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The *HER-2/neu* gene is frequently amplified and /or its protein product, p185, is frequently overexpressed in a number of human cancers. Overexpression of p185 correlates with poor prognosis and low survival rates in breast and ovarian cancer patients. Downregulation of *HER-2/neu* gene is a way to reverse the transformation phenotype induced by *HER-2/neu*. In this project, the effects of PEA3, a member of *ets* oncogene family of transcriptional regulatory factors, on *HER-2/neu* expression were investigated to learn whether PEA3 plays a role in *HER-2/neu* induced transformation and could be developed to a gene therapeutic agent. This progress report includes our recent data which demonstrating that, PEA3 is indeed a trans-repressor of *HER-2/neu* promoter in *HER-2/neu*-overexpressing human cancer cells. Expression of PEA3 also blocked *HER-2/neu*-mediated transformation in mouse fibroblast and suppressed the growth of *HER-2/neu*-overexpressing human cancer cells. Therefore PEA3 has tumor suppression activity in breast cancer cells in vitro. The consensus sequence of PEA3 binding site on *HER-2/neu* promoter, a DNA element for efficient transcription, is required for PEA3-mediated transcriptional repression. That PEA3 competes for binding to *HER-2/neu* promoter with another ETS-related *trans*-activator may explain the mechanism of *trans*-repression of the *HER-2/neu* promoter by PEA3. The *in vivo* studies about effects of PEA3 on the tumor growth in orthotopic breast cancer model is under investigation. This project will develop a HER-2/neu targeting gene therapy agent for breast cancer.

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BACKGROUND

Breast Cancer Cause High Death Rate for Women in the United States. It was estimated that more than 180,000 new cases of breast cancer are diagnosed and 46,000 women die from this disease in the United States each year. It was also recently estimated that one in eight women will have breast cancer in their life span in the United States. Therefore, there is an urgent need to develop novel agents for breast cancer diagnosis, prevention and therapy.

Amplification/overexpression of the *HER-2/neu* oncogene was first found in human breast cancer (approximately 30%) (1-3) and then ovarian cancer (4-6). It was also reported that the overall survival rate and time of relapse for those breast and ovarian cancer patients, with *HER-2/neu* overexpression, is significantly shorter than those without *HER-2/neu* overexpression, indicating that *HER-2/neu* overexpression is an excellent prognostic factor (7-10). A large number of literature demonstrates that *HER-2/neu* overexpression is a cause (and not just a consequence) of human cancer. In addition to breast and ovarian cancer, *HER-2/neu* overexpression has recently been found also in lung, gastric, bladder, and oral cancers with high frequency (11-14), suggesting that *HER-2/neu* overexpression most likely plays a critical role in the development of human malignant cancer cells. So *HER-2/neu* oncogene is a good target for development of therapeutic agent for *HER-2/neu* overexpressing human cancers.

We have previously demonstrated that mutation-activated *HER-2/neu* oncogene can enhance experimental metastasis in mouse embryo fibroblast (15). More recently, by transfecting normal *HER-2/neu* gene into a lung cancer cell line with very low level of *HER-2/neu* expression, we further showed that overexpression of normal *HER-2/neu* gene also enhances metastatic potential by promoting multiple steps associated with metastasis (16). The results have provided convincing evidence that increased *HER-2/neu* expression can enhance the metastatic potential of human lung cancer cells. Interestingly, *HER-2/neu* overexpression was also found to cause chemoresistance in human cancer cell lines. (17, 18). **Taken together, these findings suggest that *HER-2/neu* overexpression can enhance metastatic potential and induce chemoresistance in human cancer cells, and**

provide a plausible interpretation for the poor clinical outcome for patients with *HER-2/neu*-overexpressing cancers. Transcriptional repression of HER-2/neu may be a very effective way of downregulating the HER-2/neu gene expression and its resultant transformation.

PEA3 (Polyomavirus Enhancer Activator 3) is a member of the ETS gene family. It has DNA binding domain capable of binding the PEA3 motif (5'-AGGAAG-3') (19). PEA3 binding site occurs in the upstream regions of a number of genes, whose products are involved in cell growth, migration and differentiation, suggesting that this protein plays a regulatory role in mammalian development and differentiation (20, 21). Our recent results show that PEA3 protein specifically binds to *HER-2/neu* promoter, highly represses *HER-2/neu* expression in *HER-2/neu*-overexpressing human ovarian cancer cells. **The results prompted us to hypothesize that the PEA3 may function as a tumor suppressor for the *HER-2/neu*-overexpressing cancer cells by repression of *HER-2/neu* overexpression. The current research will aim at development of PEA3 gene therapy for *HER-2/neu*-overexpressing breast cancer.**

The purposes

The current proposal will develop a tumor suppressing agent of PEA3 targeting at HER-2/neu-overexpressing breast cancer cells.

1. Testing the transcriptional repression activity of PEA3 in human breast cancer cells.
2. Testing the effects of PEA3 on growth and transformation activity of human breast cancer cells.
3. Investigating the mechanism of PEA3 on regulating the *HER-2/neu* gene expression, and the mechanism for PEA3 mediated cell growth arrest in human cancer cells.
4. Constructing Adenovirus expressing PEA3 and testing the tumor suppression activity of PEA3 in orthotopic breast cancer model, using both liposome and adenovirus gene delivery system.

Body

Experimental procedures

Plasmids - For the *HER-2/neu* promoter driven luciferase expressing vector, the promoter region of human *HER2/neu* was amplified with a pair of primers (Sense: 5'-GATAGGATCCGGGGGTCCTGGAAGCC and antisense: 5'-GGGCAGATCTGGTTTTCCGGTCCCAATGGA). The amplified DNA fragment was treated with BamHI and BglII, and ligated into the BamHI site of pGL2-Basic (Promega, Madison, WI); the resultant plasmid was named pNeulit. The sequence of this insert was confirmed, as described (21).

