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Review

Epigenetic Corruption of VDR Signalling in Malignancy

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Abstract. *Background: The ligand-mediated switch from binding co-repressor to co-activator complexes is central to the transcriptional actions of the vitamin D receptor (VDR) and other nuclear receptors. The capacity of deregulated co-repressors to attenuate the responsiveness of VDR signalling in cancer models was examined. Materials and Methods: Proliferation and gene regulation studies were undertaken in non-malignant and malignant cell line and primary models. Results: Both primary tissue models and cancer cell lines displayed a spectrum of suppressed responsiveness towards 1α, 25 hydroxy vitamin D_3 ($1\alpha25(OH)_2D_3$) which correlated with elevated co-repressor content: specifically, elevated silencing mediator of retinoid and thyroid hormone receptors/nuclear co-repressor 2 (NCoR2/SMRT) in prostate cancer cell lines and primary tumour cultures, and elevated nuclear receptor co-repressor 1 (NCoR1) in breast cancer cell lines. Interestingly, whilst the cancer cell lines frequently also displayed reduced VDR content, the primary tumour material retained and/or elevated VDR mRNA, correlated with co-repressor content. Functional approaches towards NCoR2/SMRT (siRNA) in prostate cancer cells or NCoR1 (overexpression) in non-malignant breast epithelial cells confirmed a role in suppressing VDR transcriptional and cellular actions. Targeted co-treatments of $1\alpha25(OH)_2D_3$ plus HDAC inhibitors (TSA, NaB) resulted in re-expression of antiproliferative target genes (e.g., GADD45α, $p21^{(waf1/cip1)}$) and synergistic inhibition of proliferation. Conclusion: These data suggest that VDR actions in solid tumours are retained, but were skewed by epigenetic mechanisms to suppress selectively antiproliferative target gene promoter responses. This molecular lesion provides a novel chemotherapy target for acceptable doses of $1\alpha25(OH)_2D_3$ plus HDAC inhibitors.*

Abbreviations: AR, andregen receptor; ER, estrogen receptor; ERR, estrogen-related receptor; FXR, farnesoid X receptor; HDAC, histone deacetylase; LCA, lithocholic acid; LXR, liver X receptor; NaB, sodium butyrate; NCoR1, nuclear receptor co-repressor 1; NCoR2/SMRT, silencing mediator of retinoid and thyroid hormone receptors/nuclear receptor co-repressor 2; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; RE, response element; RXR, retinoid X receptor; TSA, trichostatin A; VDR, vitamin D receptor; $1\alpha,25(OH)_2D_3$, 1α,25dihydroxyvitaminD_3.

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Breast, Prostate and Colon are Self-renewing Tissues which Give Rise to Common Cancers

The underlying causes for these three high profile cancers are still not clearly understood. Only the minority of cases are strongly associated with a positive family history. For example, about 5% of all breast cancer cases have been linked to high penetrant genetic mutations at the *BRCA1* and *BRCA2* gene loci. Similarly the *APC* gene, whose product is a key regulator of the Wnt/β-catenin pathway, is frequently mutated in blood relatives with aggressive colon cancer (55). Historically, this exclusive genetic causality has provided a paradigm for investigating the aetiology of cancer, although other strong penetrance genes have not been easily identified.

An alternative contemporary view of these and other common cancers is that they include a contribution from an ill-defined combination of genetic factors with weak penetrance, acting in response to a multitude of environmental factors (59). Statistically, the single greatest risk factor for most cancers is age, with the average age of onset of breast, prostate and colon cancer being in the sixth

and seventh decades of life. The sporadic, temporal acquisition of a cancer phenotype is compatible with multi-factorial models which require disruption of mechanisms of cell restraint and tissue organisation (42).

Prostate and mammary glands and the lining of the gastro-intestinal tract, all typify self-renewing tissues containing stem cell populations, which give rise to committed progenitors and, in turn, the multiple cell lineages required for tissue function (23, 27, 85, 87). Stem cells are relatively rare and frequently quiescent, but long-lived. Furthermore, they are uniquely able to undergo asymmetric division, to give rise to both other stem cells and transiently amplify populations of progenitor cells, which in turn give rise to the differentiated cell types. By contrast, differentiated epithelial cells are functional, but short-lived, and are lost through programmed cell death processes, to be replaced by newly differentiated transiently amplifying cells. Control of the intricate balance of the processes of division, differentiation and programmed cell death in these different cell types include common roles for Wnt, Hedgehog and other developmental signal transduction processes (10, 85). Convergent targets for these signals include key regulators of cell proliferation such as the cyclin-dependent kinase inhibitor $p21^{(waf1/cip1)}$.

