Fanconi anemia (FA) is a rare genetic disorder caused by defects in DNA damage repair. FA patients often develop squamous cell carcinoma (SCC) at sites where high-risk human papillomaviruses (HPVs) are known to cause cancer, including the cervix. However, SCCs found in human FA patients are often HPV negative, even though the majority of female FA patients with anogenital cancers had preexisting HPV-positive dysplasias. We hypothesize that HPVs contribute to the development of SCCs in FA patients but that the continued expression of HPV oncogenes is not required for the maintenance of the cancer state because FA deficiency leads to an accumulation of mutations in cellular genes that render the cancer no longer dependent upon viral oncogenes. We tested this hypothesis, making use of Bi-L E7 transgenic mice in which we temporally controlled expression of HPV16 E7, the dominant viral oncogene in HPV-associated cancers. As seen before, the persistence of cervical neoplastic disease was highly dependent upon the continued expression of HPV16 E7 in FA-sufficient mice. However, in mice with FA deficiency, cervical cancers persisted in a large fraction of the mice after HPV16 E7 expression was turned off, indicating that these cancers had escaped from their dependency on E7. Furthermore, the severity of precancerous lesions also failed to be reduced significantly in the mice with FA deficiency upon turning off expression of E7. These findings confirm our hypothesis and may explain the fact that, while FA patients have a high frequency of infections by HPVs and HPV-induced precancerous lesions, the cancers are frequently HPV negative.

FA patients are at high risk for developing squamous cell carcinoma (SCC) at sites where high-risk human papillomaviruses (HPVs) frequently cause cancer. Yet these SCCs are often HPV negative. FA patients have a genetic defect in their capacity to repair damaged DNA. HPV oncogenes cause an accumulation of DNA damage. We hypothesize, therefore, that DNA damage induced by HPV leads to an accumulation of mutations in patients with FA deficiency and that such mutations allow HPV-driven cancers to become independent of the viral oncogenes. Consistent with this hypothesis, we found that cervical cancers arising in HPV16 transgenic mice with FA deficiency frequently escape from dependency on the HPV16 oncogene that drove their development. Our report provides further support for vaccination of FA patients against HPVs and argues for the need to define mutational profiles of SCCs arising in FA patients in order to inform precision medicine-based approaches to treating these patients.
To test this hypothesis, we used our animal model for HPV16-associated cancers of the female reproductive tract. Specifically, we put Bi-L E7/K14-ITATransgenic mice, in which we can temporally regulate expression of the dominant HPV16 oncogene, on either the FabD2-sufficient background or the FabD2-deficient background and chronically treated the mice with estrogen to induce cervical cancer. We observed that E7 induced cervical cancers and that FabD2 deficiency increased the incidence of cancer. When E7 expression was repressed on the FabD2-sufficient background, cancers and high-grade dysplasia regressed, consistent with our earlier studies (18). However, on the FA pathway-deficient background, cancers and precancerous lesions remained even though E7 was no longer expressed. This result indicates that cervical cancers do not require the continuous expression of E7 on the FA pathway-deficient background. These findings may reconcile the paradoxical findings that, while FA patients are highly susceptible to HPV infection and develop cancers at sites known to be associated with HPVs, these cancers are not found to be positive for HPVs.

### RESULTS

The incidence of HPV-associated cancers was increased in FabD2-deficient mice. To examine if HPV16 E7 increases the incidence of cervical cancer, Bi-L E7 and K14-ITATransgenic mice were crossed to FabD2 heterozygous mice to generate Bi-L E7/FabD2+/− and Bi-L E7/FabD2−/− mice. These mice were then crossed to each other to create FabD2+/−, Bi-L E7/K14-ITATransgenic mouse and FabD2+/−, FabD2−/−, and Bi-L E7/K14-ITATransgenic mouse. To induce cervical cancer, mice were treated with 17β-estradiol, which synergizes with HPV oncogenes, for 10 months. At the end of 10 months, we sacrificed the mice, harvested their reproductive tracts, fixed the samples in 4% paraformaldehyde, embedded the samples in paraffin, and sectioned the tissues. We stained tissue sections with hematoxylin and eosin (H&E) and scored every 10th section histopathologically for the worst grade of disease, classifying the tissues as representing hyperplasia, low-grade dysplasia (cervical intraepithelial neoplasia 1 [CIN I]), midgrade dysplasia (CIN II), high-grade dysplasia (CIN III), or cancer.

As summarized in Table 1, no cervical cancers were observed in FabD2+/+ and FabD2−/− mice, indicating that FabD2 deficiency alone is not sufficient to cause cervical cancer, supporting our previous observations (23). Nine percent of the Bi-L E7/K14-ITATransgenic mice developed cancer. The severity of disease (this assesses all stages of disease from hyperplasia to cancer) in the

| Genotype (n) | Dox treatment | Hyperplasia | CIN I | CIN II | CIN III | Cancer (%) |
|--------------|---------------|-------------|------|-------|---------|------------|
| FabD2+/+     | 3             | 4           |      |       |         |            |
| Bi-L E7/K14-ITATransgenic (23) | 2 | 10 | 9 | 2 (9) |
| Bi-L E7/K14-ITATransgenic (19) | 1 | 12 | 6 | 3 (56) |
| FabD2+/− | 1             | 2           |      |       |         |            |
| Bi-L E7/K14-ITATransgenic (16) | 3 | 3 | 1 | 9 (56) |
| Bi-L E7/K14-ITATransgenic (15) | 3 | 3 | 1 | 9 (56) |

a n, total number of mice examined for each genotype. 

b P = 2.644 × 10−5 (comparing the average severity of disease of Bi-L E7/K14-ITATransgenic to that of Bi-L E7/K14-ITATransgenic [Dox] using a two-sided Wilcoxon rank sum test). 

c P = 0.085 (comparing the average severity of disease of Bi-L E7/K14-ITATransgenic [Dox] using a two-sided Wilcoxon rank-sum test), P = 0.0158 if we do the same comparison but exclude cancers.

d P = 0.2852 (comparing the incidence of cancer of Bi-L E7/K14-ITATransgenic to that of Bi-L E7/K14-ITATransgenic [Dox] using a two-sided Fisher exact test).

Table 1 Incidence of cervical disease in mice
Bi-L E7/K14-tTA/FancD2+/− mice was significantly worse than in the FancD2+/+ mice (P = 0.008). In contrast, 56% of the Bi-L E7/K14-tTA/FancD2−/− mice developed cancer. Again, their disease was significantly more severe than that in the FancD2−/− mice (P = 0.013). The cancer incidence in Bi-L E7/K14-tTA/FancD2−/− mice was significantly higher than in the Bi-L E7/K14−tTA/FancD2+/+ mice (p = 0.009), indicating that FA deficiency facilitates development of cervical cancer.

Severity of disease and cancer incidence in the cervix are independent of continued expression of HPV16 E7 on the FancD2-deficient background but not on the FancD2-sufficient background. We have previously shown that, on the FancD2-sufficient background, cervical cancers are addicted to E7; turning off expression of E7 in the context of Bi-L E7/K5-tTA mice by administering doxycycline (Dox) led to regression of cervical neoplasia (18). We found the same to be true in our hands in the context of Bi-L E7/K14−tTA/FancD2+/+ mice, upon administration of doxycycline for the last month before the endpoint (Table 1). The decrease in cancer incidence and in the severity of disease was highly significant. Examples of histology results are shown in Fig. 1. To examine if cervical cancers arising in Bi-L E7/K14−tTA mice on the FancD2-deficient background remain dependent upon continued expression of E7, we likewise administered doxycycline to Bi-L E7/K14−tTA/FancD2+/− mice for the last month on estrogen to repress E7 expression. On the FA pathway-deficient background, cancers still remained even though E7 was no longer expressed, indicating that these cancers had become independent of E7. However, the overall severity of cervical disease, representing scores for all stages of neoplastic disease, was decreased though this decrease did not reach the point of being statistically significant. This difference between retention of cancers versus a marginal decrease in the severity of disease may suggest that precancerous lesions have not yet become completely independent of E7.

MCM7 expression patterns confirm that cancers retained in Bi-L E7/K14-tTA/FancD2+/− mice treated with doxycycline are not dependent upon continued expression of HPV16 E7. The gene encoding mouse anti-minichromosome maintenance protein 7 (MCM7) is an E2F-responsive gene which is negatively regulated by pRb and is used as a surrogate for E7 expression/function in humans and mice (13, 25, 26). MCM7 is normally expressed in the basal cells rather than in the suprabasal cells in our mouse models (25). To determine whether the expression pattern of MCM7 is changed in mice when E7 is repressed, we performed immunohistochemistry on sections of female reproductive tracts (Fig. 2). In Bi-L E7/K14−tTA/FancD2+/+ mice, MCM7 expression was upregulated in cancers and epithelium as evidenced by expression of MCM7 throughout the epithelial cells within these tissues, but when E7 expression was repressed by doxycycline, MCM7 was limited in its expression in the poorly differentiated cells within the epithelium (no cancers were present in this population of mice, which is reflective of their continued dependence on E7—see Table 1). In Bi-L E7/K14−tTA/FancD2−/− mice, we likewise observed expression of MCM7 throughout the epithelial cells within the cancers and the cervical epithelium. However, in Bi-L E7/K14−tTA/FancD2+/+ mice treated with doxycycline, expression of MCM7 was restricted in its expression to the poorly differentiated cells not only within the epithelium but also in the cancers, confirming that E7 is no longer expressed in those cancers. These findings indicate that the cancers retained
in Bi-L E7/K14-tTA/FancD2+/− mice treated with doxycycline do not depend on E7 activity. Our results confirm that the cancers persisting in the Bi-L E7/K14-tTA/FancD2+/− mice treated with doxycycline no longer depend upon the continued expression of HPV16 E7.

Proliferation is upregulated in E7-expressing mice and in bi-l e7/k14-tta/FancD2+/− mice treated with doxycycline. We were interested in knowing whether cell proliferation is affected by the presence or absence of E7 and the FA pathway. To study this, mice were injected with the nucleotide analog 5-bromo-2′-deoxyuridine (BrdUrd) 1 h before sacrifice. We carried out immunohistochemical staining for BrdUrd to measure newly synthesized DNA, and BrdUrd-positive cells in the suprabasal layer were counted and quantified as indicated in Fig. 3. Bi-L E7/K14-tTA/FancD2+/+ mice showed increased cell proliferation compared to FancD2−/− mice. We observed a decrease in proliferation in Bi-L E7/K14-tTA/FancD2+/− mice treated with doxycycline that was marginally significant (P = 0.056) compared to that observed in Bi-L E7/K14-tTA/FancD2+/+ mice not treated with doxycycline. However, in FancD2-deficient mice, turning off expression of E7 did not lead to a decrease in cell proliferation (P = 0.6286). Comparing cervical tissues from FancD2+/+ and FancD2−/− mice, we saw a trend toward higher proliferation rates in the FancD2−/− group, consistent to what we observed in our prior study (23). These results indicate that FancD2 deficiency substitutes for the need of E7 to induce cell proliferation.

DISCUSSION

HPV-associated cancers become independent of HPV16 oncoprotein E7 in the absence of a functional FA pathway. In the current study, we addressed whether HPV16 E7 is required to maintain HPV-associated cancers when the FA pathway is disrupted. To investigate this issue, we utilized Tet-regulatable Bi-L E7/K14-tTA transgenic mice to control E7 expression via administration of the tetracycline analog doxycycline (19). Consistent with our previous studies (18, 19), E7 was required for cervical cancers in the presence of a functional FA pathway. However, we observed that E7-driven cancers in FancD2-deficient mice become independent of continued expression of E7. Turning off expression of E7 led to only marginal regression of overall disease severity in the study of the lower reproductive tract (for Bi-L E7/K14-tTA/FancD2−/− versus Bi-L E7/K14-tTA/FancD2+/− [Dox], P = 0.08; P = 0.16 if we exclude cancers). In contrast, the difference in the overall severities of disease when E7 was turned off in the FancD2-sufficient background was highly significant (for Bi-L E7/K14–tTA/FancD2−/− versus Bi-L E7/K14–tTA/FancD2+/− [Dox], P = 2.644 × 10−6, P = 7.9 × 10−6 if we exclude cancers). This indicates that not only the cancers but even some precancerous lesions must escape from their dependency on E7 in the FancD2-deficient mice.

We also observed that FA deficiency marginally increased the disease severity (for Bi-L E7/K14–tTA/FancD2−/− versus Bi-L E7/K14–tTA/FancD2+/−, P = 0.11) while greatly increasing the incidence of cancers (for Bi-L E7/K14–tTA/FancD2+/− versus Bi-L E7/K14–tTA/FancD2−/−, P = 0.0026). This suggests that E7 expression and FA deficiency synergize to worsen the stage of neoplastic disease in the female reproductive tract, further demonstrating that the FA pathway counteracts the contribution of E7 to carcinogenesis. A simple explanation for this synergy is that E7 induces DNA damage that fails to be repaired on the FA-deficient background (27). What genetic/epigenetic alterations accumulate
in the FA-deficient context that render cancers or even precancerous lesions no longer dependent upon HPV oncogenes is an important issue that remains unresolved both in this preclinical animal model setting and in humans. With the development of emerging technologies for deep sequencing of archival, paraformaldehyde-fixed, paraffin-embedded tissues at even the single-cell level, we and others in the FA field hope to be able to resolve this issue in the near future. Such knowledge could lead to identifying pathway-targeted therapies for use in treating cancers arising in FA patients, for whom traditional chemotherapy and radiotherapy cannot be used. It may also help us learn if there are genetic and/or epigenetic signatures left behind by HPV oncogenes that could allow us to identify HPV-negative cancers that initially arose from an HPV infection. Such cancers could occur even in an FA-sufficient context due to the ability of HPV oncogenes to drive mutagenesis through their induction of DNA damage (28, 29), aneuploidy (28), and genome editing (30) and their ability to reprogram the cell epigenetically (31) as well as to influence of the microenvironment such as by the mutagenic effects of reactive oxygen species elicited due to local inflammatory responses. Having a signature for HPV-initiated cancers could allow us to learn if there is a broader role for this highly oncogenic virus than is currently appreciated.

Escape from dependency on an oncogene has been reported in other cancer contexts. Gunther et al. observed that mammary tumors were dependent on the continuous expression of Wnt1 in wild-type mice but that, on the p53−/− background, repression of Wnt1 did not lead to regression of tumors (32). Similarly, in the study by D’Cruz et al., mammary tumors in mice regressed when c-Myc expression was silenced but tumors bearing the de novo mutation of Ras did not regress, indicating that these tumors had become independent of c-Myc (33). In these examples, it is likely that the direct effect of the p53 heterozygous state (and/or loss of heterozygosity [LOH] at the p53 allele in the tumors) or the activating mutation in ras contributes to the loss of addiction to Wnt1 or c-Myc, respectively. In our studies, the FA-deficient state potentially indirectly contributed to the loss of addiction to E7 through an accumulation of mutations in cellular genes that then supplanted E7’s oncogenic activities. In this regard, E7 may induce DNA damage at least in part through its inactivation of the tumor suppressor pRb and of related pocket proteins, p107 and/or p130 (23). Importantly, this induction of DNA damage by E7 is accentuated in the FA-deficient context both in vitro (20) and in vivo (27). These observations may provide a mechanistic underpinning to our observation that whereas FancD2+/− mice that are not transgenic for HPV16 E7 are not more susceptible to cervical carcinogenesis than FancD2+/+ mice, the FancD2−/− mice that are transgenic for HPV16 E7 are more susceptible to cervical carcinogenesis than their FancD2+/+ counterparts. That is, there is a synergy between E7 and FA deficiency in causing cervical cancer.
The relationship between HPV and Fanconi anemia: implications for the clinical treatment of FA patients. FA patients are at a high risk of being infected with HPVs and developing cancers known to be associated with HPVs in the general population. However, the association of HPVs with SCCs in FA patients has been highly debated. Whereas Kutler et al. (34) reported that 84% of head and neck cancers were HPV positive, van Zeeburg et al. (35) observed that 0 of 16 head and neck cancers were HPV positive. Recently, Alter et al. (36) detected only 1 HPV16-positive vulvar squamous cell carcinoma among 4 anogenital cancers and 0 of 4 head and cancers from FA patients. Those studies used different HPV detection methods and were performed on samples from patients living in different geographical areas of the world; however, the data as a whole would suggest that for many FA patients, the SCCs that they develop are HPV negative.

Our current studies could provide an explanation for why FA patients, while frequently infected with HPVs and frequently developing cancers at sites known to be associated with HPV-induced carcinogenesis, often do not contain HPVs in their cancers arising at such sites. Specifically, we posit that, in FA patients, the paucity of HPV found in anogenital tract and head and neck cancers is a consequence of their cancers becoming independent of a need for continued expression of HPV oncogenes. Our data, however, also show that in the absence of HPV16 E7, FA pathway deficiency is not sufficient to cause these cancers. This observation, which confirms our earlier observations, suggests that HPV may still be the driver of SCCs arising in FA patients, even if the cancers that arise then become independent of the virus. Thus, we think that it remains prudent to vaccinate FA patients as a means of preventing the onset of anogenital tract and head and neck region cancers.

MATERIALS AND METHODS

Transgenic mice. Bi-L E7 (18), K14-tTA (37), and FancD2−/− (38) transgenic mice have been described previously. One hour before sacrifice, mice were injected with 0.3 ml 5-bromo-2'-deoxyuridine (BrdUrd; 12.5 mg/ml). Female lower reproductive tracts were harvested, fixed for 24 h at 4°C in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with H&E for histologic analysis of neoplastic and dysplastic disease.

Treatment with doxycycline for repression of expression of E7 in mice. Doxycycline-containing chow (Bio-Serv) (2 g/kg of body weight) was used to repress the expression of the Bi-L E7 transgene at the indicated time points.

Immunohistochemistry and immunofluorescence. Immunohistochemistry and immunofluorescence procedures were performed as described previously (25). The following primary antibodies were used: mouse anti-BrdUrd (Calbiochem Immunohchemicals) (1:50) and mouse anti-minichromosome maintenance protein 7 (MCM7; NeoMarkers Corp.) (1:200).

Quantification of BrdUrd-positive cells. Sections from at least 3 mice from each genotype were scored for positivity for BrdUrd (using at least 8 frames/sample at ×20 magnification).

Statistical analysis. The MSTAT software program (http://mcar-dile.wisc.edu/msstat/) was used for determining statistical significance in this study. The Wilcoxon rank sum test was used to determine the average severity of disease and the number of BrdUrd-positive cells in comparisons between groups of mice. The Fisher exact test was used to determine the significance of cancer incidence in comparisons between groups of mice.

ACKNOWLEDGMENTS

We acknowledge the expert advice of Norman Drinkwater in the statistical analysis of data described in this report and MyeongKyun Shin and Kathleen Makielski for help in preparing figures and/or analyzing data.

This work was supported by a grant from the National Cancer Institute (CA022443) to P.F.L.

FUNDING INFORMATION

This work, including the efforts of Paul F. Lambert, was funded by HHS | National Institutes of Health (NIH) (CA022443).

REFERENCES

1. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, Auerbach AD. 2003. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood 101:1249–1256. http://dx.doi.org/10.1182/blood-2002-07-1270.

2. Auerbach AD. 1988. A test for Fanconi’s anemia. Blood 72:366–367.

3. German J, Schonberg S, Caskie S, Warbrick D, Falk C, Ray JH. 1987. A test for Fanconi’s anemia. Blood 69:1637–1641.

4. Sasaki MS. 1975. Is Fanconi’s anemia defective in a process essential to the repair of DNA cross links? Nature 257:501–503. http://dx.doi.org/10.1038/257501a0.

5. Moldovan GL, D’Andrea AD. 2009. How the Fanconi anemia pathway guards the genome. Annu Rev Genet 43:223–249. http://dx.doi.org/10.1146/annurev-genet-102108-134222.

6. De Winter JP, Joenje H. 2009. The genetic and molecular basis of Fanconi anemia. Mutat Res 668:11–19. http://dx.doi.org/10.1016/j.mrfmmm.2008.11.004.

7. De Araujo MR, Rubira-Bullen IR, Santos CF, Dionisio TJ, Bonfim CM, De Marco L, Gillio-Tos A, Merletti F. 2011. High prevalence of oral human papillomavirus infection in Fanconi’s anemia patients. Oral Dis 17:572–576. http://dx.doi.org/10.1111/j.1601-0825.2011.01803.x.

8. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19. http://dx.doi.org/10.1002/(SICI)1096-3835(199909)189:1<12::AID-PATH431>3.0.CO;2-F.

9. Zar Hauenst J. 2009. Papillomaviruses in the causation of human cancers—a brief historical account. Virology 384:260–265. http://dx.doi.org/10.1016/j.virol.2008.11.046.

10. Dyson N, Howley PM, Münger K, Harlow E. 1989. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243:934–937. http://dx.doi.org/10.1126/science.243.4903.934.

11. Riley RR, Duensing S, Brake T, Münger K, Lambert PF, Arbet JM. 2003. Dissection of human papillomavirus E6 and E7 function in transgenic mouse models of cervical carcinogenesis. Cancer Res 63:4862–4871.

12. Strati K, Lambert PF. 2007. Role of RB-dependent and RB-independent functions of papillomavirus E7 oncogene in head and neck cancer. Cancer Res 67:11585–11593. http://dx.doi.org/10.1158/0008-5472.CAN-07-3057.

13. Strati K, Pitot HC, Lambert PF. 2006. Identification of biomarkers that distinguish human papillomavirus (HPV)-positive versus HPV-negative head and neck cancers in a mouse model. Proc Natl Acad Sci U S A 103:14152–14157. http://dx.doi.org/10.1073/pnas.0606698103.

14. Hwang ES, Naeger LK, DiMiao D. 1996. Activation of the endogenous p53 growth inhibitory pathway in HeLa cervical carcinoma cells by expression of the bovine papillomavirus E2 gene. Oncogene 12:795–803.

15. Hwang ES, Riese DJ, II, Settleman J, Nilson LA, Honig J, Flynn S, DiMiao D. 1993. Inhibition of cervical carcinoma cell line proliferation by the introduction of a bovine papillomavirus regulatory gene. J Virol 67:3720–3729.

16. Goodwin EC, Yang E, Lee CJ, Lee HW, DiMiao D, Hwang ES. 2000. Rapid induction of senescence in human cervical carcinoma cells. Proc Natl Acad Sci U S A 97:10978–10983. http://dx.doi.org/10.1073/pnas.97.20.10978.

17. Wells SJ, Francis DA, Karpova AY, Dowhanick JJ, Benson JD, Howley PM. 2000. Papillomavirus E2 induces senescence in HPV-positive cells via pRB- and p21(CIP)-dependent pathways. EMBO J 19:5762–5771. http://dx.doi.org/10.1093/emboj/19.21.5762.

18. Jabbar SF, Abrams L, Glick A, Lambert PF. 2009. Persistence of high-
22. Hoskins EE, Morreale RJ, Werner SP, Higginbotham JM, Laimins LA, Lambert PF. 2012. Cervical cancers require the continuous expression of the human papillomavirus type 16 E7 oncoprotein even in the presence of the viral E6 oncoprotein. Cancer Res 72:4008–4016. http://dx.doi.org/10.1158/0008-5472.CAN-11-3085.

23. Park JW, Shin MK, Lambert PF. 2010. Deficiencies in the Fanconi anemia DNA damage repair pathway increase sensitivity to HPV-associated head and neck cancer. Radiother Oncol 113:337–344. http://dx.doi.org/10.1016/j.radonc.2014.08.026.

24. McLaughlin-Drubin ME, Crum CP, Münger K. 2011. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. Proc Natl Acad Sci U S A 108:2130–2135. http://dx.doi.org/10.1073/pnas.1009331010.

25. Gunther EJ, Moody SE, Belka GK, Hahn KT, Innocent N, Dugan KD, Cardiff RD, Chodosh LA. 2003. Impact of p53 loss on reversal and recurrence of conditional Wat-induced tumorogenesis. Genes Dev 17:488–501. http://dx.doi.org/10.1101/gad.1103403.

26. D’Cruz CM, Gunther EJ, Boxer RB, Hartman JL, Sintasath I, Moody SE, Cox JD, Ha SL, Belka GK, Golant A, Cardiff RD, Chodosh LA. 2001. c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. Nat Med 7:235–239. http://dx.doi.org/10.1038/nm0491.

27. Van Zeeburg HJ, Snijders PJ, Wu T, Gluckman E, Soulier J, Surralles J, van Zijl MC, Gunther EJ, Boxer RB, Hartman JL, Dreyer B, Philbrick WP, Wysolmerski JJ. 2001. Temporally regulated overexpression of para- thyroid hormone-related protein in the mammary gland reveals distinct fetal and pubertal phenotypes. J Endocrinol 171:403–416. http://dx.doi.org/10.1677/joe.0.1710403.

28. Spardy N, Duensing A, Hoskins EE, Wells SI, Duensing S. 2008. HPV-16 E7 reveals a link between DNA replication stress, Fanconi anemia D2 protein, and alternative lengthening of telomere-associated promyelocytic leukemia bodies. Cancer Res 68:9954–9963. http://dx.doi.org/10.1158/0008-5472.CAN-08-0224.

29. Park JW, Nickel KP, Torres AD, Lee D, Lambert PF, Kimple RJ. 2014. Human papillomavirus type 16 E7 oncoprotein causes a delay in repair of DNA damage. Radiother Oncol 113:337–344. http://dx.doi.org/10.1016/j.radonc.2014.08.026.

30. Vieira VC, Leonard B, White EA, Starrett GJ, Temiz NA, Lorenz LD, Lee D, Soares MA, Lambert PF, Howley PM, Harris RS. 2014. Human papillomavirus E6 triggers upregulation of the antiviral and cancer genome DNA deaminase APOBEC3B. mBio 5 pii:02234–14. http://dx.doi.org/10.1128/mBio.02234–14.

31. Dartre T, Dhermain B, Istasse A, Soulier J, Dancey J, Leemans CR, Joenje H. 2007. Impact of p53 loss on reversal and recurrence of conditional Wat-induced tumorogenesis. Genes Dev 17:488–501. http://dx.doi.org/10.1101/gad.1103403.