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TITLE: Photodynamic Therapy Oxidative Stress as a Molecular Switch Controlling Therapeutic Gene Expression for the Treatment of Locally Recurrent Breast Carcinoma

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13. ABSTRACT ***(Maximum 200 Words)***

Photodynamic therapy (PDT) is a developing therapeutic modality which continues to show promise in the clinical treatment of cancer, including locally recurrent breast carcinoma. Our application is directly related to using novel molecular technologies to improve the effectiveness of PDT for treating locally recurrent breast cancers. In PDT, properties of photosensitizer localization in tumor tissue and photochemical generation of reactive oxygen species are combined with precise delivery of laser generated light to produce a procedure offering local tumoricidal activity. We have demonstrated that PDT mediated oxidative stress is a strong transcriptional inducer of stress proteins belonging to the heat shock protein (hsp) and glucose regulated protein (grp) families. We have also shown that the hsp and grp promoters can drive inducible expression of heterologous genes following PDT mediated oxidative stress. Inducible expression and function of p53 as well as inducible expression, secretion and biological activity of TNF-a have been documented in human tumor cells. We have also demonstrated PDT inducible expression of the suicide gene HSV-thymidine kinase and enhanced tumoricidal action when PDT is combined with inducible HSV-TK gene therapy. These studies address a critical problem associated with improving treatments for locally recurrent breast cancer using new approaches which will minimize systemic toxicity and maximize a patient's quality of life.

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Army Grant Progress Report

Introduction:
Photodynamic therapy (PDT) is a therapeutic modality which has recently obtained FDA approval for the treatment of solid tumors. This therapy continues to show progress in clinical trials for a variety of malignancies including locally recurring breast cancer. Our research efforts have been directed at combining laser inducible gene therapy techniques with photodynamic therapy in an effort to improve the effectiveness of PDT for treating breast cancers. Photodynamic therapy involves the localization of photosensitizer within tumor tissue and the photochemical generation of reactive oxygen species such as singlet oxygen following the delivery of laser light specifically to the tumor tissue. The activating non-thermal light is normally generated by lasers and delivered via fiber optic cables. The procedure is highly localized and produces minimal systemic toxicity when compared to conventional chemotherapy or radiation therapy. Tumor responses following PDT have been positive but there remains considerable need for improvement in this therapeutic modality. At a molecular level photodynamic therapy mediated oxidative stress is a strong transcriptional inducer of variety of genes including those including stress proteins such as heat shock proteins (hsp) and glucose regulated proteins (grp). Elements within the promoter regions of genes encoding stress proteins can be used to efficiently drive inducible expression of therapeutic genes following photodynamic therapy. This is the type of combined treatment PDT plus inducible gene therapy) observations that we wish to exploit in enhancing the therapeutic response of PDT for breast cancer. These studies address a critical problem associated with improving treatments for locally recurring breast cancer using new approaches which will minimize toxicity and maximize quality of live.

Body:
The two specific tasks approves in our Statement of Work involve: 1) To transfect breast cancer cells with PDT inducible expression vectors and evaluate the gene expression, and 2) to determine the tumorcidal efficacy of photodynamic therapy in mice implanted with breast cancer cells containing PDT inducible expression vectors. During the preceding year, we focused our efforts on the refinement of PDT parameters and gene therapy protocols needed for providing enhanced, localized and selective therapeutic gene expression following photodynamic therapy. We have shown that the glucose regulated protein promoter provided excellent inducible expression of therapeutic genes such as the herpes simplex thymidine (HSV-TK) kinase suicide gene following photodynamic therapy. During the previous funding period we performed extensive kinetic analysis to determine the appropriate timing associated with PDT inducible heterologous gene expression. At a cellular level, we found that the expression of thymidine kinase was a function of photodynamic therapy treatment conditions (photosensitizer dose, light dose, incubation conditions) as well as the actual time intervals between light exposure and analysis. These studies have been evaluated utilizing Western Immunoblot analysis with a polyclonal HSV antibody. Our results have demonstrated that thymidine kinase expression occurs between 8 and 36 hours after photodynamic therapy treatment with maximal expression occurring 12 to 24 hours after treatment. Minimal expression is observed immediately after photodynamic therapy. Likewise, the temporal nature of our PDT inducible gene expression system is evident by the fact that HSV thymidine kinase expression returns to background levels at 72 hours following treatment. Expression of the thymidine kinase protein was evaluated in direct comparison to actin expression.

