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14. ABSTRACT

Scope: Prostate cancer (CaP) is characterized by unique prostate-associated antigens; hence, it has been considered a prime candidate for immunotherapy. Despite numerous laboratory advances, clinical outcomes have been partial and transient.

Purpose: The overall goal of the proposed studies is to optimize the effectiveness of therapeutic whole-cell CaP vaccines by taking into consideration tumor-associated hypoxia as a relevant determinant of tumor antigenicity.

Major findings: Transcriptome studies revealed that gene expression in hypoxically cultured cells is more akin to that in tumor cells *in situ* than are cells grown normoxically. Transcripts of hypoxia-associated genes DLG7, CCNB1 and HMMR were associated with Gleason score and with disease prognosis suggesting their potential as CaP biomarkers with prognostic value. By 2D-gel electrophoresis, we screened patient sera and detected novel hypoxic-cell reactive autoantibodies (under validation).

Significance: Our data suggest that hypoxically cultured CaP cells are more akin to tumor cells *in situ* than are cells grown normoxically. We have identified hypoxia-reactive proteins, pathways and autoantigens with potential value as biomarkers or therapeutic targets. Introduction of pO_2 as a variable can constitute a tool for the development of more effective immunotherapy for CaP.

15. SUBJECT TERMS

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Introduction

Prostate cancer (CaP) remains among the most common causes of cancer-related deaths in men. Because CaP is characterized by unique prostate-associated antigens, it has been considered among prime candidates for immunotherapy. Despite numerous laboratory advances, clinical outcomes have been partial and transient. One plausible reason for the incomplete response is that vaccine cells, prepared under standard tissue culture conditions, can drastically differ in expression of macromolecules *in situ*, and thus may immunize against less complete antigen spectrum. The purpose of the proposed studies is to optimize the effectiveness of therapeutic whole-cell CaP vaccines by taking into consideration tumor-associated hypoxia as a relevant determinant factor of tumor antigenicity. We hypothesize that hypoxically cultured CaP cells are more similar in their antigen landscape to CaP cells *in situ* than are normoxically cultured CaP cells. The following Tasks were defined in the approved statement of work; Task 1. Identify oxygen-tension responsive genes and proteins in the cells comprising a clinical-grade prostate cancer (CaP) cellular vaccine; Task 2. Validate differentially expressed molecules in CaP in association with tissue hypoxia. Introduction of oxygen tension (pO_2) as a variable can constitute a tool for the development of more effective allogeneic vaccines for CaP. If the proposed studies demonstrate that CaP cells grown under low pO_2 are more antigenically similar to cells *in situ*, this will justify the evaluation of their therapeutical value in a preclinical model.

Body

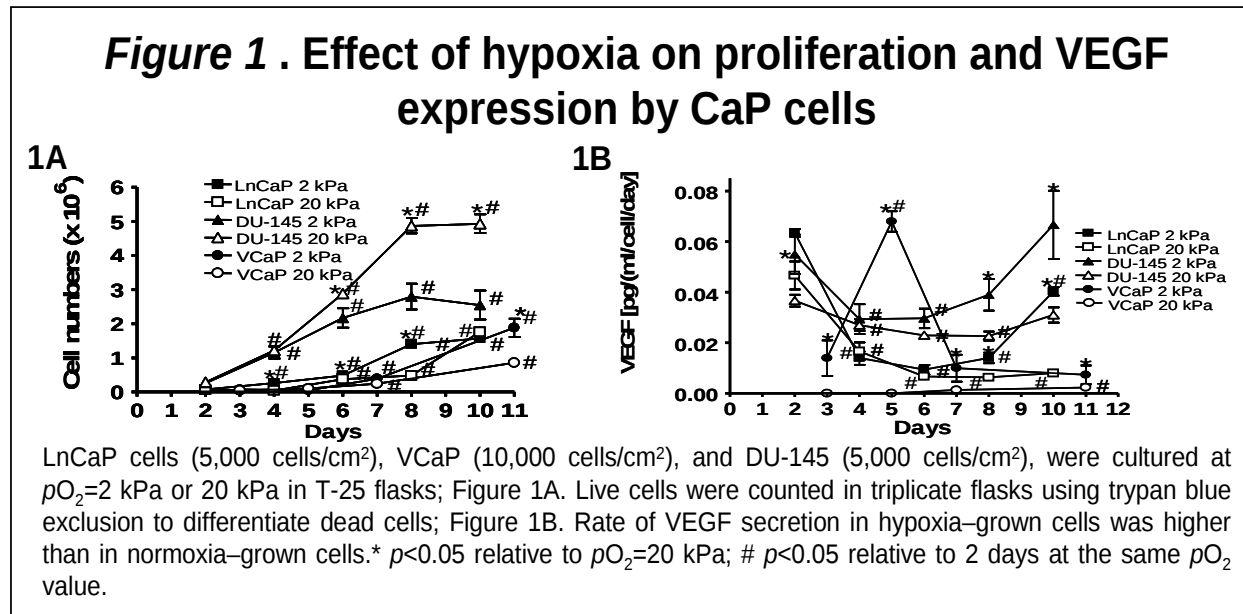
Task 1. Identify oxygen-tension responsive genes and proteins in the cells comprising a clinical-grade CaP cellular vaccine.

Approach: Identification of specific candidate genes with pO_2 -dependent expression in CaP cells has not been established yet in the context of their antigenic relevance. In Task 1, CaP cells grown at different pO_2 were tested by state-of-the-art high throughput genomics and proteomics techniques. This approach was designed to identify pO_2 -regulated tumor-associated pathways and macromolecules. To determine the antigenic potential of pO_2 -regulated tumor-associated macromolecules, we tested their reactivity with the spontaneous antibodies from CaP patients and the sera of age-matched non-cancerous controls, other cancers and an autoimmune disease.

Task 1a. To propagate LnCaP and VCaP cells under pO_2 -controlled conditions.

Prompted by the evidence that pO_2 modulates the biological properties of CaP tumor cells, we initiated studies aimed at using pO_2 as a tool to manipulate the antigenic signature of cells used as cellular vaccines. As a model, we used LnCaP cells, originated from a lymph node metastasis [1]. These cells have been used as a component of an allogeneic whole cell vaccine tested in a phase 2 clinical trial of androgen-independent CaP [2]. VCaP cells were generated from a vertebral metastatic lesion and harbor the TMPRSS2-ERG fusion (present in 40–60 percent of CaP patients) [3, 4]. These two cell lines were selected because their comparison could represent a broad spectrum of CaP patients, and may thus result in a better vaccine. For contrast we included DU-145 cells, derived from a brain metastatic tumor [5] into initial experiments. The cells were routinely maintained in culture medium (RPMI-1640 for LnCaP and DU-145 and Dulbecco's modified MEM for DU-145 cells) supplemented with 10 percent fetal bovine serum. LnCaP cells were propagated at different pO_2 levels in a BioSpherix chamber (Lacona, NY) with adjustable oxygen partial pressure. Humidified atmosphere was maintained at 37 °C and equilibrated with a mixture of 2% O_2 and 5% CO_2 using controlled N_2 and CO_2 gas intake. Controls were placed in a standard cell culture incubator at 37 °C in a humidified atmosphere.

containing 21% O₂ and 5% CO₂. Hypoxic LnCaP and VCaP cells ($pO_2=2$ kPa) proliferated faster than at standard cell culture conditions, however hypoxia reduced the proliferation of DU-145 cells (**Figure 1A**). Independent of the cell proliferation rate (as observed earlier by others [6]), hypoxic cells secreted more VEGF (**Figure 1B**).



Task 1b. cDNA gene microarrays and data analysis.

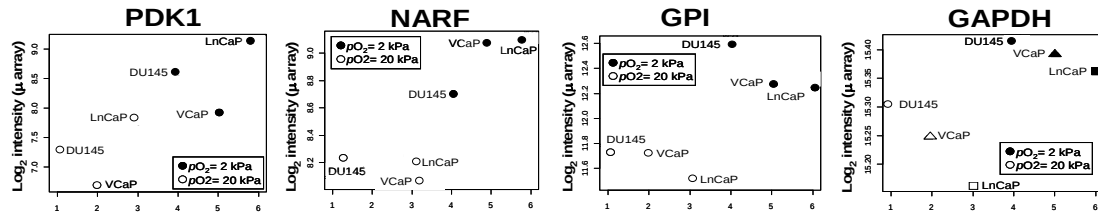
In response to changes in oxygen availability, cells differentially regulate a vast array of genes involved in diverse pathways such as apoptosis, metabolism or angiogenesis [7, 8]. In preliminary experiments, we observed hypoxia-associated increase in VEGF expression by CaP and ovarian cancer cells (Knutson, G.J., Vuk-Pavlovic, S.; unpublished observations). In addition, we found numerous differences between 2-D electropherograms of lysed LnCaP cells propagated at $pO_2=2$ kPa and to 20 kPa. Altogether, this evidence suggests that hypoxia profoundly affects gene expression relative to normoxia. Only a handful of studies, however, have analyzed the effect of hypoxia on gene expression in CaP, especially in the context of pO_2 impact on malignant progression [9-11]. We cultured LnCaP, VCaP, and DU-145 at $pO_2=2$ kPa or 20 kPa and lysed them when data showed pO_2 -related differences in VEGF secretion (4, 7 and 4 days for LnCaP, VCaP and DU-145; respectively). We isolated total RNA, verified its quality using an Agilent 2100 Bioanalyzer, and assessed the transcriptome by Affymetrix Human U133 Plus 2.0 array. Genes expressed differently between experimental groups were identified as probe sets with at least a twofold hypoxia-related expression change. We selected approximately 1450, 3700 and 1400 probe sets in DU-145, LnCaP, and VCaP cells, respectively. To relate functions, pathways, networks, and unique features to genes differently expressed between two pO_2 s, we used Ingenuity Pathway Analysis (IPA; Ingenuity Systems). Benjamini-Hochberg correction was used for multiple comparisons. Transcriptome studies revealed different gene expression in cells grown in hypoxia relative to those in normoxia. Correlation analysis between expression profiles, for all probes or hypoxia-sensitive probes revealed that LnCaP and VCaP cells were similar in gene expression changes and different to DU-145 cells (data not shown). This last finding and the similar effect of hypoxia on cell growth in LnCaP and VCaP cells could be related to the presence of androgen receptor in these cell lines and its absence in DU-145 cells [12]. Interestingly, regardless of cell-specific changes in gene

expression profiles, hypoxia-modified genes in molecular pathways associated with cancer and urologic diseases (**Table 1**) were overexpressed in comparison to normoxic cells ($p<0.001$) in all cell lines. These data suggest an association of low pO_2 and aggressive features of CaP.

