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Viewpoint

LncRNA: a new player in 1α, 25(OH)$_2$ vitamin D$_3$/VDR protection against skin cancer formation

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Abstract: Sunlight, vitamin D and skin cancer form a controversial brew. While too much sunlight exposure causes skin cancer, it is the major source of vitamin D from skin. We propose that these processes can be balanced. Vitamin D signalling (VDS) protects against skin cancer as demonstrated by the susceptibility of the skin to tumor formation in VDR null mice and protection from UVB-induced mutations when VDR agonists are administered. The question is how is protection afforded. Previously, we have focused on the Wnt/β-catenin/hedgehog and DNA damage repair (DDR) pathways. As VDR regulates hundreds of genes with thousands of VDR response elements (VDRE) throughout the genome, and many VDREs are in non-coding regions, we decided to explore long non-coding RNAs (lncRNA). LncRNAs are mRNA-like transcripts ranging from 200 bases ~100 kb lacking significant open reading frames. They are aberrantly expressed in human cancers and involved in a spectrum of tumorigenic/metastatic processes (cell proliferation/apoptosis/angiogenesis). We discovered that VDS regulated the expression of certain lncRNAs in a manner consistent with VDS protection against skin cancer. Given the huge variation in genes actively regulated by 1,25(OH)$_2$D from different cell types, it is conceivable that our results could apply to personalized medicine based on the distinctive lncRNA profiles. These lncRNAs could also serve as skin cancer biomarkers secreted into the blood or urine via exosomes as demonstrated in other cancer types (breast, prostate). Modulation of lncRNA profile by VDS may also provide insight into regulating pathways such as Wnt/ß-catenin and hedgehog.

Key words: lncRNA – skin cancer – vitamin D signalling

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Introduction

Skin cancer is the most common cancer in the world. The association of non-melanoma skin cancer (NMSC) with sunlight exposure is compelling. However, sunlight via the UVB portion of the spectrum (280–320 nm) is required for the photoconversion of the epidermal 7-dehydrocholesterol (7-DHC) to vitamin D (1). This process provides the major source of vitamin D for most people (2). In animal studies, vitamin D has been shown to have antitumor actions in many tissues including the skin. In particular, mice lacking VDR are predisposed to either chemical- or UVB-induced skin tumor formation (3–5), whereas topical application of the active vitamin D metabolite 1,25(OH)$_2$D protects against the UVB-induced mutations in skin (6). Moreover, an increased risk of basal cell carcinoma is associated with VDR polymorphisms leading to decreased VDR activity (7). To understand the mechanism for this protective role of vitamin D signalling, we have previously examined two mechanisms: (i) the role of Wnt/beta-catenin and Hedgehog pathways, pathways that when abnormally activated result in epidermal tumor formation; (8,9) and (ii) the role of VDR in facilitating DDR (10). Although regulation of these pathways no doubt plays an important role in the protection against skin cancer by vitamin D signalling, consideration of the vast number of genes potentially regulated by vitamin D and the even vaster number of potential vitamin D response elements (VDRE) in the genome, leads us to conclude that these previously identified pathways are but the tip of the iceberg. As such we initiated a study of vitamin D regulation of long non-coding RNAs (lncRNA) in the skin as a number of these lncRNAs are associated with susceptibility to or protection from malignancy in other tumor types. Indeed, our data reveal that keratinocytes lacking VDR have altered expression of lncRNAs in a pattern associated with malignant transformation in other tissues (11). This viewpoint article will outline the established roles for these VDR-regulated lncRNAs in imprinting, tumor suppression and invasion/metastasis, supporting our hypothesis that lncRNAs play a key role in the means by which vitamin D signalling protects the skin from tumor formation and providing a rationale for assessing lncRNA profiles in the skin of individuals at high risk of NMSC (Fig. 1).

VDR, lncRNA and genomic regulation

The human genome only encodes ~20 000 protein-coding genes, representing ~2% of the total genome sequence, while a much larger percentage of the genome is actively transcribed without protein-coding potential (12). These non-coding transcripts can be broadly categorized into short and long non-coding RNAs. The arbitrary size delineation is at 200 bases in length: small non-coding RNAs are less than 200 bases, including tRNAs, microRNAs, small nuclear RNA (snoRNAs). In contrast, lncRNAs are endogenous cellular RNAs of larger than 200 bases and can even be greater than 100 kb in length (13). LncRNAs account for 80% of the transcriptome (12); they are spliced and contain polyadenylation signals, much like messenger RNAs (14). LncRNAs are expressed across all mammalian genomes and have emerged as master regulators of embryonic pluripotency, differentiation and body axis patterning, promoting developmental transitions (14,15) and regulating histone modifications hence influencing the epigenetic programmes of the transcriptome (16). So far, an estimated 7000–23 000 lncRNAs have been identified in the human genome, representing an enormous component of normal cellular networks in which aberrant expression will lead to various diseases including human cancers (Table S1) (13,17,18).

