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Chronic exposure to dexamethasone induces hypomethylation of ornithine decarboxylase genes in a human myeloma cell line

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Chronic exposure of a human myeloma cell line to dexamethasone resulted in a selection of cells resistant to the growth-inhibitory action of the glucocorticoid. Upon acute exposure of the parental myeloma cells to dexamethasone growth inhibition was associated with depression of ornithine decarboxylase (ODC, EC 4.1.1.17) activity. However, in cells adapted to grow in the presence of micromolar concentrations of dexamethasone, ODC activity was fully comparable to that in the parental cells. Restriction enzyme analyses with the two isoschizomers *Hpa*II and *Msp*I as well as with the methylation-sensitive *Cfo*I, indicated that the otherwise heavily methylated ODC gene(s) were rendered hypomethylated in the myeloma cells resistant to dexamethasone. This hypomethylation within and/or around ODC genes was associated with a 2–4-fold enhancement of accumulation of ODC mRNA.

Dexamethasone; Ornithine decarboxylase; Gene methylation; mRNA; (Sultan myeloma)

1. INTRODUCTION

Ornithine decarboxylase (ODC) belongs to those enzymes whose activity is not only strikingly stimulated by almost any growth-promoting stimulus, but is likewise profoundly depressed upon exposure of cells or tissues to catabolic or growth-inhibitory agents. One of the best examples of the tissue-specific responses of ODC is the action of glucocorticoids. Natural glucocorticoids and their synthetic congeners, such as dexamethasone, elicit large increases in ODC activity in parenchymal organs, such as in liver and kidney, while profoundly depressing the enzyme activity in tissues and cells of lymphatic origin [1].

Although the molecular mechanisms of these tissue-specific responses are not known, it appears obvious that they are in line with the generally accepted view that ODC is a strictly growth-related enzyme.

Even though many controversies exist regarding the importance of gene methylation for its transcriptional activity [2], there are many recent examples indicating that hypomethylation in or around a given gene is associated with enhanced expression of the gene [3–7]. We recently found that a distinct methylation polymorphism exists between different human tumor cell lines [8]. In comparison with human leukemia cells, the Sultan myeloma cells appeared to be much more methylated [8]. Here, we report that chronic exposure to dexamethasone renders human myeloma cells resistant to the growth-inhibitory actions of the glucocorticoid. This resistance was associated with hypomethylation of ODC genes and enhanced accumulation of the message of the enzyme.

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2. MATERIALS AND METHODS

2.1. *Cell cultures and selection of dexamethasone-resistant cells*

The human myeloma cell line (Sultan) was originally obtained from an IgG-myeloma patient. The myeloma cells were grown in RPMI 1640 medium supplemented with 5% (v/v) pooled human serum (Transfusion Service, Finnish Red Cross, Helsinki) and antibiotics (penicillin and streptomycin). The cells were exposed to increasing concentrations of dexamethasone (10–1000 nM) over a period of several weeks resulting in the generation of a cell population readily growing in the presence of 1 μM dexamethasone.

2.2. *Chemicals*

Dexamethasone was obtained from Sigma (St. Louis, MO). [^{32}P]dCTP (spec. act. >400 Ci/mmol) was purchased from Amersham International (Amersham, England). The restriction enzymes *Eco*RI and *Hpa*II were purchased from Amersham International, and *Msp*I and *Cfo*I from Boehringer Mannheim.

2.3. *Preparative and analytical methods*

Genomic DNA was isolated and extracted by the method of Blin and Stafford [9]. The DNA was digested with restriction enzymes according to the instruction of the suppliers. The restriction fragments were electrophoresed in 0.9% agarose gels, transferred to nitrocellulose filters [10] and hybridized with nick-translated [11] pODC10/2H complementary to the human ODC mRNA [12]. The specific activity of the probe was 1×10^8 cpm/μg.

For cyto-dot analyses of the ODC mRNA levels, the cells were lysed in 10 mM Tris-HCl, 1 mM EDTA (pH 7.5) buffer containing 1% NP-40. The lysates were applied with a manifold (Bethesda Research Laboratories) to an mRNA affinity paper (Hybond-mAP, Amersham) using the loading and washing conditions described in [13]. The affinity paper was then hybridized with nick-translated pODC10/2H and autoradiographed.

