

Lawrence Berkeley National Laboratory

LBL Publications

Title

TDF- β signaling engages an ATM-CHK2-p53-independent RAS-induced senescence and prevents malignant transformation in human mammary epithelial cells

Permalink

<https://escholarship.org/uc/item/50v4w5j0>

Authors

Stampfer, MR

Cipriano, R

Kan, CE

et al.

Publication Date

2023-12-11

Peer reviewed

TGF- β signaling engages an ATM-CHK2-p53-independent RAS-induced senescence and prevents malignant transformation in human mammary epithelial cells

Rocky Cipriano^a, Charlene E. Kan^b, James Graham^a, David Danielpour^c, Martha Stampfer^d, and Mark W. Jackson^{a,c,1}

Departments of ^aPathology and ^bGenetics and ^cCase Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106; and ^dLife Sciences Divisions, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Edited* by George R. Stark, Lerner Research Institute NE2, Cleveland, OH, and approved April 14, 2011 (received for review October 7, 2010)

Oncogene-induced senescence (OIS), the proliferative arrest engaged in response to persistent oncogene activation, serves as an important tumor-suppressive barrier. We show here that finite lifespan human mammary epithelial cells (HMEC) undergo a p16/RB- and p53-independent OIS in response to oncogenic RAS that requires TGF- β signaling. Suppression of TGF- β signaling by expression of a dominant-negative TGF- β type II receptor, use of a TGF- β type I receptor inhibitor, or ectopic expression of MYC permitted continued proliferation upon RAS expression. Surprisingly, unlike fibroblasts, shRNA-mediated knockdown of ATM or CHK2 was unable to prevent RAS-mediated OIS, arguing that the DNA damage response is not required for OIS in HMEC. Abrogation of TGF- β signaling not only allowed HMEC lacking p53 to tolerate oncogenic RAS but also conferred the capacity for anchorage-independent growth. Thus, the OIS engaged after dysregulated RAS expression provides an early barrier to malignant progression and is mediated by TGF- β receptor activation in HMEC. Understanding the mechanisms that initiate and maintain OIS in epithelial cells may provide a foundation for future therapies aimed at reengaging this proliferative barrier as a cancer therapy.

breast cancer | tumor suppressor

Cancer cells acquire errors that impart behaviors not present in their normal cells of origin. Such altered capacities include (i) loss of sensitivity to antigrowth and/or proapoptotic signals, (ii) constitutive growth signaling, (iii) unlimited replication potential, and (iv) invasive potential (1). Early studies using normal mouse cells indicated that a limited set of genetic manipulations could confer neoplastic potential (2). However, normal human cells have been more difficult to transform to malignancy, indicative of their more stringent tumor-suppressive pathways. Extensive study of cultured human mammary epithelial cells (HMEC) has identified two senescence barriers. One involves the stress-associated induction of the cyclin-dependent kinase inhibitor p16 before attaining critically short telomeres. This stasis barrier can be overcome by inhibiting p16, allowing continued proliferation, which results in agonescence, a proliferative barrier mediated by telomere depletion (3). Additionally, the ability of dysregulated oncogenic signaling to induce senescence in human cells has implicated oncogene-induced senescence (OIS) as an important tumor-suppressive barrier. A number of recent studies have demonstrated the physiological relevance of OIS in human tumorigenesis and in vivo tumor mouse models (4). Additionally, the presence of senescent cells in benign but not advanced tumors argues that OIS serves as an early tumor-suppressive barrier that needs to be dismantled for full oncogenic progression (4). In human fibroblasts, OIS could be bypassed by disabling p16 or molecular components of the DNA damage response (DDR), including ATM, CHK2, or p53, before RAS, MOS, or STAT5 overexpression (5–9). However, OIS in HMEC has been shown to be independent of p53 and the p16-RB pathway after oncogenic RAF-1 expression (10). The contrasting responses between epithelial and fibroblast cells argue that the signaling networks responsible for OIS have tissue specificity. Indeed, fibroblasts and epithelial cells can have markedly

different responses to cytokines such as TGF- β , which inhibits HMEC growth while promoting the growth of isogenic fibroblasts (11). Furthermore, there are significant cell-type differences in requirements for RAS effector signaling for malignant transformation (12). Understanding the tumor-suppressive pathways that prevent breast cancer is therefore best performed using HMEC, the cell of origin for the majority of human breast cancers.

