interaction networks can be constructed from KEGG PATHWAY database. KEGG PATHWAY contains pathway maps, which are manually created from published materials, of metabolism, genetic information processing, environmental information processing, cellular processes, human diseases and drug development.

Cell Designer

All the simulations in this research work were performed using Cell designer software, whose networks are able to link

with simulation and other analysis packages through the systems biology workbench. Cell Designer is a process diagram editor for drawing gene-regulatory and biochemical networks (http://www.celldesigner.org/). Cell Designer also supports simulation and parameter search, which is supported by integration with SBML ODE (SBML Ordinary Differential Equation) Solver, enabling us to simulate through our sophisticated graphical user interface.

Methods

The First step of Pathway modeling is a careful curation of models from existing pathway databases and biochemical data buried in literature. The Information about individual pathways has been taken from Kegg database and drawn in Cell designer. For each and every species we set up the kinetic equations and performed simulation. Vibrant modeling of the disease mechanism is often limited to mechanistic details of the entities available within the pathways. Thus using the quantitative information of reaction rates and the molecular concentration of such entities, we have developed a mathematical model of the ErbB oncogenic pathway. The kinetic parameters and the chemical equations along with the concentration of the each entities obtained from the literature were used to simulate the oncogenic pathways. Simulation was carried out through Cell Designer. For the purpose of simulation, rate constant for Costunolide in the pathway was given a default value 1.0. All other parameters obtained from literature.

Result and Discussion

ERbBR mediated Raf, PI3K signaling pathways are shown in Fig.1. The kinetics (*i.e.* the transient and steady-state behavior) of the cellular response to ErbBR depends on many factors, including the number of receptors displayed on the cell surface, cytoplasm; the concentration of the growth factor, docking, and target proteins; and their initial activity states. The kinetic model emphasizes that the dynamic pattern of signal propagation strongly depends on the relative abundance of molecular factors involved in the ErbBR pathway. Table.1 shows the initial concentration each protein in the pathway. Rate constants of step in the reaction have been depicted in Table 2. Ordinary Differential Equations of each step in the reaction was given in Table3. Table 4 showed the concentration of each protein in cancer condition after a time of 100 sec. Table 5 represented the concentration level of each protein in the cancer pathway after introducing Costunolide.

Fig (1) represented the pictorial representation of ErbB mediated Raf, PI3K signaling pathway. Graphical representation of each protein level present in the diseased pathway was given in Fig (2). In Fig (2), the expression level of Ras, Sos, Mek, PI3K and Erk were not clearly differentiated, which will be clearly depicted in Fig(3). After introducing Costunolide, the expression level of ErbB got decreased and shown in Fig4. But at the same time the concentration of Grb2 and that of Shc increased (Fig5 and Fig12 respectively). Fig 6 showed the inhibition of Raf after administration of Costunolide. Inhibition of PKB/Akt shown in Fig7. Expression level of Erk, Sos, Ras and Mek, after introducing the compound had been given in Fig.8, Fig.9, Fig.10 and Fig.11 respectively.

To evaluate the dynamics of signal transduction pathways, we developed a computational model of the ErbB signaling pathways and also modeled the effect of a phytochemical compound Costunolide isolated from *C.speciosus*, on it. To our knowledge, this is first model to take into account the Costunolide and ErbB receptor

simultaneously.

Table.1 Initial concentration of each protein in the pathway

S.I.	Entities in the pathway	Initial Concentration	References
S1	ErbBR	80	9
S2	Shc	100	17
S3	PI3K	0.006	17
S4	Grb2	0.1	10
S5	SOS	0.5	10
S6	Ras	2	17
S7	Raf	0.5	10
S8	Mek	0.6	10
S9	Erk	0.018	17
S10	PKB/Akt	0.05	10
S11	Costunolide	20	11

Table.2 Rate constant of each reaction in the pathway

Rate Constants	References
K1 = 1.2	12
K2 = 0.01	13
K3 = 1	13
K4 = 50	13
K5 = 0.1	13
K6 = 20	13
K7 = 60	9

