

Cell Stress and Biophysical Therapies

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Overview

Cells respond to environmental or physiological stresses through a multitude of highly conserved mechanisms that can be protective or pathological. These stresses can be chemical, physical or biological in nature, and include oxidative stress and heat shock. The unifying theme of the Program is the exploitation of oxidative and thermal stress as target mechanisms for therapies. The broad goals are to: (a) understand the molecular and cellular basis for these mechanisms and identify new targets for treatment and diagnosis; (b) develop novel cancer treatment strategies based on cell stress mechanisms and biophysical principles, especially photodynamic action, heat and radiation; and (c) bring these to clinical evaluation by facilitating collaborations among clinicians and scientists. The Program is highly multidisciplinary; Program members have expertise in molecular biology of oxidative stress and tumor hypoxia, thermal stress proteins, mitochondrial dysfunction, DNA replication stress, radiation/oxidative stress DNA damage, photodynamic reactions and therapy, anti-tumor vaccines, nanotechnology, drug discovery, and functional gene target discovery.

Themes

The Program is focused on developing novel strategies for cancer therapy based on cell stress mechanisms and biophysical principles. The hypothesis driving research in the program is that cell stress elicits responses in the cell/organism that can be exploited for cancer therapy or diagnosis.

The program has three themes:

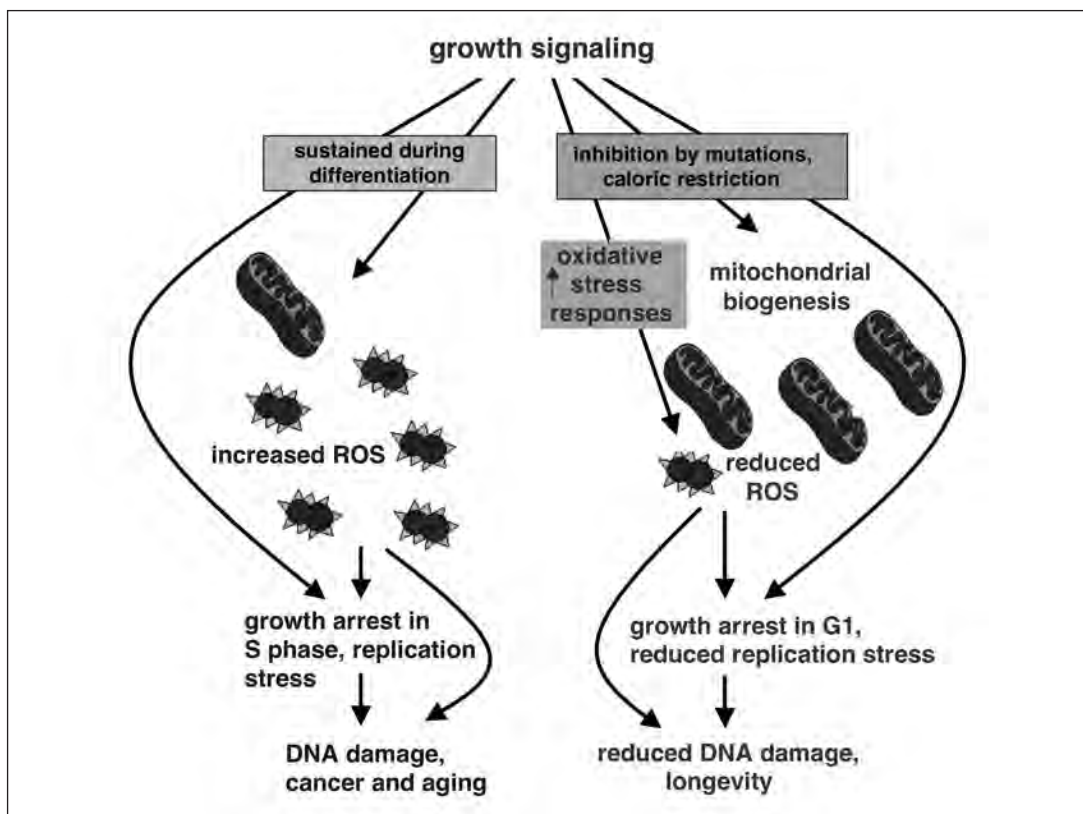
1. Molecular mechanisms of cell stress and identification of new targets
2. Cell stress mechanisms and host responses
3. Rational treatment design and optimization

