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Review

Where is the vitamin D receptor?

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ABSTRACT

The vitamin D receptor (VDR) is a member of the nuclear receptor superfamily and plays a central role in the biological actions of vitamin D. VDR regulates the expression of numerous genes involved in calcium/phosphate homeostasis, cellular proliferation and differentiation, and immune response, largely in a ligand-dependent manner. To understand the global function of the vitamin D system in physiopathological processes, great effort has been devoted to the detection of VDR in various tissues and cells, many of which have been identified as vitamin D targets. This review focuses on the tissue- and cell type-specific distribution of VDR throughout the body.

Introduction

Vitamin D_3 undergoes sequential 25- and 1α-hydroxylation to become the active hormone, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) [1]. $1,25(OH)_2D_3$ is an important modulator of calcium and phosphate absorption in intestine, calcium re-absorption in kidney, and calcium mobilization in bone [2]. In addition to maintaining calcium/phosphate homeostasis, it promotes differentiation and inhibits proliferation of certain cells, suggesting a potential role in cancer chemoprevention [3]. $1,25(OH)_2D_3$ has also been shown to suppress autoimmune diseases in several animal models [2,4]. $1,25(OH)_2D_3$ exerts its functions by binding to VDR,[1] a member of the steroid hormone receptor superfamily [5–9], leading to transcriptional regulation of target genes [10]. Many genes are directly upregulated (e.g., CYP24A1, CaBP-D_{9k}, CaBP-D_{28k}, osteocalcin, and Rankl) or downregulated (e.g., PTH and CYP27B1) via activation of VDR [11–19]. Thus, VDR plays a central role in the biological actions of vitamin D.

Accurate identification of VDR in tissues is critical to understand the physiopathological significance of vitamin D and could be key to the development of novel therapeutic modalities targeting the receptor. Since VDR was discovered three decades ago, more than 50 targets have been identified involving a broad realm of vitamin D functions [2,20–22]. However, contradictory results have been reported perhaps due to selection of methods. Although VDR immunohistochemistry was developed as a new powerful tool for determining the presence of VDR in tissues, great care must be exercised using appropriate positive and negative controls [23]. This review focuses on the tissue distribution and cellular localization of VDR.

Assays for VDR detection in tissues

Selection of tissues

Human and animal tissues are widely used for addressing VDR expression. A number of factors such as age, vitamin D status, calcium, and health can affect the expression of VDR gene in certain tissues. For example, vitamin D status and calcium regulate VDR expression in kidney [24] and bone [25], but not in intestine. In addition, VDR expression in tumors does not necessarily reflect that in normal tissues as seen in some breast and colon carcinomas, which seem to lose VDR expression [26–28].

Freshly isolated cells, such as immune cells and osteoblasts, are often used to determine expression of VDR. However, a recent study by Ahn et al. using pancreatic beta cells suggests that the isolation process itself can alter gene transcription [29]. It is also possible that cells can acquire VDR during *in vitro* culture, which was reported in human articular chondrocytes [30]. Hepatocytes do not express VDR *in vivo*, whereas low levels of VDR mRNA and protein were detected in freshly cultured hepatocytes from human, rat, and mouse [31]. Thus, using cultured cells may provide misleading information in regard to VDR expression.

Ligand binding assay

The ligand binding assay was used to identify the presence of receptor in a tissue preparation using either centrifugation or chromatographic analysis [8,9]. Historically, this was used successfully to identify VDR in chicken intestinal tissue [8]. However, this lacks

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[1] *Abbreviations used:* VDR, vitamin D receptor; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; ELISA, enzyme-linked immunosorbent assay.

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sensitivity and cannot identify the exact cell types containing the receptor.

Autoradiography

Autoradiography uses radiolabeled ligands (usually with tritium) to determine tissue distribution of VDR following administration of the ligand into circulation and subsequent tissue removal and sectioning. To assure that the radiation signal in the nucleus is receptor-specific, a radiolabeled analogue with low affinity to VDR (e.g., 25-hydroxyvitamin D_3 ($25(OH)D_3$)) is often administered as well, which should not result in any specific nuclear signals. Alternatively, unlabeled ligand (e.g., $1,25(OH)_2D_3$) can be administered to compete with the labeled ligand and abolish the specific signal. Many tissues and cells have been successfully identified as vitamin D targets using this technique (*vide infra*) [20,21].

Although the autoradiographic technique is highly sensitive, errors in VDR detection still occur as a result of receptor stability, ligand/receptor dissociation, difference in cellular uptake and metabolism of ligand, or the presence of endogenous ligand [32]. Because this technique measures not only [^{3}H]-$1,25(OH)_2D_3$ but also its metabolites and kidney is the major organ involved in the catabolism of $1,25(OH)_2D_3$, interpretation of the autoradiograms of kidney is difficult [24,33]. More importantly, VDR determination in vitamin D deficient animals may not represent VDR expression under normal physiological conditions. For example, VDR was detected in proximal renal tubules in vitamin D sufficient animals but not in the deficient animals [21,34]. In addition, vitamin D binding protein (DBP) in monocytes and lymphocytes may cause non-receptor uptake of $1,25(OH)_2D_3$ [35], interfering with the interpretation of autoradiography.

Assays for VDR transcript

Methods such as *in situ* hybridization and PCR/qPCR analyze gene transcripts and are often used to evaluate VDR expression in target tissues [36,37]. Because *in situ* hybridization uses labeled complementary DNA or RNA probes to localize specific DNA or RNA sequences, this technique can spatially determine VDR mRNA in target tissues [36,37]. Although these techniques are presumably specific and sensitive, they do not directly measure VDR protein.

