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TITLE: Relative Contribution of Ornithine Decarboxylase (ODC)
Versus S-adenosylmethionine Decarboxylase (SAMDC) to
Human Breast Cancer Progression and Development

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Introduction:

Considerable evidence indicates that polyamines (putrescine, spermidine, and spermine) play an important role in breast cancer biology (1). In particular, it appears that they may contribute to mammary carcinogenesis (2,3) as well as breast cancer progression to a more aggressive and metastatic hormone-independent phenotype (4). However, the relative contribution of the two polyamine biosynthetic enzymes, namely ornithine decarboxylase (which primarily controls the synthesis of the diamine putrescine) and S-adenosylmethionine decarboxylase (which mediates the production of the more distal polyamines spermidine and spermine) is not known. Therefore, our concept proposal planned to address this issue using the MCF-7 breast cancer cell line as well as a transgenic approach.

Body:

In contrast to the large body of evidence supporting an important role of ODC, very limited information is available on the possible influence of SAMDC on breast cancer phenotype. Therefore, our first priority was to investigate the effects of this enzyme on breast cancer behavior using SAMDC overexpressing MCF-7 breast cancer cells which were generated in our laboratory (5). Since SAMDC promotes the formation of the distal polyamines spermidine and spermine, we hypothesized that induction of its overexpression would adversely affect breast cancer phenotype. Indeed, some preliminary evidence generated in a previous publication (5) from our group suggested that SAMDC-MCF-7 cells were more clonogenic and bypassed the need of serum and estrogens for growth in soft agar. More detailed analysis of the properties of these cells, however (as described in our attached publication [6]), did not confirm these initial findings and indicated instead that SAMDC overexpression conferred a more benign phenotype. As described in detail in our publication (6), SAMDC overexpressing MCF-7 cells manifested reduced invasiveness in matrigel and were less tumorigenic in nude mice. Furthermore, the growth of established xenografts was slower. In addition, control and SAMDC overexpressing cells did not differ with regard to sensitivity to estrogens and anti-estrogens, thus indicating that activation of this enzyme did not lead to hormone independence. In

retrospect, these results are not surprising since SAMDC overexpression is associated with a compensatory downregulation of ODC and activation of SSAT which results in near total suppression of putrescine and an approximately 50% reduction in cellular spermidine level (5,6). Cellular spermine content is increased *in vitro* but is not different from control *in vivo* (6). Therefore, this polyamine profile is similar to that induced by inhibition of ODC with alpha-difluoromethylornithine, a drug which inhibits breast cancer cell proliferation and reduces invasiveness and metastasis. SAMDC overexpression in our experimental system did not modify activation of MAPK, or STAT signaling in response to EGF administration (6). In contrast, we have observed, in a different experimental system (the immortalized MCF-10A human breast epithelial cell line), that ODC overexpression increases phosphorylation of MAPK (2,3) and tyrosine phosphorylation of STAT-3 (unpublished observations). Unfortunately, we have been unable to extend the characterization of our ODC overexpressing MCF-7 cells since, in order to maintain elevated levels of ODC, we needed to chronically select the cells with DFMO which was found to have effects unrelated to the polyamine pathway. This was surprising to us since DFMO selection has been extensively used in the literature to induce ODC overexpression. Nevertheless, these extraneous effects of DFMO made our experimental system unusable. Therefore, we have used a different experimental approach to address the role of ODC in breast cancer progression. In recently published work, we have observed that inhibition of ODC activity with DFMO reduced *in vitro* invasive features of aggressive hormone independent MDA-MB-435 and MDA-MB-231 breast cancer cells and, most importantly, nearly totally abolished pulmonary metastasis from MDA-MB-435 breast cancer xenografts in nude mice (7). In summary, our up-to-date results indicate that ODC does, indeed, play a major role in breast cancer progression, contributing in a major way to development of metastasis, while SAMDC (at least when overexpressed) favorably influences breast cancer behavior.

We have placed considerable effort in trying to generate transgenic mice with targeted overexpression of ODC to the mammary gland. We obtained from Dr. Dean Selcher at UCSF, CA,

FVB-MMTV-Tet-Ta mice which are homozygous for the Tet transactivator driven by the MMTV promoter. Tet-Ta binds to the tetracycline responsive element (TRE) and activates transcription of the target gene (ODC in this case) in the absence of doxycycline (tet/off). In order to create mice overexpressing ODC in the mammary gland, the above mice were crossed to homozygous C57BL/6 transgenic mice carrying ODC under the control of a minimal CMV promoter attached to a multimerized TRE (obtained from Dr. Tom G. O'Brien, Lankenau Medical Center, Wynnewood, PA). Although we were able to generate mice with the appropriate phenotype, we were unable to detect ODC overexpression in their mammary gland. We are now trying a different promoter, C3(1) which has been reported to be successful in inducing gene expression in the mammary gland. These studies are currently funded by a Dean's Feasibility Grant at our institution.

Key Research Accomplishments:

- SAMDC overexpression reduces invasiveness of breast cancer cells.
- SAMDC overexpression reduces tumorigenicity in nude mice of MCF-7 breast cancer cells and retards the growth of established tumors.
- SAMDC overexpression does not influence the hormone responsiveness of MCF-7 breast cancer cells.
- ODC mediates *in vitro* invasiveness of hormone independent MDA-MB-435 and MDA-MB-231 breast cancer cells.
- ODC mediates the development of pulmonary metastasis from MDA-MB-435 human breast cancer xenografts in nude mice.

Reportable Outcomes:

Manni, A., Fischer, S., Franks, M., Washington, S., DeArment, R., Griffith, J., Demers, L., Verderame, M., Leiby, B., Mauger, D. S-adenosylmethionine decarboxylase (SAMDC) overexpression reduces invasiveness and tumorigenicity in nude mice of MCF-7 breast cancer cells. Int J Oncology, 19:317-323,2001.

Manni A, Washington S, Griffith JW, Verderame MF, Mauger D, Demers LM, Samant RS, Welch DR.

Influence of polyamines on *in vitro* and *in vivo* features of aggressive and metastatic behavior by human breast cancer cells. Clin Experimental Metastasis 19:95-105, 2002.

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Conclusions:

The observation that SAMDC overexpression has a favorable influence on breast cancer phenotype has important clinical implications since inhibitors of SAMDC are actually currently being developed as anticancer agents (8). Although found to be effective in reducing proliferation of breast cancer cells, our preliminary data in SAMDC overexpressing MCF-7 cells suggests that the antitumor effects of these compounds may be mitigated by the marked compensatory increase in ODC and putrescine levels induced by these drugs. Therefore, they may best be utilized in combination with inhibitors of ODC activity such as DFMO.

A major finding is that ODC is involved in distant metastasis from breast cancer. If this observation is confirmed in future studies, it would provide a strong rationale for testing the feasibility of inhibitors of ODC such as DFMO in the adjuvant therapy of breast cancer. It is worth noting that

inhibition of polyamine biosynthesis is effective in hormone independent tumors, i.e., those with the most aggressive behavior and for which treatment options are limited.

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