As a result of their long life cycle and high proliferative capacity, stem cells, rather than the differentiated cells, are the candidates for tumorigenesis (86). To counter this, there appear to be a range of mechanisms in place in stem cells to maintain genomic integrity (reviewed in (90)). These controls notwithstanding, the transformation of stem cells has given rise to the concept of cancer stem cells. Accumulating evidence supports the presence of these cells in prostate, breast and colon cancers (4, 20, 28, 88).

Emerging Roles for Diet Impacting on Malignancy

Recently, the appreciation of the impact of diet on either the initiation or progression of cancer has come significantly to the fore (reviewed in (6, 44, 65)). Reflecting on the accumulation of these data, the World Health Organisation has now stated that diet forms the second most preventable cause of cancer (after smoking). This impact will increase due to demographic factors and, quite possibly, due to changing dietary habits worldwide.

Aspects of these relationships are found in breast, prostate and colon cancer, where the aetiology of the disease reflects the cumulative impact of dietary factors over an individual's life time (12). Equally, these relationships have the potential to be exploited clinically, for example in the SELECT trial to assess the chemopreventive potentials of vitamin E and selenium in prostate cancer (78).

Despite the significance and potential clinical benefits of these relationships the critical time frame remains unclear, when dietary factors may be protective against cancer development, for example during embryogenesis, childhood development or adult life. Resolving this is, understandably, highly challenging. Considerable resources were required to elucidate what is now established as a clear causal relationship between cigarette smoke and lung cancer (26). To address this, the emerging field of nutrigenomics aims to dissect the impact of dietary factors on genomic regulation, and, thereby, physiology and pathophysiology utilising a range of post-genomic technologies (73).

The Vitamin D Receptor and Other Nuclear Receptors Allow a Local, Integrated Response to Lipophilic Nutrients

The nuclear receptors form one of the largest human families of transcription factors and frequently bind, with a range of affinities, to lipid-derived hormonal, dietary and environmental factors to regulate gene targets, and can be classified broadly according to ligand affinities. The first group of receptors bind ligands with high affinity and are typified by the sex steroid hormone receptors, *e.g.,* the estrogen receptors (ERα and β). Equally a number of micronutrient ligands are also bound with high affinity by specific receptors. For example, the active metabolites of vitamins A and D are bound, respectively, by the retinoic acid and retinoid X receptors (RARs and RXRs) (15) and vitamin D receptor (VDR) (91). The second group of receptors, the adopted orphan receptors (71), bind with broader affinity more abundant macronutrients such as polyunsaturated fatty acids and bile acids, for example the peroxisome proliferator activated receptors (PPARs) (72, 92), liver X receptors (LXRs (31)) and farnesoid X receptor (FXR). Finally, a group of orphan receptors exists, for which no ligands have been identified. Phylogenetic classification, by contrast, has defined seven subfamilies and, within these, the VDR is in the group I sub-family, sharing homology with the LXRs and FXR and more distantly with the PPARs.

Both high and broad affinity receptors appear to work in concert. For example, in the case of the group I receptors, originally the VDR had been described for its central endocrine role in the maintenance of serum calcium levels. Similarly, the FXR and LXRs were described for their roles in regulating cholesterol and fatty and bile acids metabolism in the enterohepatic system. However, these and the related RXRs can equally mediate the local response to fatty and bile acids (39). Thus, the VDR can also respond to the secondary bile acid lithocholic acid (LCA), to induce the cytochrome P450, CYP3A4 (1, 67). Thus, the finding that oxysterols, bile acids, and some polyunsaturated fatty acids can bind and collectively activate LXRs, FXR, PPARs, pregnane X receptor (PXR), RXR and VDR providing a strong molecular link between tissue homeostasis and the

type and availability of nutrients, at least in the colon. The protection provided by PXR, FXR and VDR activation may become compromised when the detoxification pathway is overwhelmed (*e.g.*, by increased levels of LCA due to sustained high-fat diets), or where other nuclear receptor ligands are deficient, for example reduced signalling *via* the VDR in cases of vitamin D deficiency and where diets have reduced levels of dietary-derived fatty acids which are known to be ligands for the PPARs. Equally the ERs and the estrogen-related receptors (ERRs) respond to estrogenic hormones and a range of phytoestrogens and flavenoids, with different affinities, and collectively play a role in the maintanance of the mammary gland (9, 11, 17, 36).

