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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 hypoxic 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 autotigens with potential value as biomarkers or therapeutic targets. Introduction of pO2 as a variable can constitute a tool for the development of more effective immunotherapy for CaP.

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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 (pO2) 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 pO2 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 pO2-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 pO2 were tested by state-of-the-art high throughput genom ics and proteomics techniques. This approach was designed to identify pO2–regulated tumor–associated pathways and macromolecules. To determine the antigenic potential of pO2–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 pO2–controlled conditions.

Prompted by the evidence that pO2 modulates the biological properties of CaP tumor cells, we initiated studies aimed at using pO2 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 TM PRSS2-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 pO2 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% O2 and 5% CO2 using controlled N2 and CO2 gas in take. Controls were placed in a standard cell culture incubator at 37 °C in a humidified atmosphere.
containing 21% O\textsubscript{2} and 5% CO\textsubscript{2}. Hypoxic LnCaP and VCaP cells (pO\textsubscript{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).

![Figure 1. Effect of hypoxia on proliferation and VEGF expression by CaP cells](image)

LnCaP cells (5,000 cells/cm\textsuperscript{2}), VCaP (10,000 cells/cm\textsuperscript{2}), and DU-145 (5,000 cells/cm\textsuperscript{2}), were cultured at pO\textsubscript{2}=2 kPa or 20 kPa in T-25 flasks; Figure 1A. Live cells were counted in triplicate flasks using trypan blue exclusion to differentiate dead cells; Figure 1B. Rate of VEGF secretion in hypoxia–grown cells was higher than in normoxia–grown cells.* p<0.05 relative to pO\textsubscript{2}=20 kPa; # p<0.05 relative to 2 days at the same pO\textsubscript{2} value.

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\textsubscript{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\textsubscript{2} impact on malignant progression [9-11]. We cultured LnCaP, VCaP, and DU-145 at pO\textsubscript{2}= 2 kPa or 20 kPa and lysed them when data showed pO\textsubscript{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\textsubscript{2}s, we used Ingenuity Pathway Analysis (IPA; Ingenuity Systems). Benjamin-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.

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<td>Immunological Disease</td>
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In collaboration with Drs. George Vasmatzis 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 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 \( (p<0.005) \) (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 \( \{\text{“the Warburg effect”;} \ [14]\} \) that led to the assumption of tum or 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 \( [15-18] \).
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).

