[CANCER RESEARCH 46, 5406–5408, October 1986]

Significance of 1,25-Dihydroxyvitamin D_3 Receptor in Primary Breast Cancers[1]

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ABSTRACT

Specific high affinity receptors for 1,25-dihydroxyvitamin D_3 are present in several human breast cancer cell lines, and this hormone can regulate the replication of these cells. These receptors are also present in primary breast carcinomas. The present study has resulted from the follow-up for up to 68 mo of 263 women, who had had 1,25-dihydroxyvitamin D receptor (1,25-DR) levels measured in their primary tumors. Survival data on 191 women were correlated with the levels of 1,25-DR and other steroid hormone receptors, menopausal status, and age by life table analysis. Survival was not affected by 1,25-DR level in either absolute terms or relative levels. However, the late development of lymph node metastases in eight of 47 individuals was correlated with the 1,25-DR level ($P = 0.05$). There was no correlation between 1,25-DR or other hormone receptor levels and the development of hypercalcemia or bone metastases in the small number of individuals so affected. As we had observed previously, there was no correlation between the level of 1,25-DR and that of the other steroid hormones. These data show that the presence of 1,25-DR in primary breast cancers is independent of other prognostic indicators and, inasmuch as it correlated with late lymph node metastasis, may be an adverse prognostic indicator.

INTRODUCTION

Breast cancer remains the most common cancer in women and a significant cause of death in Western societies. The occurrence of metastases is of grave significance despite improvements in both hormonal and chemotherapeutic approaches. The presence of ER[3] indicates a good prognosis as well as the implication of a better chance of response to hormonal therapy (1, 2). These predictions, though improved by measurement of PGR or other steroid hormone receptor levels, are not sufficiently accurate clinically, and improvements are necessary (1, 3). We have demonstrated previously that human mammary cancer cell lines in culture and primary breast cancers possess high affinity, highly specific receptors for the vitamin D_3 hormone 1,25-dihydroxyvitamin D_3 (4–8). Moreover, the replication of these cell lines *in vitro* is regulated by this hormone and certain of its metabolites and analogues (7, 9–11). The 1,25-dihydroxyvitamin D_3 has also been demonstrated in other cancer cell lines, and in certain of these, effects on cellular replication *in vitro* have been reported (6, 9, 12). In an *in vivo* model with a murine leukemic cell line, 1,25-$(OH)_2D_3$ has been shown to prolong survival (13). We have also demonstrated the effects of 1,25-$(OH)_2D_3$ on the growth of xenografts of certain malignant melanoma, and colonic cancer cell lines in immune suppressed mice.[4] For these reasons it seemed that breast cancers bearing the 1,25-DR could have a better prognosis by analogy with those breast cancers bearing ER. The outcome in 263 women, who had had 1,25-DR receptor levels determined on their primary breast cancers, was examined. We report here the analysis of the correlation between survival and levels of 1,25-DR, other receptors, and metastases to lymph nodes, bone, and other sites.

MATERIALS AND METHODS

Breast tumors, which had been sent for ER level, were obtained from laboratories at the Prince Henry's Hospital Medical Centre and at the Department of Surgery, St. Vincent's Hospital, Melbourne, Australia. ER levels were determined at the above laboratories according to the previously described methods by single saturating dose analysis (8). The levels of PGR, AR, and in some cases GlucoR receptors were measured at Prince Henry's Hospital Medical Centre also by single saturating dose analysis as previously described (8). We determined the 1,25-DR levels on the tumor pieces which were stored at −20°C at these laboratories. Frozen pulverization was used to disrupt the tumors prior to homogenization as this improves receptor recovery (14). Frozen weighed tumors were immersed in liquid nitrogen and powdered. The powdered tissue was then homogenized in buffer containing potassium phosphate (0.05 M; pH 7.2), ethyleneglycol-bis(β-aminoethylether)-*N,N'*-tetraacetic acid (0.002 M), dithiothreitol (0.002 M), potassium chloride (0.3 M), and Trasylol (aprotinin) (1000 KIU/ml). Single saturating dose analysis for 1,25-DR level was performed on high speed supernatants (cytosol) as previously described (4, 6, 8, 14, 15). We have shown previously (8, 14, 15) that this method of single saturating dose analysis measures high affinity 1,25-DR; also competition by 100-fold molar excess of 25-hydroxyvitamin D_3, which is part of each analysis, excluded binding to the serum vitamin D binding protein. Analysis of the levels of the various receptors in relation to date of surgery did not show any drift in these measurements over time.

