Inhibition of the Transition of Ductal Carcinoma In Situ to Invasive Ductal Carcinoma by a Gemini Vitamin D Analog

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Abstract

Ductal carcinoma in situ (DCIS) is a nonmalignant lesion of the breast with the potential to progress to invasive ductal carcinoma (IDC). The disappearance and breakdown of the myoepithelial cell layer and basement membrane in DCIS have been identified as major events in the development of breast cancer. The MCF10DCIS.com cell line is a well-established model, which recapitulates the progression of breast cancer from DCIS to IDC. We have previously reported that a novel Gemini vitamin D analog, 1α,25-dihydroxy-20R-21(3-hydroxy-3-deuteromethyl-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-cholecalciferol (BXL0124) is a potent inhibitor of the growth of MCF10DCIS.com xenografted tumors without hypercalcemic toxicity. In this study, we utilized the MCF10DCIS.com in vivo model to assess the effects of BXL0124 on breast cancer progression from weeks 1 to 4. Upon DCIS progression to IDC from weeks 3 to 4, tumors lost the myoepithelial cell layer and basement membrane as shown by immunofluorescence staining with smooth muscle actin and laminin 5, respectively. Administration of BXL0124 maintained the critical myoepithelial cell layer as well as basement membrane, and animals treated with BXL0124 showed a 43% reduction in tumor volume by week 4. BXL0124 treatment decreased cell proliferation and maintained vitamin D receptor levels in tumors. In addition, the BXL0124 treatment reduced the mRNA levels of matrix metalloproteinases starting at week 3, contributing to the inhibition of invasive transition. Our results suggest that the maintenance of DCIS plays a significant role in the cancer preventive action of the Gemini vitamin D BXL0124 during the progression of breast lesions. Cancer Prev Res; 7(6); 1–10. ©2014 AACR.

Introduction

Breast cancer is one of the most common cancers, and the second highest cancer-related cause of death among women (1). Regardless of its high prevalence in society, the etiology and pathogenesis of breast cancer have yet to be elucidated (2–5). Genetic, hormonal, and environmental factors have been suggested as causes of breast cancer (6, 7). Early development of breast cancer consists of noninvasive lesions such as ductal carcinoma in situ (DCIS), and approximately 25% of breast abnormalities detected during screening are DCIS (8). DCIS is defined by enhanced proliferation of intraductal epithelial cells (9). DCIS is a precancerous lesion and if left untreated, approximately 30% to 50% of cases will progress to invasive disease (10–12). Therefore, the transition from DCIS to invasive ductal carcinoma (IDC) is a crucial event in the advancement of breast tumors. The human breast ductal structure is made up of an inner layer of luminal epithelial cells, surrounded by an outer layer of myoepithelial cells and is separated from the stroma by the basement membrane (13). In order for tumor cells to invade and metastasize, they must penetrate these 2 protective layers (14). The disappearance and breakdown of the myoepithelial cell layer and basement membrane in DCIS have been identified as major events in the progression from DCIS to IDC (15). Thus, it is important to find chemopreventive agents, such as vitamin D, that could inhibit the progression to later stages of breast cancer.

Exposure to sunlight has shown an inverse relationship to breast cancer risk (16). This is linked to the increased production of vitamin D3 upon exposure to ultraviolet light (17–20). Since this discovery, many studies have linked 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], the active metabolite of vitamin D, and vitamin D analogs to the suppression of cancer cell invasion, proliferation, and metastasis (21–24). However, the pharmacologic dose of 1,25(OH)2D3 required to elicit a response on breast tumors can induce hypercalcemic toxicity (24, 25). Therefore, non-calcemic vitamin D analogs have been of interest in the inhibition of breast cancer (21, 25–27). We have previously demonstrated that a Gemini vitamin D analog BXL0124 significantly decreased tumor growth of MCF10DCIS.com.
xenografts in vivo without hypercalcemic toxicity (25, 28). However, the preventive effects of BXL0124 on the early transition from DCIS to IDC have not been elucidated. We utilized the MCF10DCIS.com progression model to investigate whether BXL0124 blocks or delays the early transition of DCIS to IDC in vivo.

