

Dual expression of α -tocopherol-associated protein and estrogen receptor in normal/benign human breast luminal cells and the downregulation of α -tocopherol-associated protein in estrogen-receptor-positive breast carcinomas

Sharlin Johnykutty¹, Ping Tang¹, Hongwei Zhao², David G Hicks¹, Shuyuan Yeh³ and Xi Wang¹

¹Department of Pathology and Laboratory Medicine, University of Rochester Medical School, Rochester, NY, USA; ²Department of Epidemiology and Biostatistics, Texas A&M Health Science Center, College Station, TX, USA and ³Department of Urology, University of Rochester Medical School, Rochester, NY, USA

The hormonal carcinogenesis of breast cancer involves hormone-driven cell proliferation and genetic alterations, including oncogene activation and suppressor gene inactivation. However, the predominant genes involved in these processes are currently unknown. Our previous studies identified a gene, namely α -tocopherol-associated protein, which is preferentially expressed in normal/benign breast and prostate tissue, but its expression is downregulated in breast and prostate carcinomas. To further examine its function in hormone-induced carcinogenesis, we examined if there is an association between α -tocopherol-associated protein and estrogen-receptor expression in normal/benign breast tissue and in human breast carcinomas. We found that α -tocopherol-associated protein is coexpressed with estrogen receptor in the luminal cells of normal/benign breast tissue in a scattered manner by immunohistochemical staining of consecutive tissue sections of 20 cases, whereas α -tocopherol-associated protein expression is downregulated in 46% (45 of 98) of estrogen-receptor/progesterone-receptor-positive, so-called luminal type A or B human breast carcinoma. This is similar to the association of α -tocopherol-associated protein and androgen receptor expression in normal/benign prostate and prostate carcinomas. In contrast, α -tocopherol-associated protein expression is mostly negative in basal, Her2 and triple-negative nonbasal subtypes of high-grade breast carcinomas. These findings are consistent with α -tocopherol-associated protein acting as an antiproliferative factor in estrogen-receptor-positive luminal cells in normal/benign breast tissue. α -Tocopherol-associated protein downregulation may have triggered hormonal carcinogenesis in at least some of the breast carcinomas, providing further, albeit indirect evidence to support a role for vitamin E in breast cancer prevention.

Modern Pathology advance online publication, 20 March 2009; doi:10.1038/modpathol.2009.24

Keywords: TAP; ER; breast cancer

It is well recognized that a major category of breast cancers are regulated by steroid hormone receptors. Approximately 60–80% of breast carcinomas are estrogen receptor (ER) and/or progesterone receptor (PR) positive, and this is particularly true for

non-high-grade tumors.¹ A recent model of molecular subclassification based on gene expression profiling has demonstrated that ER-/PR-positive breast carcinomas can be further divided into luminal type A (ER/PR+, Her2-) and B (ER/PR+, Her2+), whereas ER-/PR-negative carcinomas can be subclassified as Her2 overexpression type (ER/PR-, Her2+, any CK5/6 and EGFR), basal-like type (ER/PR/HER2-, CK5/6 and/or EGFR+) and triple-negative nonbasal type (ER/PR/Her2-, CK5/6 and EGFR-).^{2–4} On the other hand, ER and/or PR are expressed in only scattered normal/benign breast

Correspondence: Dr X Wang, Department of Pathology and Laboratory Medicine, University of Rochester Medical School, 601 Elmwood Ave, Box 626, Rochester, NY 14642, USA.

E-mail: xi_wang@urmc.rochester.edu

Received 5 December 2008; revised 9 February 2009; accepted 10 February 2009; published online 20 March 2009

epithelial cells. In a given terminal duct lobular unit, it was reported that only approximately 10% of the luminal cells are ER and/or PR positive.⁵ If we hypothesize that ER- and/or PR-positive cells in normal/benign breast tissue serve as the progenitor cells of ER-/PR-positive luminal type breast carcinomas, it is reasonable to speculate that in the normal setting, some factors must be preventing those ER-/PR-positive luminal cells in normal/benign breast tissue from undergoing malignant transformation.