Table 1 . Transcripts for diseases and disorders preferentially affected by hypoxia		
DU-145		
Pathway	p-value	# molecules
Genetic Disorder	$1.11 \times 10^{-9} - 5.16 \times 10^{-3}$	558
Cancer	$2.98 \times 10^{-9} - 5.50 \times 10^{-3}$	354
Reproductive System Disease	$2.79 \times 10^{-7} - 4.40 \times 10^{-3}$	232
Developmental Disorder	$3.04 \times 10^{-7} - 3.97 \times 10^{-3}$	103
Immunological Disease	$1.00 \times 10^{-6} - 5.16 \times 10^{-3}$	253
LnCaP		
Pathway	p-value	# molecules
Cancer	$2.11 \times 10^{-7} - 9.17 \times 10^{-3}$	800
Reproductive System Disease	$5.03 \times 10^{-6} - 8.63 \times 10^{-3}$	440
Genetic Disorder	$6.90 \times 10^{-6} - 7.92 \times 10^{-3}$	1393
Inflammatory Disease	$2.31 \times 10^{-5} - 9.32 \times 10^{-3}$	665
Connective Tissue Disorders	$6.42 \times 10^{-5} - 9.32 \times 10^{-3}$	433
VCaP		
Pathway	p-value	# molecules
Organismal Injury and Abnormalities	$7.42 \times 10^{-5} - 3.23 \times 10^{-2}$	28
Cancer	$1.13 \times 10^{-4} - 3.20 \times 10^{-2}$	184
Hematological Disease	$3.50 \times 10^{-4} - 2.93 \times 10^{-2}$	62
Genetic Disorder	$4.42 \times 10^{-4} - 3.06 \times 10^{-2}$	346
Respiratory Disease	$4.42 \times 10^{-4} - 2.53 \times 10^{-2}$	71

In collaboration with Drs. George Vasmataz and Farhad Kosari (Mayo Clinic Cancer Center) we compared the analyzed transcriptomes with those of CaP resected tissues previously used to identify prognostic biomarkers [13]. Samples of fresh frozen tissue [(CaP, n=32), benign prostatic tissue adjacent to CaP (BPC, n=40), and benign prostate tissue from CaP-free men (BP, n=28); and epithelial cells collected from an independent patient set by laser-capture microdissection (LCM) [CaP, n=68; BPC, n=31; BP, n=11; BPH, n=5]. We compared the data to those from CaP cells cultured at $pO_2=20$ kPa or $pO_2=2$ kPa. Notably, hypoxia increased transcript levels for pyruvate dehydrogenase kinase isozyme 1, nuclear prelam A recognition factor, glucose phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase in all three cell lines ($p<0.05$) to levels comparable to those found in primary bulk tissue and LCM isolated cells ($p<0.005$) (**Figure 2**). This finding suggests that gene expression in hypoxically cultured cells is more akin to that in tumor cells *in situ* than are cells grown normoxically. Our results challenge the long standing idea suggesting that tumors develop independence of oxygen [“the Warburg effect”; [14]] that led to the assumption of tumor insensitivity to oxygen. Also our findings add to recent data suggesting that in CaP, tumor-associated hypoxia associates to malignant progression, metastasis, resistance to therapy, and poor clinical outcome [15-18].

Figure 2. Different gene expression by CaP cells in hypoxia and normoxia



Expression in LCM and bulk tissue:

symbol	p-Bulk	p-LCM
PDK1	0	0
NARF	0	0.001
GPI	0.4903	0
GAPDH	0.0045	0.004

Transcription profiles revealed different gene expression in cells grown in hypoxia relative to those in normoxia. Interestingly, transcripts for pyruvate dehydrogenase kinase isozyme 1 (PDK1), nuclear prelamin A recognition factor (NARF), glucose phosphate isomerase (GPI), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were overexpressed in hypoxia in all three cell lines ($p < 0.05$), in bulk tissue and LCM isolated cells ($p < 0.005$).

By complementary data mining [19] we identified 88 known HIF-1 targets and 500 hypoxia associated target genes [20]; 23 genes of the conserved core hypoxia signature [21]; twelve HIF-1 targets tested in CaP [22]; and 708 genes in Ingenuity hypoxia signaling pathway [23]. This approach identified 24 hypoxia-associated genes significantly overexpressed in CaP ($p \leq 0.02$), both in bulk tissue and LCM cells (Table 2).

Table 2. Hypoxia-associated genes significantly overexpressed in CaP bulk tissue and samples isolated by Laser-capture microdissection

Symbol	Name	Bulk tissue		LCM	
		Ca/N ratio	p value	Ca/N ratio	p value
ACACA	acetyl-Coenzyme A carboxylase alpha	1.5	0	1.2	0.002123
CDCA3	Cell division cycle-associated protein 3	1.7	0	1.5	0
CEP55	centrosomal protein 55kDa	1.8	0	1.4	0
CCNB1	cyclin B1	1.8	0	1.3	0
CKS2	Cyclin-dependent kinases regulatory subunit 2	1.1	0.000043	1.2	0.000001
DLG7	discs, large (Drosophila) homolog-associated protein 5	1.8	0	1.5	0
SLC7A1	High affinity cationic amino acid transporter 1	1.6	0	1.2	0.000023
HMMR	Hyaluronan-mediated motility receptor	2.1	0	1.7	0
HIG2	hypoxia-inducible protein 2	1.5	0	1.2	0.000001
LOX	lysyl oxidase	1.1	0.000044	1.5	0
MMP10	matrix metalloproteinase-10	1.2	0.021521	1.4	0.000066
MCOLN2	mucolipin 2 (cation channel protein)	1.1	0.000332	1.4	0
NLN	neurolysin (metallopeptidase M3 family)	1.2	0	1.2	0.000012
PDLIM5	PDZ and LIM domain 5 (Scaffold protein)	1.1	0	1.2	0.000121
PSD3	pleckstrin and Sec7 domain containing 3	1.1	0.000001	1.2	0
C20orf74	Ral GTPase-activating protein subunit alpha-2	1.3	0	1.2	0.000092
FAM80A	ribosomal modification protein rimK-like family member A	1.7	0	1.2	0.000013
SDK1	sidekick homolog 1, cell adhesion molecule	1.5	0	1.5	0
STC2	Stanniocalcin-2 (secreted)	1.2	0.009053	1.3	0.000059
SOX4	Transcription factor SOX-4	1.2	0	1.2	0.000001
TMEM200A	transmembrane protein 200A	1.3	0	1.3	0.000886
TFF3	trefoil factor 3 (intestinal, stable secretory protein)	1.2	0.001889	1.2	0.001276
UBE2C	Ubiquitin-conjugating enzyme E2 C	1.4	0	1.2	0
UBE2E3	Ubiquitin-conjugating enzyme E2 E3	1.8	0	1.3	0.000079

Abbreviations: Ca, prostate cancer; N, Normal

Among hypoxia-associated genes, the disc large (Drosophila) homolog-associated protein 5 [DLG7], cyclin B1 [CCNB1], and hyaluronan-mediated motility receptor [HMMR] (Figure 3A) were associated with Gleason score and disease prognosis (Figure 3B). Since the products of

CCNB1 [24] and HMMR [19] genes have been recently identified as molecular markers of CaP progression, our results suggest the potential utility of hypoxia-associated genes as a criterion to identify CaP biomarkers with prognostic value. In additional studies we found a high correlation between DLG7 and DNA topoisomerase 2 α (TOP2A) transcript levels (Pearson Coefficient=0.816) (**Figure 3C**). As TOP2A is the strongest predictor of outcome for high-risk CaP [25], a predictive value of DLG7 for outcome in men at high-risk CaP can be anticipated.

Figure 3 . Three hypoxia-controlled genes are associated with Gleason score and disease prognosis

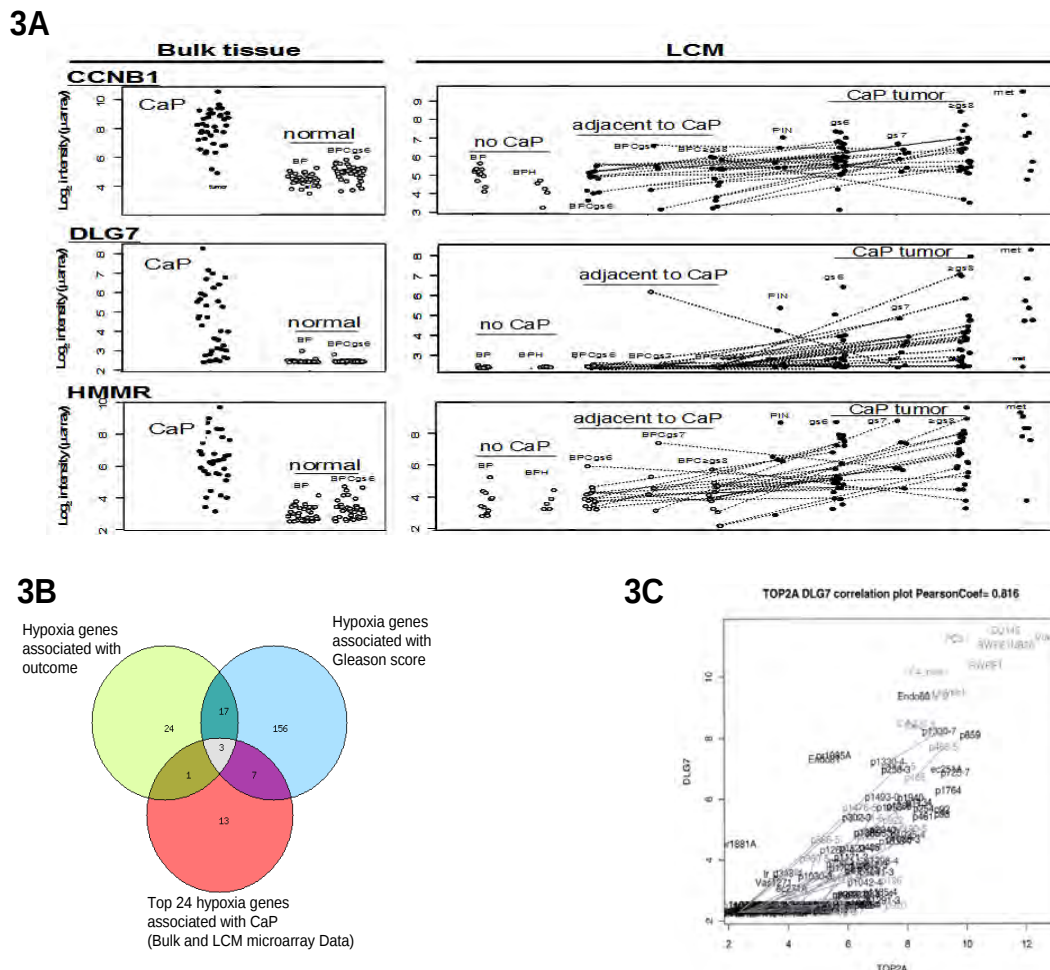


Figure 3A. Abbreviations: BP: benign prostate tissue from men who were free of CaP, BPC: benign prostate tissue in prostates that contained cancer, BPH: Benign prostatic hyperplasia, CaP: prostate cancer, CCNB1: Cyclin B1, DLG7: Disc large (Drosophila) homolog-associated protein 5, HMMR: hyaluronan-mediated motility receptor, LCM: laser-capture microdissection, met: metastasis, PIN: prostatic intraepithelial neoplasia. Figure 3B: Of the 24 hypoxia-controlled genes significantly overexpressed in CaP tissue, three of them: cyclin B1 [CCNB1]; disc large (drosophila) homolog-associated protein 5 [DLG7], and hyaluronan-mediated motility receptor [HMMR] were associated with Gleason score and with disease prognosis. Figure 3C: correlation between the RNA levels of DLG7 and TOP2A in CaP patients (Pearson Coefficient = 0.816)

Recognizing the potential of *in vitro* culture for studies of hypoxia-modulated malignant and survival properties of CaP cells (see above and [6]) we analyzed the transcript levels of DLG7, CCNB1 and HMMR genes in CaP cells grown in normoxia and hypoxia. Hypoxic cells

expressed 30 to 60 percent more CCNB1 and DLG7 transcripts. DLG7 is a cell-cycle-regulated [26], microtubule-associated protein and a Ran GTPase effector involved in mitotic kinetochore fiber stability [27]. Biological function of DLG7 is compatible with its role in cancer; however, pertinent information on its role in CaP is limited [26]. In one study, DLG7 was detected in nearly 90 percent of transitional cell carcinoma (TCC) of the bladder, but not in benign urological diseases; a higher level of DLG7 was found in recurrent TCC [28]. Overall, our results indicate that the DLG7 expression is higher in CaP and hypoxic CaP cells and correlated with disease outcome. These studies show the feasibility of identifying biomarkers linking CaP hypoxia and prognosis and establishing the contribution of hypoxia-associated genes to CaP progression. Identification of hypoxia-related biomarkers might help identify the patients who could benefit from hypoxia-modulating therapies [18]. These findings constituted the preliminary data for the application entitled “Hypoxia-regulated DLG7 in CaP carcinogenesis and prognosis”. This proposal was recently awarded a Treatment Sciences Creativity Award from the Prostate Cancer Foundation and is aimed at validating the role of DLG7 role in tumor progression.