Fig.1 Pictorial representation of ERBBR mediated Raf, PI3K signaling pathways

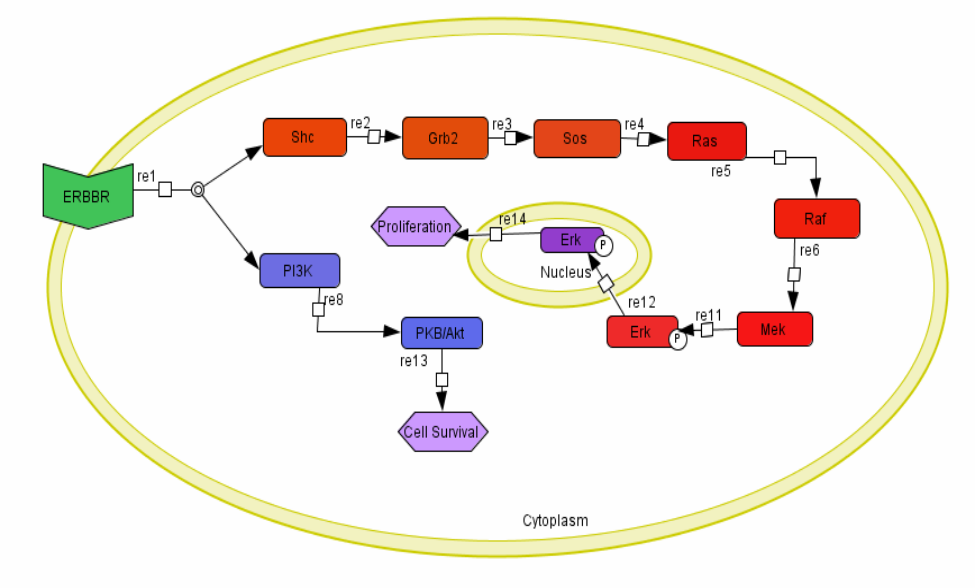


Fig.2. Mutant ErbB (shown in red) Signaling Pathway

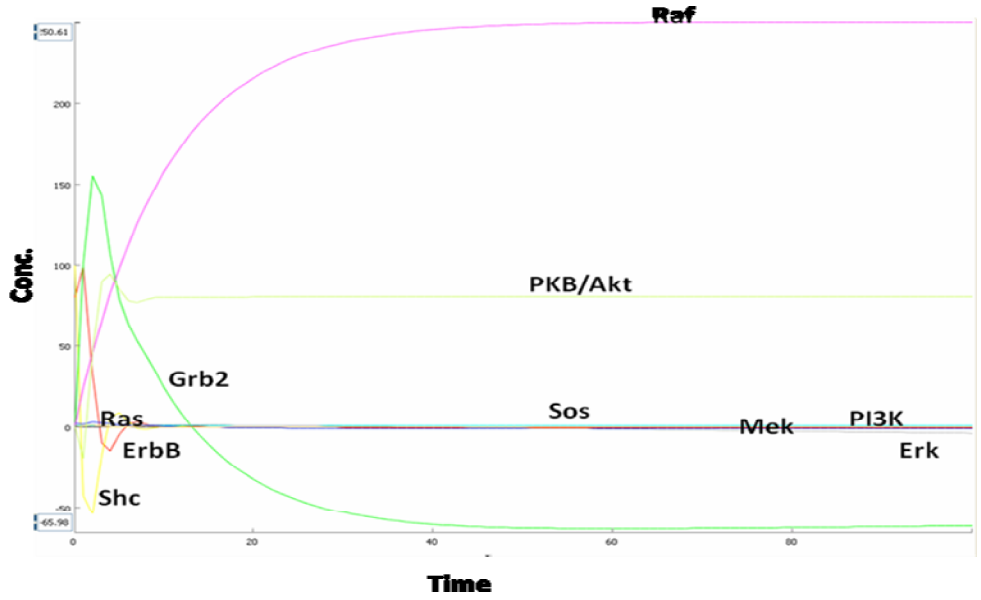


Fig.3 Expression level of Ras, Sos, Mek and Erk protein in ErbB signaling pathway

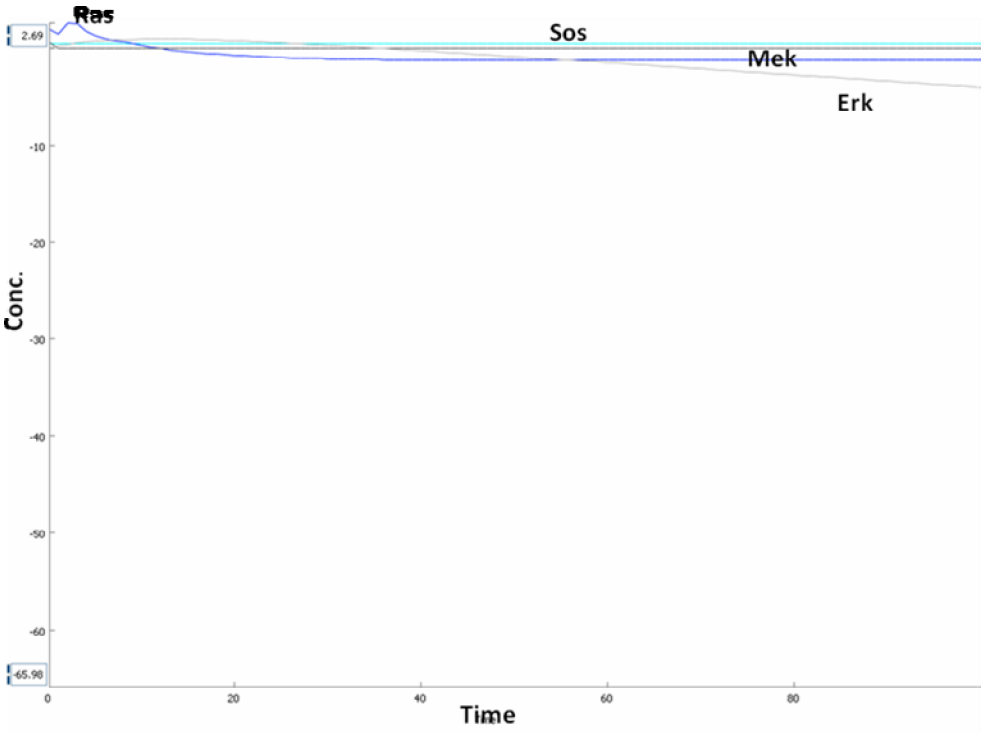


Table.3 ODEs of each reaction in ErbBR pathway

d [ErbBR]/dt = -k1{ [Shc] + [PI3K]}
d [Shc]/dt = -k2 [Grb2] + k1{[ErbBR] – [PI3K]}
d [Grb2]/dt = k2 [Shc]- k3 [SOS]
d [SoS]/dt = k3[Grb2] –k4[Ras]
d[Ras]/dt = k4 [Sos] – k5 [Raf]
d [Raf]/dt = k5 [Ras] – k6[Mek]
d [Erk]/dt = k6 [Mek]
d [Akt/PKB]/dt = k7 [PI3K]
d [Mek]/ dt = k6 [Raf] – k7[Erk]

