Identification of Key Genes Influencing Aggressiveness and Metronomic Treatment for Prostate and Breast Cancer

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Prostate and breast cancers are among the leading causes of cancer deaths in the United States. Treatment options for prostate and breast cancer are surgical resection, sterilizing radiation, hormone ablation therapy, and/or chemotherapy. Chemotherapeutic drugs alone or in combination remain the primary treatment options for aggressive and metastatic cancers; however, dose-limiting toxicity and drug resistance limits their clinical utility. Therefore, novel treatment strategies that improve survival or delay disease progression are needed. Our overall goal is to tailor therapies by using tumor specific molecular signatures for individual patients with aggressive metastatic cancers.

Using human prostate cancer cell lines, PC-3 (metastatic castration resistance; mCRPC) and LNCaP (androgen-sensitive) were used to determine the antitumor activity of conventional (CONV) and metronomic (METRO) dosing of the anticancer agent topotecan (TOPO) by protein staining (SRB) and metabolic activity (MTT). Concentration-dependent decrease in SRB and MTT were observed following increased drug concentrations. Changing the media/drug daily to mimic METRO increased potency (IC$_{50}$) ~8-fold (p<0.05) compared to CONV dosing after 72hr exposure. These data demonstrated that METRO dosing resulted in similar anticancer activity as CONV dosing, but at lower drug concentrations. Cancer pathway gene-based expression studies following METRO and CONV dosing vs. control (no-drug treatment) were performed. We identified several genes as signatures for aggressiveness in prostate cancer (PLAU, TGFB1, SERPINE1, MET, TIMP1, CXCL8, MCAM, ITGA3) and altered the following genes METRO therapy (CDKN1A, ERBB2, SERPINB5, ITGA3, PLAU, GZMA and ITGA4).

Although differences in gene expression were observed, their translation into proteins needed to be verified. The specific goal for this project was to validate the expression of a number of candidate genes via protein immunoblotting (Western analysis). Protein was isolated from a diverse panel of prostate cancer cell lines (PC-3M, PC-3, DU-145, LNCaP, and 22RV-1 (arranged most to least aggressive; Figure 1A). Immunoblotting was achieved using a 4-14% gradient gel and Actin-Beta as a housekeeping (control) protein. My research focused on validation of top significant genes with immunoblotting. Here we show the effect of treatment schedule (CONV- vs METRO-TOPO) on SERPINE1, a gene associated with aggressiveness in prostate cancer. SERPINE1 is a serine proteinase inhibitor gene that is important in making plasminogen activator inhibitor 1 (PAL-1), which promotes tumor progression. Research has shown that SERPINE1 controls plasmin and initiates migration and remodeling of body tissues. In summary, METRO administration of TOPO increased its antitumor activity in comparison to CONV high-dose chemotherapy. Unique gene signatures were identified in aggressive cancer and following METRO dosing. Immunoblot analysis verified that SERPINE1 was downregulated significantly (p<0.05) following METRO dosing (Figure 1B). The finding supports the hypothesis that METRO dosing schedules could be utilized to personalize patient therapy, based on an individual's molecular signature. Future studies will continue validation of treatment-mediated effects on the genomics and transcriptomics associated with various molecular signatures using molecular (CRISPR-Cas9 gene editing) and conventional pharmacological techniques in both aggressive prostate and breast cancers. The effect of tumor stroma (e.g., macrophages or fibroblasts) in 2D and 3D-tumor spheroids on treatment efficacy will be determined.

Statement of Research Advisor

The development of advanced “omic” tools have ushered in a new era of research that will provide greater mechanistic insights into disease progression and treatment response. Elena has worked to validate
molecular signatures that we have identified for aggressive prostate cancer and therapeutic response to metronomic therapy. Her research will support development and optimization of novel dosing strategies that may be tailored for a patient's specific cancer.

– Robert “Rusty” Arnold, Drug Discovery & Development

References


![Figure 1. In vitro validation - Immunoblotting. (A) SERPINE-1 was identified as the top marker for aggressiveness in mCRPC (unpublished data) and its expression was increased according to aggressiveness. The most aggressive cell lines (PC-3M and PC-3) showed greatest amount of SERPINE-1 protein. (B) METRO-TOPO treatment downregulated SERPINE-1 compared to CONV.](image-url)