We show here that activated RAS expression induces HMEC to undergo a p16- and p53-independent senescence that requires the TGF- β receptor. In contrast to studies performed using fibroblasts, shRNA-mediated knockdown of ATM or CHK2 did not prevent RAS-mediated OIS in HMEC lacking p53. However, suppression of TGF- β signaling by expressing a dominant-negative TGF- β type II receptor, use of a TGF- β type I receptor inhibitor, or ectopic expression of MYC prevented RAS-mediated OIS, and together with loss of p16 and p53 function, permitted the expansion of HMEC with a malignant phenotype. Understanding the unique tumor-suppressive responses that are engaged in human breast epithelial cells can provide a foundation for future therapies aimed at reengaging these suppressive pathways.

Results

Recently identified cell-type-specific requirements for transformation indicate that unique tumor-suppressive mechanisms exist to protect each tissue from cancer development (12). To examine the growth-suppressive mechanisms underlying RAS-mediated OIS in HMEC, we first examined the role of p53. An shRNA targeting p53 and a control shGFP were delivered by lentiviral transduction to postselection HMEC, which lack p16 expression owing to promoter methylation (13, 14). Western analysis confirmed the knockdown of p53 protein levels in the shp53-HMEC and the abrogation of p53-dependent transactivation of target genes HDM2 and p21 in response to Nutlin-3, a p53-stabilizing compound (Fig. S1A). Furthermore, treatment of shGFP-HMEC with Nutlin-3 resulted in p53-mediated growth arrest, whereas the shp53-HMEC were unaffected (Fig. S1B). The shGFP-HMEC and shp53-HMEC were next examined for their response to activated RAS by infecting them with a retrovirus encoding RAS-G12V or a control vector (V). Both HMEC cultures were strongly growth inhibited, as determined by cell counts 5 d after infection (Fig. 1A). The RAS-expressing cells exhibited an increase in cell size, cell spreading, vacuolization, and multinucleated cellular morphology typical of senescence (15) and stained positively for the presence of senescence-associated β -galactosidase activity (Fig. 1B and Fig. S2). Western analysis confirmed the expression of RAS-G12V, the induction

Author contributions: R.C. and M.W.J. designed research; R.C., C.E.K., J.G., and M.W.J. performed research; D.D. and M.S. contributed new reagents/analytic tools; R.C. and M.W.J. analyzed data; and R.C. and M.W.J. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

¹To whom correspondence should be addressed. E-mail: mwj7@case.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015022108/-DCSupplemental.