LacZ reporter gene assay

Reporter genes are often used as indicators of whether a certain gene has been expressed in the cell or organism population. Using gene targeting, Erben et al. generated the VDR knockout mice carrying the reporter gene *lacZ* driven by the endogenous VDR promoter [38]. The gene-targeted mutant mice express a VDR with an intact hormone binding domain, but lacking the first zinc finger necessary for DNA binding. Prominent *lacZ* expression is detected in the tissues known to abundantly express VDR, such as duodenum, kidney, parathyroid glands, and the central region of pancreatic islets, indicating the specificity of the reporter gene [38,39]. Since there is no functional VDR in the tissues and the expression of VDR is autoregulated by its ligand, the *lacZ* activity may not represent the normal level of VDR expression.

Immunological assays

Immunoblotting, enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry have also been used for VDR detection. The application of antibody-based methods has provided important information regarding tissue-specific expression of VDR. However, VDR immunoassays have produced variable and even contradictory results likely derived from the use of different VDR antibodies and the lack of proper controls and standardized protocols.

A great number of VDR antibodies [23], such as rat monoclonal antibody 9A7, mouse monoclonal antibody IVG8C11, or rabbit polyclonal antibody C-20, have been developed over the years [33,40–48]. The application of these antibodies resulted in identifying VDR in many tissues/cells (*vide infra*). However, in immunoassays, antibody preparation and tissue handling may affect the specificity and sensitivity. Based on the results with VDR antibodies on tissue samples from the Demay VDR knockout mice, many VDR antibodies including the widely-used 9A7 (Affinity BioReagents) and C-20 (Santa Cruz Biotechnology) not only bind VDR but also possess non-specific interactions with other unidentified proteins, determined by both immunoblotting (Fig. 1) and IHC [23]. As a result, the utility of these antibodies to identify VDR in tissues and cells may be constrained by their limited specificity (C-20 or 9A7) and/or low immunosensitivity (IVG8C11 or H-81).

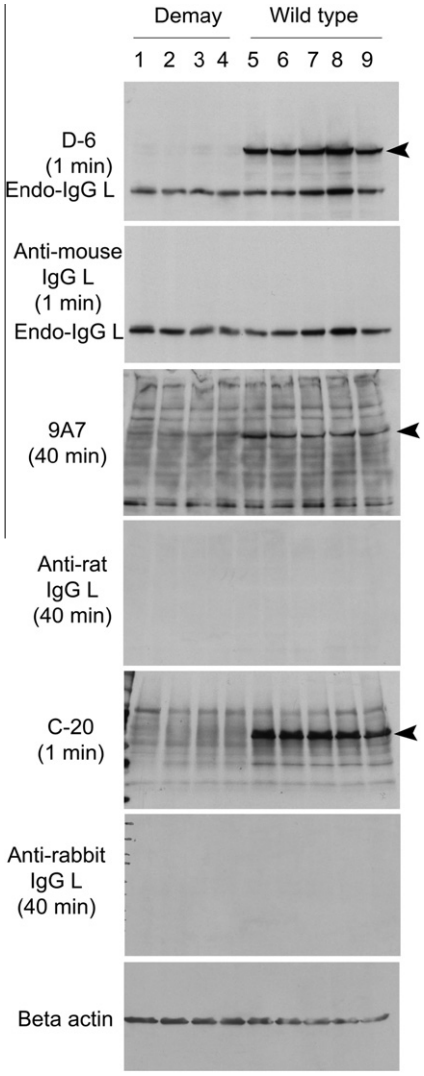

Fig. 1. Specificity and sensitivity of VDR antibodies characterized by immunoblotting of mouse duodenal lysates. Fifty micrograms of duodenal tissue lysates from the Demay VDR knockout (lanes 1–4) or wild type (lane 5–9) mice were used for immunoblotting. The blots were incubated with VDR antibodies including D-6, 9A7, or C-20. The blots were incubated with HRP-conjugated secondary antibodies. For the blots used to evaluate non-specific reactions of secondary antibodies with tissue, the primary antibody was omitted. HRP signal was developed by incubation of blot with ECL solution (Amersham) and captured by X-ray film for variable time indicated in this figure. The VDR band on each blot is denoted by an arrowhead. Blots were also reprobed with an antibody to beta actin as a loading control.

In addition, some antibodies (XVIE6E6 and 39069) are species-specific [23]. Based on the parallel comparison of a large selection of VDR antibodies, D-6 (Santa Cruz Biotechnology), a mouse monoclonal antibody against the C-terminus of human VDR, possesses the highest specificity, sensitivity, and versatility [23,34]. D-6 is capable of binding VDRs from human, monkey, pig, chicken, rat, and mouse, and can be used for multiple immunoassays including immunoblotting, immunocytochemistry, and immunohistochemistry. It can be applied to paraffin-embedded tissue samples if an antigen retrieval process is included [23].

The importance of a proper negative control for accurate evaluation of immunoassays is also highlighted in the recent studies [23,49]. Serum from animals that were not immunized, irrelevant immunoglobulins, or omission of the primary antibody has been used as negative controls. However, these controls failed to identify the non-specific interactions of the primary antibody. In addition, the validity of controls employing pre-incubation of the primary antibody with VDR-containing tissue preparations [33] or use of competitive peptides [50] is also questionable. This type of negative control does not rule out the possibility that some proteins may share an identical or similar epitope(s) to VDR. Genetically engineered animals that lack VDR represents a proper control for immunoassays. The Demay VDR knockout mice have been generated by deletion of the exon 3 of the VDR gene and do not express VDR proteins [23,51]. In contrast, a truncated form of VDR is detected in the Kato VDR knockout mice, making it unsuitable as a negative control.

The immunoblotting and ELISA methods can quantify VDR in tissues. However, they are unable to identify the exact cell types that express the receptor. The DeLuca group developed a unique ELISA to determine VDR in tissue homogenates [52]. In this system, VDR from tissue homogenates is bound between two monoclonal antibodies recognizing different epitopes on the receptor protein. The first antibody is immobilized on the plate and the second is biotinylated for quantitation of VDR via alkaline phosphatase-conjugated streptavidin. This unique assay seems to be highly specific and sensitive in VDR determination. Among all VDR immunoassays, only *in situ* immunohistochemistry can spatially determine VDR protein in target tissues.