Examination of VDR, RARs, PPARs, FXR and LXR signalling reveals that they have in common target genes (5) that regulate the cell cycle (*e.g.,* $p21^{(waf1/cip1)}$ (18, 50, 66, 93) and xenobiotic clearance *via* cytochrome P450s (*e.g.*, CYP3A4 (30, 38, 46, 56)). Furthermore, there appears to be co-regulation of the receptors. Thus, FXR has been shown to induce a range of target genes that may play other roles in controlling cell proliferation, such as the related nuclear receptor, PPARα, thus providing molecular evidence for a local, paracrine, cross-talk between the bile acid-sensing receptor and cell proliferation (80). A similar co-regulatory relationship exists between the VDR and PPAR (29).

The post-genomic description of the nuclear receptor superfamily conjoined with profiling approaches (61) reveals that not only colon epithelial cell, but also breast myoepithelial and prostate epithelial cells express a rich cohort of nuclear receptors, many of which display overt nutrient sensing capacity for micro- and macro-nutrients (2, 25, 49, 62). The expression of these receptors in tissues, such as prostate and breast (Table I), suggests a broader and integrated role as a network or conduit in the local sensing of dietary lipid molecules, providing a functional link between both hormonal and environmental and dietary cues, and tissue homeostasis (30).

Table I. *Relative expression of dietary sensing nuclear receptors in normal breast, prostate and colon epithelium and examples of dietary ligands. The relative abundance was determined in silico from publicly deposited SAGE data(61) and the gradation of colour indicates the relative mRNA abundance. The darker shades indicate higher expression.*

Nuclear receptors	Relative abundance			Dietary-derived ligand
High affinity	Breast	Prostate	Colon	
ERα				Phytoestrogens, *e.g.,* genistein and flavonoids,
ERβ				
ERRα				
ERRβ				
RARα				All *trans* retinoic acid
RARβ				
RARγ				
RXRα				9-*cis* retinoic acid, Docosahexanoic acid
RXRβ				
RXRγ				
VDR				$1\alpha25(OH)_2D_3$ and LCA
Broad affinity				
PPARα				Eicosapentaenoic acid
PPARδ,				Linoleic acid
PPARγ				5,8,11,14-eicosatetraenoic acid
LXRα				24(S) hydroxycholesterol and cholesterol derivatives
LXRβ				Oxysterols
FXR				Chenodeoxycholic acid and other bile acids

Local Remodelling of Chromatin is Central to Nuclear Receptor Transcriptional Functions

The nuclear receptors share a common architecture, which includes defined regions for DNA recognition, ligand binding and co-factor interactions. The DNA-binding domain recognises specific response elements (RE) in target gene enhancer/promoter regions. Most receptors preferentially form homo- or heterodimeric complexes; RXR is a central partner for VDR, PPARs, LXRs and FXR. Therefore, simple REs are formed by two recognition motives and their relative distance and orientation contribute to receptor binding specificity and, more recently, composite elements have been identified.

In the absence of ligand, the VDR-RXR dimer exists in an '*apo*' state, as part of large complexes (~2.0 MDa) (63, 101), associated with co-repressors (*e.g.*, NCoR2/SMRT) and bound to RE sequences. These complexes actively recruit a range of enzymes that post-translationally modify histone tails, for example histone deacetylases (HDACs) and methyltransferase and, thereby, maintain a locally closed chromatin structure, around RE sequences (75). Ligand binding induces a so-called *holo* state, facilitating the association of the VDR-RXR dimer with co-activator complexes. A large number of interacting co-activator proteins have been described, which can be divided into multiple families including the p160 family, the non-p160 members and members of the large 'bridging' DRIP/TRAP/ARC complex, which links the receptor complex to the co-integrators CBP/p300 and basal transcriptional machinery (68, 84, 96). These receptor co-activator complexes co-ordinate the activation of an antagonistic battery of enzymes such as histone acetyltransferases and, thereby, induce the reorganization of local chromatin regions at the response element (RE) of the target gene promoter. The

complex choreography of this event has recently emerged and involves cyclical rounds of promoter-specific complex assembly, gene transactivation, complex disassembly and proteosome-mediated receptor degradation (52, 84).