The MCF10DCIS.com cell line is one of the human MCF10A series of cell lines, which is unique in the fact that it is representative of breast cancer progression in vitro and in vivo (29, 30). The MCF10DCIS.com cell line consistently produces comedo DCIS-like lesions in animal xenografts, which highly resemble the histopathology of human DCIS in xenograft models. The DCIS-like lesions have been shown to reproducibly progress to invasive tumors, providing a unique in vivo model to investigate the transition of DCIS to IDC (28, 29). The MCF10DCIS.com xenograft model forms histology that resembles human DCIS, however this is not classical DCIS, which are premalignant cells confined to the mammary ducts. Therefore, from this point forward when we refer to DCIS we are referring to the DCIS-like histology that is observed in the subcutaneous xenografts. In addition, Hu and colleagues showed similarities of cell type–specific expression profiles between human DCIS samples and MCF10DCIS.com xenografts by comparing myoepithelial and epithelial cell gene expression profiles (15). The study showed a statistically significant enrichment of genes involved in the extracellular matrix, basement membrane structure, and development in the myoepithelial cell populations of both the human DCIS samples and MCF10DCIS.com xenografts. Because the enrichment patterns of genes were highly similar from human to the MCF10DCIS.com xenograft tumors, this is an excellent model to test the effects of chemopreventive agents on the structural, molecular, and phenotypic changes during the progression of DCIS to IDC. In this report, we investigated the effects of BXL0124 treatment on MCF10DCIS.com xenografts, and found that BXL0124 maintained the integrity of critical structures related to noncancerous breast lesions, which are typically lost during the progression to malignant disease.

Materials and Methods

Reagents and cell culture

Gemini vitamin D analog, 1α,25-dihydroxy-20R-21(3-hydroxy-3-deuteromethyl-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-cholecalciferol (BXL0124, >95% purity; ref. 31) was provided by BioXell, Inc. and dissolved in dimethyl sulfoxide (DMSO) solution at 37°C. BXL0124 was diluted in Cremophor EL: PBS (1:8, v/v). MCF10DCIS.com cell line was provided by Dr. F. Miller at the Barbara Ann Karmanos Cancer Institute (Detroit, MI). The MCF10DCIS.com cell line was authenticated by short tandem repeat profiling at American Type Culture Collection. MCF10DCIS.com cells were maintained in monolayer cell culture in DMEM/F12 medium supplemented with 5% horse serum, 1% penicillin/streptomycin, and 1% 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) solution at 37°C, 5% CO2.

Xenograft animal studies

All animal studies were approved by the Institutional Review Board for the Animal Care and Facilities Committee of Rutgers University. Female nude mice (5–6 weeks old) were purchased from Charles River Laboratories. They were allowed to acclimate to the facilities for 2 weeks, at which time they were injected (7–8 weeks old) with human MCF10DCIS.com cells into the dorsal flank with 10⁶ cells per site and treatment began the following day. Mice were treated with DMSO control or BXL0124 (0.1 μg/kg body weight) intraperitoneally 6 times per week over the course of 4 weeks. Tumors were palpated twice per week and total body weights were measured weekly. Tumors were measured with a vernier caliper and tumor volume (V, cm³) was calculated using the equation V = Dd²/2, where D (cm) and d (cm) are the largest and smallest perpendicular diameters. Animals were sacrificed 1 to 4 weeks after injection, at which time tumors were excised and blood drawn for further analysis. Tumors from 1 to 4 weeks were fixed in 10% formalin for 15 hours for immunohistochemical analysis. Tumors from 3 and 4 weeks were snap frozen in liquid nitrogen for qPCR analysis.

Determination of serum calcium level

The determination of calcium concentration in serum was carried out by using the calcium reagent set from Pointe Scientific, Inc., following the manufacturer's protocol. Briefly, serum (4 μL) was mixed with the diluted reagent (200 μL) in the wells of a 96-well plate and incubated at room temperature for 1 minute and absorbance read at 570 nm using a Tecan infinite M200 plate reader (Tecan). The calcium concentrations were calculated from calcium standards provided by the manufacturer.