Themes

Theme 1: MOLECULAR MECHANISMS OF CELL STRESS AND IDENTIFICATION OF NEW TARGETS

William Burhans, PhD, focuses his work on relating DNA replication stress to cancer and aging (R01 CA84086, CA81326). Replication stress arises in preneoplastic cells downstream of the activation of oncogenic growth-signaling pathways. The Burhans laboratory continues to investigate the important question of how replication stress arises at early stages of cancer using the genetically tractable model organism budding yeast (*S. cerevisiae*). Normally, nutrient depletion of budding yeast cells downregulates growth signaling pathways, which leads to a cell cycle arrest in G1. This duplicates the quiescent state in mammalian cells induced by the downregulation of homologous growth-signaling pathways during differentiation. In collaboration with Joel Huberman, PhD and Ping Liang, PhD (both GN), as well as Dr Keshav Singh, the Burhans laboratory has determined that sustained activation of growth-signaling pathways during nutrient depletion of budding yeast cells increases levels of reactive oxygen species (ROS), which inhibit growth

arrest in G1. Cells arrest growth in S phase instead of G1, where they undergo replication stress and age-dependent genome instability. In contrast, caloric restriction or mutational inactivation of growth signaling pathways leads to a more efficient G1 arrest during nutrient depletion, which protects against replication stress and genome instability. The tighter G1 arrest produced under these conditions is due in part to a reduction in ROS. These findings point to highly conserved inhibitory effects of ROS on G1 arrest leading to replication stress as an important factor in the etiology of cancer and in aging of all eukaryotes. (Marchetti *et al.*, *J Cell Sci* 2006; 119:124-131; Trabold *et al.*, *J Biol Chem* 2005; 280:12413-12421; Ramachadran *et al.*, *FEMS Yeast Res* 2006; 6:763-776; Burhans *et al.*, *BMC Evol. Biol.* 2006; 6:58; Burhans and Weinberger, *Mol Cell* 2007; 25:1-3; Weinberger *et al.*, *PLoS ONE* 2007, 2(8): e748 doi:10.1371/journal.pone.0000748; Burhans and Weinberger, *Nuc. Acids Res.* 2007 35: 7545-7556; Madia *et al.*, *J. Cell Biol.* 2008 180: 67-81.)



DNA Replication Stress, Cancer and Aging. Sustained growth signaling leads to an increase in ROS, which inhibits G1 arrest, causing cells to arrest growth in S phase instead. Cells growth-arrested in S phase undergo replication stress, which leads to DNA damage and genome instability. Inhibition of growth signaling by mutations or caloric restriction (right) reduces ROS – in some cases by stimulating mitochondrial biogenesis and more efficient respiration – thus potentiating growth arrest in G1, which protects against replication stress.

Theme 2: CELL STRESS MECHANISMS AND HOST RESPONSES

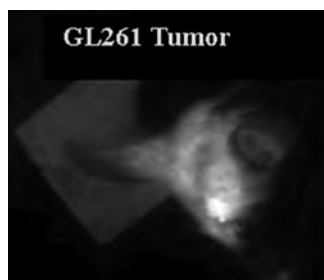
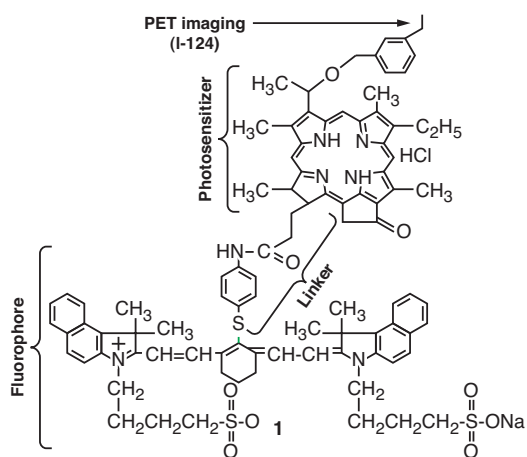
Cell stress associated with biophysical therapies such as photodynamic therapy (PDT) and thermal therapy induces complex host responses that involve the tumor microvasculature as well as innate and adaptive immunity. Efforts led by Drs. Henderson, Oseroff and Gollnick, in collaboration with Baumann (TII) and Cheney (GN) have revealed that PDT regimens can be rationally designed to achieve specific vascular and inflammatory tumor responses (Henderson *et al.*, *Cancer Res* 2004; 64:2120-26), which in turn generate different levels of stimulation of the adaptive anti-tumor immune response (Kabingu *et al.*, *Brit J Cancer* 2007, *in press*). These insights will likely change the way PDT is delivered clinically. While the post-PDT tumor milieu is highly inflammatory, these investigators discovered that PDT renders cells transiently refractory to inflammatory cytokines via loss of cytokine receptors and crosslinking of STAT-3 signaling molecules (Wong *et al.*, *Cancer Res* 2003; 63:3812-18; Liu *et al.*, *Cancer Res* 2004; 64:6579-87; Henderson *et al.*, *Cl Cancer Res* 2007; *in press*).

