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Commentary OMICS International

25-hydroxyvitamin D_3 may Function via Genomic and Non-Genomic actions

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Abbreviations

$25D_3$: 25-hydroxyvitamin D_3; $1\alpha,25D_3$: 1α,25-dihydroxyvitamin D_3; CYP27B1: 25-hydroxyvitamin D 1α-hydroxylase; VDR: vitamin D receptor; RXR: Retinoid X receptor; VDRE: Vitamin D responsive element.

Commentary

Vitamin D_3 ingested from food or produced in the skin binds to the vitamin D binding protein (DBP) in the blood. Vitamin D_3 bound to DBP is carried to the liver and hydroxylated at position 25 by CYP2R1 or CYP27A1, belonging to the cytochrome P450 (CYP) superfamily, resulting in 25-hydroxyvitamin D_3 ($25D_3$). $25D_3$ binds to DBP and circulates through the blood; in the kidney, it binds to megalin, is highly expressed in the renal proximal tubule, and is taken up by endocytosis [1]. $25D_3$ incorporated into cells together with DBP is hydroxylated at the 1α position by CYP27B1, resulting in 1α,25-dihydroxyvitamin D_3 ($1\alpha,25D_3$). $1\alpha,25D_3$ circulates through the blood and binds to vitamin D receptor (VDR) in organs with VDR expression, such as the bone, small intestine, and kidney [2]. VDR forms a dimer with retinoid X receptor (RXR) and binds to vitamin D response elements (VDRE) upstream of the target gene promoter [3,4]. As a result, vitamin D interacts with various transcription coupling factors and basic transcription factors to activate promoters and regulate gene expression [5]. $1\alpha,25D_3$ plays an important role in the maintenance of calcium homeostasis, bone formation, and differentiation of cells by genomic action, i.e., by controlling the expression of the target genes, mainly via VDR. However, in recent years, rapid, non-genomic action that does not involve protein synthesis has been reported. Specifically, $1\alpha,25D_3$ activates the intracellular signaling molecule present in the membrane via caveolae, structural functional components of the cell membrane, and membrane-associated rapid response steroid (MARRS) binding protein, and liberates a second messenger [6,7]. It has been reported that such non-genomic action leads to physiological activities, such as insulin secretion and smooth muscle cell migration. These previous findings have indicated that vitamin D exerts various physiological effects by genomic and non-genomic action.

The expression of CYP27B1, a key enzyme that makes $1\alpha,25D_3$, is suppressed by $1\alpha,25D_3$. In contrast, the enzyme CYP24A1 inactivates $1\alpha,25D_3$ and is induced by $1\alpha,25D_3$. Accordingly, the $1\alpha,25D_3$ concentration in the blood is kept fairly constant. Based on these observations, $1\alpha,25D_3$ is referred to as "active vitamin D_3" and is thought to be a true VDR ligand. On the other hand, $25D_3$ is considered a "precursor of active vitamin D_3" because its affinity for VDR is several hundredths that of $1\alpha,25D_3$. However, many observations cannot be explained if only $1\alpha,25D_3$ is regarded as active vitamin D_3. For example, the L99P mutation in CYP2R1 causes rickets [8]. The $25D_3$ concentration (approximately 10 nM) in the blood of individuals who are homozygous for this mutation is markedly lower than that of healthy people (30 to 120 nM). Blood calcium and phosphorus levels are also low, and the blood alkaline phosphatase concentration is abnormally high, i.e., 10 times that of healthy people. However, the concentration of $1\alpha,25D_3$ in the blood of patients is normal, and this result is difficult to understand.

Vitamin D is deeply involved in bone metabolism, cell differentiation and proliferation, and immunity, and is closely related to various diseases, such as osteoporosis, cancer, diabetes, arteriosclerosis, and autoimmune diseases [9]. The $25D_3$ concentration, but not the $1\alpha,25D_3$ concentration, in the blood is a disease indicator. The affinity of 25D3 for VDR is remarkably low, about 1/500 times that of $1\alpha,25D_3$, but, inversely, the blood concentration is nearly 1000 times higher. These facts suggest that $25D_3$ itself may act directly as a ligand for VDR. Additionally, several papers have suggested the importance of direct action of $25D_3$ [10,11].