Table.4 Concentration of each target in the cancer condition

Graph | Table

species | fluxes | parameters | compartments

time / names	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10
0.0	80.0	100.0	0.1	0.5	2.0	0.5	0.6	0.018	0.0060	0.05
1.0	98.7791146...	-42.419805...	141.972599...	0.77152642...	2.0	-17.805935...	0.00500000...	0.41549999...	0.80980755...	-19.532922...
2.0	32.9750983...	-53.918337...	160.069986...	1.28945554...	2.0	40.7812975...	0.00499999...	0.51550000...	1.06555004...	46.0153516...
3.0	-10.031344...	-18.030911...	124.025878...	0.66364094...	2.0	84.4702368...	0.00500000...	0.61549999...	0.36481926...	89.7225248...
4.0	-14.897529...	5.79494685...	96.6523843...	0.12614163...	2.0	93.3215569...	0.00499999...	0.71550000...	-0.1096978...	95.0632276...
5.0	-4.8681710...	8.65639834...	91.2711468...	-0.0033285...	2.0	85.8414543...	0.00499999...	0.81549999...	-0.1708550...	85.0950260...
6.0	2.27676389...	3.19284026...	96.1559554...	0.07918450...	2.0	79.0927558...	0.00500000...	0.91549999...	-0.0644109...	77.8436470...
7.0	3.35079582...	-0.7625290...	100.457681...	0.16307421...	2.0	77.4884776...	0.00500000...	1.01549999...	0.01425921...	76.6909449...
8.0	1.83431998...	-1.3836690...	101.420330...	0.18552819...	2.0	78.5409903...	0.0050	1.11549999...	0.02727500...	78.1944050...
9.0	0.65288981...	-0.5593048...	100.676784...	0.17298598...	2.0	79.5541441...	0.00500000...	1.21549999...	0.01125479...	79.3918553...
10.0	0.43290687...	0.09399996...	99.9640212...	0.15896190...	2.0	79.7476100...	0.0050	1.31549999...	-0.0017223...	79.6248155...
11.0	0.65952421...	0.21986642...	99.7789394...	0.15455526...	2.0	79.4846146...	0.0050	1.41549999...	-0.0043282...	79.4008040...
12.0	0.85375421...	0.09694889...	99.8854000...	0.15607036...	2.0	79.2053264...	0.0050	1.51549999...	-0.0019466...	79.2041924...
13.0	0.89666740...	-0.0103522...	100.000787...	0.15809370...	2.0	79.0523037...	0.0050	1.61549999...	1.82135667...	79.1591504...
14.0	0.86324284...	-0.0347371...	100.034595...	0.15864558...	2.0	78.9757528...	0.0050	1.71549999...	6.82864614...	79.1920742...
15.0	0.83147815...	-0.0166570...	100.019438...	0.15821090...	2.0	78.9050295...	0.0050	1.81549999...	3.33804774...	79.2241880...
16.0	0.82337725...	8.74167342...	100.000753...	0.15765388...	2.0	78.8148409...	0.0050	1.91549999...	-1.3570603...	79.2326363...
17.0	0.82822847...	0.00545425...	99.9946449...	0.15732110...	2.0	78.7118512...	0.0050	2.01550000...	-1.0705949...	79.2278785...
18.0	0.83339592...	0.00283891...	99.9967336...	0.15715218...	2.0	78.6073792...	0.0050	2.11550000...	-5.6791850...	79.2226608...
19.0	0.83488821...	-1.0247482...	99.9997381...	0.15700964...	2.0	78.5058742...	0.0050	2.21550000...	-4.0562989...	79.2211121...
20.0	0.83419821...	-8.5076337...	100.000823...	0.15683333...	2.0	78.4064957...	0.00500000...	2.31550000...	1.66723675...	79.2217851...
21.0	0.83336198...	-4.8036958...	100.000545...	0.15662998...	2.0	78.3074425...	0.0050	2.41550000...	9.59428102...	79.2226284...
22.0	0.83309219...	-1.9735479...	100.000065...	0.15642131...	2.0	78.2079411...	0.0050	2.51550000...	4.89318444...	79.2229073...
23.0	0.83318776...	1.31770403...	99.9998745...	0.15621768...	2.0	78.1080882...	0.00500000...	2.61550000...	-2.5777407...	79.2228148...
24.0	0.83332239...	8.07507464...	99.9999093...	0.15601848...	2.0	78.0081690...	0.0050	2.71550000...	-1.6104087...	79.2226792...
25.0	0.83337044...	6.66440890...	99.9999858...	0.15582031...	2.0	77.9083167...	0.0050	2.81550000...	-1.4783430...	79.2226297...
26.0	0.83335767...	-2.0246723...	100.000018...	0.15562141...	2.0	77.8085222...	0.0050	2.91550000...	3.95298434...	79.2226419...
27.0	0.83333611...	-1.3487698...	100.000014...	0.15542177...	2.0	77.7087406...	0.0050	3.01550000...	2.68608063...	79.2226636...
82.0	0.83333333...	-1.3974158...	100.0	0.14444343...	2.0	72.2197232...	0.0050	8.51549999...	2.73853786...	79.2226666...
83.0	0.83333333...	-1.0497275...	100.0	0.14424383...	2.0	72.1199228...	0.0050	8.61549999...	2.06909280...	79.2226666...
84.0	0.83333333...	-5.3921230...	100.0	0.14404423...	2.0	72.0201224...	0.0050	8.71549999...	1.05743267...	79.2226666...
85.0	0.83333333...	1.58306409...	100.0	0.14384463...	2.0	71.9203220...	0.0050	8.81549999...	-3.0537406...	79.2226666...
86.0	0.83333333...	8.65593053...	100.0	0.14364503...	2.0	71.8205216...	0.0050	8.91549999...	-1.6764597...	79.2226666...
87.0	0.83333333...	1.32417504...	100.0	0.14344543...	2.0	71.7207212...	0.0050	9.01549999...	-2.5924241...	79.2226666...
88.0	0.83333333...	1.81076407...	100.0	0.14324583...	2.0	71.6209208...	0.0050	9.11549999...	-3.5564505...	79.2226666...
89.0	0.83333333...	2.32536015...	100.0	0.14304623...	2.0	71.5211204...	0.0050	9.21549999...	-4.5685389...	79.2226666...
90.0	0.83333333...	2.86796328...	100.0	0.14284663...	2.0	71.4213200...	0.0050	9.31549999...	-5.6286894...	79.2226666...
91.0	0.83333333...	3.43857346...	100.0	0.14264703...	2.0	71.3215196...	0.0050	9.41549999...	-6.7369019...	79.2226666...
92.0	0.83333333...	4.01075503...	100.0	0.14244743...	2.0	71.2217192...	0.0050	9.51549999...	-7.8453294...	79.2226666...
93.0	0.83333333...	4.46736899...	100.0	0.14224782...	2.0	71.1219188...	0.0050	9.61549999...	-8.7419571...	79.2226666...
94.0	0.83333333...	4.90679307...	100.0	0.14204822...	2.0	71.0221184...	0.0050	9.71549999...	-9.6048430...	79.2226666...
95.0	0.83333333...	5.32902727...	100.0	0.14184862...	2.0	70.9223180...	0.0050	9.81549999...	-1.0433987...	79.2226666...
96.0	0.83333333...	5.73407160...	100.0	0.14164902...	2.0	70.8225176...	0.0050	9.91549999...	-1.1229389...	79.2226666...
97.0	0.83333333...	6.12192606...	100.0	0.14144942...	2.0	70.7227172...	0.0050	10.0154999...	-1.1991049...	79.2226666...

