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Critical and synergy nodes in insulin-EGF signaling network

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Abstract

Signaling pathways are not isolated from their surroundings. They are also intervened by other signaling pathways known as "crosstalk mechanism". One of the most important crosstalk mechanisms is the insulin-EGF network. Although insulin and epidermal growth factor (EGF) networks have some complexity in their isolated forms, their complexities will grow in the crosstalk network. In this study, we used the analytical tools of the systems biology workbench for elucidating some ambiguities of the insulin-EGF crosstalk. Based on sensitivity analysis, we reconstructed an elucidated model with 51 chemical reactions in comparison with the previous model with 111 chemical reactions. Interestingly, this reduced model reproduces the results of the original model in synergy conditions. We noticed two controlling pathways with direct participation of phosphorylated insulin and EGF receptors that involve Insulin Receptor Substrate (IRS) and Src kinase modules. Also, insulin pathway by producing phosphatidylinositol-3, 4, 5-triphosphate (PIP3), and EGF pathway by activation of GAB1, control the downstream events and lead to potentialities in the mitogenic signal. Surprisingly, Shc and phosphatase SHP2-dependent reactions have no significant roles in the synergy conditions and are not involved in the reduced model. Regarding sensitivity analysis, all Ras/ERK cascade reactions are crucial for signal transduction and were kept in the reduced model.

***Keywords*:** Signaling pathways, crosstalk, computational modeling, systems biology, insulin-EGF networks, sensitivity analysis, targeted drug therapy

Introduction

Since the 1990s, modeling has appeared as a novel tool to perform the abundant information on the molecular parts list and the troublesome complex interaction circuitry of signaling networks (Kholodenko, 2006). Signal is transuded along a complex pathway of molecular interactions; this leads to distinct biological responses and different functions of the cells. Signaling pathways depend very much on various species, interactions, and parameters of such busy pathways and it is difficult to identify conserved signaling modules and those specific control mechanisms that modulate the strength of any signaling. On the other hand, the crosstalk between heterologous pathways increases the complexity of the integrated signaling pathways.

Moreover, in these years, study about the mechanisms of cross talking among signaling pathways becomes an interesting research area in medicine and cell biology (Borisov et al., 2009; Sasagawa et al., 2005; SureshBabuCV et al., 2008; Yu et al., 2006; Zhu and Kyprianou, 2008). Thereby, some strategies are required to reduce the complexity of a crosstalk model and characterize the possible synergy effects. Mathematical modeling emerged as a solution to study the complex behavior of networks (HarshaRani et al., 2005; Orton et al., 2005). One of the most popular analytical tools for model reduction and identifying the controlling nods of a pathway is sensitivity analysis (Birtwistle et al., 2007; Bornheimer et al., 2007; Chen et al., 2009; Ihekwaba et al., 2004; Kinzer-Ursem and Linderman, 2007; Liu et al., 2005; Mahdavi et al., 2007; Mauch et al., 1997; Maurya et al., 2005; Zhang et al., 2009; Zheng and Rundell, 2006). Sensitivity analysis is an important tool in the studies of the dependence of a system on external parameters (Ingalls and Sauro, 2003). With this modeling technique, it is possible to predict the main routs of any pathway and reduce the complexities. This approach could be especially useful for studying the complex crosstalk mechanism.

One of the most important crosstalk mechanisms that involve such complexities is insulin-EGF network. Although insulin and EGF networks have some complexity in their isolated forms, these complexities will grow in the crosstalk network

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(Avruch, 1998; Kholodenko et al., 1999; Schoeberl et al., 2002; Taniguchi et al., 2006). Insulin is a well-described anabolic agonist. The main physiological function of insulin signaling is metabolic, involving the control of glucose metabolism and stimulation of protein and lipid synthesis (Cheatham and Kahn, 1995). Other important functions of insulin are to enhance, or potentiate, the effects of growth factors such as epidermal growth factor (EGF), particularly in relation to cell proliferation, extracellular signal-regulated kinase (ERK) activation and DNA synthesis (Chong et al., 2004; Crouch et al., 2000; Ediger and Toews, 2000). On the other hand, EGF can negatively regulate insulin signaling and in some conditions can evoke metabolic responses, e.g., GLUT4 translocation (Gogg and Smith, 2002; Gual et al., 2003; Ishii et al., 1994). The epidermal growth factor receptor (EGFR) and the insulin receptor (IR) networks share many downstream components and can be considered as integrative cellular signaling network (Borisov et al., 2009). However, this crosstalk with combinatorial complexity of molecular interactions and a variety of feedback and feed-forward loops has imposed some limitation on our ability to understand their functionality and how they affect the robustness of the overall pathway. The formulation and study of such models must also be reduced as far as possible to cope with the increasing complexity demanded and exponential of metabolic reconstruction, computed from sequenced genomes (Goryanin et al., 1999). Also identifying the synergy sites of such systems has crucial roles in development of the future studies and therapeutic usage such as targeted diabetes (Carlson et al., 2003) or cancer (hornberg et al., 2006) therapy.

