Vitamin D3 Inhibits Hedgehog Signaling and Proliferation in Murine Basal Cell Carcinomas

Jean Y. Tang1, Tony Zheng Xiao2, Yuko Oda3, Kris S. Chang2, Elana Shpall2, Angela Wu2, Po-Lin So2, Jennifer Hebert3, Daniel Bikle3, and Ervin H. Epstein Jr.2

Abstract

Constitutive Hedgehog (HH) signaling underlies several human tumors, including basal cell carcinoma (BCC). Recently, Bijlsma and colleagues reported a new biologic function for vitamin D3 in suppressing HH signaling in an in vitro model system. On the basis of that work, we have assessed effects of vitamin D3 on HH signaling and proliferation of murine BCCs in vitro and in vivo. We find that indeed in BCC cells, vitamin D3 blocks both proliferation and HH signaling as assessed by mRNA expression of the HH target gene Gli1. These effects of vitamin D3 on Gli1 expression and on BCC cell proliferation are comparable to the effects of cyclopamine, a known inhibitor of the HH pathway. These results are specific for vitamin D3, because the precursor 7-dehydrocholesterol and the downstream products 25-hydroxy vitamin D3 [25(OH)D] and 1,25-dihydroxy vitamin D3 [1,25(OH)2D] are considerably less effective in reducing either Gli1 mRNA or cellular proliferation. Moreover, these effects seem to be independent of the vitamin D receptor (VDR) because short hairpin RNA knockdown of VDR does not abrogate the anti-HH effects of D3 despite reducing expression of the VDR target gene 24-hydroxylase. Finally, topical vitamin D3 treatment of existing murine BCC tumors significantly decreases Gli1 and Ki67 staining. Thus, topical vitamin D3 acting via its HH inhibiting effect may hold promise as an effective anti-BCC agent. Cancer Prev Res; 4(5); 744–51. ©2011 AACR.

Introduction

In 1941, Apperly (1) noted that the incidence of colon cancer in the United States is considerably higher in the north than in the south, and the data favoring this "latitudinal gradient" remain strong for several cancers including in particular those of the colon, breast, and prostate. Four decades later, Garland and Garland noted that the differences in cancer incidence in different locales are inversely related to the amount of sunlight they receive and proposed that this gradient might be explained by an anticancer effect of varying amounts of vitamin D made in sun-exposed skin (2, 3). This proposal has been seminal, and 6,000 papers have been published touching on vitamin D and cancer. Approaches taken to investigate this putative relationship include comparisons between cancer risk and sun exposure and/or dietary vitamin D intake; assessments of circulating 25-hydroxy vitamin D3 [25(OH)D], the most readily available measurement of body vitamin D status, in patients with cancers and controls; comparisons of DNA polymorphisms in the genes encoding the vitamin D receptor (VDR) and the enzyme responsible for the catabolism of the VDR ligand, 1,25-dihydroxy vitamin D3 [1,25(OH)2D]; and studies of the cancer preventive effects of supplemental dietary vitamin D. The latter include one very large prospective study of the effects of 400 IU of vitamin D3 per day, which showed no effects on cancer incidence or mortality (4), albeit the compliance rate in this study was poor, and one considerably smaller prospective study of 1,100 IU vitamin D3 per day, which found a statistically significant reduction of cancer incidence in those taking the vitamin supplement (5). Taken together, the positive correlation of cancer incidence and latitude of residence seems strong and the inverse correlation of sunlight exposure and cancer seems moderately strong, but the mechanistic importance of any inverse correlation of cancer incidence and vitamin D3 and the anticancer efficacy of vitamin D3 supplementation remain uncertain (6–8).

The most studied mechanism of the effect of vitamin D3 is the 1,25(OH)2D-induced transcriptional activation of the VDR with resultant changes in cell behavior including enhanced differentiation and reduced proliferation of skin.
keratinocytes (9–11). By contrast, Bijlsma and colleagues (12) recently proposed a new biologic function for unhydroxylated vitamin D3—the inhibition of hedgehog (HH) signaling. They found that D3 binds to Smo specifically and thereby inhibits Gli reporter activity in C3H/10T1/2 fibroblasts in vitro. In addition, D3 treatment of zebrafish in vivo mimicked the Smo−/− phenotype. In fact, Bijlsma and colleagues (12) propose that Ptch1 protein accomplishes its inhibition of HH signaling by transporting vitamin D3 to Smo protein.

HH signaling was identified initially as a pathway crucial to development but more recently has come to be seen as a potentially important stimulator of carcinogenesis when dysregulated. This can occur via mutations in the genes encoding components of the pathway or by excess production of HH ligand by the tumor or stromal cells (13). Indeed, the first human inhibitor of HH signaling GDC-0449 is now in clinical trials for at least 8 human cancers (clinicaltrials.gov), and several other HH inhibitors are in varying stages of clinical development.

Of the human cancers with mutations in HH signaling pathway components, the best studied tumor—HH relationship in humans and mice is that found in basal cell carcinomas (BCC), and inhibition of HH signaling with small molecule drugs can have dramatic inhibitory effects on human BCCs (14). BCCs are the most common of all human carcinomas (BCC), and inhibition of HH signaling with vitamin D3 is constitutive activation of the HH signaling pathway in humans and mice (15). The pivotal molecular abnormality in BCCs is constitutive activation of the HH signaling pathway, in 10% to 20% of tumors by mutational activation of SMO and in the great majority of the others associated with mutational inactivation of PTCH1 (16–23). In addition to mutational activation of the HH pathway, human BCCs also frequently have mutations in p53 (22, 24). Pch1+/− mice develop BCCs after mutational insults, and the addition of conditional loss of keratinocyte p53 greatly accelerates murine BCC carcinogenesis.

On the basis of the known role of HH signaling in BCC carcinogenesis and on this newly reported HH-inhibitory function of vitamin D3, we have studied whether vitamin D3, its precursor, and its hydroxylated derivatives can inhibit cellular proliferation and downregulate HH signaling in established murine BCC cell lines and in murine BCCs in vivo. We then investigated whether the anti-BCC effects of vitamin D3 are mediated via the classic VDR pathway.

Results

In vitro studies

Vitamin D3 inhibits proliferation of BCC cell lines. We treated an established murine BCC cell line (ASZ), a murine nontumorogenic keratinocyte cell line (C5N), and a murine medulloblastoma cell line (Med1) with upregulated HH signaling (25) with cyclopamine or vitamin D3 and assessed cellular proliferation after 48 hours. As we found previously, the classical HH inhibitor cyclopamine decreases Med1 and ASZ proliferation at 5 and 10 μmol/L, respectively, but does not decrease C5N cell proliferation (Fig. 1A; ref. 26). Vitamin D3 similarly inhibits the proliferation of ASZ and Med1 cells by more than 80% although not significantly inhibiting the proliferation of C5N cells (Fig. 1A). Vitamin D3 also inhibits the proliferation of 2 other BCC cell lines (BSZ and CSZ) that were established from BCC tumors from Pch1+/− mice with keratinocyte deletion of p53 (Fig. 1B). Neither the vitamin D3 precursor 7-dehydrocholesterol (7DHC) nor the hydroxylated forms 25(OH)D and 1,25(OH)2D consistently inhibit proliferation of BCC cells. We tested lower concentrations of 1,25(OH)2D because of the nonspecific cell toxicity of concentrations greater than 1 μmol/L (27). Thus, vitamin D3 specifically inhibits the proliferation of all 3 BCC cell lines tested.

Vitamin D3 decreases Gli1 mRNA expression in BCC cell lines. To address the mechanism by which vitamin D3 inhibits ASZ proliferation, we next measured mRNA levels of the HH target gene Gli1 and of the VDR target gene 25-hydroxyvitamin D-24-hydroxylase (24-hydroxylase, CYP24) in ASZ cells incubated with vitamin D3, 7DHC, 25(OH)D, or 1,25(OH)2D for 24 or 48 hours. Vitamin D3, 25(OH)D, and 1,25(OH)2D each increased 24-hydroxylase mRNA levels. This was expected, as normal human keratinocytes and BCCs can convert vitamin D3 to 25(OH)D and then to the VDR-activating 1,25(OH)2D (refs. 9, 28; Fig. 1C). In contrast, 7DHC fails to increase 24-hydroxylase levels, because UV is required to convert 7DHC to vitamin D3. As expected, cyclopamine does not affect 24-hydroxylase mRNA levels. Similar to prior reports (26), cyclopamine at 10 μmol/L decreases Gli1 mRNA by 3- to 5-fold at 24 and 48 hours after incubation (Fig. 1D). Vitamin D3 treatment at the same concentration (10 μmol/L) also decreases Gli1 mRNA by 3- to 4-fold at 24 and 48 hours. In contrast, 7DHC (10 μmol/L), 25(OH)D (10 μmol/L), and 1,25(OH)2D (0.10 μmol/L) each fail to reduce Gli1 mRNA significantly. Our finding that 25(OH)D and 1,25(OH)2D can activate VDR (as indicated by increased expression of the VDR target gene 24-hydroxylase), without significantly reducing Gli1 mRNA is consistent with Bijlsma and colleagues’ data in other test systems indicating that vitamin D3’s inhibition of the HH pathway is independent of VDR. Importantly, our finding that concentrations of 25(OH)D and of 1,25(OH)2D that did not affect murine BCC cell growth did upregulate VDR target gene expression is consistent with the effect on proliferation being independent of the VDR.

Vitamin D3 decreases Gli1 mRNA expression independent of the vitamin D3 receptor. To investigate further the requirement of VDR signaling for the effect of vitamin D3, we incubated ASZ cells with a viral construct expressing short hairpin RNA (shRNA) against VDR (shVDR) for 3 days, and then treated these cells with 5 μmol/L of vitamin D3 for 24 hours (n = 3 experiments). This construct infected approximately 80% to 90% of cells as measured by the expression of red fluorescence (Fig. 2A). Western blot analysis indicated that shVDR infection reduced VDR protein levels by approximately 75% as compared with...
VDR protein levels in cells infected with a virus expressing a random oligo (Fig. 2B). To assess the effectiveness of shVDR on VDR function, we measured 24-hydroxylase mRNA levels by quantitative PCR (qPCR) in cells treated with vitamin D3. Vitamin D3 increased 24-hydroxylase mRNA by at least 20-fold in ASZ cells previously treated with the control shRNA. Vitamin D3 increased 24-hydroxylase levels in ASZ cells pretreated with shVDR by a much lower amount (Fig. 2C). By contrast, pretreatment with shVDR failed to affect vitamin D3’s decrease in Gli1 mRNA levels (Fig. 2C). Furthermore, vitamin D3 inhibited proliferation of ASZ cells pretreated with shVDR or control vector (Fig. 2D). These results were similar when shVDR was knocked down in a second cell line (BSZ, data not shown).

In vivo studies

**Vitamin D3 decreases proliferation but does not affect differentiation in BCC tumors in vivo.** To test the in vivo effects of vitamin D3 on BCC tumors, we next utilized our murine model in which treatment with ionizing radiation (IR) at age 8 weeks induces BCC carcinogenesis. Specifically, our IR-treated Pten1<sup>−/−</sup> K14-CreER2 p53 <sup>f/f</sup> mice treated with tamoxifen at age 6 weeks to activate Cre and thereby delete keratinocyte p53 develop multiple visible BCC tumors starting at age 5 to 6 months. These murine BCC tumors histologically resemble nodular human BCCs [Fig. 3A (a)]; and similar to BCCs in Pten1<sup>−/−</sup> p53 wild-type mice (16) these tumors express the basal cell marker keratin 14 [Fig. 3A (b)] but not the suprabasal differentiation marker keratin 10 [Fig. 3A (c)], and have a high level of proliferation as measured by positive staining for Ki67, treated with tamoxifen at age 6 weeks to activate Cre and thereby delete keratinocyte p53 develop multiple visible BCC tumors starting at age 5 to 6 months. These murine BCC tumors histologically resemble nodular human BCCs [Fig. 3A (a)]; and similar to BCCs in Pten1<sup>−/−</sup> p53 wild-type mice (16) these tumors express the basal cell marker keratin 14 [Fig. 3A (b)] but not the suprabasal differentiation marker keratin 10 [Fig. 3A (c)], and have a high level of proliferation as measured by positive staining for Ki67 [Fig. 3A (e and f)]. As expected for cells lacking p53, they do not express the apoptosis marker cleaved caspase 3 [CC3; Fig. 3A (d)].

We applied acetone without (n = 7) or with 1.3 mg/kg vitamin D3 (n = 10) to visible BCCs daily for approximately 30 days [Fig. 3A (g-i)]. Treated BCCs continue to express keratin14 [Fig. 3A (h)] but not keratin10 [Fig. 3A (i)], suggesting that vitamin D3 does not induce differentiation of BCC cells. However, topical vitamin D3 treatment markedly decreases cellular proliferation as shown by reduction of Ki67 staining [P = 0.02; Fig. 3A (k and l) and B]. Topical D3 did not affect apoptosis as assessed by the low number of brown staining CC3-positive cells relative to background staining [Fig. 3A (d vs. i)]. These topical
applications of vitamin D3 to BCCs and their surrounding skin were sufficient to increase circulating levels of 25(OH)D by approximately 4-fold ($210 \pm 160$ vs. $50 \pm 12$ ng/mL, $P < 0.01$).

**Topical vitamin D3 decreases Gli1 mRNA level in BCC tumors in vivo.** We also treated murine nodular BCCs with topical acetone ($n=9$) or with topical vitamin D3 (2.6 mg/kg; $n=24$) daily for 4 days and measured changes in mRNA expression of Gli1 and 24-hydroxylase. Topical vitamin D3 reduced Gli1 mRNA levels (Fig. 4A) and induced significant increases in skin 24-hydroxylase mRNA (Fig. 4B). We found no correlation between suppression of Gli1 mRNA levels and increase of 24-hydroxylase mRNA levels ($R=0.2$, $P=0.28$), consistent with the idea that vitamin D3 inhibition of HH signaling is independent of VDR. Topical application of a 1,25(OH)$_2$D analog (calcipotriene; Dovonex cream, Leo Pharmaceuticals) used clinically for treatment of psoriasis did not significantly alter Gli1 mRNA but induced significant increases in skin 24-hydroxylase mRNA (Fig. 4C and D). However, calcipotriene cream, similar to topical vitamin D3, also decreases Ki67 staining in BCC tumor cells ($1.7 \pm 1.0$ vs. $2.8 \pm 1.0$, calcipotriene vs. control cream, respectively; $P=0.02$).
Discussion

We have assessed the effects of vitamin D3 on carcinogenesis by using BCCs because this is the tumor in which aberrant HH signaling is most clearly the pivotal molecular abnormality. Our findings in BCC tumor cell lines in vitro and in BCC tumors in vivo are highly consistent with findings in noncancer assays used by Bijlsma and colleagues (29) to identify anti-HH effects of vitamin D3 that are independent of the VDR. They suggest that nonhydroxylated vitamin D3 may have therapeutic potential as an inhibitor of BCC carcinogenesis—as a chemopreventive and/or a chemotherapeutic agent. Others have noted the structural similarity between the Smo inhibitor cyclopamine and sterols such as vitamin D3 (30).

Furthermore, our findings suggest a more complex role for UV radiation in BCC carcinogenesis than has been considered previously. The evidence that sunlight enhances BCC risk is well accepted. In fact, a significant proportion of PTCH1 and p53 gene mutations in human BCCs are of the UVB-signature type (31–33). As an additional mechanism by which sunlight might enhance BCC carcinogenesis, UV radiation is known to be immunosuppressive, and there is some evidence for a role for the immune system in protecting against BCC carcinogenesis (34). However, details of the relationship between sunlight and BCC risk have been confusing, unlike the more straightforward relationship between sunlight and squamous cell carcinoma (SCC) risk. Thus the relative risk for BCCs peaks at a lifetime exposure of 10,000–35,000 hours whereas that for SCCs, which are not known to be driven by HH activation, continues to rise with more hours of sunlight (35, 36). Were UVB not only to stimulate BCC carcinogenesis via mutagenesis and immunosuppression but also to increase cutaneous vitamin D3, thus blocking HH signaling and thereby inhibiting BCC growth, this might provide at least a partial explanation for the less than straightforward relationship between sun exposure and BCC risk. However, other mechanisms also may contribute to the complex relationship. Thus despite our knockdown data, the VDR itself, even without the 1,25(OH)2D ligand may affect BCC carcinogenesis, as illustrated by the development of BCCs in VDR−/− mice treated with 7,12 dimethylbenz(a)anthracene (37). One possible mechanism for this could be the non–1,25(OH)2D-dependent effect of VDR on Wnt signaling, which seems to function downstream of HH signaling in BCCs (38, 39). Our data do not exclude possible anticancer effects of vitamin D3 acting via the canonical VDR pathway in tumor and/or stromal cells because the topical 1,25(OH)2D analog also decreases Ki67 measured tumor proliferation.

Collectively, our data show that pharmacologic doses of vitamin D3 can block proliferation of murine BCC cell lines in vitro and block proliferation in BCC tumors in vivo. We do not know the ultimate effect of vitamin D3 on long-term tumor proliferation studies or tumor induction, and we do not know the role of endogenous physiologic doses of vitamin D3 in the HH pathway or BCC development.

Figure 3. A, histology of BCCs treated topically with acetone (control) for 30 days (a–f) or with topical 1.3 mg/kg vitamin D3 for 30 days (g–l). β-Gal and hematoxylin and eosin staining for acetone or vitamin D3 treated BCC (a, g). BCCs from Ptch1+/−/K14-Cre-ER p53 fl/fl mice stain blue due to β-gal activity which is encoded by the lacZ gene that was inserted to replace the wild-type Ptch1 gene. Keratinocyte markers of differentiation, K14 (B, H) and K10 (c, l), are shown, and also shown is CC3, a marker of apoptosis (e, f, k, l). Scale bars, 100 μm. B, levels of Ki67 staining in BCC tumors treated with either acetone (control, n = 7) or vitamin D3 (1.3 mg/kg, n = 10) for 30 days (P < 0.05).
lines were generated from nodular BCCs from Ptch1+/−/K14CreER p53 fl/fl mice. We bred mice with the K14-Cre-ER transgene (40) and mice with a floxed p53 allele (41) with our Ptch1+/−/ mice to generate Ptch1+/−/K14-Cre-ER p53 fl/fl mice. We treated these mice intraperitoneally at age 6 weeks with 100 μg/day of tamoxifen for 3 consecutive days. At 8 weeks of age, Ptch1+/−/K14-Cre-ER p53 fl/fl mice were exposed to 4 Gy of IR. Mice were fed a 1% calcium Teklad Global 18% Protein Rodent Diet 2918 Irradiated, containing 1.5 IU/g vitamin D3 from Harlan Laboratories (Teklad Diets). Mice were housed in a barrier facility with 12 hour light–dark cycles by using fluorescent bulbs emitting undetectable UV.

**Vitamin D3 treatments**

Vitamin D3, 7DHC, 25(OH)D3, or 1,25(OH)2D3 (Sigma Aldrich) were solubilized in 100% ethanol for cell culture experiments. Stocks were stored at −80°C for up to 2 months at 33 mmol/L. KAAD-cyclopamine (Toronto Research Chemicals Inc.; catalog no. K171000) was dissolved in 100% dimethyl sulfoxide (DMSO) and stored at −80°C for up to 2 months at 10 mmol/L. For topical treatment of BCC tumors in vivo, 1.3 to 2.6 mg/kg (equivalent to 1,500–3,000 IU) of vitamin D3 was applied daily to the tumors. Approximately 200 μL of the vitamin D3 acetone solution was applied to visible BCCs on 20 cm² area of dorsal back skin for 5 days per week for 30 days (1.3 mg/kg for changes in immunohistochemistry studies, Ki67) or 2.6 mg/kg daily for 4 days (for changes in Gli1 mRNA). Commercially available 1,25(OH)2D3 analog cream (Dovonex; calcipotriene 0.005%) was donated by Leo Pharmaceuticals.

**Cell proliferation assay**

Cell numbers were assayed with the WST-1 cell proliferation assay (Roche Applied Science). A total of 7 × 10³ cells were seeded in 96-well plates and cultured overnight to 60% to 70% confluency and then serum starved overnight. Cells were incubated with vitamin D3, 7DHC, 1,25(OH)2D3, or cyclopamine at various concentrations for 48 hours and colorimetric readings were measured at 450 nmol/L on a microplate reader, SpectraMax 340PC (Molecular Devices) as previously described (26).

**Quantitative PCR for Gli1 and 24-hydroxylase mRNA expression**

RNA was collected by using the PureLink RNA mini kit (Invitrogen). Reverse transcription was done by using the Taqman reverse transcription kit (Applied Biosystems) and qPCR was done on cDNA by using TaqMan premixed primer probes and reagents from Applied Biosystems. Ribosomal 18S or GAPDH was used as a normalization control for all experiments.
Adenoviral infection of shRNA to knock down the vitamin D3 receptor

The target sequences of shRNAs produced from adenoviral constructs are VDR 5’-CCATTGAGGTCATCATGTT-3’ and the nonsilencing control 5’-TGGCTTGCTAGTACCACT-3’ (42). A total of 1.5 × 10^5 ASZ cells were seeded in T12.5 flasks and infected 4 hours later at a rate of 100 infectious units/cell and grown for 3 days in 154-CF medium containing 2% chelexed FBS, 1× penicillin and streptomycin and 0.05 mmol/L calcium chloride (42). Cells were then serum starved overnight and exposed to vitamin D3 at 5 μmol/L for 24 hours.

Western blotting

Proteins were extracted with Lysis-M reagent (Roche) and protein concentration was measured by using the micro BCA protein kit (Thermo scientific Pierce). Proteins were resolved by using 4% to 20% precast gradient SDS PAGE gels (Invitrogen), semidry transferred to polyvinylidene difluoride membrane (Millipore), blocked with 5% milk-TBST, and hybridized overnight at 4°C by using VDR-specific (1:300; Santa Cruz Biotech) or β-actin (1:15,000; Sigma) polyclonal antibody. After incubation with secondary antibody conjugated with horseradish peroxidase (1:2,000; Cell Signaling) for 1 hour at room temperature, signals were detected by enhanced chemiluminescence by using the Supersignal West Femto maximum Sensitivity Substrate Kit (Thermo Scientific Pierce). Relative protein levels were quantified by ImageJ software.

β-Gal staining and immunohistochemistry

LacZ-encoded bacterial β-galactosidase (β-gal) was detected by incubation of glutaraldehyde and formalin fixed tissue with X-gal (Roche Applied Science; ref. 43). Mouse tissues were fixed in 10% buffered formalin, embedded in paraffin, and cut into 5-μm sections. Sections were deparaffinized in xylene and antigen retrieval was carried out in Trilogy solution (Cell Marque) by heating. Mouse tissues were fixed in 10% buffered formalin, fixed tissue with X-gal (Roche Applied Science; ref. 43). Proteins were extracted with Lysis-M reagent (Roche) and protein concentration was measured by using the micro BCA protein kit (Thermo scientific Pierce). Proteins were resolved by using 4% to 20% precast gradient SDS PAGE gels (Invitrogen), semidry transferred to polyvinylidene difluoride membrane (Millipore), blocked with 5% milk-TBST, and hybridized overnight at 4°C by using VDR-specific (1:300; Santa Cruz Biotech) or β-actin (1:15,000; Sigma) polyclonal antibody. After incubation with secondary antibody conjugated with horseradish peroxidase (1:2,000; Cell Signaling) for 1 hour at room temperature, signals were detected by enhanced chemiluminescence by using the Supersignal West Femto maximum Sensitivity Substrate Kit (Thermo Scientific Pierce). Relative protein levels were quantified by ImageJ software.

Statistical analysis

Nonparametric t test and Spearman’s correlation test were used to compare the difference in mean values and correlations of Gli1 and 24-hydroxylase mRNA levels, respectively. All P values reported are 2-sided.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Allan Balmain for the C5N cell line, Pierre Chambon and Anton Iberns for supplying the K14-CreER2 and p53 fl alleles, and Loreta Chan for her assistance with the hematoxylin–eosin staining and immunohistochemistry staining.

Grant Support

This work was supported by the National Institute of Health (CA81888 and CN-95116 to E.H. Epstein) and the NIH/NCRR/OD UCSF-CTSI KL2 RR024130, NIH/NIAMS K23 AR056736-01 and Prevent Cancer Foundation to J.Y. Tang.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 15, 2010; revised December 28, 2010; accepted February 1, 2011; published OnlineFirst March 24, 2011.

References


Cancer Prevention Research

Vitamin D3 Inhibits Hedgehog Signaling and Proliferation in Murine Basal Cell Carcinomas


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-10-0285

Cited articles
This article cites 41 articles, 11 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/4/5/744.full.html#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
/content/4/5/744.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.