

## Basic Science in Medicine

# ADENOSINE DEAMINASE ACTIVITY IN ESTROGEN RECEPTOR POSITIVE AND NEGATIVE HUMAN BREAST CANCER CELL LINES

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### ABSTRACT

**Background:** The aims of this study were to assay the activity of adenosine deaminase (ADA) in estrogen receptor positive (MCF-7) and negative (MDA-MB468) breast cancer cell lines.

**Methods:** MDA-MB468 and MCF-7 breast cancer cell lines were cultured in complete medium, striped serum with and without 0.01  $\mu$ M diethylstilbestrol (DES), complete medium in the presence and absence of 1  $\mu$ M tamoxifen for 20 hr. Adenosine deaminase activity was determined using the colorimetric method described by Guisti and Galanti.

**Results:** It was found that the activity of enzyme in estrogen receptor positive (ER<sup>+</sup>) cell line (MCF-7) was significantly higher than that of estrogen receptor negative breast cancer cell line (MDA-MB468). ADA activity in MCF-7 cells cultured in the presence of tamoxifen or charcoal-striped serum was significantly lower than that of control. Furthermore addition of diethylstilbestrol (DES) to the striped serum increased the value of ADA activity to that of control.

Unlike MCF-7 cells, the activity of ADA in MDA-MB468 cells remained unchanged upon treatment with tamoxifen or striped serum.

**Conclusion:** These findings suggest estrogen responsiveness of ADA expression in MCF-7 cells.

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**Keywords:** Adenosine deaminase, breast cancer cell lines, estrogen receptor.

### INTRODUCTION

Adenosine deaminase (ADA, E.C.3.5.4.4) is a metal-

loenzyme that catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively.<sup>1</sup> Due to the irreversibility of the reaction catalyzed by ADA, this enzyme reaction seems to be one of the rate-limiting steps in adenosine degradation. ADA is present in virtually all human tissues, but the highest

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## ADA Activity in ER Positive and Negative Breast Cancer

levels are found in the lymphoid system such as lymph nodes, spleen, and thymus.<sup>2-4</sup> Adenosine deaminase deficiency was described 30 years ago and results in severe combined immunodeficiency (SCID), with virtual absence of T cells and a variable decrease in B cells.<sup>5</sup> In several studies, ADA activities were found to be increased<sup>6-8</sup> or decreased<sup>9</sup> in cancer cells. Detoxification of adenosine and deoxyadenosine is important since their high concentrations are toxic to the cells.

17 $\beta$ -estradiol ( $E_2$ ) has been shown to induce several enzymes involved in purine, pyrimidine, and DNA synthesis in MCF-7 human breast cancer cells, and this is accompanied by increased [<sup>3</sup>H]thymidine uptake and cell proliferation.<sup>10-14</sup> In addition, there is only one report demonstrating that the expression of ADA, an enzyme that decreases intracellular pools of adenosine and deoxyadenosine, is  $E_2$  responsive in the MCF-7 cells.<sup>15</sup> However, the activity of this enzyme has not been evaluated in estrogen receptor positive and negative (non-hormone responsive) breast cancer cell lines so far.

In order to clarify the hormone responsiveness of ADA and obtain a better understanding of purine metabolism in estrogen receptor positive and negative breast cancer cells, we have analyzed ADA activity in MCF-7 (ER+) and MDA-MB-468 (ER-) cell lines.

### MATERIAL AND METHODS

MDA-MB468 and MCF-7 breast cancer cell lines, obtained from National Cell Bank of Iran (NCBI), were grown in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin. They were incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> and 95% air. Cultures were regularly examined using an inverted microscope (Micros, Austria).