Munetsuna *et al.* added $25D_3$ at a final concentration of 10 nM or 100 nM to the medium of human prostate-derived PZ-HPV-7 cells, and observed incorporation of $25D_3$ into cells, metabolism of $25D_3$, and intranuclear translocation of VDR and CYP24A1. Induction of the transcription of VDR target genes and cell proliferation were measured over time. They also investigated the effects of VDR and CYP27B1 using siRNA [12]. They found that the ratio of intracellular/extracellular abundance of $25D_3$ reached an equilibrium within 10 minutes, VDR nuclear translocation was nearly complete in 90 minutes, *CYP24A1* transcription was induced beginning at 120 minutes, and significant metabolism of $25D_3$ by CYP24A1 occurred after approximately 300 minutes [13]. HPLC using tritium-labeled $25D_3$ showed a faint peak at the elution position of $1\alpha,25D_3$. Even if all of these peaks are regarded as $1\alpha,25D_3$, the amount of $1\alpha,25D_3$ produced in cells is very small, and the observed phenomena are mainly attributed to $25D_3$ itself. In addition, siRNA studies have strongly suggested that $25D_3$ directly binds to VDR and acts almost independently of CYP27B1. Furthermore, based on a comprehensive analysis of gene expression using DNA microarray and real-time PCR approaches, *cystatin D*, *cystatin E/M*, and *semaphorin 3B* are each involved in the inhibitory effects of $25D_3$ on cell proliferation. In other words, $25D_3$ is presumed to control the expression of various genes and suppress cell proliferation by genomic action, i.e., by binding to VDR as a ligand in PZ-HPV-7 cells [13]. This suggests that in humans, the direct effect of $25D_3$ can explain the risk of prostate cancer and the $25D_3$ concentration in the blood shows a negative correlation with cancer risk (Figure 1).

