System modeling of EphB4/ephrinB2 signaling pathways

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Introduction

It has recently been shown that Eph-ephrin interactions are necessary for tumor angiogenesis and essential for tumor progression. Understanding the main functions of Eph-ephrin interactions and the intricacies of related signaling pathways would be helpful for the discovery of novel antitumor drugs. Eph–ephrin interactions can trigger a wide array of cellular responses, including cell adhesion, migration, proliferation and repulsion. Currently, the exact mechanism leading to the diversity of these responses remains unclear. In order to better understand this system, we have used a kinetic modeling approach to study the intricacies of the EphB4 system.

The main aims of this study are to:

1. Reconstruct the EphB4/ephrinB2 signaling pathways from the literature

2. Develop a kinetic model for EphB4/ephrinB2 forward signaling

3. Analyze the model behavior and prediction to allow deeper insight into EphB4/ephrinB2 forward signaling and its influence on tumor progression or suppression mechanisms

Pathway reconstruction

General scheme of basic events in EphB4/ EphrinB2 signaling in epithelial cells
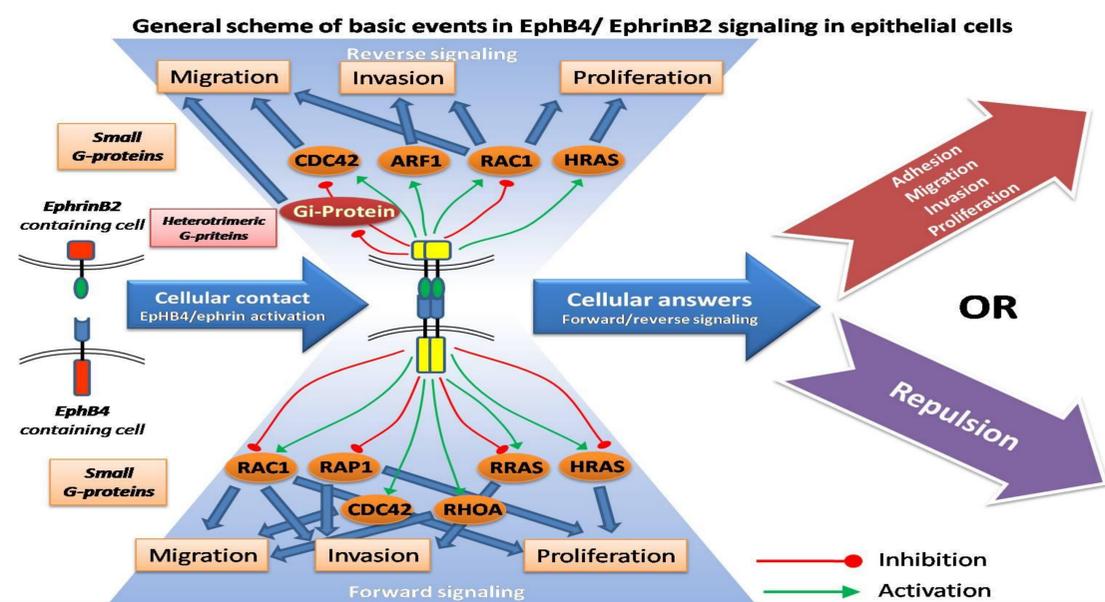
Main results of pathway reconstruction

We have ascertained all components of the EphB4/ephrinB2 regulatory system and interaction between them including reverse and forward signaling. For example, we can show that:

- Reverse EphB4/ephrinB2 signaling has strong pro-tumor action resulting in activation of angiogenic processes in arterial endothelial cells.
- The main regulatory branches of forward EphB4/ephrinB2 signaling have antitumor action caused by inhibition of migratory, adhesive and proliferative cellular responses. However, these effects appear to be very cell specific.