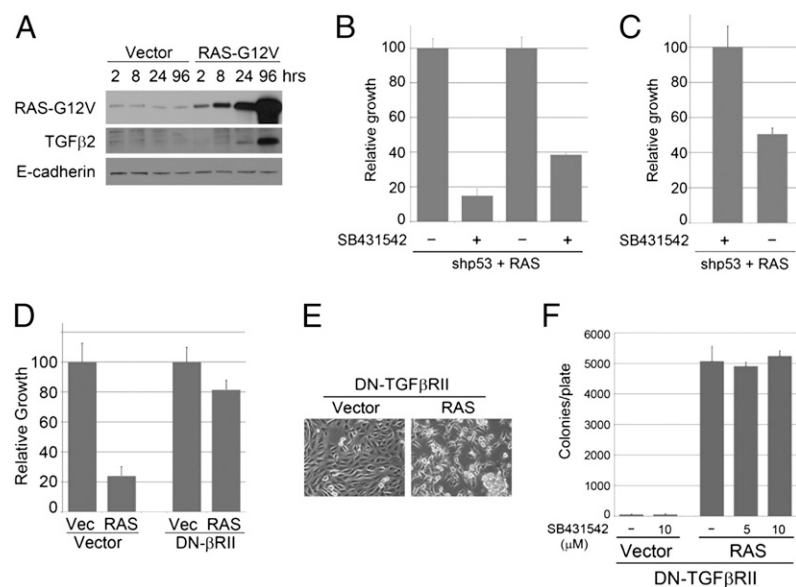


Fig. 2. TGF- β signaling is required for RAS-mediated OIS. (A) RAS-G12V-infected Shp53-HMEC were subjected to Western analysis for TGF- β 2, RAS, and E-cadherin at the indicated time points. (B) Shp53-HMEC were pretreated with SB431542, a TGF- β receptor I antagonist, infected with a retrovirus encoding RAS-G12V, plated, grown for 5 d, and counted. (C) Shp53-RAS-G12V-HMEC grown in the presence of SB431542 were plated in the presence (+) or absence (–) of the inhibitor, and relative growth was measured after 5 d. (D) Shp53/DN-TGF β RII-HMEC were infected with a retrovirus encoding RAS-G12V or control retrovirus (Vector) and relative growth determined 5 d after RAS-G12V infection. (E) Representative images of Shp53/DN-TGF β RII/RAS-G12V-HMEC and control Shp53/DN-TGF β RII/Vector-HMEC. (F) Shp53/DN-TGF β RII/RAS-G12V-HMEC or control Shp53/DN-TGF β RII/Vector-HMEC were examined for AIG in the presence or absence of SB431542.

whether HMEC deficient in three prominent tumor-suppressor pathways (p16, p53, and TGF- β signaling) and resistant to OIS had acquired properties of malignant transformation. Indeed, the shp53/DN-TGF β RII/RAS-HMEC appeared morphologically distinct from control cells, growing as aggregates with diminished attachment to the substratum (Fig. 2E). To determine whether the shp53/DN-TGF β RII/RAS-HMEC were malignantly transformed, they were plated into soft agar to assay for anchorage-independent growth (AIG). Shp53/DN-TGF β RII/RAS-HMEC efficiently formed anchorage-independent colonies, whereas control shp53/DN-TGF β RII/Vector-HMEC failed to form colonies (Fig. 2F). Addition of the TGF- β type I receptor antagonist SB431542 failed to increase AIG in the control shp53/DN-TGF β RII-HMEC. These results indicate that RAS expression promotes AIG in shp53-HMEC in the absence of functional TGF- β signaling, but inhibition of TGF- β signaling alone is not sufficient for AIG. Further, because SB431542 did not enhance the AIG observed in the shp53/DN-TGF β RII/RAS-HMEC, we conclude that inhibition of either TGF β RI or TGF β RII is sufficient to cooperate with RAS-G12V to induce a malignancy-associated phenotype in the shp53-HMEC.

We also examined a small population of shp53-HMEC that grew out of the senescent cultures after RAS expression (Fig. 3A and B). Our hypothesis was that cells capable of tolerating RAS expression would need to first dismantle OIS signaling, which involves TGF- β . However, the RAS-resistant shp53-HMEC (RAS-R cells) that grew out of the senescent cultures for 20 d expressed significantly less RAS-G12V than the senescent cultures examined 4 d after infection (Fig. 3C). In addition, the RAS-R cells remained sensitive to growth inhibition in response to exogenous TGF- β and were unable to efficiently form anchorage-independent colonies (Fig. 3D). Surprisingly, treatment of these RAS-R cells with SB431542 significantly enhanced AIG, arguing that TGF- β signaling was intact and functioning to suppress AIG. Treatment of control shp53-HMEC lacking RAS expression with SB431542 failed to promote AIG, confirming that combined expression of low levels of RAS-G12V and TGF- β receptor inhibition promotes malignant transformation. We conclude that cells expressing a low level of RAS did not engage a TGF- β -mediated senescence, because they remained sensitive to exogenous TGF- β . Our data suggest that this acquired resistance to the cytostatic effects of TGF- β is required for malignant transformation.

