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Advances in Brief

The *ZNF217* Gene Amplified in Breast Cancers Promotes Immortalization of Human Mammary Epithelial Cells[1]

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Abstract

The functional consequences of overexpression of a candidate oncogene on chromosome 20q13.2, *ZNF217*, were examined by transducing the gene into finite life span human mammary epithelial cells (HMECs). In four independent experiments, *ZNF217*-transduced cultures gave rise to immortalized cells. HMECs that overcame senescence initially exhibited heterogeneous growth and continued telomere erosion, followed by increasing telomerase activity, stabilization of telomere length, and resistance to transforming growth factor β growth inhibition. The incremental changes in telomerase activity and growth that occurred in *ZNF217*-transduced cultures after they overcame senescence were similar to the conversion pattern we have described previously in rare HMEC lines immortalized after exposure to a chemical carcinogen. Aberrant expression of *ZNF217* may be selected for during breast cancer progression because it allows breast cells to overcome senescence and attain immortality.

Introduction

The candidate oncogene *ZNF217*, predicted to encode alternatively spliced Krüppel-like transcription factors, was originally identified based on its core location in an amplicon on chromosome 20q13.2 in breast cancer cell lines and primary tumors and its recurrent pattern of expression in tumors (1). 20q amplification, common in many human cancers, is also associated with overcoming senescence and p53-independent genome instability in cultured human uroepithelial cells (2, 3). We investigated the functional consequences of *ZNF217* overexpression by transducing the gene into finite life span HMECs[3] (4). In four independent experiments, *ZNF217*-transduced cultures gave rise to immortalized cells. HMECs that overcame senescence initially exhibited heterogeneous growth and continued telomere erosion, followed by increasing telomerase activity, stabilization of telomere length, and resistance to TGF-β growth inhibition. The incremental changes in telomerase activity and growth that occurred in *ZNF217*-transduced cultures after they overcame senescence were similar to the conversion pattern we have described previously in rare HMEC lines immortalized after exposure to a chemical carcinogen (5).

Materials and Methods

HMEC Culture. Finite life span 184 HMECs were obtained from reduction mammoplasty tissue and were cultured in serum-free MCDB 170 medium (Clonetics) as described previously (6, 7).[4] Extended life span culture 184Aa cells emerged from 184 HMECs after benzo(*a*)pyrene exposure of primary cultures growing in MM medium as described previously (8). Cells that showed no evidence of growth after 6 weeks were considered to have become senescent. To assay growth heterogeneity of single cell-derived colonies in the absence or presence of TGF-β, cultures were maintained for 14–20 days after seeding 200-1000 cells/100-mm dish. [^{3}H]Thymidine (0.5–1.0 μCi/ml) was then added for 24 h 4–7 h after refeeding, and labeled cells were visualized by autoradiography as described (9). Colony forming efficiency was determined by counting the number of colonies containing >50 cells and growth capacity by counting the percentage of labeled nuclei in these colonies. Uniform good growth was defined as a labeling index of >50%. To determine growth capacity in TGF-β, 5 ng/ml TGF-β (R&D Systems) in the presence of 0.1% BSA (Sigma) were added to some cultures for 10–14 days once the largest colonies contained 100–250 cells, and the cultures were then labeled.

Retroviral Construction and Infection. A 3.1-kb cDNA encoding the complete *ZNF217* open reading frame flanked by a hemagglutinin tag (TAC CCA TAC GAC GTC CCA GAC TAC GCT) and *Eco*RI sites was constructed by PCR using a high-fidelity Taq polymerase (Expand High Fidelity Taq; Boehringer Mannheim). The PCR product was cloned into the *Eco*RI site of the retroviral vector pLXSN (10) using STBL2 competent cells (Life Technologies, Inc.). High-titer amphotropic stocks of *ZNF217* and control retrovirus were prepared using a transient packaging system (11) and used to infect parallel cultures of recipient HMECs. After 24 h in normal medium, cells were selected with G418 (400 μg/ml) medium for 10 days and subsequently maintained in 100 μg/ml G418. High *ZNF217* mRNA and protein expression in the retrovirally transduced cells, comparable with that seen in breast tumor cell lines, were confirmed by Northern and immunoblot analyses (data not shown).

