Article

Mathematical modeling deciphering balance between cell survival and cell death using insulin

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Abstract

When modelling cell signalling networks, a balance must be struck between mechanistic detail and ease of interpretation. In this paper we apply a deterministic numerical method to the analysis of a large, systematic dataset describing the dynamics of cell signalling downstream of, tumor necrosis factor-α (TNF), epidermal growth factor (EGF), and insulin receptors in human colon carcinoma cells. Deterministic modeling is useful as a means to assemble and test what we know about proteins and networks. We extensively study the space of parameters to show that the model is structurally stable and robust over a broad range of parameter values. We have made the biological view of the main paths of the insulin input showing cell survival and cell death. Than with the help of different parameters relating to that protein present in the model we have designed scheme of the biochemical paths/deterministic model. With those parameters equations were formed which vary with time i.e. differential equation. Thus, our model is suitable for implementation in multi-scale simulation programs that are presently under development to study the behavior of large tumor cell populations.

Keywords tumor necrosis factor-α; epidermal growth factor; insulin; signaling; biological view.

1 Introduction

Large datasets for biological systems are becoming increasingly available due to ongoing improvements in high-throughput measurement methods. However, similarly improved computational methods are needed if we are to gain useful insights from these datasets. Among prominent examples of this current challenge in biology is in understanding how extra cellular cues influence highly interconnected and complex cell signalling pathways to yield cell behavioural responses (Kitano, 2002). In the case of programmed cell death, cytokines such as tumor necrosis factor-alpha (TNF-α) (Wajant et al., 2003) function as a pro-death cue, whereas growth factor such as epidermal growth factor (EGF) and insulin (Avruch, 1998) exert pro-survival effects. The magnitudes of the responses vary with cell type, but the pathways downstream of cytokine receptors are conserved and highly interconnected. It appears that the determination of whether a cell will live or die

involves a balance between pro-death and pro-survival signals (Xia et al., 1995). Thus, the intracellular signal transduction network stimulated by TNF, insulin, and other cytokines acts as a signal processor that converts opposing cues into a functional response that controls cell fate.

Although many of the components of cellular signalling systems have now been identified, it is not yet known how binary decisions, such as death–survival, are made. Moreover, the complexity of cell signalling networks precludes a simple protein-by-protein assignment of function. Increasingly, systematic methods are applied to the interpretation and computational analysis of cell signalling (Brent, 2000; Kitano, 2002). These computational methods lie on a spectrum of approaches that vary in their level of abstraction and specificity (Ideker et al., 2003). The most abstract methods, such as multivariate analysis and clustering (Rives et al., 2003), are powerful because they can handle empirical data and prior knowledge about the underlying phenomena in a flexible fashion. At the other hand, differential equation-based models (Schoeberl et al., 2002) are useful for coding existing prior knowledge in pursuit of *in silico* predictions.

Current high-throughput experimental protocols allow us to measure an increasingly large number of variables describing cells or cellular populations. For example, large scale phosphorylation screens (Lund-Johansen et al., 2000), protein–protein binding screens (Cagney et al., 2000) and migration assays (Maliakal, 2002) have demonstrated that it is possible to simultaneously measure the activity of tens to thousands of variables in a biological system. Future advances in micro fluidics (Burns et al., 1998) and high throughput mass spectrometry (Morand et al., 2001), for example, promise even more measurements will be possible at a fraction of their current cost. Analysis of these data has generally focused on identifying a small number of pathway components involved in governing a particular cell behaviour. Growing interest is focusing on how to use these multivariate data to determine integrated pathways in, for instance, signal transduction (Gardner et al., 2003) and metabolic pathways (Price et al., 2003).

In this paper, our purpose is to determine whether numerical model such as deterministic model can be used to uncover important aspects of cellular signals deciphering the fate of cell. Specifically, we examine the TNF-, EGF- and insulin-mediated death-versus-survival response by deterministic approach. We wish to attract the interest of other experimental molecular cell biologists, so we emphasize the conceptual details of deterministic method as a practical modelling technique. A noteworthy feature of deterministic model applied to biological data, is that biological measurements reflect tangible molecular entities with known, mechanistic roles in intracellular processes. Thus, these data-driven models of signalling are empirical, but not phenomenological, and suggest mechanistic dependencies. We show here that numerical and related methods can uncover key contributors to death–survival decisions. These contributions always involve multiple proteins working in concert, but the informative proteins consist of only a fraction of the original protein dataset. Thus, our results suggest that within the entire cellular signalling system lies a reduced set of information-rich protein measurements that together constitute an efficient model of the signalling network state and the relevant signal–response relationships.