MYC is an oncoprotein frequently overexpressed in breast cancer and is shown to suppress the cytostatic effects of TGF- β (24). Therefore, we examined whether elevated expression of

MYC could rescue HMEC from RAS-mediated OIS. Shp53-HMEC were infected with a retrovirus encoding MYC or an empty vector and subsequently infected with a retrovirus encoding RAS-G12V (Fig. 4A and B). Control shp53/Vector-HMEC underwent RAS-mediated OIS, whereas shp53/MYC-HMEC continued proliferating in the presence of RAS-G12V (Fig. 4B). As expected, HMEC expressing either MYC or DN-TGF β RII were also resistant to the growth-inhibitory properties of exogenous TGF- β treatment (Fig. 4C). Our data thus far define a significant role for TGF- β signaling in (i) OIS induction and (ii) suppressing malignant transformation in the absence of p16/RB and p53 signaling. However, although p53 loss was unable to overcome the OIS barrier, p53 may still have a role in suppressing AIG. To

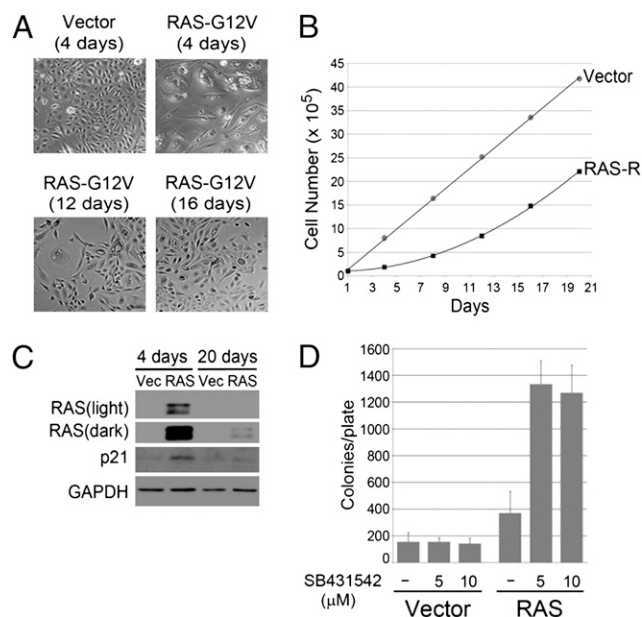


Fig. 3. TGF- β signaling must be inhibited for HMEC transformation. (A–C) Representative images (A), growth curves (B), and Western analysis (C) for RAS, p21, and GAPDH of shp53-HMEC infected with a retrovirus encoding RAS-G12V or a control retrovirus at the indicated time points after infection. (D) Vector and RAS-R cells were examined for AIG in the absence or presence of SB431542.

test this, HMEC and shp53-HMEC were infected with retroviruses encoding MYC and RAS-G12V, either alone or in combination. Western analysis of each of the HMEC cultures failed to identify known senescence-associated signaling as candidates responsible for the OIS. For example, p53 was phosphorylated and stabilized, CHK2 was phosphorylated, and p14ARF was induced by MYC expression alone but not by RAS-G12V expression alone (Fig. 4D) (25). Despite the elevated levels of p14ARF, p53, and DNA damage-responsive signaling, MYC expression in HMEC with or without shp53 does not induce a senescent phenotype (Fig. 4E). In contrast, RAS-G12V expression did not induce p14ARF, p53, or DNA damage-responsive signaling, yet induced a senescent phenotype in the both the presence or absence of p53 (Fig. 4E).