Therefore, in this paper we used the robust and new model of this crosstalk mechanism constructed by Brisov et al. (Borisov et al., 2009), and employed sensitivity analysis to identify those reactions that exert the greatest control on the activation of ERK. Using obtained results, we reproduced a reduced model that contains controlling reactions in synergy condition. Then, with parameter variation, we signalized the roles of synergic and essential reactions of this mechanism.

Materials and Methods

Model reconstruction

As a template for analysis, we use the new and robust insulin-EGF crosstalk model of Borisov et al. (Borisov et al., 2009). This model contains 111 processes and several of these processes have some sub processes, too. Therefore, they have named their model "minimal" (Borisov et al., 2009). We converted the processes of the template model into the ordinary differential equations (ODEs) form. The construction and further analysis of the model was carried out by Matlab simbiology toolbox. This toolbox can perform time-dependent sensitivity analysis.

Sensitivity analysis

We considered one simplification in calculating sensitivity coefficients and did not consider the mass balance for EGF (Borisov et al., 2009). Other aspects of the template model are conserved. For each process, we computed the time-dependent sensitivity coefficients for the parameters such as the rates of reaction, forward and backward rate constants, k_{cat}, and Michaelis-Menten constants during activation of signal. The number of the studied sensitivity coefficients depends on the choice of system variables and system parameters. These coefficients also vary with both time and stimulus dosage. For illustrative figures, we assigned sensitivity coefficients in two time domains: *i)* maximum sensitivity of each reaction before and after maximal activation of ERK *ii)* in maximal activation of ERK (figure 1). Dose-dependencies may lead to the wrong results in calculation of sensitivity coefficients (Liu et al., 2005). Some reactions have a high flux but not a high sensitivity coefficient while these reactions may be critical in the signaling pathway. Moreover, in particular concentration of stimulus, addition of excessive dose does not change the maximal activation and only leads to increment of the flux. To handle this problem, we calculated the dose-response curves for insulin and EGF network and then assigned the doses in which the maximal activation is not saturated. Therefore, for computation of all sensitivities, we used 0.05 nM and 30 nM doses for EGF and insulin, respectively (figure 2).

Results

Reduced model of Insulin-EGF network

Using sensitivity analysis we were able to reconstruct a reduced replica of the original model of insulin-EGF crosstalk mechanism. Browsing in time-dependent sensitivity coefficients showed that more than one-half of reactions have the sensitivity coefficients equal or near to zero. With some simplifications, reactions that have non zero sensitivity coefficients, are gathered in a reduced model. Thus, in this model, only the necessary

reactions that have a noticeable role in the activation of phosphorylated ERK are considered. The graphical representation of this reduced model shown in figure 3 and its SBML file (HarshaRani et al., 2005) is provided. The model is initiated by ligand binding to Insulin and EGF receptors and autophosphorylation of the ligand-receptor complexes. Further details, approximations and explanations of isolated or crosstalk models of insulin and EGF are discussed in the literatures (Avruch 1998; Borisov et al., 2009; Cheatham and Kahn, 1995; Crouch et al., 2000; Johnston et al., 2003; Kholodenko et al., 1999; Schoeberl et al., 2002).

Validation and scope of reduced model

Not with standing all changes in the original model that were discussed above, simulation of reduced model versus original model (Borisov et al., 2009) in different doses of insulin and EGF confirm the reliability of the model (figure 4). For simulations, we utilized the same doses as used in original model (Borisov et al., 2009). On the other hand, sensitivity coefficients were obtained in the synergy conditions (Co-stimulation of both stimuli), because we are interested in the study of synergy effects. So, the model is reconstructed for the study of the synergy effects and only works correctly by co-stimulation. Also, at high concentrations of each stimulus, variations from the original model are not negligible (figure 4 (1 nM EGF and 100 nM insulin)). This is due to notability of deleted reactions (especially inhibition reactions) in regulation of high concentrations of each stimulus.

