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Role of Ornithine Decarboxylase Suppression and Polyamine Depletion in the Antiproliferative Activity of Polyamine Analogs

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SUMMARY

Two transfected cell lines, one carrying a mammalian ornithine decarboxylase (ODC) that is suppressed by polyamines and one carrying a trypanosomal ODC that is not, were used to ask whether ODC suppression is necessary for the antiproliferative activities of two polyamine analogs, N^1,N^8-bis(ethyl)spermidine (BES) and N^1,N^{11}-bis(ethyl)homospermine (BE444). Both analogs accumulated within cells and suppressed S-adenosylmethionine decarboxylase, as well as polyamine-sensitive mouse ODC activity. Neither drug was able to suppress the activity of the polyamine-refractory trypanosome ODC. But, whereas BE444 was able to inhibit growth of both cell lines, BES could inhibit only growth of cells carrying the polyamine-sensitive ODC, under conditions that cause prolonged depletion of endogenous polyamines. We conclude from these studies that the antiproliferative activity of BES, a less potent drug, requires the suppression of ODC. The efficacy of BE444 is enhanced by its ability to suppress ODC. However, it can function without ODC suppression, whereas BES cannot.

Polyamines are cationic molecules produced in all living organisms and are essential for life (for reviews, see Refs. 1 and 2). Synthesis of polyamines is associated with cell proliferation, making this process an attractive target for chemotherapeutic intervention. Specific inhibition of polyamine-biosynthetic enzymes results in depletion of intracellular polyamine pools and inhibition of growth (1, 2). The first enzyme of the polyamine-biosynthetic pathway, ODC, is a highly regulated enzyme, which, together with AdoMetDC, another tightly regulated enzyme of this pathway, controls the *de novo* synthesis of polyamines. ODC activity and protein increase in response to various physiological stimuli and are suppressed in response to elevated intracellular levels of the polyamines (3–6). In mammalian cells, AdoMetDC is similarly regulated in response to polyamines, so that elevated intracellular spermine and spermidine pools greatly reduce AdoMetDC protein (7) (for review, see Ref. 8).

ODC has been identified as a potential target in the chemotherapy of cancer and parasitic disease (for reviews, see Refs. 9–11). DFMO, an irreversible suicide inhibitor of ODC, has been used clinically against the trypanosome parasite responsible for African sleeping sickness (12). Despite its success in the therapy of trypanosomiasis, DFMO has shown limited potential in the cure of proliferative diseases such as cancer. As an alternative to the use of inhibitors, analogs of polyamines have been developed. These analogs are designed to mimic polyamines in certain regulatory functions, such as suppression of ODC and AdoMetDC. However, the analogs, while replacing natural polyamines within cells, appear unable to substitute for them in growth-related functions (for review, see Ref. 13).

BE444 is a spermine analog that shows promise as a cancer chemotherapeutic agent (14–16). Another compound, BES, is a spermidine analog with less potent antiproliferative activity than BE444, although it also appears to regulate the levels of polyamine-biosynthetic enzymes (17–19). To understand better the basis for this difference in antiproliferative activity, we have tested these compounds against two transfected cell lines that differ only in the nature of their ODC. One expresses an ODC that responds to suppression by polyamines, the other an ODC that does not (20). We have expressed cloned mouse ODC or trypanosome ODC DNA in the C55.7 CHO cell line, which lacks endogenous ODC activity (21). Although the mouse and trypanosome enzymes are remarkably similar in amino acid sequence, they differ markedly in certain properties. When expressed in CHO cells, trypanosome ODC is not degraded

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ABBREVIATIONS: ODC, ornithine decarboxylase; AdoMetDC, S-adenosylmethionine decarboxylase; DFMO, α-difluoromethylornithine; BES (also known as BESPM), N^1,N^8-bis(ethyl)spermidine; BE444 (also known as BE4SPM), N^1,N^{11}-bis(ethyl)homospermine; CHO, Chinese hamster ovary; AdoMet, S-adenosylmethionine.

