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TITLE: Pilot Study Testing the Technical Feasibility and Toxicity of High Dose Rate Brachytherapy Combined with Hyperthermia to Treat Prostate Cancer Recurrences after External Beam Irradiation or Permanent Seed Implant Failure

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Pilot Study Testing the Technical Feasibility and Toxicity of High Dose Rate Brachytherapy Combined with Hyperthermia to Treat Prostate Cancer Recurrences after External Beam Irradiation or Permanent Seed Implant Failure

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Introduction

The principal objective of this research is the development of the combination of hyperthermia (HT) and high dose rate (HDR) brachytherapy as a therapy for locally advanced, recurrent prostate cancer after failure using front line external beam definitive radiation therapy (EBRT). We had previously developed a system for low dose rate systems (LDR), however technological advances in HDR systems make the application of LDR essentially obsolete. There are several fundamentally different aspects to HDR practice and dosimetry, which render the LDR technology developed here obsolete as well. As a result the first three tasks in the statement of work are the development of a new template system, new software to control power deposition in the tumor and phantom testing before beginning patient treatment. As is demonstrated in the body of this report all three tasks have been completed on schedule. The new HDR template design differs radically from the older LDR system is easier to set up and more comfortable for the patient. The software was completely re-written to accommodate the fundamentally different HDR dosimetric approach. The combination of the hardware and software was then extensively tested on phantoms. Once satisfied that the system was safe for human application one patient was treated in accordance with the approved protocol during year 1. The final task is the development of heat activated gene therapy as an adjuvant to HDR brachytherapy specifically applied to this proposal but which should have considerably broader applicability in cancer treatment.

Body of Report

Task 1. Complete the ongoing constructions of an electronic template interface compatible with HDR brachytherapy and hyperthermia systems, permitting simultaneous operation.
Completed during year 1 (see 2002 annual report)

Task 2. Complete the ongoing development of the computer code required to effectively drive the "random placement" needle patterns associated with current HDR brachytherapy practice.
Completed during year 2 (see 2003 annual report)

Task 3. Test the system on phantoms that are constructed to mimic patients that have undergone HDR prostate treatment and estimate how effectively it will generate the desired hyperthermia heating patterns.
Completed during year 2 (see 2003 annual report)

Task 4. Recruit and treat patients with recurrent prostate cancer that meet the patient selection criteria outlined in the approved clinical protocol.

The patient treated during year 1 has responded well and shown no evidence of any chronic toxicity. This patient has now been followed for approximately 30 months and the PSA values from this follow-up period are as follows:
The patient is alive with no evidence of disease. This result represents an excellent response but is inconclusive in itself. Changes in the protocol reported have resulted in increased interest but no additional patients were treated during the reporting period. Since the target number of 15 patients was not achieved this task was not completed successfully.

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**Task 5.** Investigation and integration of gene therapy into the treatment scheme.

**EXPERIMENTAL RESULTS**

This grant has supported research that has helped develop heat-activated gene therapy using a highly toxic transgene to a point that it will now be tested in an orthotopic brain tumor model. If the latter is successful in improving local control of the model brain tumor, the results will be reviewed very carefully to determine if this targeted form of gene therapy should progress on to human clinical trials to treat recurrent prostate cancer and primary brain tumors.

**Bystander Killing with CdtB**

Earlier, we reported that the Cytolethal distending toxin (CdtB) transgene, which is being tested as the therapeutic proteotoxin, exhibited bystander killing. CdtB is a DNAse I homolog that causes DNA double strand breaks in cells that express it. Bystander killing refers to the phenomenon where cells that did not express CdtB died simply because they were grown adjacent to other cells that expressed CdtB. The magnitude of the bystander killing increased with the time that the CdtB-expressing cells were incubated in the presence of the nonexpressing cells (Fig. 1). Figure 1 shows the results for Dut-145 human prostate carcinoma cells, but similar results were obtained for U-87 MG (human glioblastoma), HeLa (human cervical carcinoma), and VX2 (rabbit tumor) cells.

Bystander killing was most prominent when cells were grown as three-dimensional aggregates. Figure 2 shows further evidence for the CdtB bystander effect. U-87 MG cells infected with the HSP70B-CdtB adenovirus vectors and then seeded to form monolayer cultures that would be nearly confluent 24 h later. Non-virus infected cell were treated identically to serve as controls.

Cells were heat shocked for varied times at 41.0°C, 24 h after infection. Some flasks were trypsinized immediately following heat shock for the colony formation assay while others were maintained for 36 h after heat shock before being used for colony formation.

