Title:

**Interaction between signaling pathways in malignant prostate cancer: Understanding via mathematical modeling**

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Current prognosis and treatment stratification for prostate cancer do not accurately predict clinical outcome. Hence there is an urgent need for improved biomarkers to determine prognosis and appropriate treatments. We identified two key signaling pathways (PI3K-Akt and NF-κB) whose constitutive activation correlates with prostate cancer progression. Using an integrated approach of quantitative experimentation and mathematical modeling, we seek to develop a multi-level, hierarchical, quantitative systems biology model - where the lower level captures the dynamic molecular processes of the signaling pathways and the higher level models the cancer phenotype. Firstly, we analyzed p-Akt (Ser473) and NF-ĸB/p65 protein expression and their co-localization in benign and cancer specimens of various Gleason grades. We also utilized androgen-responsive LNCaP cells (possessing increased Akt activity due to mutation in PTEN gene) and androgen-refractory PC-3 cells with constitutive activation of Akt and NF-ĸB and their treatment with specific inhibitors. Western blotting was performed to determine the expression of native/active forms of Akt and NF-ĸB and their effector proteins. Subsequent work include connecting the model of cell signaling to the physiologically “higher” levels using mechanistic details or statistical models. Compared to benign tissue, cancer specimens exhibited constitutive activation of p-Akt (Ser473) and NF-ĸB/p65 which was more pronounced in high-grade cancer (Gleason grade 7-10). Immunohistochemical analyses further demonstrated co-localization of these proteins in a subset of aggressive cancer tissues. Individual treatment of cell lines with Akt Inhibitor VIII and NF-ĸB inhibitor Parthenolide (for 24-72 h demonstrated partial suppressive effect in cancer cell growth; whereas concurrent blocking of Akt and NF-κB/p65 resulted in potentiated toxicity and inhibition of downstream effector proteins in both cell lines. The categorical behaviors were explored by extensive simulations conducted to explore possible parameterizations of the model and to define the range of potential responses. A differential equation based (mass action and Michaelis-Menten) mathematical model was constructed and structurally calibrated/validated using experimental data. The resulting behaviors were identified and categorized according to the severity of pathway activity. Model parameterization (numerical values of reaction rates and basal protein levels) was accomplished by adopting portions of the models estimating the remaining unknowns from available experimental data. Our results suggest that Akt activation provides long-term cell survival by activating pathways that influence NF-kB-dependent gene transcription, and hence plays a role in prostate cancer aggression.