The laboratory of Dr. Subjeck, in collaboration with Drs. Wang (PR), Repasky (TII), and Evans (TII) continues to be focused on mammalian stress proteins, i.e. heat shock proteins

(HSPs) and glucose-regulated proteins (GRPs), and their capacity to protect cells against environmental stress and interact with the immune response. Clinical trials have been reported using stress proteins that are biochemically purified from a patient's tumor specimen after surgery. These trials have encountered difficulties, in part due to the complexities associated with the usually limited quantities of tumor material available for protein purification. The approach employed by the Subjeck laboratory uses recombinant tumor proteins antigens (e.g. melanoma antigens gp100 and Trp2) which are complexed to recombinant hsp110 or grp170 using the strong molecular (chaperoning) functions of these stress proteins (Park *et al.*, *Cancer Res* 2006; 66:1161-1168; Facciponte *et al.*, *Immunol Invest* 2005; 34:325-342; Facciponte *et al.*, *Cancer Immunol Immunother* 2006; 55:339-346; Wang *et al.*, *J Immunol* 2006; 3:1543-1551). These molecular studies form the basis for an awarded RAID grant (to Dr. Kane) from the NCI to prepare a hsp110/gp100/Trp2 vaccine for the treatment of melanoma. The preparation of clinical grade recombinant vaccine at the NCI is at an advanced stage and a clinical trial may be expected to commence by 2008.

Theme 3: RATIONAL TREATMENT DESIGN AND OPTIMIZATION

The broad drug development program pursued by the PDT Center group, spearheaded by Dr. Pandey, and including Drs. Henderson, Bellnier, and Gollnick continues to address questions of photosensitizer optimization. Nine Phase I and II clinical trials involving the Departments of Medicine and Surgery have been completed or are ongoing with the second-generation photosensitizer HPPH, including studies in lung cancer, esophageal cancer, and high grade dysplasia associated with Barrett's esophagus, and head and neck cancer. This agent lacks the drawback of general skin photosensitization associated with Photofrin, the only FDA approved PDT drug for oncologic use (Bellnier *et al.*, *Cancer Res* 2003; 63:1806-13; Bellnier *et al.*, *Cancer Chemother Pharmacol* 2006; 57:40-45). Joint studies with other institutions using this drug are being initiated and licensing negotiations are ongoing. NIH Roadmap initiatives are addressed with the development of multi-functional agents that combine therapeutic capabilities with MR, PET, SPECT and optical imaging (Gryshuk *et al.*, *J Med Chem* 2006; 49:1874-1881; Rosenfeld *et al.*, *Photochem Photobiol* 2006; 82:626-634). Funding for these activities is provided by RO1 and R21/R33 mechanisms.



Multifunctional Agent for Tumor Imaging (Fluorescence/PET) and Photodynamic Therapy (PDT). Fluorescence images of C57BL/6 mouse bearing GL261 tumors in the brain with conjugate 1 (Ex: 780nm, Em: 865nm). The images were taken at 24h post injection. Drug dose: 0.3 μ mol/kg.