For site-directed mutagenesis construction of pm-pNeulit, two pairs of primers were used to do overlapping PCR, including the primers mentioned above and the other pair of primers spanning the PEA3 site on the *HER-2/neu* promoter(antisense: 5'-CATTCTTATACGAGCTCAAGCTCCTCC and sense: 5'-GGAGGAGGAGGGCTGCTTGAGCTCGTATAAGAATG). PCR fragment was treated with BamHI and BglII, ligated and sequenced as constructing pNeulit.

The full length PEA3 cDNA was cloned into the ClaI/BamHI site of the vector pSRα to make pSRαPEA3 and PEA3 CDNA was cut out from pSRαPEA3 by HindIII and BamHI and cloned into the same sites of pCDNA3 vector to get pCDNA3-PEA3.

Cells- Mouse fibroblast NIH3T3 and human cancer cells, MDA-MB-453, MDA-MB-435, SK-BR-3, SK-OV-3.ip1, 2774 c-10 were maintained in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum and 10% fetal bovine serum, respectively.

Focus forming assay- $2x10^5$ NIH3T3 cells per petri dish were plated to 100-mm culture dished 24 hrs before transfection. Using a modified calcium phosphate precipitation technique, cells were transfected with 0.5 μg of pSV2neo, 1 μg of cNu104 plus 5 μg of pSRαPEA3 or pSV2E, or 0.5 μg pSV2neo plus 6 μg of pSRαPEA3. Or with 0.5 μg of pSV2neo, 1 μg of pSV2*neu** plus 5 μg of pSRαPEA3 or pSV2E. Two days after transfection, cells were split to 4 plates, two were selected in 500 μg/ml of G418

(Life Technologies, Inc), and the other two maintained in normal medium. Media were changed every 3 days until foci appeared. The number of foci was normalized by the corresponding number of neomycin resistant colonies.

Gel mobility shift assay- The oligonucleotide derived from *HER-2/neu* promoter (with wide type PEA3 site: 5'-GGAGCTCGAGGGCTGCTTGAGGAAGTATAAGAATG-3'; with PEA3 site mutated: GGAGCTCGAGGGCTGCTTGAGGTCGTATAAGAATG-3') was end labeled by [^{32}P]-γATP. Binding of the protein factors to DNA sequences was achieved in a mixture containing 1 X binding buffer (20 mM HEPEs, pH 7.9, 5 mM MgCl2, 5% glycerol, 0.1 M KCl, 0.2 mM EDTA, 2 mM DTT), 1 μg PEA3-GST, 2-8 μg poly d(I) d(C), 1 μg of BSA, and 20,000 cpm of [^{32}P]-labeled DNA binding fragment. The binding reaction was performed at room temperature for at least 10 min. These samples were electrophoresed in 1 X TBE in a 4% or 5% polyacrymide gel that had been pre-run at 100V for at least 30 min. Gel were dried for 45 min under vacuum and autographed.

Transient transfection and luciferase assay- 100 mm petri dish or 6-well cell culture plate was seeded the human cancer cells for transfection to get a 60-80% of confluency. Transfection was done by using the amount of DNA (indicated in the figure) complexed with DC-chol liposome in a ratio of 13 nmol lipid/1 μg of DNA in serum free condition for 2-5 hrs. Cell extracts were harvested 48 hrs later, using 800 μl/100 mm dish (or 130 μl/ 6-well) of 1 X Reporter Lysis Buffer (Promega, Madison, WI). Luciferase activity was measured by taking 50 μl of the cell extract and injected 100 μl of assay substrate (Promega, Madison, WI), and light output was measured for an integration time of 10 seconds using the monolight 2010 Luminometer (Analytical Luminescence Laboratory). To normalize for variations in transfection efficiency, the values obtained from luciferase values were divided by ß-galactosidase values. The ß-galactosidase assays to control for transfection efficiency were performed by mixing equal amounts of cell extract and 2 X Buffer (120 mM Na_2HPO_4, 80 mM NaH_2PO_4, 2 mM $MgCl_2$, 100 mM ß-mercaptoethanol, 1.33 mg/ml orthonitrophenyl galactose), incubating at 37^{o} until a faint yellow color has developed (usually 30 min- 2 hrs), and reading the absorbance at 420 nm.

Stable transfection and colony formation assay-Different human cancer cells were transfected 1 μg of PEA3 expression plasmids or its backbone plasmid pCDNA3, using DC-chol liposome as a carrier, and 48 hours later selected with G418 500μg/ml-800 μg/ml. Media were changed every 3-4 days until the colonies formed. Transfection in each cell line was repeated 2-3 times with cells of different passages and DNA from separate extractions.

RT-PCR for gene expression- The colonies derived from cancer cells transfecting the PEA3 or backbone plasmids were developed to cell lines, total RNA was extracted and the reverse transcription and PCR were done using the SuperScript preamplification system (Life Technologies, Inc). The primers for PCR are sense: 5'-TGAATTATGACAAGCTGAGCCG, and antisense: 5'TCAGCGAGCTCTAGCATTTAGG. GAPDH primers(sense: 5'-AAGGTGAAGGTCGGAGTCAAC, and antisense: 5'-TCCATTGATGACAAGCTTCCC) were used as PCR control. Transiently transfection of PEA3 to Cos1 cells was a positive control for RT-PCR procedure.