2 Theoretical Model

2.1 Binding and internalization of insulin/IRS1 complexes

Current biochemical data show that the IRS receptors rapidly self-trimerized at the cell membrane and interact with insulin homo trimers. Thus, the mechanism of EGF binding to IRS can in principle be viewed as the result of the monomeric interactions between one molecule of insulin and one molecule of receptor. This simplification is further supported by the following considerations:

(1) The mechanism of receptor self-trimerization followed by ligand binding can be modeled by a set of 4

differential equations with 6 parameters. The model can fit experimental data of insulin binding, but one finds that the kinetics of receptor trimerization are much faster than the binding kinetics, and thus the trimerized receptor behaves as an effective monomer. In addition, experimental determinations of the parameter values for intermediate binding reactions are not available;

(2) The model by Bajzer et al. (1989) fitted experimental data well; we use it to describe the early events of TNF interactions with cells. However the original model of Bajzer et al. must be updated to account for some novel aspects of TNF biology. In particular, Bajzer et al. (1989) assumed that internalized ligand/receptor complexes could be recycled back at the cell surface. It is highly probable that TNF/TNF-R1 complexes do not recycle at all but are finally degraded into lysosomes. Therefore we modify the model by Bajzer et al. (1989) and Chignola et al. (2009) model as follows:

$$\begin{aligned}
\frac{d[R]}{dt} &= V_r - k_d[R] - k_{on}[L][R] + k_{off}[N_c] \\
\frac{d[L]}{dt} &= -k_{on}[L][R] + k_{off}[N_c] \\
\frac{d[N_c]}{dt} &= k_{on}[L][R] - (k_{off} + k_{in})[N_c] \\
\frac{d[N_{in}]}{dt} &= k_{in}[N_c] - k_{\deg}[N_c]
\end{aligned} \qquad (1)$$

where square brackets denote molar concentrations of free IRS receptors (R), free insulin (L), insulin/IRS complexes bound at the cell membrane (N_C) and internalized complexes (N_{in}). Here k_{on} and k_{off} are the association and dissociation rate constants for insulin binding to IRS, respectively, k_{in} is the internalization rate constant of insulin/IRS complexes and k_{deg} is the rate constant of lysosomal degradation of the complexes.

The two parameters V_r and k_d where introduced by Bajzer et al. although with a slightly different notation, to describe “the zero-order rate of insertion of receptors into the membrane and the turnover (internalization) rate constant of ligand-free receptors (Bajzer et al., 1989; Grell, 1998; Vuk-Pavlovic et al., 1989) respectively.

2.2 Modeling the intracellular signaling pathways triggered by insulin

Cell signaling induced by insulin leading to cell survival and cell death has been represented in Fig. 1*A*. The approximation of this model used for mathematical implementation is shown in Fig.1*B*. We define seven molecular species, *H, G, F, E, C, B* and *A*, that collectively summarize the various reactions leading to cell survival and death. The molecules *H, G, F, E, C, B* and *A* can be loosely identified with mTOR, JAK/STAT, FKHR, MEK/ERK, JNK/FLIP, NF-κB/FLIP and caspase-3 respectively. We assuming that after the initial triggering of the pathway, it precedes irreversibly to its endpoint the equations for *A, B, C, E, F, G* and *H* can be written as:

$$\frac{d[A]}{dt} = \alpha[N_{in}] - \gamma[B][A] - \varphi[C][A] - \varepsilon[E][A] + \rho[F][A] - \psi[G][A] - \xi[H][A] - k_{A\deg}$$
$$\frac{d[B]}{dt} = \beta[N_c] - k_{B\deg}[B]$$
$$\frac{d[C]}{dt} = \delta[N_c] - k_{C\deg}[C]$$
$$\frac{d[E]}{dt} = \eta[N_c] - k_{E\deg}[E]$$
$$\frac{d[F]}{dt} = \mu[N_c] - k_{F\deg}[F] \ (Death)$$
$$\frac{d[G]}{dt} = \chi[N_c] - k_{G\deg}[G]$$
$$\frac{d[H]}{dt} = \lambda[N_c] - k_{H\deg}[H] \qquad (2)$$