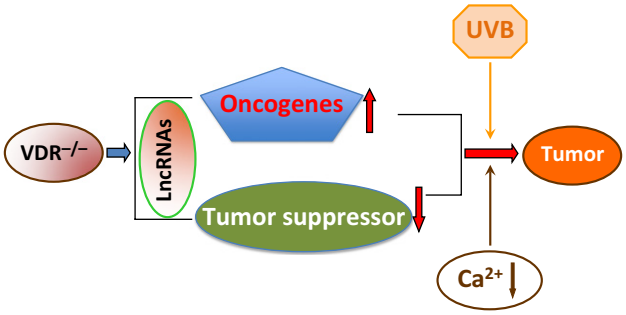

Figure 1. The emerging role of lncRNAs in VDR protection against skin tumor formation. This schematic shows our working hypothesis that when vitamin D signalling is disrupted, there is an alteration in lncRNA-expression profiling such that oncogenic lncRNAs are increased in parallel with a decrease in tumor suppressing lncRNAs. This disturbed balance in lncRNA expression then results in a predisposition to cancer induced by environmental stressors such as UV irradiation and chemical carcinogens.

VDR is one of the members of the nuclear hormone receptor superfamily. Its function generally requires heterodimerization with RXR for effective DNA interaction (19). The VDR interacts with specific motifs in the genome called VDRE. ChIP-chip and more recently ChIP-Seq analyses have revealed thousands of VDR binding sites (VDREs) in hundreds of genes in the genomes of different cells, with surprisingly modest overlap from cell type to cell type (20,21). Many cell specific factors including lncRNAs likely control which VDRE is available and which gene can be regulated in that specific cell. VDR is encoded by a relatively large gene encompassing two promoter regions, eight protein-coding exons and six untranslated exons (22). It has an extensive promoter region capable of generating multiple tissue-specific transcripts. In addition, VDR signalling is regulated directly or indirectly by interacting with a variety of mediators including lncRNAs. One example is steroid receptor RNA activator (*SRA*). SRA is a lncRNA found in the steroid receptor coactivator-1 (SRC-1) complex (23) that binds to SLIRP (21,22) (*SRA* stem-loop interacting RNA binding protein), a small *SRA* binding protein functioning as a nuclear receptor co-repressor (24).

LncRNAs and cancer

Recent studies indicate that lncRNAs function as master regulators of cancer development, by sustaining tumor cell proliferation, evading growth suppressors, enabling replicative immortality, stimulating angiogenesis and promoting invasion and metastasis. Several of the mechanisms of lncRNAs potentially involved in skin cancer development in VDR null keratinocytes (8) (Table 1) are described below. The mechanisms for specific lncRNAs are summarized in Table S1 and illustrated in Figure S1.

LncRNA and imprinting

Imprinting is a process of epigenetically silencing one copy of a gene inherited from one parent. In cancer biology, loss of imprinting often leads to alteration in gene expression. *H19* is one of best-known imprinted lncRNAs. It encodes a 2.3 kb lncRNA that is expressed exclusively from the maternal allele, which is localized nearby the insulin-like growth factor-2 gene. While *H19* is highly expressed during vertebrate embryo development, its expression level decreases shortly after birth (exception of skeletal tissue and cartilage). However, loss of imprinting at the *H19* locus leading to the re-expression of *H19* has been observed in various cancers including colon, liver, oesophagus and bladder (18), and is essential for human cancer growth (25,26). The gene expression of *H19* can be directly activated by the oncogenic transcription factor *c-myc*, and downregulated by the tumor suppressor p53 (27). Increased *H19* expression is also found in bladder cancer with metastasis, associated with EZH2, which leads to Wnt/β-catenin activation and subsequent downregulation of E-cadherin (28).