The process of hormonal carcinogenesis involves hormone-driven cell proliferation and the accumulation of gene mutations, including tumor suppressor gene downregulation and oncogene activation.⁶ In breast cancer, although alterations of HER2 oncogene and *p53* tumor suppressor gene have been identified in about 20–25% of breast carcinomas,^{7,8} no dominant gene mutations have been identified in the majority of sporadic carcinomas. Notably, HER2 and *p53* alterations have been found mostly in ER-/PR-negative breast carcinomas, such as basal subtype or triple-negative nonbasal type,^{9,10} but their alterations are uncommon in ER-/PR-positive luminal type tumors. These findings indicate that *p53* and *Her2/neu* are not the dominant genes in the hormonal carcinogenesis in breast cancer.

In addition, breast cancers are considerably heterogeneous with respect to their biological and clinical behavior. Among the ER-/PR-positive luminal type breast carcinomas, there is a broad range of tumor histologies, nuclear grade and significant variability in responsiveness to adjuvant endocrine treatment and chemotherapy. Only 50% of ER-/PR-positive tumors are hormone dependent and show a positive response to endocrine therapy.¹¹ The mechanisms underlying these dramatic clinical and biological variations are currently unknown.

Our previous study showed that α -tocopherol-associated protein (TAP), an α -tocopherol-binding protein, is universally expressed in normal/benign breast epithelium in a scattered manner.¹² However, it is downregulated in breast cancer cell lines and in 57% of breast carcinomas, suggesting that this protein may have a tumor suppressor-like function through a vitamin E-dependent or -independent pathway. To further explore this hypothesis, we wanted to observe if there is an association between TAP and ER expression in normal/benign breast tissue and breast carcinoma, to help further define a potential function of TAP in the hormonal carcinogenesis of breast cancer. In the current study we have applied immunohistochemical staining for TAP and ER on consecutive tissue sections of normal/benign breast core biopsy specimens to compare their distributions in breast epithelium. Furthermore, we compared TAP expression with that of ER/PR, Her2/neu, CK5/6 and EGFR in breast carcinomas. We found that although TAP is consistently coexpressed with ER in normal/benign duct luminal cells, its expression is downregulated

in 46% of ER-/PR-positive breast carcinomas, further supporting its tumor suppressor-like function in hormonal carcinogenesis.

Materials and methods

Twenty core biopsy specimens of human breast with normal morphology or benign changes were identified from the file in the Pathology Department at Strong Memorial Hospital (Rochester, NY, USA). Ten of them were from patients without breast cancer and ten were from patients with a history of breast cancer. Ten patients were younger than 50 years and the other ten were 50 years or older. Consecutive 3.0 μ m thick sections were taken.

A total of 141 cases of invasive mammary carcinomas were identified, as previously reported.¹² A Bloom–Richardson grade was given to each case by two pathologists after reviewing the H&E slides. Of them, 59 were high-grade (Bloom–Richardson grade 3) and 82 were non-high-grade (grade 1 and 2) carcinomas. One tissue block that had both invasive carcinoma and normal breast tissue was selected from each case.

Immunohistochemical staining for TAP and ER was performed on the consecutive sections of normal/benign breast core biopsy specimens. A total of 141 cases of invasive carcinomas were stained for TAP and ER/PR. Among them, 92 cases were also stained with antibodies to Her2/neu, CK5/6 and EGFR. Pretreatments consisted of enzyme digestion or pressure cooker method. Staining was performed on Dako Autostainer with antibodies incubation at room temperature for 60 min, then with secondary antibodies 30 min, washed and then incubated with streptavidin–HRP for 30 min and AEC for 10 min followed by Meyer's hematoxylin. The methodology, including the source, dilution and procedures of these immunohistochemical stains, has been published previously.^{12,13}

α -Tocopherol-associated protein staining of tumor cells was classified as positive or negative under light microscopy by comparing it with the adjacent normal/benign breast tissue. Nuclear and/or cytoplasmic staining in tumor cells with the same intensity as the normal/benign breast tissue was considered positive, whereas markedly reduced or absence of staining was considered negative. Positive staining for ER/PR was defined as nuclear staining in >10% of tumor cells, for Her2/neu and EGFR as >10% of tumor cells with 3+ complete membrane staining and for CK5/6 strong cytoplasmic staining.^{13,14}

Fisher's exact test and χ^2 -test were used in statistic analysis of the data.