Task 1c. 2-D gel analysis, in gel enzyme digestion and mass spectrometry.

In our preliminary experiments, we observed hypoxia-associated increase in VEGF production in CaP cells and ovarian cancer cells. In addition, we observed differences in spots in CaP cell propagated at $pO_2 = 2$ kPa relative to 20 kPa, as detected by 2-D electrophoresis. This suggests that hypoxia affects protein expression relative to normoxia. To date, identification of specific candidate genes with pO_2 -dependent expression in CaP cells in the context of their antigenic relevance has not yet been established.

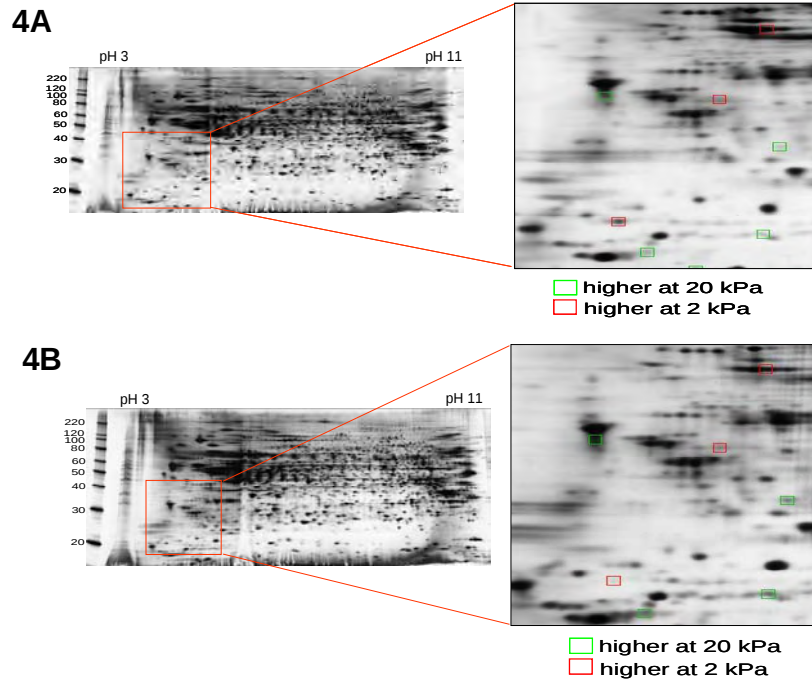
To characterize the effects of hypoxia on the proteome of CaP cells further, LnCaP and VCaP cells were cultured at $pO_2 = 2$ kPa or 20 kPa. Following four or seven days of incubation for LnCaP cells or seven or eleven days for VCaP cells, their lysates were loaded onto nonlinear pH 3–10 strips and subjected to isoelectrofocusing according to manufacturer’s instructions (Bio-Rad, Hercules, CA) and published protocols [29, 30]. We ran multiple strips in the first dimension and simultaneous second dimension assuring the highest possible reproducibility. Gels were silver stained, scanned and analyzed by PDQUEST software (Bio-Rad Laboratories). The proteome revealed multiple spots that differed in intensity and/or position between VCaP cells grown at $pO_2 = 2$ kPa and at 20 kPa (**Figure 4**). Surprisingly, the difference in pO_2 affected the proteome mostly quantitatively (i.e., by change in spot intensity). Using a threshold of fivefold change we found that in VCaP cells cultured for seven days, levels of only 13 proteins decreased and of 4 proteins increased during hypoxia. The results were similar for all cells and culture duration (data not shown). Our results are in line with the reports showing that hypoxia affects expression only of a small fraction of total cellular protein and that the content of total protein is not altered significantly [31, 32]. In addition, our findings rule out translational modification as an important response to hypoxia in cancer cells.

Task 1d. Association between gene-specific changes in mRNA and hypoxic proteome.

In Tasks 1a and 1b we established that hypoxia affects expression of particular genes in CaP and that the effects on proteome are mostly qualitative. However, establishing an association between changes in transcriptome and proteome demands additional effort. Along with similar studies [11, 31, 32], we set to identify the most affected protein spots on 2D-gels and find how they compare with the most affected gene transcripts. We sequenced spots from the 2D gels in Task 1e; the spots were selected as potential tumor-associated antigens (TAAs). In a preliminary analysis of six transcripts and proteins, we found no correlation between changes in levels of

transcripts and protein (data not shown). The data suggest the possibility that change in protein levels was not transcription dependent, in line with the findings suggesting that the changes affecting the proteome during hypoxia may be governed by posttranscriptional mechanisms rather than by changes in transcription or translation [11, 31, 32].

Figure 4. Oxygen pressure affects VCaP cell proteome

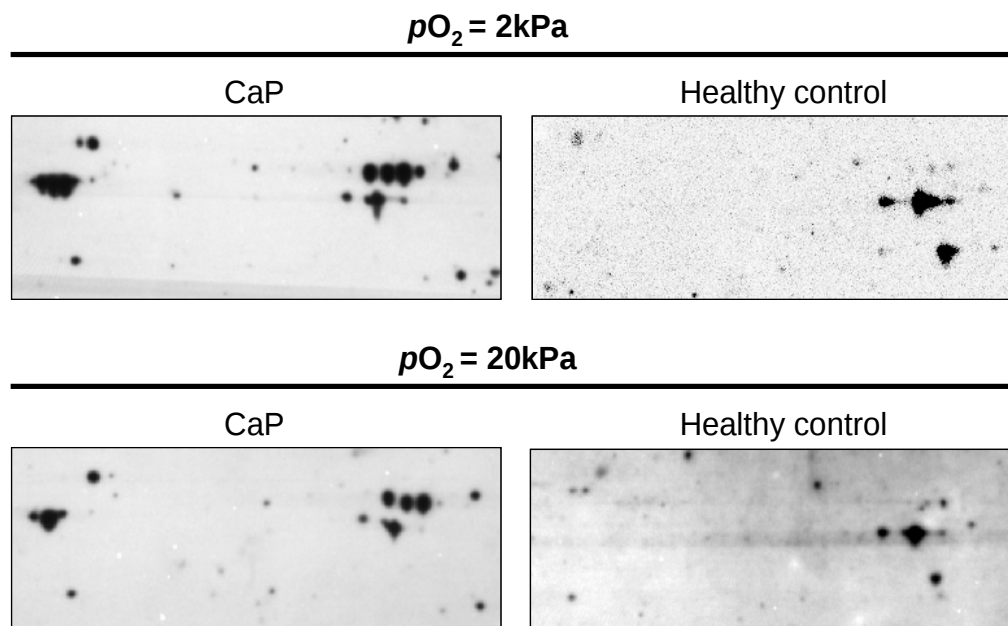


2D SDS electropherograms of lysed VCaP cells cultured at $pO_2=2$ kPa (Figure 4A) or $pO_2=20$ kPa (figure 4B) for 7 days were prepared. First dimension: Thirty μ g protein were loaded on pH 3-10 NL IPG strips for isoelectric focusing. Second dimension: 10.5-14 % SDS-PAGE gel. Similar results were observed in LnCaP cells (data not shown).

Task 1e. Identification of CaP antigens by 2D–Western blots.

The finding that patients harbor autoantibodies against tumor antigens has been used as to identify new autoantibody-binding peptides derived from CaP and other tumors [33, 34]. First we studied the reactivity of autoantibodies in CaP patient plasma. Following published protocols [29], we prepared total cell lysates of VCaP and LnCaP cells cultured at $pO_2=2$ kPa or 20 kPa, resolved them by 2D electrophoresis, transferred onto nitrocellulose membranes, and incubated with pooled plasma (1:300) from 25 patients and 25 controls. Plasma of 17 patients with autoimmune diseases (rheumatoid arthritis), 10 with colorectal cancer and 10 with lung cancer was used to validate specificity of potential candidates. Following incubation with goat anti-human Ig–HRP conjugate (1: 3000), bound antibodies were detected by chemiluminescence, followed by detection in autoradiographic films. Sera from patients with CaP reacted with numerous spots, some of which were observed in the control groups and thus considered nonspecific (Figure 5).

Figure 5 . Spontaneous autoantibodies in plasma from newly diagnosed CaP patients against VCaP cells

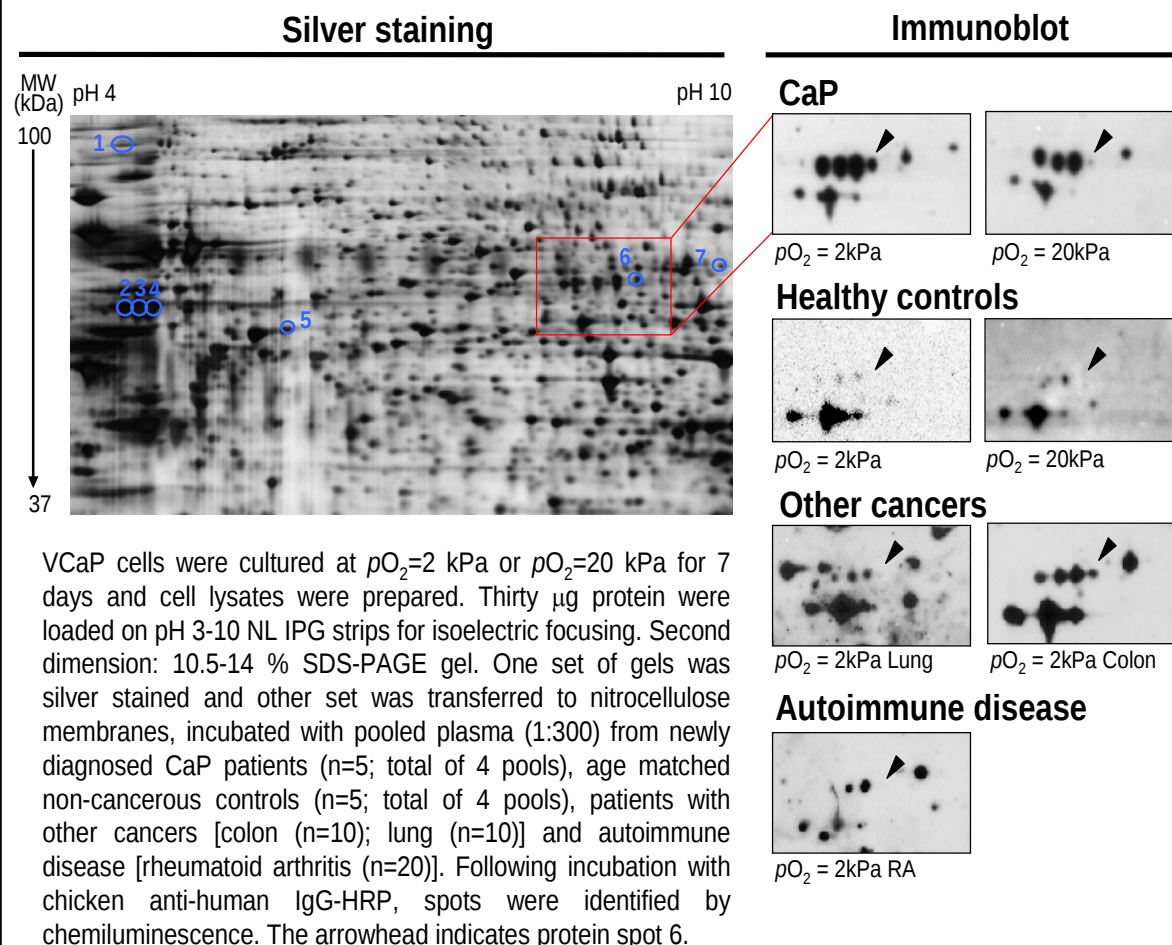


Thirty μ g protein were loaded on pH 3-10 NL IPG strips for isoelectric focusing (pH range 4–10 is shown). Second dimension: 10.5-14 % SDS-PAGE gel, transferred to nitrocellulose membranes, incubated with pooled plasma (1:300) from newly diagnosed CaP patients (n=5; total of 4 pools) or age matched non-cancerous controls (n=5; total of 4 pools). Following incubation with chicken anti-human IgG-HRP, spots were identified by chemiluminescence.