Fig.4 Inhibited mutant ERBBR kinetics

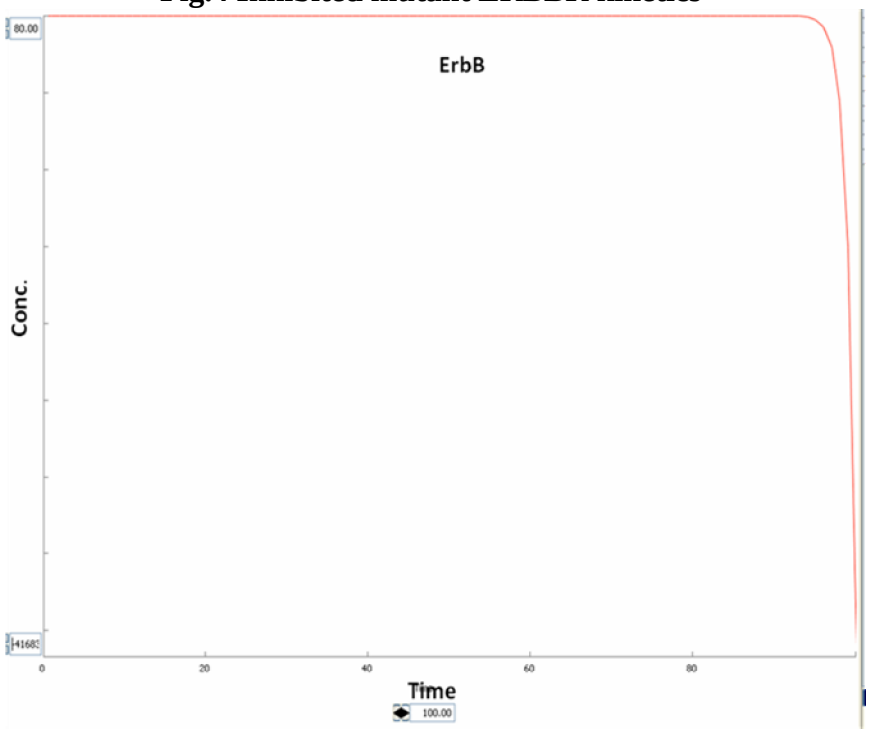


Fig.5 Activation of Grb2 after administration of Costunolide

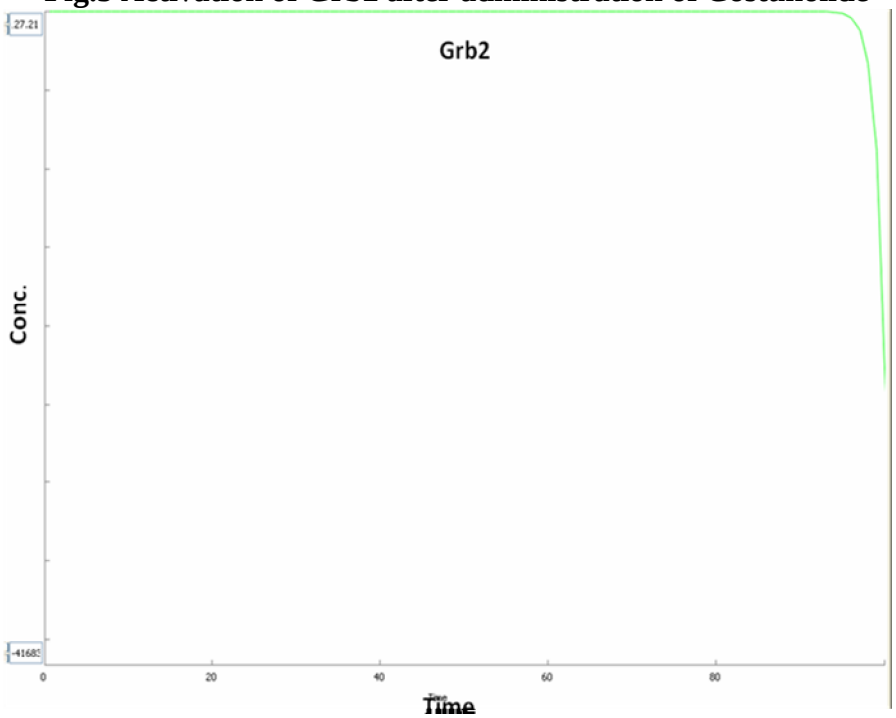


Fig.6 Inhibiton of mutant Raf

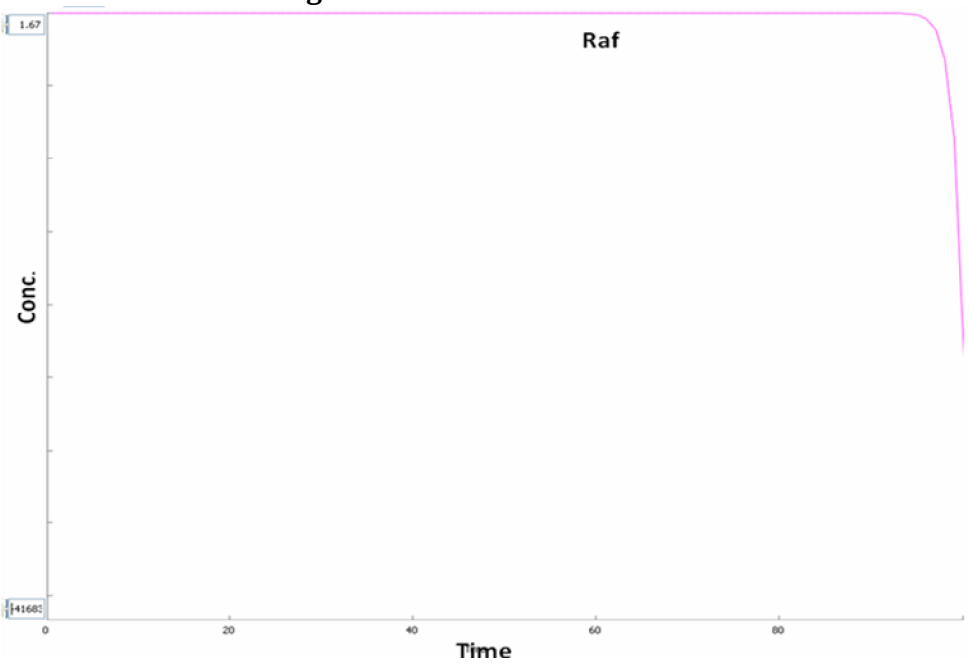


Fig.7 Inhibition of PKB/Akt in the kinetics

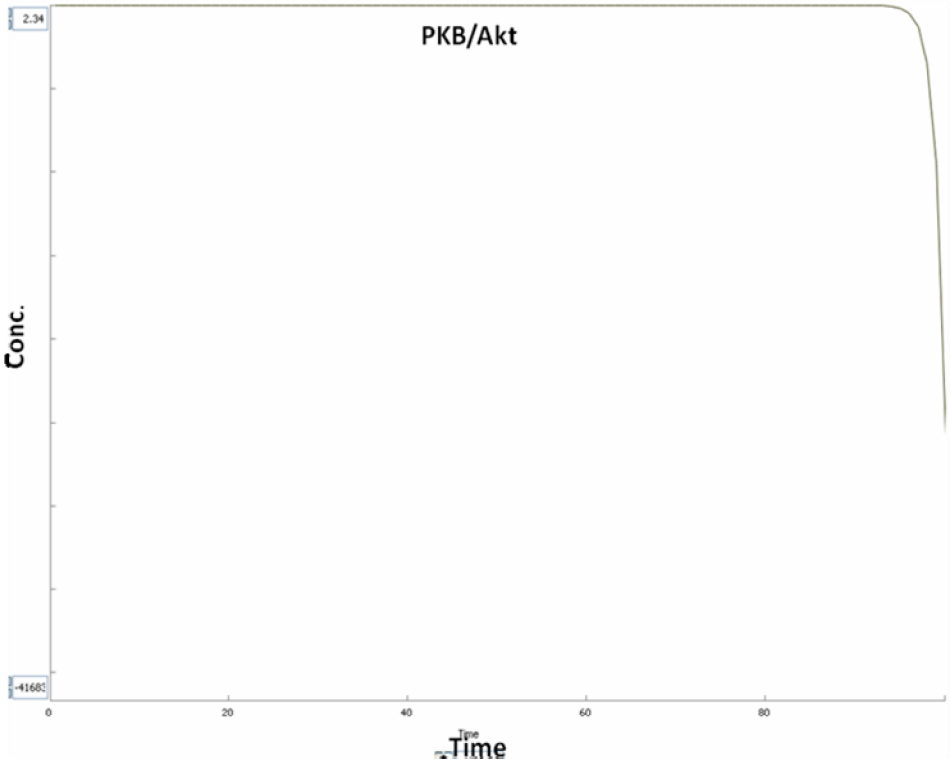