Adenovirus vector-PEA3 cDNA was cut out from the pCDNA3-PEA3 vector at HINDIII and NotI sites and was inserted to the adenovirus shuttle vector at the same sites. This shuttle vector will be cotransfected with JM17 to 293 cells to get recombined PEA3-Adenovirus.

Results

Repression of HER-2/neu transcription in HER-2/neu-*overexpressing breast cancer cells by PEA3* To examine whether PEA3 regulates transcription of the *HER-2/neu* gene in Human breast cancer cells, we did a CAT co-transfection experiment using the reporter plasmid pHUNeu2-CAT in which the 530-bp promoter region of the *HER-2/neu* gene was linked to the CAT gene. When pHUNEU2-CAT was transfected into a *HER-2/neu*-overexpressing human breast cancer cell line MDA MB453 with the effector plasmid to express the PEA3 protein, the level of CAT activity was decreased compared to with the control effector plasmid expressing no protein. The repression of PEA3 on human *HER-2/neu* promoter activity exhibits a dose dependent manner.(Figure 1A). Thus, PEA3 protein dramatically represses the transcriptional activity of human *HER-2/neu* promoter.

PEA3 suppresses the transforming activity of the activated genomic rat neu To further demonstrate whether the PEA3 repression of *HER-2/neu* transcription may result in suppression of the transformation caused by *HER-2/neu*, focus forming assay was performed in mouse fibroblast NIH3T3. cNu104 is a cosmid containing mutation-activated genomic rat *neu* under its own promoter. NIH3T3 cells were transformed by expression of *neu* through transfection of cNu104, and foci formed on the top of the monolayer cells. This foci formation was dramatically reduced while PEA3 expression plasmids was co-transfected with cNu104 (Figure 1B). However, transfection of PEA3 was not able to affect the formation of foci caused by a plasmid expressing the activated *neu* under the SV40 promoter (data not shown). These data indicated that PEA3 gene suppresses the transformation of activated *neu* through transcriptional regulation.

PEA3 binds directly to HER-2/neu *promoter* To examine whether PEA3 binds directly to the *HER-2/neu* promoter, gel shift analysis was performed using the bacterially expressed PEA3 and oligonucleotide sequence derived from *HER-2/neu* promoter spanning the consensus sequence of the PEA3 binding site. A protein-DNA

complex was detected which could not be competed out in the presence of excess amount specific competitors(Figure 2A). However, PEA3 protein was not able to form a complex with the mutated oligo in which the core sequence of PEA3 binding site was changed from AGGAAG to AGCTCG(Figure 2B). These results clearly demonstrate that PEA3 protein specifically binds to *HER-2/neu* promoter at AGGAAG consensus site *in vitro*.

Binding to neu *promoter is required for PEA3-mediated transcriptional repression*

A reporter plasmid pNeulit was constructed in which the 530-bp promoter region of the *HER-2/neu* gene was linked to the luciferase gene. Site-directed mutagenesis was used to make plasmid bearing mutant *HER-2/neu* promoter driven luciferase gene (called pm-pNeulit), in which the PEA3 binding core sequence, AGGAAG was changed to AGCTCG. PEA3 would not bind to the mutated PEA3 sites according to the gel shift assay. Transient expression of PEA3 in the breast cancer cells did not affect the activity of pm-pNeulit in a transfection assay(Figure 3A). Directly DNA binding is thus required for PEA3 mediated transcriptional repression of *HER-2/neu* gene. The transcriptional activity of pm-pNeulit is dramatically decreased compared with wild type *HER-2/neu* promoter construct pNeulit(Figure 3B), the only difference between which is the PEA3 binding site, suggesting that the PEA3 site is originally a targeting sequence of a transactivator in *HER-2/neu*-overexpressing cancer cells, the abolishment of which caused a lower transcriptional activity. Another study suggested that an ETS-related protein binds to *HER-2/neu* promoter in breast cancer cells (22). ETS family transcriptional factor share DNA binding site through their highly homologous ETS DNA binding domain. Our study further suggested that the transcriptional activation of *HER-2/neu* gene by this ETS-related protein might contribute to *neu*-induced transformation, the effects of PEA3 on *HER-2/neu* gene transcription and transformation are likely mediated through competition between PEA3 and this activator.

PEA3 expression suppresses the growth of HER-2/neu-*overexpressing human cancer cells* *HER-2/neu* overexpression contributes to the transformation and oncogenesis of human breast and ovarian cancer. To test whether expression of PEA3 suppresses the growth of cancer cells, *HER-2/neu*-overexpressing human breast cancer cells MDA MB 453 and SK-BR-3 and ovarian cancer cells SK-OV-3.ip 1 were transfected with the PEA3 expression plasmids and were placed under G418 selection. Two to four weeks later, drug resistant colonies were stained with crystal violet. Transfection with the PEA3 expression plasmids produced very few number of colonies. In contrast, many drug-resistant colonies formed following transfection with the backbone expression plasmid pCDNA-3. Same transfections were also performed in other cell lines expressing basal level of *HER-2/neu* gene, a breast cancer cell line MDA MB435 and an ovarian cancer cell line 2775 c-10. However, transfection with PEA3 and its backbone expression plasmid formed similar number of drug-resistant colonies in these two cell lines. Together, these experiments indicate that reintroduction of PEA3 can specifically suppress the growth of *HER-2/neu*-overexpressing human cancer cells (Figure 4 and table 1).