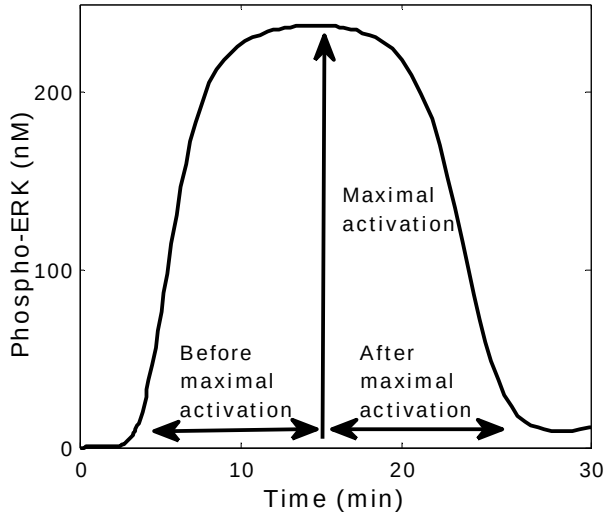


Figure 1. Time regions for calculation of sensitivity coefficients.

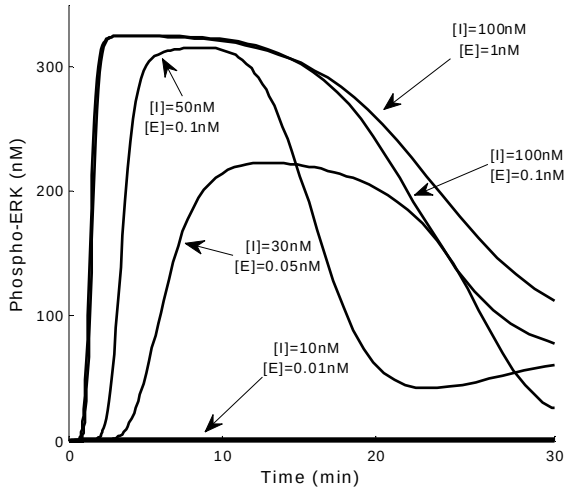


Figure 2. Dose response curve of ERK activation. [E] And [I] represent concentrations of EGF and insulin respectively.

