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Vitamin D as a promising anticancer agent

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Abstract

Presence of vitamin D receptors in noncalcemic tissues and subsequent identification of its involvement in growth factor(s)-mediated cellular function suggested its probable beneficial role in genesis, progression and survival of cancerous growths. Data collected from both in vitro and in vivo studies are highly optimistic regarding its potential in prevention and regression of colorectal, prostate and breast cancers. The vitamin has been found to interfere with the transduction pathways of various growth factor(s)-activated receptors (receptor tyrosine kinases) thereby modulating transcription and alteration of genomic functions resulting in inhibition of cell proliferation and angiogenesis and facilitation of cell differentiation and apoptosis. It also increases the level of an endogenous protein - cystatin D, which possesses antitumor and antimetastatic property, by facilitation of the expression of the gene coding for it. Though not as a primary anticancer agent, this vitamin may be used for the prevention of cancer and included as an adjuvant in combination chemotherapy for the treatment of cancer.

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Full Text

Introduction

Vitamin D is well known as being essential for bone health. [1] It is a group of fat-soluble prohormones, which are converted in the body into a number of biologically active metabolites that function as true hormones, circulating in the blood and regulating the activities of various cell types - both calcemic and noncalcemic. Their known important action is the maintenance of plasma Ca 2+ concentration by increasing Ca 2+ absorption in the intestine, mobilizing Ca 2+ from bone and lowering its renal excretion.

The nuclear calcitriol (the metabolite of vitamin D which mediates its action) receptors have been detected in both calcemic and noncalcemic tissues (except liver) which play important roles in the functioning of many cell types. [2] When activated, they not only control calcium metabolism but also elicit a wide variety of biological responses,

which influence cellular growth, proliferation, apoptosis, and functioning of immune system. [1] Recently, this growth modulating property of calcitriol is subjected to intense research in the field of cancer prevention and inhibition. [3] In this review article, attempt has been made to present the results of research work undertaken by several investigators using vitamin D as an anticancer agent with particular emphasis on its mechanism(s) of anticancer action.

Anticancer Actions of Vitamin D

Anticancer property of vitamin D has been studied in a wide variety of commonly occurring cancers (both in vitro and in vivo) of which the actions on colorectal, breast and prostate cancers have been found to be most promising. [4],[5],[6],[7],[8],[9],[10],[11],[12],[13],[14]

Mechanism of Anticancer Action of Vitamin D

The principal methods of treatment of cancer include surgery, irradiation and chemotherapy, usually given in combination depending on the type and stage of the disease. Though early treatment is curative in many of the cancers, early diagnosis is not possible in majority of cases and the disease still remains incurable in spite of all sincere and rational approaches for treatment. Moreover, the after effects of surgery and irradiation along with serious adverse effects of chemotherapy further limit the initiation and continuation of therapy.

Recent approaches for cancer treatment

The rapidly growing understanding of biological processes governing carcinogenesis has helped to exploit biological derangements unique to cancer cells and to identify novel cellular targets for anticancer drugs. Some of these include - matrix metalloproteinase inhibitors to inhibit invasion and prevent metastasis, inhibitors of angiogenesis, which prevent new blood vessel formation essential for tumor growth, signal transduction inhibitors to interrupt the critical signaling pathways essential for cell growth and proliferation, differentiation agents to keep the neoplastic cells in a stage where they have little or no proliferative potential, designer molecules, designed to inhibit dysregulated tyrosine kinase hyperactivity and proapoptotic agents which possess direct lethal effect on cancer cells or enhance the cytolytic effects of anticancer agents. [15]

Chemoprevention of cancer

Since many cancers are currently incurable, attempts may be made to prevent their occurrence, if possible. In this respect, life style change may significantly reduce the risk of developing certain types of cancer. Moreover, chemoprophylaxis may be considered for the population as a whole, or for groups at high risk of a specific cancer. Intake of some vitamins and derivatives and dietary micronutrients like beta-carotene, isotretinoin, folic acid, ascorbic acid, and alpha-tocopherol, may inhibit the development of cancer. Large-scale trials in this respect are in progress. [16]

Cancer and vitamin D

It has been observed that calcitriol is not only synthesized (from its precursor) in the kidney but also in several other tissues of the body including prostate, colon and breast with the help of the enzyme 1-OHase (for the conversion of calcifediol to calcitriol) which is expressed on them. Such an observation indicates that besides calcium mobilizing function, this vitamin also influences other body functions like regulation of cellular proliferation and differentiation, modulation of immune functions and vascular tone and influences on renin and insulin secretion which are unrelated to calcium metabolism.