Telomerase and Telomere Length Assays. Cell extracts for telomerase assays were prepared by a modification of the detergent lysis method (12). Telomerase activity was measured using the TRAP-EZE telomerase detection kit (Oncor) using 2 μg of protein/assay. The Sybr Green-stained telomerase products were detected using a Storm 860 fluorescence imager (Molecular Dynamics). DNA for mean TRF analysis was isolated using a genomic DNA isolation kit (Qiagen), and the TRF analysis was performed as described previously (13) with the following modifications. Two μg of genomic DNA were restriction digested and resolved on a 0.5% agarose gel. The DNA was then transferred to a nylon membrane and hybridized with the ^{32}P-labeled telomere-specific oligonucleotide $(CCCTAA)_4$. The ^{32}P signal was detected using a PhosphorImager (Molecular Dynamics). Mean TRF length was calculated as described (14).

p53 Analysis. Protein lysates were collected in 2× SDS lysis buffer (4% SDS, 20% glycerol, and 0.126 M Tris-Cl, pH 6.8) with protease inhibitors (20 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin A). The lysates were boiled for 10 min and sheared by several passages through 23-gauge needles. Thirty μg of each protein sample were resolved on a 10% Tris bis-polyacrylamide Nupage minigel (Novex). Protein was transferred to polyvinylidene difluoride membrane, and total p53 protein was detected using the anti-p53 antibody Ab-6 (Oncogene Research). To test for p53-dependent GADD45 expression, subconfluent HMECs were exposed to UV irradiation

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[3] The abbreviations used are: HMEC, human mammary epithelial cell; TGF, transforming growth factor; CGH, comparative genomic hybridization; DAPI, 4′,6′-diamidino-2-phenylindole; SA β-gal, senescence-associated β-galactosidase; TRF, terminal restriction fragment.

[4] Internet address: http://www.lbl.gov/~mrgs.

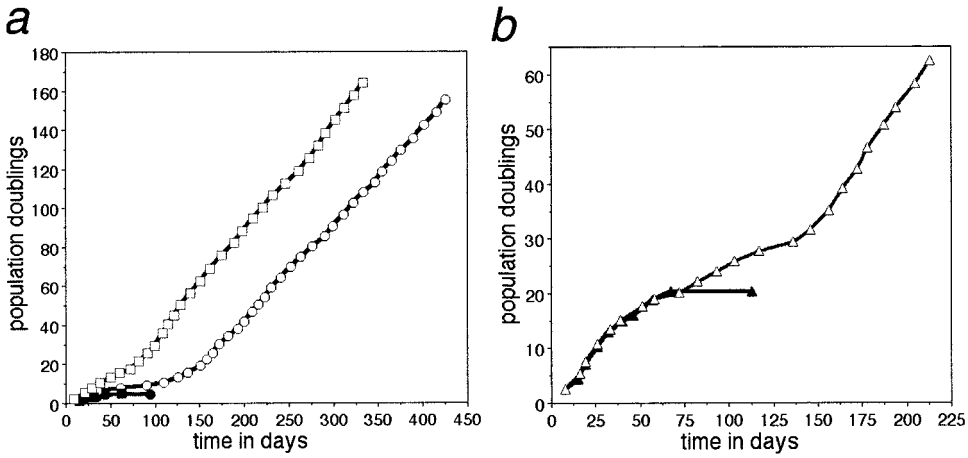

Fig. 1. *ZNF217*-transduced HMECs continue to grow indefinitely after control cultures have senesced. The cumulative population doublings of 184Aa (*a*) or 184 (*b*) cells infected with either vector alone (LXSN; ●, ■, ▲) or ZNF217 (○, □, △) were plotted against time in days. The LXSN controls senesced and were discarded after >60 days in culture without net increases in cell numbers. In one 184Aa experiment shown, a single immortal clone grew out of an otherwise senescent LXSN-infected population and is not plotted; this clone was distinct from ZNF217-transduced immortal clones in both morphology and growth characteristics. Note that the population doublings indicated are underestimates, because they do not take plating efficiencies into account.

(37 joules/m^2). Samples were then collected at 0- and 4-h time points by lysis directly into buffered guanidine thiocyanate solution. Total cellular RNA was then purified, and Northern blots were prepared as described previously (9). Blots were then hybridized with a ^{32}P-labeled, GADD45-specific probe (15). GADD45 signal was measured and quantitated using a PhosphorImager. The values for relative hybridization were normalized by subsequent hybridization of the blot to a ^{32}P-labeled probe specific for a constitutively expressed human acidic ribosomal protein transcript (16).