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rapidly, whereas the mouse enzyme is (22, 23). This reflects accurately the behavior of each when expressed in its native environment (24–26). In addition, the trypanosome ODC does not respond to the negative regulatory stimulus of polyamines, whereas mouse ODC does, in CHO cells. This difference allows us to use the two transfectants of the C55.7 cell line to ask whether regulatory reduction of ODC protein levels by polyamine analogs contributes to the antiproliferative activity of these compounds.

Materials and Methods

Polyamine analogs. BES and BE444 were generous gifts of Raymond J. Bergeron (University of Florida, Gainsville, FL).

Cells. Growth and maintenance of ODC-deficient C55.7 CHO cells have been described in detail elsewhere (21, 23). Transfection and selection of cells carrying either the mouse ODC (c461) or trypanosome ODC (Try II) have been published previously (22, 23).

Growth studies. Cells at desired cell densities (see legend to Fig. 1) were seeded in triplicate in tissue culture flasks. Analogs, prepared in Hanks' balanced salt solution and sterilely filtered, were added 1 day after cell seeding. Cell growth was followed by trypsinization and counting, using electronic particle counters, following the procedure described previously (20).

ODC and AdoMetDC assays. Duplicate plates of 1×10^6 cells were left untreated or treated with drug 24 hr after seeding. After a 24-hr exposure to the drugs, cells were washed in cold saline and harvested by scraping; cells were lysed in 200 μl of buffer containing 50 mM phosphate (pH 7.4), 10 mM EDTA, and 5 mM dithiothreitol. ODC activity was assayed in duplicate for each plate, as described previously (26). [1-^{14}C]Ornithine used in the assays was purchased from New England Nuclear. Final activity was corrected for protein content as described previously (22, 23).

AdoMetDC assays were carried out on similarly treated lysates. Assays were performed as described previously (17). [*carboxy*-^{14}C] AdoMet was purchased from New England Nuclear.

Determination of polyamine and polyamine analog levels in cells. Cells ($0.5–1.0 \times 10^6$) were harvested by trypsinization, washed twice with cold (4°) phosphate buffer, and sonicated in 8% sulfosalicylic acid. Polyamine and polyamine analog levels were measured in triplicate by high performance liquid chromatography, as described (27).

Results

Growth of c461 and Try II cells in the presence of the polyamine analogs BE444 and BES. c461 and Try II cells are transfectants of ODC-deficient C55.7 CHO cells carrying the mouse ODC cDNA and trypanosome ODC DNA, respectively. We have shown that the ODC enzymes expressed in these transfected cells differ in their ability to respond to the negative regulatory stimulus of polyamines (24). To test whether ODC suppression plays a role in the antiproliferative activity of these drugs, cells were cultured in the presence of 10 μM BE444 and 100 μM BES and the cell number was determined each day, in two experiments. In the first experiment, cells were seeded at high cell density (2×10^5 cells/plate) and cell growth was followed for 4 days. Alternatively, cells were seeded at lower cell density (1×10^5/plate) and the cell number was determined for 6 days.

When cells are seeded at a higher cell density, BE444 inhibits growth of both cell lines to a similar extent (Fig. 1, A and B). Little inhibition of growth is seen with BES against either cell line under these conditions. Thus, under these conditions, there is no difference in growth inhibition between cell lines; however, there is a difference in antiproliferative activity between analogs. BE444 is an effective inhibitor of growth, whereas BES is not.

With lower cell densities and longer exposures, BE444, again, inhibits growth of both cell lines (Fig. 1, C and D). BE444 may be slightly more effective against c461 than against Try II. BES is as potent an inhibitor of growth of c461 as is BE444 under these conditions, whereas BES is significantly less effective against Try II. Thus, when cells are seeded at lower cell densities, the effectiveness of BES, and to some extent BE444, as a growth inhibitor differs between the two cell lines. However, under these conditions, we observe a difference in growth-inhibitory activities between drugs only against Try II cells.