Each HMEC culture was next assessed for AIG. HMEC and shp53-HMEC expressing MYC or RAS-G12V alone formed colonies inefficiently. In contrast, expression of both MYC and RAS together promoted AIG, with a significant increase in colony number when p53 was abrogated (Fig. 4F). We confirmed that p53 remained fully functional in the MYC/RAS-HMEC using Nutlin-3, which induced a potent p53-dependent growth arrest (Fig. S9). Interestingly, these data demonstrate that acquisition of AIG in HMEC can occur independent of human telomerase reverse transcriptase (hTERT) expression or immortality, because the HMEC used in this study were not previously immortalized by exogenous hTERT or reactivation of endogenous hTERT. However, both the MYC/RAS-HMEC and shp53/MYC/RAS-HMEC cultures formed significantly fewer colonies at later passages and, despite their capacity for AIG, eventually stopped proliferating. These data support the contention that telomerase activation is a key rate-limiting step in malignant progression, and further analysis is currently underway (26).

The postselection HMEC used thus far have overcome stasis, the stress-associated senescence barrier, by selecting for p16 promoter methylation (3). In that process, they also acquired additional aberrant properties, including numerous DNA methylation changes (3, 27). We thus extended this work by examining normal, prestasis HMEC transduced with an shRNA targeting p16; this population does not express the aberrant methylation seen with the postselection HMEC (3). Shp16-HMEC were infected with shp53 or control vector, and shp16-HMEC and shp16/shp53-HMEC were further transduced with MYC and RAS-G12V alone or in combination. The results for AIG capacity were similar to what was seen with postselection HMEC (Fig. 4 *G* and *H*). These data indicate that the ability of MYC and RAS to promote AIG in HMEC lacking p16 and p53 does not depend on the particular aberrations present in the postselection cultures.

Prior studies in HMEC have correlated the level of RAS-G12V expression with AIG (28). Therefore, we altered the level of RAS-G12V expression using different retroviral vectors in postselection MYC-HMEC and shp53/MYC-HMEC. Increasing RAS levels increased the AIG of shp53-HMEC until a critical threshold was surpassed, resulting in reduced AIG (Fig. 5A). Again, HMEC with wild-type p53 exhibited significantly less AIG in the presence of MYC and RAS than shp53-HMEC (Fig. 5A). Thus, we conclude that p53 is not required to suppress OIS but remains a suppressor of malignant transformation. Interestingly, each population of cells recovered from soft agar expressed a level of RAS-G12V that was significantly reduced from that of the initial population (Fig. 5B), similar to previous studies using RAF-1 (10). These findings argue that, in tumor-derived cells harboring a RAS mutation, the OIS response may be abrogated by elevating MYC expression. To examine whether MYC depletion in tumor-derived cells harboring activating RAS mutations would reactivate a senescence program, we infected epithelial

