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Improved Kinetic Model of Short Term EGFR Signaling Network

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Abstract. Short Term Signaling by the Epidemical Growth Factor Receptor (EGFR) plays important roles in the process of cell growth, differentiation and replication. The kinetic model was developed by Kholodenko et al. in 1999 and reconstructed by Atef Suleiman in 2006. The simulation results agreed to a certain extent with experimental analysis of the effect of Epidermal Growth Factor (EGF) on the activation of downstream proteins, but the model deviated from experimental results of EGFR signaling at EGF concentrations below saturating level. In this paper, we improve the model by considering the degradation of protein in EGFR signal pathways. To evaluate effectively the error between simulation results and experimental data, we present a new definition (namely as acceptance degree) and calculate a reasonable fitness value, so that the genetic algorithm is used to obtain optimized degradation rates. The comparative research between the simulation results and experimental analysis show that the improved model is better than the kinetic model developed by Kholodenko et al. and Atef Suleiman. Our work can provide valuable insight into the dynamics of the signal-response relationship between the activated EGFR and the activation of downstream proteins.

Keywords: EGFR, degradation, acceptance degree, genetic algorithm.

1. Introduction

Cell signaling networks are very complicated in terms of organization and regulation. Quantitive research can make us view the whole network systematically and make related experiments controllable and repeatable, which may contribute to know about cells and cure several disease. The Epidermal Growth Factor (EGF), which is a signaling molecule that affects gene expression, binds with another transmembrane receptor--the Epidermal Growth Factor Receptor (EGFR)--a messenger protein positioned at the surface of the cell. The binding of the ligand EGF to two adjacent EGFR monomers forms an active EGFR dimer which is a tyrosine kinase, an enzyme that attaches phosphate groups to certain tyrosine residues converting them into an active state. Some of the tyrosine kinase will spread into cell nucleus and affect the expression of gene ultimately. Thus EGF and EGFR play a critical role in the growth of a cell, and research of the EGFR signaling network is of great importance. Kholodenko et al. developed the kinetic model in 1999 [1] and Atef Suleiman reconstructed the model [2] in 2006, which provides an integrative and quantitative description of EGFR signal transduction. However, at EGF concentrations below saturating level, these models can not fit experimental results of EGFR signaling. In this paper, we modify the model to capture the quantitative and qualitative behavior of EGFR signaling for a wide range of EGF stimulation. Moreover, we use the genetic algorithm to search the parameters in the modified model to fit experimental results in different EGF stimulation, and obtain good results.

2. Model and Method

2.1. The degradation of proteins

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Biological experiments show that proteins in cells can be degraded in two ways[3][4]: (1) the lysosome in a cell contains many acidic enzymes like protease which can degrade the proteins. (2) Ubiquitination. In ubiquitination, a protein is inactivated by attaching ubiquitin to it. Ubiquitin acts as a tag that signals the protein-transport machinery to ferry the protein to the proteasome for degradation. Although the speed of degradation inside a cell is very slow, this mechanism may have a great influence on the density of the protein and should be considered in the model.

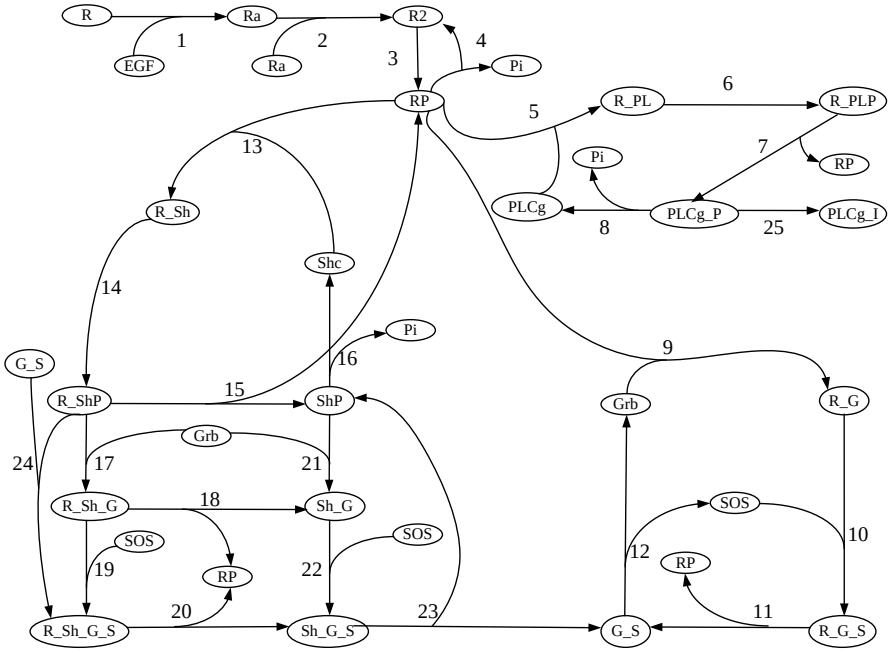

Fig.1: Kinetic scheme of EGFR signaling pathways.