Effect of BE444 and BES on ODC and AdoMetDC. To verify that BE444 and BES have the ability to suppress mouse ODC but not trypanosome ODC, cells were harvested and assayed for ODC activity before and after exposure to the analogs (Table 1). Treatment of c461 with BE444 or BES causes a profound decrease in ODC activity, resulting in approximately 1% or less activity remaining after 24 hr of treatment. Unlike c461, the mouse ODC cDNA-transfected cells, the ODC activity of Try II, the trypanosome ODC DNA-transfected cells, is only slightly inhibited (85% of control with BES) or not inhibited at all (in the case of BE444) (Table 1). Both ODC activities are inhibited by DFMO (data not shown).

Depletion of polyamine stores by treatment with DFMO causes an induction of AdoMetDC in both c461 and Try II cells (data not shown). In contrast, treatment of cells with BE444 or BES causes AdoMetDC levels to fall in both cell types (Table 1), presumably by mimicking a state of natural polyamine excess.

Polyamine and polyamine analog levels in cells treated with BE444 or BES. To verify that the polyamine analogs are entering the cells, extracts of c461 cells and Try II cells treated with BE444 or BES at high or low cell densities were analyzed, by high performance liquid chromatography, for intracellular analog levels (Table 2). Significant amounts of both analogs accumulate in both cell lines. BES, a spermidine analog, reaches concentrations equal to or higher than control spermidine levels, whereas levels of BE444, a spermine analog, are approximately equal to control spermine concentrations in 3–4 days.

Short term treatment (1–4 days), with BE444 or BES, of cells plated at higher cell density results in reduction, but not complete depletion, of spermidine in both c461 and Try II cells (Fig. 1, A and B, *insets*). Spermine levels are also similarly depleted by both analogs, but less so than spermidine, in both cell types. Early and nearly complete depletion of putrescine, to undetectable levels, occurs only in BES-treated c461 cells (Fig. 1A, *inset*), whereas putrescine levels of BE444-treated c461 cells are elevated to greater than control putrescine levels. Reduction of putrescine levels in Try II cells occurs with both BE444 and BES. This reduction is never complete (50% control) and occurs between days 3 and 4. In general, BES seems to be a better polyamine-depleting agent than BE444 during short term treatment of both cell lines.

Long term treatment (4–6 days) of c461 cells plated at lower cell density results in rapid and virtually complete depletion of all three polyamines by both BE444 and BES (Fig. 1, C and D, *insets*). In Try II cells, however, both analogs only partially reduce polyamine levels. Only spermine is completely depleted