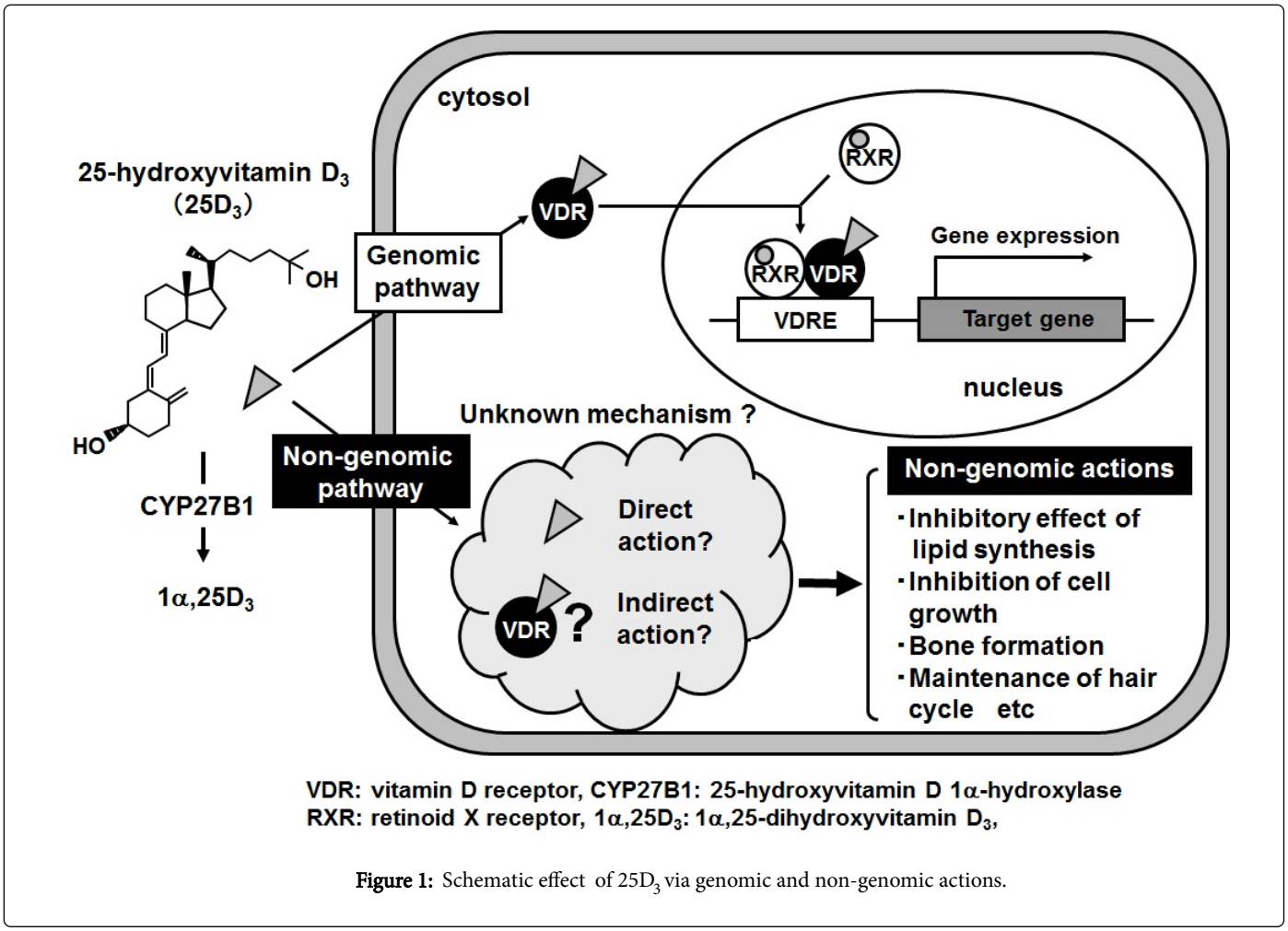

Figure 1: Schematic effect of $25D_3$ via genomic and non-genomic actions.

$1\alpha,25D_3$ is not detected in the blood of *Cyp27b1* knockout mouse, which exhibit an osteogenesis deficiency, uterine hypoplasia, immune dysfunction, etc. *Cyp27b1* knockout mice can be treated successfully by the administration of $1\alpha,25D_3$, indicating that $1\alpha,25D_3$ plays important roles in bone formation, uterus formation, immune function, and other processes. Rowling *et al.* found a remarkable increase in the $25D_3$ concentrations, blood calcium concentration, and bone density after the administration of vitamin D_3 to *Cyp27b1* knockout mouse, suggesting a direct effect of $25D_3$. However, since the weight, size, and capacity of the spinal column are considerably smaller than those of wild-type mice, they deduced that it is not possible to compensate for the effects of $1\alpha,25D_3$ with only $25D_3$ [14,15]. Asano *et al.* reported a non-genomic action in which $25D_3$ suppresses the expression of the *sterol regulatory element-binding protein (SREBP)* gene, an important transcription factor in lipid cholesterol and fatty acid biosynthesis, directly, without VDR [16]. In addition, in a comparative analysis, Hirota *et al.* found that hair loss observed in *Vdr* knockout mice was not observed in *Cyp27b1* knockout mice. These results suggest that $25D_3$ has a genomic action responsible for the maintenance of the hair cycle in the hair matrix via Vdr. Furthermore, abnormal chondrogenesis was less frequent in *Vdr* knockout mice than in *Cyp27b1* knockout mice. *Vdr* knockout mouse phenotypes are thought to be explained by non-genomic effects in which $1\alpha,25D_3$, which does not interact with Vdr, but is abundant in the blood, directly controls the proliferation of chondrocytes [17].

Based on previous findings, Sakaki proposed that the physiological action of vitamin D is the sum of the effects of the "strong hormone 1α, $25D_3$ and weak hormone $25D_3$, and the contribution of both is different depending on the organ and cell" [9]. Additionally, $1α,25D_3$ is a powerful hormone produced in small quantities in an emergency, assuming that $25D_3$ is responsible for vitamin activity. In future vitamin D research, it is necessary to elucidate its comprehensive functions *in vivo* by clarifying the genomic and non-genomic actions of $25D_3$.

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