Sera from CaP patients specifically bound to seven spots (**Figure 6**); four were hypoxia-specific. All selected spots were excised from the gel, trypsin-digested, and analyzed by MALDI-TOF mass spectrometry. We identified them as heat shock 70 kDa protein 4; 60 kDa heat shock protein; protein disulfide isomerase A3; heterogeneous nuclear ribonucleoprotein L; U1 small nuclear ribonucleoprotein 70kDa and leucine-rich repeat-containing protein 47. With the exception of the latter molecule, identified proteins have been identified or validated as TAAs before [see references in (**Table 3**)]. However, to the best of our knowledge none of the proteins has been validated as a TAA in CaP.

Interestingly, the sequence of spots 2, 3 and 4 in Figure 6 corresponded to the 60 kDa heat shock protein, identified by us as a hypoxia-insensitive TAA. As there are three Hsp60 isoforms [35], it is likely that we identified the three isoforms as potential TAAs. Additional research will clarify the relevance of Hsp60 isoforms as relevant TAAs in CaP. Lysates from LnCaP cells grown at $pO_2=2$ kPa interacted with specific antibodies in plasma from CaP patients (data not shown); however, overall reactivity was lower than in VCaP cells. Interestingly, a series of spots consistent with spots 2-4 in VCaP cells was also recognized by plasma from patients blotted against lysates from LnCaP cells. It is evident from those experiments that hypoxic LnCaP cells exhibit similar reactivity to hypoxic VCaP cells. We are currently sequencing identified proteins from LnCaP cells with the expectation that they will validate the spots in VCaP cells and identify additional novel hypoxia-sensitive TAAs.

Figure 6. Identification of hypoxia-specific protein spots from CaP patients



Among the most conspicuous spots recognized by plasma from CaP patients in VCaP lysates was spot 6; this hypoxia-sensitive spot was strongly reactive with the pooled sera of CaP patients (**Figure 6**). This spot contains the U1 small nuclear ribonucleoprotein 70kDa and the heterogeneous nuclear ribonucleoprotein L. Both these proteins have been identified as TAAs [36-38]; their validation in CaP is our next immediate priority. For validation we will narrow the pH range of isoelectric focusing from 7 to 10 and use specific antibodies to confirm the identity of the molecules. Protein spot 6 exhibited little or no reactivity with sera of healthy controls, lung cancer or rheumatoid arthritis, but we detected some reactivity (in a hypoxia-dependent manner) with plasma from colorectal cancer (CRC) patients. The relevance of hypoxia has been recognized in CRC [39] and numerous CRC-associated-TAAs have been identified [40]; however, establishing the relevance of hypoxia-sensitive U1 small nuclear ribonucleoprotein 70kDa and heterogeneous nuclear ribonucleoprotein L is a new and interesting aspect in CRC as it could expand the use of hypoxia to the identification of TAAs in other tumors.

Table 3. Potential TAAs identified in VCaP cells						
Spot number	Protein name	Hypoxia specific	Accession number	MW (kDa)	Peptides matched	TAA [Refs]
1	Heat shock 70 kDa protein 4	yes	P34932	94.3	28-39	Esophageal [41], hepatocellular carcinoma [42, 43]
2	60 kDa heat shock protein	no	P10809	61.3	43-63	Breast [29], hepatocellular carcinoma [43], colorectal [44], oral [45], gastric lymphoma [46]
3	60 kDa heat shock protein	no	P10809	61.3	59-80	
4	60 kDa heat shock protein	no	P10809	61.3	50-105	
5	Protein disulfide isomerase A3	yes	P30101	56.8	16-26	Breast [29, 47], hepatocellular carcinoma [43]
6	Heterogeneous nuclear ribonucleoprotein L	yes	P14866	64.1	18-52	Acute leukemia [36], healthy [37]
6	U1 small nuclear ribonucleoprotein 70kDa	yes	P08621	51.4	11-52	Lymphoma [38]
7	Leucine-rich repeat-containing protein 47	yes	Q8N1G4	63.5	16-16	

Task 2. Validate differentially expressed molecules in CaP in association with tissue hypoxia

Approach: The presence of a hypoxic cancer microenvironment correlates with increased tumor invasiveness, metastases, resistance to radio- and chemotherapy, and poor clinical outcome [15, 41]. It is well established that CaP cells are found under hypoxic conditions *in vivo* [42] and that numerous proteins are modified in their expression by hypoxia [11]. Although many endogenous markers have been associated with the hypoxia response in cancer they are not all unregulated in primary CaP tissue [22]. This may be because the evaluation of potential markers has not been made taking into consideration the hypoxic environment in first place. Task 2 (planned for the second period of the award) is aimed at assessing the expression of select candidate genes identified in Task 1 in CaP tissue. Real time PCR and RNA *in situ* hybridization added to immunodetection will allow detection of specific candidate genes in CaP tissue.

The proposed sub Tasks are:

2a. RNA extraction and real time quantitative PCR in CaP tissue (months 13-16)

2b. mRNA *in situ* hybridization (months 16-22)

2c. Immunohistochemistry Staining in CaP tissue (months 16-24)

Ongoing experiments are addressing the methodological concerns in experimental approaches to this Task. As part of the recently awarded project “Hypoxia-regulated expression of DLG7 gene in prostate cancer prognosis and progression” (funded by The Prostate Cancer Foundation), assessment the relationship of DLG7 expression and cancer-specific outcomes is a most relevant Task. For this project, we are studying gene expression in conjunction with a histopathology evaluation CaP tissue. Next will follow and analysis of the association of transcript levels and protein levels with clinical parameters. We will apply a similar strategy to accomplish Task 2. It is expected that the completion of Task 2 will occur as proposed.

Key research accomplishments

- Hypoxic LnCaP and VCaP cells proliferate more effectively than at standard cell culture conditions.
- Hypoxic cells secrete more VEGF.
- Hypoxia induced overexpression of molecules involved in intracellular signaling networks in cancer and urologic diseases in comparison to normoxic cells.
- Hypoxia increased transcript levels for some genes in cell lines to levels comparable to those in CaP tissue.
- Hypoxia-associated DLG7, HMMR and CCNB1 genes were significantly overexpressed in CaP and associated with Gleason score and disease prognosis.
- Hypoxic cells expressed 30 to 60 percent more CCNB1 and DLG7 transcripts.
- The change in pO_2 , affected the proteome mostly quantitatively (*i.e.*, by change in spot intensity).
- There was no correlation between changes in protein levels and mRNA induction among a group of select genes tested.
- Protein lysates from cells exposed to hypoxia revealed novel potential TAAs (currently under validation) in sera from CaP patients (heat shock 70 kDa protein 4; protein disulfide isomerase A3; heterogeneous nuclear ribonucleoprotein L; U1 small nuclear ribonucleoprotein 70kDa and leucine-rich repeat-containing protein 47).

Reportable outcomes

Abstracts

1. **Gomez, C.R.**, Kosari, F., Schreiber, C.A., Knutson G.J., Vasmatazsis, G., Vuk-Pavlović S. 2010. Hypoxic exposure as a novel strategy to identify tumor-associated antigens in prostate cancer. Presented at "Tumor Immunology: Basic and Clinical Advances." Miami Beach, FL.
2. **Gomez, C.R.**, Knutson G.J., Schreiber, C.A., Kosari, F., Vasmatazsis, G., Vuk-Pavlović S. 2011. Hypoxia affects gene expression and proteome of prostate cancer cells. Presented at the 102th AACR Annual Meeting, Orlando, FL.
3. **Gomez, C.R.**, Kosari, F., Schreiber, C.A., Knutson G.J., Vasmatazsis, G., Vuk-Pavlović S. 2011. Hypoxic cell culture for more effective cancer vaccines. To be presented at the 7th ISABS Conference on Forensic, Anthropologic and Medical Genetics, Bol, Island of Brač, Croatia.

Funding applied

1. Title: Hypoxia-regulated DLG7 in prostate cancer carcinogenesis and prognosis (P.I.). Date: Dec 2010. Submitted to: Prostate Cancer Foundation. Treatment Sciences Creativity Awards.. Status: Funded. Project goals: We found the transcripts of the disc's large homolog-associated protein 5 (DLG7), a hypoxia-regulated gene, overexpressed in human primary prostate cancer and human prostate cancer cell lines. The overall goal of the proposed studies is to validate the role of DLG7 role in tumor progression. Specific aims: 1) To measure the levels of DLG7 transcripts in resected CaP tissues and study the association with survival. 2) To overexpress DLG7 in prostate cells (normal epithelium and tumorigenic cells) and compare tumorigenesis in

the context of hypoxia. Key personnel receiving salary support from this project: 50% effort CR Gomez. 100% effort postdoctoral fellow

2. Title: A Method for Prognosis of Prostate Cancer Based on Cellular Markers of Hypoxia (P.I.). Date: Jan 2011. Submitted to: Center for Translational Science Activities (CTSA) - Novel Methodology Development Award (NMDA). Level of Funding: Status: Not funded.

Research opportunities

Active collaboration has been established with John Cheville, M.D., Professor of Pathology; Jeffrey Karnes, M.D., Director, Mayo Prostate Cancer SPOR Clinical Core and Chair, of Mayo Clinic Dept. of Urology Radical Prostatectomy Registry; Farhad Kosari, Ph.D., College of Medicine; and George Vasmatzis, Ph.D., Department of Pathology and Laboratory Medicine, Center of Individualized Medicine and Department of Molecular Medicine, Mayo Clinic. This effort has identified hypoxia-sensitive genes as a new parameter for detecting aggressive prostate cancer with prognostic relevance. Access to tissue samples and data from a 150-pair case-control study will allow studies of the relationship between hypoxia-sensitive gene expression and cancer-specific outcomes. In addition to providing archived tissue samples, collaborators assist with histopathology evaluation and analysis and interpretation of the data with respect to the association of transcript and protein levels with clinical parameters.

Employment applied

Based on this work, in June 2011 the P.I. has been offered a tenure-track associate professor position at the Department of Pathology, University of Mississippi Cancer Center, Jackson, MS.