VDR is abundant in intestine

The nuclear component bound to $1,25(OH)_2D_3$ was first identified in chick intestinal mucosa in 1975 [8] and later identified as VDR [5,6,53]. Since then, many studies have shown high abundance of the receptor in intestinal mucosa from animals and humans by autoradiography [21], *in situ* hybridization, and immunological methods [23,33,42]. VDR is widely distributed throughout small intestine from duodenum to ileum and large intestine including cecum and colon [21,23,33,42,44,54–58].

More specifically, VDR is localized in intestinal epithelial cells (Figs. 2 and 3), consistent with the vitamin D function in intestinal calcium absorption. In contrast, goblet cells and lymphatic tissue have no receptor at all [21,23,54]. Although nuclei of some cells in the underlying muscle layers are also positive for VDR as detected using the rat monoclonal antibody 9A7 [42], no receptor is found in the smooth muscle by autoradiography and a highly specific and sensitive immunohistochemistry method [21,23,49].

VDR is undetectable in liver

Liver is generally considered negative for VDR [2], even though estrogens have been shown to induce the appearance of VDR in rat livers [59,60]. Autoradiography failed to detect VDR in the hepatocytes [61], but an extremely low level of VDR was detected in normal rat liver (1300-fold lower than that in intestine) [44]. We found no detectable levels of VDR in human and mouse liver by immunohistochemistry (Fig. 3 and unpublished data), whereas others showed inconsistent expression of VDR in fetal and adult hepatocytes [33,42,62], suggesting extremely low levels of VDR if any or lack of antibody specificity in the studies.

In addition to hepatocytes, some smaller populations of liver cells such as sinusoidal cells contain weak VDR immunological signals [62]. Stumpf et al. found that these sinusoidal cells in animal liver concentrate retained variable amounts of radiolabeled compound in the cytoplasm but not in the nuclei after administration of $[^3H]$-$1,25(OH)_2D_3$ and $[^3H]$-$25(OH)D_3$, suggesting probable sites of specific storage, similar to that of vitamin A [61].

VDR is strongly expressed in pancreatic beta cells

VDR was first detected by the ligand binding assay in normal chick pancreas [57]. A follow-up study showed concentrated autoradiographic signals in the nuclei of cells that were centrally located in adult rat pancreatic islets [63]. Using autoradiography and co-immunostaining, Clark et al. reported that the target cells of vitamin D were the insulin-containing cells, not those producing glucagon or somatostatin [64]. We also showed that VDR is only detected in the insulin-positive cells of pancreatic islets (unpublished data).

Stumpf et al. studied VDR in digestive organs/tissues from rat, mouse, hamster, and zebra finch by autoradiography and concluded that VDR is predominantly expressed in the beta cells of pancreas [61]. The site-specific expression of VDR is in good agreement with the findings by Erben et al. using the *lacZ* reporter system under the control of VDR gene promoter, which detected *lacZ* activity in the central region of pancreatic islets [38].

VDR is expressed in kidney tubular epithelial cells

In situ determination of VDR in kidney by immunohistochemistry and various other methods have shown that VDR is expressed in distal renal tubules and collecting ducts [21,23,33,36,37,65–69], but at low levels in proximal renal tubules (24-fold lower than that in the distal tubules in mouse) (Figs. 3 and 4) [34]. This is consistent with the VDR function in regulating calcium re-absorption from pre-urine, and the low abundance of VDR in proximal tubules seems to exercise a critical role in the transcriptional regulation of CYP24A1 and CYP27B1.

Historically, VDR in the proximal tubule was not detected by autoradiography in the vitamin D deficient animals [21,65,69,70], probably due to the effect of the vitamin D deficiency on VDR expression at this particular site. The measurement of VDR protein or transcript in laser dissected renal samples using methods such as ligand binding assay [68], RT-PCR/qPCR [37,67], *in situ* hybridization [36,71], and immunohistochemistry [33,36,40,66] was inconsistent likely resulted from low specificity and sensitivity of certain methods [23,72].

VDR is also found in several special tissues of kidney such as macula densa and glomerular podocytes, which are considered vitamin D targets as well, although the expression level is low in podocytes (Fig. 4) [21,34]. In contrast, VDR is not detected in the juxtaglomerular cells, renal interstitial fibroblasts, mesangial cells and endothelial cells of glomeruli (Fig. 4) [21,22,33,34,36,66,73].

VDR is selectively expressed in bronchial epithelial cells

VDR is found in bronchial epithelial cells in human (Fig. 3), mouse (unpublished results), and rat fetus, but absent in alveolar cells in human lung as shown by immunohistochemical staining (Fig. 3) and autoradiography [33,74].