where the variables N_C and N_{in} are the same as in the differential system (Eq.(1)). We see from the differential system (Eq.(2)), that the cell survival signal, modeled phenomenologically by means of the chemical species *B*, *C*, *E* and *H* depends on the number of insulin/IRS complexes at the cell surface (e.g. N_C), with rate constant parameter β, δ, η, χ and λ. On the other hand, the apoptotic signal, modeled phenomenologically by means of the chemical species *F* that denotes FKHR pathway, with a rate constant μ. While *A*, depends on the number of internalized ligand/receptor complexes (e.g. N_{in}) with rate a constant α. Induction of cell survival pathway inhibits the apoptotic pathway by destroying *A* with rates $\gamma[B]$, $\varphi[C]$, $\varepsilon[E]$, $\psi[G]$, $\xi[H]$. Finally, *H*, *G*, *F*, *E*, *C*, *B* and *A* can be degraded by means of ubiquitination and proteasome cleavage and/or irreversibly inhibited by other molecular species, and these processes are described by the rate constants $k_{H\deg}$,$k_{G\deg}$, $k_{F\deg}$, $k_{E\deg}$, $k_{C\deg}$, $k_{B\deg}$ and $k_{A\deg}$ respectively.

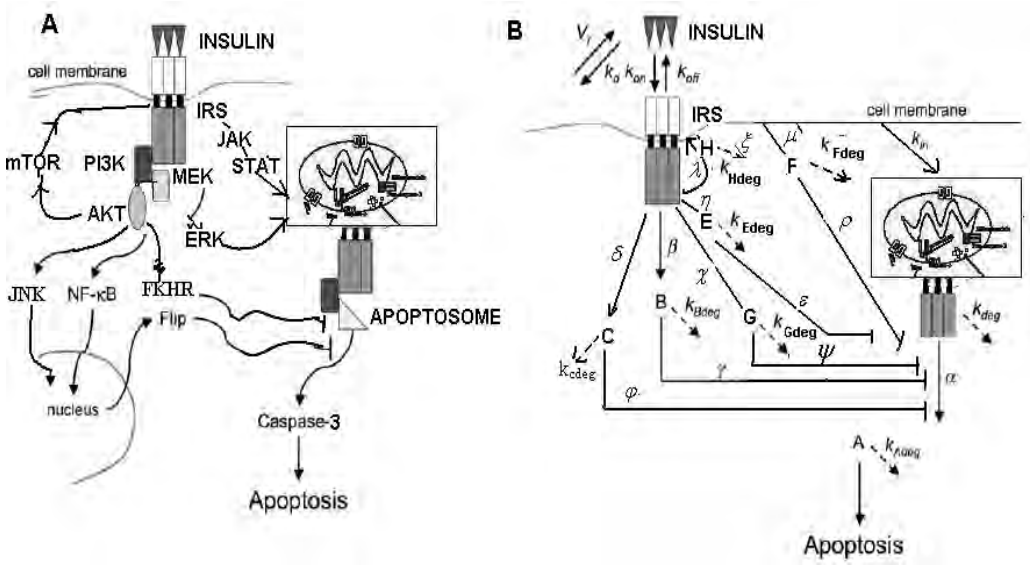

Fig. 1 Modeling insulin mediated signaling. A. Biological view of the main insulin paths. B. Scheme of the biochemical pathways that have been considered in the present model.

3 Numerical Methods

Let us consider a differential equation as

$$\frac{d\,y}{dx} + P\,y = Q \tag{3}$$

$\frac{d}{dx}$ can be written as D, replacing in Eq. (3) we get

$$D\,y + P\,y = Q \tag{4}$$

Now, we know the integrating factor is

$$e^{\int P\,dx}$$

Multiply Eq. (4) with integrating factor both sides, we get

$$e^{\int P dx}.\left(D\,y + Py\right) = e^{\int P dx}.Q$$

$$D\,y\,e^{\int P dx} + P\,y\,e^{\int P dx} = e^{\int P dx}.Q$$

$$D\,y\,e^{\int P dx} = e^{\int P dx}.Q$$

Integrating both sides

$$y\,e^{\int P dx} = \int Q.e^{\int P dx}\,dx + c$$

Finally the solution of equation is

$$\mathbf{y} = \frac{\int \mathbf{Q.e^{\int P dx}\,dx + c}}{\mathbf{e^{\int P dx}}} \tag{5}$$

Now our main equation is $\frac{d[B]}{dt} + k_{B\deg}[B] = \beta[N_c]$ (6)

If we compare the above equation i.e Eq. (6) with Eq. (4) we get

$$y = B; P = k_{B\deg}\ ;\ Q = \beta[N_c]$$

Putting all these values in Eq. (5), we get

$$B = \frac{\int \beta\,N_c.e^{\int k_{B\deg}\,dt}\,dt + c}{e^{\int k_{B\deg}\,dt}} \tag{7}$$

$$B = e^{-k_{B\deg}t}\left[\left(\int \beta\,N_c.e^{k_{B\deg}t}\,dt\right) + c\right]$$

