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13. ABSTRACT (Maximum 200 Words)

High levels of Wilms' Tumor 1 (WT1) mRNA in breast tumors have been linked with poor prognosis for breast cancer patients. However, the function of WT1 protein in breast cancer was not known. We reported the expression of WT1 protein in 9 out of 10 human breast cancer cell lines. The levels of WT1 protein were increased by the HER2/neu oncogene and 17β-estradiol. We demonstrated that WT1 protein is vital to the proliferation of breast cancer cells since down regulation of WT1 protein expression led to breast cancer growth inhibition and apoptosis, which was correlated with decreased cyclin D1 and Bcl-2 levels. WT1 has been shown to undergo two splicing events, which result in four different isoforms. Stable transfection of the different WT1 isoforms was performed in MCF-7 cells. Our data indicate that the WT1 isoforms enhance the in vitro proliferation of MCF-7 breast cancer cells, but do not modulate the sensitivities of MCF-7 cells to doxorubicin, taxol, or tamoxifen. WT1 protein enhances breast tumorigenesis induced by other oncogenes or growth factors, such as HER2/neu and estradiol, but its over expression alone is not sufficient to induce breast tumorigenesis.

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INTRODUCTION

The Wilms' Tumor 1 (WT1) protein and mRNA is expressed in human breast tumors and breast cancer cell lines. Loeb et al. [1] demonstrated that *WT1* mRNA and protein are expressed in nearly 90% of breast cancers, but not in most normal breast tissues. Silberstein et al. [2] suggested that there was an association of WT1 protein expression with a biologically aggressive phenotype of breast cancer. Recently, Miyoshi et al. [3] correlated high levels of *WT1* mRNA with poor prognosis in breast cancer patients. But it is not known how WT1 protein contributes to breast tumorigenesis. One aim of this project is to determine if the WT1 protein contributes to breast tumor progression by deregulating cell proliferation and apoptosis. The deregulation of proliferation and survival pathways has been associated with chemoresistance in many tumors. Therefore, it is hypothesized that WT1 regulates chemoresistance in breast cancer cells. This project seeks to determine the mechanisms and the isoforms by which WT1 deregulates breast cancer cell proliferation and apoptosis.

BODY

Specific Aim 1: To determine whether WT1 overexpression increases the proliferation and survival of breast cancer cells in cell culture models

We observed that the levels of WT1 protein correlated with the proliferation of breast cancer cells [4]. When the proliferation of breast cancer cells was stimulated by 17β-estradiol, WT1 protein expression increased. But when the proliferation of breast cancer cells was inhibited by tamoxifen or all-*trans* retinoic acid, WT1 protein expression decreased [4]. We also observed that MCF-7 breast cancer cells stably transfected with the *HER2/neu* oncogene express approximately 3-fold higher levels of WT1 protein than parental MCF-7 cells [5]. Conversely,

inhibition of HER2/*neu* with the anti-HER2/*neu* trastuzumab (Herceptin™) antibody or inhibition of Akt with an Akt inhibitor decreased WT1 protein levels in HER2/*neu*-overexpressing BT-474 and SKBr3 breast cancer cells, indicating that HER2/*neu* engages Akt to increase WT1 protein expression [5]. Since WT1 protein levels were increased by 17β-estradiol and HER2/*neu*, two factors known to stimulate breast tumor proliferation, we hypothesize that WT1 protein plays a role in regulating breast cancer cell proliferation. Using liposome-incorporated WT1 antisense oligodeoxynucleotides, we found that downregulation of WT1 protein expression led to cell cycle arrest at the G1 phase and increased apoptosis, which is correlated with decreased cyclin D1 and Bcl-2 levels, in breast cancer cells [4, 5]. These results indicate that WT1 protein plays a vital role in mediating the proliferative and anti-apoptotic signals in breast cancer cells. Our data support earlier observations that WT1 may play a vital role in the aggressive phenotypes of breast cancer cells [1, 3].

WT1 has been shown to undergo two splicing events, which result in four different isoforms. These isoforms are able to bind to different DNA promoter elements and different protein partners. Plasmids encoding the four isoforms “A”, “B”, “C”, and “D” of the wild type *WT1* gene were transfected into human MCF-7 breast cancer cells to prove that overexpression of the WT1 gene will increase breast cancer cell proliferation and survival. Stable transfectant clones were selected. The transfection remained stable for up to at least 6 months. Western blot confirmed that WT1 protein was expressed about 2-3 levels higher in the transfectants than in the parental cells. The CellTiter 96 Aqueous nonradioactive proliferation (MTS) assay was used to determine the proliferative rates of these transfectants. Compared to parental and vector-transfected cells, all four isoforms increase MCF-7 cell proliferation by about 130-150%. But

flow cytometric analysis does not show any difference in the distribution of cells in the different phases of the cell cycle between the WT1 transfectants and the control cells.