LncRNA promoting cell proliferation

LncRNAs are involved in cell proliferation during tumorigenesis. One such lncRNA is *SRA*, which plays an important role in nuclear receptor-mediated, hormone-dependent cancers (29,30) and potentially may play that role for VDR as alluded to earlier. *SRA* was originally identified as a transcriptional coactivator of steroid hormone receptors. It is an RNA component of the SRC-1 complex, which selectively enhances the AF-1 activity of class I nuclear receptors, but also VDR unlike other class II nuclear receptors (29). *SRA* interacts with SLIRP as a general corepressor for various nuclear receptors including VDR to suppress transcription (9,29). Additionally, *SRA* acts as co-activator in the Notch signalling pathway (31), which functions as a 'tumor suppressor' in mouse skin keratinocytes in that Notch1 ablation results in spontaneous and inducible skin cancer (32,33). Although mice over expressing human *SRA* display increased proliferation, these mice do not develop malignancies (30), indicating that *SRA* alone is insufficient to induce tumorigenesis.

Table 1. Human cancer-associated lncRNAs regulated by VDR in mouse keratinocytes (MKs)

LncRNA	Changes	Cancer type	Action mode	Clinical relevance	Genomic coordinates
SRA-1/SRA	Increase	Breast, uterus, ovary	Co-activator of steroid Receptors & other transcription Factors; associate with metastasis	Oncogenic	AF092039 (m), AF092038 (h)
lincRNA-p21	Decrease	Various tumors (breast, colon)	Global gene regulation p53 repression via hnRNP-K; inducing cellular apoptosis	Tumor suppressor	AK144811 (m)
HOTAIR	Increase	Breast	Epigenetically silences gene expression via LSD1/CoREST & PRC2; metastasis	Oncogenic	AK035706 (m), AK123741 (h)
H19	Increase	Bladder, lung, liver	Control of imprinting breast, cervix, oesophagus prostate, endometrial, colon	Oncogenic or tumor suppressor	AK003142 (m), AK056774 (h)
Malat1	Increase	Breast, lung, uterus	Invasion & metastasis pancreas, colon, prostate liver, cervix, neuroblastoma osteosarcoma	Oncogenic	AK141413 (m), BX538238 (h)

h, human; m, mouse; PRC2, polycomb repressor complex 2.

LncRNAs that block tumor suppression

In addition to directly promoting cell growth, cancer cells evade growth suppression by inhibiting the expression, activation or function of tumor suppressors such as P53, PTEN, cyclin-dependent kinase inhibitors and RB. *LincRNA-p21* fits in the latter category (34). The complete length and structure of human *lincRNA-p21* have not yet been defined. It appears to be regulated by p53 (35). In the mouse, this lncRNA is a direct p53 transcriptional target gene residing next to the p21 gene on chromosome 17; p53 can be readily upregulated upon DNA damage and in different tumor models. Loss-of-function studies using mouse embryonic stem cells reveal a broad *LincRNA-p21* action in controlling pluripotency and differentiation via global gene expression modification, pluripotent state maintenance, repression of lineage programmes and binding to diverse chromatin proteins (36). *LincRNA-p21* acts as a tumor suppressor by association with hnRNP-K, a well-known RNA binding protein and a tumor suppressor by itself (37,38). *LincRNA-p21* exerts its tumor suppressor function by mediating the binding of the hnRNP-K to its target genes, leading to gene silencing and apoptosis induction.

LncRNAs that promote invasion and metastasis

The most significant feature of a malignant tumor is the capability to invade and form distant metastases. Under such circumstances, cancer cells must undergo a multistep process leading to invasion and metastasis, including morphological alterations and changes in their cell–cell or cell–matrix interactions through a developmental regulatory programme known as 'epithelial–mesenchymal transition' (EMT), acquiring the ability to invade into the healthy tissue, followed by invasion into and spread by the blood and lymphatic systems (39–41). Cancer cells also must escape immune surveillance, extravasate from the vessels into their target tissues to form micrometastases and eventually produce a secondary tumor. LncRNAs are involved in all of these mechanisms. Two such examples are metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1)* and HOX antisense intergenic RNA (*HOTAIR*).

Metastasis-associated lung adenocarcinoma transcript 1, also called nuclear-enriched abundant transcript 2 (*NEAT2*), is a prognostic marker for high metastatic potential and poor patient survival in non-small cell lung cancer (42). It is widely expressed in normal human tissues (42) and upregulated in a variety of human cancers including breast, prostate, colon, liver and uterus (43–45). The human *MALAT1* gene is localized on chromosome 11q13.1, the same region that harbours chromosomal translocation breakpoints associated with cancer (46–48). In the mouse, this gene is localized on chromosome 19.5. The 8-kb *MALAT1* transcripts undergo post-transcriptional modification to produce the 5′ end of a short, tRNA-like molecule *mascRNA* and the 3′ end of a long *MALAT1* transcript with a poly(A) tail-like moiety (49). Depletion of *MALAT1* alters the processing of a subset of pre-mRNAs that play important roles in cancer biology (44). Additionally, *MALAT1* interacts with the unmethylated form of CBX4, hence controlling relocation of growth-control genes between polycomb bodies and interchromatin granules, that is, from silent to active regions of gene transcription (50). A recent study suggests that *MALAT1* supports proliferation and invasion of cervical cancer cells. Knockdown of *MALAT1* in CaSki cells led to an upregulation of caspase-8 and caspase-3 and Bax and the downregulation of Bcl-2 and Bcl-x_L (43) thus promoting apoptosis.