Maintaining cells as high-density cultures for 36 h after heat-induction of CdtB expression yielded greater killing, potentially due to the bystander effect. Cells were refractory to 41.0°C, with no detectable killing until nearly a 2 h heat shock. Thus the cell killing in the virus-infected cells was due to CdtB.
spheroids rather than two-dimensional monolayer cultures. This result showed that tighter cellular packing promoted bystander killing, and the spheroid model better represented the cellular proximity in tumors and normal tissues better than monolayer cultures.

In one set of experiments, cells that expressed enhanced green fluorescent protein (EGFP) were used to distinguish cells that did not express EGFP from cells that did. Then cells were mixed together to form spheroids, the spheroids were retained in suspension culture for varying time periods, and the spheroids were then seeded into 35 mm culture dishes so that the cells could spread out for analyses by immunocytochemistry.

Cells in the spread spheroids were treated with several different antibodies to help elucidate the mechanism of the bystander killing. When cells were stained with an antibody for phosphorylated gamma histone 2 AX (pγH2AX) to observe foci of DNA double strand breaks, positive staining was observed in the CdtB-expressing cells (as expected). However, most of the cells that did not express CdtB unexpectedly also stained positive for pγH2AX (Fig. 2). This result indicated that the bystander killing was mediated by a molecule or signal that triggered events in the CdtB nonexpressors that ultimately resulted in the formation of DNA double strand breaks.

This result was reminiscent of the radiation bystander effect. The operational hypothesis then became that the formation of DNA double strand breaks in a cell resulted in a transmissible signal that resulted in DNA double strand break formation in adjacent cells that were not themselves exposed directly to the double strand break forming treatment. The next things to determine were how the signal was transmitted and what the signal was.

When medium from cultures expressing CdtB was transferred to cultures of the same cell type that were not expressing CdtB, no cell killing was observed in the latter. Hence, it was concluded that the bystander killing was mediated primarily via a mechanism that involved cell to cell contact, and that any contribution from a signal that diffused from CdtB expressing cells to nonexpressing cells across the intervening culture medium was minimal or nonexistent. Consequently, subsequent experiments focused on mechanisms that required cell-cell contact.

Some experiments have suggested that radiation bystander killing requires intercellular communication through gap junctions. However, there is concern regarding how important the gap junction mechanism for bystander killing would be since it has been reported that many tumor cell types exhibit lower levels of gap junction communication than normal tissues. While this may be true, the cell types listed above all exhibited significant and readily detectable levels of gap junction communication when tested using a standard calcein transfer assay, as illustrated for U-87 MG cells in Fig. 3. The ability of the potent gap junction inhibitor,
carbeneoxylone, to inhibit intercellular calcein transfer demonstrated conclusively that calcein transfer did indeed occur across gap junctions.

Initially, we postulated that whatever signal mediated CdtB bystander killing would be a relatively small molecule that somehow triggered apoptosis or some other mechanism in cells that did not express CdtB. This is because the size of molecules that can be transferred across gap junctions has an upper limit of 1.5 kDa to 2.5 kDa. However, we wanted to test the possibility that larger molecules, including CdtB itself, could be transferred between cells by some cytoplasmic bridges, intercellular membrane exchange, or some form of coupled exocytosis and then phagocytosis.

**INTERCELLULAR TRANSPORT OF LARGE MOLECULES**

In the course of running the mixed spheroid experiments described above (some cells tagged with a fluorescent protein or chimera), the more sensitive light detection of flow cytometry revealed a phenomenon that was overlooked by microscopic observations. Within 24-36 h after forming the mixed spheroids, more than 60% of the cells that were not expressing the fluorescent protein now contained low, but detectable quantities of the fluorescent protein. This was detected after the spheroids were dissociated into individual cells using trypsin and other proteolytic enzymes, and the cells were analyzed with the flow cytometer (Fig. 4).

Extensive trypsinization was used to ensure that fragments of the cells expressing the fluorescent protein were not adhering to the nonexpressing cells, and confocal microscopy was used to confirm this. Since the fluorescent EGFPs and EGFP chimeric proteins had molecular weights of 27 kDa to 56 kDa they could not diffuse cell to cell via gap junctions, hence some other transfer mechanism must be involved. Potential mechanisms for intercellular transfer of such large molecules include: cytoplasmic bridges, coupled exocytosis-phagocytosis, and intercellular membrane exchange. One or more heretofore-undiscovered mechanisms also remain a possibility.

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**Fig. 4: Intercellular Transfer of Large Molecular Weight Proteins in Cancer Cells**

Wild-type HeLa cells (A) were mixed equally with HeLa cells that constitutively expressed a fluorescent chimera of EGFP and HSP27 (C) in order to form a mixed spheroid (B). The spheroids formed within 6 h and were maintained in suspension culture for up to 160 hours. Within 48 h, flow cytometry of individual cells dissociated from the spheroids showed that some of the fluorescent chimera had transferred into the wild-type HeLa cells (B). This was determined from the shift to the right (increased fluorescence) for the wild-type HeLa cells that were in the spheroid, and an increase in the number of cells with fluorescence that was intermediate between the wild-type HeLa cells and those expressing the chimera (B).