Specific Aim 2: To determine whether WT1 overexpression increases breast tumor growth in animal models

MCF-7 wild type cells and MCF-7 transfectants (vector control, WT1 isoform "A", WT1 isoform "D") were implanted into the mammary fat pad of nude mice that had 0.72 mg of 17β-estradiol pellets. We chose WT1 isoforms "A" and "D" for the *in vivo* studies because these isoforms were believed to regulate different genes since they were found to bind to different DNA elements [6]. Four weeks later, tumors were found from mice implanted with the control wild type and vector cells. Six out of nine mice implanted with MCF-7wild type cells form tumors, and eight out of eight mice implanted with MCF-7/vector cells form tumors. However, no tumor was found in mice implanted with the MCF-7 cells stably transfected with the WT1 "A" isoform or the WT1 "D" isoform. Our data support those of Zhang et al. [7] who found that WT1 protein inhibited tumor growth of MDA-MB-231 breast cancer cells in nude mice.

Specific Aim 3: To determine whether WT1 regulates chemoresistance in breast cancer cells

MTS assay was used to compare the chemosensitivity of WT1 transfectants with control cells. No difference is observed between the doxorubicin and the taxol sensitivity of any of the isoforms and the control cells. Similarly, no difference is observed between the tamoxifen sensitivity of any of the isoforms and the control cells.

KEY ACCOMPLISHMENTS

- Published our data that WT1 protein increases the expression of cyclin D1 protein, and increases the proliferation of breast cancer cells.

  Zapata-Benavides, P., Tuna, M., Lopez-Berestein, G., and Tari, A. M. Downregulation of Wilms' Tumor 1 Protein Inhibits Breast Cancer Proliferation. Biochem. Biophys. Res. Commun., *295:*784-790, 2002.

- Published our data that the *HER2/neu* oncogene engages the Akt pathway to increase the expression of WT1 protein. WT1 protein stimulates the transcription of *cyclin D1* and *Bcl-2* genes. WT1 protein plays a vital role in mediating proliferative and anti-apoptotic functions in HER2/*neu*-overexpressing breast cancer breast cancer cells.

  Tuna, M., Chavez-Reyes, A., and Tari, A. M. HER2/*neu* increases the expression of Wilms' Tumor 1 (WT1) protein to induce S-phase proliferation and inhibit apoptosis in breast cancer cells. Oncogene, *24:*1648-1652, 2005.

REPORTABLE OUTCOMES

Abstract

1. Tuna, M. and Tari, A. M. HER2/*neu* uses Akt to increase WT1 expression in breast cancer cells. Proc. Amer. Assoc. Cancer Res., *44 (1st ed):* 1939, 2003.

The abstract was selected for oral presentation. Dr. Tuna received an AACR research award for the abstract.

Manuscripts

1. Zapata-Benavides, P., Tuna, M., Lopez-Berestein, G., and Tari, A. M. Downregulation of Wilms' Tumor 1 Protein Inhibits Breast Cancer Proliferation. Biochem. Biophys. Res. Commun., *295:*784-790, 2002. (See attached)
2. Tuna, M., Chavez-Reyes, A., and Tari, A. M. HER2/*neu* increases the expression of Wilms' Tumor 1 (WT1) protein to induce S-phase proliferation and inhibit apoptosis in breast cancer cells. Oncogene, *24:*1648-1652, 2005. (See attached)

CONCLUSIONS

We are very surprised that the four WT1 isoforms, which have been shown to bind to different partnering proteins and different DNA sequences, appear to behave quite similarly *in vitro* in the MCF-7 breast cancer cell background. Furthermore, it was very disappointing that the WT1 transfectants did not form tumors in nude mice. Thus, we believe that WT1 protein enhances breast tumorigenesis induced by oncogenes and growth factors, such as HER2/*neu* and estradiol. But its overexpression alone is not sufficient to induce breast tumorigenesis

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LIST OF PERSONNEL RECEIVING PAY FROM THE RESEARCH EFFORT

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