487f Systematic Analysis of Erbb Induced Signaling, Proliferation, and Migration

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Cellular decision processes are governed by a complex interplay between extracellular cues and intracellular signals. To control cell behavior (i.e. create drugs to hinder or aid the cell in proliferation, migration, or programmed death), we need to understand how the cell synthesizes and responds to information from these cues and signals. Here, we show that cell behavior, in the context of the ErbB family, can be understood via a linear mapping technique that correlates key kinase activities and phosphorylation patterns with cell motility and cell proliferation. In particular, we have obtained through experiment large, quantitative datasets at both the cell signaling and cell behavior levels. These data were gathered within a human mammary epithelial cell system with varying levels of ErbB2, under stimulation via heregulin (HRG) or epidermal growth factor (EGF). To quantitatively measure signal activation in a temporally resolved manner, we developed and utilized a novel mass spectrometry approach which provides tyrosine phosphorylation levels for more than 80 proteins. In addition, we used a high-throughput microtiter kinase activity assay to measure ERK, AKT, JNK, and IKK activity. Further signal characterization was achieved through the use of antibody phospho-protein arrays. Cell motility measurements were obtained using a novel, high-throughput wound healing assay we developed for migration studies in real time. Cell proliferation was measured using thymidine incorporation assays. Linear mapping models connecting relevant signals to cell migration and proliferation were constructed using principal components analysis and partial least squares regression. The model was tested both computationally (i.e. jack-knifing techniques) and experimentally (i.e. inhibitor studies) and shown to be predictive. The model provides us with information about the specific quantitative characteristics of signal control over cell proliferation and cell migration, and offers clues about how to effectively control each behavior individually or both together using signal level manipulation.