The EGFP-HSP27 chimeric protein has a molecular weight of approximately 56 kDa; too large to transfer between cells via gap junctions. Confocal microscope observations showed that the increased fluorescence of the wild-type cells was not due to fluorescent portions of the EGFP-HSP27 cells adhering to the outer membrane of the former.

The fluorescence of the wild-type cells continued to increase the longer the mixed spheroids were incubated (data not shown). However, the wild type cells never
became as fluorescent as the cells expressing the EGFP-HSP27 chimera. Thus, the two cells could never be confused with each other under a fluorescent microscope, e.g., in the experiment depicted in Fig. 2.

CdtB is a potent enzyme that causes DNA double strand breaks due to its DNase I-like activity. Hence, a very small amount of CdtB within a cell could prove lethal. It is plausible that CdtB bystander killing occurs and is propagated by the intercellular transfer of CdtB itself from cells expressing it to adjacent cells that do not. This would explain the apparent appearance of DNA double strand breaks in cells that do not express CdtB (Fig. 2).

Interestingly, the intercellular transfer of the fluorescent chimeric proteins was never observed in two, normal human cell lines (PZHPV7 normal human prostate cells and 18-LU normal human lung fibroblasts) (data not shown). Consequently this phenomenon may only occur between tumor cells, and might mean that CdtB bystander killing would only occur amongst tumor cells and would not involve normal tissues.

**HSP70B PROMOTER EXPRESSION INDUCED BY HYPOXIA.**

Dut-145 cells were infected with the adenovirus vector carrying the CdtB transgene under control of the HSP70B heat shock promoter and then incubated under normoxic or hypoxic (0.7% O2) conditions. Fig. 5 shows that no DNA double strand breaks were observed under normoxic conditions until the cells were heat shocked to induce CdtB expression from the HSP70B promoter. However, DNA double strand breaks were observed (by pulse field gel electrophoresis) in unheated cells that were incubated under hypoxic conditions. These data suggest that CdtB cytotoxicity will be targeted to hypoxic tumor regions since the CdtB transgene will be preferentially expressed in hypoxic tumor regions without heat shock induction.
Key Research Accomplishments and Conclusions: Year One

- A hyperthermia template compatible with high dose rate brachytherapy (HDR) was constructed and tested.
- Software was written and tested which is compatible with the quasi-random needle placement used in HDR.
- The combination of the new template and software underwent extensive testing using phantoms to qualify the system for use in patient therapy.
- A patient was treated using the system was treated according the the approved protocol for this project.
- A combination promoter system that is activated by ionizing radiation and hyperthermia was constructed for use in gene therapy. This promoter system is to be used to construct vectors that contain cytotoxic genes (e.g. the cytolethal distending toxin).
- The new HDR template was constructed and works as anticipated. Some cracking difficulties were encountered with the original plastic components constructed of Lucite but a switch to another plastic Delrin solved the problem.
- The software as written and extensively tested is ready for patient therapy. It is anticipated that some modifications will be necessary after further experience is gained with actual patient therapy.
- One patient was treated during this granting period. The patient tolerated the procedure well and the target temperature parameters were achieved. The patient, to date (4 months follow-up), has shown no significant short term toxicities and his PSA has decreased to zero from a pretreatment value of 5.4. Obviously, the follow-up period is short and no conclusions can be drawn on long term tumor control and late toxicities. The results indicate that we are now ready for continued patient accrual under the protocol.
- The gene therapy project is progressing well and we are now prepared to proceed with vector construction using the heat and ionizing radiation induced promoter. Cytotoxic transgenes can be added to the construct (e.g. CdtB and Diphtheria) and subsequently testing can begin in \textit{in vitro} and rodent model systems.
- The combination of the progress to date suggests that this approach may result in a viable treatment for recurrent prostate cancer. Whether or not that is true will require several years more work.

Key Research Accomplishments and Conclusions: Year Two

- Modifications to the software controlling clinical therapy were completed during this period and are described in detail in the body of the report. These modifications were subjected to the same rigorous testing as the original program and found to be operating within nominal parameters. These modifications simplified the system, making operation of the system much more ‘user friendly’ for the therapists. It also reduced the maximum number of computers required from three to two.
Follow-up of the prostate cancer patient treated during year one of the research work continues with no evidence of recurrence.

An adenoviral vector containing the DNA for a potent proteotoxins (Cytolethal Distending Toxin B (CdtB)) was constructed and produced in sufficient quantities to carry out both in vitro and in vivo experimentation. This was a major hurdle to overcome and was done without the necessity of including the double heat/radiation promoter switch described in the year one progress report.

