

MINI-REVIEW

Ovarian Cancer: Interplay of Vitamin D Signaling and miRNA Action

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Abstract

Increasing attention is being devoted to the mechanisms by which cells receive signals and then translate these into decisions for growth, death, or migration. Recent findings have presented significant breakthroughs in developing a deeper understanding of the activation or repression of target genes and proteins in response to various stimuli and of how they are assembled during signal transduction in cancer cells. Detailed mechanistic insights have unveiled new maps of linear and integrated signal transduction cascades, but the multifaceted nature of the pathways remains unclear. Although new layers of information are being added regarding mechanisms underlying ovarian cancer and how polymorphisms in VDR gene influence its development, the findings of this research must be sequentially collected and re-interpreted. We divide this multi-component review into different segments: how vitamin D modulates molecular network in ovarian cancer cells, how ovarian cancer is controlled by tumor suppressors and oncogenic miRNAs and finally how vitamin D signaling regulates miRNA expression. Intra/inter-population variability is insufficiently studied and a better understanding of genetics of population will be helpful in getting a step closer to personalized medicine.

Keywords: miRNA - signaling - ovarian cancer - apoptosis - vitamin D - VDR

Asian Pac J Cancer Prev, **15** (8), 3359-3362

Introduction

Ovarian cancer is a multifaceted and genomically complicated disease. *In vitro* studies have shown that there are wide ranging biological mechanisms which underpin ovarian cancer development. Epigenetic mutations of tumor suppressor genes (Wu et al., 2014), overexpression of oncogenes, loss of apoptotic cell death and dysregulated signaling pathways are some of the widely studied mechanisms (House et al., 2014). Chemotherapeutic resistant ovarian cancer further confounds standardization of therapy. *In vitro* studies have shown that diallyl trisulfide induced apoptosis in cisplatin resistant ovarian cancer SKOV-3 cells (Wan et al., 2013). Moreover, Galectin-3 has been shown to be an underlying cause of drug resistance. It was reported that Gal-3 competitive inhibitor considerably improved paclitaxel mediated ovarian cancer cell killing (Hosseini et al., 2013).

Vitamin D is a steroid-like hormone which is obtained via 2 sources: ultraviolet (UV) B radiation in sunlight that converts 7-dehydrocholesterol to vitamin D₃ in skin, and ingestion of vitamin D (D₂ or D₃) in food and supplements. Vitamin D induced signaling is reported to be involved in modulation of different cellular activities

(Patel et al., 2012). It has recently been reviewed that VDR FokI polymorphism correlated with increased risks for ovarian cancer (Xu et al., 2014). Moreover, statistically non-significant and inverse association of circulating 25 (OH) D with OC incidence was reported in a meta-analysis (Yin et al., 2011). However, recently beneficial effects of Vitamin D₃ supplementation were reviewed and it was concluded that moderate doses particularly ranging from 30-80 ng/ml, can prove to be beneficial with reference to decreasing risk of cancer development (Walentowicz-Sadlecka et al., 2013).

VDR Gene Polymorphism and Ovarian Cancer

VDR BsmI polymorphism in ovarian cancer development has also been studied in various populations, but the results are discordant. One meta-analysis states that there is no association between VDR BsmI polymorphism and susceptibility to ovarian cancer in Caucasians while another meta-analysis states that VDR BsmI G/A gene variant might be a moderate risk factor of OC development in the European population instead of North America or Asian population (Qin et al., 2013; Zhang et al., 2013).

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The results of studies on VDR gene polymorphisms in ovarian cancer development are contradictory. This may be due to ethnicity. Therefore more studies in different ethnic populations are needed to identify the role of VDR gene polymorphisms in ovarian cancer development.

in vitro and in vivo studies

It is becoming progressively more understandable that non-hypercalcemic anti-cancer vitamin D analogs are effective anticancer agents as evidenced by *in vitro* and *in vivo* studies. Mechanistically it was noted that PT19c, a nonhypercalcemic Vitamin D₂ Derivative considerably induced regression of tumor growth in nude mice xenografted with SKOV-3 cells. Additionally it was shown that PT19c induced remarkably enhanced actin remodeling such as actin retraction, tear, and condensation (Kawar et al., 2013). It has been experimentally shown that MT19c effectively reduced tumor growth in nude mice inoculated with SKOV-3 cells and in syngeneic animal models without causing hypercalcemia or acute toxicity. Detailed mechanistic insights revealed that MT19c exerted its inhibitory effects via reducing cellular levels of malonyl CoA in SKOV-3 cells and inhibited EGFR induced intracellular signalling axis (Moore et al., 2012). Another contemporary study indicated additional targets of MT19c in cancer cells. These targets included IGFR/IRS-1/2-MEK-ras-ERK1/2-pathway via activated TNF α -receptor/SAPK/JNK components which were found to be suppressed in MT19c treated cancer cells (Brard et al., 2011).

Certain hints have emerged suggesting that 1,25-dihydroxyvitamin D₃ (1,25 D₃) signals through VDR to upregulate hCAP18/LL-37 in monocytes, macrophages, keratinocytes. VDR antagonist ZK159222 treated cells did not show increase in hCAP18/LL-37 expression. Laboratory findings underscored the fact that co-culture of ovarian cancer cell lines, HO-8910, OV-90, SKOV3, and 3AO with monocytes induced proliferation of cancer cells. However, neutralization of hCAP18/LL-37 considerably suppressed proliferation rate of cancer cells when co-cultured with monocytes. It is relevant to mention that versican V1, a macrophage activator transduces the signals intracellularly through TLR2 and TLR6. Additional set of experiments revealed that SKOV3 cell lines stably expressing shRNAs against versican V1 did not induce activation of TLR2 and TLR6 when co-cultured with macrophages. Altogether it was shown that versican utilized an intricate protein network to modulate expression of hCAP18/LL-37 (Li et al., 2013).