A suspension of 5% charcoal and 0.5% dextran T70 was prepared. The suspended dextran coated charcoal, at a volume equal to that of serum to be stripped, was centrifuged at 2500 rpm for 20 min. The serum was then added to the pellet and the mixture remained suspended by rolling at 4 cycle/min, 37°C for 1 hr. The suspension was centrifuged at 2500 rpm for 20 min and the supernatant was filtered using a 0.2  $\mu$ m filter and stored at -20°C.

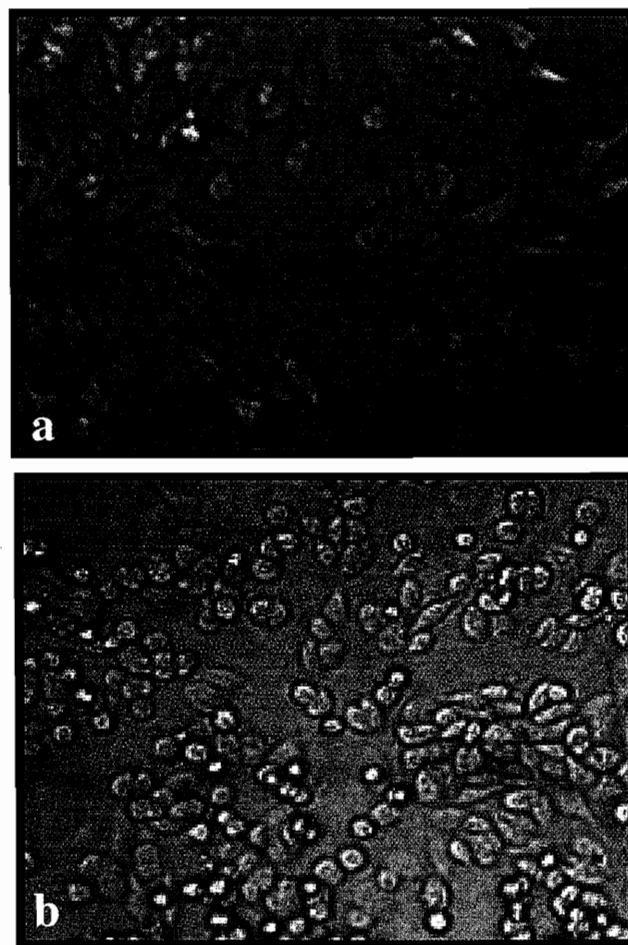
The cells were grown in the stripped serum, with and without 0.01  $\mu$ M diethylstilbestrol (DES) and complete medium in the presence and absence of 1  $\mu$ M tamoxifen for 20 hr. The cells were trypsinized and centrifuged at 400 g for 5 min at 4°C and washed (twice with PBS). They were resuspended in phosphate buffer (50 mM, PH= 6.5) and then vortexed with pre-chilled glass beads for 3-5 times, each time for one minute while keeping the cells on ice for one minute intervals. The homogenate was centrifuged at 20,000 g for 30 min at 4°C and the super-

natant used for the assay. Adenosine deaminase activity was determined using the colorimetric method described by Guisti and Galanti.<sup>16</sup> The protein content was estimated by the Bradford method.<sup>17</sup>

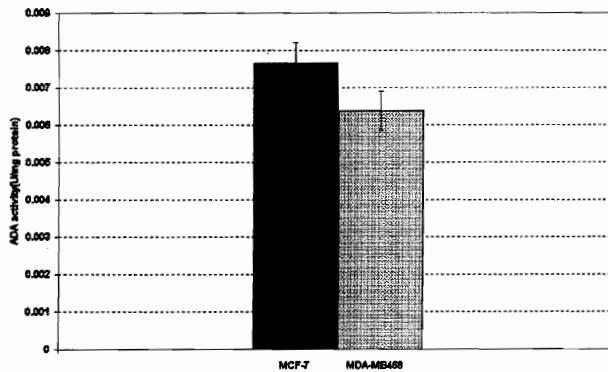
Results are expressed as means $\pm$ SD of 5 repeats each in duplicate.  $p < 0.05$  was considered significant using one way ANOVA.

