Vitamin D in the Context of Evolution

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For at least 1.2 billion years, eukaryotes have been able to synthesize sterols and, therefore, can produce vitamin D when exposed to UV-B. Vitamin D endocrinology was established some 550 million years ago in animals, when the high-affinity nuclear receptor VDR (vitamin D receptor), transport proteins and enzymes for vitamin D metabolism evolved. This enabled vitamin D to regulate, via its target genes, physiological process, the first of which were detoxification and energy metabolism. In this way, vitamin D was enabled to modulate the energy-consuming processes of the innate immune system in its fight against microbes. In the evolving adaptive immune system, vitamin D started to act as a negative regulator of growth, which prevents overboarding reactions of T cells in the context of autoimmune diseases. When, some 400 million years ago, species left the ocean and were exposed to gravitation, vitamin D endocrinology took over the additional role as a major regulator of calcium homeostasis, being important for a stable skeleton.

Keywords: vitamin D ; evolution ; energy metabolism ; immune system

1. Introduction

Evolution is a dominant driver of the development of biological processes and their adaption to changes in the environment. The statement “Nothing in biology makes sense except in the light of evolution” [1] underlines that evolution is an essential component for understanding the mechanisms of these processes.

It was exactly 100 years ago that vitamin D was named a vitamin, because it is able to cure experimentally induced rickets in dogs and rats [2]. Since rickets is a bone malformation disorder in children, this and many other studies linked vitamin D to calcium homeostasis and bone remodeling [3]. However, calcium homeostasis is only one of multiple biological processes being regulated by vitamin D, such as detoxification, energy metabolism as well as innate and adaptive immunity [4]. In fact, the relationship between vitamin D and bone remodeling developed as one of the most recent evolutionary functions of vitamin D.

Furthermore, the consequences of the migration of modern humans from equatorial East Africa to regions of higher latitude will be reflected in relation to vitamin D’s possible role in skin lightening, particularly in European populations [5].

2. Evolution of Vitamin D Endocrinology

The core protein of an endocrine system is its receptor. A high-affinity receptor for vitamin D, the transcription factor VDR evolved some 550 million years ago [6]. However, contrary to its name, VDR is activated neither by vitamin D_2 nor by vitamin D_3 [7]. Carrying only one hydroxy group, both secosteroids are not polar enough to bind VDR. In fact, two hydroxylation reactions are required to form with 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) a vitamin D metabolite that offers three hydroxy groups for specific high-affinity binding to the ligand-binding domain (LBD) of VDR. This implies that the 25-hydroxylases cytochrome P450 (CYP) 2R1 and CYP27A1, as well as the 1α-hydroxylase CYP27B1, are key components of vitamin D endocrinology. They transform vitamin D_3 into 25-hydroxyvitamin D_3 ($25(OH)D_3$) and $25(OH)D_3$ into $1,25(OH)_2D_3$, respectively. Furthermore, as described for other hormones, the levels of $1,25(OH)_2D_3$ need to be tightly regulated. This happens via the 24-hydroxylase CYP24A1 that converts $1,25(OH)_2D_3$ to $1,24,25(OH)_3D_3$ and inactivates the VDR ligand in this way [8]. Despite their hydroxyl groups, all vitamin D metabolites are lipophilic and need to be carried in hydrophilic serum and cellular liquids by transport proteins. Thus, for a functional endocrine system, specific receptor(s), metabolizing enzymes and transport proteins need to evolve [9].

VDR belongs to the transcription factor family of NRs, which in humans is formed by 48 genes [10]. Comparative genomics demonstrates that the closest relatives to VDR are the NR1I subfamily members pregnane X receptor (PXR) and constitutive androstane receptor (CAR), and the NR1H subfamily members liver X receptor (LXR) α and b, as well as the farnesoid X receptor (FXR) [11]. This indicates that VDR and its five relatives have a common ancestor and that the individual receptor genes developed by whole genome duplications in early vertebrate evolution [12]. Interestingly, the six

NRs function as sensors for cholesterol derivatives, such as 1,25$(OH)_2D_3$, oxysterols and bile acids [13]. Moreover, FXR, VDR, CAR and PXR detect toxic secondary bile acids, such as lithocholic acid, and get activated by them [14][15][16][17]. This suggests that the prime function of the common ancestor of NR1H- and NR1I-type NRs was to act as a bile acid sensor. Accordingly, one of the first functions of VDR and its relatives was the regulation of genes encoding for enzymes of marine biotoxin degradation [4][18].

Detoxification reactions represent a specialized form of metabolism that allows a response to environmental conditions, such as the rise in toxic compounds. However, the most dominant environmental challenge of species is their diet, which is primarily composed of macro- and micronutrients. This created an evolutionary pressure, with the push of which the sensing of the levels of nutritional molecules like fatty acids, cholesterol and vitamins became the main function of NRs, such as peroxisome proliferator-activated receptors (PPARs), LXRs, retinoid acid receptors (RARs) and VDR [19][20]. This function is closely linked to the control of energy metabolism, which was and still is a prime task of many NRs, including VDR [21]. Accordingly, a significant proportion of the hundreds of VDR targets are metabolic genes [22][23][24][25].