ODC activity was assayed by the method of Jänne and Williams-Ashman [14].

3. RESULTS

As shown in fig.1, dexamethasone clearly inhibited the growth of the human myeloma cells. However, cells chronically exposed to the glucocorticoid grew at least as fast as the parental cells (fig.1). Growth inhibition in response to acute exposure to 1 μM dexamethasone, although not complete, was highly reproducible, varying between 40 and 60%. The antiproliferative effect was usually apparent after an exposure of 24 h. In another experiment, the increase in cell density between 24 and 48 h was $0.756 \pm 0.032 \times 10^6$/ml in untreated cells while in cultures exposed to 1 μM dexamethasone the cell number increased to $0.507 \pm 0.0012 \times 10^6$/ml ($p < 0.001$). The ODC activity, as measured 24 h after the change to fresh medium, was 59.4 and 55.4 pmol/10^6 cells in parental cells and in cells chronically exposed to dexamethasone respectively, and 32.8 pmol/10^6 cells in cells following acute exposure to the glucocorticoid.

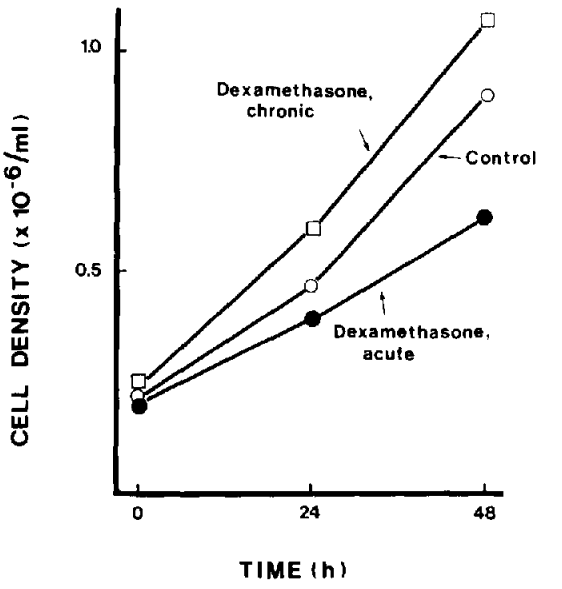

Fig.1. Growth of Sultan myeloma cells in the absence and presence of dexamethasone. The parental cell line was grown in the absence (control) or in the presence of 1 μM dexamethasone (dexamethasone, acute) added at time point zero. The dexamethasone-resistant cell line (dexamethasone, chronic) was grown in the presence of the glucocorticoid for several months prior to the experiment. Each time point represents duplicate cultures.

Restriction enzyme analyses of genomic DNA isolated from the parental Sultan cells and