The expression, localisation and isoforms of co-repressor complexes have emerged as being critical to determine the spatio-temporal equilibrium between the antagonistic actions of the *apo* and *holo* nuclear receptor complexes and, thus, determine target gene promoter responsiveness in a range of physiological and pathological settings. For example, in regulating nuclear receptor function during neural cell differentiation, in determining cell-specific responses to steroid hormones and in the inappropriate silencing of nuclear receptor actions associated with neoplasia (45, 58, 89).

It remains unclear to what extent the various histone modifications initiated by the *apo* and *holo* nuclear receptor mega-complexes around gene promoter regions influence the subsequent transcriptional responsiveness of the promoter. It has been proposed that these modifications may form a stable and heritable "histone code", that determines the assembly of factors upon the chromatin template and controls individual promoter transcriptional responsiveness (51, 95). Functional studies of the SANT motif contained in the co-repressor NCoR2/SMRT supports this latter idea (43), thus recognising and sustaining specific histone modifications.

Cellular Resistance to the Actions of the VDR

The role for VDR actions in non-calcaemic tissues has been the subject of intensive investigation and a consistent theme that emerges is the regulation of target genes, which subsequently control cell growth, differentiation and programmed cell death. *In vitro* $1\alpha25(OH)_2D_3$ is able to regulate the proliferation of a wide range of normal tissues including epithelial cells from the prostate, breast and colon and myeloid CD34-positive precursors (Table II). Complimentary studies using the background of the *vdr*-deficient mice has revealed an important role for this receptor to influence mammary gland morphology by regulating differentiation (105) and integrate with estrogenic programs. Importantly, the *vdr* ablated backgrounds have now been crossed into a range of tumourogenic models and support anti-tumour chemoprevention roles for VDR (106, 107).

A major limitation in the therapeutic exploitation of these anti-proliferative and pro-differentiative signals of $1\alpha25(OH)_2D_3$ is the resistance of cancer cells towards $1\alpha25(OH)_2D_3$; cancer and leukemic cell lines often display a spectrum of sensitivities including complete insensitivity to $1\alpha25(OH)_2D_3$, irrespective of VDR expression (Table II) (69, 104). The molecular mechanisms for $1\alpha25(OH)_2D_3$-insensitivity in cancer are emerging. We and others have demonstrated that the VDR is neither mutated, nor is there a clear relationship between VDR expression and growth inhibition by $1\alpha25(OH)_2D_3$ (13, 14, 58). The lack of an anti-proliferative response is reflected by a suppression of the transcriptional responsiveness of anti-proliferative target genes such as *p21(waf/cipi1)*, *p27(kip1)*, *GADD45α* and *BRCA1* (24, 85, 103). It is interesting to note that the levels of *p21(waf/cipi1)* and *p27(kip1)* mRNA expression play roles in the terminal differentiation of committed progenitor cells and thus $1\alpha25(OH)_2D_3$ may play an integrated role in regulating self-renewal.

Paradoxically, VDR transactivation is sustained or even enhanced, as measured by induction of the highly $1\alpha25(OH)_2D_3$-inducible *CYP24* gene (70, 83). Together, these data suggest that the VDR transcriptome is skewed in cancer cells to disfavour antiproliferative target genes, and that a lack of functional VDR alone cannot explain resistance.

