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OVERPRODUCTION OF ORNITHINE DECARBOXYLASE CONFERS AN APPARENT GROWTH ADVANTAGE TO MOUSE TUMOR CELLS

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SUMMARY: We have selected mouse myeloma and leukemia cell lines overproducing ornithine decarboxylase (ODC) under the pressure of α-difluoromethylornithine (DFMO), a mechanism-based inhibitor of the enzyme. Two of the tumor cell variants overproduced ODC by virtue of an amplification of transcriptionally active ODC genes. In one case the overproduction of the enzyme was based on an enhanced transcription of the enzyme's message at normal gene copy number. The DFMO-resistant cells exhibited ODC activity that was 8 to 25 times higher than the enzyme activity in the parental cells. When plated into soft agar, the parental mouse myeloma cells failed to form any colonies, whereas the ODC overproducing variant cells grew soft agar at a plating efficiency of about 16 %. The difference between parental and ODC overproducing cells was even more striking in case of mouse leukemia L1210 cells. The parental LI210 cell formed colonies in soft agar at an efficiency of 1.9 % while two overproducer variant cell lines formed colonies at up to 60 % plating efficiency. These results clearly indicate that an overproduction of ODC offers a distinct growth advantage to tumor cells.

Ornithine decarboxylase (ODC; EC 4.1.1.17), the key enzyme of the biosynthetic pathway of the polyamines, is a growth-related protein, the inhibition of which invariably leads to severe disturbances of cellular proliferation (1, 2).

ODC belongs to those about dozen mammalian proteins the genes for which easily undergo amplification (3). Thus both mouse (4, 5, 6) and human (7) tumor cells acquire resistance to DFMO, an irreversible inhibitor of ODC (18), through amplification of transcriptionally active ODC sequences. An overproduction of the enzyme can likewise occur of transcriptional level without changes in gene copy number (9, 10).

We recently selected a DFMO-resistant mouse L1210 cell line by exposing the cells to DFMO in the presence of micromolar concentrations of cadaverine (9). These cells overproduce ODC at normal gene dosage. Upon omission of cadaverine, we selected another

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The abbreviations used are: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithhine; AdoMetDC, S-adenosylmethionine decarboxylase.

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L1210 variant cell line in which the overproduction of the enzyme was based on a marked amplification of hypomethylated ODC sequences (11). We have now used these cell lines as well as a mouse myeloma cell line overproducing ODC as a result of gene amplification to compare their growth properties with those of the parental cells. It appears clear that the overproduction of ODC confers a distinct growth advantage as judged by a strikingly effective colony formation of these cells in semisolid culture medium.

MATERIALS AND METHODS

Cell lines

The selection of the L1210 mouse leukemia cells overproducing ODC without gene amplification (designated as L1210/10DC) has described in (9). Similarly, the selection of the L1210/10D cell line derived from L1210/10DC overproducing ODC thgrough gene amplification has been described earlier (11). A mouse myeloma cell line (X63 Ag8 653) was exposed to increasing concentrations of DFMO (starting from 0.1 mM) over a period of about 6 months resulting in a cell line fully resistant to the antiproliferative actions of 20 mM DFMO. These cells overproduced ODC based on a more than 50-fold gene amplification. The DFMO-resistant cell line was designated as X63/20D.

Cell cultures and soft agar cloning

All the cell lines were maintained in suspension cultures in RPMI 1640 (Gibco Ltd, Paisley, Scotland) medium supplemented with 5 % pooled human serum (Finnish Red Cross Transfusion Service, Helsinki, Finland) and with antibiotics (penicillin and streptomycin).

To test the colony forming ability, the tumor cells were grown in a two-layer soft agar system. The lower layer consisted of one part of 2.4 % agar (Nepto-agar, Difco) in 0.15 M NaCl and 4 parts of fresh RPMI 1640 medium (with 5 % human serum) or the fresh medium was replaced by conditioned medium obtained from actively growing cell cultures. The upper layer contained 1 part of 2.4 % agar in 0.15 M NaCl, 4 parts of RPMI 1640 medium (or conditioned medium) with 5 % human serum and 1.7 parts of cell suspension dilution. Visible colonies were counted 11-21 days after plating.

Analytical methods

The activities of ODC and adenosylmethionine decrboxylase (AdoMet DC; EC 4.1.1.50) were assayed by published methods (12, 13). For statistical analyses two-tailed t-test was used.

Chemicals

DFMO was generously given by Centre de Recherche Merrell International (Strasbourg, France). DL-[I-^{14}C] ornithine (sp. radioactivity 61 Ci/mol) was purchased from Amersham International (Amersham, U.K.).