2.2. Kinetic equations

All the kinetic interactions of short term EGFR signaling pathways are illustrated in Fig.1, which is derived from [2]. Ri denotes the kinetic equation of reaction i, ki and kbi are forward and backward rate constant of reaction i respectively, fi is the degraded rate constant of protein i. All proteins' reactions are listed in Table 1. This EGFR network comprises 23 variables which participate in 25 kinetic reactions with 73 associated rate constants. The model of the signalling network can be described by the 23 ordinary differential equations which are listed on Table 2, and all the initial concentrations of the corresponding components, forward and backward rate constants are the same as in [2].

Table.1: Rate equation

Reaction	**Symbolic Equation**	**Rate Equation**
R1	R+EGF→Ra	k1[R][EGF] – kb1[Ra]
R2	Ra + Ra→R2	k2[Ra][Ra] – kb2[R2]
R3	R2→RP	k3[R2] – kb3[RP]
R4	RP→R2+Pi	V4[RP]/(k4+[RP])
R5	RP+PLCg→R_PL	k5[RP][PLCg] – kb5[R_PL]
R6	R_PL→R_PLP	k6[R_PL] – kb6[R_PLP]
R7	R_PLP→RP+PLCg_P	k7[R_PLP] – kb7[RP][PLCg_P]
R8	PLCg_P→PLCg+Pi	V8[PLCg_P]/(k8+[PLC_g])
R9	RP+Grb→R_G	k9[RP][Grb] – kb9[R-G]
R10	R_G+SOS→R_G_S	k10[R_G][SOS] – kb10[R_G_S]
R11	R_G_S→RP+G_S	k11[R_G_S] – kb11[RP][G_S]
R12	G_S→Grb+SOS	k12[G_S] – kb12[Grb][SOS]
R13	RP+Shc→R_Sh	k13[RP][Shc] – kb13[R_Sh]
R14	R_Sh→R_ShP	k14[R_Sh] – kb14[R_ShP]
R15	R_ShP→ShP+RP	k15[R_ShP] – kb15[ShP][RP]

R16	ShP→Shc+Pi	V16[ShP]/(k16+[ShP])
R17	R_ShP+Grb→R_Sh_G	k17[R_ShP][Grb] – kb17[R_Sh_G]
R18	R_Sh_G→RP+Sh_G	k18[R_Sh_G] – kb18[RP][Sh_G]
R19	R_Sh_G+SOS→R_Sh_GS	k19[R_Sh_G][SOS] – kb19[R_Sh_G_S]
R20	R_Sh_G_S→Sh_G_S+RP	k20[R_Sh_G_S] – kb20[Sh_G_S][RP]
R21	ShP+Grb→Sh_G	k21[ShP][Grb] – kb21[Sh_G]
R22	Sh_G+SOS→Sh_G_S	k22[Sh_G][SOS] – kb22[Sh_G_S]
R23	Sh_G_S→ShP+G_S	k23[Sh_G_S] – kb23[ShP][G_S]
R24	R_ShP+G_S→R_Sh_G_S	k24[R_ShP][G_S] – kb24[R_Sh_G_S]
R25	PLCg_P→PLCg_I	k25[PLCg_P] – kb25[PLCg_I]

