Tumor Endothelial cells as a Targetable Gateway That Modulates Access of Drugs to Cancer Cells

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Abstract MP29-13

Introduction

- Human endothelial cells (EC) lining the tumor vasculature of human prostate adenocarcinoma are preserved in primary xenografts of intact surgical remnant tissue.
- Androgen deprivation (AD) causes apoptosis in AR-expressing endothelial cells.
- Testosterone-deprivation (T-D) leads to a rapid loss of 40-60% of Ecs resulting in de-endothelialization of the vasculature of primary xenografts.
- De-endothelialization is followed by re-endothelialization with human endothelial cells in the absence of T.
- This study determined the consequences of targeted perturbation of human prostate endothelium on access of chemotherapeutic agents to prostate cancer (CaP) cells.

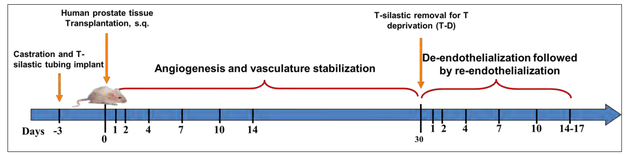

Fig 1. Model overview - SCID or nude mice were "humanized" to mimic human circulating T. Mice were castrated and implanted with T-silastic tubing 3-7 days before subcutaneous (s.q.) transplantation with 8-10 pieces of fresh clinical prostate tissue. Over the initial 14 days after transplantation there was a burst of angiogenesis by the human endothelial cells resulting in a 5-10 fold increase in MVD. Endothelium stabilized by 2 weeks after implantation. Removal of T-silastic tubing (T-D) induced acute endothelial cell involution that can be monitored over the 14-17 days after T-D.

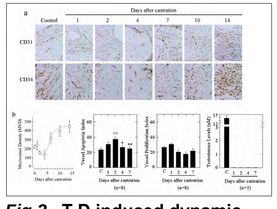

Fig 2. T-D induced dynamic vascular changes.

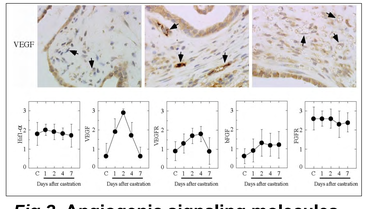

Fig 3. Angiogenic signaling molecules were upregulated after T-D.

Experimental Procedures

- Fresh clinical prostate tissue and tissue transplanted onto "humanized" mice were used.
- Vasculature leakage was measured using MRI (contrast dye), MS (nano particles), IHC (lectins), and photo-acoustic imaging (hemoglobin).
- Cell isolation: Tissue specimens were disaggregated enzymatically. Epithelial cells and endothelial cells were enriched using human EpCAM- and CD31-conjugated magnetic beads, respectively. Cells in the final flow-through after the cell type-specific enrichment steps was considered as stromal cells.
- Transcriptomes of isolated cells of each cell type were acquired using RNASeq.
- Cisplatin (Cis-Pt) was investigated due to its membrane transporter/pump regulated uptake and efflux, and efficiency in targeting slow-growth cancer cells.
- Cis-Pt DNA adducts were analyzed using specific antibody or Cytof.

Results

Compromised Endothelial Barrier Upon T-D

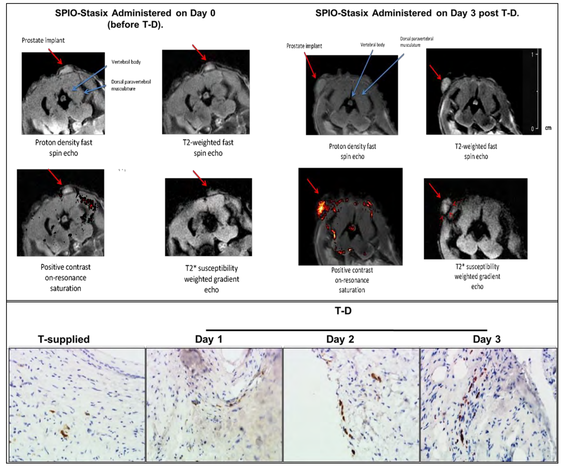

Fig 4. Increased tissue localization of Stasix® particles (platelets) in prostate tissue following T-D. Upper panel shows MRI signals. Lower panel shows IHC staining using the platelet specific marker CD42b. Platelets were injected via the tail vein on Day 3 post T-D and allowed to circulate for 15 minutes. Stasix® particles were from Entegrion, Inc., Research Triangle Park, NC. Imaging was performed by Dr. Christopher Lascola, MD, Duke University Medical Center with a 5T Brucher MRI.