RESULTS

ODC and AdoMetDC activities in the different cell lines are depicted in Table 1. As shown, ODC activity was 8 to 25 times higher in the DFMO-resistant cell lines in comparison with the parental lines. AdoMetDC was also markedly elevated in the DFMO-resistant L1210 cells but not in the resistant myeloma (X63/20D) cells (Table 1).

Table 1. The activities of ODC and AdoMetDC in parental and DFMO-resistant mouse myeloma and leukemia cells. Duplicate cultures

Cell variant	(pmol/10^6 cells per 60 min) ODC	AdoMetDC
L1210	68	129
L1210/10DC	567	1850
L1210/10D	1700	502
X63	58	125
X63/20D	1530	110

We did our initial soft agar cloning experiments with the L1210/10DC variant cell line, which overproduces ODC through an apparent transcriptional activation without an increase in gene dosage for ODC (9). Table 2 shows that the parental cells (L1210) failed to form any colonies in soft agar over a period of 3 weeks. The overproducer cells (L1210/10DC), however, formed visible colonies at a plating efficiency of nearly 20 % (Table 2). In this experiment the soft agar system was constructed with fresh RPMI 1640 medium. In the experiment, the results of which are depicted in Table 3, conditioned medium was used. The parental cells (L1210) still poorly formed colonies whereas the two overproducer variants (L1210/10DC and L1210/10D); the latter being derived from the former and showing a marked ODC gene amplification) gave rise to colonies at a very high efficiency.

Table 2. Growth of parental (L1210) and ODC overproducing (L1210/10DC) mouse leukemia cells

Cell variant	Number of cells plated	Number of colonies (±S.D.)	% of cells plated
L1210	500	0	0
L1210/10DC	500	98±8***	19.6

***$p < 0.001$

The incubation time was 21 days. Triplicate cultures.

Table 3. Growth of parental (L1210) and two ODC overproducing variant (L1210/10DC and L1210/10D) mouse leukemia cell lines in soft agar

Cell variant	Number of cells plated	Number of colonies (±S.D.)	% of cells plated
L1210	700	13± 6	1.9
L1210/10DC	700	439±30***	62.7
L1210/10D	700	333±14***	47.6

***$p < 0.001$
The incubation time was 12 days. Triplicate cultures.

Table 4 depicts the results of a further experiment, in which X63 mouse myeloma cell line and its DFMO-resistant variant (overproducing ODC through gene amplification) were used. In spite of the use of conditioned medium, the parental cells (X63) failed to form any colonies, yet the overproducer cells showed a plating efficiency of about 10 %.

We thus have three different mouse tumor cell lines overproducing ODC by different mechanisms and all these variant cell lines show an enhanced growth potential, in comparison with their respective parent lines, as judged by colony formation in semisolid culture medium.

DISCUSSION

Although a specific inhibition of ODC, by compounds such as DFMO, almost invariably leads to a marked retardation of cellular proliferation, the functions of the polyamines in

Table 4. Growth of parental (X63) and ODC overproducing (X63/20D) mouse myeloma cells in soft agar

Cell variant	Number of cells plated	Number of colonies (±S.D.)	% of cells plated
X63	500	0	0
	1000	0	0
X63/20D	500	78±40***	15.6
	1000	166±15***	16.6

***$p < 0.001$
The incubation time was 11 days. Triplicate cultures.

cell growth have remained unknown. We have earlier presented experimental evidence suggesting that an increased gene dosage for ODC and the subsequent overproduction of the enzyme could offer a distinct growth advantage to tumor cells in vivo. We selected a DFMO-resistant Ehrlich ascites carcinoma cell line overproducing ODC as a result of an amplification of transcriptionally active sequences and found that the overproducer cells behaved more aggressively in vivo when inoculated into mice (14). The present results likewise strongly support the view that not only the inhibition of ODC halts the growth of animal cells but that an overproduction of the enzyme offers a dstinct growth advantage to tumor cells. The striking ability of the overproducer cells, in comparison with the parental cells, to grow in semisolid medium, that is an unambiquous indication of strong growth potential, is a further piece of evidence linking ODC even more tightly to the growth processes.

It is, however, possible that during the selection process the tumor cells aquire other growth-related properties possibly associated with the gene amplification of ODC. The amplification may likewise lead to genomic rearrangements with unknown metabolic consequences. This possibility, however, remains somewhat unlikely as one of the variant cell lines (L1210/10DC) overproduced ODC without gene amplification and, in fact, grew most effectively in semisolid culture medium (Table 3). Even though suggestive the present results must be substantiated by direct transfection experiments before any firm conclusions of the ODC's role in cellular proliferation can be drawn. In fact, such experiments are underway in our laboratory.

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