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The c-erbB2 (or HER-2, *neu*) gene encodes a 185-kDa transmembrane glycoprotein (p185), which belongs to the EGF-r family. The c-erbB2 gene was found to be amplified and/or overexpressed in approximately 30% of human breast carcinomas. Our previous studies demonstrated that c-erbB2 overexpression can enhance metastatic potential and confer increased chemoresistance to breast cancer cells, thereby leading to poor clinical outcome in breast cancer patients. Our recent studies demonstrated that overexpression of the c-erbB2 gene can protect human breast cancer cells from apoptosis induced by the chemotherapeutic agent Taxol. One of the mechanisms is overexpression of c-erbB2 can upregulate p21^{Cip1}, which inhibits taxol-mediated p34^{Cdc2} activation, delays cell entrance to G2/M phase, and thereby inhibits taxol-induced apoptosis. This new finding provided an explanation on c-erbB2 mediated chemoresistance of breast cancer cells and also explored the importance of p21^{Cip1} in G2/M phase transition. Since we only use the 435 and its c-erbB2 transfectants in this study, we would like to further confirm this in other c-erbB2 overexpression breast cancer cell lines with different genetic background. Then we will find out whether other p34^{Cdc2} regulators contribute to taxol resistance.

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FOREWORD

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Date

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Progression Reports for 1999-2000

A.Introduction

The c-erbB2 (or HER-2, *neu*) gene encodes a 185-kDa transmembrane glycoprotein (p185), which belongs to the EGF-r family . The c-erbB2 gene was found to be amplified and/or overexpressed in approximately 30% of human breast carcinomas. Our previous studies demonstrated that c-erbB2 overexpression can enhance metastatic potential and confer increased chemoresistance to breast cancer cells, thereby leading to poor clinical outcome in breast cancer patients. Our recent studies demonstrated that overexpression of the c-erbB2 gene can protect human breast cancer cells from apoptosis induced by the chemotherapeutic agent Taxol. One of the mechanisms is overexpression of c-erbB2 can upregulate p21^{Cip1}, which inhibits taxol-mediated p34^{Cdc2} activation, delays cell entrance to G2/M phase, and thereby inhibits taxol-induced apoptosis. This new finding provided an explanation on c-erbB2 mediated chemoresistance of breast cancer cells and also explored the importance of p21^{Cip1} in G2/M phase transition. Since we only use the 435 and its c-erbB2 transfectants in this study, we would like to further confirm this in other c-erbB2 overexpression breast cancer cell lines with different genetic background. Then we will find out whether other p34^{Cdc2} regulators contribute to taxol resistance.

B. Specific Aims

The specific Aims 1 and 2 have been modified. Since we found that the activity of p34^{Cdc2} is important of taxol-induced apoptosis. In our early finding that p21^{Cip1}

play an important role in 435.eB transfectants to inhibit the activity of p34^{Cdc2}. We plan to study other c-erbB2 overexpression breast cancer cell lines to see whether this is a general phenomenon.

Aim 1. Identify p34^{Cdc2} regulators involved in Taxol resistance in breast cancer cell lines.

Aim 2. Investigate these p34^{Cdc2} regulators are direct involved in Taxol resistance in breast cancer cell lines.

C. Study Results and Significance

Aim 1. Identify p34^{Cdc2} regulators involved in Taxol resistance in breast cancer cell lines.

To determine whether the activity of p34^{Cdc2} is important in other breast cancer cell lines, we use two taxol sensitive breast cancer cell lines MDA-MB-435, MDA-MB-231 and two taxol resistant cell lines BT 474, MDA-MB-361 (Appendices Fig.1), to compare their p34^{Cdc2} activity. Since phosphorylation of tyrosine 15 residue is an indication of inhibition of p34^{Cdc2} activity, I used immunoblotting with phospho-tyr-15 specific antibody to determine the activity of p34^{Cdc2} in these four cell lines (Appendices Fig.2). The phosphorylation of tyrosine 15 is higher in taxol resistant cell lines compared with taxol sensitive cell lines. Since Wee1 and Cdc25C are two important regulators to regulate the phosphorylation status on p34^{Cdc2} tyrosine 15 residue, I screened these two factors by using immunoblotting. Interestingly, Wee1 expression level is higher in BT474 and 361 cell lines (Fig.3.). I also screened other p34^{Cdc2} regulators p21^{Cip1} and cyclin B1(1). As expected, p21^{Cip1} expression level is higher in BT 474 and 361 cell lines (Fig.4.) whereas

cyclin B1 expression level is much lower in these two cell lines(1). From above data, multiple regulators are involved in regulating the taxol sensitivity. Currently, aim 1 already been finished, I will start the aim 2 study.