After solving the above equation we get

$$B = \frac{\beta N_c}{k_{B\deg}} + c.e^{-k_{B\deg}t} \quad (8)$$

Now applying the initial condition i.e. $t = 0$ and $B = 0$; in the Eq. (8), we get

$$c = -\frac{\beta N_c}{k_{B\deg}}$$

Now putting the value of c in Eq. (7), we get

$$\mathbf{B = \frac{\beta N_c}{k_{B\deg}}\left(1 - e^{-k_{B\deg}t}\right)} \quad (9)$$

Similarly, we can solve equations of different marker proteins involved in the cell survival and cell death pathway induced by TNF, EGF and insulin.

3.1 Differential equation modeling activity of NF-κB

We have solved the differential equation of NF-κB as

$$\frac{d[B]}{dt} + k_{B\deg}[B] = \beta[N_c]$$

Solution comes out to be

$$[B] = \frac{\beta[N_c]}{k_{B\deg}}\left[1 - e^{-k_{B\deg}t}\right] \quad (10)$$

By considering values of β= 0.33 min^{-1} and $k_{B\deg}$ = 0.033 min^{-1} (Chignola et al., 2009), putting these values in Eq. (10) we get

$$[B] = 10\left[1 - e^{-0.33t}\right] \quad (11)$$

Again by considering the different values of time (Gaudet et al., 2005) and solving Eq. (11); the values of B have been obtained and get a plot between concentration of NF-κB and Time which comes out to be exponential.

3.2 Differential equation modeling activity of JNK

We have solved the differential equation of JNK as

$$\frac{d[C]}{dt} + k_{C\deg}[C] = \delta[N_c]$$

Solution comes out to be

$$[C] = \frac{\delta[N_c]}{k_{C\deg}}\left[1 - e^{-k_{C\deg}t}\right] \quad (12)$$

By assuming the values of $\delta = 0.33/\min$; $k_{C\deg} = 0.018/\min$ and applying these values in Eq. (12) we got

$$[C] = 18.33\left[1 - e^{-0.018t}\right] \quad (13)$$

Considering the different values of time (Gaudet et al., 2005) in Eq. (13) we calculated the corresponding values of *C*.

3.3 Differential equation modeling activity of MEK/ERK

We have solved the differential equation of MEK/ERK as

$$\frac{d[E]}{dt}+k_{E\deg}[E]=\eta[N_c]$$

Solution comes out to be

$$[E]=\frac{\eta[N_c]}{k_{E\deg}}\left[1-e^{-k_{E\deg}t}\right] \quad (14)$$

By assuming the values of values of $\eta=0.33/\min$; $k_{E\deg}=0.016/\min$ and putting these values in Eq. (14) we solved the equation for calculating *E* as,

$$[E]=20.62\left[1-e^{-0.016t}\right] \quad (15)$$

By taking the different values of time (Gaudet et al., 2005) and solving the Eq. (15); we calculated the values of *E*.

3.4 Differential equation modeling activity of JAK/STAT

We have solved the differential equation of JAK/STAT as

$$\frac{d[G]}{dt}+k_{G\deg}[G]=\chi[N_c]$$

Solution comes out to be

$$[G]=\frac{\chi[N_c]}{k_{G\deg}}\left[1-e^{-k_{G\deg}t}\right] \quad (16)$$

Now applying the values of $\chi=0.33/\min$; $k_{G\deg}=0.017/\min$, in Eq. (16) we resolved the final equation for *G* as,

$$[G]=19.41\left[1-e^{-0.017t}\right] \quad (17)$$

With different values of time (Gaudet et al., 2005) and using Eq. (17) the values of *G* have been calculated.

3.5 Differential equation modeling activity of mTOR

We have solved the differential equation of mTOR as

$$\frac{d[H]}{dt}+k_{H\deg}[H]=\lambda[N_c]$$

Solution comes out to be

$$[H]=\frac{\lambda[N_c]}{k_{H\deg}}\left[1-e^{-k_{H\deg}t}\right] \quad (18)$$

Now applying the values of $\lambda = 0.33/\min$; $k_{H\deg} = 0.02/\min$ in Eq. (18) we solved the equation for calculating the value of *H* as:

$$[H] = 16.5\left[1 - e^{-0.02t}\right] \quad (19)$$

Also the values of *H* with respect to different values of time (Gaudet et al., 2005) have been calculated.