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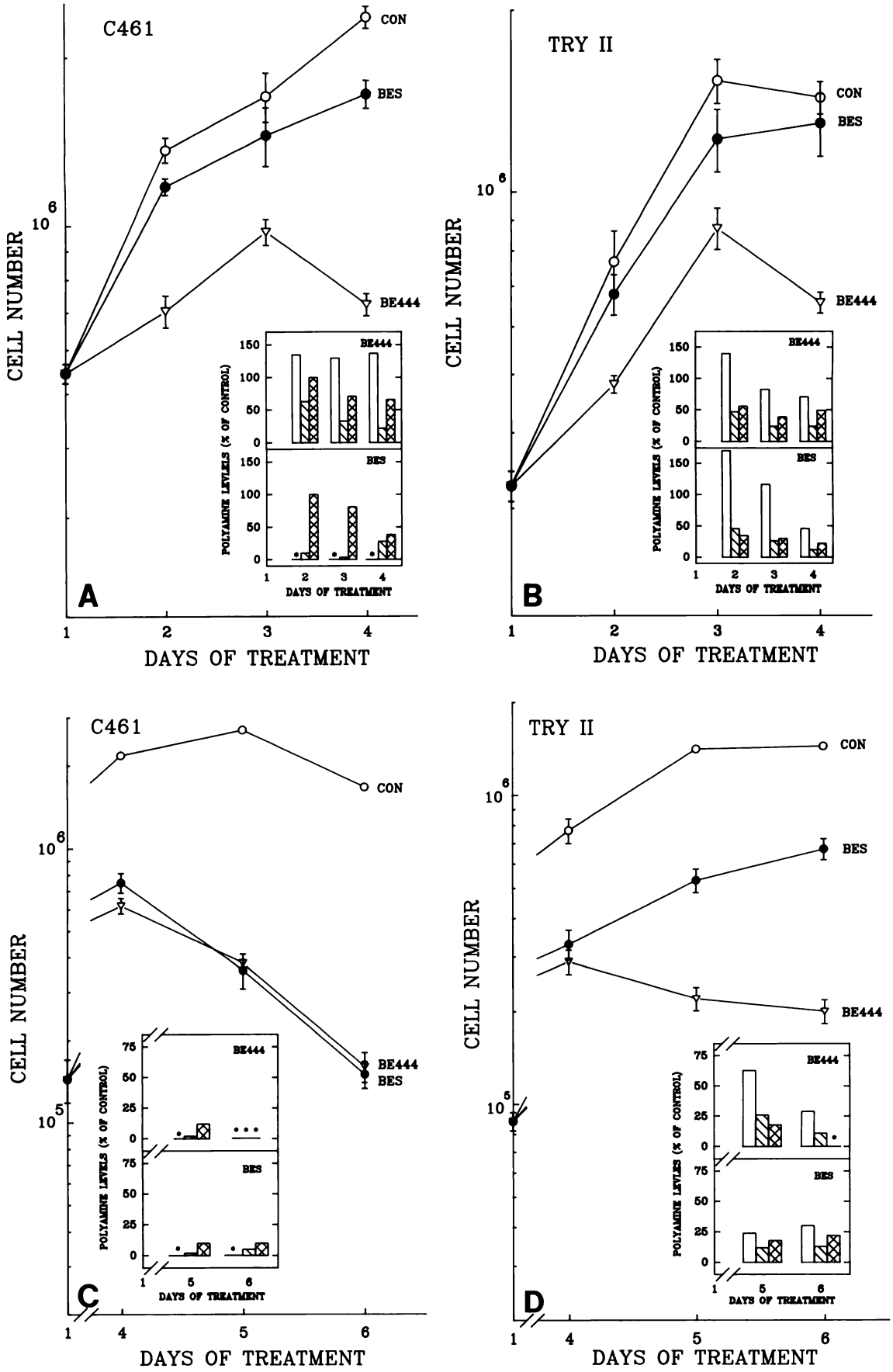

Fig. 1. Short term treatment (A and B) or long term treatment (C and D) of c461 and Try II cells with BE444 and BES. c461 cells carrying mouse ODC (A and C) or Try II cells carrying trypanosome ODC (B and D) were treated with BE444 (▽) or BES (●) or not treated (○) and the number of cells was determined. For short term treatment (A and B), cells were plated at a density of 2×10^5 cells/100-mm plate and the number of cells was determined for up to 4 days. For long term treatment (C and D), cells were plated at a density of 1×10^5 cells/100-mm plate and the number of cells was determined for 4–6 days. *Inset*, putrescine (□), spermidine (▨), and spermine (■) levels of cells treated with BE444 (*top*) or BES (*bottom*). *, Undetectable levels.

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TABLE 1

Effect of BES and BE444 on ODC and AdoMetDC activities

c461 cells or Try II cells were treated with 10 μM BE444 or 100 μM BES or not treated. After a 24-hr treatment, cells were harvested and ODC and AdoMetDC activities were determined as described previously (26).

	Activity		Control activity
	BE444	BES	
	% of control		nmol of CO_2 released/hr/mg of protein
ODC			
c461	1.1	0.2	6.14
Try II	103.7	85.3	8.54
AdoMetDC			
c461	29.3	17.4	0.29
Try II	39.3	22.7	0.15

TABLE 2

Polyamine analog levels in CHO cells treated with BE444 or BES at high or low cell density

	Analog concentration				
	2[a]	3	4	5	6
	nmol/10^6 cells				
c461					
High cell density[b]					
BE444	2.63	2.63	3.51	–[d]	–
BES	4.02	5.83	6.14	–	–
Low cell density[c]					
BE444	–	–	–	11.76	10.12
BES	–	–	–	6.25	6.56
Try II					
High cell density[b]					
BE444	3.23	3,49	4.53	–	–
BES	4.99	5.95	4.88	–	–
Low cell density[c]					
BE444	–	–	–	8.66	2.51
BES	–	–	–	4.00	4.28

[a] Days exposed to drug.
[b] Cells were plated at 2 × 10^5/100-mm plate.
[c] Cells were plated at 1 × 10^5/100-mm plate.
[d] Not determined.

in Try II; this occurs in cells treated for 6 days with BE444. Spermidine and spermine levels are 10–20% of control in all other cases.