RT-PCR was performed to further examine the expression of PEA3 gene in these colonies. One of the primers recognizes the coding region of PEA3, while the other one for the 3' untranslated sequence from derived from vector plasmid, therefore, only the exogenous PEA3 mRNA would be detected, i.e. the ones from the transfected PEA3 expression plasmids. We examined 10 colonies from PEA3 expression plasmids transfection in MDA MB 435 cells. All of these are positive in PEA3 PCR. When these colonies from PEA3 transfection in *HER-2/neu*-overexpressing breast cancer cells are analyzed, only 2 out of 10 colonies from MDA MB 453 cells are positive (data not shown). It indicates that PEA3 expression suppresses the growth of *HER-2/neu*-overexpressing cells, thus most of the PEA3 expressing cells were not able to form colonies, the few colonies remained and analyzed were those with very low or no PEA3 expression. In the cells with basal level

of *HER-2/neu*, the growth of PEA3 expressing cells was not affected and thus PEA3 positive colonies formed.

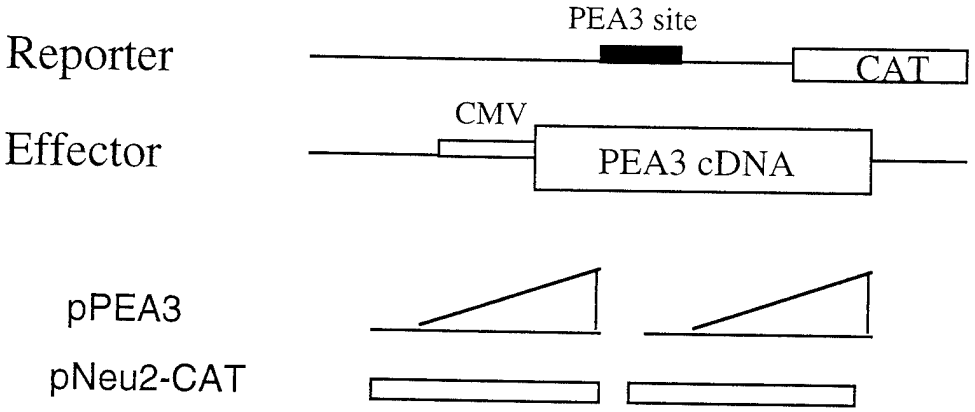
Inducing apoptosis may be the mechanism for PEA3 induced cell growth arrest-We observed that PEA3 selectively suppresses growth of human cancer cells which overexpress *HER-2/neu* proto oncogene. We would like to further pursue through which mechanism does this inhibition occur. According to the literature that ets1, another ETS family transcriptional factor, can induce apoptosis in colon cancer cells and does mediated transformation suppression function(23). We thus tested whether PEA3 could mediated apoptosis. We transfected PEA3 and its backbone vector plasmid into cos1 cells and co-transfected CMV-luciferase vector as a reporter. When we removed the serum in the cell culture plate for 24 hrs before we test the luciferase activity in the cell lysates, we found out that the cells with PEA3 transfection had much less luciferase activity in serum depletion condition. While the cells transfected with vector had similar activity under either with or without serum condition(Figure 5). This suggests that apoptosis might occur in PEA3 transfected cells in the serum-free condition, however we did need further characteristic assay to confirm this notion.

Cunstructing adenovirus expression vector of PEA3 for gene therapy preclinical trial

We have already demonstrated that PEA-3-liposome complex treatment enlongates the survival of mice-bearing *HER-2/neu*-overexpressing tumors in orthotopic ovarian cancer model. Adenovirus has the advantage of high transfection efficiency, we would like to make such expression system for PEA3. The shuttle vector-bearing PEA3 cDNA and JM17 are ready for transfecting 293 cells. We expected to see recombined PEA3-adenovirus in 2-3 months.

Figure. 1A PEA3 inhibits the *HER-2/neu* promoter activity in *HER-2/neu* - overexpressing breast cancer cell lines.

Human breast cancer cells, MDA MB453, were cotransfected with 5 μg pNeu2-CAT along with PEA3 plasmid DNA of 0, 5, 10, 15 μg. The transfection efficiency was normalized the β-galactosidase enzyme activity



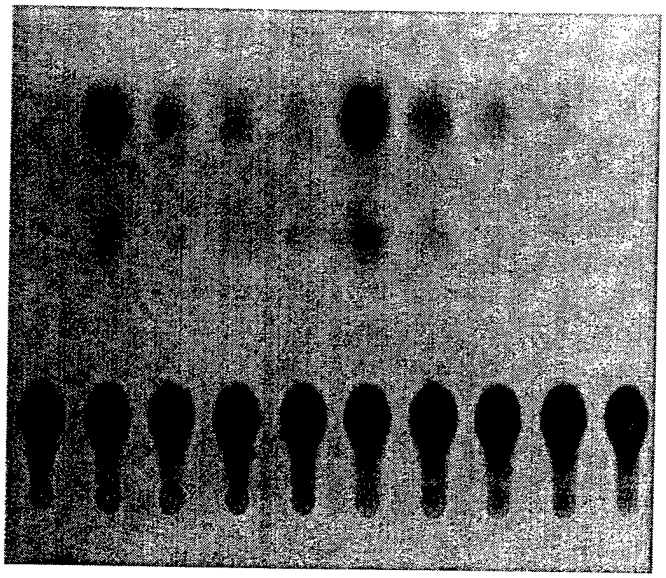


Figure. 1B Repression of the transforming activity of activated genomic rat *neu*.

cNu104 and/or pSRαPEA3 were cotransfected with pSV2-neo into NIH3T3 mouse fibroblast. Cells were split to 1:4 48 hours after transfection. duplicated plates were subsequently grown in regular medium (DMEM-F12 supplemented with 10% calf serum) or regular medium supplemented with 400 μg/ml G418. Foci and G418-resistant colonies were stained and counted after 3 weeks. Results were expressed as a ratio of foci to colonies from each transfection to normalized transfection efficiency. The ratio of transfecting cNeu104 alone was set as 100%. Bar: SD.