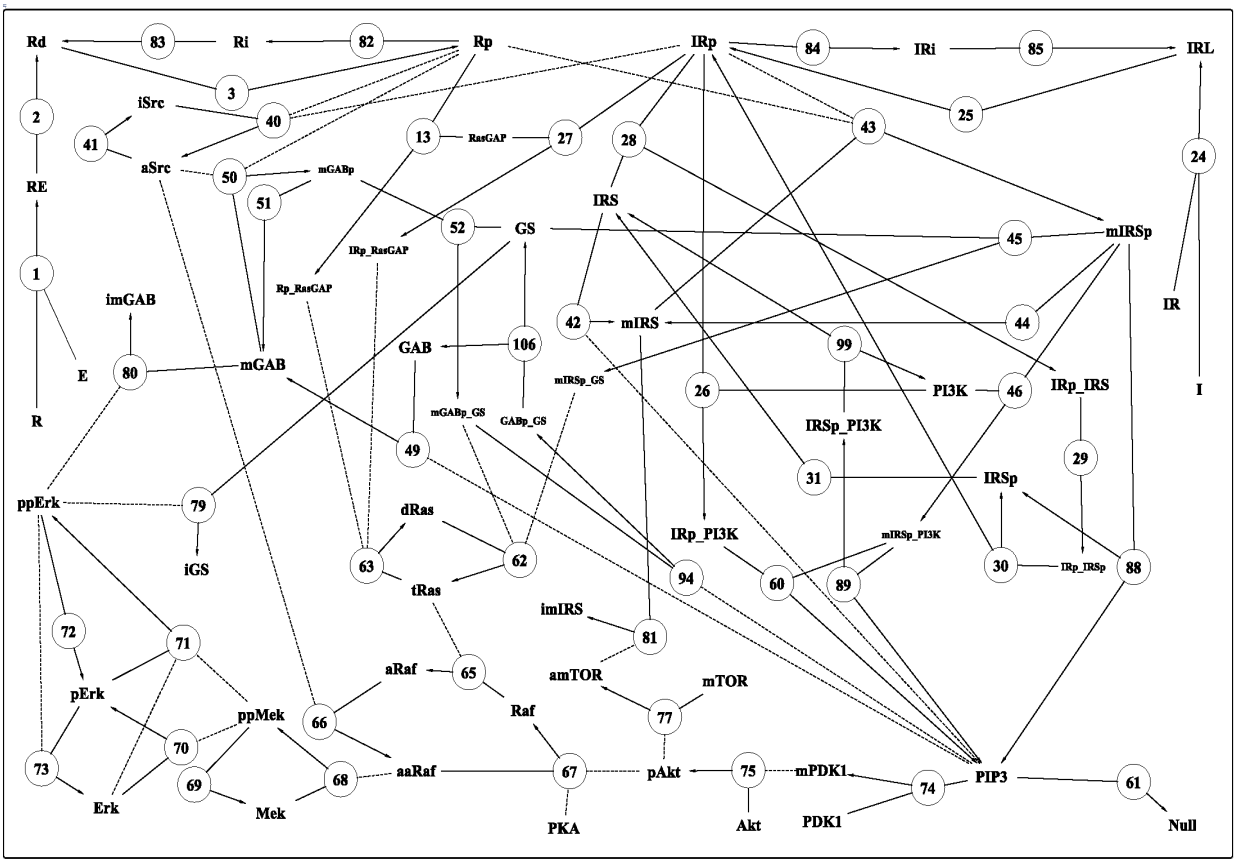


Figure 3. Graphical representation of the reduced Insulin-EGF model. The circles show the number of each reaction. Reactions are numbered according to the original model developed by Brisov et al. (Borisov et al., 2009) (All reactions, rate constant parameters and abbreviations are presented in supplementary table S1) and only operative reactions with respect to their sensitivity coefficients are considered. The dotted lines show the activation and inhibition interactions. Phosphorylation of ERK is the end point of the pathway.

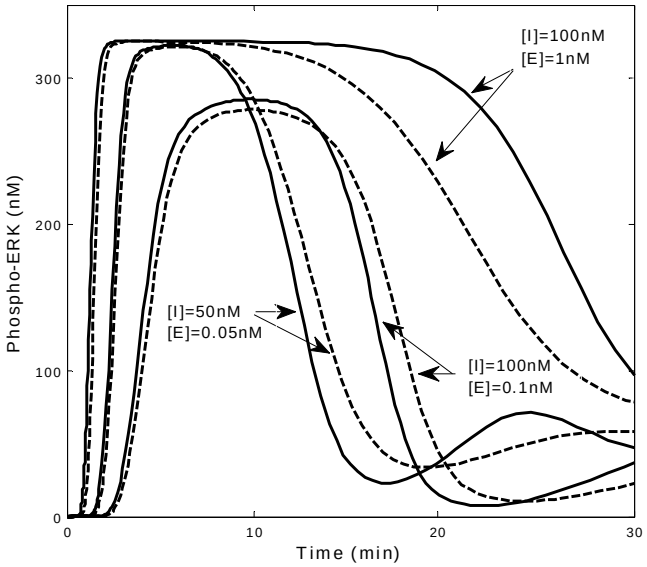


Figure 4. Dynamics of EGF and insulin-induced ERK activation. The dashed lines show the time courses calculated by the original model and the solid lines show the time courses calculated by the reduced model. Calculations were performed with different co-stimulation of EGF and Insulin. [E] And [I] represent concentrations of EGF and insulin, respectively.

Discussion

Unlike the original model (Borisov et al., 2009), the reduced model does not consider direct binding of EGFR to Shc, Grb2–SOS, PI3K, protein phosphatase SHP2 and also binding of phosphorylated insulin receptor to phosphatase SHP2. Instead, phosphorylated EGFR only participates on the activation of Src (reaction 40), phosphorylation of membrane associated IRS (reaction 43), and GAB1 (reaction 50) and binds to RasGAP (reaction 13). Also, Phosphorylated insulin receptor activates Src, phosphorylation of membrane associated IRS and binds to the IRS, PI3K and RasGAP. Surprisingly, all Shc and SHP2-dependent reactions are eliminated in the reduced model. All Ras/ERK cascade reactions are conserved in the reduced model. Activation of ERK was the main purpose in derivation of sensitivity coefficients and thus, some reactions of PI3K/AKT cascade that have no impressive role in activation of ERK, are omitted. Moreover, all degradation reactions and useless complexes are deleted. Now, the model is elucidated to some extent and therefore, it is ready for further discussion, experimentation or analysis.

Synergy nodes in the model-direct participation of insulin and EGF in the crosstalk

Two distinct synergy nodes, with direct participation of the phosphorylated insulin and EGF receptors, appear in the reduced model and involve activation of Src (reaction 40) and phosphorylation of membrane associated IRS (reaction 43, figure 3). Sensitivity coefficients of these two processes in maximal activation of ERK show the emphasis of reaction 40 in comparison with reaction 43 (figure 7A). Parameter variation in different conditions also indicate this result (figures 5 A and B). Diminution of the rate has more destructive effects for reaction 40 than that for reaction 43. Src activation ultimately leads to Raf activation (figure 3). There are some evidences indicating that the application of Src inhibitors, lead to the improvement of tumor cells in some extent (Chen et al., 2008; Koga et al., 2006). This shows the fragility and important role of Src kinase in signaling pathways in different cells which is also in agreement with our results.