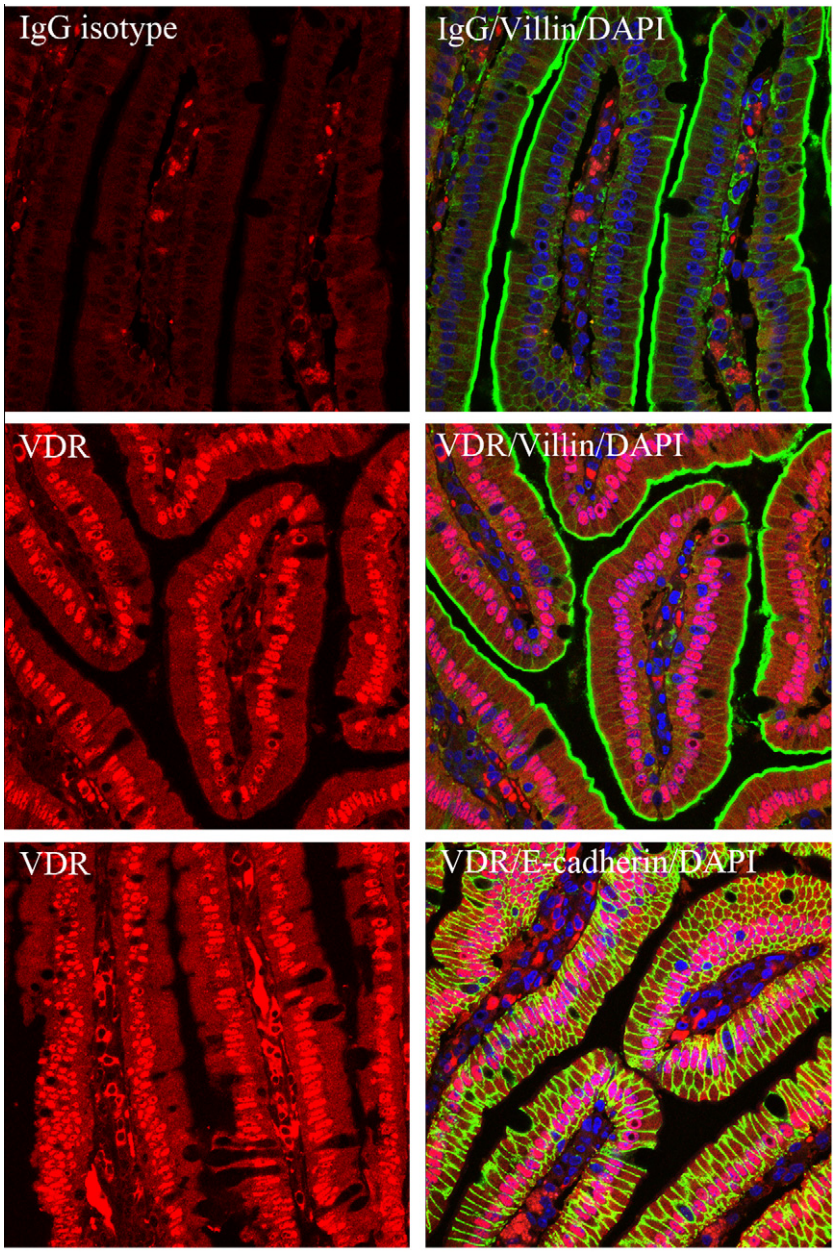

Fig. 2. Immunohistochemical staining of duodenal tissue sections from the wild type mice. The paraffin embedded tissue sections were stained with the antibodies indicated in this figure. 4′,6-Diamidino-2-phenylindone (DAPI; blue) staining was applied to locate nucleus. VDR antibody: D-6; E-cadherin: marker for epithelial cells; Villin: marker for the brush border of intestinal villi. Staining method was reported previously [23]. Images were taken using confocal microscope (at x600). VDR were only found in gut epithelial cells (E-cadherin-positive cells). The immunostaining located in the interior of microvillus and the lymphatic tissues was contributed by endogenous IgG. The smooth muscle cells remained unlabeled [23].

VDR is selectively expressed in skin epithelial cells

The presence of VDR in skin was determined by ELISA, PCR, and *in situ* hybridization [44,46,75] and the target cells were identified by *in situ* immunohistochemistry [33,72]. VDR seems to be restricted to the nuclei of epidermal epithelial cells (unpublished data). The epithelial cells of the hair root sheaths, sweat glands, and sebaceous glands also contain VDR [21,76–79].

VDR is detected in osteoblasts and chondrocytes

Bone is the first tissue identified as a target for vitamin D [80]. VDR was detected in the preparations of fetal rat bone and mature rat bone tissues by ligand binding assay and ELISA, respectively [9,44]. qPCR analysis revealed that $1,25(OH)_2D_3$ strongly induced VDR gene transcription in mouse bone [25,81]. VDR was detected in osteoprogenitor cells, osteoblasts, lining cells, osteocytes, and chondrocytes in bone [33,80,82–86]. The presence of VDR in osteoclasts, however, is still under debate [42,83–85,87,88]. To clarify if VDR is expressed in bone cells such as osteoblasts, we studied VDR expression in neonatal and adult mouse bone tissues using *in situ* immunohistochemistry and Demay VDR knockout mice as negative control (unpublished data). The result clearly showed that VDR expression is high in osteoblasts and low in hypertrophic chondrocytes while undetectable in osteoclasts and chondroclasts.

VDR is undetectable in muscle

Stumpf et al. was not able to show $[^3H]$-$1,25(OH)_2D_3$ localization in muscle by autoradiography [21,57,89–91], and no mRNA