Conclusion

We are studying the ways to optimize the effectiveness of therapeutic whole-cell CaP vaccines by tumor-associated hypoxia as a relevant determinant of tumor antigenicity. Our results show that in cultured human CaP cells hypoxia modifies expression of genes associated with cancer and urologic disease to levels comparable to those in resected human CaP tissue. These results suggest that gene expression in hypoxically cultured cells is more akin to that in tumor cells *in situ* than are cells grown normoxically. We studied the transcriptome in human primary CaP tissue. Transcripts of hypoxia-associated genes DLG7, CCNB1 and HMMR were overexpressed and associated with Gleason score and with disease prognosis; this suggests their potential as CaP biomarkers with prognostic value. 2D-gel electrophoresis experiments confirmed previous findings indicating that hypoxia affects the proteome mostly quantitatively (*i.e.*, by change in spot intensity). Nonetheless, protein lysates from CaP cells exposed to hypoxia revealed novel potential TAAs thus suggesting the relevance of antigenic landscape of hypoxic proteome. Overall, our results suggest that hypoxia modifies the cellular properties of CaP cells towards a phenotype that is more similar to tumor cells *in situ*. Introduction of pO_2 as a variable can constitute a tool for the development of more effective immunotherapy for CaP.

So what: The role of pO_2 in tumor biology has been unappreciated. Recently, tumor-associated hypoxia has been associated with malignant progression, metastasis, resistance to therapy, and poor clinical outcome. Our results validate the relevance of tumor-associated hypoxia in CaP and, more importantly, define the potential of hypoxia as a tool in the development of cellular vaccines for CaP.

We identified hypoxia-controlled genes with potential as prognostic factors in CaP. Validation of these genes anticipates applications in the clinic and research laboratory. Potential applications include refinement of the current prognostic tools for CaP and better tools to predict therapeutic outcome. In addition, a test for assessing hypoxia in tumors *in situ* could alleviate the problems of measuring pO_2 in tumor tissues. Because we found that molecular signature of tumor tissue under hypoxic conditions is retained after resection, the correlation of pO_2 and expression of hypoxia-controlled genes *in situ* could provide a surrogate method to assess pO_2 in CaP tissue. Finally, our evidence of substantial sensitivity of CaP cells to hypoxia might lead to enhanced efficacy of therapy not only for CaP, but also serve as a paradigm for other forms of cancer.

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Appendices



An AACR Special Conference

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xenografts (volume 100mm³). Weekly systemic administration of MRG partially yet significantly inhibited tumor growth, as compared to injection of carrier only. MRG1 did not stimulate Antibody Dependent Cellular Cytotoxicity mediated elimination of melanoma cells in SCID-NOD mice. Single intravenous injection of melanoma specific human T cells yielded partial inhibition of tumor growth. Importantly, the treatment with a single adoptive cell transfer clearly synergized with weekly MRG1 therapy (>75% inhibition).

In conclusion, we show that CEACAM1 directly enhances various aggressive features of melanoma and provisionally position MRG1 as a potential novel anti-melanoma drug. Importantly, 75% of clinical melanoma specimens express CEACAM1 and are thus suitable for CEACAM1-targeted therapy. This represents a novel modular line of therapy that can stand alone and also potentially synergize with other therapeutic modalities.

A3 Hypoxic exposure as a novel strategy to identify tumor-associated antigens in prostate cancer. Christian R. Gomez, Farhad Kosari, Claire A. Schreiber, Gaylord J. Knutson, George Vasmatazis, Stanimir Vuk-Pavlovic. Mayo Clinic, Rochester, MN.

Hypoxia is a hallmark of the environment of many tumors. We hypothesize that it can modulate expression of tumor-associated antigens (TAAs) and thus affect immunity-based therapeutic strategies. To test the effects of hypoxia on prostate cancer (CaP), we studied global gene expression in 100 CaP tissues and 71 samples of adjacent benign tissues. RNA was extracted from cancer cells isolated by laser-capture microdissection (LCM) or without isolation ("bulk tissue"). We identified 24 hypoxia-associated genes significantly overexpressed in CaP ($p \leq 0.02$), both bulk tissue and LCM. Among these genes, cyclin B1 [CCNB1], disc large (drosophila) homolog-associated protein 5 [DLG7], and hyaluronan-mediated motility receptor [HMMR] were associated with Gleason score and with disease prognosis. Since the products of CCNB1 and HMMR

genes have been recently identified as TAAs, our results suggest the potential of DLG7 and other candidate genes as possible TAAs. In addition, we propagated CaP cell lines LnCaP, VCaP and DU145 in hypoxia ($pO_2=2$ kPa) and compared them with normoxically grown cells ($pO_2=20$ kPa). Hypoxic cells proliferated faster and secreted more vascular endothelial growth factor (VEGF; $p < 0.05$). As expected, transcription profiles revealed differential gene expression in cells grown in hypoxia relative to those in normoxia. Interestingly, transcripts for pyruvate dehydrogenase kinase isozyme 1 (PDK1), nuclear prelamin A recognition factor (NARF), glucose phosphate isomerase (GPI), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were overexpressed in hypoxia in all three cell lines ($p < 0.05$) both in bulk tissue and LCM isolated cells ($p < 0.005$). Also, we observed a 30–60 percent hypoxia-associated increase in CCNB1 and DLG7 expression in VCaP cell line, the only one harboring the TMPRSS2-ERG fusion (present in 40–60 percent of CaP patients) among the three cell lines studied. To determine if hypoxia affects immunogenicity of CaP cells, we screened the sera from CaP patients ($n=20$) and healthy controls ($n=13$) for spontaneous antibodies cross-reactive with spots resolved on 2D electropherograms of lysates of LnCaP cells grown in hypoxia and normoxia. CaP patient sera bound to numerous spots in both electropherograms, but the binding patterns differed significantly. Overall, our data indicate that hypoxia affects gene expression and antigenic signature in CaP. Our ongoing experiments will resolve the question whether CaP cells grown in hypoxia are more akin to of tumor cells in situ than are cells grown in normoxia and will further identify and validate selected candidate TAAs in CaP patients. Support: DOD PC094680 (CRG), Minnesota Partnership for Biotechnology and Medical Genomics, Mayo Clinic Prostate SPORE 5P50CA091956 (FK, SV-P), Adelyn Luther, Singer Island, Florida (SV-P); and Mayo Clinic Cancer Center (SV-P).

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Presentation Abstract

Abstract
Number: LB-308

Presentation
Title: Hypoxia affects gene expression and proteome of prostate cancer cells

Location: Exhibit Hall A4-C, Poster Section 38

Author
Block: Christian R. Gomez, Clayford J. Knutson, Claire A. Schreiber, Farhad Kosari, George Vasmatazis, Stanimir Vuk-Pavlovic, Mayo Clinic College of Medicine, Rochester, MN

Abstract
Body: Tumor-associated hypoxia facilitates malignant progression, metastasis and poor prognosis in prostate cancer (CaP); however, effects of oxygen tension (pO_2) in CaP are just beginning to be investigated. To establish mechanisms whereby hypoxia enhances malignant properties and survival of CaP and to identify pO_2 -regulated tumor-associated macromolecules, we propagated CaP cell lines LnCaP, VCaP and DU145 in hypoxia ($pO_2=2$ kPa) and compared them with normoxically grown cells ($pO_2=20$ kPa); hypoxic cells proliferated faster and secreted more vascular endothelial growth factor ($p<0.05$). Transcriptome studies revealed different gene expression in cells grown in hypoxia relative to those in normoxia. Interestingly, in hypoxic cells transcripts associated with cancer and urologic disease were overexpressed in comparison to normoxic cells ($p<0.001$) suggesting an association of low pO_2 and aggressive features of CaP. Further, we analyzed the transcriptome in primary CaP cells isolated by laser-capture microdissection (LCM) and whole CaP tissue. We compared the data to those from CaP cells cultured at $pO_2=20$ kPa or $pO_2=2$ kPa. Notably, hypoxia increased transcript levels for pyruvate dehydrogenase kinase isozyme 1, nuclear prelamin A recognition factor, glucose phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase in all three cell lines ($p<0.05$) to levels comparable to those found in primary bulk tissue and LCM isolated cells ($p<0.005$). This finding suggests that gene expression in hypoxically cultured cells is more akin to that in tumor cells *in situ* than are cells grown normoxically. By 2D-gel electrophoresis, we found that change in pO_2 affected the proteome mostly quantitatively (*i.e.*, by change in spot intensity). Our results suggest that hypoxia affects transcriptome and proteome in CaP cells. In addition, we screened 20 patient sera and 20 healthy control sera for spontaneous antibodies cross-reactive with VCaP cells and found that the sera reacted with numerous proteins, some previously reported to elicit an immune response in CaP patients (*e.g.*, nucleoporin 62 and transitional endoplasmic reticulum ATPase). By this method we detected numerous novel autoantigens (under validation). Currently we are clarifying the pO_2 effects on the relationship of transcriptome, proteome and tumor-associated antigens in CaP cells. Support: DOD PC094680 (CRG), Minnesota Partnership for Biotechnology and Medical Genomics, Mayo Clinic Prostate SPORC 5P50CA091956 (FK), Mrs. Adelyn L. Luther, Singer Island, Florida and Mayo Clinic Cancer Center (SV-P).

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HYPOXIC CELL CULTURE FOR MORE EFFECTIVE CANCER VACCINES

Christian R. Gomez, Farhad Kosari, Claire A. Schreiber, Gaylord J. Knutson, George Vasmataz, Stanimir Vuk-Pavlović. Mayo Clinic, Rochester, MN, USA

For some malignancies, vaccines containing allogeneic cancer cells enhanced the overall survival in early clinical trials. Because the cells were cultured at $pO_2=20$ kPa, it is unclear whether they provide an adequate antigen match to tumor cells *in situ* where pO_2 is generally much lower. Thus, we postulate that hypoxically grown vaccine cells will be a better antigen match to tumors *in situ*. We are testing this hypothesis by studying the effects of hypoxia on prostate cancer (CaP) cells. We analyzed the transcriptome of 100 CaP tissues and 71 adjacent benign tissues and found 24 genes (known as oxygen-regulated) significantly overexpressed in CaP relative to control tissue ($p \leq 0.02$). Overexpression of cyclin B1, hyaluronan-mediated motility receptor and disc large (Drosophila) homolog-associated protein 5 was associated with high Gleason score and poor prognosis. As the former two genes encode tumor-associated antigens (TAAs), it is possible that the product of the latter is a TAA too. When CaP cell lines LnCaP, VCaP and DU145 were grown at $pO_2=2$ kPa, they proliferated faster and secreted more vascular endothelial growth factor than the cells grown at $pO_2=20$ kPa ($p < 0.05$). Interestingly, in hypoxia all three cell lines transcribed more pyruvate dehydrogenase kinase isozyme 1, nuclear prelamin A recognition factor, glucose phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase genes than in normoxia ($p < 0.05$); we found a similar relationship between transcript levels for those genes in CaP cells *in situ* and nonmalignant control cells ($p < 0.005$). We continue to characterize the relationship of hypoxically cultured CaP cells and CaP cells *in situ* to identify and validate TAAs for a more effective therapeutic vaccination. Support: DOD PC094680 (CR G), Minnesota Partnership for Biotechnology and Medical Genomics, Mayo Clinic Prostate SPORC 5P50CA091956 (FK); Mrs. Adelyn L. Luther, Singer Island, Florida and Mayo Clinic Cancer Center (SV-P).