The roles of phosphatidylinositol-3, 4, 5-triphosphate (PIP3) in the ERK activation

Clearly, the reduced model shows that PIP3 can negatively (reactions 74 and 94) or positively (reaction 42 and 49) induces the ERK activation. PIP3 induces conversion of cytoplasmic IRS and GAB1 to their membrane associated states that ultimately leads to activation of Ras. The extent and manner of this activation through these two reactions can be elucidated with parameter variation (figure 6A and B). Simulations in the different conditions of these reactions (without, half and complete inhibition) showed that by complete inhibition of the reaction 49, the signal is attenuated near to zero (figure 6A). However, half and non inhibition conditions produce similar results. This means that in the synergy conditions, despite the notability of reaction 49 in the pathway, the signal is promoted by a small performance of this reaction. On the other hand, the signal has some sensitivity to the variation in the rate parameters of reaction 42 (figure 6B). These results are reflected in sensitivity coefficients, too. Maximal signal has no sensitivity to reaction 49; but reaction 49 has a sensitivity coefficient of about 0.45 (figure 7A).

Variations in the rate parameters of the reaction 74 do not change the signal amplitude considerably (data not shown); instead the reaction 94 has a high regulatory role in the model (figure 6C).

The reduced model predicts that interactions of PI3K with phosphorylated Insulin receptor and IRS are responsible reactions for PIP3 production. However, the original model has also been considered the interactions of GAB1 and phosphorylated EGFR (reaction 12 and 53 in the supplement, table S1). These results reveal some key mechanistic elucidation of crosstalk pathways in the reduced model. EGF network, by activation of Src and GAB1 modules can positively regulate the ERK activation while the insulin network activates this signal by production of PIP3. This molecular trade leads to potentialities in the mitogenic signal.(Borisov et al., 2009; Chong et al., 2004; Crouch et al., 2000).

Critical nods of the model

We conserved all reactions of Ras/ERK cascade in the reduced model (reactions 63-73). This is due to the high amounts of sensitivity coefficients for the reactions of this cascade. The coefficients also show the fragility of each reaction (figure 7 A and B). Thus, like Src kinase, these fragilities lead to the appearance of new points for drug targeting (Amit et al., 2007; Roberts and Der, 2007; Scaltriti and Baselga, 2006; Zhu and Kyprianou, 2008). However, the sensitivity coefficients for the crosstalk mechanism, rather than for each isolated pathway, are to some extent decreased (Data not shown). This shows that the co-stimulation of insulin and EGF decreases the stress on the Ras/ERK cascade and corroborates the synergy

results in the integrated crosstalk mechanism (Borisov et al., 2009). Other substantial reactions are in upstream of the insulin and EGF networks (reaction 1 and 24). Reaction 1 has the highest sensitivity coefficient in the maximal ERK activation (figure 7A), whereas reaction 24 has the highest sensitivity coefficient before maximal activation of ERK (figure 7B). Parameter variation shows that the role of the reaction 1 is more critical than reaction 24 (figure 8 A and B). This confirms the valuably of sensitivity coefficients in maximal ERK activation relative to the other time regions.

