Transcriptome to Reactome Deterministic Modeling: Validation of *in Silico* Simulations of Transforming Growth Factor-β1 Signaling in MG63 Osteosarcoma Cells, TTR Deterministic Modeling

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**Abstract.** Integrated Systems Biology was used to study bone cancer via an iterative process of *in vitro* testing for validation of an *in silico* computer simulation where the transcriptome was used to derive the parameters of a kinetic model. A computer simulation model of the transforming growth factor-beta (TGF-β1) signaling pathway was obtained from Reactome®. The transcriptome of MG-63 cells was accessed from NCBI GEO GSE11414. With this method the model is not trained to match the biological system. The *in vitro* study on osteosarcoma (MG-63) cells was used to compare with the results from the computer simulation. MG-63 cells were grown in culture and exposed to TGF-β1 to identify differences in expression of a target-gene, TGF-β1-Induced 68kDa protein (TGFBI), at serial time intervals. Real-time PCR was used to measure TGFBI mRNA levels and the temporal profile was identical with that predicted by the *in silico* model. A sensitivities test was performed through the *in silico* model and a candidate target for gene-knock-down in the TGF-β1 signaling pathway, Smad3, was identified. An 80% reduction of this reactant in the model attenuated TGFBI expression by 64%, an effect that matched such knockdown of Smad3, *in vitro*, for other target genes reported in the literature. The assumption that the transcriptome drives the reactome is validated and substantiates a novel method for deriving parameters for kinetic deterministic models of biological systems.

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1 Introduction

The promise of in silico simulations in biological research was to reduce the number of actual studies by allowing researchers to control parameters in systems individually, which is normally not possible in vivo. This research integrates work by a significant number of researchers, publically available genome-wide microarray data sets, and validates one particular cell signaling collection of reactions from publically available pathway models. More importantly, a novel method of parameter determination from gene expression profiles is substantiated. This method generates kinetic models that simulate the biological systems without training and parameter estimations; all parameters are determined by derivation from the transcriptome. Phelix and coworkers [1] recently reported the utility of in situ hybridization as a source of gene expression profiles to model metabolic pathways in brain tissue; this report extends the method to the use of genome-wide microarray transcription profiles for determining model parameters and for signaling pathways.

Integrated Systems Biology includes in silico, in vitro and in vivo studies for the iterative process of validating model approaches. Such approaches for modeling the transforming growth factor-beta (TGF-β) signaling pathway have used results of the biological systems studies, including parameter estimations, to train the models to fit the biological data [2-5]. TGFβ-Induced Gene Human Clone 3 (BIGH3), also known in the literature as TGF-β Induced 68kDa protein (TGFBI) and keratoepithelin, is an integrin adhesion-class extracellular matrix protein. LeBaron, Phelix and coworkers have reported that TGFBI mediates the TGF-β1-dependent apoptosis in cultured osteosarcoma, MG-63, cells [6]. TGFBI is a widely recognized target gene of TGF-β1 signaling via the Smad protein pathway [7], but has not been included in any model system yet reported [2-5]. This study is the first to report kinetic modeling of the target gene expression in the TGF-β signaling pathway and to validate those expression dynamics by in vitro testing of temporal expression patterns and gene-knock-down studies [8,9].

The purposes of the in vitro study were to document that TGF-β1 evokes TGFBI gene expression in MG-63 osteosarcoma cells with a characteristic temporal pattern. Osteosarcoma cells were treated with TGFβ-1 to stimulate Smad signaling leading to TGFBI expression. After TGF-β1 was added to cells, TGFBI mRNA expression was measured. The result was compared to the in silico TGFBI mRNA levels in a kinetic model where the parameters were determined by the genome-wide transcription profile of MG-63 osteosarcoma [10] cells to determine if the computer model could be validated. The overall goal is to develop successful biomarker identification as novel cancer therapies for the individual patient through individualized personalized medicine. Sensitivities analyses were performed to identify a single knock-down candidate and thus Smad3 was chosen for its strongest sensitivity value. Knock-down, in silico, of Smad3 protein expression attenuated target gene