Table II. *Cellular responsiveness towards 1α25(OH) and HDAC inhibitors.*

Cell line model	ED_{50} (nM)[1]	Co-operative anti-proliferative effects with HDACi[2]	Co-operative gene regulatory effects with HDACi[3]
Myeloid (74)			
U937	4	nd	nd
HL-60	80	++ (NaB)(103)	*VDR* (NaB)
KG-1a	>1000	nd	nd
Breast epithelial (14)			
MCF-12A	20	–/+ (NaB, TSA)	nd
T-47D	15	+ (NaB, TSA)	nd
MCF-7	100	+ (NaB, TSA)	nd
MDA-MB-231	>1000	++ (NaB, TSA)	*p21(waf1/cip1)* (TSA)
Prostate epithelial (58)			
RWPE-1	10	nd	nd
PrEC	15	–/+ (NaB, TSA)	nd
LNCaP	100	+ (NaB, TSA)	nd
PC-3	450	++ (NaB, TSA)	*GADD45α, MAPK-APK2 (TSA)*
DU 145	>1000	++ (NaB, TSA)	nd
Colon cancer CaCo2	100	++ (NaB)	*p21(waf1/cip1), VDR* (NaB) (34)

[1]ED_{50} = the estimated dose required to inhibit cell proliferation by 50%.
[2]Co-operative antiproliferative interactions with HDAC inhibitors, either NaB or TSA as indicated).
+/– = moderate enhancement of effects only observed at low nM doses of $1\alpha25(OH)_2D_3$.
+ = moderate enhancement of effects observed across a dose response range of $1\alpha25(OH)_2D_3$.
++ = strong or synergistic enhancement of effects observed across a dose response of $1\alpha25(OH)_2D_3$.
[3]Gene targets identified to be co-ordinately regulated by co-treatment of HDAC with $1\alpha25(OH)_2D_3$.
nd = not determined.

Epigenetic Resistance

The gene encoding the VDR protein is known to display a number of polymorphic variations, which are associated variously with cancer incidence, degree of aggressiveness and metastasis (reviewed in (41, 47)). To date, no cytogenetic abnormalities of the *VDR* have been reported.

By contrast we and others have begun to explore epigenetic mechanisms which disrupt VDR signalling. For example, we have proposed that apparent $1\alpha25(OH)_2D_3$ insensitivity is not determined solely by a linear relationship between the levels of $1\alpha25(OH)_2D_3$ and the VDR, but rather epigenetic events skew the responsiveness of target gene promoters. We have investigated these possibilities in breast and prostate cancer.

Prostate cancer: We found frequently elevated co-repressor mRNA expression, most commonly involving *NCoR2*/*SMRT*, in malignant primary cultures and cell lines, with reduced the $1\alpha25(OH)_2D_3$ antiproliferative response, but not normal or benign disease, indicating that the ratio of VDR to co-repressor may determine $1\alpha25(OH)_2D_3$ responsiveness in cancer cells. We explored the significance of elevated co-repressor levels in both cancer cell lines and primary cultures, and reasoned that this lesion could be targeted by co-treatment of ligand ($1\alpha25(OH)_2D_3$) plus the HDAC inhibitor trichostatin A (TSA). Supportively, we demonstrated that the $1\alpha25(OH)_2D_3$-response of the androgen-independent PC-3 cells was restored to levels indistinguishable from control normal prostate epithelial cells, by co-treatment with low doses of TSA (58, 82).

This reversal of $1\alpha25(OH)_2D_3$ insensitivity provided the opportunity to examine gene expression patterns. Microarray studies demonstrated that $1\alpha25(OH)_2D_3$ plus TSA uniquely up-regulated a group of 'repressed' gene targets associated with the control of proliferation and induction of apoptosis, notably *GADD45α* and *MAPK-APK2*, a mediator of the p38 stress response pathway (58, 82).

A siRNA approach towards *NCoR2*/*SMRT* demonstrated the significant role this co-repressor plays in regulating this response, with its repression resulting in profound enhancement of the induction of *GADD45α* in response to $1\alpha25(OH)_2D_3$ (98). These data support a central role for elevated *NCoR2*/*SMRT* levels in suppressing the induction of key target genes resulting in loss of sensitivity to the antiproliferative action of $1\alpha25(OH)_2D_3$.

Roles for both GADD45α and MAPK-APK2 have been demonstrated in cell lines that retain sensitivity to $1\alpha,25(OH)_2D_3$ signalling. For example, p38/MAPK-APK2 activation regulating $1\alpha25(OH)_2D_3$-induced HL-60 myeloid differentiation and up-regulation of *GADD45α* is a functional part of the antiproliferative action of EB1089 [an analogue of $1\alpha,25(OH)_2D_3$] in SCC25 squamous carcinoma cells (3) and $1\alpha25(OH)_2D_3$ in ovarian cancer cell lines (53). These studies and our own re-expression data highlight these targets as key mediators in the anti-proliferative actions of $1\alpha25(OH)_2D_3$.

