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Upregulation of IGF-I (Insulin-like Growth Factor) receptor dependent pathways like phosphatidylinositol 3-kinase (PI 3-kinase) may diminish chemosensitivity of breast cancer cells via c-Jun N-terminal Kinase (JNK) response. In this project we have determined that IGF-I activates the survival pathway involving PI 3-kinase and Akt. Using stable transfection approaches we have confirmed the survival effect of activated Akt in MCF-7 cells. Surprisingly, we have also observed that IGF-I induces JNK in breast cancer cells. IGF-I and chemotherapy co-treatment results in additive effect on JNK activity, in contrast to our predicted results that IGF-I would antagonize chemotherapy induced JNK activity. PI 3-kinase mediates IGF-I induction of JNK in MCF-7 cells but not in T47D cells. We plan to further confirm the importance of JNK in IGF-I mediated survival effects in breast cancer cells.

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Introduction

Cancer patients experience several toxicities from chemotherapy. One frequent toxicity is peripheral neuropathy, and strategies to relieve chemotherapy-induced neuropathy have had little success. Clinical trials using IGF-I have been proposed to combat chemotherapy-related neurotoxicities. IGF-I has trophic effects on motor neurons and is used for treatment of amyotrophic lateral sclerosis (ALS or Lou Gerig's disease) and diabetic neuropathy. However, there is concern about the potential for IGF-I to stimulate tumor growth or inhibit chemotherapy effects in patients receiving various treatments.

Body

Research accomplishments: We are studying the effects of IGF-I on breast cancer cells, focusing particularly on its ability to inhibit chemotherapy activity. We hypothesized that IGF-I acts through its receptor to induce at least one cell survival pathway. Task 1 (to characterize IGF-I receptor cytoprotective effects on PI 3-kinase) of this project was essentially completed in Year 1 of funding, as discussed in previous progress report.

Task 2 of this project is to characterize the potential interactions between the PI 3-kinase survival and JNK stress-induced signaling pathways by exposure to IGF-I -/+ chemotherapy using pharmacologic and molecular techniques to confirm these interactions. Previously, we had shown that JNK activity is induced by Taxol and Taxotere treatment in MCf-7 cells. Surprisingly, IGF-I treatment of cells markedly enhanced JNK activity as well. Co-culture with a PI 3-kinase inhibitor reduced IGF-I mediated JNK activity. In addition, when cells were co-treated with IGF-I and chemotherapy, JNK response was enhanced by co-treatment, rather than abrogated by IGF-I, as we had initially predicted. In order to better delineate the function of JNK in our model, MCF-7 cells were transfected with wildtype and dominant negative forms of JNK. Stable transfectants were cultured in Taxol alone, IGF-I alone, or Taxol + IGF-I. Our results generally support a role for JNK in mediating cell death. Our data do not support a role for JNK3 in mediating IGF-I survival responses, but we may not have observed this interaction for various reasons.

In the past year, we have also been studying if IGF-I induction of JNK may be observed in other cell lines especially since other investigators have published that JNK may confer survival effects only in cell expressing mutant p53. Since MCF-7 cells express wild-type p53, we decided to study the T47D breast cancer cell line. Indeed, we observe IGF-I induction of JNK in these breast cancer cells as well. In contrast to MCF-7 cells, IGF-I induction of JNK is not dependent upon PI 3-kinase. We have also developed a mutant p53 overexpressing transfectant of the MCF-7 cells to study JNK survival effects more completely in the future. We plan to further characterize the importance of p53 and PI 3-kinase in JNK function.

In reconsideration of the other proposed work in Task 2, to identify substrate specificity of JNK induced by chemotherapy or IGF-I, we believe we can more directly address the role of IGF-I activated JNK using JNK antisense approaches. We are establishing a collaboration to develop antisense approaches to JNK isoforms to ultimately determine if IGF-I survival effects are mediated in part through JNK.

We have initiated studies included in Task 3 which is to characterize if IGF-I and chemotherapy interactions in breast cancer cell lines are p53-dependent and compare ER positive and ER negative breast cancer cells with an immortalized, noncancerous breast epithelial cell line. These studies are partially completed, as discussed above in Task 2. We will continue to characterize additional breast cell lines.

Training accomplishments: In the past several months we have been developing an ecdysone inducible expression system in our MCF-7 cells in order to more clearly identify the roles of Akt and JNK in our model. Dr. Horwitz's laboratory has successfully developed an ecdysone-inducible system in the T47D cells and has been advising us in optimizing our conditions.

Reviewer comments on our manuscript submitted to *Cancer Research* requested two important areas for additional data to accept our manuscript. First, we needed to demonstrate if our observations in MCF-7 cells could be applied to other cell lines. We believe we have done this using the T47D cells. Secondly, the reviewer wanted an additional apoptosis assay to confirm our initial work. This latter request has required extensive effort to identify an assay that may be used to measure apoptosis in MCF-7 cells. MCF-7 cells lack expression of caspase 3, a protein responsible for many of the morphological characteristics of cell apoptosis. We believe we have finally accomplished this goal and plan to resubmit our manuscript shortly.

Key Research Accomplishments:

- Additional apoptosis assay development
- In contrast to MCF-7 cells, IGF-I induction of JNK is PI 3-kinase independent in T47D cells
- Similar to MCF-7 cells, IGF-I and stress treatments of breast cancer cells lead to increased JNK activity compared to either treatment alone

Reportable Outcomes

Manuscripts

Mamay, C.L., Wolf, D.M., Molina, D.M., Van Den Berg, C.L. "The cooperative and opposing roles of JNK and Akt in IGF-I mediated survival of chemotherapy treated breast cancer cells". Submitted to *Cancer Research.*

Funding Based on Work Supported by this Award

Grants Funded:

"IGF-I survival effects on p53 induced apoptosis." Charlotte Geyer Foundation, November 2000 to May 2001. Total budget $58,000 (bridge funding mechanism).

"IGF-I survival effects on p53 induced apoptosis." National Cancer Institute, June 2001 to July 2004. Principal Investigator. Total Budget: $509,625.