Results

α -Tocopherol-associated protein positivity is present in a scattered manner in the nuclei and/or

cytoplasm of lumenally located cells in a given terminal ductal lobular unit in normal/benign human breast tissue, and it is not expressed in the myoepithelial cells. This expression pattern is similar to that of ER. Indeed, examination of consecutive sections revealed that TAP and ER are coexpressed in the same cells, whereas TAP-negative cells are also negative for ER. This coexpression was observed in all 20 core biopsy specimens, regardless of age or breast cancer history (Figures 1–3).

As we reported previously, TAP expression is reduced in 57% of all breast carcinomas, but even more so in high-grade tumors (88%). In the 139 cases with complete TAP and ER/PR information, 98 (71%) were ER/PR positive (so-called luminal type A or B); among them, 53 (54%) were both TAP and ER/PR positive, whereas 45 (46%) cases were ER/PR positive, but TAP negative (Figures 4 and 5). This finding shows that in contrast to its expression in normal/benign breast tissue, TAP expression is lost in a high percentage of ER-/PR-positive breast carci-

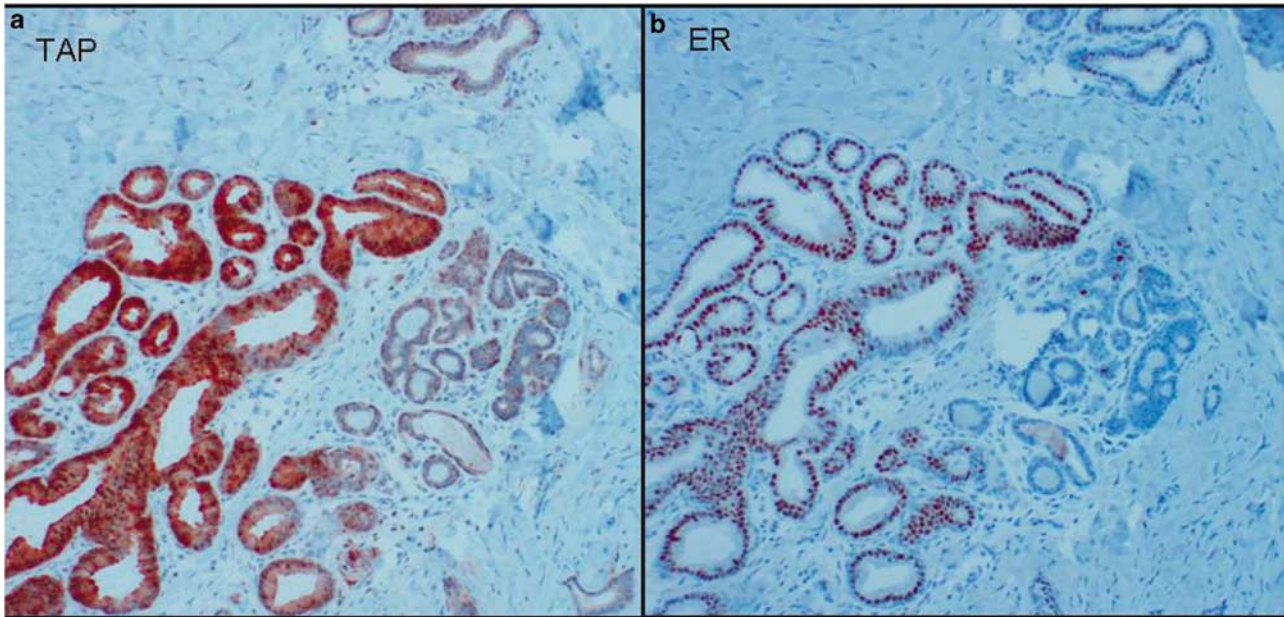


Figure 1 TAP (a) and ER (b) expression in normal/benign breast terminal duct lobular unit (original magnification $\times 40$).