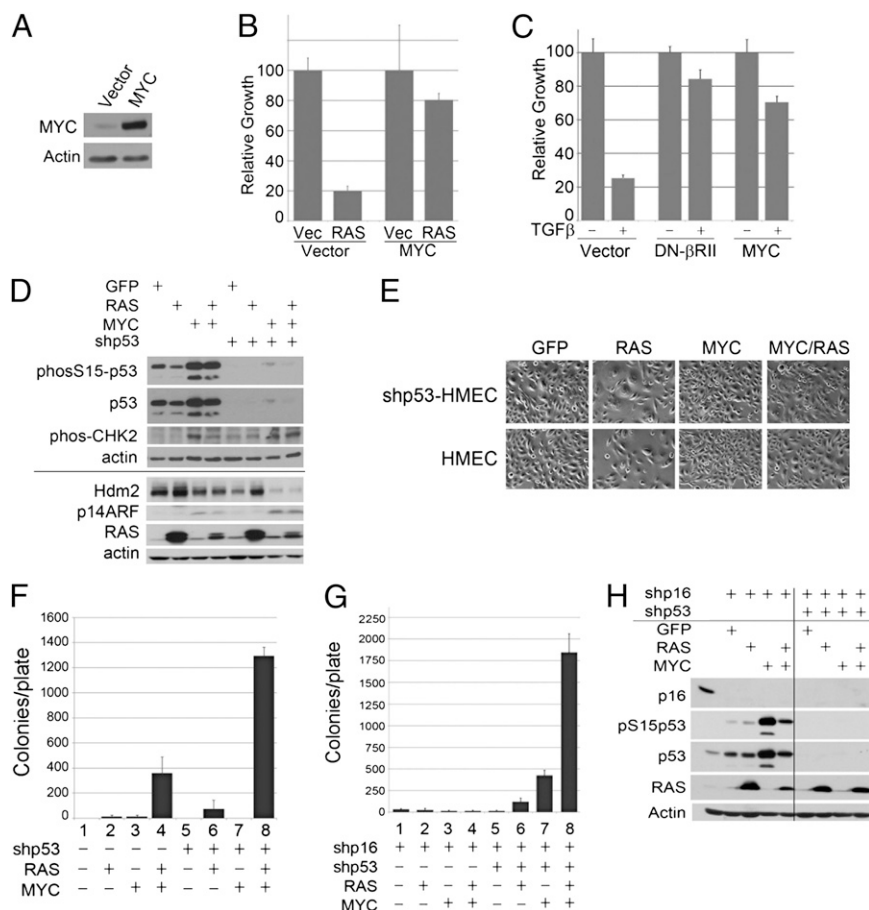


Fig. 4. Neoplastic transformation of HMEC. (A and B) Shp53-expressing HMEC were infected with a retrovirus encoding MYC or an empty vector (A) and subsequently infected with a retrovirus encoding RAS-G12V. Relative growth was determined 5 d after RAS-G12V infection (B). (C) Shp53-HMEC expressing MYC or DN-TGF β RII, or control cells (vector) were plated in the presence (+) or absence (-) of TGF- β (10 ng/mL) and relative growth determined after 5 d. (D) Western analysis of HMEC and shp53-HMEC expressing GFP, RAS-G12V, MYC, or RAS-G12V and MYC together. (E) Representative images of shp53-HMEC and shGFP-HMEC infected with a retrovirus encoding GFP, RAS-G12V, MYC, or MYC and RAS together. (F) shGFP-HMEC and shp53-HMEC were infected with retroviruses encoding MYC and RAS-G12V, either alone or in combination, and assessed for AIG. (G) Shp16-HMEC and shp16/shp53-HMEC were infected with retroviruses encoding MYC and RAS-G12V, either alone or in combination, and assessed for AIG. (H) Western analysis of shp16-HMEC and shp16/shp53-HMEC expressing GFP, RAS-G12V, MYC, or RAS-G12V and MYC together.

p16 and p53 function, acquired resistance to the cytostatic effects of TGF- β signaling, and acquisition of persistent growth signaling.

On the basis of our study, we propose that a high level of RAS signaling must be accompanied by an acquired resistance to the cytostatic effects of TGF- β , either by TGF- β receptor inhibition or elevated MYC expression (Fig. 5D). In contrast, low-level RAS signaling is incapable of engaging OIS but still cooperates with the abrogation of TGF- β signaling to promote malignant transformation. MYC is a well-known suppressor of TGF- β signaling (36) and is amplified in up to 52% of breast cancer specimens, depending on the study. Tumors that do not harbor an MYC amplification often overexpress MYC protein via additional mechanisms (36–39). RAS mutations are surprisingly infrequent in breast cancer (<5%) relative to other cancers (50% of colon and thyroid cancers and 90% of pancreatic cancers). However, the reason for the differences between cancers that tolerate RAS mutations and those that do not remains unclear (40). We speculate that mutant RAS may initiate the tumor-suppressive OIS in HMEC more potentially, or more acutely than RAS signaling elevated via overexpression of growth factor receptors or wild-type RAS, which are more frequently observed in breast cancer (41).

The observation that TGF- β signaling suppresses the growth of normal epithelial cells, yet is often required for the maintenance of a transformed phenotype, remains a paradox in the field. However, much like the differences in biological outcomes observed between fibroblasts and epithelial cells exposed to TGF- β , the response of normal, hyperplastic, and transformed epithelial cells may be explained by the diverse signals generated by TGF- β receptor activation. SMAD-dependent and -independent pathways (involving TAK1, NF κ B, JNK, MAPK, PI3K/AKT, and mTOR, among others) determine whether cells arrest, continue dividing, or un-

dergo an epithelial-to-mesenchymal transition after exposure to TGF- β (42). In our model, elevated MYC expression suppresses RAS-mediated OIS but maintains the TGF- β receptor in a functional state. We suggest that, in breast cancer, the senescence programs are simply suppressed rather than absent, leaving the option to reengage these hidden limits to proliferation as a cancer therapy.