Fig.8 Expression level of Erk in the kinetics, after Costunolide administration

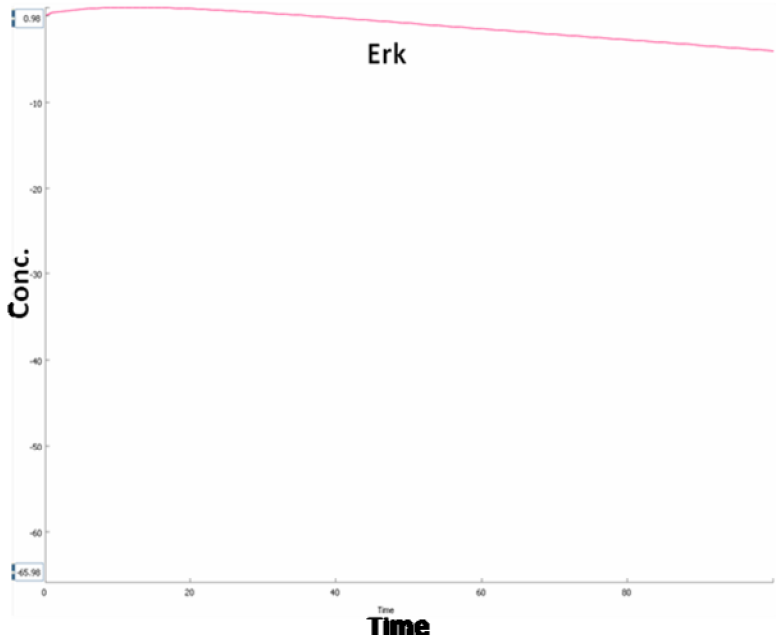


Fig.9 Level of Sos in the pathway after introducing Costunolide

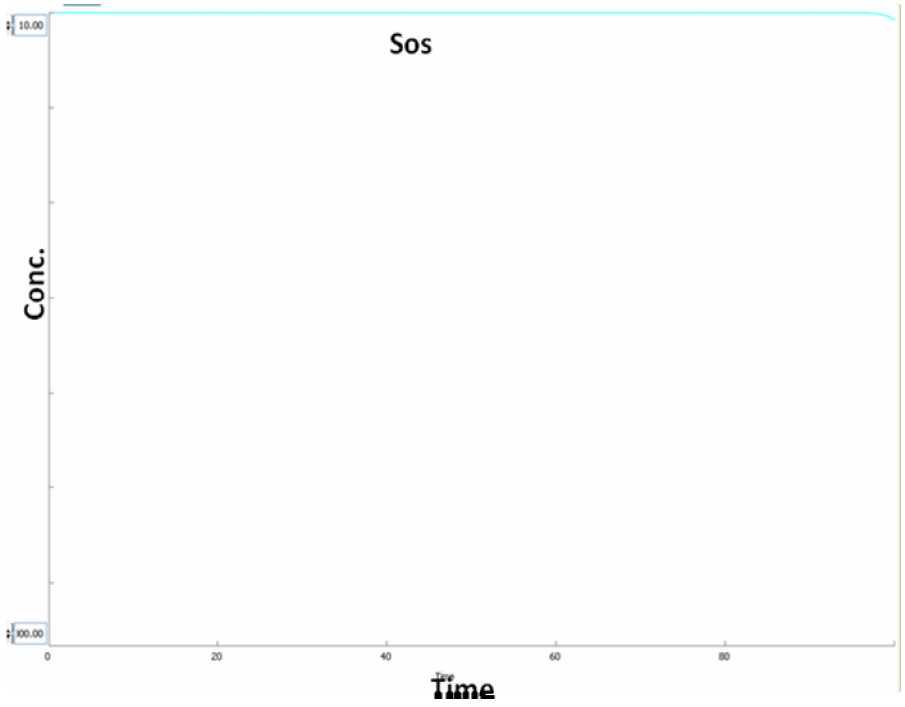


Fig.10 Expression level of Ras in the pathway after administration of Costunolide