It has been observed that regular sun exposure is associated with substantial decrease in certain cancer death rates as well as a reduction in overall cancer mortality rates, which may be related to body's vitamin D metabolic pathway. In more cloudy northern latitudes of US, a prostate, breast and colon cancer belt has been documented to occur 2-3 times more commonly than the sunnier regions. [3]

Some ecological studies indicate that sunlight may protect against prostate, colorectal, ovarian and female breast cancers. Several analytical investigations also suggest a protective association between circulating vitamin D and colorectal as well as prostate cancer. [17] Recent epidemiological data showed a strong correlation between poor

vitamin D status (i.e., serum calcifediol levels below 50 nmol/ l) and higher risk for chronic illnesses of various etiology. [18] Lack of sun exposure and vitamin D deficiency has been associated with many serious chronic diseases including some deadly cancers. [10] It is now recognized that maintaining a serum calcifediol concentration of

80 nmol/ l (32 ng/ ml) or more is useful in the prevention of osteoporosis, CVS diseases, certain autoimmune diseases and some forms of cancers. A search of primary and review medical literature published between 1970 and 2007 has supported the hypothesis that calcitriol has significant protective effect against development of cancer. Epidemiological studies have found an inverse relationship between sun exposure/ serum concentration of calcifediol/ vitamin D intake and risk of developing and/ or surviving cancer. [3] It appears that sensible sun exposure and use of vitamin D supplements are the most effective steps to prevent vitamin D deficiency. [18] In addition to cancer protection, several workers have found vitamin D to induce death of cancer cells both in vitro and in vivo. [10]

Besides cancer prevention and cell death, vitamin D can also cause tumor growth retardation as well as tumor regression because of its angiogenesis inhibitory action, which deprives the cancer cells of their nutrients and oxygen for growth and survival. Moreover, the young and immortal cancer cells never grow up, mature and die off and vitamin D derivatives are known to promote normal cell growth and maturation (differentiation) which are antagonistic to the nature of the cancer cells. [3] Because of these antitumor activities, some researchers propose that vitamin D supplementation may be beneficial in the treatment and prevention of some types of cancer. [19]

The mechanism of anticancer action of vitamin D at cellular level has not been fully understood. It has been shown that cells, including cancer cells, express specific nuclear vitamin D receptors (VDRs) for its active metabolite calcitriol. These receptors belong to the superfamily of nuclear receptors, which include receptors for steroid/ thyroid hormone. [20] Agonist (calcitriol) binding to VDR induces a conformational change that activates the VDR which in turn dimerizes with the nuclear retinoic X receptor. The heterodimer then binds to vitamin D response elements (VDREs) in the promoters of target genes and promotes their transcription resulting in alterations in phosphocalcic metabolism or regulation of cell division, differentiation and cell death. [21] When bound to VDR, calcitriol has been found to regulate the activity of more than 60 genes leading to prodifferentiating, antiproliferative and antimetastatic effects on cells in addition to effects on cell cycle and angiogenesis (antiangiogenic). [7],[22],[23],[24]

Thus the non-calcium mobilizing functions of vitamin D are associated with cell proliferation, differentiation, apoptosis and angiogenesis. In this article, an attempt has been made to discuss briefly the above cellular processes occurring in normal as well as cancer cells and the modulatory role of vitamin D on them.

Cell proliferation, differentiation and tumor growth

Cell proliferation is a physiological necessity during growth and repair but becomes pathological in certain cases of cellular hypertrophy and hyperplasia and in all cases of tumor growth, whether benign or malignant. For proliferation, the cell passes through several stages of cell cycle during which all its components are replicated and finally the cell divides itself into two identical daughter cells. Although the physiological and pathological cell proliferations are almost identical, minor differences in them do exist which permit selective drug action while treating cancers. Cancer cell growth behavior (persistent proliferation and continued cell division) and their increased susceptibility for apoptosis after chemotherapy are also responsible for such selectivity. [25]

A proliferating cell passes through highly ordered phases of G 1 , S, G 2 and M in sequence, of which 'S' (phase of DNA synthesis) and 'M' (phase of mitosis) are critical. Entry into them (onset of S and M) is strictly regulated giving rise to two 'check points' in the cycle. DNA damage due to any cause can arrest the cell cycle at any one of these phases and precise progress of the cycle through them determines the genetic stability of the cell. [26],[27]

Once adulthood is attained, though some of the cells go on dividing constantly (bone marrow cells, gastrointestinal epithelial cells), most of them do not do so; they remain in a static phase (G 0) outside the cycle for a varying period till subjected to a suitable stimulus. In response to the stimulus, the dormant cell in G 0 phase is recruited into the G 1 phase and the cycle continues. [26]

