TITLE

POTENTIAL ROLE OF VITAMIN D IN RESTORING SENSITIVITY TO mTOR INHIBITORS IN HEPATOCELLULAR CARCINOMA (HCC): 1,25(OH)VITAMIN D (VITD) REVERTS EVEROLIMUS (EVE) RESISTANCE IN A HCC CELL LINE.

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BACKGROUND AND AIM

HCC is a difficult-to-treat- cancer with poor prognosis. The recent EVOLVE-1 trial demonstrated that EVE did not improve overall survival in molecularly and clinically unselected patients with advanced HCC resistant to sorafenib treatment. In selected patients, the well-established antitumor effect of EVE could make this drug a potential adjuvant therapy. Unfortunately, the acquired resistance to this molecule due to the tumour adaptation to chronic drug use is a current challenge. VitD has been deemed as potential regimen to treat a variety of cancers alone or in combination with other drugs. The aim of this study was to assess the antiproliferative effect of the combined treatment with EVE and VitD in JHH-6, a model of HCC cell line, and to explore the role of VitD pre-treatment in the re-sensitization to EVE in JHH-6 cell line resistant to EVE (JHH-6 RR).

METHODS

Messenger and protein VitD receptor (VDR) expression was confirmed by RT-qPCR and immunofluorescence. To verify whether VitD can have additive effect in combination with EVE, JHH6 cell line was used. Moreover, to assess whether VitD can play a role in re-sensitization to EVE treatment, JHH-6 EVE resistant (RR) were obtained after 4 months of treatment with EVE 10^{-8}M. Cell proliferation was evaluated by measurement of total DNA content after 6 days of treatment with EVE 10^{-8}M in JHH6 and in JHH6 RR preceded by 12 hours of pre-treatment with VitD. To better understand molecular mechanisms involved in re-sensitization to EVE after pre-treatment with VitD, protein levels of c-myc, a proto oncogene that is often overexpressed in many types of malignancies and known to be regulated by VitD, has been investigated.

FIGURES

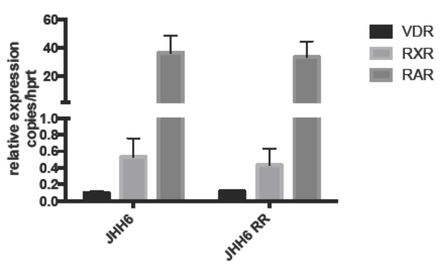

Figure 1: Expression levels of VDR, RAR, RXR in JHH6 and JHH6 RR

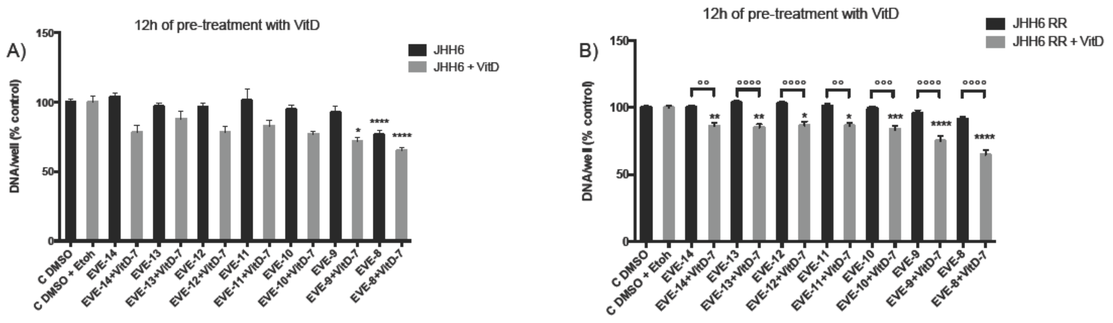

Figure 2: DNA assay in JHH6 (A) and JHH6 RR (B) after treatment with EVE with/without VitD. *p<0.05; **p<0.01; ***p<0,001; ****p<0,0001; °p<0,01; °°°p<0,001; °°°°p<0,0001

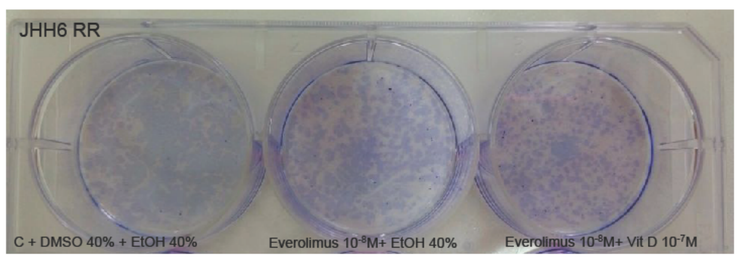

Figure 3: Proliferation of JHH6 RR after treatment with EVE with/without VitD.