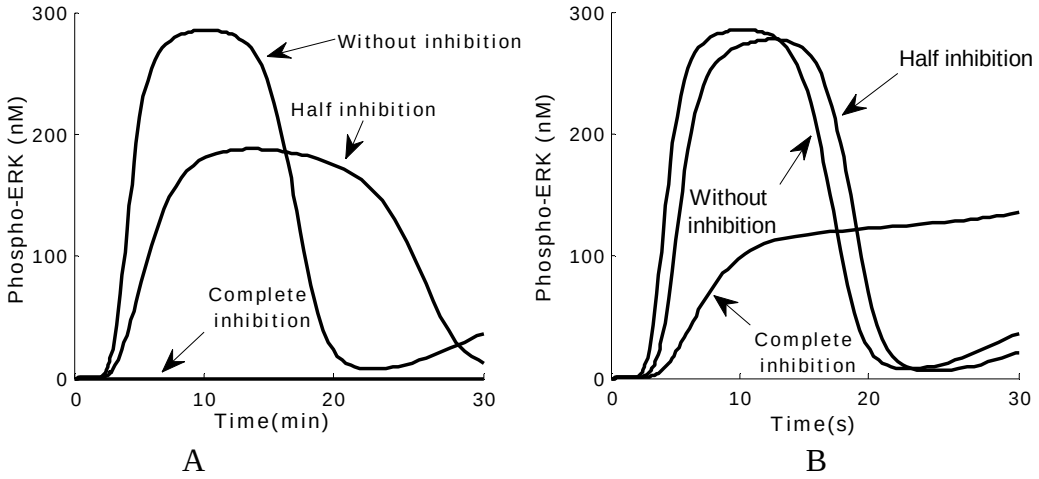


Figure 5. Activation of ERK in response to changes in the reaction 40 (A) and reaction 43 (B) in various levels of inhibition.

