

Transcriptional repression in cancer gene therapy: targeting *HER-2/neu* overexpression as an example

Review Article

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Summary

Overexpression of the *HER-2/neu* oncogene has been well-documented as a frequent event in human cancers. In clinic, overexpression of *HER-2/neu* indicates a unfavorable prognosis and highly correlated with the low survival rate of patients associated with breast and ovarian cancers. Downregulation of the *HER-2/neu* gene expression in cancer cells by attenuating the promoter activity of the gene is therefore an attractive strategy to reverse the transformation phenotype induced by *HER-2/neu* overexpression. We have identified a number of cellular and viral transcriptional regulators, including the *ets* family member *PEA3*, the SV40 large T antigen, and the adenovirus type 5 *E1A*, which are able to repress the *HER-2/neu* gene expression. Expression of these transcriptional regulators resulted in downregulation of the *HER-2/neu* promoter activity and reversed the malignant phenotype of the transformed cells *in vitro*. These observations were followed by a series of studies to investigate whether these *HER-2/neu* repressors can act therapeutically as tumor suppressor genes for cancers that overexpress *HER-2/neu*. The growth of tumors derived from *HER-2/neu*-overexpressing cancer cells was inhibited by the transcriptional repressors, accompanied by decreased *HER-2/neu* expression in tumor cells. The results of these preclinical studies clearly indicate that transcriptional repressors which downregulate *HER-2/neu* can be a promising regimen for cancer treatment in a gene therapy format.

I. Introduction

A. *HER2/neu* overexpression serves as a critical target for cancer gene therapy

The *HER-2/neu* (also known as *c-erbB2*) gene encodes a receptor tyrosine kinase (p185) with significant structural and functional homology to the epidermal growth factor receptor (EGFR) (Bargmann et al, 1986a, Hung et al, 1986, Yamamoto et al, 1986). Each protein member of the erbB receptor family contains an extracellular domain, a transmembrane domain, and an intracellular domain with intrinsic tyrosine kinase activity. Although the ligand for the HER2/neu receptor has not been identified, the HER2/neu receptor is known to mediate lateral signal transduction through all erbB receptor family members (Craus-Porta et al, 1997, Wallasch et al, 1995, Carraway et al, 1994, Sliwkowski et

al, 1994, Plowman et al, 1993), due to the preference for the HER2/neu receptor as a heterodimerization partner for all erbB receptors. After ligand binding, EGFR, HER-3 (also known as erbB3), and HER-4 (also known as erbB4) can heterodimerize with HER2/neu, and can lead to the tyrosine phosphorylation of all of these receptors (Craus-Porta et al, 1997, Wallasch et al, 1995, Sliwkowski et al, 1994).

The oncogenic property of the *HER-2/neu* proto-oncogene was originally demonstrated in the rat *neu* oncogene (Hung et al, 1989, Bargmann 1986b, Hung et al, 1986). As a matter of fact, the mutation-activated rat *neu* oncogene, which contains a point mutation in the transmembrane domain of the protein resulting in a constitutive tyrosine kinase activity, was originally isolated from rat neuroblastoma due to its ability to transform mouse cells (Hung et al, 1989, Bargmann et al,