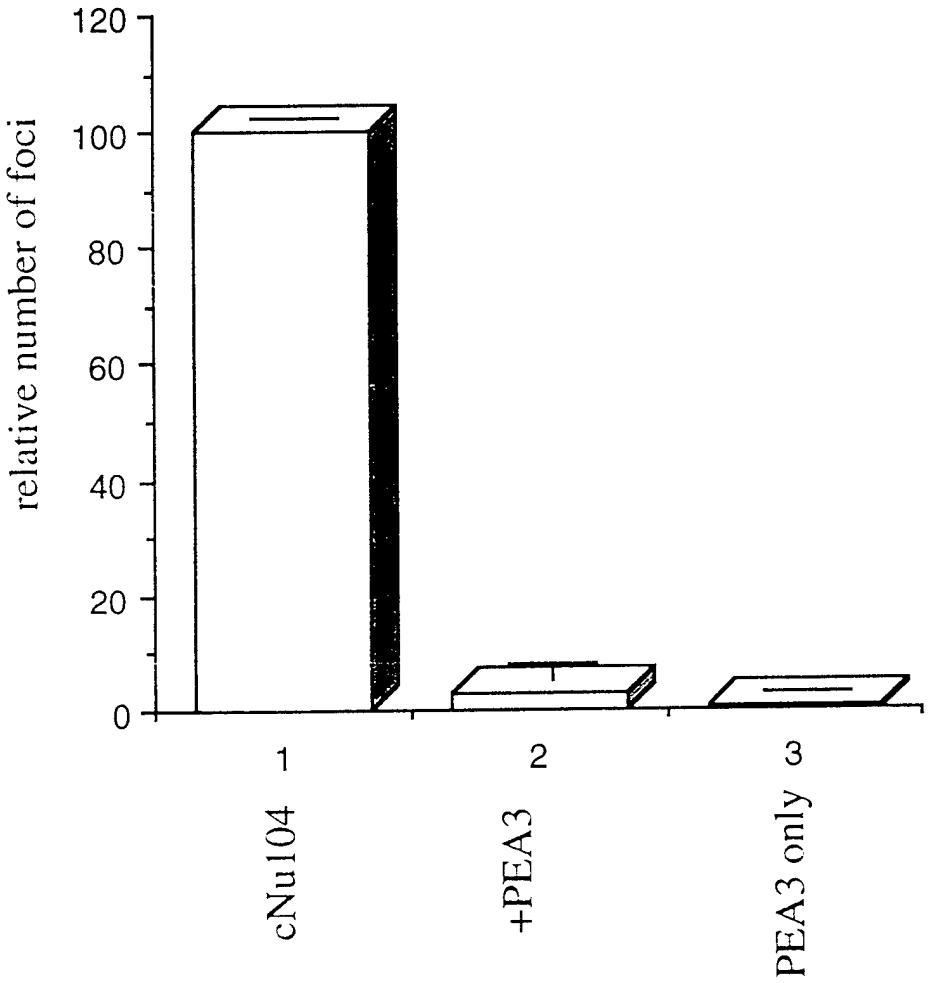


Figure. 2. Binding of PEA3 to HER-2/*neu* promoter *in vitro.*

A. Radioactivity labeled oligonucleotide probe derived from *HER-2/neu* promoter was end labeled by ^{32}p-γ ATP. 1 μg of GST-PEA3 fusion protein was used to interact with the oligonucleotide alone or with the presence of 100 fold molar excess of the same oligonucleotide, or with the presence of 100 fold excess of non-specific oligonucleotide. As controls, the buffer, and 1 μg of GST protein was also incubated with the labeled probe.

B, the same as A, except that the PEA3 protein was used to interact with both wide type oligo from HER-2/neu promoter, and the oligo with mutation at PEA3 motif.