The CdtB containing vector was tested in vitro and found to be lethal with a single viral particle infecting a cell. Furthermore, it was discovered that this agent exhibits a remarkable, as yet unexplained, bystander effect which magnifies its potency by a factor of 10 in terms of cell survival. This effect requires cell-cell contact. Such bystander effects are a key requirement for cancer therapy of existing malignancies.

Experiments were carried out that demonstrate conclusively that the CdtB toxin exhibits DNAse-1 like activity and kill by the creation of non-repaired double strand breaks. pH2AX and CHEF gel studies show that the pH2AX foci correspond with DNA double strand break formation and that these phenomena also occur under hypoxic conditions, another key requirement for human tumor therapy.

The bystander effect requirement for cell-cell contact has led us to develop assays for gap junction activity using Calcein that can be applied in this case. Preliminary results suggest that the bystander activity is transmitted by these gap junctions since pH2AX foci appear in uninfected cells. The full meaning of these observations awaits further experimentation.

**Key research Accomplishments and Conclusions: Year Three**

Follow-up of the prostate cancer patient treated during year one of the research work continues with no evidence of recurrence

Experiments carried out in year two suggested that CdtB bystander killing was being occurring by the transfer of some factor via gap junctions. Extensive experimentation was carried out to elucidate this bystander mechanism. As reported above (Figures 3 and 4) small molecules that can traverse gap junctions (2-4 kDa) were ruled out as a factor in bystander killing.

Extracellular transfer of a cytotoxic factor or signaling molecule was ruled out.

Chimeric proteins (fusion proteins) containing both EGFP and CdtB (approximately 56 kDa) were observed to transfer to uninfected cells when prolonged cell-cell contact was permitted in DUT-145 human prostate cancer cells. U87MG human glioma cells and other human tumor cells. This molecular transfer was conclusively documented to cause Cytolethal effects and double strand breaks by pH2AX immunostaining.

Transfer by gap junctions was ruled out. Potential mechanisms for this cell-cell transfer are cytoplasmic bridges, coupled exocytosis-phagocytosis and intercellular membrane exchange. One or more heretofore undiscovered mechanisms are also a possibility.

The cell-cell transfer was observed in human tumor cells but not observed when identical experiments were carried out in their normal human cell counterparts. If this observation hold up under more extensive experimentation this phenomenon may be tumor specific, greatly enhancing it's utility in human cancer treatment.
Figure 5 above demonstrates that under normoxic conditions CdtB when attached to the HSP70B promoter is silent yielding no expression of CdtB or double strand DNA breaks as evidenced by pulse field gel electrophoresis. However under chronic hypoxic conditions the gene is activated causing double strand break induction. These observations suggest that this agent can be used to target hypoxic tumor regions preferentially in the absence of heat shock adding another potential facet to the utility of this approach.

Reportable Outcomes: Year Three

Five presentations were made at national and international meetings and abstracts were published as follows.


NAHS PROGRAM

SYMPOSIA

SN BB6: Stress Signaling and Molecular Chaperone Gene Expression
(Sept 10. 14.00-16.00. Room B)

Coordinators: S. Calderwood, N. Mivechi

- 14.00-14.20: N. Mivechi (Medical College of Georgia): Ral binding protein 1 interaction with heat shock factor 1 and hsp90 links the Ral signaling pathway to the heat shock response
- 14.25-14.45: X. Wang (Dana Farber): High affinity binding of 14-3-3 to phosphorylated heat shock factor 1: role in nucleocytoplasmic shuttling and hsp gene expression
- 14.50-15.10: C. Patterson (Univ NC/Chapel Hill): The ubiquitin ligase/co-chaperone CHIP is a novel stress capacitor
- 15.15-15.35: M. Stevenson (Beth Israel): Protein kinase pkr is essential for hsp mRNA stabilization and expression of hsp after stress
- 15.40-16.00: G. Li (Memorial Sloan Kettering): DNA methylation and Ku 70 associated inhibition of heat-induced hsp70 expression

SN T2: Heat- and Other Stress-Targeted Gene Therapy
(Sept 10. 16.00-18.00. Room Les Chutes)

Coordinator: C. Li, M. Borrelli

- 16.00-16.20: M. Borrelli (Beaumont): Heat activated gene therapy with proteotoxin transgenes
- 16.25-16.45: A. Lee (Univ S Calif): Spontaneous and controllable activation of the stress-inducible Grp78 promoter in cancer gene therapy
- 16.50-17.10: C. Gomer (Univ S Calif): Photodynamic therapy mediated oxidative stress as a molecular switch for selective activation of stress gene promoters - potential role in gene therapy
- 17.15-17.35: G. Li (Memorial Sloan Kettering): TBA
- 17.40-18.00: C. Li (Duke): Targeted gene therapy directed towards tumor hypoxia