Out of several such stimuli, growth factors are most important, which upon combining with the corresponding cell surface receptors lead to the production of two types of signal transducers, one type being positive regulators of the cell cycle which regulate the changes necessary for cell division while the other type consists of negative regulators which control the activity of the positive regulators. Therefore, the functional balance between these two groups of regulators along with 'apoptosis' is responsible for the maintenance of normal cell number in a particular tissue. [25],[26],[27]

Cell surface receptors mediating the action of growth factors and cytokines belong to the family of receptor tyrosine kinases (RTKs). Ligand binding causes receptor dimerization and subsequent autophosphorylation of their intracellular tyrosine residues, which in turn phosphorylate other intracellular proteins thereby forming the signal transduction cascade leading to formation of cell cycle regulators which control cell proliferation, differentiation and division. [26],[27] Two such positive regulators are - 'cyclins' and 'cyclin dependent kinases' (CDKs). The inactive cdks get activated by cyclin and phosphorylate the necessary substrate proteins (determined by cyclin) that participate in the continuation of cell cycle. One such signal transduction system involves Ras/ Raf pathway (Ras is a proto-oncogenic product and Raf is a serine/ threonine kinase) which activates the kinase cascade by sequential phosphorylation of the kinases like Raf, Src, MAP (Mitogen-activated protein) and ERK 1/ 2 (Extracellular signal-regulated kinases 1 and 2) leading to gene expression resulting in the progression of the cell cycle and ultimate cell division. It has been observed that constitutive activation of RTK as well as other nonreceptor kinases do occur in many cancers in association with mutations in the genes coding for proteins involved in the kinase cascade. [26],[27] Activity of cyclin/ CDK complex is modulated by various negative cell cycle regulators acting at one or other of the two check points mentioned earlier. [26]

The two daughter cells, after cell division, are in G 0 stage till they are acted upon by growth factors and cytokines to enter into the G 1 phase. During this stage, another regulatory protein - the retinoblastoma (Rb) protein - is in a hypophosphorylated state due to low concentration of cyclin D. The hypophosphorylated Rb protein, by binding with the transcription factors E2F, prevents its (E2F) controlling function on gene expression that code for cyclins E and A, DNA polymerase, thymidine kinase, dihydrofolate reductase, etc. - thereby holding the cell cycle in check at check point 1 and thus depriving the proliferating cell of essentials required for DNA replication during 'S' phase. [26],[27] While in G 1 phase, the cell synthesizes mRNAs and proteins required for DNA replication during the subsequent S phase. Growth factor action increases the concentration of cyclin D via Ras/ Raf kinase cascade activation and cyclin D/ cdk 4 and 6 complex cause phosphorylation of the Rb proteins. This in turn releases the transcription factors E2F to activate the genes for synthesis of components required for DNA synthesis during 'S' phase. [25],[26],[27],[28]

In several cancer cells including breast cancer cell lines, calcitriol has been found to cause dephosphorylation of the Rb gene product, leading to blockade of cell cycle at check point 1 and hence tumor growth inhibition. [29] In a study involving carcinoma of breast, investigators have demonstrated that calcitriol inhibits Src tyrosine kinase in Ras/ Raf kinase cascade, thereby leading to subsequent decrease in activity of ERK 1/ 2 and MAP kinase, this in turn reduces the formation of cyclin/ cdk proteins, thereby leading to inhibition of cell proliferation and alteration of differentiation in G 1 and other phases of the cell cycle (MAP kinase plays an important role in cell proliferation and differentiation). [30]

During 'S' phase, cyclin/ cdk proteins - cyclin E/ cdk 2 and cyclin A/ cdks 1 and 2 , continue to phosphorylate and activate the necessary proteins/ enzymes taking part in DNA synthesis; phosphorylating action of cyclin A/ cdks 1 and 2 is further continued throughout G 2 and M phase. [25],[26],[28] As the cell cycle progresses through 'G 2 ' phase, other cellular components are duplicated with the help of newly synthesized mRNA and protein which requires the active and promoter action of cyclin/ cdk complexes (cyclin A/ cdks 1 and 2 and cyclin B/ cdk 2). Otherwise, cell proliferation will be checked at check point 2. [25],[26],[28]

The proliferating cell undergoes division during the subsequent 'M' phase giving rise to two identical daughter cells that are in G 0 phase. Out of these, those which are destined for further proliferation, (cancer cells and certain normal cells which proliferate continuously) are activated to do so by the growth factors and cytokines while the rest remain quiescent till they are activated. [25],[26],[28]