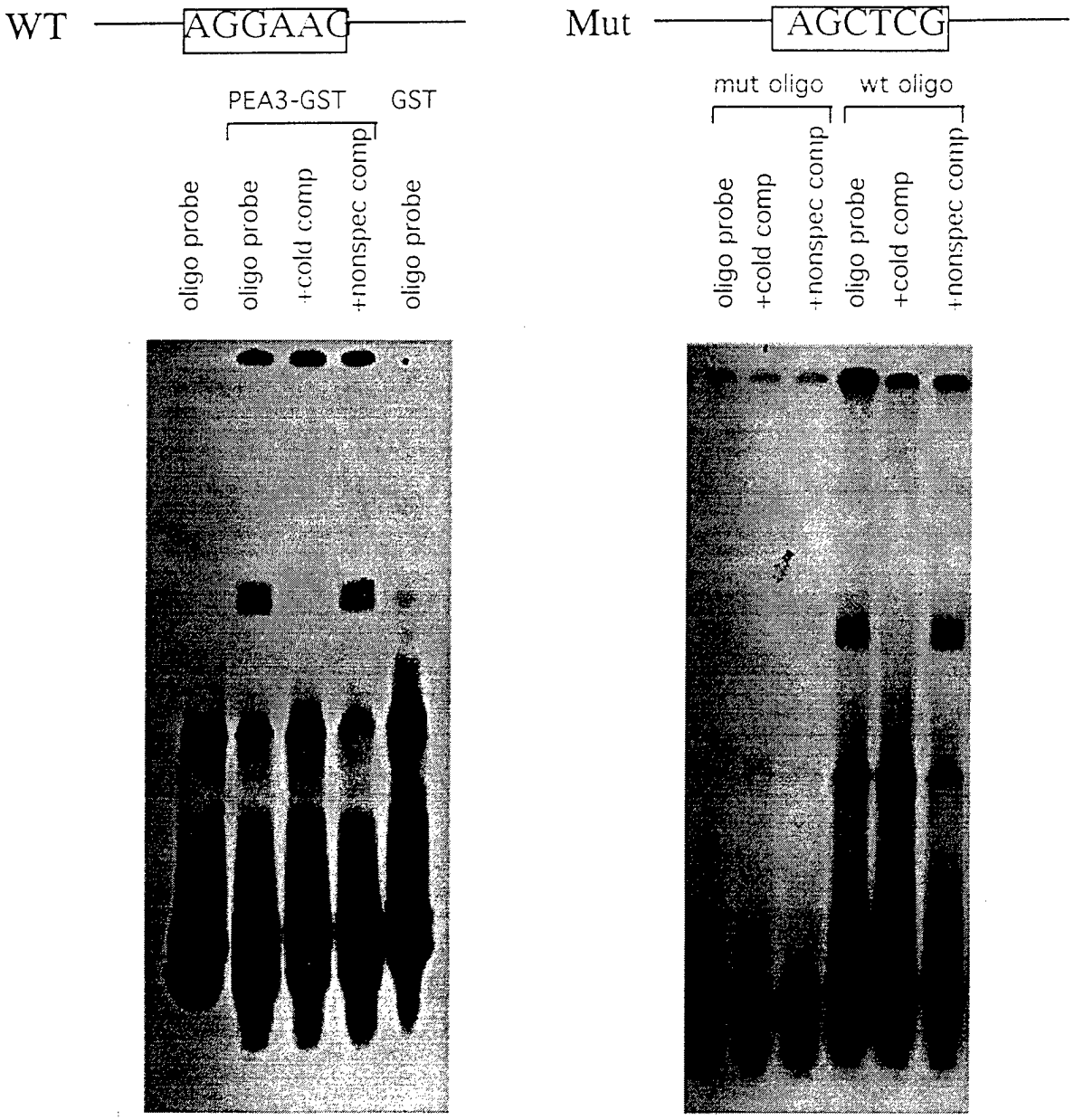
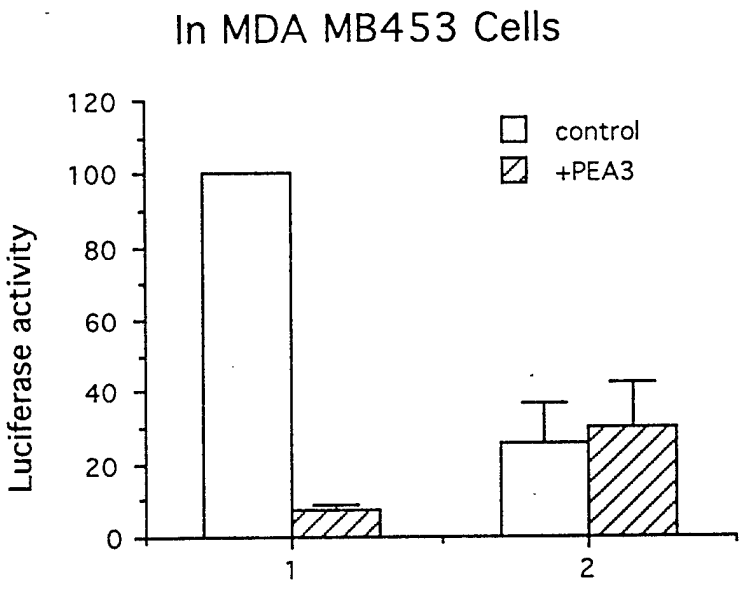


Figure. 3 PEA3 regulates HER-2/*neu* promoter through *ets* consensus site

Human breast cancer cells MDA MB453 were cotransfected with PEA3 plasmid and HER-2/*neu* promoter luciferase reporter or its counterpart that mutates the *ets* site. Bar. SD The activity of pNeulit was set to 100%.



1. Wild-type promoter

2. Mutant promoter

Figure. 4 PEA3 inhibits colony formation of breast cancer cells

Human breast cancer cells were transfected by 5 μg of either pCDNA3-PEA3 plasmid or pCDNA3 vector. Three days later, cells were split to 4 plates and were cultured in 10% FCS DMEM in the presence of 500 μg/ml of G418 for 3 weeks and the colonies that formed were stained.