Immunohistochemical and immunofluorescence analysis

Subcutaneous tumors were fixed, embedded in paraffin, and sectioned at 5-μm thickness. Individual tumors were analyzed histopathologically by hematoxylin and eosin (H&E) staining. For immunohistochemistry, sections were stained as previously described (32) with antibodies to proliferating cell nuclear antigen (PCNA; 1:8000; Dako; M0879) and vitamin D receptor (VDR; 1:200, Santa Cruz Biotechnology; sc-13133). The sections were counter-stained with Harris hematoxylin (Sigma Aldrich). The PCNA nuclear intensity and VDR total pixel intensity were quantified by blinding the samples and having a third party carry out the analysis using a Scan Scope (Aperio). For immunofluorescence staining, the slides were blocked in 10% goat serum, and then incubated sequentially overnight at 4°C with the indicated combination of primary antibodies to smooth muscle actin (SMA; 1:200; Abcam; ab5694), laminin 5 (1:200; Abcam; sc13587), pancytokeratin (panCK; 1:50; Dako; M3515), and TO-PRO-3 iodide nuclear antibody (Invitrogen; 1 μmol/L). Fluorophore-conjugated secondary antibodies (Alexa Fluor 488 or 546, 1:100; Invitrogen) were incubated...
at room temperature for 30 minutes. The images were taken using confocal microscopy with lasers at 488, 546, and 633 nm (TO-PRO-3). Immunofluorescence was visualized using a Nikon Eclipse C1 Plus confocal microscope system.

**Western blot analysis**

The procedures have been described previously (32). Five tumor samples from each group were homogenized and pooled for analysis. Primary antibodies against VDR (1:200; Thermoscientific; MA1-710) and β-actin (1:2,000; Sigma-Aldrich; A1978) were used for analysis. Secondary antibodies were from Santa Cruz Biotechnology. Western blots were quantified by using ImageJ software (US National Institutes of Health, Bethesda, MD) and calculating the relative density of the bands using the gel analyzer command.

**qPCR analysis**

These procedures have been reported previously (33, 34). The Taqman probe-based gene expression system from Applied Biosystems was used to detect the genes of interest.

**DCIS quantification**

Hematoxylin and eosin images of DCIS were quantified using ImageJ software (US National Institutes of Health). The total tumor area was selected, and then the areas of DCIS within the tumor were calculated. The percentage of DCIS within each tumor was presented as the area of DCIS within the tumor were calculated. The percentage of DCIS within each tumor was presented as the area of DCIS divided by the total tumor area.

**Statistical analysis**

Statistical significance was evaluated using the Student t-test.

**Results**

**Gemini vitamin D analog, BXL0124, inhibits the transition of ductal carcinoma in situ to IDC in MCF10DCIS.com xenografts**

Previous studies have shown that BXL0124 has an inhibitory effect on the growth of MCF10DCIS.com xenograft mammary tumors at 5 weeks (28). However, the effect of BXL0124 on DCIS progression to IDC has not been investigated. The early stages of breast cancer, specifically the DCIS to IDC transition, are of utmost importance from a prevention standpoint. Therefore, in this study, we evaluated the effects of BXL0124 on the early transition of DCIS to IDC. In our preliminary studies, we first compared the DCIS progression of MCF10DCIS.com xenografts between the mammary fat pad and subcutaneous injections. The subcutaneous xenografts formed a higher number of comedo DCIS lesions and more consistently produced DCIS lesions compared with the mammary fat pad xenografts (Supplementary Fig. S1A and S1B), in agreement with a previous report by Hu and colleagues (15). Comparison of 4 separate lesions from subcutaneous or mammary fat pad at week 3 xenografts shows that subcutaneous xenografts reproducibly form a higher number of DCIS lesions compared with the mammary fat pad xenografts (Supplementary Fig. S1B). The subcutaneous xenografts show a clear point DCIS to IDC transition so it is easy to determine whether pharmacologic agents, such as BXL0124, inhibit this transition (Supplementary Fig. S2A), whereas the mammary fat pad xenografts it is not clear when or if the transition is blocked (Supplementary Fig. S2B). Therefore, we proceeded with the MCF10DCIS.com subcutaneous xenograft model for the clarity of studying the effects of pharmacologic agents on DCIS progression to IDC. Using MCF10DCIS.com subcutaneous xenografts, we investigated the histopathologic and molecular changes that occurred during the growth of tumors in nu/nu mice over the course of 4 weeks. Animals treated with BXL0124 showed a reduction in average tumor volume over the first 3 weeks and significant repression was observed by week 4 with a 43% reduction in tumor size (P < 0.05; Fig. 1A). BXL0124 treatment did not cause any significant changes in body weight or serum calcium levels, indicating that there was no observed hypercalcemic toxicity associated with the given dose (Fig. 1B and C). H&E staining showed that MCF10DCIS.com cells subcutaneously xenografted into nu/nu mice formed lesions histologically resembling that of human DCIS. In the control group, DCIS lesions started to escape to an invasive-like stage at week 3 and the tumors advanced to IDC rapidly by week 4, at which time the tumors were 80% invasive (Fig. 1D). The tumors treated with BXL0124 formed DCIS lesions by week 3 and unlike control tumors, maintained these DCIS lesions through week 4, showing approximately 30% invasive histology (P < 0.05; Fig. 1D).