CGH. Genome copy number changes were analyzed as described previously (17). Briefly, DNA samples isolated from normal human lymphocytes and from a test cell line were labeled by nick translation with fluorescein-12-dUTP and Texas Red-dUTP, respectively. Two hundred ng of each DNA probe were mixed with 20 μg of unlabeled Cot-1 DNA and hybridized to normal lymphocyte metaphase spreads for 3 days. The preparations were washed and counterstained with DAPI for chromosome identification. DAPI, fluorescein, and Texas Red images were acquired for several metaphases for each hybridization as described previously (18). Chromosomes were segmented based on the DAPI image, and green:red ratio profiles along the segmented images were calculated for each chromosome. The results from 8 to 10 chromosomes of each type were combined for each hybridization to determine a mean ($\pm$ 1σ) for each chromosome type. Mean profiles for the 23 chromosome types (the Y chromosome was not analyzed) were arranged from short arm to long arm and from chromosomes 1 to 22, then X, to produce a genome-wide CGH profile.

Results and Discussion

Two HMEC strains were used for these experiments: 184Aa, an extended but finite life span culture obtained from reduction mammoplasty-derived HMECs exposed to a chemical carcinogen (8); and postselection 184, a population of reduction mammoplasty-derived HMECs capable of long-term growth in serum-free medium before reaching senescence (6). The cyclin-dependent kinase inhibitor p16^{INK4a}, thought to serve as one block to immortal transformation, is not expressed in either cell strain because of mutation and/or epigenetic silencing (19). Senescence occurs reproducibly after ~16 passages (~64 population doublings) in 184Aa and 20 passages (~80 population doublings) in 184 HMECs. In Fig. 1*a*, the cumulative population doublings of transduced cell cultures are plotted against time in culture for two experiments using 184Aa. In both experiments, the *ZNF217*-transduced cells showed no initial growth advantage over