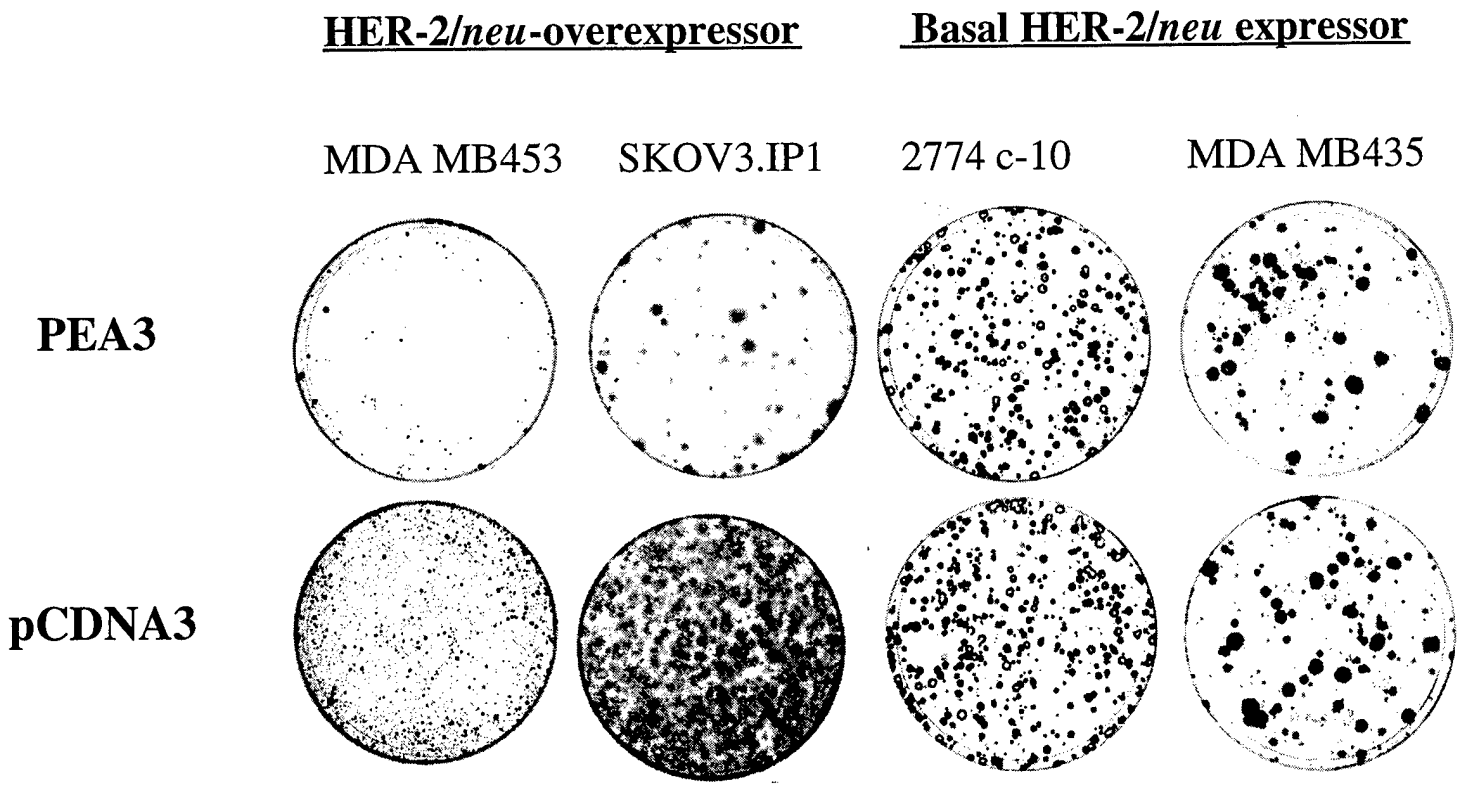
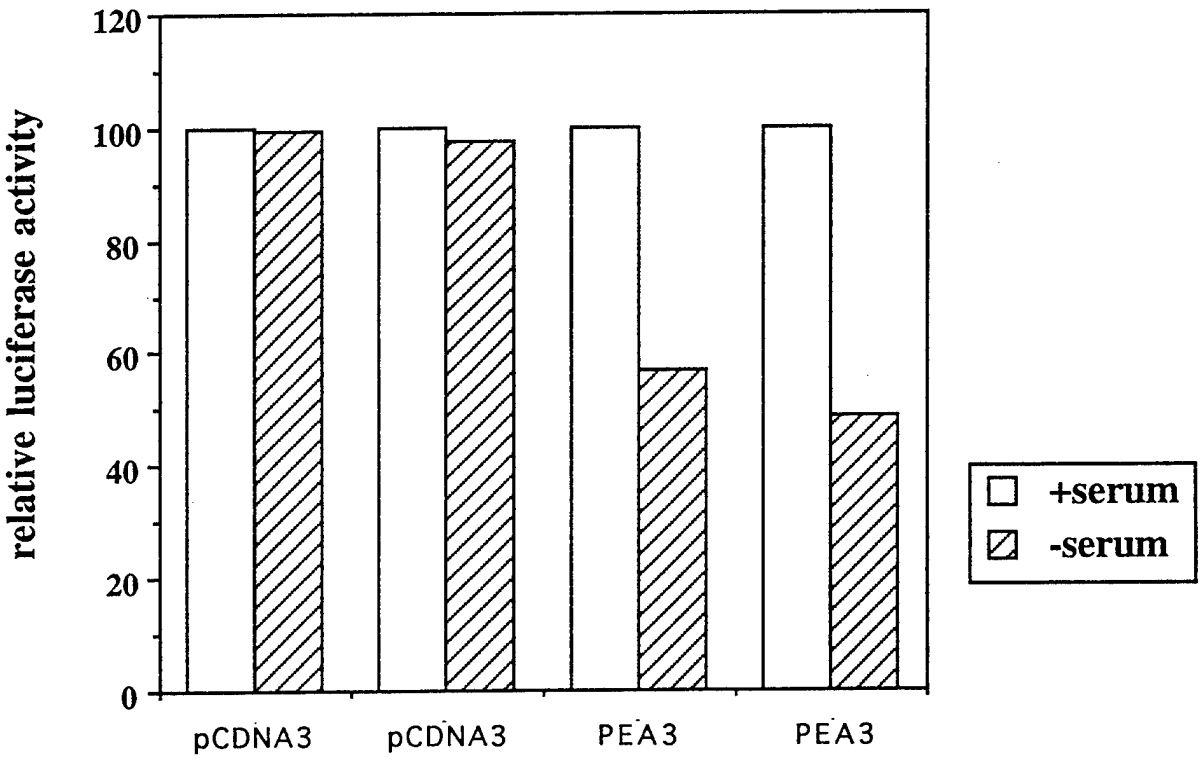


Table 1, PEA3 suppresses the growth cancer cells-overexpressing *HER-2/neu*
Transfection in each cell lines was performed 2-4 times independently, using different batches of cell and DNA. The tendency is the same among them. The data presented here is one representative experiment.