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1998 - 2000 Research assistant: CAAT/enhancer-binding protein signaling during the acute phase response of aged Fisher 344 rats, Advisor: Dr. Robin Walter, Department of Cellular and Molecular Biology, University of Chile School of Medicine, Santiago, Chile

2000 - 2004 Doctoral dissertation: Macrophage inflammatory protein 1-alpha as a modulating factor of the acute phase response: extension to the inflammatory response in aged individuals, Advisor: Dr. Felipe Sierra, Department of Cellular and Molecular Biology, University of Chile School of Medicine, Santiago, Chile

2004 - 2008 Postdoctoral Fellow: Aging and inflammatory responses. Supervisor: Dr. Elizabeth J. Kovacs, Loyola University Chicago, Stritch School of Medicine, Department of Cell Biology, Neurobiology and Anatomy and Department of Surgery, Maywood, IL

International courses

Natural Antibodies in the Maintenance of Tolerance to Self: Lessons from Physiology and Therapy, Program of Immunology, Faculty of Medicine, University of Chile, Santiago, Chile, 15-16 December 1998

International Symposium and Training Course: "Cellular Signaling From Plasma membrane to the Nucleus", Program of Cellular and Molecular Biology, Faculty of Medicine, University of Chile, Santiago, Chile, 12-23 July 1999

International Symposium and Training Course: "International Course on Techniques for the Study of Functional Genomics", Program of Cellular and Molecular Biology, Faculty of Medicine, University of Chile, Santiago, Chile, 19 June – 1 July 2000

Training Course: "Molecular Biology of Aging", Marine Biological Laboratory, Woods Hole, MA, USA, 31 July – 19 August 2006

Faculty Appointments

2004 - 2007 Research Associate, Loyola University Chicago, Stritch School of Medicine, Department of Cell Biology, Neurobiology and Anatomy, Maywood, IL

2007 - 2008 Research Associate, Loyola University Chicago, Stritch School of Medicine, Department of Surgery, Maywood, IL

2008 - Date Research Associate, Stem Cell Laboratory, Department of Oncology, Mayo Clinic Cancer Center, Rochester, MN

2009 - Date Assistant Professor of Biochemistry/Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN

2010 - Date Assistant Professor, Division of Preventive, Occupational, And Aerospace Medicine, Mayo Clinic, Rochester, MN (Pending)

Professional awards

1998-2002 Chilean National Council for Science and Technology (CONICYT) doctoral scholarship

2000 International Travel Award: "Identification of genes that are differentially expressed during the acute phase response of senescent animals", Lab. Dr. Christian Cell, Lankenau Institute for Medical Research, Thomas Jefferson University, Wynnewood, PA, USA

2002 International Travel Award: "Characterization of differential hepatic expression of the chemokines MIP-1 α , in aged rats, injected with bacterial endotoxin (LPS)", Lab. Dr. Christian Sell and Lab. Dr. Vincent Cristofalo, Lankenau Institute for Medical Research, Thomas Jefferson University, Wynnewood, PA, USA

2002 International Travel Award: "Standardization of the measurement of tissue and circulating levels of cytokines during the acute phase response of aged rats", Lab. Dr. Elizabeth J. Kovacs, Stritch School of Medicine, Loyola University, Maywood, IL, USA

2001 Distinguish Award for the "Best dissertation project on Gerontological Studies" Interdisciplinary Program for Gerontological Studies, University of Chile, Santiago, Chile

2004	Travel award to attend the Annual Meeting of the Society for Leukocyte Biology, Toronto, Canada
2004	Doctoral Medal, University of Chile, Santiago, Chile
2005	Travel award to attend the Annual Meeting of the Society for Leukocyte Biology, Oxford, England
2006	Young Investigator Travel Award to attend the Twenty-Ninth Annual Conference on Shock, Broomfield, CO, USA
2006	Travel award to attend the Annual Meeting of the Society for Leukocyte Biology, San Antonio, TX, USA
2010	AACR Minority Scholar in Cancer Research Award to attend the AACR Special Conference, Tumor Immunology: Basic and Clinical Advances. Miami, FL
2011	Prostate Cancer Foundation. Treatment Sciences Creativity Awards 2011

Professional Society Membership and Activities

2004 - 2008	Society for Leukocyte Biology, Member
2004 - 2008	Shock Society, Member
2009 - Date	American Association for Cancer Research (AACR), Associate Member
2009 - Date	Mayo Clinic Alumni Association, Member
2009 - Date	AACR, Minority Scholar in Cancer Research, Member

Media related quotes and interviews

2005	"Healthy Aging", Interview, University of Santiago Radio. Santiago, Chile
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Journal Review Activity

2010 - Date	Ad Hoc Reviewer: Journal of Leukocyte Biology, American Journal of Physiology-Advances in Medical Education.
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Teaching experience

1999, 2001	Teaching Assistant, Course of Cellular Biology for Kinesics therapy and Occupational therapy and Medical Technology (First year students), Faculty of Medicine, University of Chile, Santiago, Chile
2002-2003	Teaching Assistant, Seminars on Biotechnology for Medical technology students (second year students), Mention clinical bio-analysis, Hematology and Blood bank, Faculty of Medicine, University of Chile, Santiago, Chile
2002-2003	Teaching Assistant, Cellular Biology course for Biochemistry students (fourth year students), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile
2003-2004	Teaching Assistant, Workshop for Integration of Basic Sciences for Medical students (first year students), Faculty of Health Sciences, University Diego Portales, Santiago, Chile
2003-2006	Teaching Assistant, Course Structure and Function I for Medical students (first year students), Faculty of Health Sciences, University Diego Portales, Santiago, Chile
2003	Teaching Assistant, Course of Cell Biology for Ph.D. students, Faculty of Medicine, University of Chile, Santiago, Chile
2003	Teaching Assistant, Course of Advanced Genetics for Ph.D. students, Faculty of Medicine, University of Chile, Santiago, Chile

- 2004 Teaching Assistant, course of Cell Biology for Nursery and Medical Technology students (first year students), Faculty of Health Sciences, University Diego Portales, Santiago, Chile
- 2004 Teaching Assistant, Seminars in Molecular Biology for Medical Technology (fourth year students), Mention clinical Bio-analysis, Hematology and Blood bank, Faculty of Medicine, University of Chile, Santiago, Chile
- 2006 Teaching Assistant, Medical Histology, The Stritch School of Medicine, Loyola University Medical Center, Maywood, IL

Research Supervision

- 2005 Co-mentor: Stephanie Hirano, M.D. Student
- 2005 Mentor: Ying Peng, Ph.D. Candidate
Christine Regnell, M.S. Candidate
Shirin Birjandi, Ph.D. Candidate

All the students were at Elizabeth J. Kovacs' Laboratory at The Burn and Shock Trauma Institute, Loyola University Medical Center, Maywood, IL

- 2006-8 Mentor: Freddy Bustos, Constanza Fernández, Ana María Duhalde,
M.D. Students, Methodology in research rotation, Universidad Diego Portales, Santiago, Chile
- 2009 Co-Mentor: Freddy Bustos, M.D. Student, research rotation, Stem Cells Lab, Mayo Clinic Cancer Center, Rochester, MN
- 2010-Date Claire A. Schreiber, Luther College, Decorah, IA
Research Assistant, Stem Cells Lab, Mayo Clinic Cancer Center, Rochester, MN
- 2011 Lauren Ulbrich, St. Mary's University, Winona, MN
Summer student, Stem Cells Lab, Mayo Clinic Cancer Center, Rochester, MN

Research Grant Support:

Ongoing:

Title: Enhancing therapeutic cellular prostate cancer vaccines (PC094680) (P.I.).

Time commitment: 100%

Supporting agency: Department of Defense. New Investigator Award

Performance period: 04/15/10 – 03/31/12

Project goals: The overall goal of the proposed studies is designed to optimize the effectiveness of therapeutic whole-cell CaP vaccines. We hypothesize that hypoxically cultured CaP cells are more similar in their antigen landscape to CaP cells *in situ* than are normoxically cultured CaP cells

Specific aims: 1) To identify oxygen-tension responsive genes and proteins in the cells comprising a clinical-grade CaP cellular vaccine. 2) To validate differentially expressed molecules in CaP tissue in association with tissue hypoxia

Key personnel receiving salary support from this project: 100% effort CR Gomez. 50% effort allied staff

Title: Hypoxia-regulated DLG7 in prostate cancer carcinogenesis and prognosis. (P.I.) (Waiting for award notice)

Time commitment: proposed 50%

Supporting agency: Prostate Cancer Foundation. Treatment Sciences Creativity Awards 2011

Performance period: 05/01/11 – 05/01/13

Project goals: We found the transcripts of the discs large homolog-associated protein 5 (DLG7), a hypoxia-regulated gene, overexpressed in human primary prostate cancer and human prostate cancer cell lines. The overall goal of the proposed studies is to validate the role of DLG7 in tumor progression

Specific aims: 1) To measure the levels of DLG7 transcripts in resected CaP tissues and study the association with survival. 2) To overexpress DLG7 in prostate cells (normal epithelium and tumorigenic cells) and compare tumorigenesis in the context of hypoxia.

Key personnel receiving salary support from this project: 50% effort CR Gomez. 100% effort postdoctoral fellow

Completed:

Title: Hyperbaric oxygen as mobilizer of stem cells and progenitors in senescent mice (Stanimir Vuk-Pavlovic, P.I.). Co P.I.

Time commitment: 30%

Supporting agency: Mayo Clinic, Division of Preventive, Occupational and Aerospace Medicine Small Grant Awards

Performance period: 04/01/09 – 12/30/09

Project goals: The effects of hyperbaric oxygen (HBO) on mobilization of hematopoietic and stem and progenitor cells (HSPCs) and mesenchymal stromal cells (MSCs) from bone marrow into circulation of old mice were explored.

Specific Aims: 1) To measure the effects of HBO in young and old mice by flow cytometry after labeling white blood cells with pertinent fluorescent immunoreagents for HSPCs and MSCs. 2) To measure the levels of selected circulating cytokines involved in HSPCs and MSCs mobilization.

Key personnel receiving salary support from this project: 3% effort CR Gomez

Title: Hyperbaric oxygen as mobilizer of stem cells and progenitors in senescent mice. Extension of funds for 2010 (Stanimir Vuk-Pavlovic, P.I.). Co P.I.

Time commitment: 30%

Supporting agency: Mayo Clinic, Division of Preventive, Occupational and Aerospace Medicine Small Grant Awards

Performance period: 04/01/10 – 12/31/10

Project goals: The mechanisms of age-related impairment of mobilization of both hematopoietic stem and progenitor cells (HSPCs) and mesenchymal stromal cells (MSCs) from bone marrow by hyperbaric oxygen (HBO) into circulation of old mice were studied. Specific Aims: 1) To analyze the effects of aging and HBO on the expression of SDF-1/CXCR4 system, the critical regulator of SPCs function and homing. 2) To analyze the effects of aging and HBO on the regulation of nitric oxide ($\bullet$ NO)-mediated mechanism of MSCs mobilization by HBO synthesis.

Key personnel receiving salary support from this project: 3% effort CR Gomez

Planned:

“Hypoxia and tumor microenvironment to improve cell immunotherapy for prostate cancer”. NIH, R01.
(direct costs). Period: 07/01/12 – 06/30/16. P.I.

Research Interests

1. Immunosenescence: The effects of advanced age on inflammation, immunoregulation, and injury. Age-dependent defects in cytokine networks. Interleukin-6: age-related effects on cellular and systemic immune responses. Hormone replacement and cell mediated immunity in the aged.
2. Immunotherapy for prostate cancer: Small molecules as modulators of the tumor microenvironment. Strategies aimed at improving delivery of whole-cell cancer vaccines by improving their antigenicity.
3. Restoring immunity in the aged: Hyperbaric oxygen therapy as mobilizer of stem cells and progenitors in senescent individuals.