D. Plans

Aim 1. Identify p34^{Cdc2} regulators involved in Taxol resistance in breast cancer cell lines.

Finished.

Aim 2. Investigate these p34^{Cdc2} regulators are direct involved in Taxol resistance in breast cancer cell lines.

I will design or construct anti-sense p21 oligonucleotide, anti-sense wee1 plasmid and cyclin B1 plasmid, transfect each one of them into the taxol resistant cell lines BT474 and 361. Then to investigate whether bring down or bring up one of these factors can resensitize the breast cancer cell to taxol induced apoptosis.

E. Conclusions

With the data we already had, overexpression of c-erbB2 can confer the taxol induced apoptosis by upregulation of p21^{Cip1}, which in turn inhibits the taxol mediated activation of p34^{Cdc2}. But in other breast cancer cell lines, other p34^{Cdc2} regulators like Wee1 and cyclin B1 also play a role in p34^{Cdc2} inhibition. These leading us to find out that p34^{Cdc2} may be an important target for taxol resistance.

F. Reference

1. Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M.-C. Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of p21^{Cip1}, which inhibits p34^{Cdc2} kinase, Molecular Cell. 2: 581-591, 1998a.

Appendices

FIGURE 1. DNA ladder assay of breast cancer cell lines sensitivity to taxol induced apoptosis

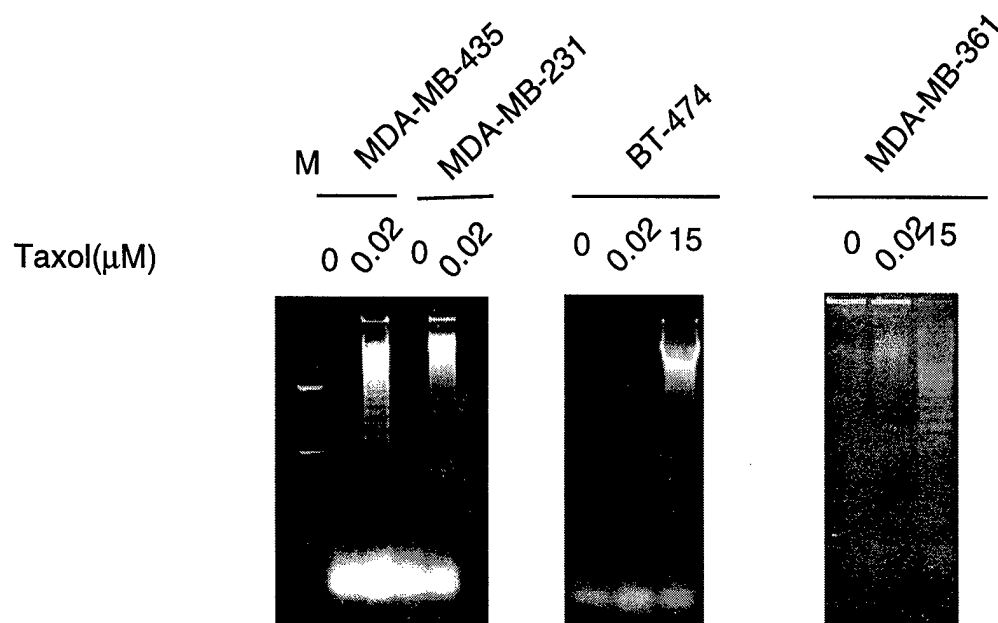


FIGURE 2. Western Blotting of phosphorylation of Cdc2 in different breast cancer cell lines