1986b, Hung et al, 1986). In human, the *HER-2/neu* proto-oncogene is frequently amplified or overexpressed in many types of cancers including breast (Gusterson et al, 1992, Toikkanen et al, 1992, Slamon et al, 1989, Slamon et al, 1987), ovarian (Slamon et al, 1989, Burchuck et al, 1991, Burchurk et al, 1990), lung (Shi et al, 1992, Weiner et al, 1990, Schneider et al, 1989), stomach (Yokota et al, 1988, Park et al, 1989), and oral (Xia et al, 1997) cancers, suggesting that *HER-2/neu* overexpression plays a critical role in the development of human cell malignancy. The overall survival rate of cancer patients whose tumors have *HER-2/neu* overexpression is significantly shorter than those patients whose tumor do not have *HER-2/neu* overexpression (Slamon et al, 1989, Slamon et al, 1987, Burchurk et al, 1990, Weiner et al, 1990, Xia et al, 1997). Furthermore, increased expression of the *HER-2/neu* gene has been shown to correlate with the number of lymph node metastases in breast cancer patients (Slamon et al, 1987), an observation consistent with many studies in that the mutation-activated *neu* gene induced metastatic potential in mouse 3T3 cells and that overexpression of the normal human *HER2/neu* gene enhanced metastatic potential in human breast, ovarian, and non-small-cell lung carcinoma (NSCLC) cells by promoting multiple steps in the metastatic cascade (Tan et al, 1997, Yu et al, 1994, Benz et al, 1993, Yu et al, 1992a, Chazin et al, 1992, Yu and Hung 1991a, Slamon et al, 1989, Slamon et al, 1987). In addition to metastasis of cancer cells, it is generally believed that *HER-2/neu* overexpression is correlated to chemoresistance of cancer cells. High level of *HER-2/neu* expression in human NSCLC appeared to result in enhanced resistance to a panel of chemotherapeutic agents (Tsai et al, 1995, Tsai et al, 1993). Similarly, overexpression of *HER-2/neu* in breast cancer cells induced chemoresistance to Taxol (Paclitaxel) (Yu et al, 1998, Yu et al, 1996). However, the expression level of *HER-2/neu* seems to be critical for the development of chemoresistance since in certain cell lines moderate p185 expression level is not accompanied with significant drug resistance (Pegram et al, 1997). It is likely that the *HER-2/neu* expression has to be higher than a threshold level to induce significant drug resistance. Furthermore, the chemoresistance developed in those *HER-2/neu*-overexpressing breast cancer cells is limited to Paclitaxel and Taxotere but not to other drugs (Pegram et al, 1997; Yu et al, 1996; Yu, D. and Hung, M. -C., unpublished results), suggesting a selective mechanism of resistance. It is not yet clear why *HER-2/neu* overexpression-mediated drug resistance behaves differently between lung and breast cancer cells. However, in the case of resistance to Paclitaxel by *HER-2/neu* overexpression in breast cancer cells, a molecular mechanism has recently been suggested (Yu et al, 1998a): upregulation of p21 by *HER-2/neu* overexpression inhibits cyclin B/cdc2 kinase activity in G2/M phase which is required for Paclitaxel induced apoptosis. This mechanism clearly indicates that *HER-2/neu* overexpression in breast

cancer cells antagonizes Paclitaxel-induced apoptosis.

Since the *HER2-neu* proto-oncogene overexpression significantly contributes to the malignant development of many types of human cancers in different aspects, molecular strategies which aim to down-regulate the *HER-2/neu* gene expression have become highly attractive approaches to fight against human cancer.

B. Transcriptional repression as an effective means to downregulate *HER2/neu* expression in cancer cells

HER-2/neu gene amplification can be detected in majority of breast tumor tissues with overexpression of the *HER-2/neu*-encoded p185 protein (Slamon et al, 1989). In established breast cancer cell lines, both gene amplification and transcriptional upregulation are common scenario accounting for the increased *HER-2/neu* gene expression in different breast cancer cells (Kraus et al, 1987, Millar et al, 1994, Boshier et al, 1996). Interestingly, it has been shown that in 10-20% of *HER2/neu* -overexpressing breast tumors and in virtually all *HER2/neu*-positive lung cancers the *HER2/neu* mRNA and protein expression can occur in the absence of increased gene copy number (Kameda et al, 1990, Kern et al, 1990, Slamon et al, 1989, King et al, 1989, Tandon et al, 1989, Berger et al, 1988). It is therefore likely that both gene amplification and transcriptional upregulation are involved in *HER-2/neu* overexpression in cancer cells.