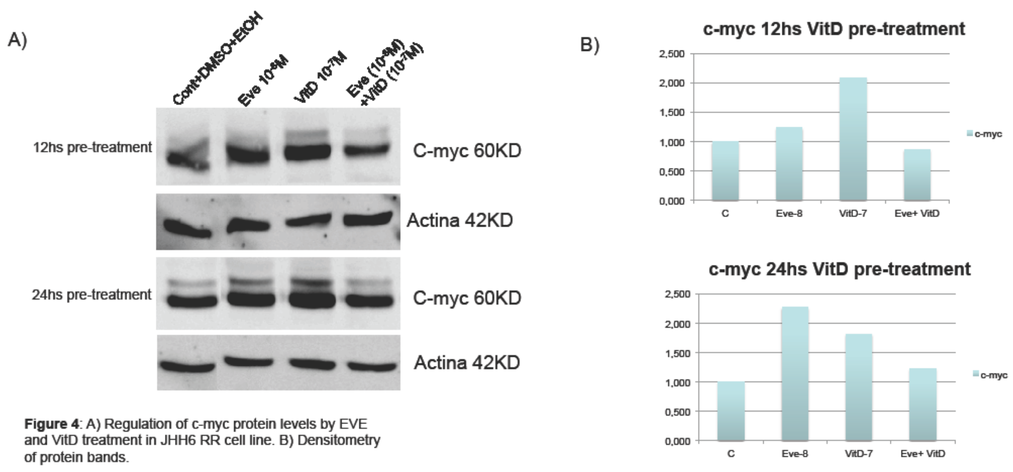

Figure 4: A) Regulation of c-myc protein levels by EVE and VitD treatment in JHH6 RR cell line. B) Densitometry of protein bands.

RESULTS

JHH6 and JHH6 RR cell lines express VDR, RXR and RAR at mRNA level (Figure 1), with no difference in the expression between JHH6 and resistant cells. In basal condition, EVE was able to significantly reduce the proliferation index in JHH6 in a dose-dependent manner after 6 days of treatment and in this setting VitD did not improve EVE effect (Figure 2A). JHH-6 RR cells no longer responded to EVE treatment in concentrations from 10^{-14}M to 10^{-8}M (Figure 2B, black bars). 12hs of VitD pre-treatment at 10^{-7}M was sufficient to significantly restore the efficacy of EVE at concentration ranging from 10^{-14}M to 10^{-8}M with a minimum effect of 14% of inhibition at 10^{-14}M and a maximum effect of 35,3% at 10^{-8}M (10^{-14}M and 10^{-13}M $p<0.01$ vs Control; 10^{-12}M and 10^{-11}M $p<0.05$ vs Control; 10^{-10} M,10^{-9}M and 10^{-8}M $p<0.001$ vs Control) as shown in Figure 3B (gray bars). In Figure 3, JHH6 RR cell growth is shown after treatment with EVE (10^{-8}M) with/without ViT D (10^{-7}M).

The analysis of c-myc protein level after drug treatment in JHH6 RR cell line revealed that after 12 and 24hs of VitD pretreatment, c-myc protein levels were reduced (Figure 4). Since some c-myc target genes are regulators of cell growth, it can be supposed that the reduced protein expression of c-myc is related to the VitD control growth and cellular proliferation.

CONCLUSIONS

VitD pre-treatment is able to revert the EVE resistance in JHH6 RR. The effect of VitD seems to be related to a decrease of c-myc protein expression. These preliminary data suggested the use of VitD to overcome the acquired resistance to EVE in HCC.

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