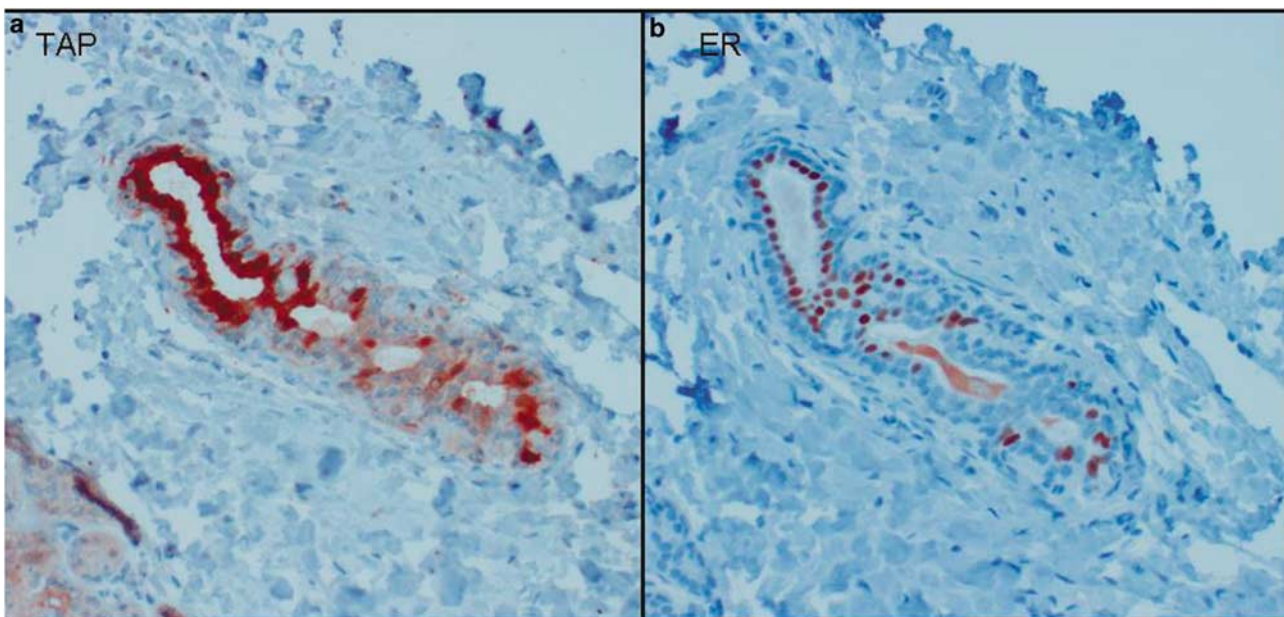


Figure 2 TAP (a) and ER (b) expression in normal/benign breast terminal ductal lobular unit (original magnification $\times 100$).