The promoter of the *HER-2/neu* gene has been well characterized. In the past few years, knowledge about the *cis*- and *trans*-acting elements regulating the transcription of the *HER-2/neu* proto-oncogene have been rapidly accumulated. A number of *cis*-acting motifs are distributed along the *HER-2/neu* promoter, including the binding sites of transcription factors Sp1, OTF1, AP2, E4TF1, and PEA3. Another 13-bp sequence in the promoter region has been identified as a positive element for *HER-2/neu* transactivation (Miller et al, 1994). The corresponding binding transcription factor(s), however, has not yet been identified. AP2 has been shown to be a strong activator of the *HER-2/neu* gene and is functionally activated in the *HER-2/neu*-overexpressing breast cancer cell lines such as MDA-MB-361, MDA-MB-175, ZR-75-1, BT-474, and SK-BR-3 (Hollywood et al, 1993). The high activity of AP2 in these cell lines has been correlated with the elevated *HER-2/neu* gene expression level in these cells. On the other hand, the *HER-2/neu* gene is subject to the negative regulation of a number of cellular or viral factors through different mechanisms. For example, PEA3, a member of the *ets* family (X. Xing, S. -C. Wang, and M. -C. Hung, unpublished results; Xing et al, 1997), and the retinoblastoma tumor suppressor (*RB*) (Yu et al, 1992b) can repress the *HER-2/neu* gene expression. Interestingly, in addition to the cellular factors, the *HER-2/neu* gene

transcription can also be repressed by a number of viral transcription factors such as the simian virus 40 (SV40) large T antigen and the adenovirus type 5 *E1A* (Yan et al, 1991a, Yu et al, 1991b). These studies have indicated that repression of transcription is an effective way to reverse the malignant transformation mediated by *HER-2/neu* overexpression, and have demonstrated the potential application of transcriptional repressors as therapeutic agents targeting *HER-2/neu*-overexpressing cancer cells.

II. Tumor suppression effects of *HER-2/neu* down-regulation mediated by genes encoding transcriptional regulators

A. Tumor suppression by viral transcriptional regulators

Both *E1A* and T antigen are viral proteins, and their ability to suppress *HER-2/neu*-mediated cell transformation is surely a surprising biological phenomenon. The adenovirus genome is about 36 kb in size. Among the proteins encoded by the adenovirus genome, *E1A* gene products are nuclear-localized phosphoproteins and have special regulatory role in the adenoviral life cycle (Berk 1986). *E1A* is the first region to be expressed after infection (Tooze 1981). Other late adenoviral genes can then be turned on by *E1A* proteins through interacting and modifying the host transcriptional apparatus. There are two types of adenovirus *E1A*. One is the transforming *E1A* carried by the adenovirus type 12. This type of *E1A* gene alone can transform normal cell lines (Schrier et al, 1983). The other type of *E1A*, such as the adenovirus type 2 or type 5 *E1A*, can not transform cells by itself alone. It is noteworthy that for the purpose of this review *E1A* refers to the type 5, non-transforming *E1A*. *E1A* was classified as an "immortalization oncogene" due to its ability to cooperate with the transforming *ras* or *E1B* genes to transform primary embryo cells (Byrd et al, 1988, Montell et al, 1984, Land et al 1983, Ruley 1983). However, expression of the *E1A* gene itself does not induce transforming phenotypes (Yu et al, 1992a). As a matter of fact, there are a number of studies indicated that *E1A* is associated with metastasis- or tumor-suppression activities (Pozzatti et al, 1988, Frisch 1991, van Groningen 1996). Recently, *E1A* has been shown to induce apoptosis under some conditions (Lowe and Ruley 1993, Rao et al, 1992). This property is similar to the well-known tumor suppressor gene *p53* that also has the ability to induce apoptosis (Subramanian et al, 1995, Symonds et al, 1994). All of these observations indicate that tagging *E1A* as an oncogene is a misconception.

We have first discovered that the adenovirus 5 *E1A* gene can repress *HER-2/neu* overexpression through both transient transfection and adenovirus delivery systems (Yu et al, 1991b, Yu et al, 1990). Transfection of the *E1A* gene into the genomic rat *neu* oncogene transformed mouse