Discussion

BE444 treatment results in inhibition of growth of CHO cells carrying either the mouse (c461) or the trypanosome (Try II) ODC, when these cells are plated at a higher cell density and the growth-inhibitory effects are monitored over a shorter period of time (Fig. 1, A and B). BES, on the other hand, is not an effective growth-inhibitory agent against either cell line under these conditions. Both drugs are taken up by the cells, and both cause a regulatory reduction in the amount of mouse ODC. Neither drug reduces the amount of trypanosome ODC, an ODC that has been shown to lack responsiveness to polyamines (Table 1). BES and BE444 reduce the amount of AdoMetDC in mouse or trypanosome ODC-carrying cells (Table 1). These analogs can, therefore, accumulate within these cells and exert regulatory effects on polyamine-biosynthetic enzymes that normally respond to natural polyamines. Specifically, the amounts of AdoMetDC and polyamine-responsive mouse ODC are affected by these analogs. The difference in the growth-inhibitory properties of BES and BE444 cannot, therefore, be explained by differences in drug uptake or by differential effects of the analogs on these two polyamine-biosynthetic enzymes. Because BES is as effective as BE444 in reducing mouse ODC activity, the suppression of ODC appears to be insufficient to account for the antiproliferative activity of BE444 under these conditions.

Quite different conclusions can be drawn under conditions where cells are plated at lower cell densities and growth is monitored over a longer period of time. In this case, c461 cells carrying the polyamine-responsive ODC (Fig. 1C) are clearly more sensitive than Try II cells to BES (Fig. 1D) in particular and, to a lesser extent, to BE444. Thus, under these conditions, ODC suppression appears to play a more prominent role in the antiproliferative activity of these analogs; cells that carry a polyamine-responsive ODC are more sensitive to growth inhibition by polyamine analogs. However, ODC suppression still cannot account for the difference in activity between BES and BE444 seen in Try II cells under these conditions.

If not ODC suppression, what is responsible for the difference in growth-inhibitory activities of these two analogs? Both drugs dramatically induce spermine/spermidine acetyltransferase activity in c461 and Try II cells, but there is no difference in induction between drugs or between cell lines (data not shown). The correlation between polyamine depletion and growth inhibition is also not straightforward. During short term treatment, BES seems to be a better polyamine-depleting agent than BE444 in both cell lines, but BE444 inhibits growth, whereas BES does not (Fig. 1, A and B, *insets*). However, during long term treatment c461 cells, whose growth is most inhibited by both drugs, lose all polyamines early (Fig. 1C, *inset*). In Try II cells, BES and BE444 both only partially deplete polyamines (Fig. 1D, *inset*), but BE444 is more effective than BES in inhibiting growth. (Although, in Try II cells, BE444 depletes spermine to nondetectable levels after 6 days of treatment, the growth-inhibitory effect of BE444 on this cell line is manifest long before spermine depletion.) Taken together, these data suggest that BE444 can inhibit cell growth in the presence of at least a residual pool of endogenous polyamines, whereas a prolonged reduction of polyamine pools to nondetectable or barely detectable levels is necessary for BES to inhibit growth.

Why can BE444 act in the presence of endogenous natural polyamines whereas BES cannot? It seems likely that the analogs themselves differ in their intrinsic properties to affect certain other critical intracellular functions. Past studies suggest several possibilities, among them being DNA-polyamine interactions (for review, see Ref. 28) and depletion of mitochondrial DNA. Our finding that BE444 exerts its antiproliferative activity in the presence of residual polyamines is consistent with the observation that BE444 binds DNA more tightly than does spermine (18, 29). In the presence of polyamines, BE444 may compete effectively for sites on DNA, whereas BES, which binds DNA less tightly than does spermine, cannot displace DNA-bound polyamines. Depletion of mitochondrial DNA from cells in culture by a homolog of BE444 [N^1,N^{12}-bis(ethyl)spermine], but not by BES, has also been reported (30, 31). It is possible that BE444 has similar effects upon mitochondrial DNA and that this effect contributes to the difference in growth inhibition exerted by BES and BE444.