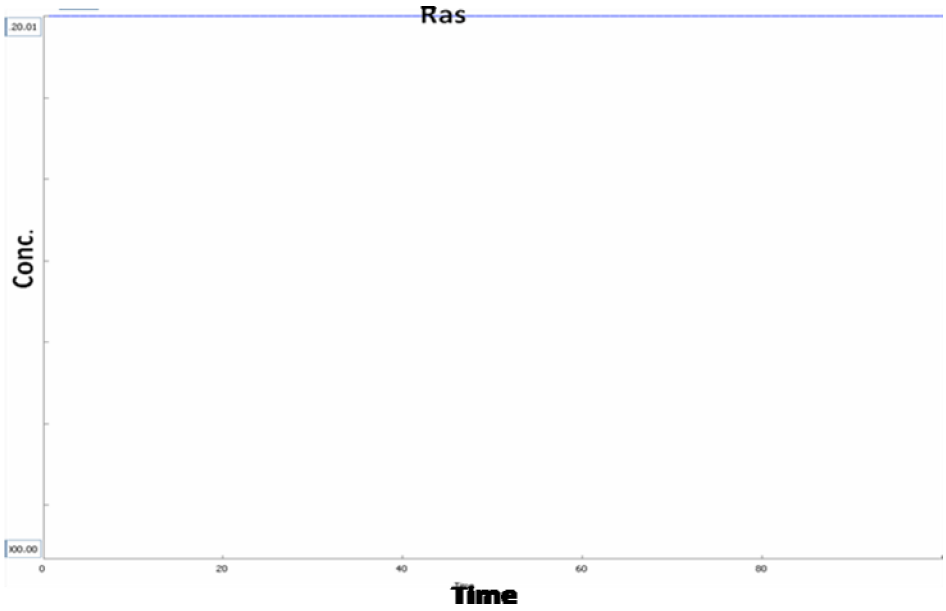


Fig.11. Expression level of Mek in the pathway after introducing Costunolide

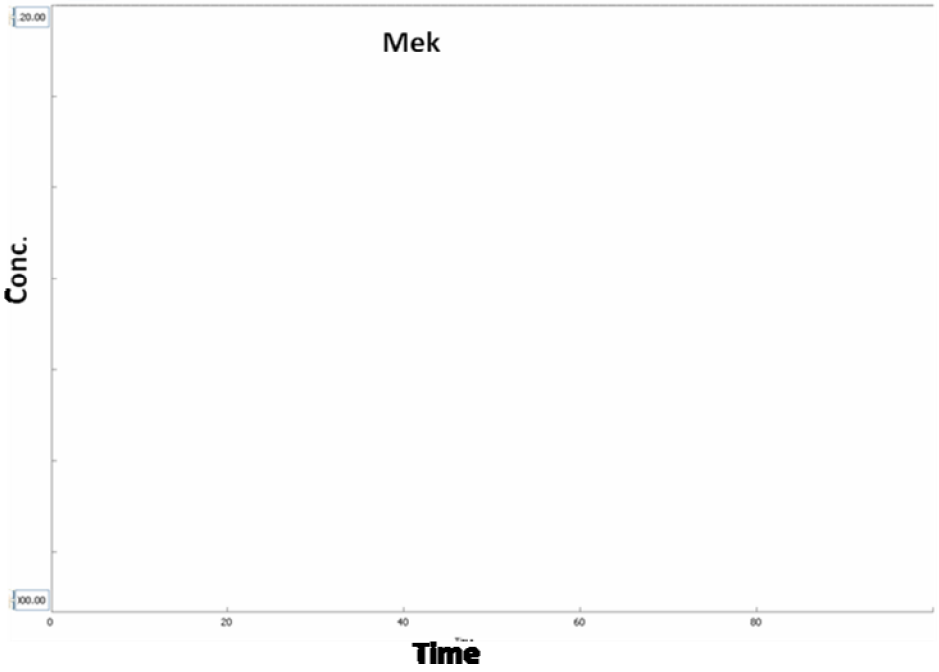


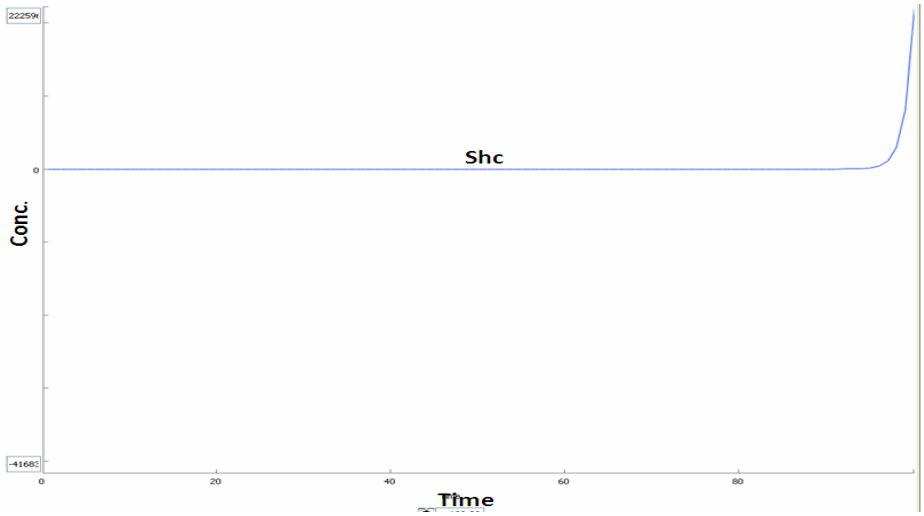
Table.5 Concentration changes after introducing Costunolide to the system