embryo fibroblast cell lines virtually abolishes the tumorigenicity and metastatic potential induced by the *HER-2/neu* oncogene through repression of *HER-2/neu* gene expression (Yu et al, 1992a, Yu et al, 1991b). Re-expression of the *HER-2/neu*-encoded p185 protein in these *E1A* transfectants by transfection of a *HER-2/neu* cDNA construct driven by a promoter that cannot be inhibited by *E1A* recovered virtually all of the transforming phenotypes including tumorigenicity, the ability to grow in soft agar, and higher *in vitro* growth rate (Yu et al, 1993a, b). Interestingly, the ability to induce experimental metastasis (measured by lung colonization through i. v. injection of the tumor cells) was only partially recovered. The incomplete regeneration of metastatic potential could be accounted for by the fact that *E1A* inhibits gelatinolytic activity that was critical for invasive activity of metastatic cells. This result indicates that the suppression of metastasis by *E1A* is through multiple molecular mechanisms in addition to repressing the *HER-2/neu* gene expression (Yu et al, 1992a). We have also demonstrated that *E1A* can indeed function as a tumor suppressor in the *HER-2/neu*-overexpressing human ovarian cancer cell line by down-regulating the expression of the *HER-2/neu* mRNA and the p185 protein product (Yu et al, 1995, Yu et al, 1993a, b, Yu et al, 1991b, Yu et al, 1990). The *E1A*-expressing ovarian cancer cell line had reduced malignancy, including a decreased ability to develop tumors in nude mice. Therefore, for the *HER-2/neu*-overexpressing transforming cells including fibroblasts and human cancer cells, *E1A* can function as tumor suppressor. And transcriptional repression of the *HER-2/neu* oncogene contributes to the tumor suppression function. However, since *E1A* is not a DNA-binding protein, the transcriptional repression of *HER-2/neu* by *E1A* has to be mediated through the targeting of other transcription factors. This is supported by our recent study demonstrating that *E1A* can abolish *HER-2/neu* overexpression by targeting the coactivator *p300*, which is required for efficient expression of *HER-2/neu* (Chen and Hung 1997).

To further investigate whether the *E1A* gene can be used as a therapeutic agent for *HER-2/neu*-overexpressing human breast and ovarian cancers in living host, a tumor-bearing mouse model was established and the *E1A* gene was delivered by the cationic liposome DC-Chol or a recombinant replication-deficient adenovirus. *E1A* treatment was able to effectively reduce the mortality of tumor-bearing mice and, in 60-80 % of the treated mice, resulted in tumor-free survival, suggesting that *E1A* gene therapy is a promising therapeutic regimen for cancers that overexpress *HER-2/neu*. In addition, the number of mice with distant metastases was significantly reduced even though a local treatment protocol by mammary fat pad injection was used in the orthotopic breast cancer model (Lane and Crawford 1979, Zhang et al, 1995). In addition to the breast and ovarian cancer animal models, we also

used a lung cancer animal model to test the therapeutic efficiency of *E1A* (Chang et al, 1996). In this case, the tumor-bearing mice were established through intratracheal inoculation of lung cancer cells and the *E1A* gene was delivered by an adenovirus vector through intravenous injection. A significant therapeutic efficacy was observed. Therefore the tumor suppression effect of *E1A* can be demonstrated through two independent gene delivery systems and three different animal models. Based on these results, a phase I clinical trial, using cationic liposome to deliver the *E1A* gene was initiated at the M. D. Anderson Cancer Center. Preliminary results suggested a down-regulation of the HER-2/neu p185 oncoprotein concomitant with the detection of the *E1A* gene expression in treated breast and ovarian cancer patients.

The simian virus 40 (SV40) large T antigen is a multifunctional protein required for the replication of the viral genome and for cell transformation (Lane and Crawford 1979, Linzer and Levine 1979). This viral protein contains transformation domains which can mediate binding to the retinoblastoma protein (pRb) and p53, respectively (Manfredi and Prive 1994). Our previous studies showed that a mutant SV40 large T antigen can repress rat *neu* transcription in mouse fibroblast NIH 3T3 cells (Matin and Hung 1993). The mutant large T antigen, named K1, contains a single amino acid change within the pRb-binding/transformation domain, which renders the viral protein unable to bind to pRb, and consequently failed to induce cell transformation (Kalderon and Smith 1984, Cherington et al, 1988, DeCaprio et al, 1988). Since the K1 mutant represses *HER-2/neu* expression as effectively as the wild-type counterpart (Matin and Hung 1993), we further tested whether K1 can function as a tumor suppressor for *HER-2/neu*-overexpressing ovarian cancer cells. K1 did suppress cancer cell growth, resulting in a significant therapeutic effect on mice with ovarian cancer with about 40% of treated mice were alive after one year (Xing et al, 1996). The autopsies showed that the mice from the control groups had larger volume of ascites and tumors within the peritoneal cavity or diaphragm or metastasis to the lungs. However, the mice that received K1-liposome complex had more locally distributed tumor nodules in their peritoneal cavities. This difference indicates that K1 suppressed the growth of *HER-2/neu*-overexpressing tumor cells so that the tumors developed with longer latency. The K1-treated mice survived for one year were sacrificed and examined for residual tumors, but no tumors were observed in the peritoneal cavity.