HOX antisense intergenic RNA is a 2.2-kb gene located in the mammalian *HOXC* locus on chromosome 12q13.13 that was first reported by Howard Chang's group (15,51). It is another metastasis-associated lncRNA that is expressed in both primary and metastatic breast tumors, and its high expression level is correlated with both metastasis and poor survival rate (52). Overexpression of *HOTAIR* in epithelial cancer cells alters H3K27 methylation leading to increased cancer invasiveness and metastasis, whereas *HOTAIR* depletion inhibits cancer invasiveness (53,54). When cells expressing high-levels of *HOTAIR* were grafted into mouse mammary fat pads, a modest increase in the rate of primary tumor growth was observed (52). *HOTAIR* is both spliced and polyadenylated. It recruits the polycomb repressor complex 2 (PRC2) to the *HOXD* locus leading to transcriptional silencing across 40 kb (52). *HOTAIR* suppression sensitizes cancer cells to TNFα-induced apoptosis and renders them more sensitive to chemotherapeutic agents (55).

LncRNAs are new players in VDR protection against skin tumor formation

We explored the potential role of lncRNAs in VDR protection against skin tumor formation by profiling 90 well-annotated mouse lncRNAs (Table S2) from mouse keratinocytes cultured *in vitro* and mouse epidermis from epidermal-specific *VDR* null mice and their normal littermates. We found that several well-known oncogenes, including *H19, HOTTIP* and *Nespas*, are significantly increased, whereas tumor suppressor lncRNAs (*Kcnq1ot1 and lincRNA-p21*) were attenuated in VDR-deleted keratinocytes (11). A similar pattern of lncRNA-expression profiling was observed in the epidermis of epidermal-specific VDR null mice versus control littermates (11). In addition to the altered lncRNAs (*H19, HOTTIP, Nespase*, *Kcnq1ot1 and lincRNA-p21)* in VDR-deleted cultured keratinocytes, there was an increase in other oncogenes (*mHOTAIR, Malat1 and SRA*) and a decrease in other tumor suppressors (*Foxn2-as, Gtl2-as and H19-as*) in VDR null mouse epidermis (11). This study reveals a novel mechanism for protection by VDR against skin cancer formation by maintaining the balance of oncogenic to tumor suppressing lncRNAs. Conceivably, lncRNAs may alter VDR function (e.g. *SRA*) and serve as upstream regulators for VDR-regulated signalling pathways such as Wnt/β-catenin, Hedgehog and DDR as has been shown at least for Wnt/β-catenin during hepatocyte proliferation (56). This will require further investigation.

Clinical implications

Sunlight causes NMSC. Sunlight is required to make vitamin D. Vitamin D signalling protects the skin and other tissues from cancer. Where is the balance? We are proposing that the balance may be determined by an evaluation of the lncRNA profile of the epidermis, an evaluation that can be performed from a skin biopsy in a patient prone to developing NMSC. Subjects with an unfavourable profile may be helped by vitamin D supplementation and/or periodic suberythemal exposure to sunlight that remains for future investigation. However, given that much of our genome is devoted to lncRNA production and that VDR regulates the expression of a number of lncRNAs including those shown to have a role in human cancers via various mechanisms, exploration

into the role lncRNAs play in the predisposition to cancer and the reversal of that predisposition by vitamin D signalling is worth pursuing and may result in better advice to patients than to avoid sunlight at all costs.

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Author contributions

YJJ performed concept and design; YJJ drafted the manuscript; DDB performed critical revision for important intellectual content; YJJ and DDB approved the manuscript. All animal experimentation in this study has been approved by the San Francisco VA Medical Center Animal Review Committee.

Conflict of interest

The authors have declared no conflicting interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cellular functions of lncRNAs.
Table S1. Human cancer-associated LncRNAs.
Table S2. The list of lncRNAs array.