

Modeling of ErbB receptor regulated G1/S transition using combinatorial RNAi

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The restriction points are the key checkpoints where the critical decisions are given for the cells during cell cycle. The G1/S restriction point, where cells are committed to enter the S-phase, is regulated by Cyclin/CDK complexes, which phosphorylate and inactivate the tumor suppressor retinoblastoma protein (pRB). Hyperphosphorylation of pRB results in the release of E2F transcription factor that provides the transcription of essential genes for DNA replication. In both normal and tumor cells, pRB oscillates between an active (hypophosphorylated) state in early G1 and inactive (hyperphosphorylated) state in late G1, S and G2/M phases. Therefore, phosphorylation and following inactivation of pRB can be accepted as key factor that makes the cell proliferate.

The ErbB family of receptor tyrosine kinases, composed of four receptors having ability to form homo-and heterodimers, couple binding of extracellular growth factor signaling to cell cycle progression through intracellular signal transduction pathways. ErbB2 was shown to regulate G1/S transition by modulating the activity of Cyclin D, Cyclin E/Cdk complex, myc oncogene and p27 kinase inhibitors. Overexpression of ErbB2 correlates with the down-regulation of p21 and p27 Cdk inhibitors. However, the interplay between different members of ErbB family has not been systematically studied on their combinatorial effect to cell cycle progression.

Here, we apply Boolean logic to our model (consisting of 18 proteins) of ErbB receptors-regulated G1/S transition (Figure 1) and simulate the network utilizing GINSim software (Gonzalez et al., 2006). The basics of Boolean logic is to use AND, OR and NOT operators to map interaction of each protein in the network. Using these operators, we have determined the type of interactions of each network elements according to literature-based information. Since the state of each protein is defined as either 1 (always ON) or 0 (always OFF), perturbation of each network element alone or in combination allowed us experimental validation of computer simulations.

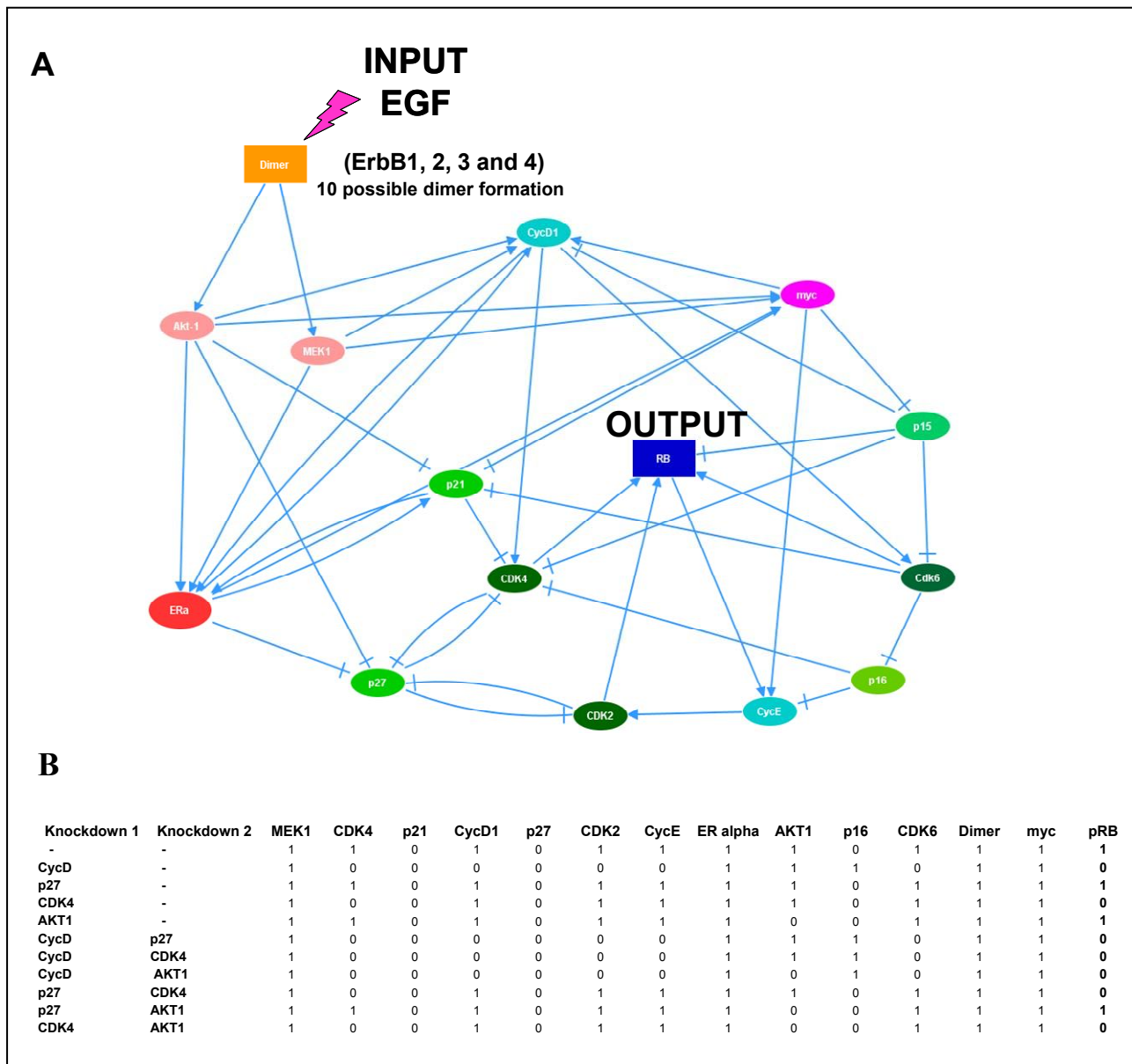


Figure 1: Network of proteins that regulate G1/S transition. **A.** EGF is given as an input for the ErbB receptors which can make several possible dimerizations upon ligand binding. All possible dimer formation was represented as “Dimer”. Phosphorylation of pRB is the output which is the marker of G1/S transition at the restriction point. All the interactions between proteins were mapped using Boolean operators. **B.** Final states of network elements after simulation of single and double knockdowns using GinSIM software. Having given an initial state for each element, outcome of the knockdowns were simulated and the final states of each protein were represented as either “1” or “0”.