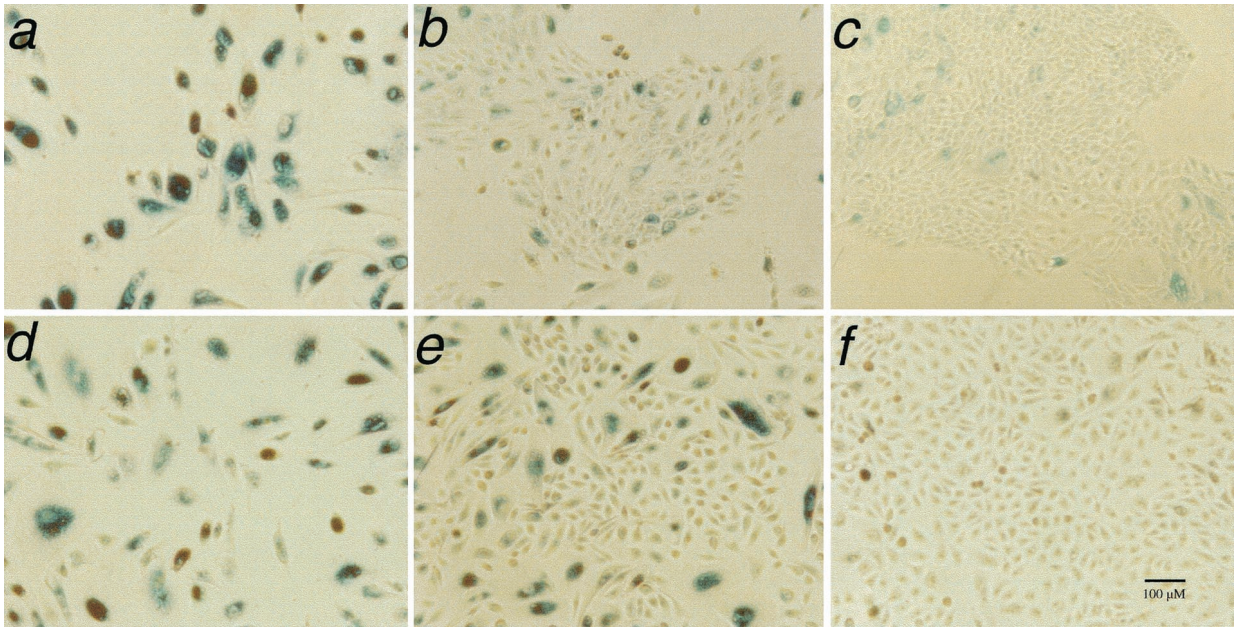

Fig. 2. *ZNF217*-transduced cultures show gradual loss of SA β-gal activity. 184Aa (*a–c*) and 184 (*d–f*) HMECs infected with LXSN control (*a* and *d*) or ZNF217-containing (*b*, *c*, *e*, and *f*) retrovirus were stained for SA β-gal activity (pH 6.0) at the following passages: *a*, p14; *b*, p17; *c*, p35; *d*, p20; *e*, p20; *f*, p25. Control cultures showed large, flat cells with abundant SA β-gal staining when they reached senescence at passages 14–16 for 184Aa cells or passage 20 for 184 cells. At this point, ZNF217 cultures began showing the presence of small, mitotic, SA β-gal-negative cells in a background of positive senescent cells. By later passages, most of the cells were SA β-gal-negative and growing well.

Table 1 *Growth of 184ZN4 and AaZN1A colonies at different passage levels in the absence or presence of TGF-β*

Single cells (200–10,000) were seeded per 100-mm dish, and the labeling index ± TGF-β in the ensuing colonies, which contained >50 cells, was determined as described in "Materials and Methods."

	Labeling index (%)								
	TGF-β (−)				TGF-β (+)				
Passage	<10	10–25	26–50	>50	<10	10–25	26–50	>50	CFE[a] (%)
184									
13									
LXSN	0	3	14	83	100	0	0	0	9.8
ZNF217	0	9	31	60	95	5	0	0	12.8
17									
LXSN	86	14	0	0	100	0	0	0	7.3
ZNF217	9	16	28	47	100	0	0	0	4.1
20									
ZNF217	4	5	35	56	93	7	0	0	4.8
23									
ZNF217	0	14	0	86	100	0	0	0	0.75
28									
ZNF217	0	0	1	99	100	0	0	0	8.4
43									
ZNF217	0	0	0	100	63	21	5	11	11.8
184Aa									
23									
ZNF217	0	0	0	100	62	11	11	16	5.5
30									
ZNF217	0	0	0	100	71	11	11	7	12.5
44									
ZNF217	0	0	0	100	53	8	13	26	9.5
50									
ZNF217	0	0	0	100	44	3	2	51	16.8

[a] CFE, colony forming efficiency.

the control cultures, but while the latter cultures senesced after 50–100 days, the *ZNF217*-transduced cells continued to grow beyond this point. The control cultures showed large, flat cells with abundant SA β-gal activity (20) when they reached senescence (Fig. 2*a*). At similar passages, the *ZNF217*-transduced cultures, termed AaZN1A and AaZN2A, began showing numerous foci of small, mitotic, SA β-gal-negative cells among SA β-gal-positive senescent cells (Fig. 2*b*). AaZN1A and AaZN2A growth was at first slow and heterogeneous but became faster and more uniform within four to six passages. By later passages, most cells were SA β-gal negative (Fig. 2*c*) and grew well.

Because 184Aa cultures have given rise to rare immortal clones spontaneously or by insertional mutagenesis (8; Fig. 1 legend),[5] we repeated the *ZNF217* transgene experiments using 184 HMECs, which have never yielded spontaneous immortal clones in numerous experiments using large numbers of cells. Similar to the *ZNF217*-transduced 184Aa cells, the *ZNF217*-transduced 184 cells (Figs. 1*b* and 2, *d–f*) showed no initial growth advantage over control cells but continued to grow after the control cells senesced, heterogeneously at first, and faster and more uniformly in later passages, producing the 184ZN4 line (Table 1). In the second experiment with 184 HMECs, a single morphologically distinct colony appeared one passage prior to senescence, and again initial passages beyond this point showed very heterogeneous, but continued, growth.

In postselection HMECs that lack p16 expression, as in other cell types, senescence has been correlated with shortened telomeres, and overcoming senescence has been correlated with derepression of telomerase (21, 22). Telomerase activity was not detectable in newly *ZNF217*-transduced 184Aa and 184 cultures, and mean TRF size, an indicator of telomere length, continued to decrease in the transduced cultures in the initial passages past control cell senescence (Fig. 3). Telomerase activity then increased incrementally, and mean TRF length stabilized at comparatively short lengths, similar to the lengths found in many carcinoma-derived cell lines (23).

