INTRODUCTION. 1,25-(OH)₂ vitamin D₃ [1,25(OH)₂D₃], the active metabolite of vitamin D, inhibits proliferation of a variety of tumor cells including prostate (reviewed in 1) as well as head and neck cancer. 1,25(OH)₂D₃ effects are mediated by the vitamin D receptor, which is necessary but not sufficient for 1,25(OH)₂D₃-mediated growth inhibition (1,2). In the human prostate cancer cell line LNCaP, 1,25(OH)₂D₃-mediated growth inhibition correlates with modestly increased levels of cyclin-dependent kinase inhibitor p27kip₁, robust inhibition of cyclin-dependent kinase 2 (CDK2) activity and consequent accumulation of cells in the G1 phase of the cell cycle (2). While 1,25(OH)₂D₃ does not affect p27 mRNA levels or the rate of p27 synthesis, pulse-chase analysis revealed that 1,25(OH)₂D₃ increased p27 protein half-life in LNCaP cells. p27 phosphorylation at Thr187 by CDK2 targets p27 for Skp2-mediated degradation. Consistent with inhibition of phosphorylation-dependent p27 degradation, 1,25(OH)₂D₃ decreased levels of phospho-Thr187 p27. Thus, stabilization of p27 is one mechanism for 1,25 D inhibition of CDK2 activity (3). Since the nuclear localization of CDK2 is necessary for its activation, we investigated the regulation of CDK2 activity more comprehensively by studying 1,25(OH)₂D₃ effects on the subcellular localization of CDK2 and assessed the relationship between 1,25(OH)₂D₃-mediated growth inhibition and CDK2 localization.

METHODS. For direct immunofluorescence imaging, LNCaP or AT-84 cells were transfected with a fusion protein YFP-Cdk2, after 48hr of 1,25(OH)₂D₃ treatment, cells were fixed and observed under a fluorescent microscope. Immunofluorescence labeling was performed as previously reported (3), and
indirect immunofluorescence imaging was performed by laser scanning confocal fluorescent microscopy. The fluorescence intensity in confocal images was determined by ImageJ software (rsb.info.nih.gov/ij/). Subcellular fractionation was performed as reported previously (3). LNCaP stable cell lines expressing a nuclear-targeted CDK2 were established by geneticin selection after transfection, and expression confirmed by western blot analysis.

RESULTS. 1,25(OH)2D3 treatment of LNCaP cells resulted in decreased nuclear CDK2 levels as assessed by subcellular fractionation and both direct and indirect fluorescence image analysis. Fluorescence imaging of head and neck squamous cell carcinoma AT-84 cells also revealed nuclear exclusion of CDK2 in response to 1,25(OH)2D3 treatment. To assess the relationship between 1,25(OH)2D3-mediated growth inhibition and CDK2 nuclear exclusion, we generated LNCaP cells stably expressing a nuclear-targeted CDK2-NLS fusion protein. The resulting LNCaP/CDK2-NLS cells were relatively resistant to growth inhibition by 1,25(OH)2D3 treatment compared to LNCaP Neo control cells. As expected, CDK2 remained predominately in the nucleus following 1,25(OH)2D3 treatment in LNCaP/CDK2-NLS. Further, CDK2 activity was only modestly inhibited by 1,25(OH)2D3 in LNCaP cells expressing the nuclear-targeted CDK2 compared to LNCaP Neo.

DISCUSSION. These data suggest that the antiproliferative effects of 1,25(OH)2D3 are mediated in large part by nuclear exclusion of CDK2 which has the two-pronged effect of increasing p27 levels and decreasing CDK2 activity.

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REFERENCES