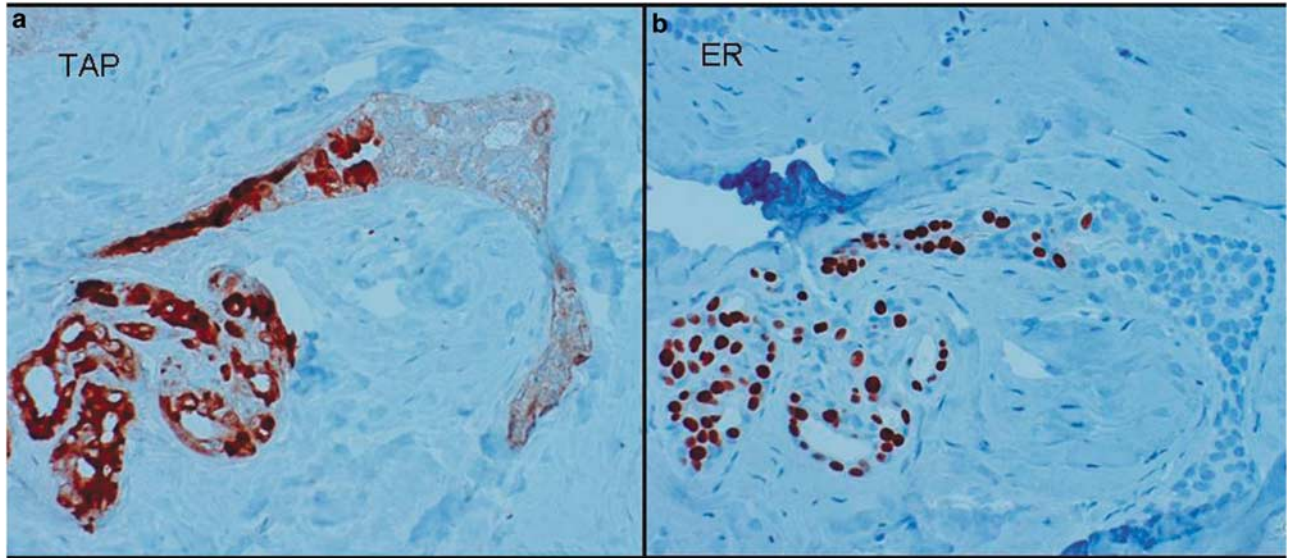


Figure 3 TAP (a) and ER (b) expression in normal/benign breast terminal ductal lobular unit (original magnification $\times 100$).

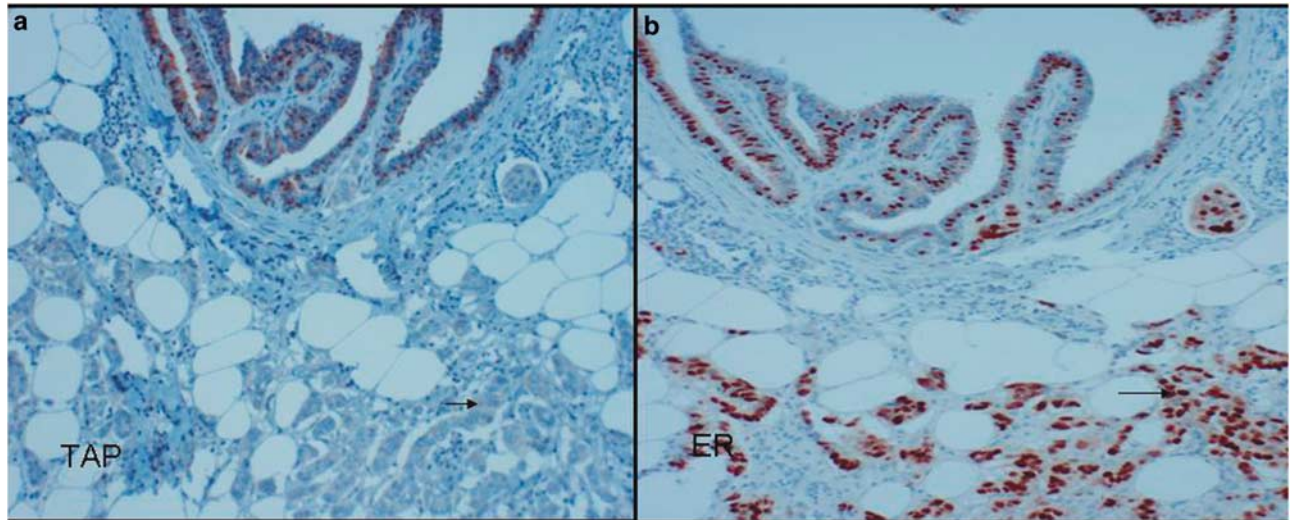


Figure 4 Negative TAP stain (a) in ER-positive (b) invasive breast carcinoma (arrow; original magnification $\times 40$).

nomas. Among the 41 (29%) ER-/PR-negative carcinomas, however, 32 (78%) were also TAP negative, whereas only 9 (22%) were TAP positive (Table 1). It is notable that TAP- and ER-/PR-double-positive tumors are mostly (89%) non-high grade (Table 2), whereas tumors that are both TAP and ER/PR negative are mostly (91%) high grade, as we would have expected, based on our previous study.¹²

In 92 cases that were also stained with the antibodies to Her2/neu and basal cell type markers, TAP was negative in all 8 tumors classified as basal-like type (CK5/6 and/or EGFR positive), 11 of 14 (79%) in triple-negative nonbasal type (ER/PR, Her2/neu, CK5/6 and EGFR negative) and 6 of 9 (67%) in Her2 over expression type (Her2/neu positive only) (Table 3). This is again in agreement with the tumor grade.