The essential physiological cell proliferation as well as the proliferation for wound healing and repair is restrictive in nature due to the activity of negative regulators of the cell cycle which regulate the activity of positive regulators (cyclin/ cdk complex action), whereas unrestricted cell proliferation is the hallmark of cancer. Besides Rb protein, there are two other families of negative regulators of the cell cycle - cdk inhibitory protein (CIP) family, comprising of proteins p21, p27 and p57 and the inhibitors of kinases (InK family) having proteins p16, p19, and p15. cdk inhibitors (CDKIs) bind with cyclin/ cdk complexes and inhibit their proliferation promoting actions at various phases of the cell cycle, particularly at check point 1 and InK proteins inhibit the kinase activation. The p53 gene codes the transcription factor p53 protein, which is present in low concentrations in healthy cells. But DNA damage due to any cause increases its concentration, which in turn activates the transcription of several other

genes including the one, which codes for the protein p21. Once formed, p21 causes inactivation of cyclin/ cdk complexes (cyclin-cdk 2 or -cdk 4) thereby preventing Rb phosphorylation and inhibition of cell cycle progression through G 1 phase resulting in arrest of cell cycle at check point 1. As the cycle does not progress, the damaged DNA gets adequate time for its repair by the DNA repair system. If successful, the cycle overcomes the blockade at check point 1 and enters into S phase. Failure of DNA repair leads to apoptosis. [26],[31],[32],[33],[34] Attempts have been made to amplify such negative regulation thereby bringing a halt to the unrestricted proliferation of cancer cells.

Vitamin D has been found to inhibit cancer cell proliferation by increasing the activity of the CDKIs p21 WAF 1/ Cip 1 and p27 Kip 1 . [21],[35] Audo et al. [21] have observed vitamin D to induce cell cycle arrest at G 1 phase which may be due to upregulation of one or both of the above-mentioned CDKIs. They have found a VDRE to be present in the promoter of p21 gene, which indicates that vitamin D may directly activate the transcription of p21. But they did not find any VDRE in p27 gene, whose upregulation seemed to be more cell-type dependent. In another study, Maruyama et al. [36] have demonstrated that VDR gene is upregulated by p53 in a variety of cancer cell lines. Their data suggest that VDR may play a novel role in p53 signaling pathway in which there may be increased sensitivity of the cancer cells to the antiproliferative action of vitamin D due to increased levels of VDR. These authors have also shown that in high-grade carcinomas, VDR is down regulated, which may be due to alteration in the activity of p53.

In estrogen receptor positive MCF-7 cells (a cell line of human adenocarcinoma), calcitriol analogues have been found to decrease the expression of c-myc (a proto-oncogene), a transient induction of c-fos gene (another proto-oncogene) and stimulation of p53. [29],[37]

Out of several growth factors, epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation and differentiation by binding to its receptor EGFR, the transduction pathway being the Ras/ Raf pathway leading to activation of kinase cascade which in turn stimulates the production of nuclear transducers (early and late response genes) resulting in the production of cell cycle regulators, both positive (cyclin and cdk) and negative (p53 protein, Rb protein, cdk inhibitors p21 and p27, etc.). [38] Besides this, enhanced tyrosine kinase signal transduction cascade also results in a variety of biochemical changes within the cell - a rise in intracellular calcium level, increased glycolysis and protein synthesis and increased expression of certain genes including the gene for EGFR. [39] Hence, EGF is considered as a procarcinogen. Cross et al. [40] applied a proliferative signal via EGF to Caco-2 cell (an epithelial colorectal adenocarcinoma cell line) and found that EGFR activation was associated with downregulation of VDR which might allow the colon carcinoma cells to escape the antimitogenic action of calcitriol. But, such downregulation was counteracted by the unique property of Caco-2 cells, which are capable of synthesizing calcitriol from calcifediol. Thus, these workers have shown that the negative effect of EGF on VDR abundancy can be counteracted by calcitriol thereby bringing a slow down of cell proliferation and tumor growth.

Moreover, it has been observed that insulin-like growth factor (IGF) stimulates the growth and multiplication of cancer cells which is interfered by vitamin D. [41]

Bouillon et al. [42] have demonstrated calcitriol to induce differentiation and inhibit proliferation in a wide variety of cells. Though the authors found vitamin D to modify the functioning of important cell cycle regulators at molecular level (functioning of cyclin/ cdk system, CDKIs, E2F transcription factors), they could not determine the precise hierarchical structure of this wide diversity of calcitriol actions.