### RESULTS

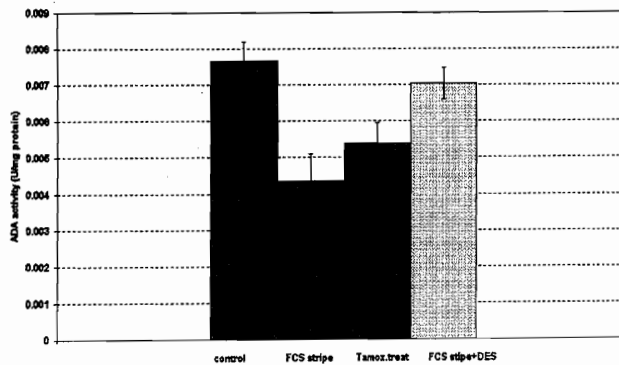
Figure 1 shows the typical morphologies of MCF-7 and MDA-MB468 human breast cancer cell lines. As illustrated in Figure 2, the activity of ADA in the MCF-7 and in the MDA-MB468 cells is  $0.0076757 \pm 0.0005212$  and  $0.0063754 \pm 0.0005322$  U/mg protein respectively. In the MCF-7 cells, the activity of ADA was significantly ( $p < 0.05$ ) higher than that observed for MDA-MB468 cells. As shown in Figure 3, the enzyme activity of MCF-7 cells cultured in the stripped serum was significantly



**Fig. 1.** Typical cell morphologies of MCF-7 and MDA-MB468 human breast cancer cell lines.



**Fig. 2.** Comparison of adenosine deaminase activity in human breast cancer cell lines, MCF-7 and MDA-MB468. Results are expressed as mean  $\pm$  SD of five repeats, each in duplicate.

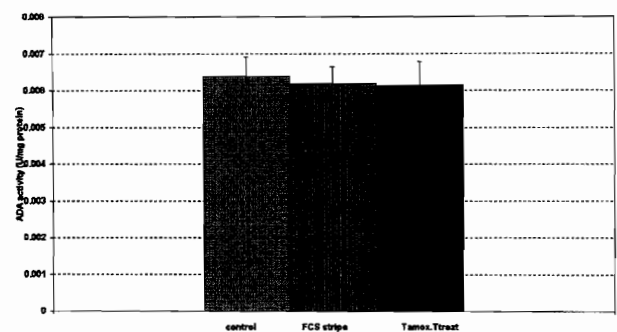


**Fig. 3.** Activity of adenosine deaminase in MCF-7 breast cancer cells. MCF-7 cells were grown in complete medium (control), stripped serum, complete medium with tamoxifen (1 $\mu$ M) and stripped serum plus diethylstilbestrol (0.01  $\mu$ M). Results are expressed as mean  $\pm$  SD of five repeats, each in duplicate.

( $p < 0.05$ ) lower than that of cells cultured in the complete medium (control). Treatment of the cells with tamoxifen also resulted in a significant ( $p < 0.05$ ) reduction in the ADA activity when compared with the value observed for the control cells, although the enzyme activity was slightly higher than those cultured in the stripped serum. Furthermore, addition of DES to the estrogen-free serum resulted in an increase in enzyme activity and the value reached the basal level (i.e. control). The activity of ADA in the MDA-MB468 cells, however, was not changed when cultured in the stripped serum or upon treatment with tamoxifen or DES (Fig. 4).

