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Review

Vitamin D compounds reduce mammosphere formation and decrease expression of putative stem cell markers in breast cancer

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ABSTRACT

Breast cancer stem cells (BCSCs) are a subset of tumor cells that are believed to be the cells responsible for the establishment and maintenance of tumors. Moreover, BCSCs are suggested to be the main cause of progression to metastasis and recurrence of cancer because of their tumor-initiating abilities and resistance to conventional therapies. Ductal carcinoma *in situ* (DCIS) is an early precursor in breast carcinogenesis which progresses to invasive ductal carcinoma (IDC). We have previously reported that a vitamin D compound, BXL0124, inhibits the progression of DCIS to IDC. In the present study we sought to determine whether this effect was mediated through an influence on BCSCs. In MCF10DCIS cells treated with vitamin D compounds ($1\alpha25(OH)_2D_3$ or BXL0124), the breast cancer stem cell-like population, identified by the $CD44^+/CD24^{-/low}$ and $CD49f^+/CD24^{-/low}$ subpopulations, was reduced. To determine the effects of vitamin D compounds on cancer stem cell activity, the MCF10DCIS mammosphere cell culture system, which enriches for mammary progenitor cells and putative BCSCs, was utilized. Untreated MCF10DCIS mammospheres showed a disorganized and irregular shape. When MCF10DCIS cells were treated with $1\alpha25(OH)_2D_3$ or BXL0124, the mammospheres that formed exhibited a more organized, symmetrical and circular shape, similar to the appearance of spheres formed by the non-malignant, normal mammary epithelial cell line, MCF10A. The mammosphere forming efficiency (MFE) was significantly decreased upon treatment with $1\alpha25(OH)_2D_3$ or BXL0124, indicating that these compounds have an inhibitory effect on mammosphere development. Treatment with $1\alpha25(OH)_2D_3$ or BXL0124 repressed markers associated with the stem cell-like phenotype, such as CD44, CD49f, c-Notch1, and pNFκB. Furthermore, $1\alpha25(OH)_2D_3$ and BXL0124 reduced the expression of pluripotency markers, OCT4 and KLF-4 in mammospheres. This study suggests that vitamin D compounds repress the breast cancer stem cell-like population, potentially contributing to their inhibition of breast cancer.

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Contents

Abbreviations: $1\alpha,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; BCSC, breast cancer stem cell; DCIS, ductal carcinoma *in situ*; GATA3, GATA binding protein 3; IDC, invasive ductal carcinoma; KLF4, Kruppel-like factor 4; MFE, mammosphere forming efficiency; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; OCT4, octamer-binding transcription factor 4; PCNA, proliferating cell nuclear antigen; SOX2, SRY (sex determining region Y)-box 2.

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1. Introduction

Ductal carcinoma *in situ* (DCIS) is an early, non-malignant lesion of the breast constituting approximately 25% of breast abnormalities [1]. The treatment of DCIS typically consists of surgery, radiation, and when necessary, hormonal therapy. Despite the effective treatment regimen and initial response to therapy, approximately 15% of patients with DCIS will relapse [2]. Additionally, 30–50% of DCIS cases will progress to invasive ductal carcinoma (IDC) if left untreated [3,4]. Evidence suggests that these processes are regulated through the interaction of the breast cancer stem cells (BCSCs) with the surrounding microenvironment via cell adhesion molecules and receptors [5]. Therefore, the progression of DCIS to IDC, along with the recurrence of DCIS or IDC after treatment are two critical events that could be driven by BCSCs and their understanding is crucial in the prevention of breast cancer.