The role of cysteine cathepsins (cysteine proteases belonging to cathepsin family) in causing colon cancer progression and metastasis has been established (cause proteolytic cleavage of matrix components, adhesion proteins and other proteases in addition to interference in cell cycle at nuclear level and resistance to chemotherapy). [43] On the other hand, the protein cystatin D (belongs to cysteine superfamily) has been found to possess multiple actions which are antitumorigenic and antimetastatic. It inhibits these endogenous cysteine proteases (endosomal/ lysosomal), has some modulatory role in cell proliferation, differentiation, survival and migration in addition to interleukin and nitric oxide production. It also inhibits migration and anchorage - independent growth, antagonizes Wnt/β-catenin signaling pathway (Wnt is an oncogene which codes for the growth factor Wnt), represses c-myc and expression of a number of epithelial-mesenchymal transition inducers (SNAI1, SNAI2, ZEB1 and ZEB2) which are involved in the progression of primary tumors towards metastasis. The protein (cystatin D) induces E-cadherin expression which is repressed during epithelial-mesenchymal transition period. [43] (E-cadherins are calcium - dependent adhesion molecules which inhibit cancer progression and metastasis by increasing the strength of cellular adhesion within a tissue, thereby decreasing cellular motility).

The gene CST5, which has been found to be downregulated during colon tumorigenesis, codes cystatin D formation. Binding of calcitriol to VDR has been found to activate CST5 promoter in the DNA leading to increased CST5 RNA and hence increased cystatin D levels in colon cancer. [43] In addition, a study conducted by Dr. S.

Chistakos reported that calcitriol has been found to induce a tumor suppressing protein (cystatin D) that can also inhibit the growth of breast cancer cells. [44]

Some investigators, in a gel shift assay, have shown that constitutively expressed proto-oncogene c-fos could hinder the association of vitamin D + VDR complex with VDRE in DNA. This observation suggests that c-fos gene product c-Fos may inhibit the binding of VDR + vitamin D complex to VDRE by making a c-Fos-VDR complex. [45]

Apoptosis and tumor growth

Apoptosis is a genetically programmed well-organized built-in self-destruct mechanism, where sequence of biochemical events lead to cell death and its removal without inducing inflammatory reactions as occurs in necrosis. Besides its physiological function in maintaining constancy of cell numbers in different tissues, apoptosis also prevents the possibility of mutational changes leading to malignancy after DNA damage by removal of such damaged cells. As dysregulated apoptosis has been found to be involved in the pathogenesis of many forms of cancers, understanding of the mechanism of apoptosis and its dysregulation processes offers scope for novel approaches to treatment of cancer. [16]

The process of apoptosis is effected by caspases, which are a family of cysteine proteases present in the cell in inactive nonfunctional form. [26],[46] When activated, they cause selective proteolysis of target proteins (enzymes, structural components, etc.), thereby activating some and inactivating others. Out of nine different caspases, some are 'initiators' while others are 'effectors' of apoptosis. Apoptotic initiating factor (AIF) is another protein, which after getting released from mitochondria, enters the nucleus and switches on the process of apoptosis. [26]

There are two pathways for caspase-mediated apoptosis; one is death receptor pathway and the other is mitochondrial pathway. In death receptor pathway, the transmembrane TNFR (tumor necrosis factor receptor) family functions as death receptors, each of which has a death domain in its cytoplasmic end. Ligand [TNF or TNF-alpha-related apoptosis-inducing ligand (TRAIL)] binding leads to trimerization of the death domains which in turn recruits and gets complexed with an adaptor protein; the complex subsequently activates the initiator caspase 8. Activated caspase 8 finally activates the effector caspases leading to apoptosis. The mitochondrial pathway is also activated when there is DNA damage or there is withdrawal of the actions of cell survival factors (tissue-specific trophic factors, cytokines, hormones, adhesion molecules, integrins, etc.). [26],[27]

DNA repair after its damage is mediated mainly by p21 proteins (mentioned earlier). If such repair does not occur, p21 protein becomes proapoptotic along with another proapoptotic protein Bcl-2 (Bcl-2 associated X protein or BAX) coded by p53.

Bcl-protein family has got another antiapoptotic branch. The proapoptotic BAX component of the Bcl-2 competes with the antiapoptotic Bcl-2 on mitochondrial cell surface for release of cytochrome c from it; the proapoptotic branch tends to stimulate the release, while the antiapoptotic branch inhibits it. Cytochrome c, released by BAX, combines with the protein apoptotic protease activating factor-1 (Apaf-1) and the complex thus formed, interact with activated procaspase 9 which in turn activates the effector Caspase pathway. [26],[27]

In normal cells, survival factors continuously oppose apoptosis by several mechanisms, out of which activation of antiapoptotic Bcl-2 is important. Withdrawal of action of these factors due to any cause leads to a loss of balance between the two antagonistic Bcl-2 proteins, making proapoptotic Bcl-2 action unopposed and hence induction of apoptosis resulting in cell death which are eventually phagocytosed by macrophages. [26],[27] Hence, dysregulated apoptosis, besides other harmful effects, can lead to cancer cell proliferation and their resistance to chemotherapy (apoptosis plays an important role in chemotherapy-induced cancer cell death). Thus, proapoptotic compounds may have a favorable role in the prevention of cancer development, growth and metastasis while aiding to its chemotherapy.