## DISCUSSION

It has long been known that there are high correla-



**Fig. 4.** Activity of adenosine deaminase in MDA-MB468 breast cancer cell line. MDA-MB468 cells were grown in complete medium, stripped serum, and complete medium with tamoxifen (1  $\mu$ M). Results are expressed as mean  $\pm$  SD of five repeats, each in duplicate.

tions between the carcinogenic process and the activities of some enzymes in cancerous tissues and cells. In this respect, ADA has drawn special attention. The reaction catalyzed by ADA is one of the rate-limiting steps of adenosine degradation. ADA has been reported to have a critical role in the proliferation and maturation of certain types of mammalian cells.<sup>18-19</sup> There are also several reports presenting high ADA activities in several types of cancerous tissues such as skin and bladder.<sup>6-8</sup> It has also been shown that the activity of ADA was higher in cancerous human kidney tissues compared with those of the noncancerous adjacent ones.<sup>20</sup> Some changes in ADA activity were also observed in gastric cancer and adjacent uninvaded gastric mucosa.<sup>21</sup>

With respect to the breast cancer there are only a few reports. Canbolat et al. investigated the activity of ADA in malignant breast tissues, irrespective of estrogen receptor status, and found a higher activity of the enzyme when compared to the non-cancer ones.<sup>22</sup> Concerning the relationship between ADA expression and estrogen receptor, to our knowledge there is only one study, so far, that has been reported by Xie et al.<sup>15</sup> They demonstrated the induction of ADA mRNA by estradiol in MCF-7 human breast cancer cells. Unexpectedly, these authors found that antiestrogen 4-hydroxytamoxifen significantly induced ADA mRNA levels. When the cells were co-treated with E2 plus 4-hydroxytamoxifen, a significant decrease in ADA mRNA levels was observed.<sup>15</sup> They did not provide a plausible explanation for their observations and the ADA activity was not evaluated.

In the present investigation the ADA activity was significantly higher in the MCF-7 cells than that of MDA-MB468, although the basal level was also high in the latter. MCF-7 cells cultured in the estrogen free (stripped) serum showed a significant reduction in the ADA activ-

ity when compared to that of control, in contrast to the report of Xie et al,<sup>15</sup> tamoxifen alone did significantly reduce enzyme activity, although not at the same magnitude as that observed for stripped serum. Furthermore, addition of DES, a potent synthetic estrogen, to the stripped culture media stimulated ADA expression and restored the activity. MDA-MB468 cells, however, exhibited no changes in ADA activity upon treatment with tamoxifen of removing estrogen from the media. These findings suggest E2 responsiveness of ADA expression in MCF-7 cells. In the MDA-MB468 cell line, the ADA gene is not an E2 target gene and there must be other nuclear transcription factors to induce ADA expression.

It has been demonstrated that tamoxifen induces a significant apoptosis in the MCF-7 cell line.<sup>23</sup> One possible mechanism might be through the inhibition of ADA and subsequent accumulation of toxic adenosine and deoxyadenosine that causes inhibition of ribonucleotide reductase and also inactivation of s-adenosyl homocysteine hydrolase, which results in apoptosis.