Our results indicate that both viral transcription factors, *E1A* and the large T antigen, can suppress tumor cell growth through a *HER-2/neu*-involved pathway. However, the possibility that *E1A* can mediate tumor suppression function through a *HER-2/neu*-independent mechanism should not be excluded.

B. Tumor suppression by cellular DNA-binding transcriptional factor, PEA3

The mouse *PEA3* (Polyomavirus Enhancer Activator 3) gene and its human homologue were first cloned from cDNA expression libraries due to the binding ability to the sequence 5'-AGGAAG-3' (the *PEA3* binding motif) within the polyomavirus enhancer promoter element (Xin et al, 1992, Higashino et al, 1993). The *PEA3* protein contains a stretch of about 85 amino acids with extensive sequence homology with the ETS domain, a conserved region shared by all *ets* family members that characteristically bind as monomers to the consensus core sequence GGAA by their ETS DNA-binding domains (Monte et al, 1994, Brown et al, 1992, Xin et al, 1992, Karim et al, 1990), and regulates the expression of target genes including genes involved in cell growth and differentiation (Ma et al, 1998, Taylor et al, 1997). The *ets* gene family currently contains at least 30 members present in a diverse spectrum of metazoan organisms (Degnan et al, 1993, Laudet et al, 1993) Subfamilies can be identified based on sequence/structure homology and the association with other accessory proteins for DNA binding. The *PEA3* subfamily is composed of three members : *PEA3* (Xin et al, 1992), *ERM* (Nakae et al, 1995, Monte et al, 1994), and *ER81* (Brown et al, 1992). In addition to the ETS domain, members of this subfamily share significant sequence similarity at an N-terminal acidic transcriptional activation domain (Nakae et al, 1995, Wasylyk et al, 1993, Macleod et al, 1992, Seth et al, 1992, Karim et al, 1990). Expression of the *PEA3* gene is ubiquitous in different species and can be identified in mouse, rat, monkey, and human cells. However, *PEA3* RNA expression is tissue-specific with highest level detected in brain, and, to a lower level, in pancreas, lung, and mammary gland (Xin et al, 1992). Most members of the *ets* family express at high levels in hemotopoietic cells. Unlike other *Ets* proteins, *PEA3* is the only member identified to date that is apparently not expressed in cells with hematopoietic origins (Xin et al, 1992). The significance of this tissue specific distribution is not clear.

There have been a number of candidate *PEA3*-regulated genes reported mainly based on the occurrence of putative *PEA3* binding motif in their promoter regions. Interestingly, a great portion of these candidates fall in the category of genes encoding matrix metalloproteinases (Higashino et al, 1995), such as collagenase (Gutman et al, 1990), stromelysin (Buttice et al, 1993), and the urokinase-type plasminogen activator (uPA), a serine proteinase (Nerlov et al, 1992). All these enzymes are believed to involve in the regulation of extracellular proteolysis, both in the normal organisms and in certain pathological conditions including tumor invasion and metastasis (Matrisian 1994). Consistent with this correlation, exogenous expression of *PEA3* in the breast cancer cell line MCF-7 resulted in enhanced tumor invasiveness and metastasis (Mitsunori et al, 1996).

Caveats should be taken to interpret these results. It is possible that members of the Ets family can have the same specificity required for DNA binding and share the same binding motif (Xin et al, 1992). As a matter of fact, it has been shown that Ets-2, an Ets protein belonging to the *Pointed* subfamily (Klamt 1993), is critical for the phorbol ester (TPA)-mediated induction of the human stromelysin gene expression through the PEA3 binding motif in the promoter (Buttice et al, 1993). A similar conclusion has been drawn for the promoter of the *uPA* gene (Pankov et al, 1994). In addition, whether the PEA3 protein directly binds to the putative PEA3 motif in the collagenase promoter is not clear due to the lack of appropriate anti-PEA3 antibodies to confirm the identity of the DNA-binding activity detected on the PEA3 motif (Gutman et al, 1990).