Selected Scientific Accomplishments

David A. Bellnier, PhD

Assistant Professor, Cell Stress Biology

The research focus of David A. Bellnier, PhD, is the improvement of PDT through combination therapies. Dr. Bellnier, in collaboration with Drs. Gollnick, Oseroff, Cheney (GN) and Mazurchuk (MTET), is continuing to develop a combination therapy approach that uses the small molecule biological response modifier 5,6-dimethylxanthenone-4-acetic acid [DMXAA] combined with Photofrin-based PDT and PDT using the pro-drug delta-amino levulinic acid (ALA-PDT) in several experimental murine tumors. These experiments were designed to expand an earlier observation of these investigators that the cytokine TNF- α enhances the antitumor activity of porphyrin-based PDT without a concomitant increase in normal tissue toxicity, i.e., there is a positive therapeutic gain using low doses of each monotherapy in combination. This work has led to the successful 5-year competitive renewal of an NIH RO1 (CA89656) grant. The most recent significant progress was the observation that DMXAA has activity against orthotopic head and neck tumor xenografts, e.g. FaDu implanted in the oral cavity. Work has now begun on the combination of PDT and DMXAA in this model, where the aim is to improve selectivity; selectivity is a critical issue since PDT has been shown to be effective against head and neck tumors but in some cases treatment-related toxicities may limit its use (Bellnier *et al.*, *Cancer Res* 2003; 63:18 06; Seshadri *et al.*, *CI Cancer Res* 2005; 11:4241; Seshadri *et al.*, *Neoplasia* 2006; 8:534). Dr. Bellnier, collaborating with Drs. Oseroff and Pandey, also continues to be highly active in the translational and clinical aspects of PDT and its drug development arm, defining important pharmacologic and toxicity parameters (Bellnier *et al.*, *Cancer Res* 2003; 63:7884; *Cancer Chemother Pharmacol* 2006; 57:40).

William Burhans, PhD

Associate Professor, Cell Stress Biology

In collaboration with Walter Lono's laboratory (University of Southern California), the Burhans' laboratory recently determined that the more efficient G1 arrest induced by inactivation of growth-signaling pathways suppresses age-dependent genome instability in cells that also harbor defects in proteins required for maintenance of genome integrity during DNA replication. Together, these findings predict that oncogenic activation of growth signaling pathways during differentiation of mammalian cells leads to replication stress, genome instability and neoplasia by allowing entry into S phase under suboptimal conditions for replicating DNA. To test this hypothesis, the Burhans laboratory contributed to the development by Steven Pruitt, PhD (GN), of a mouse in which cells expressing a protein required for entry into S phase have been "marked" by the expression of EGFP. Photomicrographs from an article describing this mouse were featured on the cover of a recent issue of the journal *Stem Cell* (Maslov *et al.*, *Stem Cell* 2007; 25:132-138).

Sandra Gollnick, PhD

Associate Professor, Cell Stress Biology

Currently Dr. Gollnick focuses on the identification of PDT generated danger signals, in particular danger-associated molecular patterns (DAMPs) and the NLR family of receptors that recognize endogenous danger signals (CA098156). One of these receptors, NALP3, is triggered by DAMPs, resulting in the formation of the NALP3 inflammasome that activates caspase-1, leading to the release of IL-1 β . IL-1 β augments inflammation through induction of IL-6 and tumor necrosis factor (TNF)- α , promoting DC activation and induction of immunity. Dr. Gollnick has now discovered that incubation of PDT treated tumor cell lysates with macrophages or dendritic cells results in increased NALP3 expression and release of IL-1 β .

Andrei Gudkov, PhD, DSc*Garman Family Chair in Cell Stress Biology**Associate Director for Basic Science*

Dr. Andrei Gudkov's lab has established a functional relationship between p53 and NF- κ B and demonstrated significant enhancement of NF- κ B function in the absence of p53 both in cell culture and in animal model in vivo (Komarova *et al.*, FASEB J 2005; 19:1030-1032). Furthermore, constitutively active NF- κ B, one of the most universal properties of the majority of tumor cell types, was found to be one of the most frequent mechanisms suppressing wild type p53 function in tumors (Gurova *et al.*, PNAS 2005; 102:17448-17453). This critically important finding not only opens a novel drug discovery opportunity – creating drugs that simultaneously target both pathways in desirable directions – but also led to a program of identification of specific cellular mechanisms of p53-NF κ B interaction. This work is anticipated to reveal new treatment targets. Several important potential targets have been identified, including a candidate tumor suppressor BTG2 (Boiko *et al.*, Genes and Dev 2006; 20:236-252) that is a subject of regulation by both pathways, and TLE2, a candidate oncogene playing a role in NF- κ B dependent p53 functional repression in RCC and other cancers. Both factors were discovered using advanced functional genomic methodologies, including negative selection of complex shRNA libraries.