Table.2: Kinetic equations

No.	Protein	Kinetic equation
1	EGF	d[EGF]/dt= – R1 – f1[EGF]
2	R	d[R]/dt= – R1 – f2[R]
3	Ra	d[Ra]/dt= + R1 – R2 – R2 – f3[Ra]
4	R2	d[R2]/dt= + R2 + R4 – R3 – f4[R2]
5	RP	d[RP]/dt= +R3 + R7 + R11 + R15 + R18 + R20– R4 – R5– R9 – R13 – f5[RP]
6	R-PL	d[R-PL]/dt= + R5 – R6 – f6[R-PL]
7	R-PLP	d[R-PLP]/dt= + R6 – R7 – f7[R-PLP]
8	R-G	d[R-G]/dt= + R9 – R10 – f8[R-G]
9	R-G-S	d[R-G-S]/dt= + R10 – R11 – f9[R-G-S]
10	R-Sh	d[R-Sh]/dt= + R13 – R14 – f10[R-Sh]
11	R-ShP	d[R-ShP]/dt= + R14 – R15 – R17 – R24 – f11[R-ShP]
12	R-Sh-G	d[R-Sh-G]/dt= + R17 – R18 – R19 – f12[R-Sh-G]
13	R-Sh-G-S	d[R-Sh-G-S]/dt= + R19 + R24 – R20 – f13[R-Sh-G-S]
14	G-S	d[G-S]/dt= + R11 + R23 – R12 – R24 – f14[G-S]
15	ShP	d[ShP]/dt= + R15 + R23 – R16 – R21 – f15[ShP]
16	Sh-G	d[Sh-G]/dt= + R18 + R21 – R22 – f16[Sh-G]
17	Sh-G-S	d[Sh-G-S]/dt= + R20 + R22 – R23 – f17[Sh-G-S]
18	PLCg	d[PLCg]/dt= + R8 – R5 – f18[PLCg]
19	PLCg-I	d[PLCg-I]/dt= + R25 – f19[PLCg-I]
20	PLCgP	d[PLCgP]/dt= + R7 – R8 – R25 – f20[PLCgP]
21	Grb	d[Grb]/dt= + R12 – R9 – R17 – R21 – f21[Grb]
22	Shc	d[Shc]/dt= + R16 – R13 – f22[Shc]
23	SOS	d[SOS]/dt= + R12 – R10 – R19 – R22 – f23[SOS]

2.3. Searching optimized degradation constants by genetic algorithm

In our model, all forward and backward rate constants are the same as in [2]. 23 degradation rate constants are obtained by using genetic algorithm, which is a modern optimization technique [5]. Therefore, the problem is converted into the following optimization problem:

(P) To find the optimal combination of 23 degradation rate constants that minimizes the errors between the simulation results and experimental data.

To make a reasonable evaluation for the errors, we propose a new definition, named as acceptance degree, which is used to describe the credibility of the simulating results. For example, an experimental result is 10, while the simulating result is 7, we use function $f(x)$ to determine the credibility of 7, where x is the relative error of 7. It is obvious that there is a negative correlation between x and $f(x)$. So, $f(x)$ can be described as:

$$f(x)=\begin{cases}1, x \le a\\ g(x), a<x<b\\ 0, b \le x\end{cases}$$

Where $g(x)$ is monotonic decreasing function, and $g(a)=1, g(b)=0$. That is, when relative error is smaller than a, the simulation result is acceptable; and when it is larger than b, the simulation result is unacceptable. Actually, a, b and $g(x)$ are settled manually according to the experimental condition. Actually, in this paper, we consider $g(x)$ as a linear function and $b=1$. According to [1], the initial density of EGF directly influences the density of different substance inside the cell. The lower initial density is, the harder to detect the real

value of downstream substance. In this paper, when the initial density of EGF is 20 nm, 2nm, and 0.2nm, *a* is set to 0.05, 0.08, and 0.1 respectively. Because the degradation of proteins is slow, we limit the searching range in 0～0.01.

Here, we use a vector w=(f1,f2,f3,…,f23) to represent 23 degradation rate constants. In [2], the experimental data at different time t=15, 30, 45, 60, 120 and three different initial density of EGF 20 nm, 2nm, and 0.2nm are given. Let xij represent the simulation result of one initial density of EGF i at time j, then the fitness for a set of degradation constants (w) is defined as follows:

$$F(w) = \sum_{i=1}^{3}\sum_{j=1}^{5} f\left(\frac{|x_{ij} - y_{ij}|}{x_{ij}}\right) \quad (1)$$

The genetic algorithm can be described as the follows:

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Step1 t=0, generate randomly an initial population P(t)={X1 X2… XN};
Step2 Calculate the fitness values of all individuals according to (1).
Step3 Repeatedly execute step4 to step7 until the termination conditions are
      satisfied;
Step4 t=t+1;
Step5 Randomly select m1 individuals to do multi-parent crossover and m2
      individuals to mutate, and to generate n new individuals;
Step6 Compare the new individuals with the worst individuals, and select the
      new individuals;
Step7 Calculate the fitness values of all individuals according to (1);
Step8 Output the all results.
```

3. Results

3.1. Optimized degradation rate constant

Based on the algorithm in Section 2.3, we obtain a set of degradation constants which are listed at Table 3. Their average acceptance degree is 11.6326, acceptance degree of the previously model in [2] is 9.1613.

Table.3: degradation constants

No.	Protein	degradation constant	No.	Protein	degradation constant
1	EGF	0.0033	13	R-Sh-G-S	0.0003
2	R	0.0010	14	G-S	0.0074
3	Ra	0.0015	15	ShP	0.0079
4	R2	0.0033	16	Sh-G	0.0084
5	RP	0.0044	17	Sh-G-S	0.0097
6	R-PL	0.0039	18	PLCg	0.0099
7	R-PLP	0.0025	19	PLCg-I	0.0059
8	R-G	0.0089	20	PLCgP	0.0088
9	R-G-S	0.0054	21	Grb	0.0079
10	R-Sh	0.0014	22	Shc	0.0093
11	R-ShP	0.0000	23	SOS	0.0043
12	R-Sh-G	0.0051			

3.2. Comparison of time course simulation and experimentally measured time course of EGFR autophosphorylation

At saturating EGF concentration (20nM), the comparison of the simulation and measured time course of phosphorylated EGFR is shown in Fig.2. From Fig.2 we can observe that there is no obvious difference between our model and the model in [2]. Both models have 4 simulation results which can be accepted. In both cases, the peak phosphorylation level of EGFR is reached within 15 seconds and constitutes approximately 70-80% of total EGFR, and the sustained level is reached within 2 minutes and constitutes approximately 10-20% of total EGFR. At EGF concentration of 2 nM and 0.2 nM, the comparisons of the simulation and measured time course of phosphorylated EGFR are depicted in Fig.3 and Fig.4 respectively. Fig.3 and Fig.4 show that our improved model is better than the model in [2], especially at time 120, our simulation results are agreement with the experiment results.

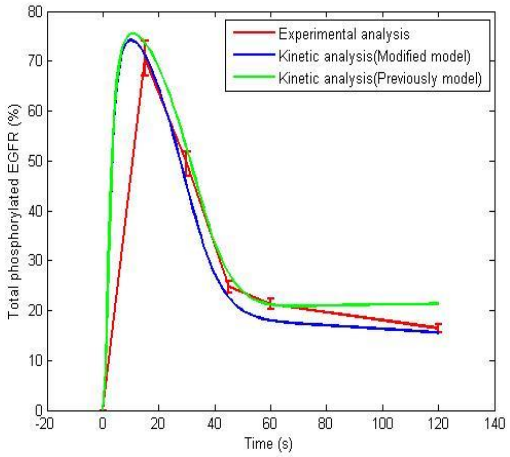

Fig.2: Comparison of simulation and experimentally measure of EGFR autophosphorylation at 20 nM EGF.

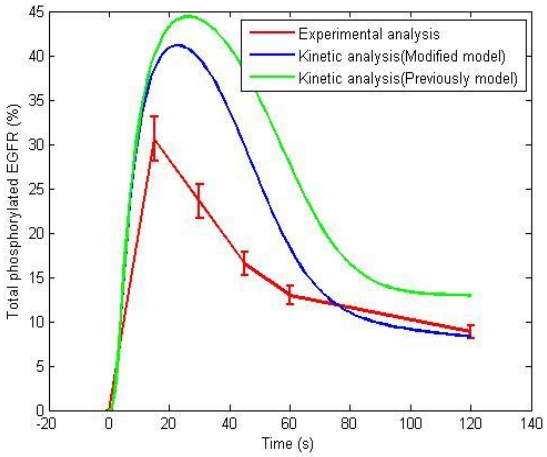

Fig.3: Comparison of simulation and experimentally measure of EGFR autophosphorylation at 2 nM EGF.

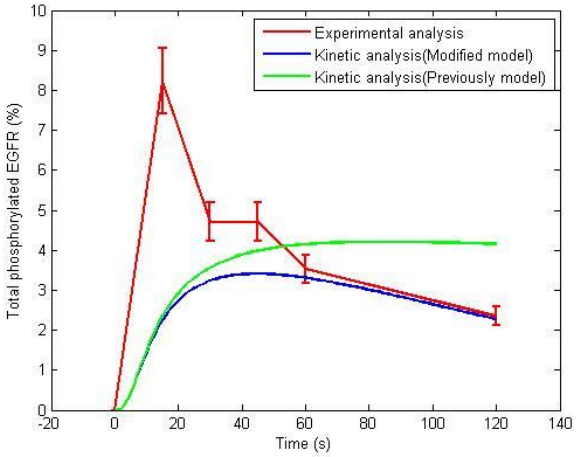

Fig.4: Comparison of simulation and experimentally measure of EGFR autophosphorylation at 0.2 nM EGF.

4. Conclusion

In this paper, we reconstruct the kinetic model of EGFR signaling pathways. We propose a new method to evaluate the simulating results and use the genetic algorithm to obtain a set of optimized degradation rate constants. The comparison study of simulating results and experimental data shows that the new model is more accurate and is considered to be acceptable in the research of cell signaling networks.

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