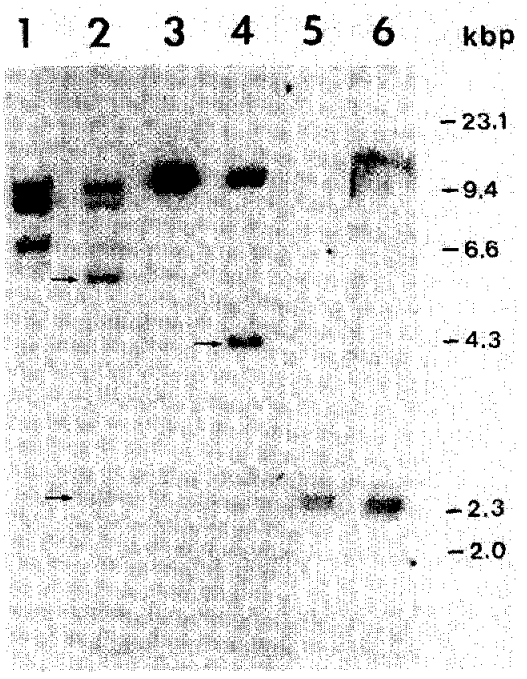

Fig.2. Restriction enzyme analysis of genomic DNA isolated from the parental cells or from dexamethasone-resistant cells. Large molecular mass genomic DNA (10 μg) from parental cells (lanes 1,3,5) or dexamethasone-resistant cells (lanes 2,4,6) was digested with *Hpa*II (lanes 1,2), with *Cfo*I (lanes 3,4) or with *Msp*I (lanes 5,6). The fragments were size fractionated by electrophoresis, blotted and hybridized with pODC10/2H as described in section 2. The molecular mass markers are shown to the right. kbp, kilobase pairs. The arrows indicate new fragments that appeared.

dexamethasone-resistant cells revealed an identical restriction pattern after *Eco*RI digestion (not shown). However, when the digestion was performed with the methylation-sensitive *Hpa*II and *Cfo*I, the former cleaving at CCGG and the latter at GCGC provided the internal cytosines are unmethylated, it became evident that the chronic exposure to the glucocorticoid resulted in distinct hypomethylation in (and around?) ODC genes. Lanes 1 (parental cells) and 2 (dexamethasone-resistant cells) in fig.2 represent genomic DNA digested with *Hpa*II and lanes 3 (parental cells) and 4 (dexamethasone-resistant cells) genomic DNA digested with *Cfo*I. As shown in fig.2, the genes were clearly less methylated in the dexamethasone-resistant cells (the arrows indicate the new fragments that appeared), yet the digestion with the methylation-insensitive isoschizomer (*Msp*I) of *Hpa*II [15] resulted in an identical digestion pattern (lanes 5 and 6 in fig.2).

Fig.3 depicts pODC10/2H with the cleavage sites for *Hpa*II and *Cfo*I. By using different probes prepared from the plasmid we found that the generation of a 2.4 kbp fragment from genomic DNA by *Hpa*II (fig.2, lane 2) indicated that one of the centrally located *Hpa*II (fig.3) sites was rendered demethylated. Moreover, our unpublished experiments have indicated that the minimum size of human ODC gene is about 5 kbp, thus suggesting that the fragment of about 4 kbp generated by *Cfo*I in dexamethasone-resistant cells (fig.2, lane 4) resulted from demethylation of a

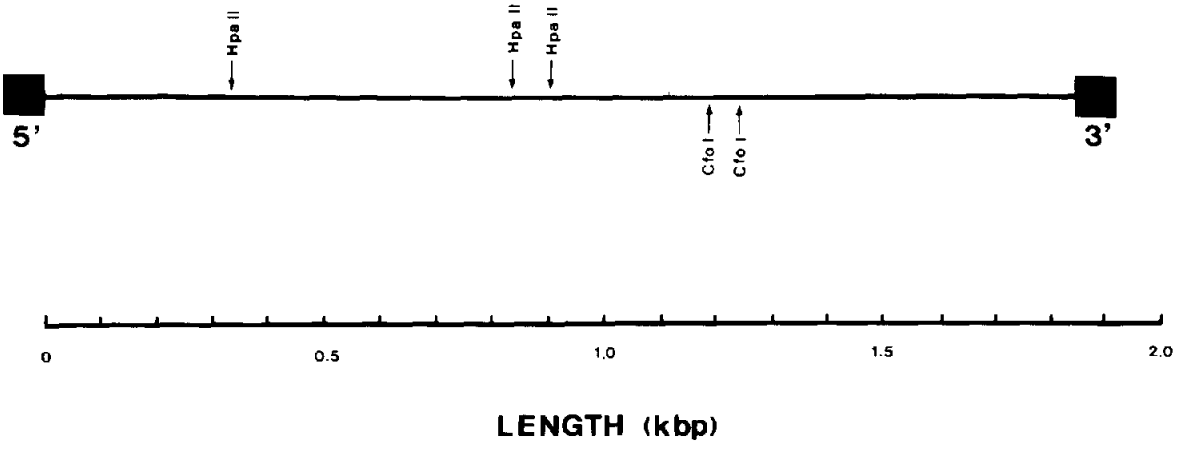

Fig.3. Partial restriction map (with *Hpa*II and *Cfo*I sites) of the human pODC10/2H. The filled boxes represent pBR322 sequences. kbp, kilobase pairs.