The occurrence of the PEA3 binding motif is not limited to those genes which potentially can enhance invasion and metastasis. Two consensus PEA3 binding motifs, distal and proximal, have been identified in the upstream regulatory region of the tumor suppressor gene *maspin* (Zhang et al, 1997a, Zhang et al, 1997b). Both motifs are positive regulatory elements for expression of the gene; the proximal site is the major functioning motif of the gene in mammary epithelial cells while both sites are equally critical for *maspin* expression in prostate cells. Functional studies have demonstrated that *maspin* functions as a tumor suppressor by inhibiting tumor invasion, metastasis, as well as tumor growth (Sheng et al, 1995, Zou et al, 1994). Even though it is still not clear if the PEA3 protein binds to the PEA3 motif in the *maspin* promoter, these observations are consistent with the prospect that tumor metastasis may be the result of imbalance between enhancing and suppressing factors (Liotta et al, 1991). This point is especially noteworthy given the large number of the ets family members and the resemblance of their DNA-binding domains and the DNA sequences of their target DNA motifs.

It is interesting to investigate the role of PEA3 in *HER-2/neu* gene expression and *HER-2/neu*-mediated transformation since a consensus PEA3-binding motif, 5'-AGGAAG-3', is present 26 nucleotide upstream from the major mRNA start site in the promoter of the human, rat, and mouse *HER-2/neu* gene (Tal et al, 1987). It has been reported that PEA3 can mediate induction of the *HER-2/neu* gene expression through the PEA3 binding motif (Benz et al, 1997). These results, however, were derived from the experiment using the COS monkey cell line. As will be mentioned below, this cell line can be characteristically different from other laboratory human breast and ovarian cancer cell lines, for which the investigation of PEA3's functions would be more biologically relevant. Furthermore, the hypothesis of PEA3-mediated *HER-2/neu* induction would predict a causal relationship between elevated PEA3 expression and *HER-2/neu* overexpression in cancer cells. However,

analysis of PEA3 gene expression in various breast cancer cells does not support this hypothesis. In fact, decreased *PEA3* RNA expression was detected in breast cancer cell lines with *HER-2/neu* overexpression (such as BT 474, SK-BR-3, MDA-MB-361), while there was no detectable *PEA3* mRNA in other *HER-2/neu*-overexpressing cell lines (such as MDA-MB-453, ZR-75-1, and MDA-MB-134-V) (Baert et al, 1997).

Nevertheless, these results suggest a negative role of PEA3 in regulating *HER-2/neu* expression. This prospect was directly tested in our laboratory and the following results demonstrate that *PEA3* is indeed a negative trans-regulator of the proto-oncogene *HER-2/neu* (Xing et al, 1997).

(1) The purified GST-PEA3 fusion protein can specifically recognize and bind to the consensus *PEA3* binding motif on the *HER-2/neu* promoter.

(2) Based on the co-transfection experiments performed on *HER-2/neu*-overexpressing human cancer cell lines, the *HER-2/neu* promoter activity can be down-regulated by *PEA3* in a dose-dependent manner. However, destruction of the PEA3-binding site on the *HER-2/neu* promoter by site-directed mutagenesis abolished the promoter activity, indicating that PEA3-induced trans-repression of the *HER-2/neu* promoter might involve competition between *PEA3* and another *ets*-related transcriptional activator(s), which contributes to the transformed phenotype of *HER-2/neu*.

(3) *PEA3* can suppress the focus forming ability of mouse embryonic fibroblast transformed by the genomic mutation-activated genomic rat *neu*.

(4) Expression of *PEA3* can suppress the growth of *HER-2/neu*-overexpressing human cancer cell lines *in vitro*, but not cell lines with basal level of *HER-2/neu* expression.