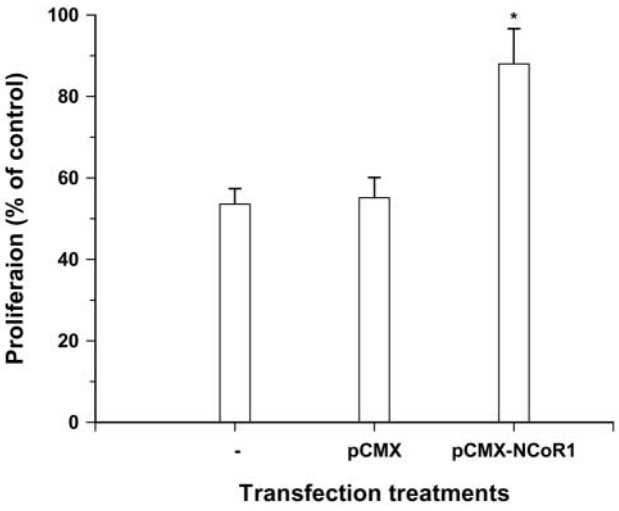

Figure 1. *Transient transfection of NCoR1 in MCF-12A cells. MCF-12A cells were plated in 96-well white-walled tissue culture-treated plates at either 4x10³ cells/well, and allowed to adhere overnight. Prior to transfection, the cells were exposed to a short trypsin exposure to enhance transfection efficiency. Briefly, immediately prior to transfection the cells were exposed to 40 µl trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA)/well for 60 seconds and then the reaction was stopped by the addition of FCS (200 µl/well), aspirated off and then washed with PBS (200 µl/well) prior to proceeding with plasmid transfection. The cells were subsequently transfected with either pCMX-NCoR1 or pCMX (empty vector control). Plasmids (200 ng/well) were mixed in a 8:1 ratio with Lipofectamine and incubated with the cells for 18 hours in a final volume of 30 µl/well final. Subsequently, the transfection mix was removed and replaced with normal MCF-12A media and the cells were allowed to recover for a subsequent 6 hour prior to dosage with $1\alpha25(OH)_2D_3$ or left untreated (control). Liquid proliferation, as measured by the changes in cellular ATP as described previously, was measured after a further 96 hours with a redose after 48 hours. ATP levels were recorded in relative luciferase units and inhibition of proliferation was expressed as a percentage of control. All experiments were repeated in triplicate wells in three separate experiments. *p<0.05.*

Breast cancer: In parallel studies, we have demonstrated a similar spectrum of reduced $1\alpha25(OH)_2D_3$-responsiveness between non-malignant breast epithelial cells and cancer cell lines. Again, this was not determined solely by a linear relationship between the levels of $1\alpha25(OH)_2D_3$ and *VDR* mRNA expression Rather elevated co-repressors mRNA levels, notably *NCoR1*, in breast cancer cell lines was common and determined sensitivity towards $1\alpha25(OH)_2D_3$ (7, 8). Transient overexpression of *NCoR1* was performed in MCF-12A cells, which display an acute response to $1\alpha25(OH)_2D_3$, to assess whether this co-repressor influences cell responsiveness to $1\alpha25(OH)_2D_3$. Transfection efficiency was routinely approximately 40% (data not shown). In untreated and mock-transfected controls the antiproliferative