4 Results and Discussions

4.1 Experimental findings

The cultures were stimulated 24 hr later by adding the stimulus diluted in 1/20 of the culture volume of serum-free medium, for final concentrations of 0, 0.2, 5, or 100 ng/ml TNF (Peprotech), 0, 1, or 100 ng/ml EGF (Peprotech), and 0, 1, 5, or 500 ng/ml insulin (Sigma). Triplicate plates were lysed at 0, 5, 15, 30, 60, and 90 min and 2, 4, 8, 12, 16, 20, and 24 hr or prepared for flow cytometry at 12, 24, and 48 hr. To explore systematically relationships between cytokine-receptor interaction, activation of intracellular signaling cascades, and apoptosis-survival cell-fate decisions, cells were exposed to a set of ten cytokine treatments shown in Table 1 and monitored over a 48-hr period. Each treatment consisted of a combination of TNF and either EGF or insulin Cells respond to TNF, EGF, and insulin in a dose-dependent manner and all three cytokines were therefore examined at sub saturating concentrations, designed to mimic physiological conditions, and at saturating concentrations, at which essentially all receptors were ligand-bound. At 13 time points after cytokine addition, three replicate dishes of cells (six for the zero time point) were harvested to measure kinase activities, changes in protein phosphorylation, caspase cleavage, and changes in protein abundance. All together, AkT signals were examined. Kinases such as Akt and ERK were maximally active 5-15 min after cytokine addition whereas caspase cleavage was evident only after 4 hr time points were spaced closely from 0-2 hr (0, 5, 15, 30, 60, 90, 120 min) and then more sparsely from 4-24 hr (4, 8, 12, 16, 20, 24 hr), where t = 0 min is the time of cytokine addition taken from Gaudet et al. (2005).

Table 1 Ten cytokine treatments

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)	(*j*)
TNF(ng/ml)	-	5	100	-	5	100	-	0.2	5	100
EGF(ng/ml)	-	-	-	100	1	100	-	-	-	-
Insulin(ng/ml)	-	-	-	-	-	-	500	1	5	500

4.2 Deterministic finding

We have presented an integrated theoretical framework for describing the balance between cell survival and cell death regulated by insulin. The theoretical models consider here for mathematical intervention is minimal in the sense it takes into consideration only those reactions that are essential to describe the action of insulin on cell survival and cell death. The results illustrated the rate constants defined in the deterministic models are biologically relevant of key proteins in the signaling pathway which decide the fate of the cell.

Moreover, the rate constant κ parameterizes the triggering kinetics of the survival signal that initiates at the cell membrane level upon binding of TNF to TNF-R1, EGF to EGFR and INSULIN to IRS. A complex of adaptor proteins (consisting of RIP-1 and TRAF-2) is formed at the cell surface transduces signal for activation