All finite life span HMECs cease proliferation in response to TGF-β. In contrast, most immortal and malignant epithelial cell lines can maintain growth in its presence, and this trait is thought to contribute to the malignant phenotype (24). We examined 184ZN4 and AaZN1A at different passages for growth capacity in TGF-β. No growth was seen prior to and just after overcoming senescence (Table 1). However, with increasing passage, some cells capable of maintaining growth in the presence of TGF-β began emerging. This gradual, heterogeneous acquisition of TGF-β resistance is similar to what is observed during conversion of our carcinogen-immortalized HMECs, where the ability to maintain growth in the presence of TGF-β is acquired incrementally in both mass cultures and clonal isolates (5). The incremental nature and reproducibility of this change suggest that it is attributable to epigenetic changes in gene expression after immortalization.

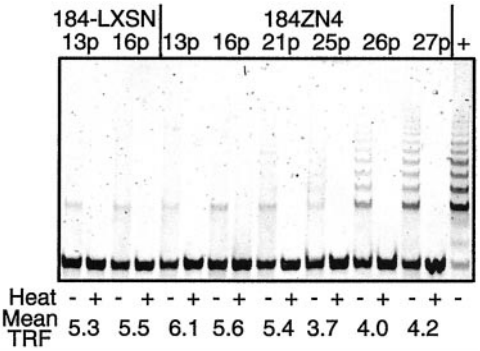

Fig. 3. Finite life span 184 HMECs transduced with *ZNF217* show gradual acquisition of telomerase activity and stabilization of telomere lengths. Telomerase activity at indicated passages was measured in 2-μg extracts of 184 HMECs transduced with LXSN alone (negative control) or LXSN containing the *ZNF217* gene (*184ZN4*). This representative telomerase assay gel reveals the characteristic 6-bp ladder indicative of enzymatic activity that is prominent in an immortalized human kidney cell line (+, positive control) and later passage (*26p* and *27p*) 184ZN4 cells. Heat-treated extracts were used as negative controls. Mean TRF size (an indicator of telomere length) was calculated from Southern blots (data not shown) of genomic DNA harvested from cells at the indicated passages.

[5] M. R. Stampfer *et al.*, manuscript in preparation.

Loss of function of the tumor suppressors, p53 and pRb, has been observed in numerous immortal cell lines and is thought to play a role in the immortalization process. To determine whether loss of p53 function contributed to the immortalization of the *ZNF217*-transduced HMECs, induction of p53 expression by the DNA-damaging agent actinomycin D was measured. Induction of p53 similar to that in the finite life span cells was observed in all three *ZNF217*-transduced immortalized HMECs tested (Fig. 4*a*). For a second confirmation of p53 activity, we analyzed p53-dependent induction of GADD45 transcripts by UV irradiation (15). GADD45 mRNA levels were increased 4 h after UV exposure in both the finite life span 184 and immortalized 184ZN4A cultures (Fig. 4*b*). pRb was also present and underwent normal cycles of phosphorylation and dephosphorylation in these cells (data not shown). Thus, as shown previously for the carcinogen-immortalized HMECs (25, 26), alterations in p53 and/or pRb are not obligatory for immortalization of the *ZNF217*-transduced HMECs.

The above data demonstrate that constitutive aberrant expression of *ZNF217* can immortalize finite life span HMECs, and that overcoming senescence is separable from subsequent changes in telomerase activity and TGF-β resistance. The precise frequency of *ZNF217*-induced immortalization remains to be determined. Southern analysis of retroviral integration sites in *ZNF217*-transduced HMECs growing past senescence suggested that these cultures were rapidly overgrown by distinct clonal populations (data not shown). In an effort to determine whether distinct chromosomal alterations might be conferring growth advantages on clones immortalized with *ZNF217*, DNA from three different immortalized cultures was used for quantitative measurement of DNA copy number using CGH (27). CGH analysis showed low-level regional DNA sequence copy number variations on chromosomes 1q and 8q common to all three cell lines (Fig. 5). The region amplified on 8q included the c-*myc* oncogene, which itself has been shown to cause HMEC immortalization when overexpressed (28). In addition, each line showed unique regions of high- and low-level DNA sequence copy number variations. These sites of regional copy number variation, some of which have also been observed frequently in breast cancer cell lines and primary tumors (27), may contain genes that cooperate with *ZNF217* in facilitating growth and immortalization.

