Supplementary Information S3 | Tumor inhibitory effects of calcitriol and vitamin D in animal models

The multiple anti-cancer actions exerted by calcitriol, analogs or dietary vitamin D in rodent models of various cancers have been extensively reviewed [1-5]. The following is a summary of the salient findings described in the animal studies.

Inhibition of Cancer Initiation and Progression/Chemoprevention	**Reference**
Diet-induced hyperplasia	
Western style diets high in fat and low in vitamin D and calcium caused hyper-proliferation of anterior and dorsal prostate epithelial cells.	[6]
Western style diets high in fat and low in vitamin D and calcium caused hyper-proliferation and hyperplasia in mouse mammary glands and prostate epithelial cells and this was suppressed by calcium and vitamin D supplementation.	[7,8]
Western style diets low in vitamin D and calcium and high in fat induced colonic tumors in mice while feeding diets supplemented with calcium and vitamin D reduced tumor incidence and multiplicity.	[9,10]
Chemical carcinogen-induced preneoplasia and cancer	
Dietary vitamin D supplementation decreased AZO-induced preneoplastic lesions in mouse colon in a dose-dependent manner. Dietary vitamin D concentrations correlated inversely with dysplasia score and maximum impact was seen when mice consumed more than 2500 IU/kg diet.	[11]
Vitamin D administered prior to a carcinogenic insult (DMH) significantly reduced the incidence of colon adenocarcinomas in rats.	[12]
Dietary vitamin D did not significantly alter incidence of colon carcinogenesis in rats when given after exposure to DMH.	[13]
The vitamin D analog (24, 25-dihydroxyvitamin D_3) diminished formation of aberrant crypt foci when administered before, after or along with DMH in rats.	[14,15]
$1\alpha(OH)D_5$ decreased NMU-induced mammary tumor incidence and multiplicity in rats and AOM-induced aberrant crypt foci in mouse colon. However, in the DMBA-induced cancer model tumor progression was inhibited with no change in the incidence of mammary tumors.	[16]
VDR ablation increased the susceptibility to DBMA-induced carcinogenesis in a tissue specific manner. Increased incidence of mammary gland hyperplasia with a higher percentage of hormone-independent tumors were observed in *Vdr* null mice.	[17]
Gemini vitamin D analogs 0097 and 0072 inhibited NMU-induced mammary tumor burden in mice without causing hypercalcemia.	[18]
Genetically engineered cancer models	
In Nkx3.1:Pten mice, a model that recapitulates the various stages of prostate cancer, calcitriol significantly reduced progression of prostatic intraepithelial neoplasia (PIN) to high grade-PIN when administered before the initial occurrence of these lesions.	[19]
A vitamin D-deficient diet increased the proliferation and severity of PIN lesions in the anterior prostate of $TgAPT_{121}$ mice.	[20]
Rxr-α null mice fed the new Western style diets high in fat and low in vitamin D and calcium developed high grade PIN.	[21]

In LH overexpressing mice EB1089 decreased the proliferation of mammary epithelial cells in preneoplastic glands and reduced growth rate of hormone-induced tumors.	[22]
MMTV-neu mice displaying haploinsufficiency of *Vdr* had shorter latency and increased incidence of mammary tumor formation.	[23]
LPB-Tag model of prostate tumors progressed faster in *Vdr* null when compared to their wild-type littermates.	[24]
The Gemini analog BXL0124 inhibited ErB2-positive mammary tumor growth in MMTV-ErB2/neu transgenic mice.	[25]
Western diets low in calcium and vitamin D increased the number of polyps in the colons of APC^{1638N} mice.	[26]
Administration of a vitamin D_2 analog decreased tumor burden in $APC^{Min/+}$ mouse.	[27]
25(OH)D_3 and two vitamin D analogs (NC and HP) failed to reduce tumor multiplicity or alter growth rates of colonic tumors in $APC^{Pirc/+}$ rats or $APC^{Min/+}$ mice.	[28]

Tumor inhibitory effects in xenograft models of cancer

Single agents

Gemini vitamin D analogs 0097 and BXL0124 inhibited growth of ER(-) MCF10DCIS cells implanted orthotopically into nude mice without causing hypercalcemia.	[18,29]
Vitamin D_2 analog decreased the growth of HT-29 human colon cancer xenografts growth in mice but not SW-620 xenografts.	[30]
EB1089 decreased growth of LNCaP human prostate cancer xenografts in nude mice.	[31]
EB1089 dramatically reduced the growth of SUM-159PT human breast cancer xenografts and increased apoptosis.	[32]
Vitamin D deficiency accelerated and Gemini analogs of vitamin D and a vitamin D-sufficient diet effectively reduced the growth of MC26 mouse colon xenografts.	[33,34]
Diets low in vitamin D but with normal calcium levels increased the growth of DU145 prostate xenografts when compared to diets containing normal or high calcium with adequate vitamin D.	[35]
Calcitriol and dietary vitamin D exhibited equivalent anti-cancer activity to inhibit the growth of MCF-7 human breast xenografts and PC3 human prostate xenografts in nude mice.	[36]

Combination Therapy

Tumor volumes were significantly lower in animals irradiated after treatment with EB1089 than those that got radiation alone suggesting that vitamin D metabolites sensitized the tumor to radiation.	[37]
Vitamin D analogs PRI 2202 and 2205 demonstrated significant inhibition of 4T1 mouse breast cancer xenografts when combined with cytostatics but not when administered individually.	[38]
Calcitriol inhibited the growth of MCF-7 xenografts in a dose-dependent manner and combination with aromatase inhibitors further enhanced this effect, especially the regulation of the gene pathways contributing to the anti-cancer activity.	[39]
Combination of dietary soy with calcitriol enhanced both anti-cancer activity as well as hypercalcemic toxicity in mice with PC3 xenografts.	[40]

Inhibition of Metastasis

EB1089 decreased total number of bone metastasis, mean surface area of osteolytic lesions and tumor burden in nude mice after intra-cardiac injections of MDA-MB-231 human breast cancer cells. [41]

Low vitamin D levels accelarated 4T1 mouse mammary tumor growth but did not affect metastasis to the Lungs. [42]

EB1089 exerted a strong inhibitory effect on PTHrP-enhanced C4-2 prostate cancer xenograft growth and metastasis to the bone. [43]

Vitamin D deficiency enhanced the growth of MDA-MB-231 breast cancer cells injected into the tibia of mice resulting in osteolytic lesions that appeared earlier and were larger than those seen in the vitamin D-sufficient mice. [44]

Vitamin D deficiency increased bone turnover, osteolytic lesions, total tumor area and total mitotic activity in nude mice receiving intra-tibial injections of PC3 prostate cancer cells. [45]

Abbreviations: AOM - azoxymethane; APC – adenomatous polyposis coli; AZO - azoxymethane; DCIS – ductal carcinoma in situ; DMBA – dimethylbenzanthracence; DMH – N,N'-dimethylhydrazine; LH – luteinising hormone; LPB-Tag - large probasin promoter directed SV40-large T-antigen; MMTV-ErB2 – mouse mammary tumor virus – HER2/neu; NMU - N-methyl-N-nitrosourea; PIN – prostate intraepithelial neoplasia; PTHrP – parathyroid hormone related protein; RXR – retinoid x receptor; VDR – vitamin D receptor;

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