At periods up to 68 min after the original surgery, patients were followed up by a questionnaire mailed to their surgeon or primary care doctor where known. Two cycles of repeat letters were sent, and where necessary, possible telephone contacts were made with those medical practitioners who did not reply. For ethical reasons, no attempts were made to contact any patient directly.

The data obtained from the questionnaire were entered into a data base (PFS file; Software Publishing Corp., Mountain View, CA) and transferred into a statistical analysis package (Minitab; Pennsylvania State University, University Park, PA) for analysis. Correlations, multiple and stepwise linear regressions, life table, and log rank analyses were performed. 1,25-DR levels below 0.3 fmol/mg of protein were considered negative (8, 9); concentrations of the other steroid hormone receptors below 3 fmol/mg of protein were considered negative. In the life table analysis 1,25-DR was grouped into three approximately equal ranges, namely, less than 0.3, 0.3 to 2.9, and greater than 3 fmol/mg of protein.

RESULTS

Of the 263 women, data on survival were available on 191 (73%). The mean age of the women at surgery was 58 ± 13 (SD) yr, and follow-up was for 35.6 ± 13 mo (median, 35 mo). Hypercalcemia was noted in 31 women, and bone metastases were noted from the time of surgery to as late as 57 mo. Of the 191 women on whom follow-up was possible, 45 (24%) had already died. 1,25-DR was present in 153 (58%) of 263 tumors with mean and maximum concentration of 2.1 and 19.7 fmol/

Received 2/24/86; revised 5/22/86; accepted 6/19/86.

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[1] These studies were supported by grants from the National Health and Medical Research Council, the Anti-Cancer Council of Victoria, Hoffmann-La Roche Inc., and the New South Wales State Cancer Council.

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[3] The abbreviations used are: ER, estrogen receptor; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 1,25-DR, 1,25-dihydroxyvitamin D_3 receptor; PGR, progesterone receptor; GlucoR, glucocorticoid receptor; AR, androgen receptor; KIU, Kalikrein inhibitory unit.

[4] J. A. Eisman, D. H. Barkla, and P. J. M. Tutton, manuscript submitted for publication.

mg of protein, respectively. ER, PGR, GlucoR, and AR were present in 174 (72%) of 273, 100 (54%) of 237, 100 (83%) of 121, and 179 (77%) of 233 tumors. The mean levels for these receptors were 93, 115, 46, and 34 fmol/mg of protein, respectively; the maximum levels were 1193, 2223, 312, and 274 fmol/mg of protein, respectively.

Analysis of the correlation between survival and receptor levels showed that 1,25-DR levels did not predict survival; the life table curves are virtually superimposable (Fig. 1). χ^2 analysis by the log rank test confirmed the lack of any correlation between survival and 1,25-DR levels up to 4 yr postoperative. The suggestion of improved survival in those with 1,25-DR-negative tumors could not be evaluated because of the small number of subjects at these long times of follow-up. At the time of surgery, 39 women had lymph node involvement, while another 8 had lymph node metastases presenting some months after the initial surgery. The event of late presentation of lymph node involvement correlated with 1,25-DR level ($r = 0.28$; $P < 0.05$). Menopausal status was also an indicator of prognosis. In the 44 women who died, the time to death was considerably shorter in the postmenopausal women, *i.e.*, 20 mo, $P < 0.01$.

The level of the 1,25-DR did not correlate with the levels of any of the other receptors, or with age or menopausal status. However, the AR level was correlated with the levels of the ER and PGR; hence, AR = 0.088 PGR + 0.089 ER + 12.4, $r = 0.70$ with 231 degrees of freedom, and $P < 0.001$. ER level correlated well with age and PGR; hence, ER = 3.84 age + 0.16 PGR − 157, $r = 0.42$ with 223 degrees of freedom, and $P < 0.001$.

DISCUSSION

Three other groups have reported on the presence of 1,25-DR in smaller numbers of human primary breast cancers (16–18). Christakos *et al.* (16) and Ullmann *et al.* (17), using sucrose density gradient analysis, found receptor in 5 of 24 and 7 of 22 tumors, respectively, positivity rates of 21 and 32%. This is considerably less than the 58% in the 263 tumors reported here. However, in the third study (18), as in the present study, single saturating dose analysis was performed, and a similar positivity rate of 75% was reported. The discrepancy between the two methods may be due to the lower sensitivity of the sucrose density gradient method. Of interest, the range of receptor level of up to 30 fmol/mg of protein found by Freake *et al.* (18) is also similar to our range of up to 19.7 fmol/mg of protein.