Discussion

Breast carcinogenesis is a complex sequential multi-step process, from the normal epithelium in terminal duct lobular units, via hyperplasia, atypical hyperplasia and carcinoma *in situ* to invasive carcinoma. Researchers have long been puzzled by the question of which cells in the terminal duct lobular unit have the potential to move on this long transformation process and become invasive carcinoma. The epithelium of terminal duct lobular unit consists of inner luminal cells and outer basal/myoepithelial cells. Recent molecular subclassification of breast carcinoma indicates that even though other types of breast carcinoma such as basal type supposedly arising from myoepithelium do exist, the majority of breast carcinomas appear to arise from luminal cells or their

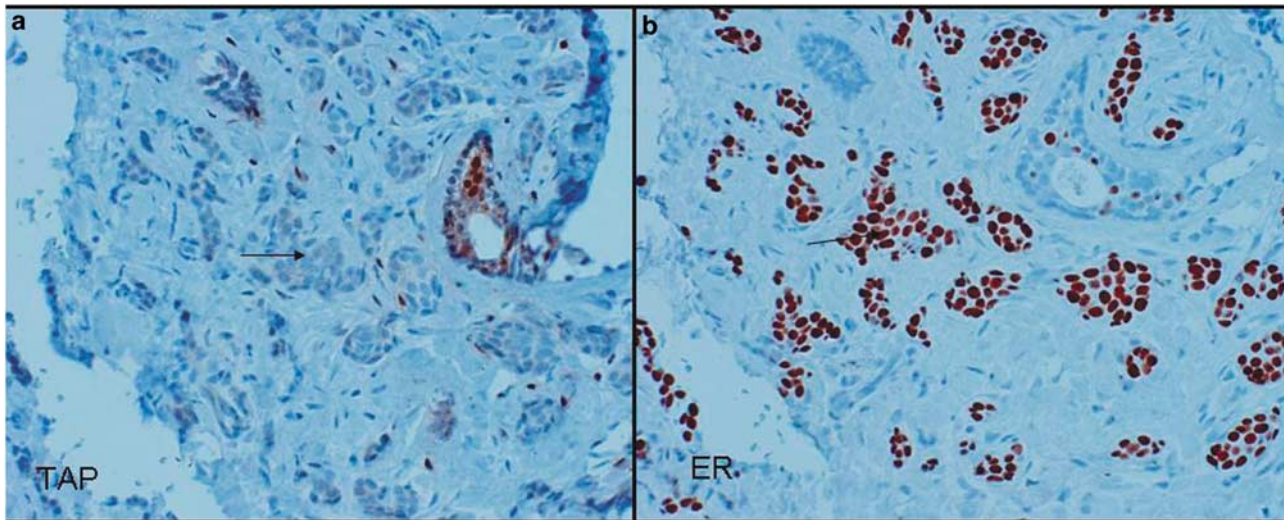


Figure 5 Negative TAP stain (a) in ER-positive (b) invasive breast carcinoma (arrow) (original magnification $\times 100$).

Table 1 TAP expression in ER/PR-positive or -negative invasive breast carcinomas

	TAP+	TAP-	Total
ER/PR+	53	45	98
ER/PR-	9	32	41
Total	62	77	139

$P < 0.05$.

