Sciences, Binghamton University, Binghamton, NY 13902, USA, [3] CPSI Biotech, Inc., 2 Court Street, Owego, NY 13827, USA, [4] Department of Surgery, State University of New York, Buffalo, NY, USA

The study of cancer biology is replete with debate and disagreement over the various analytical stratagems (*in vitro* vs. *in vivo*, animal vs. human) employed to develop therapeutic treatments. Yet, due to the highly variable nature of cancer cells and their acute sensitivity to extracellular microenvironments and culture conditions, it is critical that a culture model be chosen that closely resembles actual *in vivo* conditions in support of clinical relevance. As such, we hypothesized that the development of a three-dimensional (3D), tissue engineered human prostate cancer model would provide for a viable alternative, bridging the gap between issues with animal and *in vitro* models. In this study, we developed and tested the feasibility of such a model as well as a series of modified cell and molecular assays utilized to assess the effects of a given treatment on cell response. Human prostate cancer cells (PC3) were suspended in collagen matrices and variables such as gel thickness (1–3 mm), collagen density (0.5, 1, and 2 mg/mL), cell seeding density (1, 2.5, and 5×10^6 cells/mL), and top-seeded vs. 3D-seeded, were evaluated for their influence on cell survival, proliferation, and response to a therapeutic stress. With the engineered model we were able to assess, using modified techniques, membrane integrity, metabolic function, and various cell death mechanisms. From our evaluations it was found that PC3 cells were able to survive and proliferate under several different model parameters, but a 2 mm thick collagen gel at 2 mg/mL seeded with 5×10^6 cells/mL was chosen as the standard configuration. Additionally, the application of cryosurgical therapy to our 3D model revealed cell behavior characteristics similar to that of *in vitro* monolayer data. Through this study, we established a 3D, tissue engineered *in vivo*-like prostate cancer model along with a series of assessment techniques for utilizations in cancer research studies. Using this model, our aim is to better understand cancer cell responses to low temperatures, thus providing a new avenue for the improvement of cryosurgery.

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46. Identification and characterization of genes crucial for basal freezing tolerance in an alpine subnival plant Chorispora bungeana. Xiule Yue [1], M. Jin [1], N. Yang [2], Y. Xiang [1], Lizhe An [1], H. Zhang [1], [1] Lanzhou University, School of Life Sciences, Lanzhou, Gansu, China, [2] Northwest Normal University, School of Life Sciences Northwest Normal University Lanzhou, Gansu, China

Cold acclimation improves freezing tolerance in plants. Many advances have been made toward identifying the signaling and regulatory pathways that direct the low-temperature stress responses. However, little is known about the mechanisms for naturally freezing tolerant species such as *Chorispora bungeana* (*C. bungeana*). This novel plant grows in high attitude regions with temperature ranging from 4 °C to −10 °C. We aim to isolate genes that are crucial for basal tolerance in *C. bungeana* by suppression subtractive hybridization (SSH) with cold-treated (−4 °C) and non-cold treated plant samples combined with macroarray and real-time PCR analysis. A total of 85 genes including 52 up-regulated and 33 down-regulated genes were identified as candidates for basal freezing tolerance. These genes were sorted into broad functional categories including stress/hormone stimulus response, photosynthesis, transcription, protein metabolism, ribosomal protein, etc. This suggests that a very complex series of molecular mechanisms are involved in the response to freezing stress in *C. bungeana*. Functional analysis indicates that a fibrillin protein FBN1a and an ACTIN cross-linking protein may act as the cold sensors by their physical changes caused by cold, and DREB2A signal pathway combined with ABA signal pathway may play a critical role in regulating the *COR* genes (cold responsive genes). Meanwhile, the downstream cold responsive genes, such as *KIN2* (*COR6.6*), *ZAT10* (*STZ*) and *GR-RBP8*, also play a very important role in freezing tolerance. In addition to that, some new genes were also identified successfully which indicated that there may exist some new signal pathway or mechanisms in *C. bungeana* in response to freezing stress.

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47. In vitro assessment of apoptosis and necrosis following cold storage in Human Corneal Endothelial Cells. William L. Corwin [1,2], John M. Baust [1,3], Robert G. Van Buskirk [1,2,3], John G. Baust [1,2], [1] Institute of Biomedical Technology, State University of New York, Binghamton, NY 13902, USA, [2] Department of Biological Sciences, Binghamton University, Binghamton, NY 13902, USA, [3] CPSI Biotech, Inc., 2 Court Street, Owego, NY 13827, USA