Audo et al. [21] have found vitamin D analogues to attenuate retinoblastoma tumor growth in athymic mice by increasing apoptosis which was associated with upregulation of both p53 and p21. The same authors also applied vitamin D to cell lines of glioma and carcinoma breast and found it (vitamin D) to induce apoptosis in both of them. In glioma cell lines, there was upregulation of BAX (proapoptotic) with downregulation of antiapoptotic Bcl-2, which indicated that vitamin D-induced apoptosis was mediated through p53 pathway. But, no such involvement of Bcl-2 proteins was found in cancer cell lines, which indicated the apoptosis to be p53 independent. These observations suggested that mechanism of vitamin D-induced apoptosis varies with the cell type and can be mediated by both the pathways - p53-dependent as well as independent. They also concluded that tumor growth attenuation with vitamin D in glioma cell lines is due to apoptotic cell death rather than reduction in tumor cell proliferation.

Calcitriol has been found to decrease Bcl-2 expression in breast cancer cell lines. [29]

Angiogenesis and tumor growth

Angiogenesis refers to the growth of blood vessels from pre-existing vasculature. Although stimulators of angiogenesis are receiving growing attention for treatment of diseases of CVS, inhibitors of angiogenesis, at present, are the focus of research due to their ability to slow tumor growth and progression. Like certain other disease processes (arthritis and diabetic retinopathy), tumor growth, besides other factors, depends on neovascularization or tumor angiogenesis. Tumors (both primary and metastatic) do not grow beyond 2-3 sq.mm. and cannot metastasize unless adequately vascularized. Hence, regulation of angiogenesis carries tremendous potential for cancer therapy. [47]

Process of tumor angiogenesis begins when the 'angiogenic switch' gets 'on' due to a tilting of the balance between pro- and anti- angiogenic factors in favor of angiogenesis, the imbalance being induced by the effects of the tumor cells on surrounding environment which include hypoxia, inflammation, altered tumor cell gene expression, etc. Loss of balance between angiogenic regulators (vascular endothelial growth factor or VEGF and integrins) leads to tumor vessel structural and functional abnormality. Variable blood flow through abnormal vessels favors selection of tumor cell variants, which are resistant to hypoxia- as well as chemotherapy-induced apoptosis, often due to loss of p53 expression. Structural abnormality increases vascular permeability leading to hypertension within the tumor interstitium, which in turn hinders the delivery of chemotherapeutic agents to the tumor cells. Moreover, lack of perivascular cells as well as vascular smooth muscle cells leads to loss of vascular autoregulation inherent to normal blood vessels. [26],[27]

Tumor angiogenesis is a multistep process during which highly proliferating endothelial cells (ECs) express receptors for growth factors and other endogenous molecules required for the process. Variable blood flow in tumor vessels and increased demand of oxygen by rapidly proliferating tumor cells leads to development of hypoxia in the tumor interstitium which is a strong stimulus for secretion of trophic VEGF and other molecules by tumor cells. These trophic factors induce sprouting by proliferation and integration of EC into the tumor. Sometimes, tumor cells also grow around the host blood vessels and thrive there by coopting the local blood supply, thereby making the vessel wall inhomogeneous consisting of ECs and tumor cells (tumor derived endothelial cells or TDECs). Three important families of RTKs expressed on ECs and their ligands (VEGF, angiopoietins and ephrins) regulate angiogenesis. The process begins with the activation of ECs by growth factors followed by their proliferation and migration through the degraded extracellular matrix (by proteases) into the tumor, resulting in sprouting and finally formation of new capillary tubes. [26],[27]

Hypoxia causes transcriptional induction of the gene encoding VEGF whose receptors (VEGFR 1 , VEGFR 2 and VEGFR 3) belong to the family of RTKs, VEGF-induced angiogenesis is mediated through VEGFR 2. Besides Ras/ Raf transcription pathway, RTK-mediated cell proliferation is also mediated by activation of the serine/ threonine kinase Akt, which phosphorylates target proteins responsible for promotion of resistance to apoptosis and cell cycle progression. [26],[27]

Other ligands for RTKs are angiopoietins, secreted by stromal cells. Out of the two angiopoietins (Ang1 and Ang2), Ang1 imparts quiescence and stability to the mature blood vessel via Ang1/ Tie2 pathway and hence antagonizes tumor angiogenesis. On the other hand, Ang2 destabilizes the vasculature and thereby makes the host ECs more sensitive to the angiogenic signals coming from tumor cell-derived VEGF. Thus, Ang2 favors tumor angiogenesis. [26],[27]