## REFERENCES

1. Wilson DK, Rudolph FB, Quijcho FA: Atomic structure of adenosine deaminase complexed with a transition-state analog: Understanding catalysis and immunodeficiency mutation. *Science* 252: 1276-1284, 1991.
2. Van der Weyden MB, Kelley WN: Human adenosine deaminase. Distribution and properties. *J Biol Chem* 251: 5446-5456, 1976.
3. Adams A, Harkness RA: Adenosine deaminase activity in thymus and other human tissues. *Clin Exp Immunol* 26: 647-649, 1976.
4. Chechik BE, Schreder WP, Minowada J: An immunomorphological study of adenosine deaminase distribution in human thymus tissue, normal lymphocytes, and hematopoietic cell lines. *J Immunol* 126: 1003-1007, 1981.
5. Resta R, Thompson LF: SCID: The role of adenosine deaminase deficiency. *Immunology Today* 18: 371-374, 1997.
6. Koizumi H, Lizuka H, Auyagi T, Miura Y: Characterization of adenosine deaminase from human epidermis and squamous cell carcinoma of the skin. *J Inv Derm* 84: 199-202, 1985.
7. Camici M, Tozzi MG, Allergini Del Corso A, Sanfilippo O, Daidone MG, De Marco C, Ipata PL: Purine salvage enzyme activity in normal and neoplastic human tissues. *Cancer Biochem Biophys* 11: 201-209, 1990.
8. Sufrin G, Tritsch GL, Mittelman A, Murphy GP: Adenosine deaminase in patients with carcinoma of bladder. *J Urol* 119: 343-346, 1978.
9. Durak I, Isik AU, Canbolat O, Akyol O, Kavutcu M: Adenosine deaminase, 5' nucleotidase, superoxide dismutase and catalase activities in cancerous and non-cancerous human laryngeal tissues. *Free Radic Biol Med* 15: 681-684, 1993.
10. Aitken SC, Lippman ME: Hormonal regulation of de novo pyrimidine synthesis and utilization in human breast cancer cells in tissue culture. *Cancer Res* 43: 4681-4690, 1983.
11. Aitken SC, Lippman ME, Kasid A, Schoenberg DR: Relationship between the expression of estrogen-regulated genes and estrogen-stimulated proliferation of MCF-7 mammary tumor cells. *Cancer Res* 45: 2608-2615, 1985.
12. Aitken SC, Lippman ME: Effect of estrogen and antiestrogens on growth-regulatory enzymes in human breast cancer cells in tissue culture. *Cancer Res* 45: 1611-1620, 1985.
13. Cowan K, Levine R, Aitken S, Goldsmith M, Douglass E, Clendeninn N, Nienhuis A, Lippman ME: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexate resistant human breast cancer cells. *J Biol Chem* 257: 15079-15086, 1982.
14. Kasid A, Davidson NE, Gelmann EP, Lippman ME: Transcriptional control of thymidine kinase gene expression by estrogens and antiestrogens in MCF-7 human breast cancer cells. *J Biol Chem* 261: 5562-5567, 1986.
15. Xie W, Duan R, Safe S: Estrogen induces adenosine deaminase gene expression in MCF-7 human breast cancer cells: Role of oestrogen receptor-Spl interactions. *Endocrinology* 140: 219-227, 1999.
16. Guisti, G, Galanti, B. Colorimetric method. In: Bergmeyer HU (ed). *Methods of enzymatic analysis*. Verlag Chemie, Weinheim, pp. 315-323, 1984.
17. Bradford MM: A rapid and sensitive method for the quantities of microgram of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254, 1976.
18. Ballett JJ, Insel R, Merler E, Ronsen FS: Inhibition of maturation of human precursor lymphocyte by cofomycin, an inhibitor of the enzyme adenosine deaminase. *J Exp Med* 143: 1271-1276, 1976.
19. Havi T, Smyth JF, Allison AC, Williams SCW: Role of adenosine deaminase in lymphocyte proliferation. *Clin Exp Immunol* 23: 395-403, 1976.
20. Durak I, Beduk Y, Kavutcu M, Suzer O, Yaman O, Ozturk HS, Canbolat O, Ulutepe S: Activity of the enzymes participating in purine metabolism of cancerous and noncancerous human kidney tissues. *Cancer Invest* 15: 212-216, 1997.
21. Namiot Z, Stasiewicz A, Namiot A, Keomona A, Kraliz M, Gorski J: Adenosine deaminase activity in patients with the intestinal type of gastric carcinoma. *Cancer Letters* 109: 199-202, 1996.
22. Canbolat O, Durak I, Cetin R, Kavutku M, Dermirci S, Ozturk S: Activities of adenosine deaminase, 5'-nucleotidase, guanase, and cytidine deaminase enzyme in cancerous and non-cancerous human breast tissues. *Breast Cancer Res Treat* 37: 189-193, 1996.
23. Salami S, Karami Tehrani F: Biochemical studies of apoptosis induced by tamoxifen in estrogen receptor positive and negative breast cancer cell lines. *Clinical Biochem* 36: 247-253, 2003.