Large-Scale Simulations of Eukaryotic Cell Signaling Processes

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Abstract

A database of rate constants and related quantities has been assembled by Schoeberl et al. [1] for intracellular signaling downstream of the epidermal growth factor receptor (EGFR). This information was combined with data on metalloprotease activation [2] to build a model of autocrine signal transduction by cancer cells exposed to ionizing radiation. The model predicts prompt activation of mitogen-activated-protein-kinase (MAPK) pathways in response to a radiation-induced shift in the RasGDP↔RasGTP equilibrium toward more RasGTP. A secondary MAPK activation is predicted due to metalloprotease activity that releases transforming growth factor alpha (TGFα), an autocrine ligand of EGFR. Model predictions were compared to data by Dent et al. [3] on extracellular regulated kinase (ERK) activation following a 2 Gy exposure of carcinoma cells in vitro. Good agreement was obtained with the magnitude of prompt and secondary ERK activation; however, the experimental secondary response was delayed relative the prompt peak more than predicted by our model. A mechanistic understanding of radiation-induced growth factors is needed to improve treatment of cancer by radiation therapy. Inhibitors of the signaling pathways modeled in this study may reduce a potentially self-limiting aspect of radiation therapy whereby induced growth factors accelerate repopulation of treated tumor volumes.

Introduction

The advent of high-throughput data collection techniques in molecular biology has stimulated efforts to achieve a systems-level understanding of cellular functions [4]. While this approach has been most successful in simple systems, such as *E coli*, intense effect is being directed toward achieving similar results for eukaryotic systems directly related to disease states. Among the latter, signal transduction from EGFR clearly stands out as a paradigm for systems biology [5]. Since its discovery as a receptor tyrosine kinase, EGFR has been used in numerous studies of eukaryotic signaling processes (reviewed in [6]). Among the functions identified for EGFR family members, their role in MAPK signaling pathways is probably the most significant for cancer research and has led to new targets for treatment therapies [7].

Both *in vitro* and *in vivo* studies (reviewed in [8]) suggest that cancer-cell proliferation is regulated, in part, by autocrine-acting ligands, such as TGFα, which are synthesized on the surface of cells that also express their target receptor, EGFR. Increased expression of TGFα and activation MAPK pathways via EGFR has been proposed as a mechanism by which irradiation of cancer cells may enhance the proliferation rate of survivors within treated tumor volumes [9]. Hence, radiation therapy may contain a self-limiting effect mediated by EGFR and associated downstream signaling. This possibility has stimulated effort to find inhibitors of EGFR that will enhance the effectiveness of radiation oncology. We undertook the task of modeling autocrine signaling induced by exposure of cancer cells to ionizing radiation with the goal of contributing to this effort.

Dent et al. [3] showed that 1-2 Gy of gamma irradiation activated MAPK pathways via EGFR to levels comparable to those induced by 0.01nM of EGF. The prompt response monitored by ERK activation lasted about 30 minutes. A secondary response was observed that peaked at 2 hours and remained detectable for about 5 hours. Similar kinetics was observed for activation of cJun N-terminal kinase (JNK). Addition of an antibody, specific for binding to TGFα, eliminated the secondary response, which suggested that the prompt activation of MAPK pathways caused shedding of

TGFα, some of which was captured on EGFR to induce the secondary response.

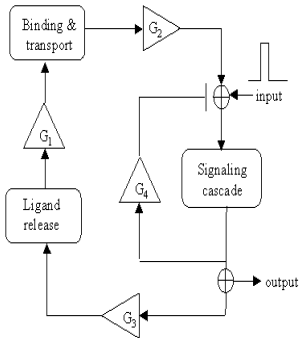

Figure 1: Schematic of autocrine-loop model developed by Shvartsman et al [10]

The model developed by Shvartsman et al. [10] to explain the experimental findings of Dent et al. [3] is illustrated by the schematic in Figure (1). The action of radiation was assumed to induce a perturbation at the first stage of a 3-level kinase cascade. The kinetics of each level was modeled by the difference between rates of phosphorylation and de-phosphorylation as developed by Goldbeter and Koshland [11]. A square pulse with duration equal to that of the radiation treatment was added to ligand-receptor complexes to shift the first stage of the cascade toward greater kinase activity. Similar shifts in the lower 2 levels rapidly followed in the simulations. A negative feedback, scaled by gain parameter G4, suppressed the input to the cascade.

