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The transcription factors Snail1 and Snail2 repress vitamin D receptor during colon cancer progression☆

María Jesús Larriba[a,*], Félix Bonilla[b], Alberto Muñoz[a]

[a] Instituto de Investigaciones Biomédicas "Alberto Sols", Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Arturo Duperier 4, 28029 Madrid, Spain
[b] Hospital Universitario Puerta de Hierro Majadahonda, Joaquín Rodrigo 2, 28222 Majadahonda, Spain

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ABSTRACT

Vitamin D receptor (VDR) mediates the antitumoral action of the active vitamin D metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). However, VDR expression is lost during colon cancer progression, possibly causing unresponsiveness to $1,25(OH)_2D_3$. Although several mechanisms responsible for resistance to $1,25(OH)_2D_3$ action in different types of cancer had been reported, none explained the loss of VDR expression. We have found that the transcription factors Snail1 and Snail2, known as inducers of epithelial-to-mesenchymal transition (EMT), inhibit VDR expression and block $1,25(OH)_2D_3$ action in colon cancer cells. Snail1 and Snail2 have an additive repressing effect on *VDR* gene promoter. These effects are specific to the Snail family, as other transcription factors that function as EMT inducers do not inhibit VDR expression in colon cancer cells. Moreover, we also found that the RNA expression of *SNAI1* and *SNAI2* is upregulated in human colorectal tumors and inversely correlates with that of *VDR*. Our results suggest that high levels of SNAIL1 and SNAIL2 are a probable cause of VDR downregulation and $1,25(OH)_2D_3$ unresponsiveness in colon cancer. In addition, they may contribute to the improvement of protocols for the clinical use of vitamin D compounds, as they indicate that advanced colon cancer patients overexpressing SNAIL1 and SNAIL2 are not suitable candidates for this therapy.

1. Introduction

1α,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and a number of less calcemic analogs are in clinical trials as anticancer agents against colon cancer and other neoplasias based on their antiproliferative, prodifferentiation, pro-apoptotic and antimetastatic activity in cultured cells and experimental animal models [1,2]. We have shown that $1,25(OH)_2D_3$ inhibits proliferation and promotes differentiation of human colon cancer cells *via* the induction of several genes such the invasion suppressor *CDH1*/E-cadherin and the candidate tumor suppressor *CST5*/cystatin D, and by the antagonism of the Wnt/β-catenin pathway that is aberrantly activated in most colon tumors [3–5].

Although preclinical studies are promising, initial clinical trials in colon cancer have shown acceptable toxicity but low activity of vitamin D compounds [1]. As it is usual in the development of new antitumoral drugs, patients enrolled in these trials had not responded to any other therapy and they were unselected in terms of putative responsiveness to $1,25(OH)_2D_3$. A better understanding of the physiology of the vitamin D system and the identification of the mechanisms responsible for resistance to $1,25(OH)_2D_3$ may help us to design future clinical trials more rationally.

2. Vitamin D receptor is downregulated during colon cancer progression

Most, if not all, $1,25(OH)_2D_3$ effects are mediated by the vitamin D receptor (VDR), a transcription factor of the nuclear receptor superfamily. Thus, cellular VDR expression is required for a clinical response to vitamin D compounds. VDR is expressed in normal colon epithelial cells and also in some colon cancer cells. Remarkably, elevated VDR expression is associated with high differentiation, absence of node involvement and favourable prognosis in colorectal cancer [6,7]. However, VDR expression is downregulated during colon cancer progression [8–10] probably causing $1,25(OH)_2D_3$ unresponsiveness.

Although VDR downregulation in colon cancer was first described more than a decade ago, its molecular basis had remained elusive. Deletions, rearrangements or point mutations affecting the coding region of the *VDR* gene had not been found in cancer. Neither

Abbreviations: EMT, epithelial-to-mesenchymal transition; VDR, vitamin D receptor; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3.

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* Corresponding author. Tel.: +34 91 5854452; fax: +34 91 5854401.
E-mail address: mjlarriba@iib.uam.es (M.J. Larriba).

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had epigenetic silencing nor transcriptional repression mechanisms been described. Several polymorphisms had been described in the *VDR* gene, some of which have been associated with increased risk of breast, prostate and colon cancer. However, their consequences for VDR expression or functionality, and therefore their implication in the development of $1,25(OH)_2D_3$ resistance remain to be established [11,12].

3. The transcription factors Snail1 and Snail2 repress VDR expression in colon cancer cells

A novel mechanism responsible for VDR downregulation in colon cancer has emerged in recent years. Our group has revealed that the transcription factors Snail1 and Snail2 (also known as Snail and Slug) encoded by *Snai1* and *Snai2* genes, respectively, bind to the promoter region of exon 1a of human *VDR* gene and repress its expression. The repressive effects of Snail1 and Snail2 on *VDR* gene promoter are quantitatively similar and are mediated by three E-boxes (CAGGTG/CACCTG, reported binding sites for Snail factors) present in the first 400 nucleotides of human *VDR* promoter. In addition, we found that both transcription factors cooperate to repress *VDR* promoter, showing an additive effect [13,14].