**BXL0124 treatment decreases the proliferation of MCF10DCIS.com tumors at week 4**

Cell proliferation was determined by measuring PCNA by immunohistochemistry staining. The cell proliferation in MCF10DCIS.com tumors remained relatively low through week 3. As control tumors progressed to an invasive phenotype at week 4, there is a marked increase in the level of PCNA expression compared with previous weeks. Treatment with BXL0124 showed a significant decrease in PCNA levels compared with the control at week 4 (Fig. 2A). Four tumors from each group were blinded and were analyzed for PCNA staining intensity. The intensities were scored from 0 (negative staining) to 3+ (strongest staining) for each individual cell. The sum of all positive staining, including 1+, 2+, and 3+, was used to calculate the percentage of PCNA-positive cells. Quantification showed that 52% of the cells were PCNA-positive in the control group at week 4, whereas only 32% of cells were positive in the BXL0124-treated group at week 4 (P < 0.05; Fig. 2B).

**BXL0124 treatment maintains VDR levels in MCF10DCIS.com tumors**

MCF10DCIS.com xenograft tumors express VDR. From weeks 1 to 3, VDR levels were similar between control and BXL0124 treatment groups. VDR was lost upon the rapid progression from DCIS to invasive tumors in the control
group in week 4. However, treatment with BXL0124 not only maintained DCIS histology but also retained VDR levels at week 4 (Fig. 3A). It is interesting to note that VDR expression is lost where epithelial and stromal cells come in contact, suggesting that this cell-to-cell interaction could potentially reduce VDR levels. Four tumors from each group were blinded and analyzed for VDR staining intensity. The intensities were scored from $0^+$(negative staining) to $3^+$ (strongest staining) based on pixel intensity of staining. The sum of all positive staining, including $1^+$, $2^+$, and $3^+$, was used to calculate the percentage of VDR-positive cells. Quantification showed $66\%$ VDR-positive staining in the control tumors, compared with $81\%$ positive staining in the BXL0124 group at week 4 ($P < 0.05$; Fig. 3B). VDR levels were also analyzed in xenograft tumors by Western blot analysis, showing a $60\%$ increase in the xenografts from

![Graphs and images showing tumor volume, body weight, serum calcium, and H&E staining progression from week 1 to week 4 for control and BXL0124 groups.](image)

Figure 1. BXL0124 inhibits tumor growth and inhibits progression to IDC in MCF10DCIS.com subcutaneous xenografts in nu/nu mice. A, average tumor volume at weekly time points is shown. $^*P < 0.05$ ($n = 5$ per group). Tumor volume ($V; \text{cm}^3$) was calculated using the equation $V = \frac{Dd^2}{2}$, where $D$ (centimeters) and $d$ (centimeters) are the largest and smallest perpendicular diameters. B, average final bodyweight at autopsy is shown ($n = 5$ per group). C, serum calcium determination to assess hypercalcemic toxicity is shown ($n = 5$ per group). D, a representative H&E staining showing the progression of MCF10DCIS.com subcutaneous xenografts in nu/nu mice from weeks 1 to 4 ($\times 40$). DCIS quantification, $^*P < 0.05$ ($n = 4$ per group).
BXL0124-treated mice compared with the control xenograft tumors (Fig. 3C).

**Treatment with BXL0124 inhibits progression to IDC by maintaining the myoepithelial cell layer**

The main diagnostic feature that distinguishes DCIS from invasive lesions is the loss of the critical myoepithelial cell layer and basement membrane (15). To investigate the integrity of the cell layers throughout progression, we analyzed the myoepithelial marker SMA and the epithelial marker panCK in xenograft tumors. Tumors at weeks 1 and 2 were composed primarily of epithelial cells as noted by panCK staining. There was a gradual establishment of the myoepithelial cell layer starting at week 2, fully forming around the epithelial cells in both the control and BXL0124-treated group by week 3 (Fig. 4). In the control group tumors, the myoepithelial cell layer spontaneoulsy dissociated from its organized structure by week 4 as the tumors progressed to IDC. However, the course of BXL0124 treatment showed the formation of the myoepithelial cell layer in week 3 and maintained organization of the myoepithelial cell layer at week 4 (Fig. 4).