Based on these results, the tumor suppression function of PEA3 is emerging. Trimble *et al*, have reported that mammary tumors derived from the transgenic mice bearing the rat *neu* gene under the control of the mouse mammary tumor virus (MMTV) promoter expressed high level of PEA3 mRNA, suggesting that PEA3 may be required for tumorigenesis and metastasis in *HER2/neu* overexpressing cells (Trimble et al, 1993). However, the data is also consistent with the possibility that there may exist a negative regulatory loop pathway in which the overexpression of *HER-2/neu* would turn on the expression of PEA3 which then act as a transcriptional repressor of the *HER-2/neu* gene and resume the homeostatic balance. The rat *neu* gene in the transgenic mice setting was driven by the heterologous MMTV promoter which is very likely not subject to the negative control by PEA3. Expression levels of both PEA3 and *HER-2/neu* would be elevated under this situation. In addition to PEA3, other Ets proteins including ERF and Net have been reported to function as transcriptional

repressors (Sgouras et al, 1995, Giovan et al, 1994). Other promoters negatively regulated by Ets binding sites have also been reported (Chen and Boxer 1995, Goldberg et al, 1994). Interestingly, the *ets* family member Ets2 has recently been reported to function as a tumor suppressor by reversing *ras*-mediated cellular transformation (Fooks et al, 1998).

To test whether *PEA3* can be used as a therapeutic agent *in vivo*, tumors were induced in nude mice (*nu/nu*) with SK-OV-3-ip1, an ovarian cancer cell line derived from SK-OV-3 and has higher *HER-2/neu* expression. For mice treated with *PEA3*-DC-Chol complex, 50% of the mice were alive and healthy without palpable tumors after 12 months. The mice of the control group, however, developed tumors and ascites, and died within 6 months. The tumor suppression activity of *PEA3* is correlated with *HER-2/neu* expression since another cell line 2774 c-10, an ovarian cancer cell line with basal level of *HER-2/neu* expressed, did not have response to *PEA3* treatment and the mice died of tumor with 5 months. Tumor samples were examined for the expression of *HER-2/neu* with immunoblot analysis. The results confirmed that *PEA3* delivered by the cationic liposome downregulated the expression of p185. The correlation between *PEA3* expression and *HER-2/neu* downregulation was further demonstrated by immunohistochemical staining of the tumor samples obtained from the *PEA3*-treated, moribund mice. Approximately 30% of the cancer cells in the tumor were positive for *PEA3* protein expression, while the p185 staining was negative for about 50% of cells. Similar level of *PEA3* expression was observed for *PEA3*-treated 2774 c-10-derived tumors while no repression of p185 was detected in these tumors. These *in vitro* and *in vivo* data clearly demonstrate the tumor suppression activity of *PEA3* and indicate the potential clinical application of *PEA3*-cationic liposome targeting the *HER-2/neu* overexpressing cancer cells.

Even though *PEA3* as well as the viral proteins *E1A* and SV40 large T can all suppress *HER-2/neu* transcription, they are very likely functioning through different mechanisms. Both *E1A* and SV40 large T may suppress *HER-2/neu* in an indirect manner. Association of *E1A* with the transcriptional co-activator CBP/p300 inhibits the p300 transactivation activity, which is required for efficient expression of the *HER-2/neu* gene (Chen and Hung, 1997). On the other hand, *PEA3* down-regulates the *HER-2/neu* gene by directly binding to its cognate binding sequence on the promoter. This feature makes *PEA3* a more attractive target for further molecular manipulation to develop therapeutic molecules with higher binding affinity and enhanced specificity.

III. Conclusions

Overexpression of the proto-oncogene *HER-2/neu* can lead to cell transformation and tightly correlated with the

development of malignant tumor growth in many tissue types. There are molecular approaches to target the promoter of *HER-2/neu*, which can downregulate the gene expression, reverses the malignant phenotype, and retards tumor growth in animal. The results of our *in vivo* and *in vitro* experiments demonstrate using viral or cellular transcriptional repressor genes transferred by safe and efficient molecular vehicles can result in significant therapeutic effects on cancer cells. Since gene overexpression is a common mechanism of cancer as well as other types of diseases such as AIDS, the therapeutic strategy discussed here can have a tremendous potential in clinical application. Finally, the studies of *E1A*- and *PEA3*-mediated *HER-2/neu* repression have unveiled new areas in cancer biology which is excitingly more complicated than what we used to expect. Studies of these areas would be critical for our understanding of cancer.

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