ER, estrogen receptor; PR, progesterone receptor; TAP, α -tocopherol-associated protein.

progenitors. It has been shown that up to 95% of breast carcinomas express phenotypic markers that are consistent with a luminal origin.¹⁵ In the search for cancer progenitor cells and the genetic changes that ultimately lead to an invasive carcinoma, the fact that breast carcinogenesis is also a hormone-driven process should not be forgotten. In a given terminal duct lobular unit, it is reported that only approximately 10% of luminal cells are ER/PR positive, whereas in contrast up to 80% of breast carcinomas are ER/PR positive. This phenomenon may indicate that those ER-/PR-positive luminal cells are the most vulnerable progenitor cells and candidates for the accumulation of genetic alterations that may ultimately give rise to ER-/PR-positive breast carcinomas. Therefore, it is important that studies should focus on ER-/PR-positive luminal cells in the terminal duct lobular unit to help us understand the early changes that could lead to initiation, promotion and progression of breast carcinogenesis.

ER is a ligand-inducible nuclear transcription factor. Its activation can trigger a cascade of signal transduction through genomic or nongenomic pathways, with the effects of increased cell proliferation and inhibition of apoptosis, and if left unchecked or unopposed, eventually tumor formation.¹ Given the multitude of powerful biological effects that ER has on the mammary epithelium, it is reasonable to postulate that

Table 2 The expression of TAP and ER/PR in high- or non-high-grade invasive breast carcinomas

	ER/PR+/TAP+	ER/PR+/TAP-	ER/PR-/TAP+	ER/PR-/TAP-	Total
Non-high grade	47	30	2	3	82
High grade	6	15	7	29	57
Total	53	45	9	32	139

$P < 0.05$.

ER, estrogen receptor; TAP, α -tocopherol-associated protein.

there are restraining mechanisms in ER-positive luminal cells, which will prevent uncontrolled growth, and that loss of these restraints may be an important early event that could eventually result in hormone-induced carcinogenesis. The works by Clarke and Russo^{16,17} identified that ER-/PR-positive epithelial cells in normal/benign breast tissue are actually Ki67 negative, indicating that they are the nonproliferating cells. This finding further supports the presence of restraining factors in ER-/PR-positive luminal cells.

Genetic alterations in carcinogenesis include oncogene activation and tumor suppressor gene inactivation. However, the specific genes involved in progression of hormone-related cancers, including breast and prostate carcinomas, are currently unknown. *p53* is a tumor suppressor gene, which has been found to be mutated in up to 50% of human cancers.¹⁸ However, its mutation rate in breast carcinomas is only 20–25%, and mutations are found primarily in ER-/PR-negative, nonluminal type carcinomas. Similarly, *Her2/neu* as an oncogene is overexpressed in only about 20% of breast carcinomas, again mostly in ER-/PR-negative tumors. It is apparent that although alterations of *p53* and *Her2/neu* may have been involved in the carcinogenesis of some of the high-grade breast carcinomas, namely *Her2* overexpression type, basal

Table 3 TAP expression in different subtypes of invasive breast carcinomas

	Luminal A	Luminal B	Her2 type	Basal type	Triple (-) nonbasal	Total
TAP+	31	3	3	0	3	40
TAP-	23	3	7	8	11	52
Total	54	6	10	8	14	92

$P < 0.05$.

TAP, α -tocopherol-associated protein.

type and triple-negative nonbasal type carcinomas, they are not the dominant genes involved in the hormone-induced carcinogenesis.

In our previous studies,^{12,19} we determined that TAP, as a tocopherol-binding protein, can facilitate vitamin E retention in tumor cells and regulate tumor cell proliferation through a vitamin E-dependent or -independent pathway. We also found that although TAP is selectively expressed in normal prostate and breast tissue, it is downregulated at the mRNA and protein levels in a large number of prostate and breast carcinomas. In current study, we first demonstrated that the expression of TAP in normal breast terminal duct lobular unit is the same as ER as a scattered pattern. The staining on consecutive tissue sections showed that TAP and ER are coexpressed in the same normal/benign luminal cells. In contrast, TAP expression is downregulated in a large number of ER-/PR-positive invasive carcinomas. More interestingly, when we reviewed the TAP status in prostate in our previous study,¹⁹ we saw that TAP is expressed in a diffuse pattern in benign prostate glands, similar to androgen receptor (AR) expression, whereas TAP expression is downregulated in AR-positive prostatic carcinomas. The corresponding findings regarding hormone receptors and TAP in breast and prostate further support a tumor suppressor-like function of TAP in hormone-induced carcinogenesis. TAP may serve as an antiproliferation factor counteracting the proliferative effect of ER or AR in normal/benign luminal cells of breast and prostate tissue to maintain tissue homeostasis. Downregulation of TAP could break up this balance and trigger the process of hormonal carcinogenesis.