Dr. Gudkov's group provided new insights in the mechanisms of p53 pathway function, some of which may create important links with the ongoing projects within the Program. Thus, p53 has been defined as a natural thermosensitive protein, the activity and stability of which vary strongly within a physiological range of temperatures (Singhi *et al.*, PNAS 2004; 101:9327-9332). Moreover, p53 growth inhibitory function was found to be under strong positive control of a heat shock protein CHIP (Singhi *et al.*, PNAS 2004; 101:9327-9332). Both findings connect p53 with thermal stress and provide potentially important mechanistic links between anticancer and immunomodulating properties of thermal therapy and will be explored. New

insight into the molecular mechanisms of mitochondria-mediated apoptosis were made in a recent paper from Dr. Gudkov's lab (Strom *et al.*, Nature Chem Biol 2006; 2:474-479) describing identification of a small molecule named PFT μ that inhibits radiation-induced apoptosis by preventing p53 binding to mitochondria. PFT μ and a previously isolated p53 inhibitor, PFT α , may serve as important experimental tools for discovery of additional components of the p53 pathway. Dr. Gudkov's studies resulted to a significant revision of the view of p53 in normal cells and in tumors (Stark and Gudkov, Hum Mol Genet 1999; 8:1925-1938). It was found that in many instances p53 can play the role of a survival factor – the conclusion that led to novel therapeutic opportunities exploring p53 as a molecular target. Dr. Gudkov's studies formed a strong methodological basis for a new avenue for integrated target and drug discovery and revealed several potential anticancer treatment targets and new candidate drugs, each targeting major stress response pathways. Each is a potential agent which may complement existing biophysical therapies.

Barbara W. Henderson, PhD*Professor, Cell Stress Biology***Allan R. Oseroff, MD, PhD***Lawrence P. and Joan Castellani Family Chair in Dermatology*

Oxidative stress induced by photodynamic treatment (PDT) of cells has long been known to induce protein crosslinking. Barbara W. Henderson, PhD and Allan R. Oseroff, MD, PhD, in collaboration with Heinz Baumann, PhD (TII), found specific covalent crosslinking of STAT3 to be highly PDT dose dependent. It was hypothesized that STAT3 crosslinking can serve as a molecular marker for the PDT reaction and a surrogate PDT dosimeter. Extensive preclinical studies as well as preliminary clinical data support this hypothesis. This discovery led to the successful application of an R21 Quick Trial grant (CA119535) extending these studies to a Phase II clinical trial of PDT of obstructive lung cancer utilizing the RPCI-developed novel photosensitizer HPPH (Liu *et al.*, *Cancer Res* 2004; 64:6579-6587; Henderson *et al.*, *CI Cancer Res* 2007; *in press*). Dr. Oseroff recently reported that certain second generation photosensitizers, such as the RPCI-developed compound HPPH, are effluxed from cells via the ATP-binding cassette protein ABCG2 (breast cancer resistance protein). ABCG2 is expressed on some tumors and importantly, on cancer stem cells. In collaboration with Maria Baer, MD (MTET), he made the further discovery that tyrosine kinase inhibitors, in particular imatinib mesylate (Gleevec®), can inhibit this efflux and enhance photosensitizer accumulation and treatment effectiveness, opening the possibility of effective combination approaches. Together with Ravindra K. Pandey, PhD, these investigators further identified photosensitizer structures that are not substrate for the ABCG2 pump, thereby explaining their improved PDT efficacy and providing a basis for the design of new agents (Liu *et al.*, *CI Cancer Res* 2007; *in press*).

Under a PO1 grant (CA055791, PDT: Mechanisms and Strategies for Optimization, Dr. Oseroff, PI) currently in its 14th year, the discovery, development and translation of novel agents for PDT continues the distinguished history of the RPCI PDT Center that started with the development of Photofrin (currently the only FDA approved photosensitizer for oncologic purposes) and includes the ongoing clinical effort for the use of the second generation photosensitizer HPPH. Negotiations are currently ongoing to license the rights to HPPH and to advance it to Phase III clinical trials. The multidisciplinary interactions among the PDT scientists continue to be essential

for this effort. In addition to developing improved photosensitizers, novel approaches towards tumor imaging are pursued. Imaging techniques are increasingly becoming an important component of early cancer detection as well as in directing surgery and other cancer treatments. Such a treatment is photodynamic therapy where the visualization of the cancer can guide the placement of optical fibers for the delivery of therapeutic light. Novel agents for such “see and treat” approaches are being developed by Dr. Ravindra Pandey, in collaboration with Drs. Henderson, Oseroff, Bellnier, and Dougherty, (Emeritus) and the entire photodynamic therapy group within the Program.