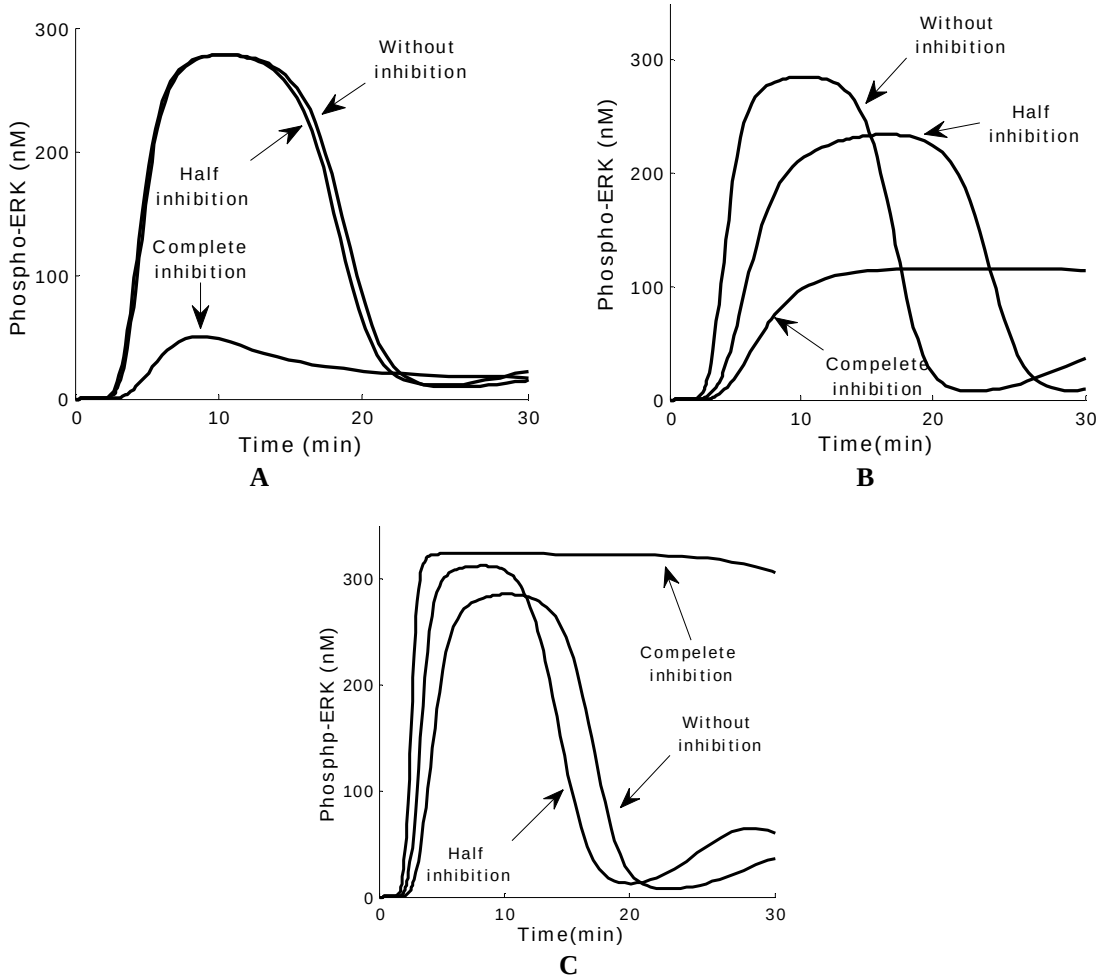
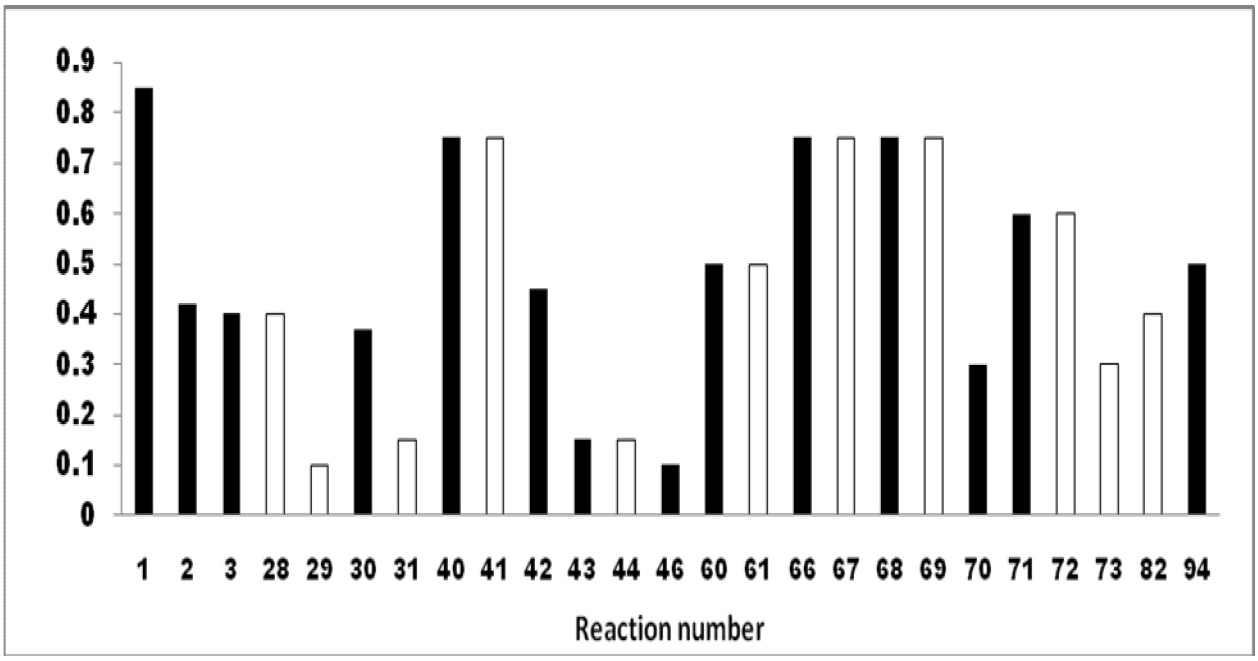
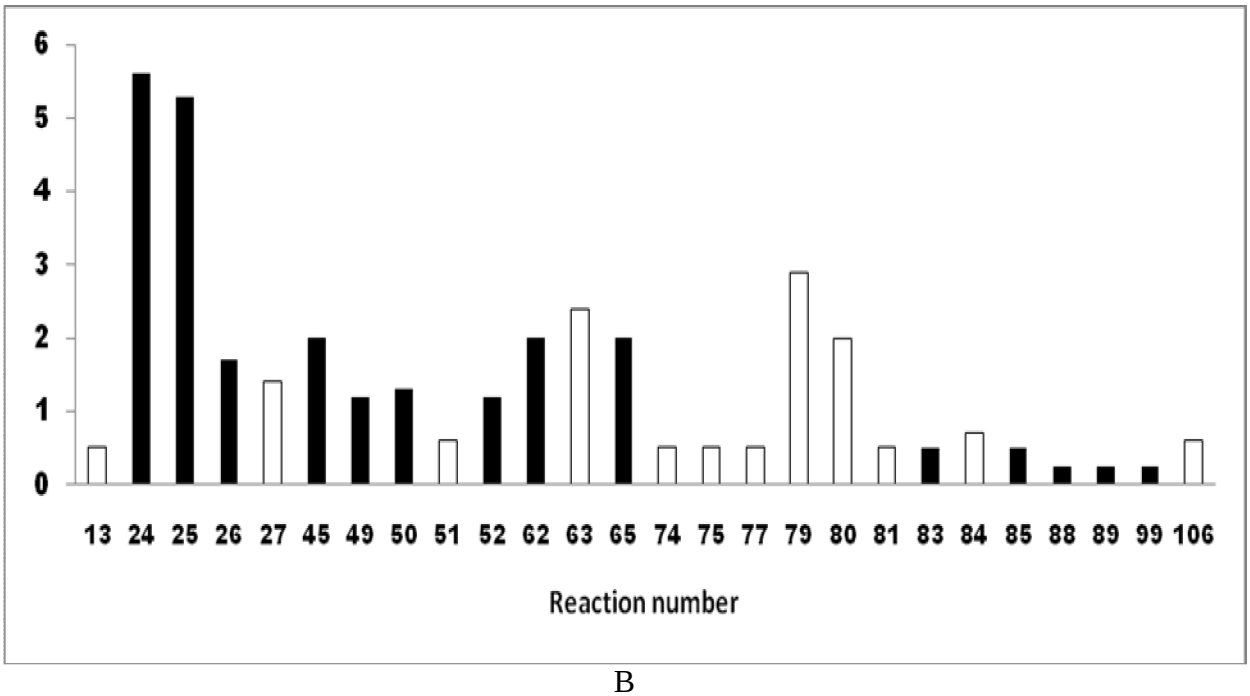


