INTRODUCTION
It is well established that the metabolically active form of vitamin D, 1α,25-dihydroxycholecalciferol [1,25(OH)₂D₃] plays a key role as a regulator in calcium and phosphate homeostasis. By definition, a vitamin is an essential nutritional factor. Therefore the term “vitamin D₃” is misleading and should be referred to as a hormone. The steroid hormone 1,25(OH)₂D₃ is synthesized in the skin from 7-dehydrocholesterol in reaction catalyzed by UV light. Thereafter vitamin D is transported to the liver where it is 25-hydroxylated to 25(OH)-D₃ and then 1-hydroxylated to 1,25(OH)₂D₃ in kidneys. Apart from its classical role, 1,25(OH)₂D₃ regulates gene expression in a variety of target cells and has been shown to regulate cell proliferation and differentiation [1−4]. These effects are mediated through binding to a specific, intracellular receptor protein, the vitamin D receptor (VDR) [5]. VDRs are expressed in many types of cancer cells, including cells derived from tumors of the breast, prostate, pancreas, colon, urinary bladder, cervix, thyroid, pituitary, melanoma, glioma, neuroblastoma, leukemia and lymphoma cells[6, 7]. When human myeloid leukemia cells are treated with 1,25(OH)₂D₃, they differentiate into functional monocytes and macrophages. Similarly, human histiocytic lymphoma cells differentiate in the presence of 1,25(OH)₂D₃[8]. Lappe at al [9] demonstrated that higher doses (1,100 IU) of vitamin D plus calcium significantly reduce cancer incidence. Epidemiological studies have correlated sun or UV light exposure with a lower incidence of a variety of malignancies, including breast, colon and prostate cancer. The biologically active 1,25(OH)₂D₃ and its analogs have been shown to have antiproliferative and differentiating effects in a variety of malignant and non-malignant cells. The effects of 1,25(OH)₂D₃ are mediated by the binding of calcitriol to a specific intracellular receptor, vitamin D receptor (VDR). The aim of this study is to review the literature concerning the role of 1,25(OH)₂D₃ and its analogs in squamous carcinoma cell lines of the head and neck (SCCHN).

KEY WORDS: 1,25(OH)₂D₃, vitamin D₃, squamous cell carcinoma of the head and neck

STRESZCZENIE
Badania epidemiologiczne wskazują na związek pomiędzy ekspozycją na światło ultrafioletowe a mniejszą częstością zapadania na nowotwory złośliwe w różnych lokalizacjach – sutka, okrężnicy czy gruczołu krokowego. Wykazane zostało antyproliferacyjne działanie czynnego biologicznie 1,25(OH)₂D₃ i jego analogów na komórki guzów złośliwych i łagodnych. Wpływ 1,25(OH)₂D₃ jest regulowany poprzez powinowactwo kalcytriolu do specyficznego wewnątrzkomórkowego receptora witaminy D. Autorzy pracy dokonali przeglądu literatury dotyczącej wpływu 1,25(OH)₂D₃ i jego analogów na komórki raka płaskonabłonkowego głowy i szyi.

SŁOWA KLUCZOWE: 1,25(OH)₂D₃, witamina D₃, rak płaskonabłonkowy głowy i szyi
loss of the VDR receptor may be one of the factors that either promote the progression of normal epithelium to a malignant phenotype or that cells lose their VDR expression as a result of epithelial dedifferentiation [4]. Heist et al [14] have shown also that the T allele of VDR Fok1 polymorphism and the G-T-C(Cdx2-Fok1-Bsm1) haplotype are associated with lower survival of patient with advanced non-small-cell lung cancer. That report demonstrated a relationship between VDR polymorphism and prognosis of patients with cancer.

**REVIEW AND DISCUSSION**

**CYTOLOGICAL EFFECTS OF 1,25(OH)2D3 IN CANCER CELLS**

Squamous carcinoma cell lines of the head and neck [HNSCC] respond to 1,25(OH)2D3 by inhibition of their proliferation and stimulation of differentiation in time- and dose-dependent manner. These effects are due to a blockade in the transition of cells from the G0 to S phases in the cell cycle, and occur through the nuclear VDR and interaction with vitamin D response elements [VDREs] in the regulatory region of the vitamin D target genes [15].