BIBLIOGRAPHY

1. Aravena, M., Perez, C., Perez, V., Acuna-Castillo, C., **Gomez, C.R.**, Leiva-Salcedo, E., Nishimura, S., Sabaj, V., Walter, R. and Sierra, F. 2005. T-kininogen can either induce or inhibit proliferation in Balb/c 3T3 fibroblasts, depending on the route of administration. *Mech Ageing Dev.* 126:399-406.
2. **Gomez, C.R.**, Boehmer, E.D. and Kovacs, E.J. 2005. The aging innate immune system. *Current Opin Immunol* 17:457-462.
3. Pérez, V., Velarde, V., Acuña-Castillo, C., **Gomez, C.R.**, Nishimura, S., Sabaj, V., Walter, R., and Sierra, F. 2005. Increased Kinin Levels and Decreased Responsiveness to Kinins During Aging. *Gerontol A Biol Sci Med Sci* 60:984-990.
4. Acuña-Castillo, C., Aravena, C., Leiva-Salcedo, E., Pérez, V., **Gomez, C.R.**, Sabaj, V., Nishimura, S., Pérez, C., Colombo, A., Walter, R. and Sierra, F. 2005. T-kininogen, a cystatin-like molecule, inhibits ERK-dependent lymphocyte proliferation. *Mech Ageing Dev* 126:1284-91.
5. **Gomez, C.R.**, Acuña-Castillo, C., Nishimura, S., Pérez, V., Escobar, A., Sabaj, V., Torres, C., Walter, R. and Sierra, F. 2006. Serum from aged F344 rats conditions the activation of young macrophages. *Mech Ageing Dev* 127:257-63.
6. Pérez, V., Leiva-Salcedo, E., Acuña-Castillo, C., Aravena, M., **Gomez, C.R.**, Sabaj, V., Colombo, A., Nishimura, S., Pérez, C., Walter, R. and Sierra, F. 2006. T-kininogen induces endothelial cell proliferation. *Mech Ageing Dev* 127:282-89.
7. **Gomez, C.R.**, Goral, J., Ramirez, L., Kopf, M. and Kovacs, E.J. Aberrant Acute Phase Response in Aged IL-6 KO mice. 2006. *Shock* 25:581-85.
8. Acuña-Castillo, C., Leiva-Salcedo, E., **Gomez, C.R.**, Pérez, V., Li, M., Torres, C., Walter, R., Murasko, D.M. and Sierra, F. T-kininogen: A biomarker of aging in Fisher 344 rats with possible implications on the immune response. 2006. *J Gerontol Biol Sci.* 61A:641-49.
9. Espinoza, I., **Gomez, C.R.**, Galindo, M. and Galanti, N. Developmental expression pattern of histone H4 gene associated to DNA synthesis in the endoparasitic platyhelminth *Mesocostoides corti*. 2007. *Gene*, 386 (1-2): 35-41.
10. **Gomez, C.R.**, Birjandi, S., Hirano, S., Cutro, B.T., Bala, H., Nomellini, V. and Kovacs, E.J., Advanced age exacerbates the pulmonary inflammatory response after lipopolysaccharide exposure. 2007. *Crit Care Med*, 35:246-51.
11. Boehmer, E.D., Meehan, M.J., Cutro, B.T., **Gomez, C.R.** and Kovacs, E.J. Aberrant TLR-mediated signal transduction in macrophages from aged mice. (ed., JD Schwarzmüller). 2006. The 6th International Cytokine Conferences. Bologna, Italy, Monduzzi Editore, pp 31-34.
12. **Gomez, C.R.**, Nomellini, V., Boehmer, E.D. and Kovacs, E.J. Signal transduction of the aging innate immune system. 2007. *Current Immunol Revs*, 3: 23-30.
13. **Gomez, C.R.**, Plackett, T.P., and Kovacs, E.J., Aging and Estrogen: Modulation of Inflammatory Responses after Injury. 2007. *Exp Gerontol*, 42:451-56.
14. Nomellini, V., **Gomez, C.R.**, and Kovacs E.J. Aging and impairment of innate immunity. 2008. *Contrib Microbiol*, 15:188-205.
15. Karavitis, J., Murdoch, E.L., **Gomez, C.R.**, Ramirez, L., and Kovacs, E.J. Acute ethanol exposure attenuates multiple macrophage functions. 2008. *J. Interf. Cytok. Res*, 28:413-22.
16. Bustos, F., Fernández, C., Duhalde, A., and **Gomez, C.R.**, Motivational profile of the students that enter and remain in the Universidad Diego Portales medical School. A retrospective study. 2008. *RECS*, 5: 33-40.
17. Nomellini, V., Faunce, D.E., **Gomez, C.R.**, and Kovacs, E.J. An age-associated increase in pulmonary inflammation after burn injury is abrogated by CXCR2 inhibition. 2008. *J. Leuk. Biol.* 83:1493-501.

18. **Gomez, C.R.**, Nomellini, V., Faunce, D.E., and Kovacs, E.J. Age and Innate immunity. 2008. *Exp Gerontol.* 43:718-728. **Note:** This article was one of the top 10 most cited papers in *Experimental Gerontology* during the period 2008 – 2010. Source www.scopus.com - September 2010.
19. Donoso, V., **Gomez, C.R.**, Orriantia, M.A., Coddou, C., Pérez, V., Torres, C., Nelson, P., Mónica Imarai, M., Huidobro-Toro, J.P., Felipe Sierra, F., Fernandez, R., Morales, B., Acuña-Castillo, C. The release of sympathetic neurotransmitters is impaired in aged Fisher 344 rats. A possible role in the changes in cytokine production. 2008. *Mech Ageing Dev.* 129: 728-734.
20. Nomellini, V., **Gomez, C.R.**, Gamelli, R.L., and Kovacs, E.J. Aging, and animal models of systemic insult: Trauma, burn, and sepsis. 2009. *Shock.* 31:11-20.
21. **Gomez, C.R.**, Acuña-Castillo, C., Pérez, C., Leiva-Salcedo, E., Ordenes, E., Oshima, K., Aravena, M., Pérez, V., Nishimura, S., Sabaj, V., Walter, R. and Sierra, F. Defective acute phase response during aging is associated with acute hepatic injury. 2009. *J. Gerontol.* 63:1299-306.
22. **Gomez, C.R.**, Nomellini, V., Baila, H., Oshima, K., and Kovacs, E.J. Comparison of the effects of aging and IL-6 on the hepatic inflammatory response in two models of systemic injury: scald injury versus i.p. LPS administration. 2009. *Shock.* 31:178-84.
23. **Gomez, C.R.**, Nomellini, V., and Kovacs, E.J. Part III-10: Immunosenescence and sex hormones, book chapter in: "Handbook on immunosenescence. Basic understanding and clinical applications". 2009. Edited by Tamas Fulop, Katsuku Hirokawa, Graham Pawelec, and Claudio Franceschi. Springer Netherlands. ISBN: 978-1-4020-9062-2. Pages: 799-831.
24. **Gomez, C.R.**, Karavitis, J., Palmer, J.L., Faunce, D.E., Ramirez, L., Nomellini, V., and Kovacs, E.J. Interleukin-6 contributes to age-related alteration of cytokine production by macrophages. 2010. *Mediators of Inflammation.* 2010:475139.
25. Salinas, D.G., Acevedo, C. and **Gomez, C.R.** Modeling a neural network for the learning of the structure-function relationship. 2010. *Adv Physiol Educ.* 34:158-61.
26. Nomellini, V., Mahbub, S., **Gomez, C.R.**, and Kovacs, E.J. Age-related defects in neutrophil chemotaxis and adhesion are markers of prolonged pulmonary inflammation in a murine model of injury. Submitted.

Manuscripts in preparation

1. **Gomez, C.R.**, Acuña-Castillo, C., Pérez, V., Nishimura, S., Sell, C., Kovacs, E.J., Sierra, F. and Walter, R. cDNA microarrays reveal a defect in the expression of chemokines during the acute phase response of aged F344 rats.
2. Nishimura, S., **Gomez, C.R.**, Acuña-Castillo, C., Pérez, V., Leiva-Salcedo, E., Pérez, C., Walter, R. and Sierra, F. Diminished MAPK activation in aged rat thioglycollate-elicited peritoneal macrophages in response to LPS.
3. Nishimura, S., Acuña-Castillo, C., **Gomez, C.R.**, Pérez, V., Leiva-Salcedo, E., Pérez, C., Manterola M., Sabaj, V., Walter, R. and Sierra, F. Exogenous T-kininogen mimics the effect of age on MAP kinase activation and TNF- α production by peritoneal macrophages.
4. **Gomez, C.R.**, Kovacs, E.J., Effects of aging and IL-6 on the oxidative stress homeostasis in aged mice.
5. **Gomez, C.R.**, Kovacs, E.J., Sierra, F., Aging, inflammation and acute stress: At a dangerous crossroad.
6. **Gomez, C.R.**, Knutson, G., Schreiber C.A., Vuk-Pavlovic S., Low oxygen tension as a tool for the development of better prostate cancer cell vaccines.
7. **Gomez, C.R.**, Knutson, G., Bulur, P., Schreiber, C., Vuk-Pavlovic S. Age-dependent mobilization of stem cells and progenitors by hyperbaric oxygen.

RESEARCH REPORTS AND ABSTRACTS

1. **Gomez, C.R.**, Walter, R. and Sierra F. 2001. Regulation of gene expression of cytokines during aging. Symposium, "Oxidative stress, cellular responses and modulator factors", XXIV annual meeting of the Chilean Society of Biochemistry and Molecular Biology. Chillán, Chile, p.23.
2. **Gomez, C.R.**, Walter, R. and Sierra, F. 2002. Expression of MIP-1 α during the acute phase response of aged rats. XVI annual meeting of the Chilean Society of Cellular Biology. Puerto Varas, Chile, p. 22.
3. **Gomez, C.R.**, Nishimura, N., Acuña-Castillo, C., Leiva-Salcedo, E., Rivas, M., Pérez, V., Sierra, F. and Walter, R. 2002. T-Kininogen (T-KG) affects the activation of macrophages in response to bacterial endotoxin. XLV annual meeting of the Chilean Society of Cellular Biology. Puyehue, Chile, R-121.
4. Acuña-Castillo, C., Aravena, M., Leiva-Salcedo, E., Pérez, V., **Gomez, C.R.**, Nishimura, S., Walter R. and Sierra, F. 2002. T-kininogen inhibits ConA-stimulated MAPKs activity in lymphocytes and macrophages. VI Latinoamerican Immunology Conference (ALAI). La Habana, Cuba.
5. Acuña-Castillo, C., Nishimura, S., **Gomez, C.R.**, Pérez, V., Sabaj, V., Aravena, M., Walter, R. and Sierra F. 2003. Relationship between differential MAPKs activation and cytokine induction in old rats. XVII Annual Meeting of the Society of Cellular Biology of Chile. Pucón, Chile. R-42.
6. Pérez, V., Velarde, V., Acuña-Castillo, C., **Gomez, C.R.**, Sabaj, V., Walter, R. and Sierra, F. 2003. The response to kinins is diminished in the aorta of old rats. XVII Annual Meeting of the Society of Cellular Biology of Chile. Chile. R-162.
7. Salinas, D.G., **Gomez, C.R.** and Montiel, J.F. 2004. Progresses of a theory-practical course for the integration on basic sciences. V workshop of Education in Health Sciences. Faculty of Medicine, University of Chile. Santiago, Chile.
8. **Gomez, C.R.**, Acuña-Castillo, C., Pérez, V., Nishimura, S., Sell, C., Kovacs, E.J., Quinn, D., Sierra, F. and Walter, R. 2004. Delay in the expression of MIP-1 α is related to a temporal defect in the acute phase response of aged F344 rats. Annual Meeting of the Society for Leukocyte Biology. Toronto, Canada.
9. **Gomez, C.R.**, Boehmer, E.D., Acuna-Castillo, C.A. and Kovacs, E.J. 2005. Serum from aged mice augments the production of TNF α by macrophages from young mice. Experimental Biology and XXXV International Congress of Physiological Sciences. San Diego, CA, USA.
10. **Gomez, C.R.**, Cutro, B.T., Fitzgerald, D.J., Goral, J., Ramirez, L. and Kovacs E.J. 2005. Aberrant Acute Phase Response in Aged IL-6 KO mice. 38th Annual Meeting of the Society for Leukocyte Biology. Oxford, England.
11. Kovacs, E.J., Cutro, B.T., Ramirez, L., Goral, J. and **Gomez, C.R.** 2005. Reduced pulmonary inflammation after injury in aged IL-6 deficient mice. 38th Annual Meeting of the Society for Leukocyte Biology. Oxford, England.
12. Nomellini, V., Ramirez, L., Cutro, B.T., **Gomez, C.R.** and Kovacs, E.J. 2005. Aberrant pulmonary pathology in aged mice may explain age-dependent differences in mortality after injury, St. Albert's day research presentations. Graduate School, Loyola University Medical Center. Maywood, IL, USA.
13. Hirano, S., Cutro, B.T., Ramirez, L., **Gomez, C.R.**, and Kovacs, E.J. 2005. Aging exacerbates pulmonary inflammation after lipopolysaccharide exposure. St Luke's Medical Research Day. Maywood, IL, USA.
14. **Gomez, C.R.**, Goral, J., Ramirez, L. and Kovacs, E.J. 2005. Acute Phase Response in Aged IL-6 knock out mice. 34th Annual Autumn Immunology Conference. Chicago, IL, USA.
15. Karavitis, J., **Gomez, C.R.**, Ramirez, L., Cutro, B.T., Faunce, D.E., Kovacs, E.J. 2005. Ovariectomy ablates the gender differences seen after ethanol plus burn injury in mice. 34th Annual Autumn Immunology Conference. Chicago, IL, USA.

