with high LeY expression. Monotherapy with CAR T cells failed to decrease tumour volume compared to vehicle control. However, CAR T cells given after a single dose of the chemotherapeutic agent carboplatin greatly and durably reduced tumour burden, with residual tumour mass being less than 1% of their original size (0.56 ± 0.23% of tumour volume at the start of treatment). Overall, these data provide preclinical evidence that: i) high membrane expression of LeY correlates with *in vitro and in vivo* CAR T cell-induced tumour cell death via the canonical perforin/granzyme B mechanism; and, ii) membrane LeY can be used as a biomarker for patient selection.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Prostate Cancer Stimulation by a Novel Liver X Receptor (LXRa)-Estrogen Related Receptor (ERRa) Axis

Chung Seog Song, PhD[1], Bandana Chatterjee, BSC,MS,PhD[2].
[1]University of Texas Health Science Center, San Antonio, TX, USA, [2]University of TX Health Science Center, San Antonio, TX, USA.

Background: Liver X Receptors (LXRα, β) are oxysterol sensing nuclear receptors that regulate lipogenesis, cholesterol homeostasis and immune cell function. While oxysterols are agonist ligands of LXRs, sulfated oxysterols, catalytically produced by the SULT2B1b sulfotransferase, are LXR-inert. Increased SULT2B1b expression leads to attenuation of LXR signaling. In a previous report, we showed that SULT2B1b is undetectable in clinical samples of castration-resistant prostate cancer (CRPC), and its level is significantly reduced in a subset of primary prostate cancer. In cell models, genetic ablation of SULT2B1b exacerbated aggressive traits of CRPC, evident from EMT-like activation, enhanced invasion, faster xenograft tumor growth and reduced cell adhesiveness and stiffness in single-cell atomic force microscopy analysis. AKR1C3, which promotes androgen biosynthesis and shows elevated expression in advanced prostate cancer, is markedly upregulated in SULT2B-depleted cells. Elevated AKR1C3 leads to activation of the ERK1/2 Map kinase survival signal in CRPC cells. **Results:** We report here that AKR1C3 upregulation is a consequence of enhanced LXRα signaling in SULT2B1b-deficient cells, since the upregulation was abolished in multiple cell models when LXRα was silenced by siRNAs or inactivated by the small molecule inhibitor SR9243, which is an LXRs-selective inverse agonist. Conversely, LXR agonism induced by an oxysterol, or by the synthetic ligand GW3965, resulted in elevated AKR1C3 expression. Consistent with a recent report that the nuclear receptor ERRα is a positive regulator of AKR1C3, we found that ERRα ablation prevented AKR1C3 upregulation in SULT2B1b-deficient cells. Notably, LXRα inactivation caused marked reduction of ERRα, indicating that ERRα functions downstream of LXRα to induce AKR1C3 and ERK1/2. Dependence of ERRα and AKR1C3 expression on LXRα was observed in both androgen receptor (AR)-positive and AR-negative CRPC cells. Elevated ERRα in prostate cancer is known to be associated with a poor disease outcome. This association may be in part due to ERRα activation by cholesterol, which is the endogenous agonist ligand for ERRα (*Cell Metab* 23: 479, 2016), and high cholesterol is a risk factor for aggressive prostate cancer. Furthermore, statins, which inhibit cholesterol biosynthesis, are beneficial to CRPC patients with elevated blood cholesterol. We identified two cholesterol-responsive ERRα-binding sites in the far upstream region of the AKR1C3 promoter. This result confirms that ERRα plays a direct role in the transcriptional upregulation of AKR1C3. **Significance:** Our study establishes a novel LXRα→ERRα→AKR1C3→ERK1/2 survival axis that is activated in CRPC cells under SULT2B1b deficiency. The LXRα→ERRα regulatory axis may be exploited for developing novel therapeutics against AR-positive and AR-negative CRPC.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Prostatic Acid Phosphatase Is a Progenitor Cell Marker That Persists After Androgen Ablation