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

| Symbol     | Name                                      | Acetyl-Coenzyme A carboxylase alpha | Cell division cycle-associated protein 3 | Cyclin B1 | Cyclin-dependent kinases regulatory subunit 2 | High affinity cationic amino acid transporter 1 | Hyaluronan-mediated motility receptor | Lysyl oxidase | Matrix metalloproteinase-10 | Mucolipin 2 (cation channel protein) | Neurolysin (metallopeptidase M3 family) | PDE5 | PDZ and LIM domain 6 (Scaffold protein) | PI3K and Sec7 domain containing 3 | Ral GTPase-activating protein subunit alpha-2 | Ral GTPase-activating protein subunit alpha-2 | Sidekick homolog 1, cell adhesion molecule | Stanniocalcin-2 (secreted) | Transcription factor SOX-4 | Transmembrane protein 200A | Tretinol factor 3 (intestinal, stable secretory protein) | Ubiquitin-conjugating enzyme E2 G5 | Ubiquitin-conjugating enzyme E2 G6 | Ubiquitin-conjugating enzyme E2 G7 | Ubiquitin-conjugating enzyme E2 G8 | Ubiquitin-conjugating enzyme E2 G9 |
|------------|-------------------------------------------|-------------------------------------|----------------------------------------|-----------|-----------------------------------------------|---------------------------------------------|---------------------------------------|----------------------------|------------------------------------------|-----------------------------------------------|---------------------------------|--------------------------------|-------------------------------------|---------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| ACACA      | Cyclin B1                                  | 1.0                                 | 0                                     | 1.3       | 0.001123                                      | 1.5                                         | 0                                     | 1.5                             | 0                                         | 1.3                             | 0.000043          | 1.2                             | 0.000001                           | 0.000444                      | 0.000332           | 0.000012                        | 0.000001                       | 0.000001          | 0.000012                        | 0.000001
| CDDC3      | Cyclin-dependent kinases regulatory subunit 2 | 1.1                                 | 0                                     | 1.5       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 0.000066                          | 0.000012 |
| CENP5      | Cyclin B1                                  | 1.8                                 | 0                                     | 1.3       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| CCNB1      | Cyclin B1                                  | 1.8                                 | 0                                     | 1.3       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| CKS2       | Cyclin-dependent kinases regulatory subunit 2 | 1.1                                 | 0                                     | 1.5       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| DLG7       | High-affinity cationic amino acid transporter 1 | 1.6                                 | 0                                     | 1.2       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| E2F5       | Hyaluronan-mediated motility receptor       | 2.1                                 | 0                                     | 1.7       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| HMMR       | Hyaluronan-mediated motility receptor       | 2.1                                 | 0                                     | 1.7       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| HIG2       | Lysyl oxidase                              | 1.1                                 | 0                                     | 1.5       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| HLOX       | Matrix metalloproteinase-10                | 1.2                                 | 0                                     | 1.4       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| MUC2       | Mucolipin 2 (cation channel protein)       | 1.1                                 | 0                                     | 1.4       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| NEMO       | Neurolysin (metallopeptidase M3 family)    | 1.2                                 | 0                                     | 1.3       | 0.000013                                      | 1.3                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| PDZ5       | PDZ and LIM domain 6 (Scaffold protein)    | 1.1                                 | 0                                     | 1.2       | 0.000013                                      | 1.2                                         | 0                                     | 1.3                             | 0.000023                                  | 1.3                             | 0.000012 |
| PDZ3       | PDK1                                      | 1.5                                 | 0                                     | 1.2       | 0.000044                                      | 1.5                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| C200774    | Ral GTPase-activating protein subunit alpha-2 | 1.3                                 | 0                                     | 1.2       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| FAM80A     | Ral GTPase-activating protein subunit alpha-2 | 1.3                                 | 0                                     | 1.2       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| SDK1       | Sidekick homolog 1, cell adhesion molecule | 1.5                                 | 0                                     | 1.6       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| STC2       | Stanniocalcin-2 (secreted)                 | 1.2                                 | 0                                     | 1.3       | 0.000059                                      | 1.3                                         | 0                                     | 1.3                             | 0.000012                                  | 1.3                             | 0.000012 |
| SOX4       | Transcription factor SOX-4                 | 1.2                                 | 0                                     | 1.2       | 0.000044                                      | 1.2                                         | 0                                     | 1.2                             | 0.000023                                  | 1.3                             | 0.000012 |
| TME200A    | Transmembrane protein 200A                 | 1.3                                 | 0                                     | 1.3       | 0.000086                                      | 1.3                                         | 0                                     | 1.3                             | 0.0001276                                  | 1.3                             | 0.000012 |
| TFPI       | Tretinol factor 3 (intestinal, stable secretory protein) | 1.2 | 0 | 1.2 | 0.0000126 |
| UBE2C      | Ubiquitin-conjugating enzyme E2 G5         | 1.4                                 | 0                                     | 1.3       | 0.000079                                      | 1.3                                         | 0                                     | 1.3                             | 0.000023                                  | 1.3                             | 0.000012 |
| UBE2E3     | Ubiquitin-conjugating enzyme E2 G6         | 1.8                                 | 0                                     | 1.3       | 0.000079                                      | 1.3                                         | 0                                     | 1.3                             | 0.000023                                  | 1.3                             | 0.000012 |

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 2α (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 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 of 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**

*4A*

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 \( \mu g \) 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).

*4B*

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

$PO_2 = 2kPa$

CaP

Healthy control

$PO_2 = 20kPa$

CaP

Healthy control

Thirty $\mu$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.
VCaP cells were cultured at \( pO_2 = 2 \) kPa or \( pO_2 = 20 \) kPa for 7 days and cell lysates were prepared. Thirty \( \mu \)g protein were loaded on pH 3-10 NL IPG strips for isoelectric focusing. Second dimension: 10.5-14 % SDS-PAGE gel. One set of gels was silver stained and other set was transferred to nitrocellulose membranes, incubated with pooled plasma (1:300) from newly diagnosed CaP patients (n=5; total of 4 pools), age matched non-cancerous controls (n=5; total of 4 pools), patients with other cancers [colon (n=10); lung (n=10)] and autoimmune disease [rheumatoid arthritis (n=20)]. Following incubation with chicken anti-human IgG-HRP, spots were identified by chemiluminescence. The arrowhead indicates protein spot 6.