We also found that the overexpression (by means of retrovirus-mediated gene transfer) of Snail1 or Snail2 in human colon cancer cells decreases VDR RNA and protein expression and strongly inhibits the regulation of $1,25(OH)_2D_3$ target genes such as *CDH1*/E-cadherin, $p21^{CIP1}$ and *CYP24A1*/$1,25(OH)_2D_3$-24-hydroxylase. Accordingly, Snail transcription factors block the epithelial differentiation induced by $1,25(OH)_2D_3$ in colon cancer cells (Fig. 1) and also the inhibitory effect of $1,25(OH)_2D_3$ on Wnt/β-catenin signaling pathway [13–15].

4. Relevance of VDR downregulation for the epithelial-to-mesenchymal transition induced by Snail1 and Snail2

Snail1 and Snail2 belong to the Snail family of zinc-finger transcription factors and modulate processes that imply cell movement during embryonic development and tumor progression. Cellular overexpression of Snail1 or Snail2 induces the epithelial-to-mesenchymal transition (EMT), which entails the loss of epithelial characteristics and the acquisition of a mesenchymal fibroblastic phenotype [16,17]. Therefore, the expression of Snail1 or Snail2 in carcinoma cells promotes their migratory and invasive properties favouring tumor invasion and metastasis. Accordingly, the aberrant overexpression of SNAIL1 and/or SNAIL2 has been observed in different types of carcinomas and is frequently associated with invasiveness, metastasis and poor prognosis [16,17].

EMT induction is not an exclusive effect of Snail factors. Other transcriptional regulators such as E47, Twist1 and E2-2 (members of the basic-helix–loop–helix family), and Zeb1 and Zeb2 (of the Zeb family) also promote this process. EMT is mainly the result of transcriptional changes induced by these factors such as the repression of *CDH1*/E-cadherin, *OCLN*/occludin, several claudins and other epithelial genes and the induction of mesenchymal genes such as *FN1*/fibronectin, *VIM*/vimentin, *LEF1* and numerous matrix metalloproteases [16,17].

As ligand-activated VDR induces epithelial differentiation and the expression of *CDH1*/E-cadherin and other intercellular adhesion genes, VDR repression by Snail1 and Snail2 guarantees the induction of EMT even in the presence of $1,25(OH)_2D_3$. This effect seems to be specific to the Snail family of transcription factors, since other EMT inducers such as Zeb1, Zeb2, E47 and Twist1 do not inhibit human *VDR* gene promoter [14].

5. Snail1 and Snail2 are responsible for VDR downregulation in colon cancer

To confirm the importance of Snail transcription factors in VDR downregulation during colon cancer progression, we analyzed *SNAI1*, *SNAI2* and *VDR* RNA expression in normal and tumoral biopsies from approximately one hundred colon cancer patients. We found that *SNAI1* RNA was not present in any normal colonic tissue but it was expressed in 60–70% (depending on the study) of colon tumors. *VDR* expression in tumors was reduced with respect to that detected in the corresponding normal tissue in a similar percentage of tumors. In contrast, basal *SNAI2* RNA expression was detected in most normal tissues and was also upregulated in 60% of colon tumors. Importantly, we observed that the overexpression of either *SNAI1* or *SNAI2* in individual tumors correlates with *VDR* downregulation [13,14,18,19]. In addition, we found that *SNAI1* and *SNAI2* RNA expression correlate directly and, therefore, a high percentage of tumors (42%) express both transcription factors. Remarkably, *VDR* downregulation was stronger in the tumors that express both *SNAI1* and *SNAI2* than in those that express only one of these genes. This is consistent with the additive effect exerted by Snail1 and Snail2 on *VDR* gene promoter in cultured human colon cancer cells [14]. Therefore, it seems that Snail1 and Snail2 collaborate in the repression of *VDR* gene in human colon cancer.

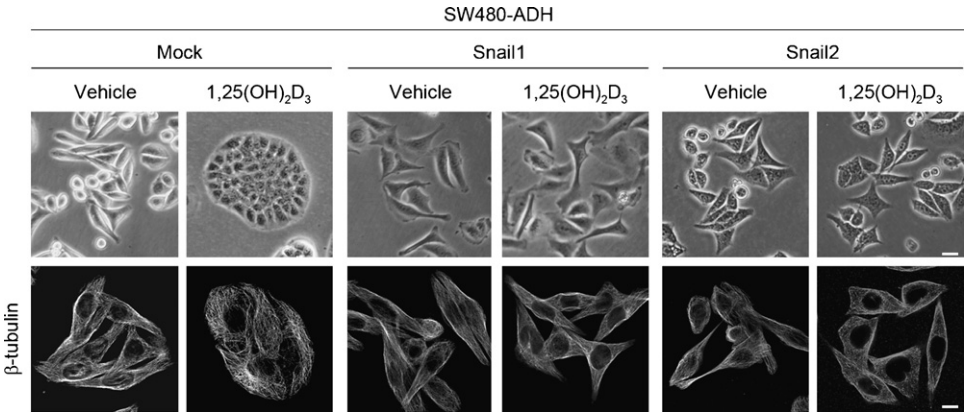

Fig. 1. The overexpression of Snail1 or Snail2 blocks the acquisition of the epithelial differentiated phenotype induced by $1,25(OH)_2D_3$ in SW480-ADH human colon cancer cells. Representative phase-contrast images (upper panels) and confocal laser immunofluorescence images showing β-tubulin staining (lower panels) of Mock, Snail1- and Snail2-overexpressing SW480-ADH cells treated with 100 nM $1,25(OH)_2D_3$ or vehicle for 48 h. Scale bar, 25 μm (upper panels) and 10 μm (lower panels).