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Cancer Biomarker Discovery by In Vitro Systems Biology

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ABSTRACT

This Thesis was made with the intention to mechanistically assess and further develop a multi-stage cell line-based (*in vitro*) model for oral cancer development. Efforts of establishing additional tumor cell lines for expanding the model were coupled with the application of systems biology technologies for characterization of the three entities of the start-up model, including: 1) normal, 2) immortal and non-tumorigenic, versus 3) immortal and tumorigenic stages. Omics data integration from assessment of cell lines as unique entities, and model-driven *in vitro* manipulations formed the basis for construction of two bioinformatics-based pipelines for this task. Altered phenotypic and genotypic characteristics and the event of non-functional cell differentiation (a hallmark of cancer development) was analyzed broadly among the transformed stages of the model relative the normal counterpart, testing the overarching hypothesis that thorough analysis of cell line data might contribute clinically useful tumor biomarkers potentially hidden in existing genome-wide assessments of clinical tissue samples.

The separate papers forming the Thesis, in order, generated: 1) a review of existing data from the start-up model under a selected standardized serum-free condition, 2) an omics-integrative tumor biomarker discovery pipeline based on the start-up model, 3) a model-driven tumor biomarker discovery pipeline based on assessment of influences of confluency (high cell density and cell-to-cell contact) in the seemingly most differentiation-deficient cell line in the start-up model, 4) a novel tumor cell line applicable to expand the number of serum-free entities of the model, 5) an expanded model-driven tumor biomarker discovery pipeline based on assessment of serum-induced influences of the extended model (now with four entities), and finally, 6) an analysis of the novel cell line under a further expanded omics-integrative tumor biomarker discovery pipeline. The overall results included broad description of the multiple alterations at gene, pathway and ontology levels that coupled with the transformed phenotypes and non-functional cell differentiation in the cell line models. The bioinformatics-driven assessment using overall six different processing tools of differential expression of 44 proteins and thousands of transcripts from these analyses suggested multiple potential biomarker signatures in head and neck squamous cell carcinoma. Overall, five *in vitro*-based signatures could be validated for clinical significance in independent data from tumor tissue analysis, including multiple oral and non-oral patient data sets as well as body-wide transcriptomics and proteomics expression databases. The taken approaches elucidated basic mechanisms of cell transformation while simultaneously generating paradigms/protocols generally applicable to cancer biomarker discovery. Proving the hypothesis under testing, the results show that the *in vitro*-derived biomarkers are complementary, often with superior accuracy, to those generated from direct assessment of cancer tissue specimens. Overall, the application of technologies and methods as described possibly generated a first description of an "*in vitro* systems biology model of oral cancer development" with potential for wide further application in experimental and translational research.

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