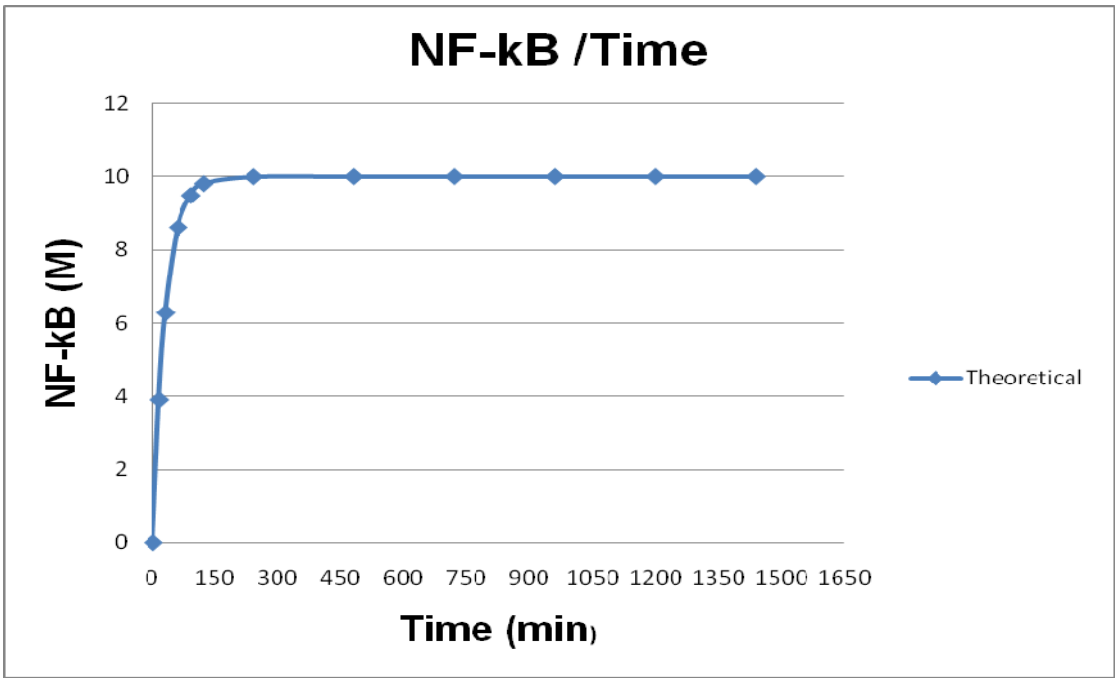

Fig. 2 Theoretical result of NF-κB.

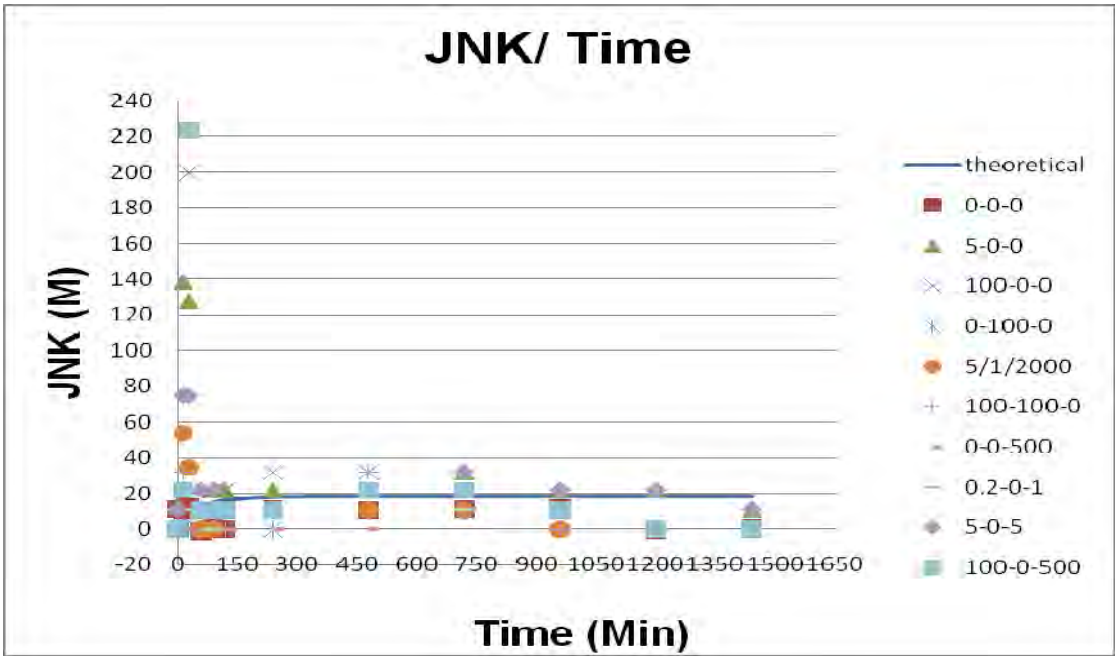

Fig. 3 Theoretical and Practical results of JNK.