Vitamin E has been widely studied as a supplement in the prevention of human cancers, especially of prostate and breast. However, the results have been not consistent, due to the limitations of epidemiological and experimental studies. In our studies, the preferential expression of TAP, the α -tocopherol-binding protein, in normal/benign breast and prostatic luminal cells indicates that there may be a preferential accumulation of α -tocopherol, the principle and most active vitamin E isoform, in the normal/benign luminal epithelial cells, whereas downregulation of TAP in *in situ* and invasive carcinomas suggests a correlation between the reduction of tocopherol and initiation of carcinogenesis. We take this as strong,

albeit indirect, *in vivo* evidence to support vitamin E as a supplement in breast cancer prevention.

References

- 1 Platet N, Cathiard AM, Gleizes M, *et al*. Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Crit Rev Oncol Hematol* 2004;51:55–67.
- 2 Sorlie T. Molecular classification of breast tumors: toward improved diagnostics and treatments. *Methods Mol Biol* 2007;360:91–114.
- 3 Cheang MC, Voduc D, Bajdik C, *et al*. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008;14:1368–1376.
- 4 Nielsen TO, Hsu FD, Jensen K, *et al*. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367–5374.
- 5 Shoker BS, Jarvis C, Clarke RB, *et al*. Estrogen receptor-positive proliferating cells in the normal and precancerous breast. *Am J Pathol* 1999;155:1811–1815.
- 6 Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis* 2000;21:427–433.
- 7 Borresen-Dale AL. TP53 and breast cancer. *Hum Mutat* 2003;21:292–300.
- 8 Osborne C, Wilson P, Tripathy D. Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications. *Oncologist* 2004;9:361–377.
- 9 Kobayashi S. Basal-like subtype of breast cancer: a review of its unique characteristics and their clinical significance. *Breast Cancer* 2008;15:153–158.
- 10 Nishimura R, Arima N. Is triple negative a prognostic factor in breast cancer? *Breast Cancer* 2008;15:303–308.
- 11 Moy B, Goss PE. Estrogen receptor pathway: resistance to endocrine therapy and new therapeutic approaches. *Clin Cancer Res* 2006;12:4790–4793.
- 12 Wang X, Jing N, Hsu C-L, *et al*. Reduced expression of tocopherol-associated protein (TAP/Sec14L2) in human breast cancer. *Cancer Invest* 2009 (in press).
- 13 Tang P, Wang X, Schiffhauer L, *et al*. Expression patterns of ER-alpha, PR, HER-2/neu, and EGFR in different cell origin subtypes of high grade and non-high grade ductal carcinoma *in situ*. *Ann Clin Lab Sci* 2006;36:137–143.
- 14 Kim MJ, Ro JY, Ahn SH, *et al*. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-over-expressing phenotypes. *Hum Pathol* 2006;37:1217–1226.
- 15 Taylor-Papadimitriou J, Lane EB, Neville MC. *The Mammary Gland: Development, Regulation and Function*. Plenum: New York, 1987, pp 181–215.
- 16 Clarke RB. Human breast cell proliferation and its relationship to steroid receptor expression. *Climacteric* 2004;7:129–137.
- 17 Russo J, Ao X, Grill C, *et al*. Pattern of distribution of cells positive for estrogen receptor alpha and progesterone receptor in relation to proliferating cells in the mammary gland. *Breast Cancer Res Treat* 1999;53:217–227.
- 18 Dey A, Verma CS, Lane DP. Updates on p53: modulation of p53 degradation as a therapeutic approach. *Br J Cancer* 2008;98:4–8.
- 19 Ni J, Wen X, Yao J, *et al*. Tocopherol-associated protein suppresses prostate cancer cell growth by inhibition of the phosphoinositide 3-kinase pathway. *Cancer Res* 2005;65:9807–9816.