Mohamed Khan, MD, PhD and Lajos Balogh, PhD*Associate Professors, Radiation Medicine*

Mohamed Khan, MD, PhD and Lajos Balogh, PhD, are focusing on the development of novel agents for radiation therapy, especially the use of nanodevices to solve specific clinical problems in cancer. Their research direction currently involves the development and characterization, *in vitro* analysis, and pre-clinical development of composite nanodevices for the improvement of tumor imaging and cancer treatment. Much of the work focuses on the angiogenic microvasculature, with nanodevices being developed to move through this leaky tumor microvasculature (and into tumors) and other nanodevices directly targeted at specific receptors present within the angiogenic tumor microvasculature. They have now completed synthesis and rigorous characterization of the first of a group of nanodevices targeted directly at the angiogenic tumor microvasculature. They have demonstrated *in vitro* that the composite nanodevices bind to the alpha-v-beta-3 target specifically using plate binding assays, and also bind to proliferating (alpha-v-beta-3 positive) human endothelial cells. The first set of detailed biodistribution studies in tumor model systems is now underway with the angiogenically targeted nanodevices. They have also completed further *in vivo* testing and analysis of composite nanodevices of differing size and surface charge, examining whether these can be sent through the leaky tumor microvasculature and into tumors. An unexpected result of this work is the fact that small changes in composite nanodevice surface charge, size, or other properties (perhaps “rigidity”) can greatly affect their biodistribution in mouse tumor model systems, producing what appears like organ selective targeting. This has ramifications for all nanodevice development internationally and suggests novel uses of the current nanodevices.

They have teamed with the pharmacokinetics-pharmacodynamics (PK/PD) Resource directed by Lakshmi Pendyala, PhD (MTET) and are currently developing the first mathematical models to describe nanodevice biodistribution. They have also now developed and conducted detailed *in vitro* and *in vivo* toxicity studies as well as with Dr. Baumann (TII) studies of inflammatory responses these new agents might elicit. Their efforts will attempt to design a multifunctional angiogenically targeted composite nanodevice platform that can be used for multi-level imaging (SPECT, Gamma detector, TEM) examining molecular targeting from the whole animal to intra-tumoral to intracellular levels with the same platform (RO1 CA10447), and then to have these angiogenically targeted composite nanodevices deliver radiation therapy prostate cancers (DOD PC061019).

The Khan laboratory has also demonstrated the use of a novel multi-hit anti-angiogenic agent, tetrathiomolybdate (TM) with radiation therapy in a head and neck cancer mouse model, and they are conducting an interprogrammatic R21 (CA096314) funded clinical trial with Dr. Nithya Ramnath (MTET) and others with this novel agent in non-small cell lung cancer patients. This study will test the safety of the combination of this agent with radiation therapy and look for novel biomarker and imaging correlates of the anti-angiogenic action of the combination therapy. These studies provide a platform on which a number of other clinical and translational studies will be developed examining new approaches in the use of ionizing radiation and new agents targeting vasculature, vaccines and radioprotectors and radiation potentiators. (Khan *et al.*, Arch Otolaryngol Head Neck Surg 2006; 32:333; Mamou *et al.*, Anticancer Res 2006; 26(3A):1753-1758).

Keshav Singh, PhD

Professor, Cancer Genetics

Keshav Singh, PhD, focuses on discovering avenues of intergenomic crosstalk between mitochondria and the nucleus, and the functional consequences of such crosstalk. All tumors examined to date contain mutations in mitochondrial DNA (mtDNA). In addition, depletion of mtDNA is reported in a variety of tumors. Mitochondrial dysfunction resulting from changes in mtDNA invokes mitochondria-to-nucleus retrograde response in human cells. Using cell lines where mitochondrial DNA has been depleted, parental control cells and hybrid cells where mitochondrial DNA has been restored, Dr. Singh in collaboration with Dr. Kazim found marked changes in the cellular proteome and identified several proteins involved

in the retrograde response. To determine a potential role for identified retrograde-responsive proteins in tumorigenesis, they analyzed the expression of UQCRC1 gene (encoding ubiquinol cytochrome-c reductase core protein I) in breast and ovarian tumors. They found that: (1) UQCRC1 was highly expressed in breast (74%) and ovarian tumors (34%); and (2) the expression positively correlated with cytochrome c-oxidase (COXII) encoded by mtDNA. These studies in collaboration with Ivan Still, PhD and Sei-Ichi Matsui, PhD (both GN) open an avenue for discovery of retrograde proteins as potential tumor suppressors or oncogenes involved in carcinogenesis (Singh *et al.*, Gene 2005; 354:140-146; Kulawiec *et al.*, Cancer Biol Ther 2006; 5:967-975).