SC&B 3: CSSI/NAHS Joint Plenary Session (See CSSI Schedule)
(Sept 10. 20.30-22.30. Room A and B)

SN T3: Heat Shock Proteins as Cancer Vaccines
(Sept. 11. 10.30-12.30. Room A)

Coordinator: J. Subjeck

- 10.30-10.50: J. Lewis (Antigenics): HSP vaccines in cancer
- 10.55-11.15: L. Mizzen (StressGen): HSP fusion proteins: therapeutic vaccines for chronic viral infection and cancer
- 11.20-11.40: M. Graner (Univ AZ): Chaperone-rich cell lysates: antigen and adjuvant in an anti-cancer vaccine
- 11.45-12.05: S. Chen (Baylor): A broadly applicable heat shock protein-mediated oncolytic tumor vaccine
- 12.10-12.30: J. Subjeck (Roswell Park): The design of chaperone vaccines, a different approach to heat shock vaccines formulation
SN C4: Clinical Hyperthermia: Recent Results and New Directions

Coordinator: M. Hurwitz

08.00-08.30: E. Jones (Duke): Trimodality therapy for cervix cancer - recent results and future plans
08.30-09.00: M. Hurwitz (Harvard): Final toxicity report of a phase II trial of trans-rectal ultrasound hyperthermia for treatment of prostate cancer
09.00-09.30: J. Bull (Univ Texas): In vivo enhancement by systemic hyperthermia of glucose-responsive protein (GRP-78) promoter CAT gen
09.30-10.00 S. Das (Duke Univ) Noninvasive Thermometry.
10.00-10.30: E. Jones (Duke): Liposomal chemotherapy and hyperthermia for breast cancer

SN P2: Current Technology for Clinical Hyperthermia and Thermal Ablative Therapies

Coordinators: P. Corry, C. Diederich, P. Stauffer

14.00-14.25: P. Corry (Beaumont): Physical aspects of thermal therapy
15.00-15.25: C. Diederich (UCSF): Deep-local hyperthermia and high-temperature thermal therapy
15.30-15.55: G. van Rhoon (Netherlands): Technology and devices for deep regional heating

NAHS WORKSHOPS

WN T4: Hyperthermia and the Inflammatory/Immune Response

Coordinators: S. Evans

14.25-14.45: L. Singh (Univ Maryland/VA Med Ctr): Differential processing of HSF-1 and regulation of TNF-alpha expression at febrile range temperatures
14.50-15.10: S. Evans (Roswell): IL-6/soluble IL-6 receptor trans-signaling activates tumor microvascular adhesion in response to fever-range thermal stress
15.15-15.35: A. Asea (Boston Univ): Active release of heat shock proteins from tumors: Role of chaperokine in hyperthermia-induced tumor killing
WN BB2: Molecular Changes to Improved Clinical Responses; Advances in the Use of Hyperthermia to Improve Radiation Therapy
(Sept. 11. 16.00-18.00. Room Les Chutes)

Coordinator: J. Roti Roti
-16.00-16.20: J. Roti Roti (Wash Univ): Introduction and overview
-16.25-16.45: J. Lepock (Univ Toronto): Role of protein dematuration and aggregation in radiosensitization by hyperthermia
-16.50-17.10: M. Xu (Wash Univ): Effects of moderate hyperthermia on the DNA proteins, MRE11 and RAD50
-17.15-17.35: E. Armour (Beaumont): Long duration hyperthermia and brachytherapy
-17.40-18.00: J. Locke (Wash Univ): Enhancement of radiosensitization by moderate hyperthermia and nonsteroidal anti-inflammatory drugs
-18.00: All participants: Discussion

WN BB4: Physiological Effects of Hyperthermia on Tumors and Normal Tissues
(Sept 12. 16.00-18.00. Room Les Chutes)

Coordinators: E. Armour, C. Song
-16.00-16.20: K. Kregel (Univ Iowa): Integrated physiological consequences of hyperthermia and the potential impact of aging
-16.25-16.45: D. Leeper (Thomas Jefferson): Acidification and oxygenation-induced sensitization of thermo-, radio-, or chemotherapy by MIBG and hyperglycemia
-16.50-17.10: C. Song (Univ Minnesota): Hyperthermia and tumor blood flow
-17.15-17.35: Z. Vujaskovic (Duke): Tumor physiology studies in breast cancer patients

WN BB1: Mechanisms of Thermotolerance (A Multi-Faceted Discussion)
(Sept 12. 16.00-18.00. Room B)