16. Nomellini, V., Ramirez, L., Cutro, B.T., **Gomez, C.R.** and Kovacs, E.J. 2005. Aberrant pulmonary pathology in aged mice may explain age-dependent differences in mortality after injury. 34th Annual Autumn Immunology Conference. Chicago, IL, USA.
17. Karavitis, J., **Gomez, C.R.**, Ramirez, L., Cutro, B.T., Faunce, D.E. and Kovacs, E.J. 2005. Does estrogen mediate the gender differences in immunity after ethanol exposure and burn injury? Alcohol and Immunology Research Interest Group (AIRIG) Meeting. Loyola University Medical Center. Maywood, IL.
18. Kovacs, E.J., Plackett, T.P., Ramirez, L. and **Gomez, C.R.** 2005. Aging, traumatic injury, and immunity: improvement following estrogen treatment. Annual Meeting of Austrian Society for Allergy and Immunology, Gratz, Austria.
19. Kovacs, E.J., Plackett, T.P., Ramirez, L. and **Gomez, C.R.** 2006. Aging and the inflammatory response after traumatic injury. 29th Annual Conference on Shock. Broomfield, CO, USA.
20. Nomellini, V., Ramirez, L., Cutro, B.T., Faunce, D.E., **Gomez, C.R.** and Kovacs E.J. 2006. Aging affects acute lung injury after burn. 29th Annual Conference on Shock. Broomfield, CO, USA.
21. Karavitis, J., **Gomez, C.R.**, Faunce, D.E. and Kovacs E.J. 2006. Gonadal hormones mediate gender differences in immune function after ethanol plus burn injury. 29th Annual Conference on Shock. Broomfield, CO, USA.
22. Murdoch, E.L., Greiffenstein, P., **Gomez, C.R.**, Nomellini, V., Ramirez, L., Morgan, M.O. and Kovacs, E.J. 2006. Short-term pulmonary consequences after ethanol exposure and burn injury. 29th Annual Conference on Shock. Broomfield, CO, USA.
23. Kovacs, E.J., **Gomez, C.R.**, Meehan, M.J., and Boehmer, E.D. 2006. Aging causes defects in macrophage signaling that are limited to TLR-mediated pathways. Joint Meeting of the International Cytokine Society and the International Society for Interferon and Cytokine Research, Vienna, Austria.
24. Kovacs, E.J. Plackett, T.P., Boehmer, E.D., Nomellini, V., and **Gomez, C.R.** 2006. Aging, estrogen, and TLR-mediated macrophage activation after injury, Symposium on Ageing Research in Immunology: the Impact of Genomics. Paris, France.
25. **Gomez, C.R.**, Baila, H., Morgan, M.O., Oshima, K., Nomellini, V., and Kovacs E.J. 2006. Elevated hepatic inflammatory response in aged mice given LPS. Joint Society for Leukocyte Biology - International Endotoxin and Innate Immunity Society. San Antonio, TX, USA.
26. Nomellini, V., Faunce, D.E., **Gomez, C.R.**, and Kovacs E.J. 2006. Increased pulmonary inflammation in aged mice subjected to burn injury. Joint Society for Leukocyte Biology - International Endotoxin and Innate Immunity Society. San Antonio, TX, USA.
27. Kovacs, E.J., Plackett, T.P., Nomellini, V., Boehmer, E.D., and **Gomez, C.R.** 2006. Advanced age alters innate immune response after injury. Joint Society for Leukocyte Biology - International Endotoxin and Innate Immunity Society. San Antonio, TX, USA.
28. Nomellini, V., Faunce, D.E., **Gomez, C.R.**, and Kovacs E.J. 2006. Pulmonary chemokine levels after burn injury in aged mice. 35th Annual Autumn Immunology Conference. Chicago, IL, USA.
29. **Gomez, C.R.**, Nomellini, V., Baila, H., Oshima, K., Karavitis, J., Ramirez, L., Morgan, M.O., and Kovacs E.J. 2007. Reduced hepatic inflammatory response in aged interleukin-6 knock out mice given lipopolysaccharide. 30th Annual Conference on Shock. Baltimore, MA, USA.
30. Nomellini, V., **Gomez, C.R.**, and Kovacs E.J. 2007. Pulmonary neutrophil accumulation after burn injury is exacerbated with age. 30th Annual Conference on Shock. Baltimore, MA, USA.
31. Bustos, F., Fernández, C., Duhalde, A., **Gomez, C.R.** and Salinas, D.G. 2007. Motivational profile of the students that enter and remain in a Medical School, University Diego Portales: A retrospective study. International IV Convention of Medical Education. Medical School, Catholic University. Santiago, Chile.

32. **Gomez, C.R.**, Karavitis, J., Ramirez, L., Nomellini, V., and Kovacs E.J. 2007. Interleukin-6 contributes to age-related alteration of cytokine production in macrophages. 40th Annual Meeting of the Society for Leukocyte Biology. Cambridge, MA, USA.
33. Nomellini, V., **Gomez, C.R.**, and Kovacs E.J. 2007. Decreased tight junction formation in lungs of aged mice following injury. 40th Annual Meeting of the Society for Leukocyte Biology. Cambridge, MA, USA.
34. Karavitis, J., **Gomez, C.R.**, and Kovacs E.J. 2007. Fc γ -receptor mediated phagocytosis is attenuated after acute *in vivo* or *in vitro* ethanol exposure. 40th Annual Meeting of the Society for Leukocyte Biology. Cambridge, MA, USA.
35. Nomellini, V., **Gomez, C.R.**, and Kovacs, E.J. 2008. Increased pulmonary inflammation in aged mice after burn correlates with increased neutrophil chemokines. Annual meeting of the American Burn Association, Chicago, IL.
36. Kovacs, E.J., Faunce, D.E., **Gomez, C.R.**, and Nomellini, V. 2008. Advanced age is associated with increased pulmonary inflammation after burn injury and can be abrogated by treatment with anti-CXCR2 inhibition. Fifth International Conference on Innate Immunity, Aegean Conferences, Chania, Crete, Greece.
37. Kovacs, E.J., **Gomez C.R.**, and Nomellini, V. 2008. Aging and injury: aberrant inflammatory responses and therapeutic interventions. US-Japan Cooperative Medical Sciences Program. Immunosenescence Workshop, San Francisco, CA.
38. Nomellini, V., **Gomez, C.R.**, and Kovacs, E.J. 2008. Aberrant neutrophil sequestration in the lungs of aged mice after burn injury is paralleled by a decrease in the neutrophil chemokine receptor, CXCR2. 41th Annual Meeting of the Society for Leukocyte Biology. Denver, CO.
39. **Gomez, C.R.**, Knutson, G., G.J., Vuk-Pavlović, S. 2008. Oxygen-tension Dependent Proteome Modulation in Human Cancer Cells. Symposium: Hypoxia, Ischemia, and Inflammation: Essential Connections. Boston, MA.
40. **Gomez, C.R.**, Kosari, F., Schreiber, C.A., Knutson G.J., Vasmatzis, G., Vuk-Pavlović S. 2010. Hypoxic exposure as a novel strategy to identify tumor-associated antigens in prostate cancer. Tumor Immunology: Basic and Clinical Advances. Miami Beach, FL.
41. **Gomez, C.R.**, Knutson G.J., Schreiber, C.A., Kosari, F., Vasmatzis, G., Vuk-Pavlović S. 2011. Hypoxia affects gene expression and proteome of prostate cancer cells. 102th AACR Annual Meeting. Orlando, FL.
42. **Gomez, C.R.**, Knutson G.J., Schreiber, C.A., Vuk-Pavlović S. 2011. Age-dependent mobilization of mesenchymal stromal cells by hyperbaric oxygen. 17th International Society for Cellular Therapy Meeting. Rotterdam, The Netherlands.
43. **Gomez, C.R.**, Kosari, F., Schreiber, C.A., Knutson G.J., Vasmatzis, G., Vuk-Pavlović S. 2011. Hypoxic cell culture for more effective cancer vaccines. International Society for Applied Biological Sciences Conference on Forensic Genetics (ISABS). Bluesun hotel ELAPHUSA, Bol, Island Brač, Croatia.

INVITED LECTURES, SEMINARS, AND PRESENTATIONS

- "cDNA Microarrays Analysis Shows Differential Expression of a Subset of Genes, During the Hepatic Acute Phase Response in Aged Fisher 344 Rats". XLIII Annual Meeting of the Biology Society of Chile. Pucón, Chile, November 2000.
- "Hepatic Response to Inflammation during Aging". International Symposium Molecular and cellular basis of Aging. ICBM, Faculty of Medicine, University of Chile. Santiago, Chile, June 2001.
- "Technologies of information and communication in a course for integrated teaching of biology". V workshop of Education in Health Sciences. Faculty of Medicine, University of Chile. Santiago, Chile, May 2004.
- "Inflammatory responses during Aging: The Good, the Bad and the Ugly", Burn and Shock Trauma Institute, Loyola University Medical Center. Maywood, IL, October 2005.
- "Inflammatory responses during Aging: From the bench to the bedside", Veteran Affairs Center of Physical Rehabilitation. La Florida, Chile, November 2005.
- "Inflammatory responses during Aging", Veteran Affairs Medical Center, Las Condes, Chile. November 2005.
- "Advanced age exacerbates the pulmonary inflammatory response after lipopolysaccharide exposure", Twenty-Ninth Annual Conference on Shock. Broomfield, CO, USA, June 2006.
- "Inflammatory responses and Aging", Faculty of Health Sciences, Diego Portales Santiago, Chile. November 2007.
- "Inflammatory responses and Aging", Faculty of Health Sciences, Burn and Shock Trauma Institute, Loyola University Medical Center. Maywood, IL, March 2008.
- "Translational studies of hyperbaric oxygen effects at Mayo", Division of Preventive, Occupational, And Aerospace Medicine Monthly Research Seminars, Mayo Clinic, Rochester, MN. March 2010.
- "Effect of aging on hyperbaric oxygen-mediated mobilization of mesenchymal stem cell and progenitors (MSCs)", Division of Preventive, Occupational, And Aerospace Medicine Monthly Research Seminars, Mayo Clinic, Rochester, MN. January 2011.

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