Graph | Table

species | fluxes | parameters | compartments

time / names	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11
0.0	80.0	100.0	0.1	0.5	2.0	0.5	0.6	0.018	0.0060	0.05	25.0
1.0	61.4223948...	-28.322747...	127.207676...	0.47908204...	2.0	-22.445962...	0.00500000...	0.41549999...	0.54214392...	-24.865595...	67.9570568...
2.0	-81.947527...	4.94572497...	96.8224558...	-0.0227317...	2.0	1.67310254...	0.00499999...	0.51550000...	-0.0675952...	2.34464681...	184.726475...
3.0	-318.91745...	149.465751...	-57.805459...	-3.1942660...	2.0	-65.590173...	0.00499999...	0.61550000...	-2.8664899...	-75.299158...	502.139107...
4.0	-848.56840...	459.241699...	-396.79724...	-10.442581...	2.0	-362.39266...	0.00499999...	0.71550000...	-8.8585309...	-402.47376...	1364.95669...
5.0	-2268.6758...	1237.97142...	-1246.8096...	-28.757456...	2.0	-1198.1708...	0.00500000...	0.81549999...	-23.887961...	-1312.7200...	3710.33993...
6.0	-6151.8393...	3343.50850...	-3539.5803...	-78.136358...	2.0	-3453.9125...	0.00500000...	0.91550000...	-64.507839...	-3764.3544...	10085.7576...
7.0	-16726.235...	9079.39568...	-9782.6172...	-212.53486...	2.0	-9568.2641...	0.00500000...	1.01549999...	-175.16554...	-10409.496...	27415.9533...
8.0	-45474.339...	24681.5869...	-26764.115...	-578.08339...	2.0	-26183.797...	0.0050	1.11549999...	-476.17144...	-28468.779...	74524.3463...
9.0	-123616.45...	67094.9916...	-72927.750...	-1571.8174...	2.0	-71351.812...	0.0050	1.21549999...	-1294.4366...	-77562.393...	202578.335...
10.0	-336025.80...	182384.829...	-198412.03...	-4273.0435...	2.0	-194133.99...	0.0050	1.31549999...	-3518.6783...	-211015.90...	550665.446...
11.0	-913414.02...	495773.643...	-539512.13...	-11615.707...	2.0	-527891.55...	0.0050	1.41549999...	-9564.7649...	-573781.22...	1496865.06...
12.0	-2482919.6...	1347653.01...	-1466718.4...	-31575.140...	1.99999999...	-1435138.8...	0.00499999...	1.51549999...	-25999.736...	-1559879.8...	4068904.33...
13.0	-6749282.1...	3663303.32...	-3987128.8...	-85830.550...	1.99999999...	-3901294.1...	0.00499999...	1.61549999...	-70674.662...	-4240375.5...	1.10604374...
14.0	-1.8346466...	9957898.68...	-1.0838320...	-233312.16...	1.99999999...	-1.0605004...	0.00499999...	1.71549999...	-192113.80...	-1.1526723...	3.00654097...
15.0	-4.9870908...	2.70683965...	-2.9461804...	-634209.08...	2.0	-2.8827591...	0.00499999...	1.81549999...	-522219.86...	-3.1333087...	8.17263214...
16.0	-1.3556329...	7.35795888...	-8.0085722...	-1723960.7...	1.99999999...	-7.8361757...	0.00499999...	1.91549999...	-1419541.9...	-8.5172410...	2.22155350...
17.0	-3.6849953...	2.00010218...	-2.1769590...	-4686215.2...	1.99999999...	-2.1300968...	0.00499999...	2.01549999...	-3858718.0...	-2.3152297...	6.03881333...
18.0	-1.0016863...	5.43684572...	-5.9175947...	-1.2738464...	1.99999999...	-5.7902100...	0.00499999...	2.11549999...	-1.0489091...	-6.2934538...	1.64152095...
19.0	-2.7228680...	1.47788905...	-1.6085704...	-3.4626763...	1.99999999...	-1.5739436...	0.00499999...	2.21549999...	-2.8512329...	-1.7107396...	4.46212010...
78.0	-1.1439913...	6.20923341...	-6.7582813...	-1.4548159...	2.00006580...	-6.6127997...	0.00500016...	8.11558163...	-1.1979228...	-7.1875370...	1.87472429...
79.0	-3.1096904...	1.68784438...	-1.8370910...	-3.9545991...	2.00006580...	-1.7975450...	0.00500016...	8.21558492...	-3.2562914...	-1.9537748...	5.09602823...
80.0	-8.4530187...	4.58803872...	-4.9937334...	-1.0749719...	2.00006580...	-4.8862362...	0.00500016...	8.31558849...	-8.8515216...	-5.3109129...	1.38524469...
81.0	-2.2977691...	1.24715844...	-1.3574377...	-2.9220773...	2.00006580...	-1.3282169...	0.00500016...	8.41559186...	-2.4060934...	-1.4436560...	3.76548615...
82.0	-6.2459869...	3.39012967...	-3.6898999...	-7.9430332...	2.00006580...	-3.6104696...	0.00500016...	8.51559516...	-6.5404431...	-3.9242658...	1.02356572...
83.0	-1.6978355...	9.21532966...	-1.0030190...	-2.1591407...	2.00006580...	-9.8142759...	0.00500016...	8.61559845...	-1.7778771...	-1.0667262...	2.78234063...
84.0	-4.6151965...	2.50498681...	-2.7264888...	-5.8691541...	2.00006580...	-2.6677973...	0.00500016...	8.71560174...	-4.8327719...	-2.8996631...	7.56318748...
85.0	-1.2545411...	6.80926393...	-7.4113692...	-1.5954023...	2.00006580...	-7.2518290...	0.00500016...	8.81560503...	-1.3136843...	-7.8821061...	2.05588865...
86.0	-3.4101967...	1.85094993...	-2.0146191...	-4.3367535...	2.00006580...	-1.9712516...	0.00500016...	8.91560832...	-3.5709645...	-2.1425787...	5.58848506...
87.0	-9.2698758...	5.03140360...	-5.4763026...	-1.1788518...	2.00006580...	-5.3584174...	0.00500016...	9.01561161...	-9.7068879...	-5.8241327...	1.51910774...
88.0	-2.5198136...	1.36767737...	-1.4886134...	-3.2044517...	2.00006580...	-1.4565689...	0.00500016...	9.11561490...	-2.6386058...	-1.5831635...	4.12936320...
89.0	-6.8495654...	3.71773356...	-4.0464720...	-8.7106052...	2.00006580...	-3.9593660...	0.00500016...	9.21561819...	-7.1724763...	-4.3034857...	1.12247760...
90.0	-1.8619049...	1.01058477...	-1.0999451...	-2.3677880...	2.00006580...	-1.0762672...	0.00500016...	9.31562148...	-1.9496812...	-1.1698087...	3.05121052...
91.0	-5.0611833...	2.74705470...	-2.9899614...	-6.4363162...	2.00006580...	-2.9255983...	0.00500016...	9.41562477...	-5.2997839...	-3.1798703...	8.29405154...
92.0	-1.3757723...	7.46726931...	-8.1275583...	-1.7495722...	2.00006580...	-7.9526011...	0.00500016...	9.51562806...	-1.4406307...	-8.6437844...	2.25455708...
93.0	-3.7397370...	2.02981432...	-2.2092995...	-4.7558306...	2.00006580...	-2.1617412...	0.00500016...	9.61563135...	-3.9160405...	-2.3496243...	6.12852180...
94.0	-1.0165659...	5.51760769...	-6.0054990...	-1.2927688...	2.00006580...	-5.8762221...	0.00500016...	9.71563464...	-1.0644902...	-6.3869413...	1.66590503...
95.0	-2.7633129...	1.49984133...	-1.6324639...	-3.5141102...	2.00006580...	-1.5973228...	0.00500016...	9.81563793...	-2.8935845...	-1.7361507...	4.52839957...
96.0	-7.5114638...	4.07699164...	-4.4374973...	-9.5523426...	2.00006580...	-4.3419739...	0.00500016...	9.91564122...	-7.8655787...	-4.7193472...	1.23094668...
97.0	-2.0418285...	1.10824177...	-1.2062374...	-2.5965971...	2.00006580...	-1.1802714...	0.00500016...	10.0156445...	-2.1380870...	-1.2828522...	3.34606163...
98.0	-5.5502657...	3.01251361...	-3.2788934...	-7.0582833...	2.00006580...	-3.2083106...	0.00500016...	10.1156478...	-5.8119233...	-3.4871540...	9.09553899...
99.0	-1.5087186...	8.18886108...	-8.9129565...	-1.9186403...	2.00006580...	-8.7210924...	0.00500016...	10.2156510...	-1.5798445...	-9.4790674...	2.47242385...
100.0	-4.1011326...	2.22596871...	-2.4227987...	-5.2154180...	2.00006580...	-2.3706445...	0.00500016...	10.3156543...	-4.2944733...	-2.5766840...	6.72076140...

Fig.12 Concentration level of Shc



In the context of this study, we used this model to help gain mechanistic insight into ligand-dependent responses of the ErbB signaling network. Studies similar to the current work hold the potential to complement the field of targeted cancer treatment. Currently, many ErbB-targeted pharmaceuticals are clinically used to treat cancer, including the small molecule ErbB1 kinase inhibitors erlotinib and gefitinib (14), and a monoclonal ErbB2 antibody, trastuzumab (15). A computational model of the ErbB signaling systems in the cancer type of interest can help predict factors that could guide the choice of when to use a particular targeted pharmaceutical, and in what combinations (16).

The kinetic parameters and chemical equations along with the concentrations of the proteins present in the pathway obtained from literature were used to simulate the ErbB signaling pathways. This simulation was carried out under a specific time scale (100 sec) in order to analyze the levels of all the components in the pathway. For the purpose of simulation, the rate constant of Costunolide was set to be 1.All the other parameters obtained from the previous work (17-20).

Results of ErbB pathway simulation showed that once ErbB protein triggered, pathway initiated, and high level of Raf expression was achieved by increasing the time scale to 100 sec. Further, it was observed that the expression of Raf was higher than other proteins in the pathway. This serves as an initiation state of the increased expression of other onco proteins such as Erk and PKB/Akt in the pathway. Thus, this computational simulation of the modeled ErbB pathway resulted in the increased expression of Grb2, Shc, Sos, Ras etc. This in turn activated the cascading proteins such as Raf, Mek, Erk which were upregulated by increasing the expression level of these proteins upon increased time scales. This activation was achieved from the ErbB mediated signaling. The concentration table of each protein in the pathway also showed as an evidence for over expression of Erk and PKB.