Coordinator: A. Laszlo
-16.00-16.20: A. Laszlo (St. Louis) *Discussion Leader
-16.25-16.45: H. Kampinga (Groningen, Netherlands)
-16.50-17.10: K. Ohtsuka (Nagoya, Japan)
-17.15-17.35: M. Borrelli (Detroit)
HEAT-ACTIVATED GENE THERAPY WITH PROTEOTOXIN TRANSGENES

Michael J. Borrelli, Diane M. Schoenherr, Laura Bernock, Sandra Galoforo, and Peter M. Corry.
Radiation Oncology Research Laboratory, William Beaumont Hospital, 3811 West Thirteen Mile
Road, Royal Oak, MI 48073

Cytolethal distending toxin B (CdtB) is highly homologous with DNase 1, and its putative toxic lesion in mammalian cells is the production of unrepaired DNA double strand breaks (DSBs). CdtB cDNA was cloned into an adenoviral vector under control of the human HSP70B heat shock promoter to be used as a heat shock-inducible, radiomimetic transgene for cancer gene therapy. The objective is to use localized heat shock to induce CdtB expression within tumors and a defined normal tissue margin to maximize CdtB cytotoxicity within tumors while minimizing normal tissue complications.

Experiments in human cell cultures have shown that CdtB expression is silent until activated by a heat shock of 41.0°C or higher. Once induced, DNA DSBs were observed in CdtB-expressing cells, as determined by neutral pulsed field gel electrophoresis and immunofluorescent staining with an antibody to phosphorylated γH2AX. All cells expressing CdtB were killed and many nearby cells that did not express CdtB were also killed by a bystander effect whose mechanism is unknown. However, preliminary data indicate that CdtB bystander killing has many features in common with radiation bystander killing. Preliminary experiments with human Xenograft tumors have shown that CdtB expression was silent until induced by heat shock. Once induced, CdtB gene therapy completely inhibited tumor growth without any detectable local or systemic normal tissue toxicity.
Current Technology for Clinical Hyperthermia and Thermal Ablative Therapies

This session has been organized to be of interest to all participants of the meeting, both CSSI and NAHS, with the view towards being generally informative as to how in vivo (mouse to man) thermal therapy can be applied. The presentations will discuss both the generalities and specifics of the issues associated with clinical hyperthermia and high-temperature thermal therapy and how they may be circumvented or improved on in the future. The session will consist of four presentations each of approximately 20 to 25 minutes with 5-10 minutes for questions. The first will be a general overview of the principles of tissue heating using various energy sources as well as invasive and non-invasive thermometry. The remaining sessions will review state-of-the-art heating systems and treatment strategies applied via superficial, interstitial, intracavitary, intraluminal, and external approaches for local and deep heating.

Presentations

Physical Aspects of Thermal Therapy
Peter M. Corry
William Beaumont Hospital
Royal Oak, MI 48073-6769
Deep-local Hyperthermia and High-temperature Thermal Therapy
Chris J. Diederich
University of California at San Francisco
San Francisco, CA 94143-1708

Superficial Hyperthermia
Paul R. Stauffer
University of California at San Francisco
San Francisco, CA 94143-1708
Technology and Devices for Deep Regional Heating
Gerard C. van Rhoon
Earsmus Medical Centre
Rotterdam, The Netherlands
Using long duration mild temperature hyperthermia (LDMH) to enhance the cytotoxicity of radiation therapy hold the possibility of improving clinical outcome. We have extended cell culture investigations by using human breast carcinoma cells (MCF7) and colon carcinoma cells (WiDr) growing as multi-cell spheroids in vitro. The role of cell to cell contact and altered metabolic state on LDMH induced cell killing and radiation sensitization was measured using this model. Relative to other human cell lines, both MCF-7 and WiDr cells growing in monolayer are resistant to being killed by both 43°C and 41°C heating. When grown as spheroids, both cell types became even more resistant to 43°C heating. Resistance to 43°C heat killing increased with increasing spheroid diameter. On the other hand, both MCF-7 and WiDr cells grown as spheroids became more sensitive to 41°C induced killing. The degree of change in 41°C sensitivity depended upon spheroid diameter and the diameter effect was different for the two cell lines. The 70 μm spheroids were most sensitive for MCF-7 cells whereas 200 μm spheroids were most sensitive for WiDr cells. For both types of spheroids, 41°C sensitivity returned to that of monolayer cells as the spheroid diameter increased. These results indicate that the in vivo milieu as modeled by spheroids results in increased 41°C sensitivity and 43°C resistance. The response of MCF-7 cells in 70, 200, and 400 μm spheroids to acute irradiation was identical to that of monolayer cells. MCF-7 cells in 700 μm spheroids were slightly more sensitive to acute irradiation expressing a partial loss of radiation survival curve shoulder. The response of WiDr spheroids to acute irradiation varied with spheroid diameter. Resistance to radiation increased with increasing spheroid diameter. Sensitization of cells in spheroids to low dose-rate irradiation was greater in WiDr cells than in MCF 7 cells. These observations are another indication that application of hyperthermia at 41°C for durations of many hours may be superior to higher temperature for durations of less than one hour.