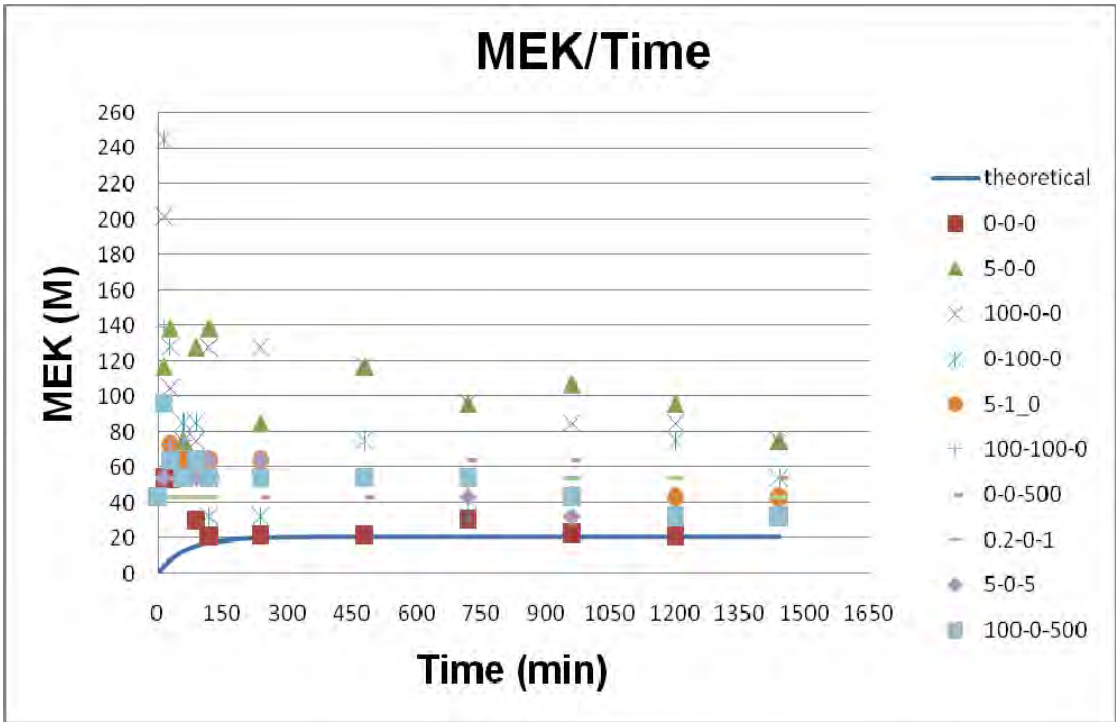

Fig. 4 Theoretical and Practical result of MEK.

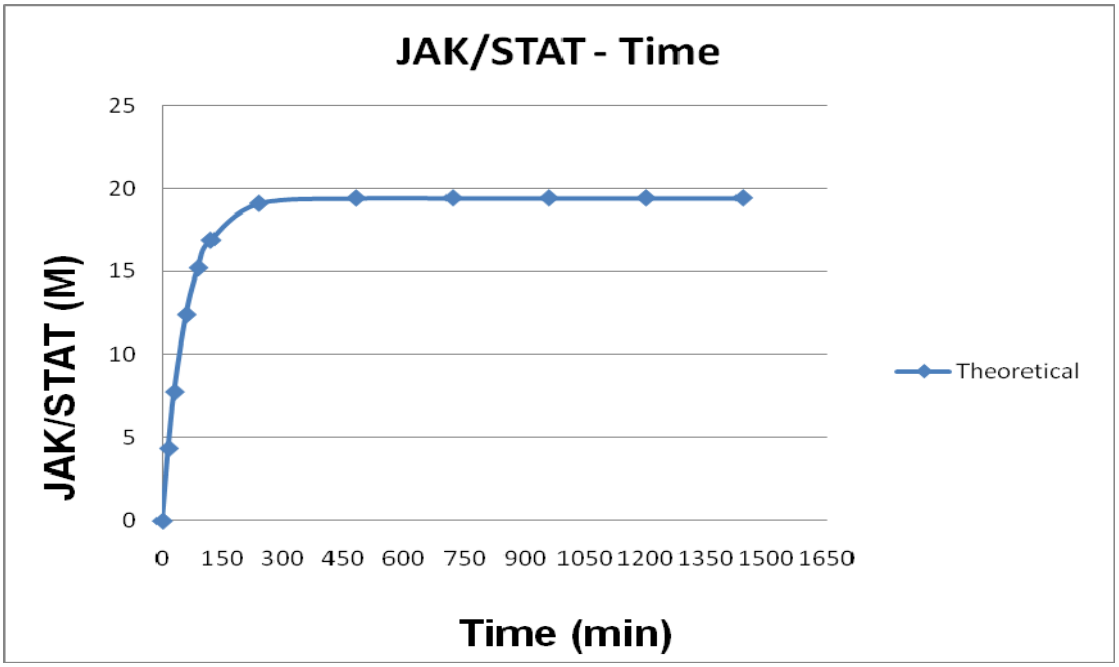

Fig. 5 Theoretical result of JAK/ STAT.