Finally, these cell lines constitute a useful method for testing the consequences of ODC suppressibility in pharmacological responses. Our data provide support for the hypothesis that ODC suppression is important in the antiproliferative activity

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of BES, which requires a prolonged and extensive depletion of polyamine pools to exert its effect. BE444, on the other hand, is aided by the suppression of ODC but does not absolutely require it for its antiproliferative activity.

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References

1. Tabor, H., and C. W. Tabor. Polyamines. *Annu. Rev. Biochem.* **53**:749–790 (1984).
2. Pegg, A. E. Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem. J.* **234**:249–262 (1986).
3. Seely, J. E., and A. E. Pegg. Effect of 1,3-diaminopropane on ornithine decarboxylase enzyme protein in thioacetamide-treated rat liver. *Biochem. J.* **216**:701–707 (1983).
4. Persson, L., J. E. Seely, and A. E. Pegg. Investigation of the structure and rate of synthesis of ornithine decarboxylase protein in mouse kidney. *Biochemistry* **23**:3777–3783 (1984).
5. Murakami, Y., K. Fujita, T. Kameji, and S. Hayashi. Accumulation of ornithine decarboxylase-antizyme complex in HMOA cells. *Biochem. J.* **225**:689–697 (1985).
6. van Daalen Wetters, T., M. Macrae, M. Brabant, A. Sittler, and P. Coffino. Polyamine-mediated regulation of mouse ornithine decarboxylase is post-translational. *Mol. Cell. Biol.* **9**:5484–5490 (1989).
7. Mamont, P. S., C. Danzin, J. Wagner, M. Siat, A. M. Joder-Ohlenbusch, and N. Claverie. Accumulation of decarboxylated *S*-adenosyl-L-methionine in mammalian cells as a consequence of the inhibition of putrescine biosynthesis. *Eur. J. Biochem.* **123**:499–504 (1982).
8. Tabor, C. W., and H. Tabor. Methionine adenosyltransferase (*S*-adenosylmethionine synthetase) and *S*-adenosylmethionine decarboxylase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **56**:251–282 (1984).
9. Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.* **48**:759–774 (1988).
10. Schechter, P. J., J. L. R. Barlow, and A. Sjoerdsma. Clinical aspects of inhibition of ornithine decarboxylase with emphasis on therapeutic trials of efluoronithine (DFMO) in cancer and protozoan diseases, in *Inhibition of Polyamine Metabolism* (P. P. McCann, A. E. Pegg, and A. Sjoerdsma, eds.). Academic Press, New York, 345–364 (1987).
11. Porter, C. W., and J. R. Sufrin. Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. *Anticancer Res.* **6**:525–542 (1986).
12. Taelman, H., P. J. Schecter, L. Marcelis, J. Sonnet, G. Kazyumba, J. Dasnoy, K. D. Haegele, A. Sjoerdsma, and M. Wery. Difluoromethylornithine, an effective new treatment of Gambian trypanosomiasis: results in five patients. *Am. J. Med.* **82**:607–614 (1987).
13. Porter, C. W., and R. J. Bergeron. Enzyme regulation as an approach to interference with polyamine biosynthesis: an alternative to enzyme inhibition. *Adv. Enzyme Regul.* 57–82 (1987).
14. Bergeron, R. J., T. R. Hawthorne, J. R. T. Vinson, D. E. Beck, Jr., and M. J. Ingeno. Role of the methylene backbone in the antiproliferative activity of polyamine analogues on L1210 cells. *Cancer Res.* **49**:2959–2964 (1989).
15. Basu, H. S., M. Pellarin, B. G. Feuerstein, D. F. Deen, R. J. Bergeron, and L. J. Marton. Effect of N^1,N^{14}-bis(ethyl)homospermine on the growth of U-87 MG and SF-126 human brain tumor cells. *Cancer Res.* **50**:3137–3140 (1990).