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

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

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 an 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


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 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 SPORE Clinical Core and Chair, of Mayo Clinic Dept. of Urology Radical Prostatectomy Registry; Farhad Kosari, Ph.D., College of Medicine; and George Vamatzis, 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 \( \text{p}O_2 \) as a variable can constitute a tool for the development of more effective immunotherapy for CaP.

So what: The role of \( \text{p}O_2 \) 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.
References


Appendices

An AACR Special Conference

TUMOR IMMUNOLOGY: BASIC AND CLINICAL ADVANCES
Presented in conjunction with the Cancer Immunology Working Group of the AACR

November 30-December 3, 2010
Renaissance Eden Roc Beach Resort & Spa
Miami Beach, FL

Conference Co-Chairpersons:
Olivera J. Finn
University of Pittsburgh School of Medicine,
Pittsburgh, PA

Dmitry I. Gabrilovich
H. Lee Moffitt Cancer Center and Research
Institute, Tampa, FL

Elizabeth M. Jaffee
Johns Hopkins Kimmel Comprehensive
Cancer Center, Baltimore, MD

Program and Proceedings

American Association for Cancer Research
www.aacr.org/meetingcalendar
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.


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<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 (pO2=2 kPa) and compared them with normoxically grown cells (pO2=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 tumor cells in situ than are cells grown in normoxia and will further identify and validate selected candidate TAAs in CaP patients.

Support: DOD PC094880 (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).
Presentation Abstract

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

Abstract: Hypoxia is a hallmark of many cancers and is thought to contribute to tumor progression and resistance to therapy. In this study, we investigated the effects of hypoxia on gene expression and proteome of prostate cancer cells (CaP).

Methods: We used quantitative proteomics and transcriptomics to compare the gene expression and proteome of CaP cells cultured in normoxia (21% O2) and hypoxia (1% O2). The proteome was measured using a mass spectrometry approach and the transcriptome was measured using RNA sequencing.

Results: We found that hypoxia induced a significant increase in the expression of certain genes, including those involved in angiogenesis and invasion. We also observed changes in the expression of several proteins, including those involved in the extracellular matrix and cell adhesion.

Conclusions: These findings suggest that hypoxia can alter the gene expression and proteome of CaP cells, potentially contributing to their aggressive behavior and resistance to therapy. Future studies are needed to further validate these findings and to explore the potential targets for therapeutic intervention.
HYPOXIC CELL CULTURE FOR MORE EFFECTIVE CANCER VACCINES
Christian R. Gomez, Farhad Kosari, Claire A. Schreiber, Gaylord J. Knutson, George Vasmatzis, 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 ($p\leq0.02$). 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 SPORE 5P50CA091956 (FK); Mrs. Adelyn L. Luther, Singer Island, Florida and Mayo Clinic Cancer Center (SV-P).
CURRICULUM VITAE

Name: Christian René Gomez Basaure Ph.D.

Present Position:
- Assistant Professor of Biochemistry/Molecular Biology, Mayo Clinic College of Medicine
- Assistant Professor, Division of Preventive, Occupational and Aerospace Medicine (Pending)
- Research Associate, Stem Cell Laboratory, Department of Oncology, Mayo Clinic Cancer Center
- 200 First Street SW, Rochester, MN 55905

Education
- 1988 - 1995 B.S. and M.S. in Biochemistry, School of Chemical and Pharmaceutical Sciences, University of Chile
- 1997-2003 Ph.D. Biomedical Sciences, University of Chile, Faculty of Medicine Date of Ph.D. completion January 2004

Research Training
- 1992 - 1994 Undergraduate thesis: Involvement of the Sodium/ATPase pump in chronic renal failure, Advisor: Dr. Miriam Alvo, Department of Physiology, University of Chile School of Medicine, Santiago, Chile
- 1995 - 1997 Research assistant: Glucocorticoid receptors in the development of Rheumatoid Arthritis: Development of a rat model, Advisor: Dr. Annelise Goecke, Department of Physiology, University of Chile School of Medicine, Santiago, Chile
- 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

**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-1α, 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

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

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), Mentions 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
Tittle: 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 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.

Completed:

Tittle: 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

Tittle: 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 (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


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.


Manuscripts in preparation


4. **Gomez, C.R.,** Kovacs, E.J., Effects of aging and IL-6 on the oxidative stress homestasis in aged mice.


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.


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.


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.


INVITED LECTURES, SEMINARS, AND PRESENTATIONS

"cDNA Microarrays Analysis Shows Differential Expression of a Subset of Genes, During the Hepatic Acute Phase Response in Aged Fish er 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.


"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.


“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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