**Treatment with BXL0124 maintains the basement membrane**

To assess the effects of BXL0124 on the integrity of the basement membrane, co-immunofluorescence staining was carried out with a basement membrane marker, laminin 5, and a myoepithelial marker, SMA. The basement membrane formed by week 2, and was maintained in week 3 in both the control and BXL0124-treated groups. At week 4, the basement membrane is disrupted in the invasive-like tumors of the control group, as indicated by reduced and fragmented staining of laminin 5. This structure remained intact in the BXL0124-treated tumors at week 4 (Fig. 5).
**BXL0124 inhibits the mRNA expression of the matrix metalloproteinases during DCIS to IDC progression**

Matrix metalloproteinases (MMP) have been implicated in increased tumor growth, invasion, and metastasis (34, 35). Consequently, we assessed the mRNA levels of specific MMPs in MCF10DCIS.com tumor xenografts at weeks 3 and 4. Analysis of MCF10DCIS.com tumors showed a significant decrease in the mRNA levels of MMP2, 9, 14, and 15 upon BXL0124 treatment at week 3 whereas MMP16 did not change (Fig. 6A). The modulation of MMP2 and MMP14 expression by BXL0124 treatment persisted to week 4. These observations suggest that reduction of MMP expression by BXL0124 contributes to the inhibition of transition from DCIS to invasive carcinomas. Interestingly, despite the changes in protein levels of VDR we did not see a significant change in mRNA expression of the VDR gene at weeks 3 and 4 upon BXL0124 treatment (Fig. 6A). We did not detect significant biologic changes in the vitamin D metabolizing genes CYP24A1 (catabolism) or CYP27B1 (synthesis) in week 3 and 4 xenograft samples (Supplementary Fig. S3). To determine if VDR is expressed in myoepithelial cells, we assessed the immunofluorescence staining of VDR together with the myoepithelial marker SMA. VDR staining did not colocalize with SMA staining in any of the samples that were analyzed, indicating that VDR is not expressed in myoepithelial cells (Fig. 6B). In addition, the loss of VDR is evident in week 4 control xenografts, whereas VDR levels are maintained upon BXL0124 treatment at week 4 (Fig. 6B).

**Discussion**

DCIS progression to IDC is defined by the escape of inner luminal epithelial cells through the outer layer of myoepithelial cells and the basement membrane, ultimately coming in contact with the stromal cell population (13). Previous reports have shown that myoepithelial cells can arise from the luminal cell population, but not vice versa (13, 36–38). In our study, the MCF10DCIS.com xenografts show growth of the epithelial cells in the early weeks followed by the formation of the myoepithelial cell layer that is consistent with those reports. BXL0124 does not seem to affect the rate of formation of the myoepithelial layer (Fig. 4; weeks 1 to 3), however it does significantly reduce the rate at which the myoepithelial cell layer is...
broken down (Fig. 4; week 4), suggesting that BXL0124 inhibits the transition from DCIS to invasive carcinoma. When the staining of laminin 5 in the DCIS and IDC lesions are examined histologically, it is clear that BXL0124 treatment helps to maintain an intact, organized structure of the critical basement membrane (Fig. 5; week 4). It is also interesting to note that the basement membrane forms first in week 2 followed by the myoepithelial cell layer at week 3. This suggests that the basement membrane might act as a scaffold for the formation of the myoepithelial cell layer, and the loss of this scaffold through enzymatic degradation could partially account for the disorganization of the myoepithelial cell layer observed in week 4 control tumors. Analyzing the effects of BXL0124 on the progression of DCIS to IDC (Fig. 1D), we found that the treatment sustains DCIS lesions and prevents progression to IDC through maintenance of the critical myoepithelial cell layer and the basement membrane.

The progression from DCIS to IDC is believed to be provoked largely by the production of proteolytic enzymes (39). MMPs degrade proteins involved in extracellular matrix structure and molecules involved in cell–cell adhesion, which releases epithelial cells from their ordered layers and deregulates cell signaling, ultimately
were higher in BXL0124-treated group when compared with
in the epithelial cells (Fig. 3A; week 4). VDR protein levels
integrity of the DCIS structure, but also retained VDR levels
our study, the BXL0124 treatment not only maintained the
progression from benign to malignant breast lesions (45). In
progression. It was previously shown that a decrease in
(28, 34), we analyzed VDR levels over the course of tumor
effects of BXL0124 are known to be dependent on VDR
animals at week 4 demonstrates the potential of BXL0124
the proliferation rate in tumors from BXL0124-treated
findings that stromal and tumor epithelial cell interactions
preservation of DCIS histology.