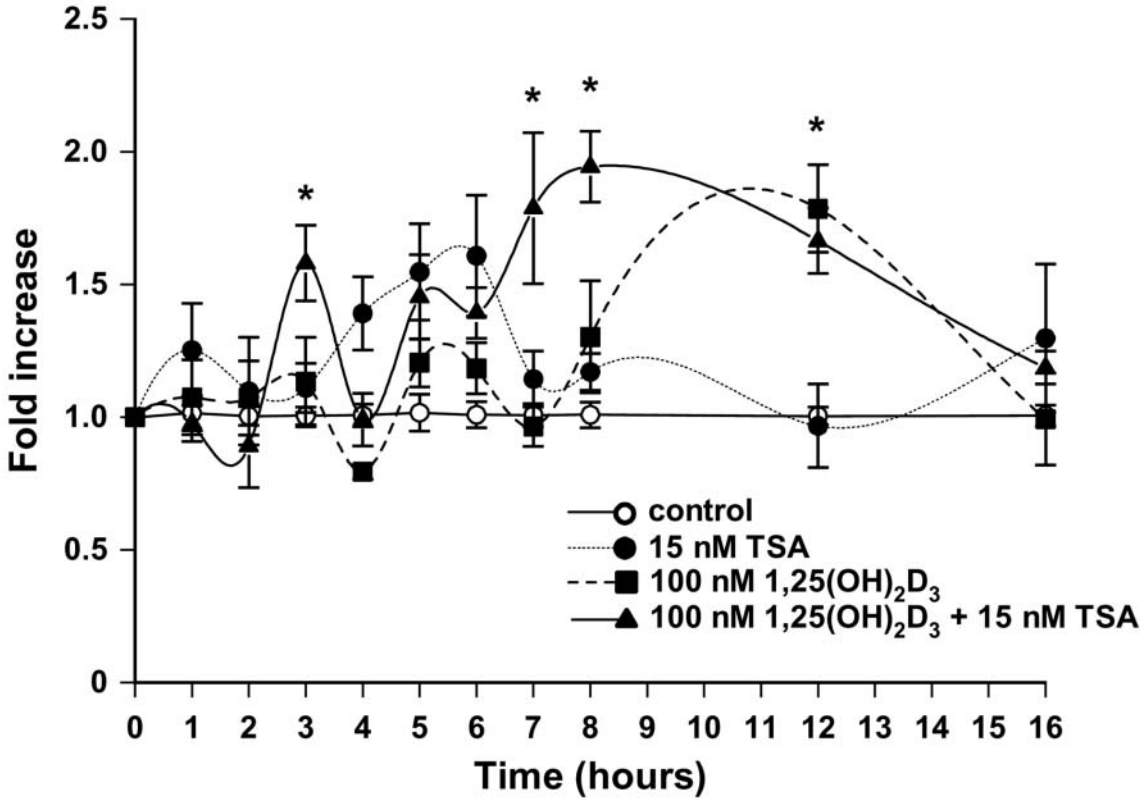

Figure 2. *Regulation of $p21^{(waf1/cip1)}$ in MDA-MB-231 cells treated with $1\alpha25(OH)_2D_3$ plus TSA. $2x10^4/cm^2$ cells were plated into 6-well plates and allowed to grow for 36 hours to ensure that the cells were in the mid-exponential phase. Total RNA was isolated after the indicated time-periods, reverse transcribed and the target genes amplified and the fold increase calculated using quantitative reverse transcription PCR, as described previously. Each data-point represents the mean of three separate experiments amplified in triplicate wells±S.E.M. *$p<0.05$.*

sensitivity in liquid culture was equal [52%±5 and 54%±4 of control proliferation in response to $1\alpha25(OH)_2D_3$ (200 nM), respectively]. In contrast, *NCoR1*-transfected cultures displayed increased resistance to 200 nM $1\alpha25(OH)_2D_3$, having 80%±9.4 proliferation compared to untreated controls ($p<0.01$ compared to both mock and non-transfected controls) (Figure 1).

We have attempted to target this molecular lesion through co-treatments of $1\alpha25(OH)_2D_3$ with HDAC inhibitors. These data also support the concept that elevated co-repressors such as NCoR1 reduce the capacity of the VDR to act as a transcription factor for antiproliferative target genes. Thus, treatment of MDA-MB-231 cells with $1\alpha25(OH)_2D_3$ plus TSA appears to co-ordinately regulate the $p21^{(waf/cipi1)}$ mRNA expression; notably up-regulating the target in a unique manner between 5 and 10 hours post-treatment (Figure 2).