	# of colonies	
	PEA3	pCDNA3
HER-2/neu overexpresser		
MDA-MB-453	47	235
SK-BR-3	3	228
SK-OV-3.ip1	55	382
basal level *HER-2/neu* expresser		
MDA-MB-435	181	290
2774 c-10	100	126

Figure 5. Expression of PEA3 might induce apoptosis

The baby monkey kidney cell Cos1 was transfected CMV luciferase together with PEA3 or pCDNA3 vector plasmids. Cells were cultured in the presence or absence of 10 % FCS for 24 hours before measuring the luciferase activity. Bar. SD.



CONCLUSION:

1. PEA3 represses HER-2/neu transcription and activated neu-mediated transformation.

In addition to the data that PEA3 represses HER-2/neu expression in HER-2/neu - overexpressing ovarian cancer cells, we demonstrated that PEA3 could mediated transcriptional repression function in HER-2/neu-overexpressing human breast cancer cells. PEA3 suppresses the foci formation of genomic neu, but not the cDNA neu driven by SV40 promoter, thus PEA3 suppresses neu-induced transformation through transcriptional regulation(figure 1A and figure 1B).

2. PEA3 represses neu through DNA-binding competition.

We proposed that the transactivation of *neu* expression through the ETS sequence by an Ets family transcriptional factor contributes to transformation of *HER-2/neu*. The expression of PEA3 may compete its DNA binding and might mediate a transcriptional repression by the occupation of a binding site for a transactivator(figure 3, & figure 4).

3.PEA3 suppresses growth of HER-2/neu-overexpressing cancer cell growth.

Reintroduction of PEA3 in human cancer cells by stable transfection, We demonstrated that PEA3 gene highly inhibits the colony formation of HER-2/neu-overexpressing cancer cells(Figure 4 and table 1).

4. Expression of PEA3 in cells suppresses cell growth, might through the induction of apoptosis.

Using RT-PCR, we examined the expression of PEA3 in colonies from HER-2/neu-overexoressing cells and from basal level HER-2/neu expressing cells and found out that the most of the colonies (80%) developed from PEA3 transfection do not express PEA3 while all the HER-2/neu basal level cells have PEA3 expression, indicating that PEA3 expression block cell growth(data not shown). Apoptosis might be the mechanism of PEA3 mediated growth arrest(Figure 5).

Task 1. Examination of tumor suppression activity of PEA3 in breast cancer cell lines. When we were testing the effects of tumor suppression activity in breast cancer cells described in specific aim 1, we found reintroduction of PEA3 to human breast cancer cells-overexpressing HER-2/neu resulted in the growth arrest and the colony formation is much less than the cells-expressing basal level of HER-2/neu(fig.4, table1). We also observed that the expression of PEA3 represses HER-2/neu gene transcription in breast cancer cells, and suppresses neu-mediated transformation(fig.1a, 1b) . These strongly indicate tumor suppression activity of PEA3 in HER-2/neu-overexpressing breast cancer cells. The mechanisms of the PEA3 effects was also investigated(fig. 2, 3a, 3b) .

Task 2. Examination of the anti-tumor effects of PEA3 by using liposome-mediated gene transfer. To set up orthotopic breast cancer animal models, HER-2/neu-overexpressing cell MDA MB453, MDA MB361 and basal level HER-2/neu expresser MDA MB435, MDA MB 231 had been tested for tumorigenecity in M.P.F. MDA MB 361 could develop 0.5 cm in diameter of tumor nodules after 2 months of inoculation while and MDA MB 231 can develop similar tumor nodules in a month, both will be used for PEA3-liposome and Adenovirus PEA3 gene therapy preclinical study.

Task 3. Construction of Adenovirus vector carrying PEA3, and control without PEA3. We have already finished subcloning of PEA3 cDNA and vector into shuttle plasmid. We are doing cotransfection of recombinant plasmid and pJM17 into 293 cells to generate infectious viral vector by recombination.
The resultant virus will be used for testing the effects of PEA3 on human cancer cells in vitro and perform (task 4) the preclinical trial study in orthotopic breast cancer model developed in task 2.

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EDUCATION/TRAINING

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Jilin University, China	B.S.	1990	Biochemistry & Molecular Biology(3.82)
Peking (Beida) University, China	M.S.	1993	Biophysics(3.80)
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RESEARCH AND PROFESSIONAL EXPERIENCE:

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1997	Special Commendation, UT GSBS
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1992	Excellent Study Award, Peking University
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Research experience after degree of bachelor in science:

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Publications:

1. **Xing, X**. The Tertiary Structure of Glucose Isomerase.(1990) Thesis for B.S.

2. **Xing, X**. The Effects of Temperature Sensitive Liposome on Targeting and Selectively Drug-Releasing to Liver Cancer Cells. (1993) Thesis for M.S.

3. Hung, M-C., Matin, A., Zhang, Y., **Xing, X.**, Sorgi, F., Huang, L., and Yu, D. (1995) *HER-2/neu* targeting gene therapy-a review. ***Gene***, 159:65-71.

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   9th International Congress On Breast Disease of Senologic International Society, Houston, 1996.
   And
   Regulatory Mechanisms in Growth and Differentiation-49th Annual Symposium on Fundamental Cancer Research, Houston, 1996.

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