This work was supported by DAMD 17-1-01-1-0117.
ICHO
St. Louis, MO USA
Gateway to Improved Cancer Treatment
Scientific Program and Abstracts
Adam's Mark Hotel • April 20-24, 2004 • St. Louis, Missouri USA
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<td>3:30 pm - 5:30 pm</td>
<td><strong>Workshop: Heat Shock Factor in Biological Systems</strong></td>
<td>IJ Benjamin (USA) &amp; G Santoro (IT) - Co-Chairs</td>
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<td>The Biology of Heat Stress Through Heat Shock Transcription Factors</td>
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<td>Mammaliam Heat Shock Transcription Factors 1 and 2: Lessons From Knockout Mice</td>
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<td>Heat Shock Factors and the Control of the Stress Response in Inflammation and Cancer</td>
<td>G Santoro (IT)</td>
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<td>Depletion of Hsp72 Sensitizes Cancer Cells to Radiation, Proteasome Inhibitors and Conventional Drugs by Downregulation of Exk and Nf-kb Signaling Pathways</td>
<td>MY Sherman (USA)</td>
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<td>DNA Strand Breaks by Heat</td>
<td>A Takahashi (JP)</td>
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<td>Phosphorylation of Ser121 by MAPKAP K-2 Inhibits Transcription Activity of Heat Shock Factor 1</td>
<td>X. Wang (USA)</td>
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<td>DISCUSSION</td>
<td>All Participants</td>
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**Friday, April 23rd**

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<td>H-S Kou (TW) &amp; MD Sherar (CN) - Co-Chairs</td>
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<td>High Resolution Thermal Modeling; Status and Developments</td>
<td>JJW Lagendijk (NL)</td>
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<td>Uses of an Accurately Derived Bio Heat Transfer Equation and its Simplified Form</td>
<td>R Roemer (USA)</td>
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<td>8:00 am - 8:50 am</td>
<td><strong>Special Lectures: Hyperthermia Dependent Delivery of Therapeutic Agents</strong></td>
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<td>Pre-Clinical and Clinical Evaluation of Liposomal Drug Delivery Agents as Augmented by Hyperthermia</td>
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<td>P Corry (USA)</td>
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Special Lectures: Advances in Modeling & Treatment Planning  
Friday, April 23rd, 8:00 am – 8:50 am

High Resolution Thermal Modelling: Status and Developments

Department of Radiotherapy, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

The discrete vasculature (DIVA) thermal model has been developed to calculate the 3D temperature distribution of hyperthermia treatments. This model is able to take into account the full thermal impact of a vascular network and anatomy at a sub-mm scale.

Using DIVA with generic tissue models is highly successful for the investigation into the capabilities of heating methods. We constructed different generic anatomy models: the brain (designed for rf safety studies and the evaluation of interstitial hyperthermia), the prostate pelvic (the evaluation of regional and interstitial hyperthermia of the prostate), the orbit (evaluation of the treatment of melanoma and retinoblastoma and for cataract safety studies, both rf and infrared), and in preparation: skin and breast (hyperthermia and ablation studies). These generic models are constructed using data of patients, anatomy handbooks and microtome slices of corpses.

The application of DIVA for individual patients is still limited by data acquisition and segmentation The current limited status of perfusion measurements and angiography, using both MRI and multi-slice CT, combined with limited segmentation, still hampers the clinical introduction for individual patients.

A new application of DIVA is the test of the reliability of bioheat continuum (heatsink) models for individual patient treatment planning. The generic anatomy models are being used for the test of bioheat transfer theory using the discrete vessel model.

Obtaining 3D spatial control of the temperature distribution and understanding the tumour control probability (TCP) concept in hyperthermia are both holy grails in hyperthermia. The DIVA technology and generic models will greatly help in designing heating technology and understanding the clinical results.