Increasing evidence supports the notion that the initiation and maintenance of breast tumors are sustained by the BCSCs (or tumor initiating cells) [6]. These cells have the ability to self-renew through symmetrical division or differentiate through asymmetrical division [7]. A tumor can be viewed as a heterogeneous mass of cells comprising cells at various stages of the differentiation process, all originating from an initial BCSC, or TIC [8]. Local recurrence and distant metastases demonstrate the resistance of BCSCs to radio- and chemotherapies [9]. Studies have shown that cells that remain after chemotherapy are enriched for putative BCSCs and these cells were capable of higher mammosphere forming efficiencies compared to cells tested before treatment [10,11]. The interaction of BCSCs with the microenvironment results in crosstalk of signaling and regulation of BCSCs via growth factors and cytokines from the microenvironment [12]. However, it is unclear how BCSC signaling can interact with the surrounding microenvironment, such as the stroma, to influence breast cancer progression. Signaling crosstalk with surrounding cells along with other emerging biological properties of BCSC-like cells provide strategies and targets to effectively develop therapies against the BCSC population. Elimination of the BCSC population may improve the treatment outcomes for breast cancer patients.

Initial studies identified the CD44^{high}/CD24$^{-/low}$ subpopulation of breast cancer cells from breast tumors to be enriched in cancer stem cells [6]. Tumor initiation studies with various subpopulations have also been used to assess the tumorigenicity of putative BCSCs [13,14]. Previous studies have shown that 1α25(OH)$_2$D$_3$ promoted the differentiation of colon cancer cells through increased E-cadherin expression and the inhibition of β-catenin [15]. Another study demonstrated antiproliferative effects of calcitriol on adult prostate progenitor and stem cells, however these effects have not been proven in BCSC-like cells [16]. It has also been found that a Gemini vitamin D analog decreased the expression of CD44, which has been identified as a cancer stem cell marker [17]. These studies have stimulated further investigation of the inhibitory effects of vitamin D and its analogs on the putative BCSC population.

Recent studies demonstrated that a cancer stem cell-like population identified within basal-like DCIS has the capacity to drive malignant progression to IDC [18]. Previously, we have shown that a Gemini vitamin D analog, BXL0124, can inhibit the transition from DCIS to IDC *in vivo* [19]. We have also demonstrated that BXL0124 was capable of reducing the CD44^{+}/CD24$^{-/low}$ subpopulation of MCF10DCIS cells [17]. Recent review on cell culture and animal models of cancer support a role of vitamin D compounds in decreasing cancer development and progression [20]. Since BCSCs have the potential to drive DCIS progression, we investigated the effects of vitamin D compounds on the DCIS breast cancer stem cell population, and determined their potential to inhibit stem cell-like properties. We utilized the MCF10DCIS basal-like breast cancer cell line in mammosphere forming assays. These assays have been used in various tissue types for the quantification of stem cell activity and self-renewal [21]. The formation of primary mammospheres is a measure of stem cell and early progenitor activity [22]. The present study will examine the ability of vitamin D compounds to target the putative BCSCs, which have important implications in the treatment and prevention of breast cancer.

2. Materials and methods

2.1. Cell culture and reagents

1α25(OH)$_2$D$_3$ and a Gemini vitamin D analog (BXL0124; 1α,25-dihydroxy-20R-21(3-hydroxy-3-deuteromethyl-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-cholecalciferol, >95% purity) (Fig. 1) were provided by BioXell, Inc. (Nutley, NJ) [23]. The MCF10DCIS human breast cancer cells (MCF10DCIS) were provided

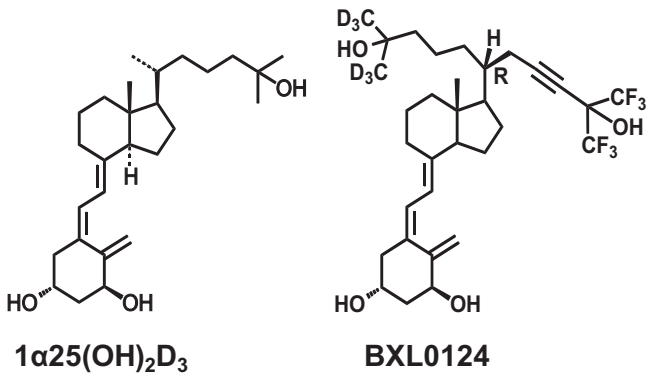

Fig. 1. The structures of 1α25(OH)$_2$D$_3$ and the Gemini vitamin D analog, BXL0124, are shown.

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