Corneal endothelial cells serve as a limiting factor in the ability to preserve whole cornea for proper utilization, such as transplantation. Previous reports have demonstrated the importance of proper endothelial maintenance during preservation for improved corneal function. As such, analyses into the modes of cell death will allow for future in-depth investigations of the complex stresses and resultant changes cells experience during the entirety of the preservation process. To this end initial studies focused on assessing the extent of cellular demise via apoptosis and necrosis following cold exposure. We hypothesized that apoptosis would play a significant role in preservation-induced delayed cell death associated with exposure to hypothermic conditions. Human Corneal Endothelial Cells (HCECs) were stored for 18 h to 8 days at 6 °C in complete media, HBSS, ViaSpan and OptiSol. Viability was assessed 0, 24 and 48 h post-storage using the metabolic indicator alamarBlue™ and flow cytometry (ViaCount Assay). Apoptotic and necrotic involvement was assessed via flow cytometry and fluorescent microscopy using the Vybrant® apoptosis assay at 1, 4, 8 and 24 h post-storage. Mitochondrial potential was assessed via flow cytometry using the fluorescent dye JC-1. Western Blots were performed to evaluate alterations in apoptotic proteins including caspases, PARP, Bid, and the Bcl-2 family proteins. In addition to these analyses, the targeted inhibition of caspases using specific caspase inhibitors (8, 9 and pan) was conducted to evaluate their overall effects on cell tolerance to cold stress. Analysis of the sample sub-populations revealed that a peak in apoptosis was seen 4–8 h after 1 day of hypothermic storage, approaching 10% under certain conditions. This analysis revealed that the necrotic population continues to increase in all conditions tested, peaking at 24 h post-storage. Viability analysis demonstrated a significant impact by caspase inhibition on cell survival. Cells stored for 4 days in ViaSpan with and without a pan-caspase inhibitor revealed that while immediately post-thaw a similar level of viability remained between the two conditions (∼65%), analysis at 24 h post-storage revealed a ∼30% improvement in cell retention post cold exposure. Samples stored in ViaSpan without inhibitor decreased to around 40–45% survival while the incorporation of a pan-caspase blocked delayed-onset cell death allowing cells to remain around 70% viable. Western blot analysis revealed a correlation between decreased viability and increased protein changes associated with apoptotic activation (i.e. cleavage of caspases, Bid, and PARP). These data are important because they are the first step in a more complete understanding of cold induced stress pathway activation.

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48. In vitro assessment of apoptosis and necrosis following cold storage in human airway cells. William L. Corwin [1,2], John M. Baust [1,3], Robert G. Van Buskirk [1,2,3], John G. Baust [1,2], [1] Institute of Biomedical Technology, State University of New York, Binghamton, NY 13902, USA, [2] Department of Biological Sciences, Binghamton University, Binghamton, NY 13902, USA, [3] CPSI Biotech, Inc., 2 Court Street, Owego, NY 13827, USA

As advances in medical technology improve the ability and efficacy of cells and tissues to be transplanted, there remains a void in our knowledge of the specific molecular response of cells to low temperature storage. While much focus has been given to the formulation of solution to perfuse the tissues during storage to increase viability, investigations into the complex molecular changes that occur during cold exposure and their subsequent effect on outcome remain limited. An understanding of cellular stress on a molecular level is of fundamental importance as such information proves critical to tissue storage and transplantation as well as to the development of new technologies and processes in non-related areas of cell biology, bioprocessing, etc. The intent of this study was to begin to quantify the levels of cell death following hypothermic storage in a lung cell model in order to establish a foundation for future in depth molecular studies in support of improved lung transplantation. Normal human lung fibroblast (IMR-90) cells were stored statically for 1, 2 and 3 days at 4 °C in basal media, complete media and ViaSpan®. Post-storage viability was assessed at 1, 2 and 3 days post-storage using the metabolic indicator alamarBlue. To determine the level of apoptotic and necrotic involvement, flow cytometry was performed and corroborated via visualization under fluorescent microscopy at 1, 4, 8, and 24 h post-storage using the Vybrant apoptosis assay. Sample analysis revealed that cells stored in ViaSpan® were 68% (±4%) viable after 24 h of storage at 4C and repopulated to 103% (±6%) within 3 days. The other solutions resulted in complete cell loss after 24 h of hypothermic storage with no re-growth observed. Extension of the storage interval to 3 days resulted in complete cell loss in all conditions. Analysis of the apoptotic and necrotic populations in the ViaSpan® stored samples revealed that 5% of the population was apoptotic at 8 h post-storage while around 20% of the same population was found to be necrotic. The data revealed a high level of sensitivity to cold storage for this cell system, resulting in a significant amount of cell death, through both apoptosis and necrosis. These data highlight the critical need for a more in depth understanding of the molecular changes that occur as a result of cold exposure in cells.

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49. Regulation of PTEN function and structural stability in hibernating thirteen-lined ground squirrels. Cheng-Wei Wu, Ryan Bell, Kenneth B. Storey, Carleton University, Institute of Biochemistry & Department of Biology. Ottawa, ON, Canada