As in this and our previous study (8), there was no correlation between 1,25-DR and ER levels. In our 263 patients there was no correlation between 1,25-DR and any of the four other steroid hormone receptor levels measured. Freake *et al.* (18) found no correlation between 1,25-DR and ER, while Ulmann *et al.* (17) found no correlation between 1,25-DR and either ER or PGR. Hence, it is clear that something quite independent of the other steroid hormone receptors is being quantitated, and as such, 1,25-DR may have quite distinct prognostic implications. Another interesting correlation in the present study was that of the level of the AR with ER and PGR; these correlations were strong separately and together, $P = 0.001$. Miller *et al.* recently reported a similar correlation between the presence of AR and of ER and PGR in 122 tumors (19). The significance of the correlation between ER levels and age is not clear.

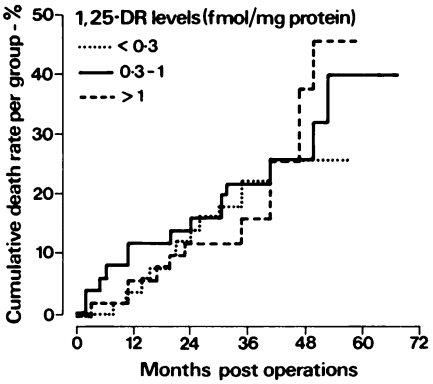

Fig. 1. Life table analysis of the survival of women with primary breast cancer in relation to 1,25-DR level. The number of deaths per group as a percentage of the number of deaths overall is plotted against months of follow-up. The three curves are not significantly different by log rank analysis.

Freake *et al.* were able to follow 56 of their patients for a short time and did not find any clinical or survival correlates of the 1,25-DR levels (18). Ulmann *et al.* also found no correlation between 1,25-DR and clinical course by 1 yr of follow-up (17). Our study, however, has clinical data on 191 women over periods of up to 68 mo, with a median of about 3 yr for those alive at the time of follow-up. Our data provide clear, relatively long-term data on the prognostic implications of 1,25-DR in primary tumors. The association of the late occurrence of lymph node metastasis with 1,25-DR could have expected to result in an association of 1,25-DR with a worsened prognosis. However, this was not observed at up to 48 mo of follow-up.

The effects of 1,25-dihydroxyvitamin D_3 on the replication of human cancer cells in culture is relevant to these clinical studies. In *in vitro* studies, 1,25-dihydroxyvitamin D_3 was able to stimulate the replication of human breast cancer cells (T47D) and malignant melanoma (MM96) at low concentrations and inhibit at higher concentrations (7, 9–12). Although *in vitro* concentrations cannot be directly related to the *in vivo* situation, the circulating level *in vivo* lies between these two extremes and has no net effect on replication *in vitro*. Thus, those breast cancers which possess the 1,25-DR may be responsive to 1,25-$(OH)_2D_3$, yet be neither stimulated nor inhibited by the normal circulating levels of this vitamin D hormone. In this regard the data on the inhibition of tumor growth by high levels of the hormone *in vitro* (7, 9–12) and our initial *in vivo* data are particularly interesting.[4] The analogy to the effects of estrogen on human breast cancer is clear. The development of compounds which inhibit tumor growth *in vivo* without unacceptable toxicity, *i.e.*, analogous to the antiestrogens, is clearly of considerable potential and interest.

In summary, in 263 women with primary breast cancers the presence of the 1,25-dihydroxyvitamin D_3 receptor has been found to correlate with the late occurrence of lymph node metastases. The 1,25-DR did not correlate with survival, age, menopausal status, or other hormone receptors. The possibility of effects of exogenous 1,25-dihydroxyvitamin D_3, as suggested by *in vitro* and mouse xenograft studies, remains to be elucidated. This cohort of women will be followed so that the longer term prognosis related to 1,25-DR levels can be determined.

ACKNOWLEDGMENTS

We thank Dr. J. Funder and Professor R. C. Bennett and their hormone receptor laboratories at the Prince Henry Hospital Medical Centre and the Department of Surgery, St. Vincent's Hospital, Melbourne, respectively, for their information on the other steroid hormone receptors of these tumors and for their valuable assistance in providing the tumors for our assays. We thank G. Ashleigh-Ng for technical assistance and S. Bella for her untiring efforts in collecting and collating

the clinical data. We also thank P. Compton and G. Egan for their invaluable assistance with the statistical analysis of the data.

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