Materials and Methods

Cell Lines and Culture Conditions. Postselection HMEC [48R batch 5 (27)] were grown in a humidified atmosphere containing 5% CO₂ in Medium 171 with mammary epithelial growth supplement (Cascade Biologics). Prestasis HMEC (specimen 48R, batch T) were grown in a humidified atmosphere containing 5% CO₂ in M87A media as previously described (27). MDA231, Hs578T, H1299, and LoVo cells were grown in a humidified atmosphere containing 5% CO₂ in DMEM supplemented with 5% FBS; A549 cells were grown in RPMI supplemented with 5% FBS.

Soft Agar and Relative Growth Assays. For AIG assays, HMEC (2×10^5) were suspended in 0.6% type VII agarose (Sigma) and plated onto a bottom layer of 1.2% agar in a 60-mm plate in triplicate. For relative growth assays, HMEC (1×10^5) or MDA231, Hs578T, A549, H1299, and LoVo (5×10^4) cells were plated in triplicate in six-well plates, and cell number was determined on a Beckman Coulter counter after 5 d of growth. Quantification of soft agar colonies and inhibitor treatments are described in detail in *SI Materials and Methods*.

Additional materials and methods are described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This work was supported by National Institutes of Health Grant T32CA059366 (to R.C.) and Department of Defense Breast Cancer Research Program Grant BC074072 (to M.W.J.).

- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100:57–70.
- Parada LF, Land H, Weinberg RA, Wolf D, Rotter V (1984) Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature* 312:649–651.
- Novak P, Jensen TJ, Garbe JC, Stampfer MR, Futscher BW (2009) Stepwise DNA methylation changes are linked to escape from defined proliferation barriers and mammary epithelial cell immortalization. *Cancer Res* 69:5251–5258.
- Courtois-Cox S, Jones SL, Cichowski K (2008) Many roads lead to oncogene-induced senescence. *Oncogene* 27:2801–2809.
- Bartkova J, et al. (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* 434:864–870.
- Bartkova J, et al. (2006) Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 444:633–637.
- Gorgoulis VG, et al. (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* 434:907–913.
- Mallette FA, Gaumont-Leclerc MF, Ferbeyre G (2007) The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence. *Genes Dev* 21:43–48.
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88:593–602.
- Olsen CL, Gardie B, Yaswen P, Stampfer MR (2002) Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. *Oncogene* 21:6328–6339.
- Hosobuchi M, Stampfer MR (1989) Effects of transforming growth factor beta on growth of human mammary epithelial cells in culture. *In Vitro Cell Dev Biol* 25:705–713.
- Rangarajan A, Hong SJ, Gifford A, Weinberg RA (2004) Species- and cell type-specific requirements for cellular transformation. *Cancer Cell* 6:171–183.
- Brenner AJ, Aldaz CM (1995) Chromosome 9p allelic loss and p16/CDKN2 in breast cancer and evidence of p16 inactivation in immortal breast epithelial cells. *Cancer Res* 55:2892–2895.
- Brenner AJ, Stampfer MR, Aldaz CM (1998) Increased p16 expression with first senescence arrest in human mammary epithelial cells and extended growth capacity with p16 inactivation. *Oncogene* 17:199–205.
- Matsumura T, Zerrudo Z, Hayflick L (1979) Senescent human diploid cells in culture: Survival, DNA synthesis and morphology. *J Gerontol* 34:328–334.
- Ossovskaya VS, et al. (1996) Use of genetic suppressor elements to dissect distinct biological effects of separate p53 domains. *Proc Natl Acad Sci USA* 93:10309–10314.
- Di Micco R, et al. (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* 444:638–642.
- Courtois-Cox S, et al. (2006) A negative feedback signaling network underlies oncogene-induced senescence. *Cancer Cell* 10:459–472.
- Tremain R, et al. (2000) Defects in TGF- β signaling overcome senescence of mouse keratinocytes expressing v-Ha-ras. *Oncogene* 19:1698–1709.
- Halder SK, Beauchamp RD, Datta PK (2005) A specific inhibitor of TGF- β receptor kinase, SB-431542, as a potent antitumor agent for human cancers. *Neoplasia* 7:509–521.
- Hahn WC, et al. (2002) Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Mol Cell Biol* 22:2111–2123.
- Di Micco R, et al. (2011) Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. *Nat Cell Biol* 13:292–302.
- Kosar M, et al. (2011) Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16(INK4a). *Cell Cycle* 10:457–468.
- Alexandrow MG, Kawabata M, Aakre M, Moses HL (1995) Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor beta 1. *Proc Natl Acad Sci USA* 92:3239–3243.
- Ries S, et al. (2000) Opposing effects of Ras on p53: Transcriptional activation of mdm2 and induction of p19ARF. *Cell* 103:321–330.
- Garbe JC, Holst CR, Bassett E, Tlsty T, Stampfer MR (2007) Inactivation of p53 function in cultured human mammary epithelial cells turns the telomere-length dependent senescence barrier from agonescence into crisis. *Cell Cycle* 6:1927–1936.
- Garbe JC, et al. (2009) Molecular distinctions between stasis and telomere attrition senescence barriers shown by long-term culture of normal human mammary epithelial cells. *Cancer Res* 69:7557–7568.
- Elenbaas B, et al. (2001) Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes Dev* 15:50–65.
- Zhuang D, et al. (2008) C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. *Oncogene* 27:6623–6634.
- Ancliffe BB, O'Hayer KM, Counter CM (2008) Oncogenic ras-induced expression of cytokines: A new target of anti-cancer therapeutics. *Mol Interv* 8:22–27.
- Wang SE, et al. (2006) HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell* 10:25–38.
- Kadota M, et al. (2010) Delineating genetic alterations for tumor progression in the MCF10A series of breast cancer cell lines. *PLoS ONE* 5:e9201.
- Worsham MJ, et al. (2006) High-resolution mapping of molecular events associated with immortalization, transformation, and progression to breast cancer in the MCF10 model. *Breast Cancer Res Treat* 96:177–186.
- Chen CR, Kang Y, Massagué J (2001) Defective repression of c-myc in breast cancer cells: A loss at the core of the transforming growth factor beta growth arrest program. *Proc Natl Acad Sci USA* 98:992–999.
- Chen CR, Kang Y, Siegel PM, Massagué J (2002) E2F4/5 and p107 as Smad cofactors linking the TGF β receptor to c-myc repression. *Cell* 110:19–32.
- Butt AJ, et al. (2008) Cell cycle machinery: Links with genesis and treatment of breast cancer. *Adv Exp Med Biol* 630:189–205.
- Deming SL, Nass SJ, Dickson RB, Trock BJ (2000) C-myc amplification in breast cancer: A meta-analysis of its occurrence and prognostic relevance. *Br J Cancer* 83:1688–1695.
- Escot C, et al. (1986) Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas. *Proc Natl Acad Sci USA* 83:4834–4838.
- Jamerson MH, Johnson MD, Dickson RB (2004) Of mice and Myc: c-Myc and mammary tumorigenesis. *J Mammary Gland Biol Neoplasia* 9:27–37.
- Bos JL (1989) ras oncogenes in human cancer: A review. *Cancer Res* 49:4682–4689.
- Clark GJ, Der CJ (1995) Aberrant function of the Ras signal transduction pathway in human breast cancer. *Breast Cancer Res Treat* 35:133–144.
- Moustakas A, Heldin CH (2005) Non-Smad TGF- β signals. *J Cell Sci* 118:3573–3584.