VDR has previously been shown to bind VDRE present in intron 1 of the EGFR. Overexpressing functionally active EGFR notably compromised 1,25 (OH) (2) D₃-mediated inhibitory effects in vitamin D sensitive ovarian cancer cells (Shen et al., 2011).

miRNA Action

General

There is a progressive enrichment in existing information related to ovarian cancer biology and it has been convincingly revealed that Vitamin D induced

expression of miRNA. Recently emerging evidence has started to shed light on vitamin D mediated inhibitory effects on cell proliferation. It has recently been shown that miR-22, miR-93, miR-106b, miR-451 were aberrantly expressed in the serum of ovarian cancer patients (Ji et al., 2014). There is rapidly accumulating evidence suggesting that miR-103 and miR-107 restore sensitivity of resistant ovarian cancer cells to chemotherapeutic drugs via targeting of RAD51 and RAD51D Huang et al. (2013). It has previously been shown that enhancer of zeste 2 (EZH2) promoted cellular proliferation and enhanced invasive potential of epithelial ovarian cancer cells (Li et al., 2010). It is also intriguing to note that gene silencing of EZH2 restored expression of Dicer (an enzyme that processes miRNA) in cancer cells (Kuang et al., 2013). Enforced expression of let-7e in epithelial ovarian cancer cells considerably reduced expression of EZH2 and cyclin D1 (CCND1), thus re-sensitizing cells to cisplatin (Cai et al, 2013). miR-222 negatively regulated P27Kip1 thus promoting cellular proliferation (Sun et al., 2013).

DGCR8 is also a regulator of miRNA biogenesis and constitutively overexpressed in ovarian cancer cells. *In vitro* studies revealed that silencing of DGCR8 in cancer cells simultaneously inhibited ERK1/2 and PI3K/Akt signaling axis (Guo et al., 2013). Drug resistance has also been noted to be reduced substantially via miR-199a mediated negative regulation of mTOR (Wang et al., 2013).

Tumor suppressor

New lines of evidence are providing latest insights of regulation of NKG2D Ligands via miRNAs in cancer cells. It has been shown that cancer cells escape from natural killer cell mediated cytotoxicity via miRNA mediated negative regulation of NKG2D Ligands. miR-302c and miR-520c negatively regulated MICA/B and ULBP2 and it was shown that 1,25 (OH) 2D₃ upregulated MICA/B and ULBP2 via suppressing miR-302c and miR-520c (Min et al., 2013). 1,25 (OH) (2) D (3) has also been shown to upregulate expression of miR-22 in SW480-ADH and HCT116 cells. Transfecting miR-22 inhibitor in cancer cells remarkably impaired 1,25 (OH) (2) D (3) mediated inhibitory effects on cell proliferation and cell migration (Alvarez-Diaz et al, 2012).

Laboratory methodologies provided persuasive evidence of miRNA mediated negative regulation of human telomerase reverse transcriptase mRNA. Expression analysis of cancer cell lines indicated substantially reduced expression of miR-498 (Kasiappan et al., 2012). Therefore it will be interesting to note if miR-498 reconstitution will be helpful in suppressing ovarian cancer.

Growing body of evidence substantiates the fact that calcitriol (1 α , 25-dihydroxyvitamin D₃) is involved in regulation of miRNA. It has recently been experimentally verified that calcitriol induced expression of miR-627 in HT-29 cells. Nude mice inoculated with HT-29 cells developed tumors however, inoculation of miR-627 transfected HT-29 cells in nude mice induced regression of tumor. Laboratory methodologies revealed that miR-627 negatively regulated Jumonji domain containing 1A

(JMJD1A). JMJD1A encodes a histone demethylase and transfection studies indicated that miR-627 reconstituted cancer cells had remarkably reduced JMJD1A (Padi et al, 2013). 1 α , 25-dihydroxyvitamin D3 is getting considerable appreciation because of its involvement in regulation of miRNA. Interestingly, a recent report highlighted presence of vitamin D response element (VDRE) in-2066/-2042bp of pre-let-7a-2. 1 α , 25-dihydroxyvitamin D3 treated lung cancer A549 cells displayed a marked increase in expression of hsa-let-7a-2 (Guan et al., 2013). In line with this notion, a contemporary study suggested that 1 α , 25-dihydroxyvitamin D3 triggered expression of miR-100 and-125b in prostate cancer cells. Prostate cancer cells reconstructed with miR-100 and-125b represented considerably reduced cellular proliferation. Therefore, it was concluded that 1 α , 25-dihydroxyvitamin D3 exerted its inhibitory effects on cell proliferation via upregulation of miR-100 and-125b (Giangreco et al., 2013).

Oncogenic

1,25 (OH) 2D3 has also been shown to upregulate expression of miR-32 in AML cells. It was shown that miR-32 negatively regulated pro-apoptotic gene Bim in AML cells and induced resistance arabinocytosine, a chemotherapeutic drug. Antisense miR-32 treatment was noted to be effective in overcoming resistance against arabinocytosine (Gocek et al., 2011).

Conclusions

Testing for VDR gene polymorphism may help to identify women susceptibility to ovarian cancer so that they can be closely followed up for developing ovarian cancer. However more research on vitamin D and VDR VDR gene polymorphisms in different ethnic populations have to be done before drawing any conclusion.

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