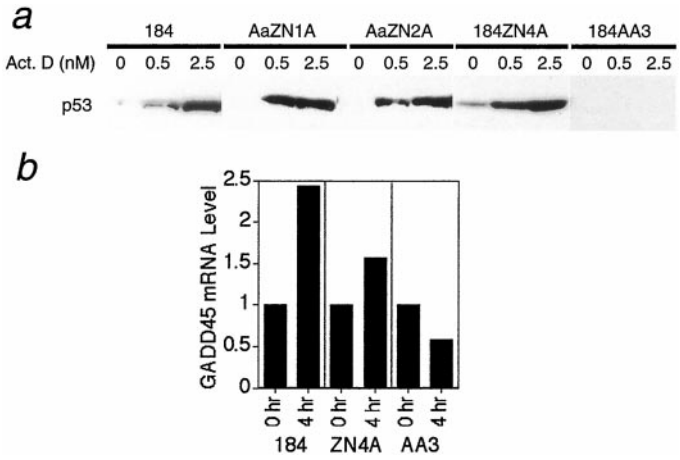

Fig. 4. p53 expression and function are intact in HMECs immortalized after *ZNF217* transduction. *a*, immunoblot of p53 expression in response to DNA damage by 24 h treatment with indicated concentrations of actinomycin D. 184 has wild-type p53. 184AA3 is a negative control HMEC line in which one *TP53* allele has been inactivated by insertional mutagenesis, and the other allele has been inactivated by unknown means.[5] The cells were assayed at passages 14 (184), 52 (AaZN1A), 49 (AaZN2A), 39 (184ZN4A), and 45 (184AA3). *b*, the relative abundance of GADD45 mRNA in indicated cell types 4 h after exposure to UV irradiation (37 joules/m^2) was measured by Northern analysis, normalized to the levels of a ribosomal protein transcript, and is presented in graphical form as induction relative to that in the same cells at 0 h.

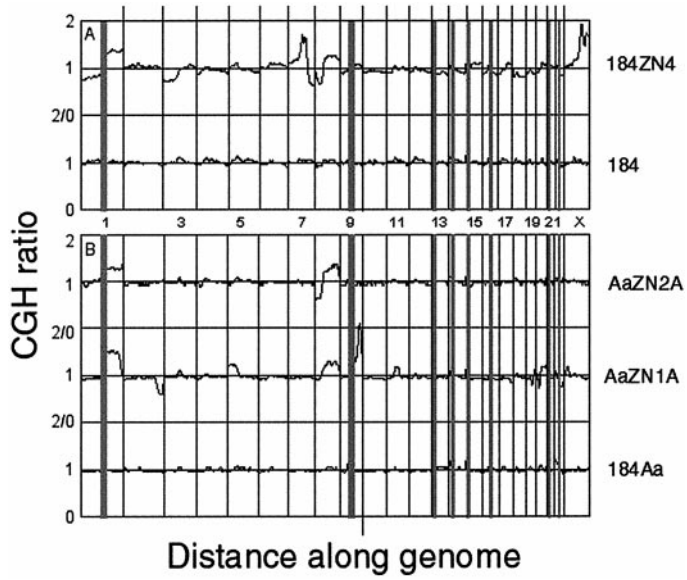

Fig. 5. CGH analyses of genome copy number in HMECs before and after *ZNF217*-induced immortalization. CGH ratios are arranged from short arm to long arm and from chromosomes 1 to 22, then X. *Thin vertical black lines*, chromosome boundaries. *Heavy gray vertical bars*, regions containing repeated sequences that were not reliably analyzed in these CGH analyses. Locations of the odd-numbered chromosomes are indicated between *A* and *B*. *A*, CGH profiles for finite life span normal 184 HMEC at passage 13 and *ZNF217*-immortalized cell line 184ZN4 at passage 37. *B*, CGH profiles for extended life span 184Aa HMEC at passage 8 and *ZNF217*-immortalized cell lines AaZN1A and AaZN2A at passages 55 and 54, respectively.