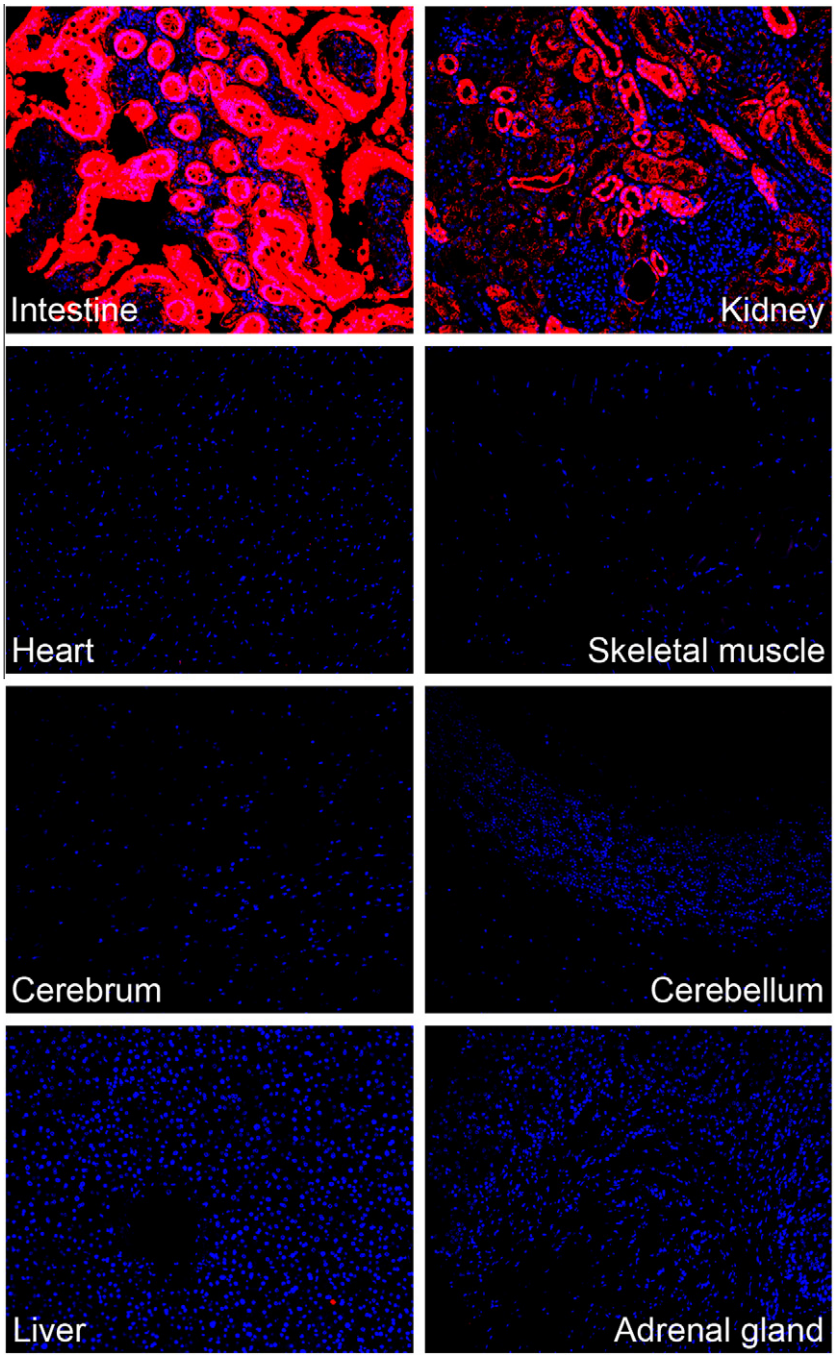

Fig. 3. Distribution of VDR in normal human organs/tissues. The experiment used multiple organ normal tissue microarray (catalogue number BN961, US Biomax, Rockville, MD) to evaluate relative levels of VDR expression among human organs/tissues. Duplicated cores per case, 24 normal human organs were included. Most organs contained more than one case. The representative data are included in this figure. Staining method was reported previously [23]. DAPI staining (blue) was applied to locate nucleus. Note that the slide was stained for VDR and E-cadherin (green). The images for colon, lung bronchus, and prostate were merged with E-cadherin. There was no significant background from secondary antibody staining or autofluorescent signal in intestine, spleen, muscle and kidney, examined using single tissue section slides (data not shown). The intensity of immunostaining provided relative levels of VDR in selected tissues. Original magnification ×200.

encoding VDR was detected in heart and liver [5]. Immunoassays also failed to detect VDR in mature skeletal and heart muscles [44]. Using immunoblotting, qPCR, and immunohistochemical staining, we showed that VDR was not detected in smooth muscle, heart muscle or skeletal muscle in human (Fig. 3) and in vitamin D deficient, normal and $1,25(OH)_2D_3$ treated animals [49].

Historically, low levels of VDR were found in skeletal muscle cells, myoblasts, cardiomyocytes, and smooth muscle cells [50,92–95]. The first *in situ* detection of VDR in human skeletal muscle was reported by Bischoff et al. using immunohistochemistry [96]. However, the VDR antibody 9A7 employed in the study can react with non-VDR related proteins in Western blot and cause false positive in the extracts prepared from the Demay VDR knockout mice [23].

VDR is found in cells of the immune system

T-lymphocytes

Nuclear intake of $[^3H]$-$1,25(OH)_2D_3$ by lymphocytes is evident for the presence of VDR in these cells [21,97,98]. VDR has been