Transcriptomes were distinctive among cell types but similar in cells of the same cell type isolated from fresh tissue and tissue transplant

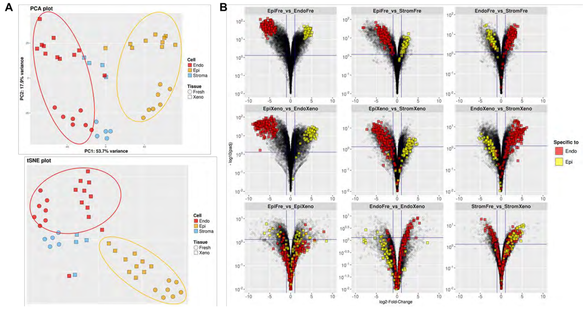

Fig 5. RNASeq data demonstrated successful isolation of the 3 cell types. Epithelial cells and endothelial cells were clustered separately by global genes, whereas stromal cells showed gene profiles overlapping epithelial and endothelial cells (A). Epithelial cell-specific and endothelial cell-specific gene sets each maintained similar in general between fresh tissue (Fre) and pre-castration transplant (Xeno) (B).

Cell type-specific expression of AR-related genes and drug uptake/efflux membrane transporter/pumps genes

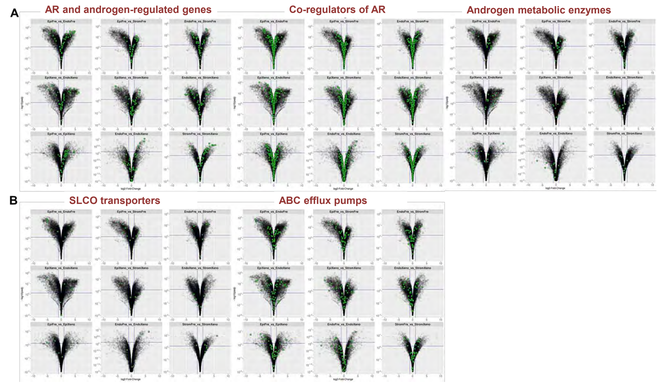

Fig 5. RNASeq data analysis compared AR-related genes (A) and drug uptake/efflux-related genes (B). Both cell type-specific and ubiquitous genes were noted. Same type of cells from fresh tissue and tissue transplant shared similar gene profiles.

Global gene expression showed dynamic changes in cells in response to T-D

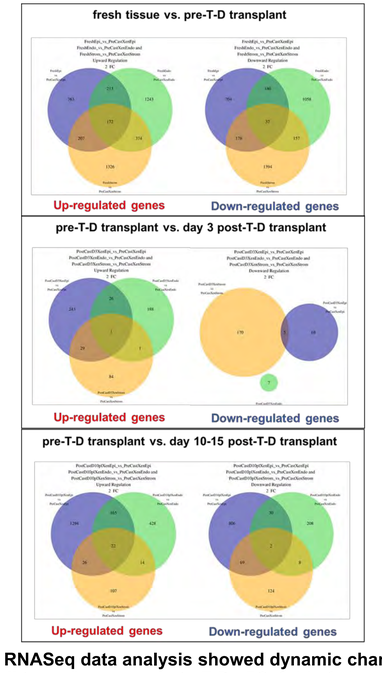

Fig 6. RNASeq data analysis showed dynamic changes in all 3 cell types in response to T-D. FC, fold of change ≥2.

T-D increased tissue exposure to Cis-Pt

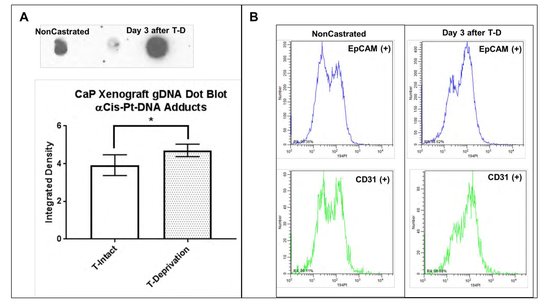

Fig 7. Mice were treated with Cis-Pt before T-D or 3days after T-D. (A) Genomic DNA was prepared using tissue transplants. Cis-Pt DNA adducts were assessed using dot blotting and an antibody specific to DNA-Cis-Pt. (B) Genomic DNA was prepared from epithelial cells (EpCAM +) or endothelial cells (CD31+). DNA-Cis-Pt adducts were measured using mass cytometry.

Summary of key findings

- T-D induced a "window" for increased access of therapeutics to prostate tissue.
- T-D increased tissue and cellular exposure to Cis-Pt.
- Cell type-specific enrichment was efficient.
- Transcriptomes were comparable within the same cell type isolated from fresh tissue and pre-T-D tissue transplant.
- T-D induced dynamic changes in transcriptomes in epithelial, endothelial and stromal cells.
- Transcriptomes evolved over the time after T-D.
- Transcriptomes of endothelial cells before and after T-D were different markedly.
- Profiles of uptake transporters and efflux pumps varied among cell types, and evolved in response to T-D.

Conclusions

- Primary tissue transplants provide a unique tool for analysis of the roles of targeted microvascular damage in organ-specific therapies.
- Evolution of the endothelial compartment and stromal compartment in response to iatrogenic intervention may be important to cancer biology and delivery of therapeutics.

Support for research

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