Sudeh Izadmehr, PhD, Alexander Kirschenbaum, MD, Shen Yao, MD, Alice C. Levine, MD.
Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Introduction: Prostatic Acid Phosphatase (PAP), a protein phosphatase and 5'ecto-nucleotidase, is expressed in prostate cancer (PCa) bone metastases and correlates with poor survival. Growing evidence suggests that PAP is *not* regulated by androgens, but rather by factors in the tumor microenvironment.
Hypothesis: We hypothesized that PAP is a marker for a more progenitor type PCa cell and its expression is androgen-independent, persisting in castration-resistant disease.
Methods: Protein expression of PAP and three androgen-regulated proteins, the Androgen Receptor (AR), Prostate-Specific Antigen (PSA), and ETS-related gene (ERG) protein, was assessed with immunohistochemistry in human fetal prostate (9.5 - 20 weeks of gestational age), archival human PCa bone metastases, and human PCa cell lines. VCaP cells were treated *in vitro* with dihydrotestosterone (DHT) and the effects on AR and PAP protein expression determined with Western Blotting. PAP-expressing PCa cell lines (LNCaP, C42B, and VCaP) were inoculated subcutaneously (s.c.) into SCID mice. To model tumor-bone interaction, LNCaP and MC3T3 osteoblast cells were co-inoculated s.c. into SCID mice. A VCaP castration study with surgical or sham castration was performed after tumors were palpable and effects of castration on tumor growth and protein expression determined.
Results: PAP expression was observed in the fetal prostate as early as 11.5 weeks of gestational age prior to PSA and AR expression. Strong PAP expression was noted in all human PCa bone metastases examined, both treatment-naive and castrate-resistant (n=10). *In vitro*, VCaP cells expressed high levels of AR and PAP protein and DHT treatment increased AR and decreased PAP protein expression. *In vivo*, PAP expression was observed in all tumor models; LNCaP (low PAP expression), C42B (moderate PAP

expression) and VCaP (high PAP expression). Castrated VCaP tumors underwent tumor stasis, were significantly smaller compared to intact mice, had decreased AR, PSA and ERG expression but persistent expression of PAP. Double staining of tumors for PAP and AR demonstrated a population of cells that were positive for PAP but negative for AR expression in hypoxic areas near necrosis. Inoculation of LNCaP cells with MC3T3 osteoblastic cells increased PAP expression *in vivo*.

Conclusions: PAP is expressed early in human fetal prostate development prior to the secretion of significant androgens or expression of AR. In mouse xenograft tumors and human PCa bone metastases, androgens did not significantly regulate PAP expression. Both hypoxia and stroma increased PAP expression. These data demonstrate that PAP is a marker of early progenitor cells, is persistently expressed after castration and is upregulated by tumor microenvironmental factors. PAP may be a suitable target for the treatment of castration-resistant metastatic disease.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Protein Signatures of Parathyroid Carcinomas Using Proteomic Analyses

Sung Hye Kong, MD[1], Jung Hee Kim, MD[1], Do-Hyun Han Han, PhD[1], Jeong Mo Bae, MD, PhD[1], Namki Hong, MD[2], Joon-Hyop Lee, MD[3], SO YOUNG PARK, MD[4], Yong Jun Choi, MD[5], Sihoon Lee, MD,PhD[3], Yumie Rhee, MD,PhD[2], Sang Wan Kim, MD[1], Chan Soo Shin, MD[1].
[1]Seoul National University College of Medicine, Seoul, Korea, Republic of, [2]Yonsei University College of Medicine, Seoul, Korea, Republic of, [3]Gachon University College of Medicine, Incheon, Korea, Republic of, [4]Kyung Hee University Hospital, Seoul, Korea, Republic of, [5]Ajou University Hospital, Suwon, Gyeonggi-do, Korea, Republic of.