leading to extensive changes in gene transcription (40).
MMP2 and MMP9, the gelatinases, are responsible for the
degradation of type IV collagen as well as laminin 5,
components of the basement membrane (39, 41). MMP2 is
secreted in a latent form and requires activation by MMP14
to its pro-MMP2 form (42). Aside from its activating
function, MMP14 as well as MMP15 and MMP16 have been
shown to directly affect cell invasion by remodeling the
basement membrane in vivo (43). Thus, reduction of MMP2,
MMP9, MMP14, and MMP15 by BXL0124 in week 3 before
the transition of DCIS to IDC likely contributes to the
maintenance of the basement membrane in the tumor
xenografts. This suggests that the downregulation of MMPs
by BXL0124 treatment could play a major role in the
preservation of DCIS histology.

As tumors progress to IDC, there is a significant increase
in cell proliferation, which is consistent with previous
findings that stromal and tumor epithelial cell interactions
can enhance proliferation (44). The significant reduction of
the proliferation rate in tumors from BXL0124-treated
animals at week 4 demonstrates the potential of BXL0124
to slow the growth of DCIS epithelial cells. Because the
effects of BXL0124 are known to be dependent on VDR
(28, 34), we analyzed VDR levels over the course of tumor
progression. It was previously shown that a decrease in
protein levels of VDR have been correlated with the pro-
gression from benign to malignant breast lesions (45). In
our study, the BXL0124 treatment not only maintained the
integrity of the DCIS structure, but also retained VDR levels
in the epithelial cells (Fig. 3A; week 4). VDR protein levels
were higher in BXL0124-treated group when compared with
the control group at week 4. However, mRNA expression of
VDR was unchanged in weeks 3 or 4, suggesting that loss of
VDR in week 4 control tumors is likely because of protein
degradation or possible posttranslational regulation.
Interestingly, loss of DCIS architecture seems to be a determining
factor in the loss of VDR expression, suggesting that the
interaction with the surrounding stromal cells could play a
major role in the downregulation of VDR. Whether adjacent
stromal-to-epithelial cell contact or factors secreted from
stromal cells contribute to the loss of VDR needs to be
further investigated. Analysis of VDR and SMA colocaliza-
tion studies show that VDR is not expressed in the myoe-
pithelial cell layer or stroma but within the luminal cells.
In invasive tumors, VDR negative cells include both tumor and
stroma cells. Taken together, it seems that VDR is expressed
in the luminal cell population and this expression is lost
upon progression to IDC. This further suggests that
BXL0124 does not act directly on the myoepithelial cell
layer but may exert its antitumor effects through a paracrine
mechanism from the luminal cells. The analysis of DCIS
revealed increased VDR levels and decreased cell prolifer-
ation, providing a prevention strategy to inhibit the early
progression of DCIS to IDC with the treatment of vitamin
D or its analogs. These data suggest a novel mechanism
in which downregulation of VDR is synchronized with the
loss of the critical myoepithelial cell layer and basement
membrane.

Breast cancer progression is indicated by the loss of
normal tissue architecture allowing invasion into surround-
ing tissue (46). Our study shows that BXL0124 has the
potential to exert its effects indirectly on the myoepithelial

Figure 6. BXL0124 inhibits the mRNA expression levels of the MMPs during DCIS to IDC progression. A, qPCR analysis of MMPs and VDR mRNA levels in MCF10DCIS.com tumor samples from weeks 3 and 4 is shown, mean ± SEM. Cycle numbers are shown in parenthesis: MMP2 (25), MMP9 (28), MMP14 (23), MMP15 (28), MMP16 (25), and VDR (26). Statistical significance refers to the respective week control, *P < 0.05, **P < 0.01, ***P < 0.001 (n = 3–5 per group). B, a representative immunofluorescence staining with the myoepithelial cell marker, SMA (shown in red), and VDR (shown in green) on tumors from weeks 3 and 4 are shown (×200). Nuclei were stained with TO-PRO-3 (blue). Scale bars represent 100 μm.
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Wahler, Y.C. Kim, N. Suh

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Wahler, F. Liu, N. Suh

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Study supervision: N. Suh

Other: H. Maehr contributed synthesis of drug substance.

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Authors’ Contributions

Conception and design: J. Wahler, Y.Y. So, F. Liu, N. Suh

Development of methodology: J. Wahler, N. Suh

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