Dr. Singh is also co-investigator on a joint grant with Lionel J. Coignet, PhD (GN), which explores the loss of a new mitochondrial protein gene, FLJ13639, in acute leukemia and whether restoration of its expression might offer a new therapeutic approach (CA116430). Dr. Singh's studies using mitochondrial gene knockout cells from a variety of tissue types also demonstrate that loss of mitochondrial function leads to cell cycle arrest, cellular senescence, and tumorigenic phenotype. In light of these and earlier studies they hypothesize the existence of a mitochondria damage checkpoint (mitocheckpoint) in human cells (Napolitano and Singh, Mitochondrion 2004; 4:755-762; Singh, FEMS Yeast Res 2004; 2:127-132; Singh, Ann NY Acad Sci 2006; 1067:182-190). Dr. Singh also utilizes yeast genetics to discover new pathways for mitochondrial DNA mutagenesis. The MtArg8 reversion assay, which measures point mutation in mtDNA, indicates that in budding yeast *Saccharomyces cerevisiae*, DNA polymerase zeta and Rev1 proteins participate in the mitochondrial DNA mutagenesis. Supporting this evidence, both polymerase zeta and Rev1p were found to be localized in the mitochondria. This is the first report demonstrating that the DNA polymerase zeta and Rev1 proteins function in the mitochondria (Zhang *et al.*, Genetics 2006; 172:2683-2688).

John Subject, PhD

Professor, Cell Stress Biology

Currently in the 4th year of an NCI-funded program project grant entitled "Heat Shock Proteins and Thermal Therapy of Cancer," research in the laboratory of John Subject, PhD continues to be focused on mammalian stress proteins, i.e. heat shock proteins (HSPs) and glucose-regulated proteins (GRPs), and their capacity to protect cells against environmental stress and interact with the immune response. This group continues to work on the "large" stress proteins called hsp110 and grp170 that it initially cloned and characterized several years ago. Others have carried out clinical trials using stress proteins that are biochemically purified from a patient's tumor specimen after surgery. These trials have encountered difficulties, in part due to the complexities associated with the usually limited quantities of tumor material available for protein purification. The approach employed by the Subject laboratory uses recombinant tumor protein antigens (e.g. melanoma antigens gp100 and Trp2) which are complexed to recombinant hsp110 or grp170 using the strong molecular (chaperoning) functions of these stress proteins (Park *et al.*, *Cancer Res* 2006; 66:1161-1168; Facciponte *et al.*, *Immunol Invest* 2005; 34:325-342; Facciponte *et al.*, *Cancer Immunol Immunother* 2006; 55:339-346; Wang *et al.*, *J Immunol* 2006; 3:1543-1551). These molecular studies form the basis for an awarded RAID award (to Dr. Kane, P.I.) from the NCI to prepare a hsp110/gp100/Trp2 vaccine for the treatment of melanoma.

Recent molecular and cellular studies of these recombinant vaccines have focused on the mechanisms of uptake and processing of hsp110- (grp170-) protein antigen complexes in antigen presenting cells (APCs). This includes the 1) identification of hsp110 specific receptors, 2) definition of the fate of the internalized hsp110-antigen complexes in APCs, and 3) their role in the maturation of APCs. Drs. Subject and Xiang-Yang Wang, (PR) have identified a diverse family of receptors on APCs which specifically bind to hsp110 and grp170. This group of receptors are referred to as "scavenger receptors" (SRs) and

have been intensively studied in the cardiovascular field as receptors for modified lipoproteins. Studies in the Subject laboratory have focused on two major SRs expressed by APCs, referred to as SR-A (scavenger receptor A) and SREC (scavenger receptor on endothelial cells) and have shown that both bind hsp110 (and grp170) in a receptor-like manner. They have demonstrated that fucoidan or PolyI (general SR ligands) interfere with stress protein binding and the cross presentation of tumor antigen (Her-2/neu) bound to hsp110, thus implicating SRs in cross presentation of antigen and vaccine activity (Wang *et al.*, *Cancer Res* 2007; *in press*).

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