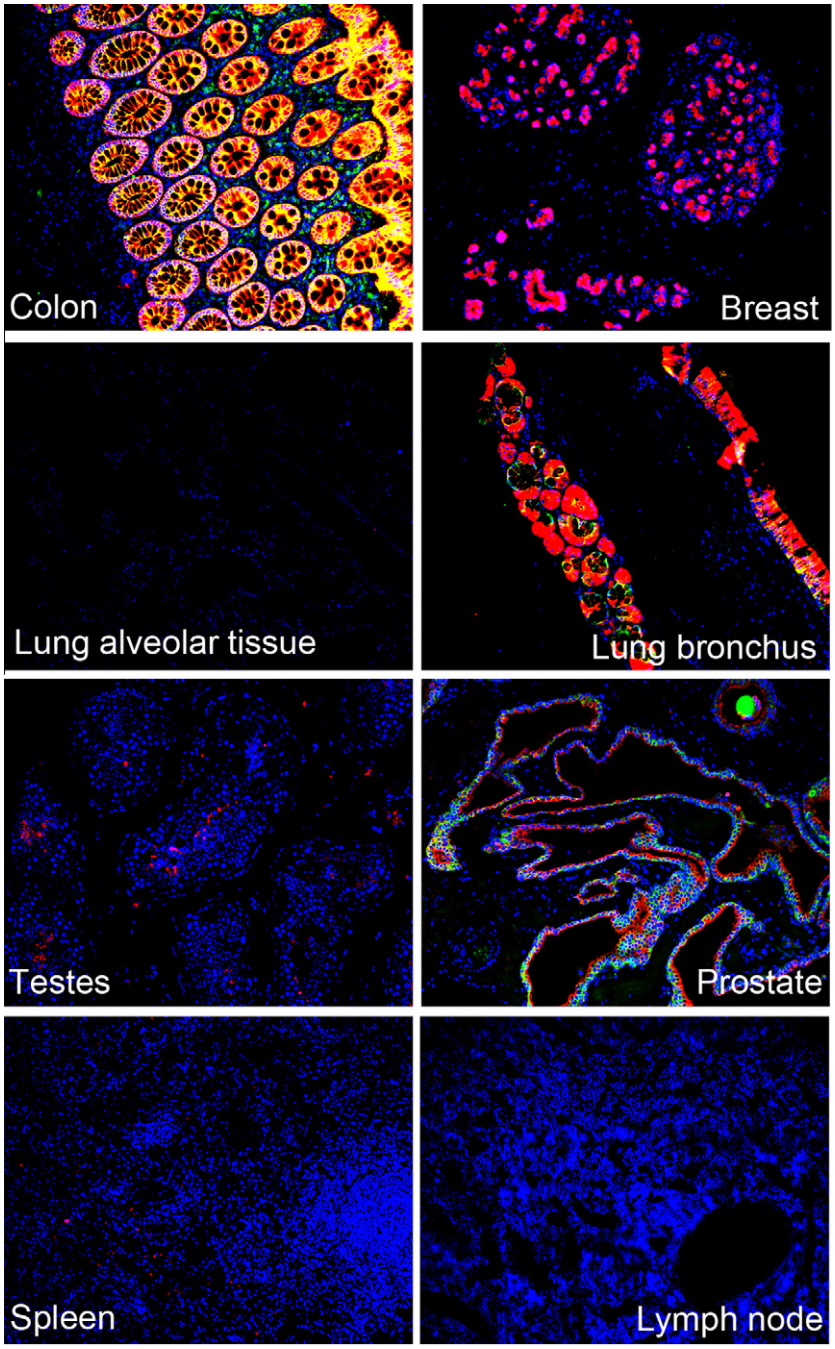

Fig. 3 (*continued*)

shown present in T-lymphocytes after activation, but absent in isolated resting human T cells [99,100]. An early clinical study reported that 76% seropositive patients with rheumatoid arthritis had lymphocytes that possessed VDR (without *in vitro* activation) compared to only 18% (3 of 17) in normal individuals [101]. Presumably, these patients have more activated T-lymphocytes. The first direct measurement of VDR in T-lymphocytes by immunohistochemical staining showed that the VDR was present in a small population of T cells located at the insulitic islets and lymph nodes from non-obese diabetic mouse pancreas (unpublished data). Majority of the T cells had no detectable VDR. The VDR-positive lymphocytes remain to be defined. A recent *in vitro* study showed that activation-associated VDR expression in T-lymphocytes was related to T cell receptor and mitogen-activated protein kinase p38 signaling pathways [102]. However, an *ex vivo* study on isolated primary cells reported that CD_8 T-lymphocytes had the highest concentrations of VDR regardless of activation and CD_4 T-lymphocytes contained relatively low but significant amount of VDR. As expected, the presence of $1,25(OH)_2D_3$ increased the amount of measurable receptor [103]. In the meantime, there are also studies suggesting that T-lymphocytes are not direct target for $1,25(OH)_2D_3$ and that $1,25(OH)_2D_3$ regulates the concanavalin-A induced lymphocyte proliferation through monocytes [104,105].

Promyelocytes, monocytes, macrophages and dendritic cells

Nuclear uptake of $[^3H]$-$1,25(OH)_2D_3$ was also observed in promyelocytes, suggesting the presence of VDR [21,97]. In addition, $1,25(OH)_2D_3$ was shown to suppress proliferation of