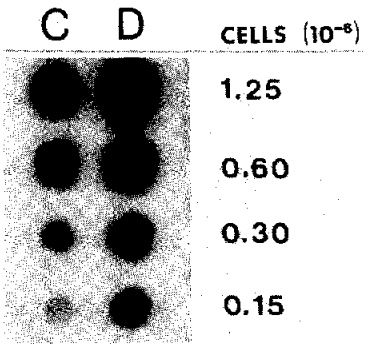

Fig.4. Cyto-dot analysis of ornithine decarboxylase mRNA levels in parental cells and dexamethasone-resistant cells. Parental cells (C) and cells chronically exposed to dexamethasone (D) were lysed and applied to an mRNA affinity paper as described in section 2. The number of cells applied is shown to the right.

cleavage site within the ODC gene. Accordingly, it is unlikely that the observed changes represent any overall genomic demethylation. The latter view was also supported by the identical pattern of ethidium bromide-stained *Hpa*II and *Cfo*I digests of genomic DNA obtained from sensitive and resistant cell lines.

Hypomethylation was associated with chronic exposure to the glucocorticoid, as acute exposure to dexamethasone (48 h) induced no changes in the cleavage pattern by *Hpa*II or *Cfo*I (not shown).

A cyto-dot analysis performed on polyuridylated mRNA affinity paper (fig.4), revealed that the accumulation of ODC mRNA was enhanced by a factor of 2–4 in the dexamethasone-resistant cells (column D). Thus in this case, the apparent hypomethylation of ODC genes led to an increased accumulation of the message of the enzyme.

4. DISCUSSION

In connection with analyses involving several human-derived cells and cell lines, we found that there exists a methylation polymorphism regarding ODC genes among different cells [8]. In comparison with human leukemia cells or even with peripheral mononuclear leukocytes, the Sultan myeloma cells appeared to be heavily methylated [8]. In these studies, we did not attempt to correlate the extent of hypomethylation with the expression rate of the gene. In the case of these dexamethasone-resistant cells there seems to be a link between hypomethylation of the gene(s) and the amount of the respective mRNA. Of course, there is no evidence to claim that these human myeloma cells acquire resistance to dexamethasone by hypomethylation of ODC genes resulting in enhanced expression of the genes.

Many, especially very recent observations suggest that hypomethylation in or around a given gene enhances its activity. Thus methylation controls the inducibility of mouse metallothionein-I gene [4], the expression of an interferon gene [16], rat growth hormone gene expression [6] and gene activity of the human β-globin cluster [7], to mention just a few examples. However, many examples with no correlation between gene methylation and activity are also available [2].

More relevant to the present findings are the reports indicating that steroid hormones can bring about changes in gene methylation. It has been shown that estrogen induces demethylation at the 5′-end of the chicken vitellogenin gene irrespective of whether the gene is expressed [17]. Similarly, a mouse mammary tumor cell line which exhibits a positive proliferative response to androgens appears to lose the response upon prolonged culture in the absence of the steroid [18]. The loss of this proliferative response is accompanied by increased methylation of mouse mammary tumor virus (MMTV)-related sequences [18].

Even more relevant to the present results are the findings of Mermod et al. [19] who found that the selection of a dexamethasone-resistant mouse lymphoid cell line (in the presence of 10 μM dexamethasone) was specifically associated with demethylation of the 5′-long terminal repeats of MMTV in the absence of any MMTV genome rearrangements or overall genome demethylation.

The fact that dexamethasone induces hypomethylation of human ODC genes may also serve as an explanation for our recent findings indicating that ODC genes in a human B cell leukemia line as well as in mononuclear leukocytes obtained from leukemia patients are less methylated than those in mononuclear leukocytes obtained from healthy volunteers [8]. The latter finding thus may indicate a development of glucocorticoid-resistant clones as a result of a recent treatment with glucocorticoids.

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