A second issue of considerable importance at the cellular level was to determine the actual PDT doses which were associated with maximal expression of thymidine kinase. In this regard, we observed that photodynamic therapy doses in the range of 315 to 420 J/cm^2 (with a 25 ug/ml Photofrin photosensitizer incubation) were associated with maximal thymidine kinase expression. Positive expression controls used the grp inducible calcium ionophor A23187. These data provide new and essential information regarding the parameters associated with effective inducible gene therapy initiated by photodynamic therapy.

An additional set of studies were performed during the previous funding period involving the in-vivo analysis of inducible gene expression parameters. Specifically, the questions of PDT dose and time intervals following treatment were analyzed using stably transfected mammary carcinoma cells transplanted as tumors in mice. The ability of in-vivo PDT treatments to induce expression of thymidine kinase was fairly uniform. PDT light doses ranging from 50 J/cm^2 up to 300 J/cm^2 all appear to be effective in the expression of thymidine kinase within solid tumor masses when a 5 mg/kg Photofrin drug dose was used. Above the dose of 300 J/cm^2 thymidine kinase expression diminished. We assume that this decrease in thymidine kinase expression correlates with a near complete killing of all cells and/or disruption of protein synthesis apparatus within the treated tumor tissue. Our studies of PDT controlled induction of thymidine kinase expression within tumors also illustrated that the kinetics for thymidine kinase expression plateau approximately 12 hours following treatment and remained steady up to 36 hours. Time periods after 36 hours were not obtainable due to the minimal amount of viable cells that one could collect. Concomitant studies documented localization of thymidine kinase expression using immunohistochemical analysis. These studies are still being completed. We have observed during our initial experiments selective expression of thymidine kinase within tumor tissues which corresponded to areas of PDT treatment.

Maximizing the treatment parameters for the most effective therapeutic gains is of utmost importance and is ongoing research. In addition to providing quantitative information regarding PDT treatment parameters such as time intervals and dosages necessary for maximal gene expression with the use of suicide gene such as thymidine kinase, the optimal administration parameters for ganciclovir are also required and such studies are, again, in progress.

Key Research Accomplishments:

1. Quantitative information regarding maximal expression profiles of thymidine kinase following photodynamic therapy have been obtained.
2. The kinetics and treatment parameters associated with maximal inducible expression of thymidine kinase with in-vivo tumors have also been obtained.
3. Immunohistochemical analysis of thymidine kinase expression within solid tumors have been started.

Reportable Outcomes:

Manuscript: Rucker, N., Ferrario, A. and Gomer, C.J. Constitutive over-expression of HSP-70 in thermal resistant tumor cells does not alter sensitivity to porphyrin, chlorin or purpurin mediated PDT. **J. Porphyrins and Phthalocyanines**, 5, 143-146, 2001.

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None

Degrees Obtained:
None

Development of Cell Line Tissues and Serum Repositories:
None

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Employment and Research Opportunities:
None

Conclusions:
During the second year of this research project we have been successful in obtaining quantitative information related to photodynamic therapy inducible expression of the herpes simplex thymidine suicide gene controlled by the grp promoter. Expression in breast cancer cells and tumors have been observed and the kinetics of expression and parameters of PDT treatment required for maximal expression have been identified. The information that we have obtained during this recent funding period refines the combination PDT plus inducible gene therapy protocol. The ability to have reproducible treatment parameters to control both the temporal and spatial activation of genes should be of significant benefit when the genes are expressed for therapeutic purposes.

References:

None

Appendices:

None