To predict a secondary MAPK response to the perturbation, output from the cascade module was coupled to a model of regulated protease activity through gain parameter G3. Elevated ERK activation increased the rate of conversion of inactive protease to the active form. The rate of TGFα shedding was assumed to be proportional, through gain parameter G1, to amount of active protease on the cell surface. Shedding added to the balance between ligand-receptor association and dissociation to create a flux of TGFα at the cell surface. This flux was one of the boundary conditions on the solution of diffusion-reaction equations that determined whether TGFα escaped into the bulk medium, where it could react with antibodies, or was captured by EGFR and coupled to secondary ERK activation through gain parameter G2.

The model developed by Shvartsman et al. [10] was qualitatively successful. In agreement with experiment, it predicted a secondary response with a maximum level about half as large as the peak of the prompt response. The predicted secondary response diminished as the concentration of TGFα-binding antibodies in the bulk medium increased. However, the kinetics of the secondary response was too rapid. The observed secondary ERK activation was separated in time from the prompt response, was largest at 2 hours post exposure, and lasted for 5 hours. The secondary response predicted by Shvartsman's model [10] overlapped the prompt ERK activation and returned to control levels in about 2 hours.

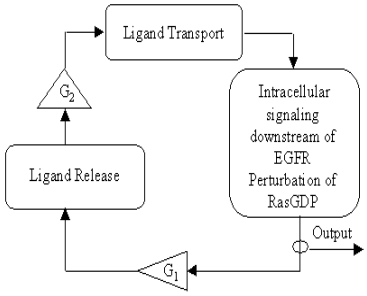

Figure 2: Model of autocrine loop using database assembled by Schoeberl et al [1]

Our model, illustrated by Figure (2), differs from Shvartsman et al. [10] in several significant aspects. First, we treat ligand transport by the method of Oehrtman et al. [12], which allows us to model anchorage-dependent cell cultures of the type used Dent et al. [3] and to include effects of plating density. Second, and most important, we use the model of Schoeberl et al. [1] for intracellular signaling downstream of EGFR. In addition to being the most complete mathematical description of ERK activation published thus far, Schoeberl's model [1] includes signaling from internalized receptors. Since endocytosis and receptor trafficking are relatively slow processes [13], signaling from internalized receptors extends the duration of ERK activation from low-levels of EGFR binding and, consequently, might explain the slow secondary response observed by Dent et al. [3].

Methods

Modules that make up our model of an autocrine loop are shown in Figure (3). Each module gives rise

to a set of ordinary differential equations (ODEs) and related rate constants. Equations for ligand transport were taken from Oehrtman et al. [12]. They not only describe the diffusion of TGFα but also antibodies added to the medium that block EGFR and allow more autocrine ligands to escape into the bulk. Equations for ligand release were taken from Shvartsman et al. [10], and include the coupling of ERK activation to metalloprotease activity and shedding, which involves gain parameters G1 and G2 in Figure (2). Equations describing the kinetics of ERK activation in response to autophosphorylation of EGFR were taken from the work of Schoeberl et al. [1]. In total, the model involved 104 ODEs coupled by 148 chemical reactions. Rate equations, rate constants, and other model parameters are available from the author (JHM) upon request.

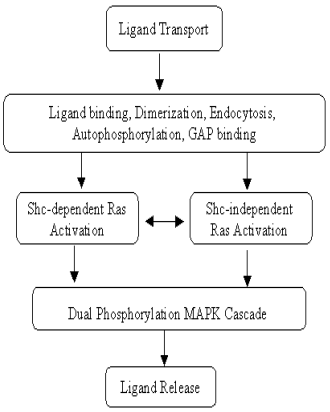

Figure 3: Components of our autocrine-loop model.

As illustrated in Figure (3), activation of the G-protein Ras by replacing guanosine diphosphate (GDP) with guanosine triphosphate (GTP), is a critical intermediate step in MAPK activation. We modeled the perturbation of cancer cells by radiation exposure that stimulates growth factor release as a small shift in the RasGDP ↔ RasGTP equilibrium toward more RasGTP.

The flow chart in Figure (4) shows the structure of the MATLAB code developed to perform our kinetic simulations. The ODEs of the model are solved twice, first to generate a steady state of the system in the absence of radiation exposure and then to simulate the transit response when the steady state is perturbed. At the beginning of the equilibration stage, the user is asked to input the total number of EGFRs per cell and whether the shc-dependent mode of Ras activation is to be included. After a subroutine call to obtain additional parameters of the model that are infrequently changed, the ODE solver is called to generate the steady state.

Values of the dynamic variables in the steady state are used as initial conditions for simulating the response to radiation exposure. The user is asked if receptor-blocking antibodies are present. After the characteristics of the perturbation caused by the radiation exposure are obtained, the ODE solver is called again to generate the transient response. The output subroutine processes the solution matrix to graph selected quantities for model interpretation and comparison with experimental data.