These results support the hypothesis that *ZNF217* gene amplification is found frequently in breast cancers because it enables breast cells to overcome senescence, allowing the cells to continue growing and accumulating other changes necessary for malignant progression. The slow gradual changes in telomerase activity and growth in *ZNF217*-transduced cells after they have overcome senescent resemble the changes seen during the conversion process in carcinogen-immortalized HMECs, where measurable telomerase reactivation follows rather than precedes the overcoming of senescence. *ZNF217* now can be added to the small list of cellular (*e.g.*, c-*myc*; Ref. 28) and viral (HPV16 *E6* and *E7*; Ref. 29) oncogenes that cause HMEC immortalization. Unlike *E6*, which may alter several cellular functions simultaneously, both *ZNF217* and c-*myc* immortalize HMECs inefficiently and are likely to require additional changes for immortalizing activity. Further studies are needed to elucidate the mechanism(s) by which *ZNF217* acts. However, one possibility is that *ZNF217* overexpression interferes with one or more checkpoint functions that normally operate to eliminate senescing cells [*e.g.*, the p53-dependent, DNA damage-sensing pathway that prevents continued proliferation with short telomeres (30)].

Acknowledgments

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References

1. Collins, C., Rommens, J. M., Kowbel, D., Godfrey, T., Tanner, M., Hwang, S., Polikoff, D., Nonet, G., Cochran, J., Myambo, K., Jay, K. E., Froula, J., Cloutier, T., Kuo, W-L., Yaswen, P., Dairkee, S., Giovanola, J., Hutchinson, G. B., Isola, J., Kallioniemi, O-P., Palazzolo, M., Martin, C., Ericsson, C., Pinkel, D., Albertson, D., Li, W-B., and Gray, J. W. Positional cloning of *ZNF217* and *NABC1*: genes amplified at 20q13.2 and overexpressed in breast carcinoma. Proc. Natl. Acad. Sci. USA, *95:* 8703–8708, 1998.
2. Savelieva, E., Belair, C. D., Newton, M. A., DeVries, S., Gray, J. W., Waldman, F., and Reznikoff, C. A. 20q gain associates with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. Oncogene, *14:* 551–560, 1997.
3. Cuthill, S., Agarwal, P., Sarkar, S., Savelieva, E., and Reznikoff, C. A. Dominant genetic alterations in immortalization: role for 20q gain. Genes Chromosomes Cancer, *26:* 304–311, 1999.