Calcitriol has been found to interfere with EC activation, proliferation, migration, sprouting and tube formation with participation of intracellular signaling pathways. [23],[48] Bernardi et al. [23] have found calcitriol to inhibit the growth of tumor-derived endothelial cells (TDECs) in two tumor models at nanomolar concentrations. This may help to restore the homogeneousness of ECs in vessel wall thereby bringing the vessel architecture and function more towards normalcy and hence prevention of tumor angiogenesis and facilitation of chemotherapy- as well as hypoxia-induced tumor-cell apoptosis. They also found calcitriol to increase the number of VDRs and the level of apoptogenic protein p27 (Kip 1) in TDECs, thereby making them more vulnerable to its antiangiogenic and apoptotic action. The same authors have also investigated the action of calcitriol on VEGF-induced TDEC proliferation and found the vitamin to reduce phospho-ERK 1/ 2 and phospho-Akt levels in them. Such observation shows that calcitriol interferes with both the proliferation - transduction pathways induced by RTKs, thereby inhibiting angiogenesis. These researchers also studied the effect of calcitriol on angiogenic signaling molecule Ang2 in squamous cell carcinoma and radiation-induced fibrosarcoma-1 cells where the vitamin caused a reduction in Ang2 level. From all these study results, Bernardi et al. [23] have concluded that calcitriol and its analogues directly inhibit TDEC proliferation at concentrations comparable to those required for inhibition of tumor cells,

thereby modulating the cell cycle as well as cell survival signals in TDEC, resulting in inhibition of tumor angiogenesis.

Matrix metalloproteinases (MMPs), secreted by stromal cells and tumor associated macrophages, play an important role in tissue remodeling associated with various physiological and pathological processes and play a major role on cell behavior like proliferation, migration, differentiation, angiogenesis, apoptosis and host defense. They mediate these functions by activation/ inactivation of the chemokines, cleavage of cell surface receptors and release of apoptotic ligands (FAS ligand). [26],[27]

During tumor angiogenesis, initially, MMPs cause degradation of extracellular matrix (ECM) and disruption of capillary basement membrane which is essential for EC migration and invasion into the ECM followed by other steps of the process.

Interleukin-8 (IL-8 or CXCL8), secreted by several cells including the tumor cells, interacts with its receptors CXC-chemokine receptors 1 and 2 (CXCR1 and CXCR2) expressed on both normal and tumor cells. Activation of these receptors on ECs modifies the process of angiogenesis. [49] Bao et al. [24] have found PC cells to increase human umbilical vein endothelial cell migration and tubule formation (two critical steps in angiogenesis) which was due to secretion of IL-8 by them. By mechanistic dissection, they have shown calcitriol to inhibit IL-8 formation by PC, the action being exerted at the nuclear level where the vitamin inhibits the upstream signal nuclear factor kappa-light chain-enhancer of activated B cells (NF- k B) for IL-8 synthesis. From PC tissue microarray analysis, these authors have found that IL-8 expression is elevated during PC progression. Such finding made them to suggest that IL-8 may play a role in tumor progression mediated through its stimulatory action on angiogenesis and PC progression can be halted by interrupting IL-8 signaling pathway with the help of calcitriol administration.

Vitamin D-binding protein (DBP), a transporter of vitamin D in the plasma, is also the precursor for the principal macrophage activating factor (maf) and is converted to DBP-maf. [50] Macrophages, besides being proinflammatory, are also strong protagonists of angiogenesis and hence their activation by DBP-maf is expected to stimulate tumor angiogenesis. But, macrophages, in addition to their proinflammatory action, also possess phagocytic action, which is exerted on ECs and tumor cells leading to their destruction and hence inhibition of angiogenesis and tumor growth. DBP-maf, in addition to macrophage activation, has also been shown to inhibit tumor growth in vivo, which is thought to be due to its antiangiogenic action. The compound has been found to inhibit human endothelial cell (HEC) proliferation by significantly inducing S- and G 0 / G 1 phase arrest in 72 h. This action was found to be due to inhibition of VEGF signaling, where DBP-maf decreases the VEGF-mediated phosphorylation of VEGFR 2 and ERK 1/ 2, leading to inhibition of kinase cascade activation. It was also found to inhibit DNA synthesis during S phase. These studies collectively demonstrate that antiangiogenic property of DBP-maf is due to blockade of critical steps such as HEC proliferation, migration, tube formation and microvascular sprouting which seems to be due to its direct inhibitory action on EC and EC destruction mediated by macrophage activation. [48] Tube formation assay, conducted by Fannon, [51] has shown a synergistic inhibitory action on endothelial tube formation when vitamin D was administered with DBP-maf. This may be due to formation of a more efficient moiety of vitamin D with DBP-maf resulting in its better cell surface binding leading to amplified antiangiogenic action.