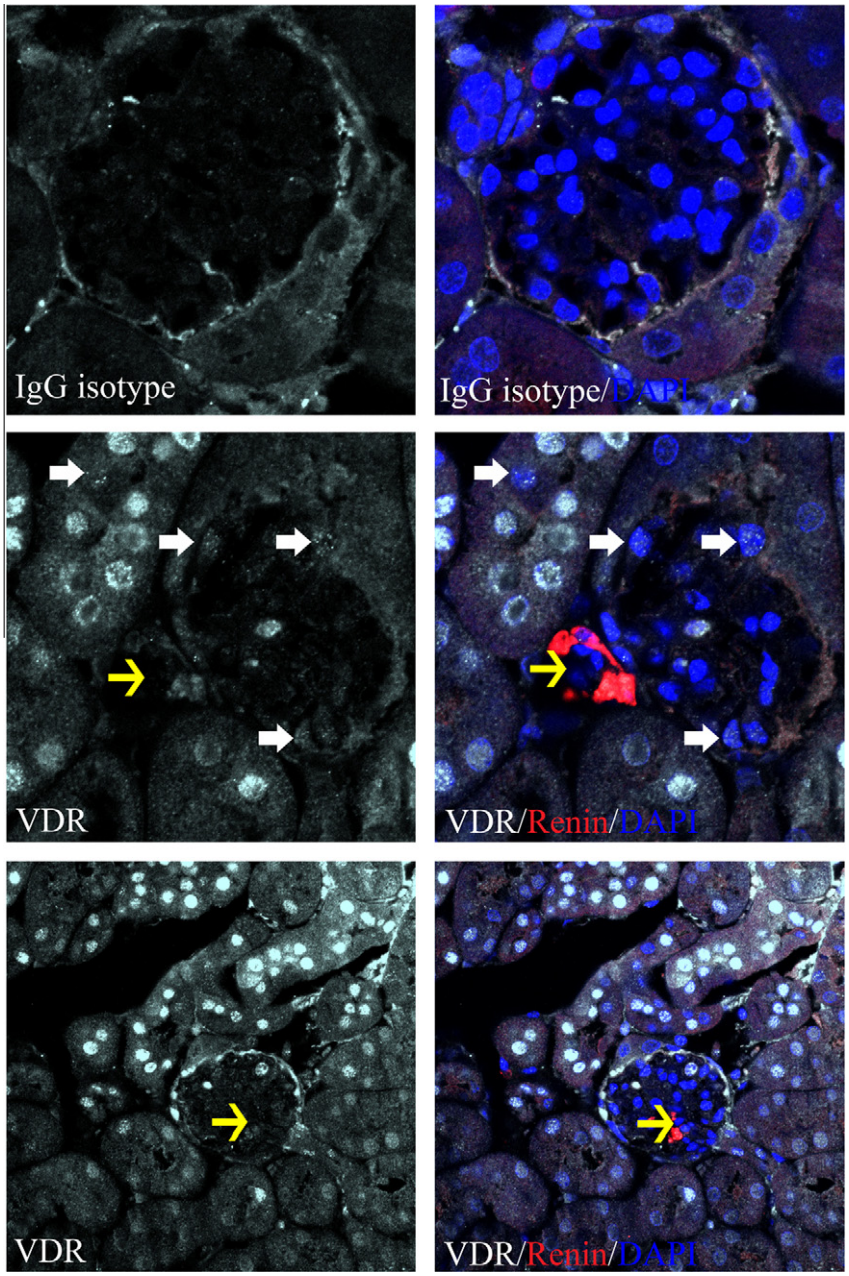

Fig. 4. VDR in mouse proximal renal tubule, glomerulus and juxtaglomerular apparatus (original amplification ×600; 3A-3D were digitally enlarged to ×1200). Kidney samples were stained with the antibodies indicated in the images. DAPI (blue) was used to stain cellular nuclei. VDR staining (D-6 antibody; white) was seen in the nuclei of both distal and proximal tubular cells and glomerular podocytes. The cells with a low level of VDR are indicated by white broad arrows. The renin staining (marker for juxtaglomerular cells; red) was selectively seen in juxtaglomerular cells. The VDR staining was not seen in the nuclei of juxtaglomerular cells (yellow arrows). Note that the interstitial fibroblasts were not stained by the VDR antibody. No nuclear staining by D-6 VDR antibody was seen in any renal cells from the glomerulus of Demay VDR knockout mouse kidney [34]. Some white staining could be seen in the cytoplasm of the proximal renal tubular cells or the outside of renal cells. The VDR knockout kidney samples stained with only the secondary antibody showed a similar pattern (data not shown), suggesting that it was most likely the staining for mouse endogenous IgG. The IgG could be detected in the proximal tubular cells because of protein and peptide re-absorption at this particular site.

promyelocytes and cause their differentiation into monocytes *in vitro* [106,107].

VDR was detected by ligand binding assay in isolated human peripheral monocytes [99,100]. Using a highly specific and sensitive VDR antibody, we revealed a sparsely stained pattern in human spleen but not in the lymph nodes (Fig. 3), which co-localized with monocytes/macrophage-specific staining (unpublished data). In addition, nuclear uptake of $[^3H]$-1,25$(OH)_2D_3$ was detected in freshly isolated human monocytes from patients with autosomal dominant osteopetrosis and sex- and age-matched controls [108]. Western blotting analysis compared disease-associated expression of VDR and showed 2-fold greater VDR level in peripheral blood monocytes in patients with idiopathic hypercalciuria than that in the age-matched control group [109]. VDR was also detected in macrophages in pancreatic insulitic islets and lymph nodes using a diabetic mouse model (unpublished data). These findings suggest that the monocytes/macrophages are potential targets of vitamin D, particularly under certain pathological conditions.

Furthermore, VDR expression in monocytes/macrophages is regulated during differentiation and activation or induced by 1,25$(OH)_2D_3$ [110,111,103,112]. VDR seems to be constitutively

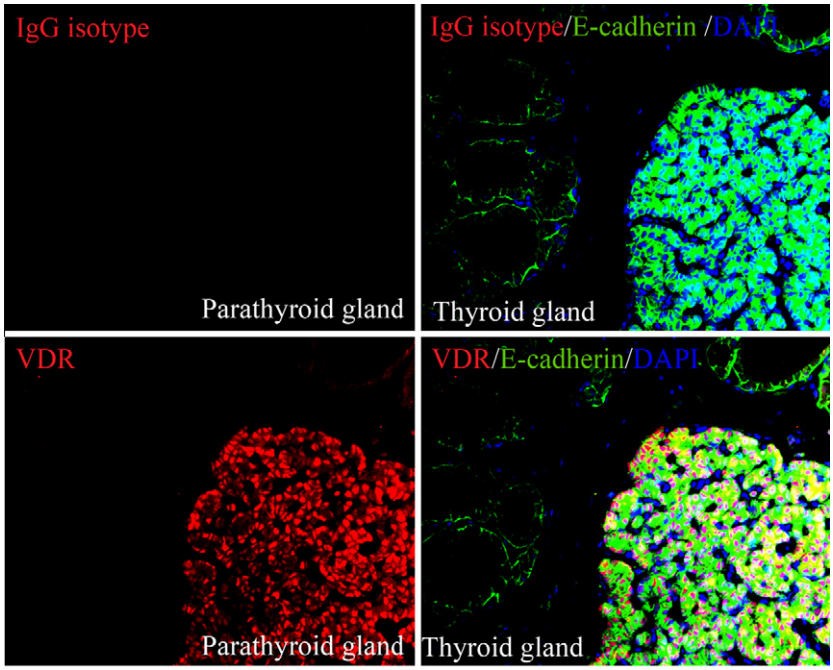

Fig. 5. Immunohistochemistry of rat thyroid and parathyroid tissue sections using D-6 antibody. The paraffin embedded sections were stained with the antibodies indicated in this figure. DAPI staining was applied to locate nucleus. Staining method was reported previously [23]. Images were taken using fluorescence microscope (at ×200). VDR were only found in the parathyroid epithelial cells (E-cadherin-positive cells). The thyroid epithelial cells remained unstained.

expressed in monocytes, but not in mature macrophages [110]. Induction of VDR by $1,25(OH)_2D_3$ occurs in monocytes and, to a lesser degree, in mature macrophages [110]. Interestingly, $1,25(OH)_2D_3$ seems to induce the differentiation of monocyte to macrophage *in vitro* [99,113,114] and enhances the monocyte function in antigen presentation [106,107,115]. It has been reported that $1,25(OH)_2D_3$ hampers maturation of fully active immature dendritic cells from monocytes [116]. Several studies showed the presence of VDR in dendritic cells [117], suggesting that dendritic cells may be another vitamin D target [118–120].