For the experimental part of the project, we have chosen a cell system (HCC1954) that expresses high amounts of ErbB2/EGFR, but resistant to Herceptin treatment. In this cell line, we first characterized the G1/S transition point when EGF is given to the cells as stimulus after synchronization with Dif-3. Phosphorylation of pRB protein was examined over 24 hours and the time point where G1/S transition takes place was determined. Knockdowns were produced and their effect on the phosphorylation of pRB was determined by reverse phase protein arrays (RPPA (Loebke et al., 2007)) (Figure 2). Single and double knockdown efficiencies at both mRNA and protein levels were assayed by qRT-PCR and RPPA, respectively (Sahin et al., 2007). Initial (after synchronization/before stimulation) and final (time point where G1/S

transition completed) states were also determined by RPPAs. The effect of knockdowns on G1/S transition at DNA level was detected by 7-AAD staining (Figure 2). Finally, we compared computer simulations of the knockdowns with the experimental counterparts to validate our model.

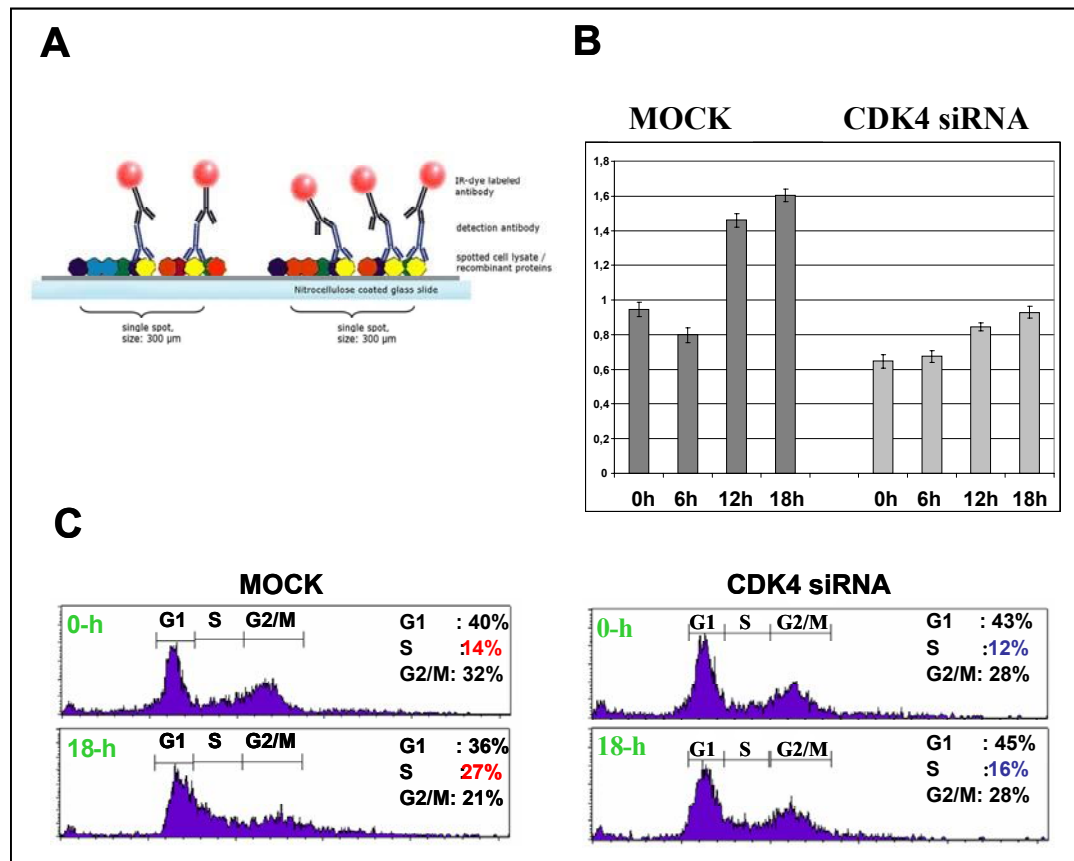


Figure 2: Experimental validation of knockdown simulations. **A.** Principle of RPPAs. The total cell lysate was printed on a glass slide and protein of interest (or phospho-state of the protein) was recognized by detection antibodies. Infrared-dye coupled secondary antibody bound to Fc region of detection antibody and signal intensity was scanned at IR-range. **B.** Effect of knockdown of CDK4 on pRB phosphorylation (G1/S transition). In the MOCK sample, transition for pRB phosphorylation takes place between 6- and 12 hours. On the other hand, for CDK4 knockdown, transition does not take place. **C.** 7-AAD staining shows the effect of knockdown of CDK4 on G1/S transition at DNA level. In the MOCK sample, the percentage of cells in S-phase twice as much of CDK4

In summary, we aim to understand the role of each ErbB family members on G1/S transition to compensate for the effects of down regulation of ErbB2 through RNAi or inactivation by cancer therapeutics using the systems biology approach described in this paper.

References

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