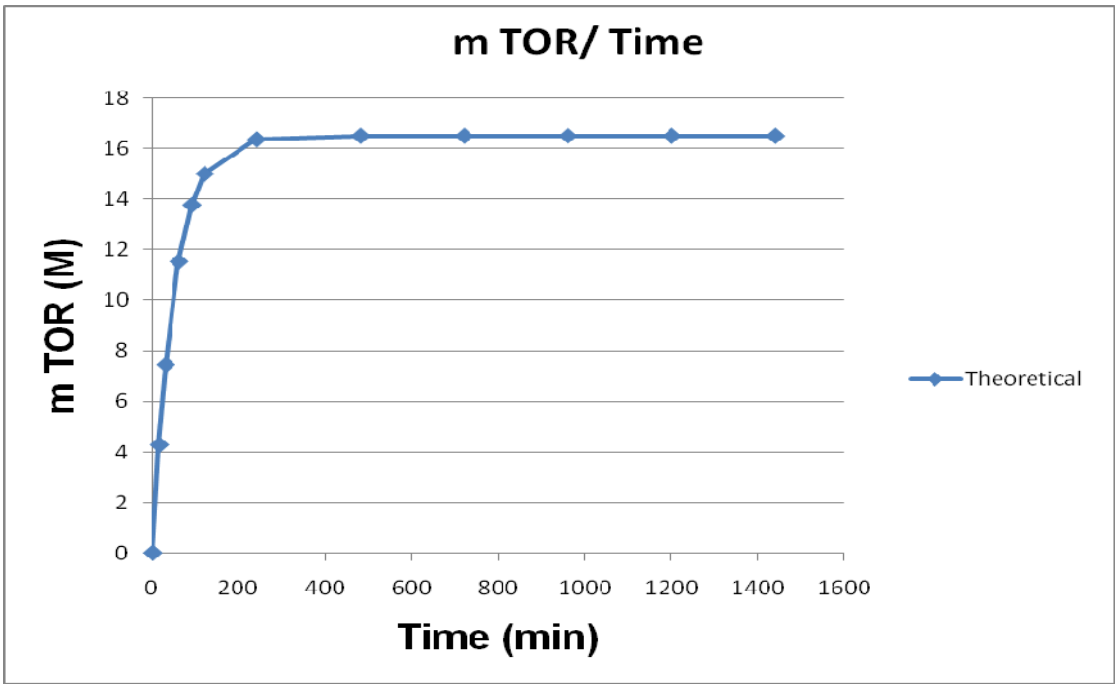

Fig. 6 Theoretical result of mTOR.

of NF-κB and JNK leading to cell survival. Insulin induced activation of proteins such as NF-κB, JNK, MEK/ERK, JAK/STAT and mTOR leading to cell survival, while NF-κB/ FLIP, FKHR, ERK/AP1, JAK/STAT to Bax and JNK/FLIP leads to cell death. Thus it has been revealed that survival and death signals induced by insulin are temporarily separated and this is reflected in our model by the differences between the values of the parameters used. Simulations based on deterministic model recapitulate most features of the data and generate several predictions involving pathway crosstalk and regulation. We uncover a relationship between the key proteins involved in insulin cellular signalling pathways that might account for the cell survival and cell death decision of the cells. More generally, deterministic models are flexible, able to incorporate qualitative and noisy data, and powerful enough to produce quantitative predictions and new biological insights about the operation of signalling networks.

The quality of fit between theoretical and experimental values (ten cytokine combinations) for the proteins such as NFκB, JNK, MEK, JAK-STAT and mTOR respectively revealed good accuracy of the model shown in Figures 2 to 6 respectively.

5 Conclusion

We have made the biological view of the main paths of the insulin input showing cell survival and cell death. We have successfully made a deterministic model for insulin which verifies experimental and theoretical data. Thus, our model is suitable for implementation in multi-scale simulation programs that are presently under development to study the behavior of large tumor cell populations.

Abbreviations

AP-1, Activation Protein 1; ASK1, Apoptosis signal-regulating kinase 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; FADD, Fas-Associated protein with Death Domain; FKHR, Forkhead transcription factor; Grb2, growth factor receptor-bound 2; IGF, insulin-like growth factor; IκB, I Kappa B (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor); IKK, IκB kinase; IR, insulin receptor; IRS1, insulin receptor substrate 1; JNK1, c-jun NH_2 terminal kinase 1; MAP kinases, mitogen-activated protein kinases; MEK, mitogen-activated protein kinase and extracellular-regulated kinase kinase; MK2, mitogen-activated protein kinase-activated protein kinase 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PLADD, pre-ligand assembly domain; PDK, Phi Delta Kappa; PI3K, phosphatidylinositol 3-kinase; p38, P38 mitogen-activated protein kinases**;** Rac, Ras-related C3 botulinum toxin substrate; SAPK/JNK , Stress-activated protein kinase/Jun-amino-terminal kinase; SH2, Src homolgy 2; SODD, Silencer of death domains; SOS, Son of Sevenless; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TRADD, TNFR associated via death domain; TRAF2, TNF receptor associated factor 2, XIAP, X-linked Inhibitor of Apoptosis Protein.

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