Special Lectures: Hyperthermia Dependent Delivery of Therapeutic Agents  
Friday, April 23rd, 8:00 am – 8:50 am

Stress Activation of Therapeutic Genes

Peter M. Corry, William Beaumont Hospital, Royal Oak, MI, USA
Michael J. Borrelli, William Beaumont Hospital, Royal Oak, MI, USA

The introduction and activation of exogeneous genetic material intended to produce gene products for therapeutic purposes, often referred to as gene therapy, is an area with exceptional, but modestly realized promise to date. While some benign conditions, such as severe combined immune syndrome, are enjoying some success, these applications do not involve the use of controlled promoters. Unfortunately there are presently no successful routine clinical applications of this technology for application to neoplasms. One of the major stumbling blocks has been the lack of the ability to control expression of the exogeneous genes. Hyperthermia, combined with the use of a modified heat shock promoter from HSP70, offers a unique and exceptionally effective method of controlling therapeutic gene expression. A number of approaches to the heat controlled treatment of malignancies have been developed by several investigators. These include the introduction of genes for very toxic products, the production of enzymes that convert nontoxic (pro-drugs) to toxic drugs as well as the production of cytokines and the targeting of the hypoxic component of the tumor. Other investigators have used the approach of activating genes by heat induces oxidative stress and have exploited some unique properties of of the Grp78 promoter. Another approach has been the introduction of genes that can controllably enhance the effects of ionizing radiation by intererering with normal repair pathways. Application of stress inducible promoters are also being developed for the controlled therapy of a number of benign conditions. All of these approaches will be discussed in this review presentation and specific examples will be used as illustration for each case.
Saturday, February 28, 2004

1st Buffalo Winter Weekend Thermal Biology and Medicine Symposium “HSPs and Thermal Therapy” – Supported by Educational Grants from Coley Pharmaceutical Group & Seppic

Co-chairs: Sharon Evans, PhD, Julie Ostberg, PhD and John Subjeck, PhD

5:30-5:35
Introduction: Julie Ostberg, Ph.D.

5:35-5:50
Thermal regulation of lymphocyte/endothelial adhesion
Sharon S. Evans, PhD Buffalo NY

5:55-6:10
Thermal regulation of antigen presenting cells; Dendritic Cells and Macrophages
Michele Pritchard, PhD Case Western Reserve University, Cleveland, OH

6:15-6:30
HSP 110 and GRP170: The design of new chaperone vaccines
Co-presenters: Xiang Wang, PhD and Masoud Manjili PhD Buffalo NY

6:35-6:45
Development of a new HSP-110/Her-2/neu vaccine trial for breast and ovarian cancer
Gary Yang, MD Buffalo NY

6:50- 7:05
Role of HSP in regulation of MHC expression
Thomas Tomasi, MD, PhD, Buffalo, NY

7:10-7:25
Heat-Activated Gene Therapy With A Radiomimetic Proteotoxin
Mike Borrelli, PhD William Beaumont Hospital, Royal Oak, MI

7:30
CLOSING KEYNOTE ADDRESS:
Molecular pathways involving HSP 70 expression and function in antigen presentation and consequences in cancer treatment
Stuart Calderwood, PhD
Beth Israel Deaconess Medical Center, Harvard University School of Medicine
Introduced by Dr. John Subjeck

8:30 PM
A very “warm” evening reception featuring Ice Wines from the Ontario and New York State Wineries (Pillars Hotel Library, 2nd floor)

Sunday, February 29, 2004

Leap Year Sunday morning breakfast trip to the Winter Niagara Falls (please contact Diane Thompson: Diane.Thompson@roswellpark.org) if you would like to take this trip to see the Falls.
HEAT-ACTIVATED GENE THERAPY WITH A RADIOMIMETIC PROTEOTOXIN

Michael T. Borrelli, Diane M. Schoenherr, Laura Bernock, Sandra Galoforo, and Peter M. Corry.
Radiation Oncology Research Laboratory, William Beaumont Hospital, 3811 West Thirteen Mile Road, Royal Oak, MI 48073

Cytolethal distending toxin B (CdtB) is highly homologous with DNase 1, and its putative toxic lesion in mammalian cells is the production of unrepaired DNA double strand breaks (DSBs). CdtB cDNA was cloned into an adenoiviral vector under control of the human HSP70B heat shock promoter to be used as a heat shock-inducible, radiomimetic transgene for cancer gene therapy. The objective is to use localized heat shock to induce CdtB expression within tumors and a defined normal tissue margin to maximize CdtB cytotoxicity within tumors while minimizing normal tissue complications.

Experiments in human cell cultures have shown that CdtB expression is silent until activated by a heat shock of 41.0°C or higher. Once induced, DNA DSBs were observed in CdtB-expressing cells, as determined by neutral pulsed field gel electrophoresis and immunofluorescent staining with an antibody to phosphorylated H2AX. All cells expressing CdtB were killed and many nearby cells that did not express CdtB were also killed by a bystander effect whose mechanism is yet unknown. However, preliminary data indicate that CdtB bystander killing has many features in common with radiation bystander killing. Preliminary experiments with human xenograft tumors have shown that CdtB expression was silent until induced by heat shock. Once induced, CdtB gene therapy completely inhibited tumor growth without any detectable local or systemic normal tissue toxicity.