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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 $[^3H]$-$1,25(OH)_2D_3$ 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).

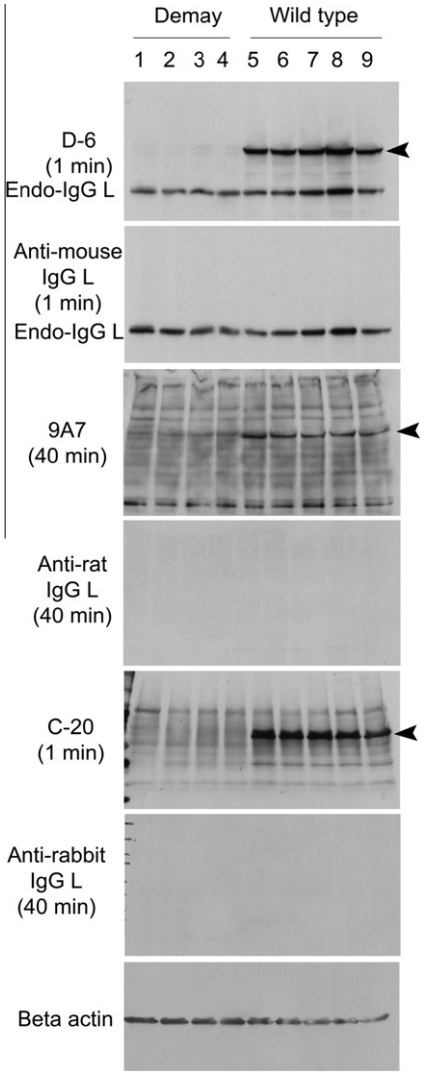

Fig. 1. Specificity and sensitivity of VDR antibodies characterized by immunoblotting of mouse duodenal lysates. Fifty micrograms of duodenal tissue lysates from the Demay VDR knockout (lanes 1–4) or wild type (lane 5–9) mice were used for immunoblotting. The blots were incubated with VDR antibodies 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.