Archetypical NRs were orphan receptors, as some members of the NR superfamily still are [26]. Comparative genomics suggests that in a stepwise evolutionary adaption, orphan, NRs changed critical amino acids within their LBD, so that a ligand-binding pocket got accessible to potential small lipophilic ligands. The 40 or more amino acids forming this pocket are specifically adapted to the shape and polarity of the ligand. Some 550 million years ago, this evolutionary adaptation process resulted in the first known VDR that binds 1,25$(OH)_2D_3$ at sub-nanomolar concentrations was found in the early jawless vertebrate sea lamprey (*Petromyzon marinus*) [6], meaning that VDR had evolved into a classical endocrine receptor, such as those for the steroid hormones estrogen, testosterone and progesterone. Crystal structure analysis of lamprey's VDR ligand-binding domain [27] confirmed similar binding of 1,25$(OH)_2D_3$ as identified for human VDR [28]. In vertebrate evolution, amphibians, reptiles, bony fish, birds and mammals also learned to express functional VDR proteins [29]. Most species have only one *VDR* gene, but the genome of teleost fishes underwent a third whole genome duplication and contains even two *VDR* genes [30].

Since the levels of 1,25$(OH)_2D_3$ in lamprey are similar to that in higher vertebrates, respective enzymes, such as CYP2R1 and CYP27B1, must have co-evolved with VDR [6]. Similar co-evolution also happened for the vitamin D transport protein vitamin D binding protein (encoded by the *GC* gene) [9]. This indicates that some 150 million years before the first species left the ocean and had the need for a stable skeleton, vitamin D endocrinology was already established. Thus, from an evolutionary perspective, the control of calcium homeostasis was rather a secondary than a primary goal for establishing the vitamin D endocrine system.

3. Evolution of the Physiological Functions of Vitamin D

Possible harming invaders created since the early times of life on Earth a strong evolutionary pressure for developing defense mechanisms, such as an immune system. The innate immune system is evolutionarily older and found already in many non-vertebrate species, such as insects. It involves a number of barriers, such as skin and mucosa, and uses a limited set of pattern recognition receptors that detect only general features of possible pathogens. In contrast, the adaptive immune system developed some 500 million years ago in ectothermic cartilage fishes and uses antigen receptors, such as B and T cell receptors, that have a very high affinity and specificity to their antigens [31][32].

The growth of immune cells and their function in defense and tissue repair takes significant amounts of energy [33]. Key vitamin D target genes in this context are *PFKFB4* (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4) in dendritic cells [34] and *FBP1* (fructose-bisphosphatase 1) in monocytes [35]. Therefore, the regulatory function of vitamin D and its receptor on energy metabolism were essential during the development of the immune system. Moreover, vitamin D modulates innate immunity through further target genes, such as those encoding for the antimicrobial peptide CAMP (cathelicidin) [36] and the toll-like receptor 4 co-receptor CD14 [35] in monocytes. Furthermore, in dendritic cells, which present antigens to T cells of the adaptive immune system, many genes respond to vitamin D [37]. In this way, vitamin D was and still is involved in efficient responses to pathogens, such as the intracellular bacterium *Mycobacterium tuberculosis* [38]. Moreover, the cluster of *HLA* (human leukocyte antigen) genes on human chromosome 6, many of which are vitamin D targets [25], is a "hotspot" of vitamin D-induced chromatin accessibility [39]. Thus, most non-skeletal functions of vitamin D, like the modulation of the immune system, developed before its regulation of calcium homeostasis and bone remodeling had been established.

Some 385 million years ago, the next important step in vertebrate evolution happened: some species moved from the ocean onto land and had to develop a skeleton supporting locomotion under gravitational forces [9]. At earlier times, calcified cartilage and dermal bone had already been developed by cartilage fishes like sharks. Bone fishes even had

replaced this cartilage with bone [40]. In the calcium-rich environment of water (approximately 10 mM), this transformation was not limited by calcium abundance. However, the calcium-poor conditions on land created an evolutionary pressure to tightly regulate the concentration of calcium in intra- and extracellular compartments of the body. Since the largest amounts of calcium are stored in bones, they serve as reservoirs to balance variations in the supply of the mineral by diet. In this process, vitamin D, as well as the peptide hormone PTH (parathyroid hormone), took the lead role. For example, the calcium channel TRPV6 (transient receptor potential cation channel subfamily V member 6), as well as the calcium-binding proteins CALB1 (calbindin 1) and CALB2 are encoded by vitamin D target genes [41].

Vitamin D regulates the activity of bone-resorbing osteoclasts by the cytokine RANKL, which is encoded by the vitamin D target gene *TNFSF11* (TNF superfamily member 11) [42]. Moreover, also bone mineralization is controlled by proteins encoded by vitamin D target genes, such as SPP1 (osteopontin) and BGLAP (bone gamma-carboxyglutamate protein, also called osteocalcin). Bone remodeling, i.e., the resorption of extracellular matrix by osteoclasts as well as bone formation by osteoblasts, requires, like immune functions, also a lot of energy. [43]. Thus, bone remodeling and immunity are connected via their dependency on energy metabolism [44]. Moreover, hematopoietic stem cells find in the interior of large bones, the bone marrow, a niche, i.e., a place where proliferating immune cells are effectively shielded from radiation and, in parallel, supported by calcium. Interestingly, already in bone fishes like zebrafish (*Danio rerio*) vitamin D regulates hematopoietic cell growth during embryogenesis [45]. Thus, the close connection of calcium homeostasis and bone remodeling to immunity, i.e., the co-evolution of both systems, illustrates why vitamin D shifted into this additional task.

Taken together, vitamin D evolved from one of the multiple factors controlling (energy) metabolism and immunity to a dominant regulator of calcium homeostasis and bone remodeling. This explains why bone malformations were observed as the first symptom of vitamin D deficiency.

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