The antiproliferative activity of 1,25(OH)2D3 is caused by expression of the cell cycle inhibitory proteins p21 and p27. These proteins inhibit the activity of cyclin-dependent kinases in the G0/G1 cell-cycle phase, resulting in dephosphorylation of the retinoblastoma protein and cell-cycle arrest [16]. Different results have been published concerning the interaction of p21 and vitamin D3 cell-cycle regulation. It was found in several studies that vitamin D3 either had no effect on p21 protein expression [17] or was accompanied by downmodulation of p21 [18]. Other studies [19, 20] linked the antiproliferative effect of 1,25(OH)2D3 to overexpression of this important cell-cycle regulatory protein. Further investigations must be conducted to clarify this process.

Immune regulatory activities of vitamin D is also very important, because HNSCC can inhibit immune defenses. HNSCC patients have an accumulation of immune inhibitory CD34+ progenitor cells and a defect in dendritic cell differentiation [21, 22]. Furthermore, T-cells from about one-third of HNSCC patients are unresponsive to stimulation through the CD3/T-cell receptor [23]. The immune depression is caused also by suppressive mediators produced by the HNSCC cells and by the immune suppressive cells that they induce [24]. Many studies have shown that treatment with active metabolite of vitamin D-1,23(OH)2D3 delayed carcinogenesis in the hamster buccal pouch tumor model [25]. In mouse models 1,25(OH)2D3 therapy can reduce the extent of metastatic disease [26]. Vitamin D3 can activate the immune system in cancer patients and stimulate intratumoral immune infiltration [27].

Many studies showing that the inflammation is protumorigenic. Vitamin D can reduce levels of the pro-inflammatory cytokines IL-6, IL-17 [28], TNF-alfa [29] it was the aim of the study to establish the role of 1α,25-dihydroxyvitamin D3 (vit. D and COX-2 [30].

**THE VITAMIN D ANALOGUES – A WAY OF SIDE-EFFECTS PREVENTION**

The main barrier to the clinical use of 1,25(OH)2D3 has been its hypercalcemic effect. More than 800 analogues were developed in an attempt to maintain the inhibitory effect on tumor cell proliferation while reducing hypercalcemia [31,32]. One such analogue is EB1089. It is 60 times more potent than 1,25(OH)2D3 in inhibiting the growth of MCF-7 breast cancer cells in vitro and 100 times more potent than 1,25(OH)2D3 in inhibiting tumor growth in animal models of breast cancer, with only half the hypercalcemic activity [33]. Lianjun Lu et al have shown in the in vitro study that EB1089 inhibits the growth of human laryngeal SCCs. They suggested that EB1089 inhibits human laryngeal SCC via protein p57. p57 is a potent inhibitor of several G1 cyclin/CDK complexes and its overexpression leads to cell cycle arrest in G1 [34] whereas Cdk inhibitors (CKIs, p57 also controls cell cycle exit and the differentiation of lens fiber cells, placental trophoblasts, chondrocytes, and human skeletal myoblasts [35–37]. Although p57 appears to be a critical terminal effector of signal transduction pathways that control cell differentiation and proliferation, its precise involvement in differentiation and proliferation in response to cytotoxic drugs or during tumorigenesis is unknown. Interestingly, p57 gene expression is also reduced in human laryngeal cancers [38], reinforcing a possible function of this protein in tumorigenesis.

In an in vitro model Chiang et al. have shown that the new generation of vitamin-D analog MART-10 has higher VDR transactivation activity than D-1,23(OH)2D3. MART-10 was also approximately 100-fold more potent in repressing cells growth through cell cycle arrest at the G0/G1 phase. They have revealed that the telomerase expression in the experimental cell line was significantly reduced after MART-10 and D-1,23(OH)2D3 treatment. In the in vivo study they have shown higher anti-cancer effectiveness of MART-10 in comparison to D-1,23(OH)2D3 [39].

Yang et al. have proved that MART-10 and D-1,23(OH)2D3 can effectively inhibit a metastatic process of HNSCC. This aim may be achieved by repressing Snail and Twist expressions. Both molecules play a role in EMT process (epithelial-mesenchymal transmission), which is an important step for cancer cells to become invasive [40]. In addition EMT process is partially responsible for the resistance to chemotherapy and radiotherapy [41–43]. Zhao et al. have demonstrated the increased level of Snail in the laryngeal squamous cell carcinoma (LSCC) cells and proved that knockdown of Snail can significantly inhibit the ability of adhesion, migration and invasion of the cancer cells. In the same study they have investigated the level of parameters, which are crucial for the EMT process such as Vimentin, E-cadherin, N-cadherin, Integrin, β-catenin. Increased level of all of the factors was detected in LSCC cells. Moreover they have proved that Snail silencing can lead to remarkable inhibition of expression of all of them.