4. Stampfer, M. R., and Yaswen, P. Culture models of human mammary epithelial cell transformation. J. Mam. Gland Biol. Neo., *5:* 27–40, 2000.
5. Stampfer, M. R., Bodnar, A., Garbe, J., Wong, M., Pan, A., Villeponteau, B., and Yaswen, P. Gradual phenotypic conversion associated with immortalization of cultured human mammary epithelial cells. Mol. Biol. Cell, *8:* 2391–2405, 1997.
6. Hammond, S. L., Ham, R. G., and Stampfer, M. R. Serum-free growth of human mammary epithelial cells: rapid clonal growth in defined medium and extended serial passage with pituitary extract. Proc. Natl. Acad. Sci. USA, *81:* 5435–5439, 1984.
7. Stampfer, M. R. Isolation and growth of human mammary epithelial cells. J. Tissue Culture Methods, *9:* 107–116, 1985.
8. Stampfer, M. R., and Bartley, J. C. Induction of transformation and continuous cell lines from normal human mammary epithelial cells after exposure to benzo(*a*)pyrene. Proc. Natl. Acad. Sci. USA, *82:* 2394–2398, 1985.
9. Stampfer, M. R., Pan, C. H., Hosoda, J., Bartholomew, J., Mendelsohn, J., and Yaswen, P. Blockage of EGF receptor signal transduction causes reversible arrest of normal and transformed human mammary epithelial cells with synchronous reentry into the cell cycle. Exp. Cell Res., *208:* 175–188, 1993.
10. Miller, A. D., and Rosman, G. J. Improved retroviral vectors for gene transfer and expression. Biotechniques, *7:* 980–990, 1989.
11. Finer, M. H., Dull, T. J., Qin, L., Farson, D., and Roberts, M. R. *kat*: a high-efficiency retroviral transduction system for primary human T lymphocytes. Blood, *83:* 43–50, 1994.
12. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L. C., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. Specific association of human telomerase activity with immortal cells and cancer. Science (Washington DC), *266:* 2011–2015, 1994.
13. Bodnar, A. G., Kim, N. W., Effros, R. B., and Chiu, C-P. Mechanism of telomerase induction during T cell activation. Exp. Cell Res., *228:* 58–64, 1996.
14. Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., Greider, C. W., and Harley, C. B. Telomere length predicts replicative capacity of human fibroblasts. Proc. Natl. Acad. Sci. USA, *89:* 10114–10118, 1992.
15. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.
16. Laborda, J. 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. Nucleic Acids Res., *19:* 3998, 1991.
17. Kallioniemi, A., Kallioniemi, O-P., Sudar, D., Rutovitz, D., Gray, J. W., Waldman, F., and Pinkel, D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science (Washington DC), *258:* 818–821, 1992.
18. Piper, J., Rutovitz, D., Sudar, D., Kallioniemi, A., Kallioniemi, O. P., Waldman, F. M., Gray, J. W., and Pinkel, D. Computer image analysis of comparative genomic hybridization. Cytometry, *19:* 10–26, 1995.
19. Brenner, A. J., Stampfer, M. R., and Aldaz, C. M. Increased p16INK4a expression with onset of senescence of human mammary epithelial cells and extended growth capacity with inactivation. Oncogene, *17:* 199–205, 1998.
20. Dimri, G. P., Lee, X., Basile, G., Roskelley, C., Medrano, E. E., Rubelji, I., Pereira-Smith, O. M., Peacocke, M., and Campisi, J. A novel biomarker identifies senescent human cells in culture and in aging skin *in vivo*. Proc. Natl. Acad. Sci. USA, *92:* 9363–9367, 1995.
21. Harley, C., and Villeponteau, B. Telomeres and telomerase in aging and cancer. Curr. Opin. Genet. Dev., *5:* 249–255, 1995.
22. Wright, W. E., and Shay, J. W. Time, telomeres and tumors: is cellular senescence more than an anticancer mechanism. Trends Cell Biol., *5:* 293–297, 1995.
23. Chu, C-T., Piatyszek, M. A., Wong, S. S. Y., Honchell, C., Holeman, L. A., Wunder, E. W., Trees, N., Palencia, M. A., Li, S., and Chin, A. C. Telomerase activity and telomere lengths in the NCI cell panel of human cancer cell lines. Proc. Am. Assoc. Cancer Res., *41:* 534, 2000.
24. Arteaga, C. L., Dugger, T. C., and Hurd, S. D. The multifunctional role of TGF-β on mammary epithelial cell biology. Breast Cancer Res. Treat., *38:* 49–56, 1996.
25. Lehman, T., Modali, R., Boukamp, P., Stanek, J., Bennett, W., Welsh, J., Metcalf, R., Stampfer, M., Fusenig, N., Rogan, E., Reddel, R., and Harris, C. p53 mutations in human immortalized epithelial cell lines. Carcinogenesis (Lond.), *14:* 833–839, 1993.
26. Sandhu, C., Garbe, J., Bhattacharya, N., Daksis, J. I., Pan, C-H., Yaswen, P., Koh, J., Slingerland, J. M., and Stampfer, M. R. TGF-β stabilizes p15INK4B protein, increases p15INK4B/cdk4 complexes and inhibits cyclin D1-cdk4 association in human mammary epithelial cells. Mol. Cell. Biol., *17:* 2458–2467, 1997.
27. Kallioniemi, A., Kallioniemi, O-P., Piper, J., Tanner, M., Stokke, T., Chen, L., Smith, H. S., Pinkel, D., Gray, J. W., and Waldman, F. M. Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc. Nat. Acad. Sci. USA, *91:* 2156–2160, 1994.
28. Wang, J., Xie, L. Y., Allan, S., Beach, D., and Hannon, G. J. Myc activates telomerase. Genes Dev., *12:* 1769–1774, 1998.
29. Wazer, D. E., Liu, X-L., Chu, Q., Gao, Q., and Band, V. Immortalization of distinct human mammary epithelial cell types by human papilloma virus 16 E6 or E7. Proc. Natl. Acad. Sci. USA, *92:* 3687–3691, 1995.
30. Artandi, S. E., Chang, S., Lee, S. L., Alson, S., Gottlieb, G. J., Chin, L., and DePinho, R. A. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice [see comments]. Nature (Lond.), *406:* 641–645, 2000.