Discussion and Conclusion

Both in vitro (cell lines) and in vivo (clinical trials) study results arrive at the conclusion that vitamin D not only has got preventive effect on colorectal, prostate and breast cancers but also is capable of causing their regression and death of the constitutive cancerous cells up to various degrees. The mechanism(s) involved in such anticancer actions include its antiproliferative, prodifferentiating, apoptotic and antiangiogenic properties. For convenience of the reader (without taxing their memory) as well as convenience of writing, the basic physiological (noncancerous) and pathological (cancerous) mechanisms involved in the above-mentioned cellular processes have been discussed as briefly as possible along with the modulatory role of vitamin D on them to produce its anticancer action. It has been established that (the nuclear receptors for this vitamin) VDRs , in addition to calcium metabolism related cells, are also present in most of the noncalcemic body cells, where they modulate cellular proliferation, differentiation, apoptosis and angiogenesis - the processes involved in cancer genesis, progression and survival. [17],[21],[22],[23],[24] All these processes are initiated and maintained by the continued activity of several growth factors which mediate their action through corresponding RTKs (receptor tyrosine kinases) leading to the necessary alteration (activation or inhibition) in genomic functions and subsequent formation of required transcription factors. [39],[40] As discussed earlier, vitamin D (calcitriol) has been found to interfere (activation or inactivation) with these subcellular transduction pathways at one or more steps, thereby inhibiting cellular proliferation and angiogenesis and stimulating the process of apoptosis and cell differentiation. [21],[23], [36],[42],[48] Moreover, the tumorigenic and prometastatic activities of cathepsins have also been found to be

counteracted by calcitriol through increased production of the protein cystatin D that increases the expression of the concerned gene.

All these anticancer actions of vitamin D have been found with chronic administration of high doses, ranging between 2000-4000 IU/ day, which may lead to vitamin D toxicity. Opinion differs with respect to dose; some workers have demonstrated the anticancer action with a dose of 1000 IU/ day without any adverse effect, [14] while others have used 4000 IU/ day, which is supposed to produce toxicity. In this respect, to avoid the high dose, sunlight (UV radiation) exposure for a certain time period daily has been recommended which is thought to supplement and make up the dose deficit without toxicity. In addition to making up the deficit, sunlight exposure may reduce plasma cholesterol concentration by diverting more cholesterol for synthesis of the vitamin D precursor 7-dehydrocholesterol in humans. [3] Further work involving dose-response relationship and toxicity is awaited.

Vitamin D is known to increase plasma calcium concentration by its calcium mobilizing property. Naturally, when administered in high doses for prolonged periods for its anticancer action (either for prevention or tumor growth inhibition and regression), hypercalcemia is bound to occur. The role of plasma concentration of calcium on cancer genesis, prevention and regression and growth inhibition is controversial and needs further investigation. Besides other adverse effects, hypercalcemia is known to favor progression of atherosclerosis, which may be dangerous in susceptible individual. Hence, necessary precautionary measures in this respect have to be undertaken to avoid this side effect of the vitamin.

One of the anticancer actions of vitamin D is inhibition of tumor angiogenesis, which is highly beneficial in causing tumor regression, and inhibition of tumor growth. But this action is not tumor specific and may involve vascular beds in other parts of the body - particularly those of the coronary, cerebral, peripheral and wound areas. The necessary beneficial role of angiogenesis in coronary artery disease, stroke, peripheral vascular diseases and wound healing is well established. Hence, chronic administration of vitamin D in high doses for cancer prevention or regression to such patients may cause more harm than good. In this respect, it may be wise to collect a proper history of these diseases (if present) before administering vitamin D for anticancer purpose. Further work on antiangiogenic effect of this vitamin on nontumor vascular beds, particularly on those of coronary and cerebral, seems to be highly essential.

From the mechanisms involved in anticancer action of vitamin D, it appears that the compound is not suitable for use in cancer as a primary anticancer agent. It may be used as an adjuvant in combination chemotherapy, where it can aid to the actions of other cytotoxic agents, particularly those of the alkylating agents (which are not cell cycle specific), by causing tumor regression and inhibition of tumor growth, in addition to counteracting their mutagenic effect by inducing apoptotic cell death. [52] As documented by several workers, this vitamin may be used in high doses as a prophylactic agent for prevention of colorectal, prostate and breast cancer development.

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