Thymic reticular epithelial cells

Thymus is a specialized organ of the immune system. It produces and educates T-lymphocytes, critical for the adaptive immune system. For a long time, bovine thymus has been considered a good source of VDR protein. Autoradiography and immunohistochemistry indicated that VDR is restricted to the nuclei of thymic reticular epithelial cells and no receptor was seen in thymocytes (unpublished data) [121].

VDR is found in certain endocrine glands

Parathyroid and thyroid glands

VDR is highly expressed in the parathyroid epithelial cells, detected by ligand binding [57], autoradiography [69,89,122], *lacZ* reporter assay [38], and immunohistochemistry (Fig. 5). $1,25(OH)_2D_3$ acts on VDR in parathyroid to increase VDR expression and suppress transcription of PTH gene [123–125]. VDR is undetectable in thyroid (Fig. 5) [38,122].

Pituitary gland

The presence of VDR in pituitary gland has been demonstrated by co-localization with the pituitary hormones [126], autoradiography [21,126], ligand bind assay [57,127–129] and immunohistochemistry [33,130].

Adrenal gland

VDR was undetectable in human adrenal gland by our immunohistochemistry method (Fig. 3). However, autoradiography [131] and immunocytochemical staining [33] were able to detect VDR in the adrenal medulla. Therefore, the presence of VDR in this gland remains uncertain.

VDR is undetectable in brain tissue

ELISA and immunohistochemical staining showed undetectable levels of VDR in cerebrum and cerebellum in rat and human (Fig. 3) [44]. Similarly, VDR was not detected in mouse neurons and glia (unpublished data). Historically, reports of VDR expression in brain were mainly derived from autoradiographic studies [132,133], *in situ* hybridization [134], and immunohistochemistry [42,134–140]. But be aware that the antibodies used for direct measurement of VDR such as 9A7 [33,42] and C-20 [135,137,139] are not VDR-specific [23] and confirmative studies will be required in the future.

VDR is found in certain reproductive tissues

Spermatozoa

VDR has been detected in testes by autoradiography [141–144], RT-PCR [145], and immunohistochemistry [146,147]. Our study showed that in human testes, VDR is predominantly expressed in the cells located at the center of efferent ducts, where more matured spermatozoa exist (Fig. 3), consistent with the previous finding that VDR is mainly located on the head/nucleus of human sperm and mid-piece [147].

Table 1
Distribution of VDR in normal tissues/cells.

Organ/tissue	Expression level	Cell types
Digestive system		
Small intestine	++++++++++	Epithelium
Large intestine	+++++++	Epithelium
Liver	–	
Pancreas	+++	Epithelium
Kidney		
Distal tubule	++++++++	Epithelium
Proximal tubule	++	Epithelium
Glomerular podocytes	+	Podocytes
Respiratory system		
Lung alveolar cells	–	
Bronchus	+++++	Epithelium
Bone		
Osteoblasts	+++++	Osteoblasts
Chondrocytes	+	Chondrocytes
Muscle system	–	
Immune system		
Thymus	+++++	Epithelium
Spleen/lymph node	++	Monocyte/macrophage/T-cell
Endocrine system		
Thyroid	–	
Parathyroid	++++++++	Epithelium
Pituitary gland	+++	Epithelium
Adrenal gland	–	
Brain		
Cerebrum	– ?	
Cerebellum	– ?	
Spinal cord	– ?	
Reproductive system		
Testis	++	Germ cells
Prostate gland	++++	Epithelium
Mammary gland	++++	Epithelium

? – Not completely defined.

Prostate gland secretory epithelial cells

Numerous studies have revealed the presence of VDR in prostate gland [33,142–146,148,149]. We further located the receptor in the secretory epithelial cells of human prostate glands (Fig. 3). VDR expression in human prostate gland seems to be age-related [149].

Mammary gland epithelial cells

VDR was predominantly found in the nuclei of lobule and ductal epithelial cells in mammary glands (Fig. 3) [33,150]. It is also noticed that VDR content increases during pregnancy and lactation [151].

Discussion

VDR is clearly present in cells of the intestinal epithelium, renal tubules, parathyroid gland cells, skin (keratinocytes), mammary epithelium, pancreas (beta islet cells), pituitary gland, skeleton (osteoblasts and chondrocytes), immune system (monocytes, macrophages, and T-lymphocytes), and germ tissues (Table 1) [2]. The tissues with the highest VDR content are intestine, kidney, parathyroid gland, and bone, all of which are associated with maintenance of calcium homeostasis. The functions of VDR in many other tissues and cells (e.g., the immune system) remain to be determined [2].

An important question is how much VDR is needed to be of functional significance? Certainly, even the most specific and sensitive antibodies have their limitation. Because functions of vitamin D are known for cells of the intestine, bone, kidney, and parathyroid, a high level of VDR is expected. However, there is a high level of VDR in the islet cells of the pancreas where its function is yet to be satisfactorily determined. On the other hand, even the most specific and sensitive antibodies fail to detect VDR in liver and both smooth and skeletal muscle. Further no function of vitamin D in these tissues has been found. Even in these tissues mRNA for VDR might be detected by highly sensitive RT-PCR. To recognize a vitamin D responsive tissue, both a function and the vitamin D signaling system must be present including VDR. In this sense, the specific detection of VDR is but one criterion of a vitamin D function. This report underscores the need to define the function of vitamin D in the cells and tissues where VDR is found such as islet cells of the pancreas, keratinocytes and cells of the immune system.

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