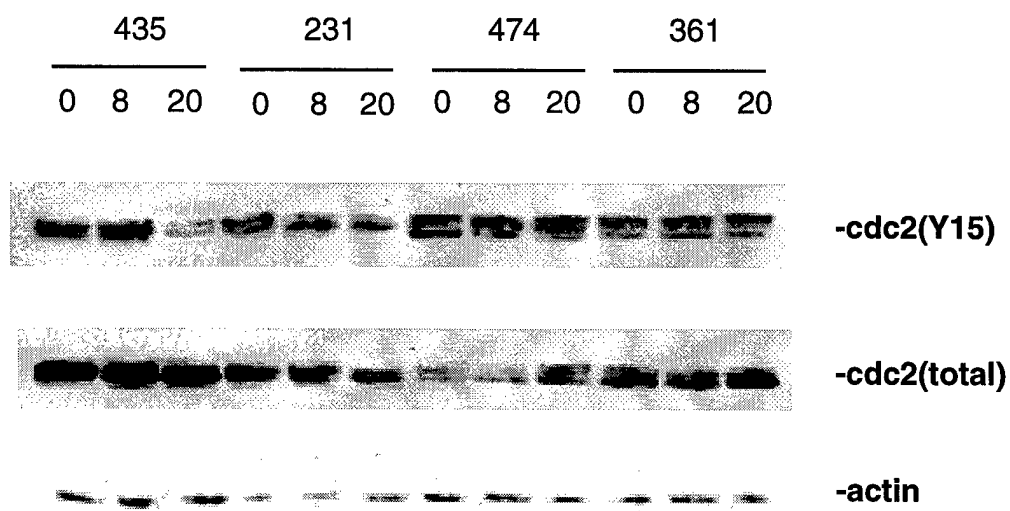


FIGURE 3. Western blotting of Wee1 and Cdc25C expression level in breast cancer cell lines

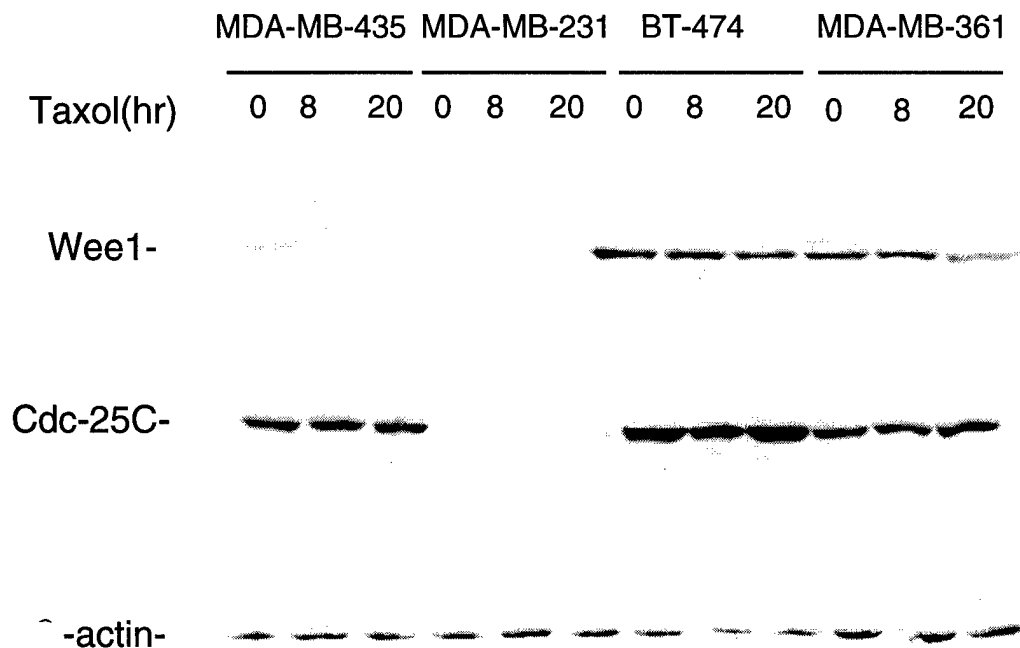


FIGURE 4. Western blotting of p21 expression level in breast cancer cell lines