Yang et al. have noticed that the inhibition of metastatic process was implemented by reversing cadherin switch...
(upregulation of E-cadherin and downregulation of N-cadherin). Low E-cadherin and high N-cadherin expression was previously linked with increased invasiveness of cancer cells [44] throughout adult life and in some pathological conditions. Cadherins, and more specifically the neural cell adhesion molecule N-cadherin, play an important role in migration. In embryogenesis, N-cadherin is the key molecule during gastrulation and neural crest development. N-cadherin mediated contacts activate several pathways like Rho GTPases and function in tyrosine kinase signalling (for example via the fibroblast growth factor receptor. Authors have also confirmed that MART-10 and D-1,23(OH)D₃ inhibit the expression of collagenases MMP -9 and MMP-2 in HNSCC cells which is another factor responsible for invasive potential of cancer cells.

THE CORRELATION BETWEEN VITAMIN D SERUM LEVEL AND CLINICAL DATA IN HNSCC PATIENTS

Bochen at al. have compared vitamin D serum level in 231 HNSCC patients with 232 healthy controls matched for age and sex. The results have shown that vitamin D serum level in HNSCC patients was notably lower. The authors have excluded influence of general malnutrition status on that result. In addition they have revealed a significant correlation between low vitamin D serum level and a positive lymph node status. Follow up time has manifested an influence of Vitamin D on overall survival (OS), which was significantly shorter in HNSCC patients with low vitamin D.

Bochen at al. have also investigated the influence of Vitamin D serum level on the anti-tumor activity of the immune system. Significantly higher infiltration of CD3+ T cells, helper T cells, cytotoxic T cells, NK cells, CD 86+ macrophages and M1 macrophages was observed in the tumor tissues of vitamin D high patients. On the other hand the infiltration of M2 macrophage (CD 163+) was notably lower in the tumor tissue of the Vit D high HNSCC patients [45] it is known that vitamin D can stimulate the patients’ antitumor immunity. However, valid epidemiological data for head and neck squamous cell carcinoma (HNSCC). It is well known that all of the immune cells play an important role in the anti-tumor activity of the immune system [46–48]. In addition Bochen at al. have shown that the infiltration of CD3+ T cells, helper T cells, cytotoxic T cells, NK cells, CD 86+ macrophages and M1 macrophages is associated with longer overall survival, whereas the infiltration of the of M2 macrophage (CD 163+) is correlated with shorter OS.

INDIRECT EFFECTS OF 1,25(OH)₂D₃ IN THE ANTI-TUMOR THERAPY

As mentioned above one of the potential actions of vitamin D in the therapy of HNSCC is its role in the augmentation of chemotherapy and radiotherapy. Latest studies have shown that this effect may be achieved also in case of Erlotinib therapy, which is currently undergoing clinical evaluation for its chemopreventive potential in head and neck cancer patients [49–51]. Activation of epidermal growth factor (EGFR) is an early stage of the neoplastic process in HNSCC [52] the epidermal growth factor receptor (EGFR, 53). Erlotinib acts through inhibiting EGFR and due to this fact has a significant potential in chemoprevention [54]. In mouse model D-1,23(OH)D₃ has been shown to augment the inhibition of the EGFR activation in a combination treatment including erlotinib and D-1,23(OH)D₃. The mechanism of this action depends on inhibition of phosphorylation of Erk and Akt, which is one of the stages in EGFR activation cascade [49].

CONCLUSIONS

Vitamin D due to its pleomorphic effect can play an important role in many different processes. An influence on all of the mentioned prooncogenic processes gives a possibility to use Vitamin D in the additional therapy of HNSCC. A supplementation of Vitamin D may improve a therapy primarily in those patients, whose natural production of D-1,23(OH)D₃ is low. It is important to take into account, that high doses of Vit D may lead to clinically relevant side effects. The correlation between serum level of Vit D and the aggressiveness of HNSCC is not precisely defined. More research is required to assess an impact of Vit D on HNSCC patients who are currently undergoing a standard therapy.

REFERENCES

THE ROLE OF 1,25(OH)₂D₃ AND ITS ANALOGS IN PROLIFERATION AND DIFFERENTIATION OF SQUAMOUS CELL...

Conflict of interest
All authors declare no conflict of interest

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