Kinetic model development

General scheme of EphB4/ EphrinB2 forward signaling kinetic model
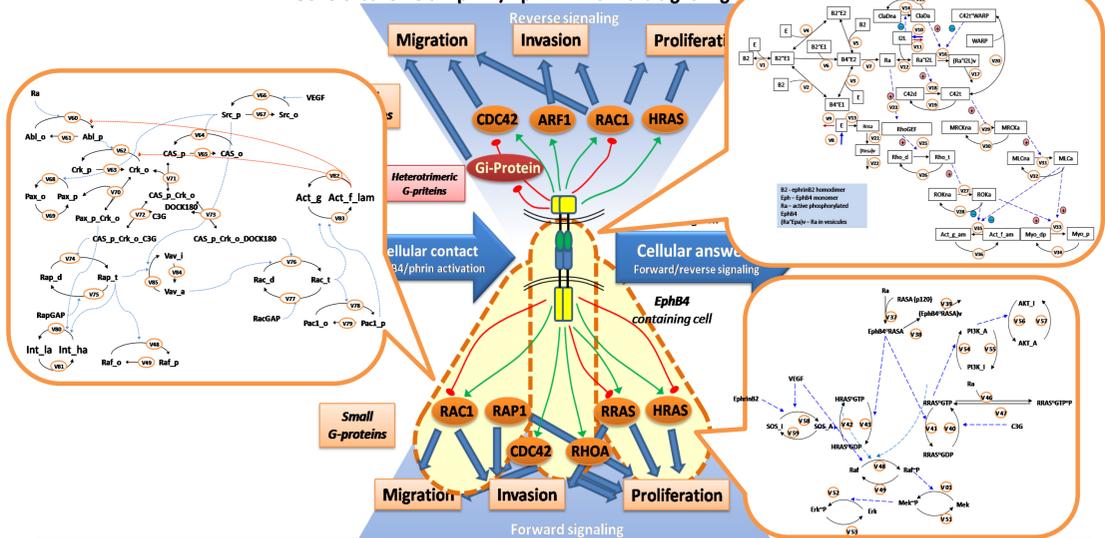
Purpose of the modeling

- We have developed a kinetic model of EphB4/ephrinB2 forward signaling for determination of molecular mechanisms causing specificity of cellular responses.
- Such modeling approaches can in principle be used for *in silico* testing of novel types of antitumor drugs targeting inhibition of tumor angiogenesis.

Strategy for kinetic model development
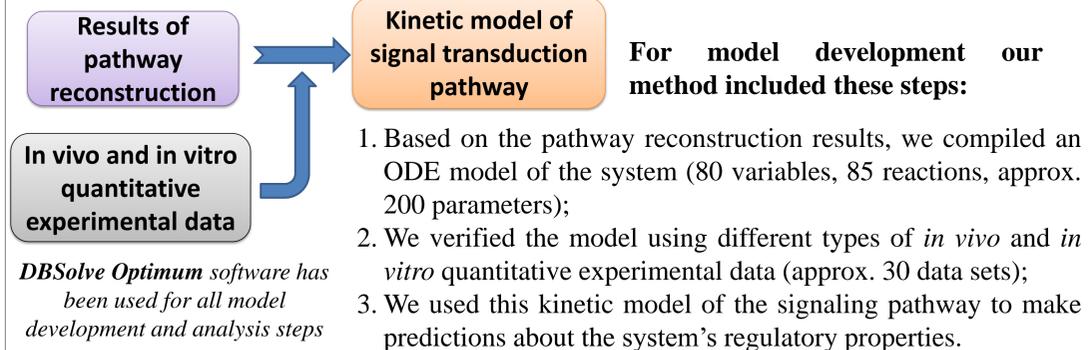
DBSolve Optimum software has been used for all model development and analysis steps

For model development our method included these steps:

1. Based on the pathway reconstruction results, we compiled an ODE model of the system (80 variables, 85 reactions, approx. 200 parameters);
2. We verified the model using different types of *in vivo* and *in vitro* quantitative experimental data (approx. 30 data sets);
3. We used this kinetic model of the signaling pathway to make predictions about the system's regulatory properties.

Kinetic model verification

For model verification analyzed different types of literature and in-house experimental data:

- Data about receptor phosphorylation, internalization and degradation against treatment of MCF7 cells with ephrin-B2-Fc.
- Various in vitro data characterizing different components of the system.
- In vivo data about different cellular responses such as migration or proliferation.
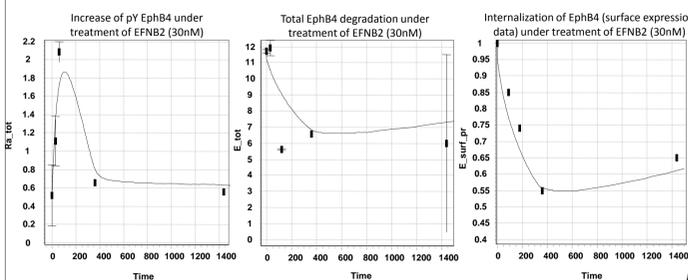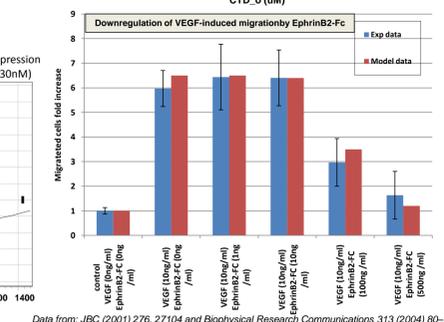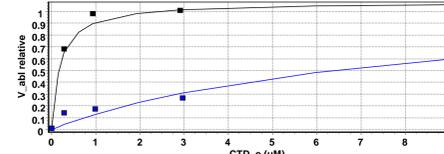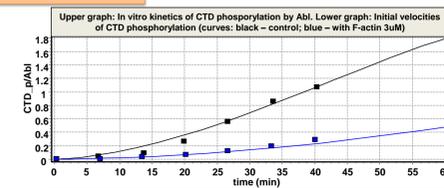
Kinetic model predictions
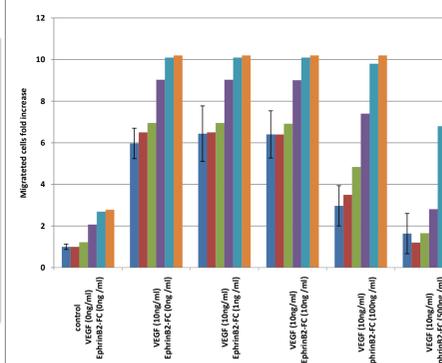
- It seems that the Abl/Rac/Rap branch of EphB4-induced forward signaling is cell specific
- One of the main reasons for it is an inhibition of Abl functioning by filament actin.
- It follows that cell types with high concentration of filament actin will be less sensitive to EphB4 inhibition of migration.

- The graph on the right shows the time dependences of the proliferation function (relative Erk_p concentration) with or without sEphB4 or VEGF. In each case, it can be seen that cell proliferation has low sensitivity to EphB4 stimulation at 10-20 h.
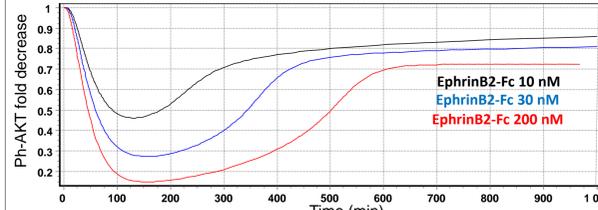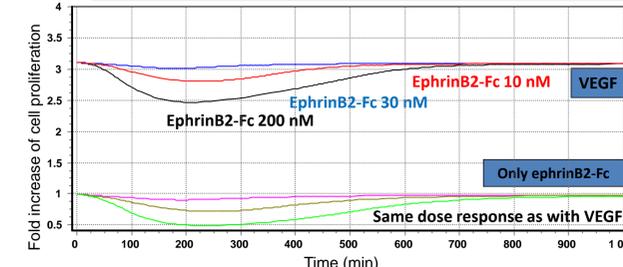
- The graph on the left shows the influence of EphB4 activation on AKT_p. It can be seen that EphB4 decreases the level of AKT_p and decreases the possible antiapoptotic cellular response. This effect is cell type dependent and should depend on level of direct activation of PI3K by different growth factors.

Conclusions

In this study, we have reconstructed the signaling pathways of EphB4-ephrinB2 interactions. Based on these results, a kinetic model of EphB4-ephrinB2 forward signaling has been developed which has been verified with different types of experimental data characterizing various components of the system. Analysis of the model behavior allows us to predict system regulation and possible cellular responses at different physiological conditions. For example, it was shown that EphB4-ephrinB2 forward signaling has a negative effect on cell proliferative, survival and migratory potential. However, all these effects are cell specific and can be weakened in particular cell types. In such a case, we can shown that increasing concentration of filament actin can completely inhibit the Rac/Rap branch of the EphB4-ephrinB2 signaling.