Parathyroid carcinomas are rare endocrine tumors derived from the parathyroid glands with poor prognosis. Moreover, parathyroid carcinomas are resistant to radiation or drug therapy, and surgical resection is the only treatment option. Understanding the molecular pathogenesis of parathyroid carcinomas may pave the way for early diagnostic biomarkers and therapeutic targets. Therefore, we aimed to elucidate the protein signatures for parathyroid carcinomas through quantitative proteomic analyses. We performed liquid chromatography with tandem mass spectrometry (LC-MS/MS) technique with formalin-fixed paraffin-embedded (FFPE) samples and reached a quantitative depth of more than 5,000 proteins per sample. For the analyses, 23 parathyroid carcinoma and 15 adenoma samples were collected from five tertiary hospitals in Korea. Patients' mean age was 52 years, and 24 (63%) were female. Patients with parathyroid carcinoma had higher parathyroid hormone (PTH) and serum calcium level than adenomas (PTH, 1077.6 ± 760.7, 181.8 ± 139.8 pg/mL; calcium 13.0 ± 2.8, 11.3 ± 1.0 mg/dL, respectively). From the proteomic expression profiling, there were 137 differentially expressed proteins with the cutoff of both $p < 0.05$ and fold change > 1.5. Using the Ingenuity Pathway Analysis (IPA), top enriched canonical pathways in parathyroid carcinomas included glycoprotein-6 signaling related to the coagulation pathway, acute phase response, mTOR, and clathrin-/caveolar-mediated endocytosis signaling. In transcription factor analysis, TGFβ and TP53 were activated in carcinoma, and these factors were up-regulators of CD44 antigen and Annexin A2 (ANXA2) proteins. In network analysis, α-1-acid glycoprotein 1 (ORM1), laminin subunit β-2 (LAMB2), and Serpin family (SERPIN) proteins were derived as essential proteins and correlated to the AKT complex. Also, with the support vector machine (SVM)-based classification method, we derived a set of proteins that can discriminate carcinomas from adenomas, which consists of Carbonic anhydrase 4 (CA4), α/β hydrolase domain-containing protein 14B (ABHD14B), CD44, LAMB2, phosphatidylinositol transfer protein β isoform (PITPNB), and ORM1, with the lowest error rate of 11.1%. In conclusion, from the proteomics analyses of parathyroid neoplasms, newly recognized pathways - signaling related to coagulation, acute phase response, and endocytosis - were enriched in parathyroid carcinoma in addition to the known mTOR signaling pathway. The proteins such as α-1-acid glycoprotein and laminin subunit β-2 from SVM classification and network analyses could be the distinctive signature of carcinoma and may provide insights into the therapeutic target.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Small Molecule Modulation of MEMO1 Protein-Protein Interactions

Julie A. Pollock, PhD, Courtney L. Labrecque, B.S., Cassidy N. Hilton, B.S., Justin Airas, B.S., Alexis Blake, B.S., Kristen J. Rubenstein, B.S., Carol A. Parish, PhD.
University of Richmond, Richmond, VA, USA.

MEMO1 (mediator of ErbB2-driven cell motility) is upregulated in breast tumors and has been correlated with poor prognosis in patients. As a scaffolding protein that binds to phosphorylated-tyrosine residues on receptors such as estrogen receptor and ErbB2, MEMO1 levels can influence phosphorylation cascades. Using our previously developed fluorescence polarization assay, we have identified small molecules with the ability to disrupt the interactions of MEMO1. We have performed limited structure-activity-relationship studies and computational analyses to investigate the molecular requirements for MEMO1 inhibition. The most promising compounds exhibit slowed migration of breast cancer cell lines (T47D and SKBR3) in a wound-healing assay emulating results obtained from the knockdown of MEMO1 protein. To our knowledge, these are the first small molecules targeting the MEMO1 protein-protein interface and therefore, will be invaluable tools for the investigation of the role of the MEMO1 in breast cancer and other biological contexts.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY