

Transcriptional Repressor Kaiso Promotes Metastasis through Epithelial to Mesenchymal Transition.

Kaiso, a novel member of the BTB/POZ (Broad Complex, Tramtrak, Bric-a-brac/Pox virus and Zinc finger) superfamily (1). Unlike any of the previously characterized POZ proteins, Kaiso recognizes sequence-specific Kaiso binding sites or methyl-CpG dinucleotide pairs to influence gene expression. Epigenetic silencing of tumor suppressor genes is a contributing factor to the pathogenesis of various cancers. Reversal of epigenetic silencing is therefore a potentially desirable modality of targeted therapy for cancer, and DNA methyltransferase inhibitors have been shown to be effective in certain types of tumors. Preliminary data showed that the progression of prostate cancer in a large tissue cohort is closely associated with the over-expression and nuclear localization of Kaiso, with significant elevations for African Americans prostate cancer patients. Further it was observed that Kaiso nucleo-cytoplasmic shuttling is regulated by EGFR signaling, which when signaled to the nucleus promotes direct binding of Kaiso to methylated sequences in the promoter region of E-cadherin. sh-Kaiso, in multiple aggressive prostate cancer cell lines have decreased proliferation, cell migration and invasiveness. Additionally, sh-Kaiso cells have decreased expression of EMT related factors, N-cadherin, fibronectin and ZEB 2 expression, further supporting a reversal of EMT. ZEB 1/2 both have CpG methylation sites in the promoter region, and although not determined yet are likely directly regulated by Kaiso. Interestingly recent reports have suggested that ZEB 1/2 expression and AR expression are regulated in a reciprocal manner in LnCaP and PC-3 cells, and this is enhanced during androgen deprivation therapy (2). Indeed, AR and ZEB1/2 have Kaiso binding sites in their respective genomic sequences, thus implicating a regulatory process. Similar observations were observed in a novel non-malignant and primary tumor paired model (RC-77N/E and RC-77T/E) where ZEB 1/2 and vimentin are increased, and E-cadherin and AR expression are decreased in the tumor cells compared to non-tumor cells. Thus several questions remain as to the role of Kaiso in these processes.

The hypothesis to be tested is that Kaiso, a member of the BTB/poxvirus family of zinc finger transcription factors, is an essential inducer of the progression of tumor cells, through methylation dependent transcriptional regulation of EMT related genes, which in reciprocal manner influences AR expression levels. Understanding the molecular links between these cell-signaling pathways in prostate cancer cells could rapidly promote the development of gene-directed therapeutics for the treatment of hormone-refractory tumors and/or metastasis.. By exploring the molecular mechanisms and widespread effects on Kaiso associated transcriptional repression, novel therapeutic approaches can be developed to target lethal metastasis. By exploring the molecular mechanisms and widespread effects on Kaiso associated transcriptional repression, novel therapeutic approaches can be developed to target lethal metastasis. **To test this hypothesis three Specific Aims are proposed:**

Specific Aim 1 Test whether Kaiso related EMT influences AR expression through methylation dependent transcriptional silencing. In this aim the hypothesis to be tested is that Kaiso is a central mediator of hormone dependency and EMT through direct regulation of AR and EMT associated gene expression. To accomplish this we will characterize the role of Kaiso in promoting EMT during the transition of prostate cancer cells from a hormone sensitive state coupled with the associated change in epithelial to mesenchymal phenotype during androgen deprivation therapy. Established hormone sensitive cell culture models and LnCaP cells will be utilized to determine the Kaiso related EMT response during treatment with Casodex or siRNA AR treatments. Furthermore, in hormone-insensitive cell lines sh-Kaiso will be utilized cells lines to determine the expression of AR and downstream AR related gene expression. Subsequently the expression of growth factors receptors will be evaluated as well.

Specific Aim 2 Establish a role for Kaiso expression/localization utilizing an experimental *in vivo* metastasis model. In this aim the hypothesis that will be tested is Kaiso is a central mediator of castration resistance in two clinically relevant xenograft models of AR sensitive and insensitive tumors. Xenograft tumors from hormone sensitive RC-77T and LnCaP cells or Kaiso overexpression RC-77T and LnCaP

cells will be inoculated subcutaneously and orthotopically, and after six weeks mice undergo surgical castration. Tumor volume and metastasis will be measured by IVIS xenogen imaging. Expression of EMT related genes will be performed by IHC on tumors once removed. These studies will determine the role of Kaiso during castration resistance and relate these findings to the onset of cells acquiring the ability to undergo EMT.

Specific Aim 3 Evaluate the clinical significance of Kaiso subcellular localization and EMT related gene expression in hormone-refractory metastatic tumors. In this aim the hypothesis to be tested is that transition from cytoplasmic to nuclear Kaiso expression in prostate cancer patients will have clinical correlation hormone insensitivity and metastatic prostate cancer. To determine this a series of prostate cancer progression TMAs that contain primary T1 and T2 tumors as well as hormone-refractory metastatic tumors will be utilized. TMA's will be stained for Kaiso and other EMT related genes. Furthermore, we will evaluate and determine the correlative expression of these markers.

Innovation and Impact

A central aspect of the current proposal, derived from the multi-disciplinary expertise, is that the current team will test the molecular signaling and clinical relevance that is associated with cells acquiring castration resistant. Retrospective examination of Kaiso and EMT related gene expression in hormone-refractory metastases and compared cytoplasmic to nuclear transition of Kaiso as a transition point to tumor aggressiveness. The hypothesis will be evaluated using invitro and invivo assays and patient tumors. The innovation in this proposal is the conceptual design that these two established mechanism of acquiring lethal disease is mediated by a central signaling mechanism. These findings can be used to augment existing treatments and develop more effective molecular driven therapies for advanced disease.

Overarching Challenge and Focus Areas

The current proposal will establish the capacity of Kaiso to promote castration resistant tumors and metastases. This fits with the overarching aim of understanding the tumor biology that will lead to molecular targets for therapeutic intervention of advanced prostate tumors.