16. Basu, H. S., M. Pellarin, B. G. Feuerstein, D. F. Deen, and L. J. Marton. Effect of N^1,N^{14}-bis(ethyl)homospermine on the growth of U-251 MG and SF-188 human brain tumor cells. *Int. J. Cancer* **48**:873–878 (1991).
17. Porter, C. W., F. G. Berger, A. E. Pegg, B. Ganis, and R. J. Bergeron. Regulation of ornithine decarboxylase activity by spermidine analogue N^1,N^8-bis(ethyl)spermidine. *Biochem. J.* **242**:433–440 (1987).
18. Basu, H. S., B. G. Feuerstein, D. F. Deen, W. P. Lubich, R. J. Bergeron, K. Samejima, and L. J. Marton. Correlation between the effects of polyamine analogs on DNA conformation and cell growth. *Cancer Res.* **49**:5591–5597 (1989).
19. Porter, C. W., J. McManis, R. A. Casero, and R. J. Bergeron. Relative abilities of bis(ethyl) derivatives of putrescine, spermidine, and spermine to regulate polyamine biosynthesis and inhibit L1210 leukemia cell growth. *Cancer Res.* **47**:2821–2826 (1987).
20. Ghoda, L., D. Sidney, M. Macrae, and P. Coffino. Structural elements of ornithine decarboxylase required for intracellular degradation and polyamine regulation. *Mol. Cell. Biol.* **12**:2178–2185 (1992).
21. Steglich, C., and I. E. Scheffler. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. *J. Biol. Chem.* **257**:4603–4609 (1982).
22. Ghoda, L. Y., M. A. Phillips, K. E. Bass, C. C. Wang, and P. Coffino. Trypanosome ornithine decarboxylase is stable because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. *J. Biol. Chem.* **265**:11823–11826 (1990).
23. Ghoda, L., T. van Daalen Wetters, M. Macrae, D. Ascherman, and P. Coffino. Prevention of rapid intracellular degradation of ODC by a carboxyl-terminal truncation. *Science (Washington D. C.)* **243**:1493–1495 (1989).
24. Phillips, M. A., P. Coffino, and C. C. Wang. Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei*. *J. Biol. Chem.* **262**:8721–8727 (1987).
25. Russell, D. H., and S. H. Snyder. Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase. *Mol. Pharmacol.* **5**:253–262 (1969).
26. Clark, J. L. Specific induction of ornithine decarboxylase in 3T3 mouse fibroblasts by pituitary growth factors: cell density-dependent biphasic response and alteration of half-life. *Biochemistry* **13**:4668–4674 (1974).
27. Kabra, P. M., H. K. Lee, W. P. Lubich, and L. J. Marton. Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phased liquid chromatography: improved separation systems for polyamines in cerebrospinal fluid, urine and tissue. *J. Chromatogr. Biomed. Appl.* **380**:19–32 (1986).
28. Feuerstein, B. G., L. D. Williams, H. S. Basu, and L. J. Marton. Implications and concepts of polyamine-nucleic acid interactions. *J. Cell. Biochem.* **46**:37–47 (1991).
29. Basu, H. S., H. C. A. Schweitert, B. G. Feuerstein, and L. J. Marton. Effect of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. *Biochem. J.* **269**:329–334 (1990).
30. Bergeron, R. J., A. H. Neims, J. S. McManis, T. R. Hawthorne, J. R. T. Vinson, R. Bortell, and M. J. Ingeno. Synthetic polyamine analogues as antineoplasitcs. *J. Med. Chem.* **31**:1183–1190 (1988).
31. Vertino, P. M., T. A. Beerman, E. J. Kelly, R. J. Bergeron, and C. W. Porter. Selective cellular depletion of mitochondrial DNA by the polyamine analog N^1,N^{12}-bis(ethyl)spermine and its relationship to polyamine structure and function. *Mol. Pharmacol.* **39**:487–494 (1991).

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