Report

Expression of a vitamin D-regulated gene (VDUP-1) in untreated- and MNU-treated rat mammary tissue

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Summary

Previous studies showed that the expression of an mRNA corresponding to VDUP-1 was decreased within MNU-induced rat mammary tumors. RNA from mammary tissue was DNase treated and reverse transcribed, and the resulting cDNA was amplified using primers designed to amplify VDUP-1 (382 bp fragment) and glyceraldehyde-6-phosphate dehydrogenase (GAPDH) (416 bp fragment). A nalysis of mammary cDNA derived from untreated or MNU-treated rats indicated that VDUP-1 expression within tumor tissue was significantly decreased, a finding which agrees with previous Northern blotting experiments. The differential expression was confirmed in tissue sections using an antisense VDUP-1 riboprobe for in situ hybridization studies which demonstrated that VDUP-1 staining in all cell types within tumor tissue was greatly decreased. VDUP-1 mRNA was expressed to a greater extent within epithelial cells and to a much lesser extent within stromal cells, including endothelial cells, in untreated mammary tissue. A significant decrease in VDUP-1 expression was detected as early as six weeks after MNU treatment, before tumors had formed. Bilateral ovariectomy did not alter VDUP-1 expression in untreated mammary tissue and ovariectomy prior to MNU treatment prevented tumor formation, as well as the associated decrease in VDUP-1 expression. The relative expression of VDUP-1 was higher in lung tissue than in adrenal, heart, kidney, liver, mammary, muscle, and ovary. Treatment of a cell line derived from an MNU-induced rat mammary tumor (MNU cells) with 1,25-dihydroxyvitamin D\(_3\) resulted in a significant increase in VDUP-1 expression and also inhibited cell growth in the absence of serum. The data are consistent with a role for VDUP-1 in mediating the inhibitory effects of 1,25-dihydroxyvitamin D\(_3\) on tumor cell growth.

Introduction

Treatment of rats with a single intravenous dose of MNU (N-methyl-N-nitrosourea) results in the formation of mammary gland tumors 12–20 weeks after treatment. These tumors harbor specific genetic alterations which may be involved in regulating tumorigenesis within the mammary gland. For example, increases in the expression of cyclins D1, A, and E, as well as CDK 2 (cyclin-dependent kinase 2) and CDK 4, occur frequently in rat mammary tumors [1], and overexpression of cyclin D1 in transgenic mice results in tumors of the mammary epithelium [2]. These results suggest that cyclins and CDKs, which are important regulators of progression of the cell through the cell cycle [3], play a role in mam-
mary tumorigenesis. Most MNU-induced tumors also contain an activated H-ras oncogene [4], although there is some evidence to suggest that these ras mutations exist prior to MNU treatment [5].

We have recently identified several genes whose expression was either increased or decreased in MNU-induced rat mammary tumors [6]. mRNA corresponding to a rat endogenous retrovirus and calcyclin were increased in mammary tumors while mRNA for transferrin and clone #27 were decreased. Clone #27 corresponds to VDUP-1 (96.9% amino acid homology), a gene originally isolated from the HL-60 human promyelocytic cell line [7]. Treatment of HL-60 cells with 1,25(OH)2D3 (1,25-dihydroxyvitamin D3) is known to cause differentiation of the cells along the monocyte/macrophage pathway. This treatment also results in a 4–5 fold increase in VDUP-1 mRNA suggesting that VDUP-1 has a possible role in regulating 1,25-(OH)2D3-induced HL-60 differentiation [7].

Although 1,25-(OH)2D3 and several analogues have been shown to inhibit the proliferation of human breast tumor cell lines [8–10] and inhibit tumor progression of chemically-induced rat mammary tumors [11], the mechanism of these effects is not known. This report describes studies conducted to further characterize the decrease in the expression of the rat homolog of VDUP-1 that occurs during MNU-induced tumorigenesis. An assay utilizing RT-PCR (reverse transcriptase-polymerase chain reaction) was developed to examine the time course of VDUP-1 expression following MNU treatment, the effects of ovariectomy on VDUP-1 expression, the tissue distribution of VDUP-1, and the effects of 1,25-(OH)2D3 on VDUP-1 expression. Additionally, the cellular localization of VDUP-1 mRNA within the mammary gland was determined using in situ hybridization.

**Materials and methods**

**Animals and treatments**

MNU (Pfaltz and Bauer, Inc., Waterbury, CT) was freshly dissolved at a concentration of 10 mg/ml in saline buffered to pH 5.0 with 3% acetic acid. Fifty-day-old female Sprague-Dawley rats (Ace Animals, Inc., Boyertown, PA) were given a single 50 mg/kg dose of MNU by tail vein injection. The treated animals developed mammary tumors in 3–5 months. A group of 39-day-old rats were anesthetized with a combination of acepromazine (0.75 mg/kg), ketamine (50 mg/kg), and xylazine (5 mg/kg) and bilaterally ovariectomized prior to MNU treatment on day 50. Mammary tissue was removed from the animals at the appropriate time points and frozen in a dry ice-ethanol bath for storage at −80 °C prior to RNA isolation. Other tissues, including adrenal gland, heart, kidney, liver, lung, muscle, and ovary, were removed from untreated female rats and quick-frozen for RNA preparation for tissue distribution studies.

**Cell lines**

The MNU rat mammary tumor cell line was obtained from American Type Culture Collection (Rockville, MD) and cultured in T-75 (75 cm2) flasks in DMEM:F-12 medium (Mediatech, Inc., Herndon, VA) containing 10% fetal bovine serum (Atlanta Biologicals, Norcross GA), 5 µg/ml gentamicin, and 2 mM l-glutamine. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO2-air. MNU cells were plated at 1 × 106 cells/T-75 flask and allowed to attach overnight. The attached cells were treated with 1,25-(OH)2D3 (Calbiochem-Novabiochem International, La Jolla, CA) concentrations ranging from 25 nM to 400 nM for 48 hours. Some flasks were treated with an equal volume of vehicle (ethanol). Following the incubation, the cells were scraped and collected for RNA isolation. MNU cells were adapted to grow in serum-free medium by gradually reducing the amount of serum present in the growth medium. The % serum was decreased by 50% after every second passage until the cells were able to grow in medium containing 0.1–0.2% serum. At this point the cells were cultured in DMEM:F-12 medium supplemented with glutamine, gentamicin, insulin (5 µg/ml), transferrin (5 µg/ml), and sodium selenite (5 µg/ml). Growth curves were carried out using a CyQUANT cell pro...