These findings compliment a number of parallel studies undertaken by others, which have established cooperativity between $1\alpha25(OH)_2D_3$ and butyrate compounds, such as sodium butyrate (NaB) (21, 22, 32, 33, 35, 76, 94) (Table II). These compounds are short-chain fatty acids produced during fermentation by endogenous intestinal bacteria and have the capacity to act as HDAC inhibitors (16). Stein and co-workers have also identified the effects in colon cancer cells of $1\alpha25(OH)_2D_3$ plus NaB co-treatments to include the co-ordinate regulation of the VDR itself. In our studies, in the time-frame studied (0-24 hours), no evidence for changes in VDR mRNA levels upon co-treatment with $1\alpha25(OH)_2D_3$ plus TSA were seen. However, together these studies further underscore the importance of the dietary-derived milieu to regulate epithelial proliferation and differentiation beyond classic sites of action in the gut.

Summary

These findings suggest that in cancer the VDR is not overtly disrupted by either genetic mechanisms, such as mutation, or appears to be the subject of cytogenetic re-arrangements. Rather, epigenetic mechanisms attenuate and selectively skew the transcriptional responsiveness. The combination of either VDR or other nuclear receptor ligands with potent HDAC inhibitors has the potential to deliver a more focused and sustained 'anticancer' regime for a range of solid tumours.

Thus, we propose that deregulated, possibly tissue-specific, patterns of co-repressors inappropriately sustain histone deacetylation around the VDRE or target gene promoter regions, and shifts the dynamic equilibrium between *apo* and *holo* receptor conformations to favour transcriptional repression of key target genes such as $p21^{(waf1/cip1)}$ or *GADD45α*. Thus, VDR gene targets are less responsive in $1\alpha25(OH)_2D_3$-insensitive cancer cells compared to non-malignant counterparts. Furthermore, targeting this molecular lesion with co-treatments of vitamin D_3 compounds plus HDAC inhibitors generates a temporal window where the equilibrium point between *apo* and *holo* complexes is shifted to favour a more transcriptionally permissive environment and allows gene targets to be modulated in a unique and significantly greater manner than either agent alone; notably $p21^{(waf1/cip1)}$ and *GADD45α*. The *apo*-VDR complex may, in turn, form a template for subsequent more stable epigenetic silencing of these regions. Equally, the silencing of nuclear transcriptional actions may allow cytoplasmic VDR actions to be sustained, some of which have recently been shown to suppress apoptosis *via* non-transcriptional interactions (97). These studies add to a growing body of data which places the expression of the CoA/CoR mileu as being critical to determine nuclear receptor actions (19, 37, 40, 45, 48, 54, 57, 60, 64, 79, 81, 100).

Epigentic mechanisms which disrupt VDR signalling most probably disrupt other receptors resulting in reduced sensitivity to a wide range of dietary-derived macro- and micro-nutrient ligands. It is interesting to note that the stem cells in prostate and breast tissues do not appear to express either the androgen receptor (AR) or ERα, respectively. It remains unknown to what extent these cells express the VDR or its related receptors and, if so, what their function is. Addressing these questions will therefore address what role deregulated co-repressor function may play in cancer initiation.

Future Perspectives

Historically, researchers have studied the abilities of single nuclear receptors to regulate a discrete group of gene targets and influence cell function. This has led to substantial knowledge concerning many of these receptors, individually. Cell and organism function, however, depends on the dynamic interaction of a collection of receptors, through the networks that link them. The current lack of an integral view of how these interactions bring about function and dysfunction of the aging human individual can be attributed to the relatively recent limitations of available techniques and tools to undertake such studies. The implementation of the new post-genomic techniques, together with bioinformatics and systems biology methodology, will generate an integral view of the processes by which cells, tissues and organisms interact with diet (99). This transition will allow VDR processes to be described in the dynamic interaction with other nuclear receptors and cell signal transduction pathways to identify critical modes of control. An equally important component will be to define how these receptors are expressed and regulated during epithelial tissue self-renewal.

A common goal of many researchers around the world is to understand how dietary intervention, based on the knowledge of nutritional requirements, nutritional status and genotype, can be used to prevent, ameliorate or cure chronic disease. Thus, as pharmacogenomics has led to the concept of "personalized medicine", so nutrigenomics may open the way for "personalized nutrition". A critical step to promote this understanding is to construct mathematical models, using system biology approaches, to define how these interactions can work. Ultimately, this will deliver a predicative, preventative and personalized understanding of the dietary interactions in the individual.

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