ErbB signaling pathway was drawn in cell designer and was given in Fig (1). Table (3) represented the concentration level of each proteins present in the above pathway, in which S_8 denoted Erk and S_{10} PKB/Akt. So, it was clear from Table (3) that concentration level of Erk increased from 0.018 to 10.0155 where as that of PKB/Akt 0.05 to 79.226. This indicated that once ErbB signaling pathway initiated, it will affect the series of proteins and finally cause the over expression of Erk and PKB/Akt to lead cell proliferation and survival.

In this work, we also incorporated the simulation of effect of Costunolide isolated from *C.speciosus* on ErbB signaling pathway. It was already reported that (11), Costunolide induces ROS- mediated mitochondrial permeability and apoptosis. Hence, this compound has been taken and model its effect on ErbB pathway. After introducing the compound, changes happened to the expression levels of each protein in the pathway.

Initially, signaling pathways were viewed as linear relay routes, which simply transmitted and amplified signals. Now it is increasingly appreciated that signaling responses are shaped by multiple interactions of many components of signaling networks (22). A subtle difference in input signals and/or interaction kinetics may result in differential response patterns and, eventually, in alterations in gene expression by signal-regulated transcription factors. Graphs of the expression levels of all the proteins in the

pathway before and after treatment with Costunolide vary differently.

It was observed that when the Costunolide was added to inhibit the ErbB signaling pathway, the expression of ErbB reduced (Fig.2) followed by the decrease in the levels of downstream signaling proteins, except Shc (Fig.12) and Erk (Fig.8). Hence, the reduced signaling of oncogenes may decrease cell proliferation. Thus it is evidently recognized that cell survival could be decreased by inhibiting PKB/ Akt (Fig.7). Similarly, the computational simulation showed that the decreased concentration of Grb2, Raf, and Sos (Fig.5, Fig. 6, Fig. 9 respectively). But still Shc was overexpressed even after the introduction of Costunolide. This in turn activated Erk, which were up-regulated by increasing the expression levels of these proteins upon increased time scales (Fig.8).

From this study, we propose that combined targeting of these proteins will provide new insights in cancer therapy. Thus, there arises a highly demanding need to develop compounds favoring multiple targeting (21) that would be a promising therapeutic agent in future.

Acknowledgement

The authors thankfully acknowledge the PKN college of arts and Science for providing intuitional facilities for performing this work.

References

1. Sawyers C. Targeted cancer therapy. *Nature*. (2004) 432: 294–7.
2. Liu ET and Karuturi KR. Microarrays and Clinical Investigations. *N Engl J Med*. (2004) 350:1595–7.
3. Ebert BL and Golub TR. Genomic approaches to hematologic malignancies. *Blood*. (2004) 104:923–32.
4. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. (1987) 235:177-182.
5. Yarden Y and Sliwkowsk MX. Untangling the ErbB signalling network. Nature Rev. Mol. Cell Biol. (2001) 2: 127-137.
6. Olayioye MA, Neve RM, Lane HA and Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J. (2000) 19: 3159-3167.
7. Yarden Y and Sliwkowski M X.Untangling the ErbB signalling network. Nature Rev. Mol. Cell Biol. (2001) 2:127-137.
8. Robinson AG, Turbin D, Thomson T, Yorida E, Ellard S, Bajdik C, Huntsman D and Gelmon K. Molecular predictive factors in patients receiving trastuzumab-based chemotherapy for metastatic disease. Clin Breast Cancer. (2006) 7: 254–261.
9. Mariko Hatakeyama, Shuhei Kimura, Takashi Naka, Takuji Kawasaki, Noriko Yumoto,Mio Ichikawa, Jae-Hoon Kim, Kazuki Saito, Mihoro Saeki, Mikako Shirouzu, Shigeyuki Yokoyama and Akihiko Konagaya. A computational model on the modulation of MAPK and Akt pathways in heregulin induced ErbB signaling. Biochemical Journal Immediate Publication. (14 Apr 2003) as manuscript BJ20021824.
10. Hatakeyama M, Kimura S, Naka T, et al., A computational model on the modulation of MAPK and Akt pathways in heregulin-induced ErbB signaling. Biochem J. 2003; 373.

11. Min-Goo LEE, Kyung-Tae LEE, *a* Sung-Gil CHI, and Jae-Hoon PARK. Costunolide Induces Apoptosis by ROS-mediated Mitochondrial Permeability Transition and Cytochrome C Release. *Biol. Pharm. Bull.* (2001) 24(3): 303—306.
12. Tzahar E, Pinkas-Kramarski R, Moyer JD, Klapper LN, Alroy I, Levkowitz G,Shelly M, Henis S, Eisenstein M, Ratzkin BJ, Sela M, Andrews GC and Yarden Y. Bivalence of EGF-like ligands drives the ErbB signaling network EMBO J. (1997) 16:4938-4950.
13. Kholodenko BN. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. Eur. J. Biochem. (2000) 267:1583-1588.
14. Harari PM, Huang S. Radiation combined with EGFR signal inhibitors: head and neck cancer focus. Semin Radiat Oncol (2006) 16: 38–44.
15. Adams GP,Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol (2005) 23: 1147–1157.
16. Marc R Birtwistle, Mariko Hatakeyama, Noriko Yumoto, Babatunde A Ogunnaike, Jan B Hoek and Boris N Kholodenko. Ligand-dependent responses of the ErbB signaling network: experimental and modeling analyses. Molecular Systems Biology 3; Article number 144; (2007) doi:10.1038/msb4100188.1-16.
17. Gise VA, Lorenz P, Wellbrock C, Hemmings B, Berberich-Siebelt F, Rapp UR and Troppmair. Apoptosis suppression by Raf-1 and MEK1requires MEK- and phosphatidylinositol 3-kinase-dependent signals. *J Mol Cell Biol.* (2001) 21:2324–36.
18. Hatakeyama M, Kimura S, Naka T, et al. A computational model on the modulation of MAPK and Akt pathways in heregulin-induced ErbB signaling. *Biochem J.* (2003) 373.
19. Ivaska J, Nissinen L, Immonen N, Eriksson JE, Kähäri VM, Heino J. Integrin 2β1 Promotes Activation of Protein Phosphatase 2A and Dephosphorylation of Akt and Glycogen Synthase Kinase 3β. *Mol Cell Biol.* (2002) 22:1352–9.
20. Kalisch BE, Demeris CS, Ishak M, Rylett RJ. Modulation of nerve growth factor-induced activation of MAP kinase in PC12 cells by inhibitors of nitric oxide synthase. *J Neurochem.* (2003) 87:1321–32.
21. de Philippe M, Gilles F, Mare P. Multiple targeting by the Antitumor Drug Tamoxifen: A Structure- Activity Study. Current Medicinal Chemistry- Anti-Cancer Agents. (2004) 4:491-508.
22. Bhalla US and Iyengar R.*Science* (1999) 283: 381–387.