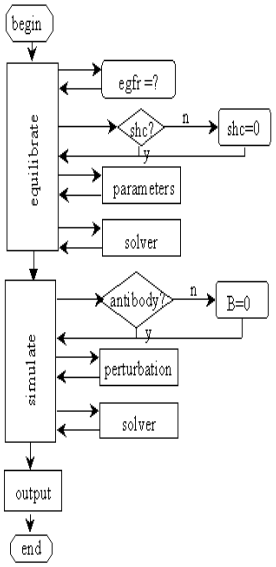

Figure 4: Flow chart of MATLAB code developed to solve the kinetics of ERK activation.

Results

Even in the absence of perturbation by ionizing radition, our model is subject to positive feedback if gain parameters G1 and G2 in Figure (2) are sufficiently large to increase the rate of metalloprotease activation above the basal level. We found that gain parameters in this range were required to predict a secondary response of the magnitude observed by Dent et al.[3]; consequently, the dynamic variables are only quasi-stable at the end of the equilibration phase of the simulation. In this quasi-stable state, the dynamic variables have

different rates of change but were below levels of experimental detection in all cases.

The magnitude of the perturbation, 0.25% of the RasGDP in the quasi-steady state converted to RasGTP, was chosen to give agreement with the prompt ERK activation reported by Dent et al. [3] when carcinoma cells were exposed to 2 Gy of gamma irradiation. Figure 5 shows that with this perturbation and gain parameters G1 and G2 equal to 3.172 and 0.008, respectively, model predictions are in good agreement with the observed magnitudes of the prompt and secondary MAPK activation, as well as the rate of decay of the secondary response. However, our best fit to the data Dent et al. [3] is still poor at the four data points that immediately following the peak of the prompt response.

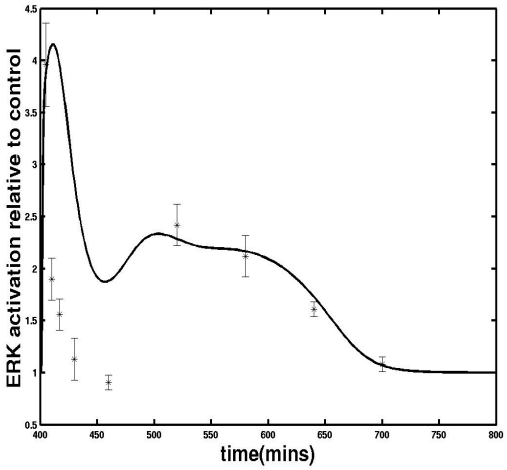

Figure 5: Fit of model to data of Dent et al. [3]

Our model predicts no secondary EFK activation when we include a high concentration of EGFR-blocking antibodies that allows essentially all of the shed TGFα to escape into the growth medium. Figure (6) shows that predicted kinetics under these conditions are a little slower than the observed prompt ERK activation. However, these results clearly show that the main source of the disagreement between our model and experiment data is the overly rapid development of secondary ERK activation.

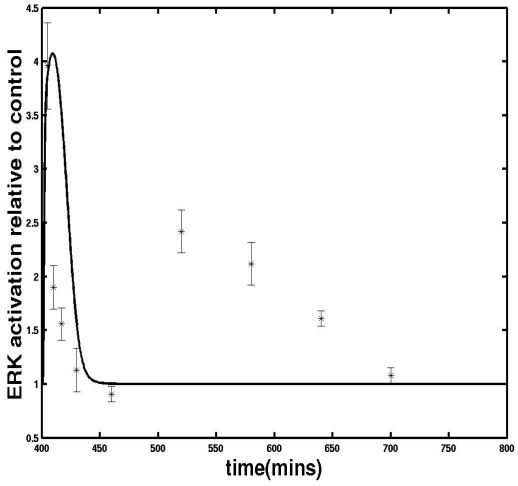

Figure 6: Fit to prompt ERK activation with EGFR blocking antibodies present.

Several explanations for the delay of protease activity are possible. First, gene expression may be required in radiation-induced shedding. This mechanism would be similar to the positive feedback in *Drosophila* where MAPK activated by EGFR induces transcription of Rhomboid, an intracellular protease that processes the EGFR-ligand Spitz [14]. Second, other signaling pathways may be involved. The intermediate role of ERK activation in the protein kinase C-regulated cleavage of TrkA [15] is an example of this type of delay between MAPK activation and shedding. Work is in progress to model these delay mechanisms.

Acknowledgements

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