Figure 6. Activation of ERK in response to changes in the reaction 49 (A), reaction 42(B) and reaction 94 (C) in various levels of inhibition.



A



B

Figure 7. Sensitivity coefficients of the operative reactions of insulin-EGF crosstalk. (A) Reactions that have some sensitivity in maximal ERK activation. (B) Reactions that have no sensitivity in maximal ERK activation but have some sensitivity before and after it. (The bars with black and white colors represent minus and plus sensitivity coefficients, respectively.

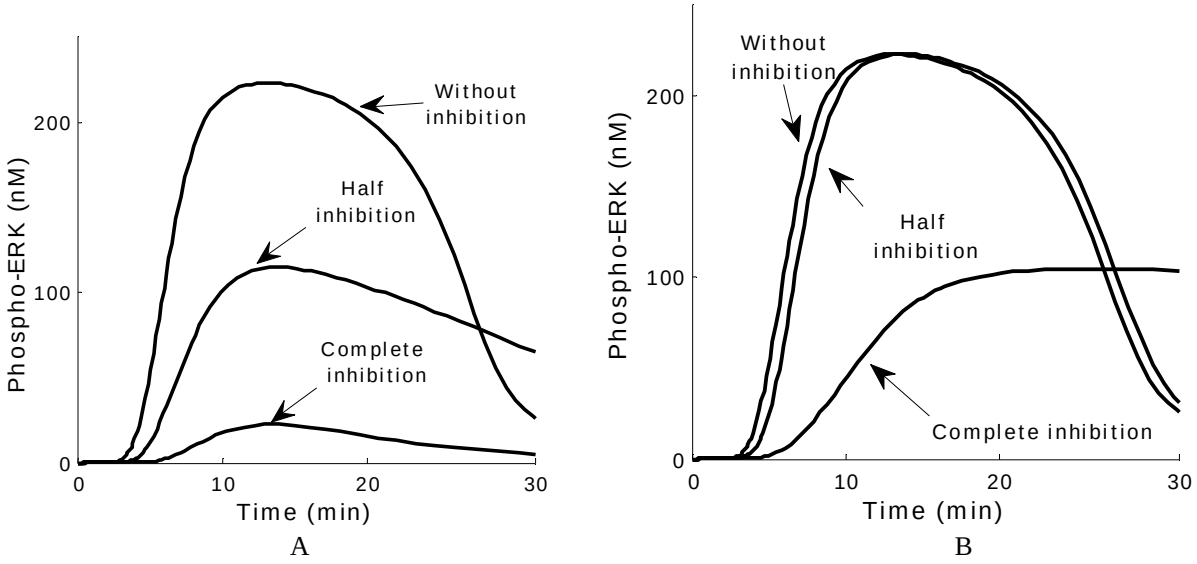


Figure 8. Activation of ERK in response to changes in the reaction 1 (A), reaction 24 (B) in various levels of inhibition.

In summary, the main aims of the current study are to reproduce an elucidated model of insulin-EGF networks and reliably determine the controlling and synergy nods of this crosstalk. Major effects of EGF pathway appear in participation of the phosphorylated EGF receptor in the activation of Src kinase and GAB1. Src kinase is a crucial and synergic nod of the crosstalk. Activation of Src leads to Raf activation and its downstream events. Thereby, signal is destructed by complete inhibition of Src activation. Roles of insulin pathway are reflected in recruitments IRS and PIP3 by phosphrylated insulin receptor. These two modules influence Ras and GAB1 activation, respectively. Finally, all of these solidarities are emerged with regard to activation of Ras–Raf–MEK–ERK cascade. The high amounts of sensitivity coefficients of the cascade reactions confirm these objectivities. Also, the recent therapeutic reports indicated that the targets of many anti-cancer drugs are within the Ras–Raf–MEK–ERK cascade. These reports are repeated for Src and GAB1, too. We hope that the results of this paper and other similar studies would elucidate some ambiguities of the drug targeting mechanisms.

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