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Preclinical report

Hydroxyurea treatment enhances metastatic cell adhesiveness: a possible chemotherapy-induced increase in metastatic potential?

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Treatment of tumor cells with certain chemotherapeutic agents, including hydroxyurea, increases their metastatic potential. In the present study the adhesiveness of SW620 colonic metastasis cells treated with hydroxyurea was compared with untreated cells by measuring their ability to remain attached to tissue flask walls during gentle saline washes. Treatment of SW620 cells was found to significantly increase their adhesiveness, suggesting a mechanism by which hydroxyurea may enhance the metastatic potential of tumor cells. [© 1999 Lippincott Williams & Wilkins.]

Key words: Cell adhesiveness, hydroxyurea, metastasis.

Introduction

Metastasis is a multistage process in which a cell must successfully detach itself from the main tumor, enter the vasculature, survive passage in the circulation and then establish itself in the capillary network of a distant site. The difficulty of this process is reflected in the fact that only about 0.01% of tumor cells in circulation establish metastasis.[1] It is well known that certain chemotherapy agents[2] including hydroxyurea[3] enhance the metastatic potential of tumor cells. A number of studies have investigated the possible mechanisms involved in this enhancement.[3,4] One such study[4] found that increased motility is associated with tumor cells in G_1/S where the hydroxyurea block occurs. Evidence is presented here showing that hydroxyurea treatment of metastatic cells enhances their adhesiveness compared with untreated cells.

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Methods

SW620 cells, which were originally derived from a colonic tumor metastasis, are adherent cells. However, they bind loosely to tissue flask walls and when washed with saline a proportion of cells become detached. Cells (0.3×10^6) were seeded into 15 tissue flasks (25 cm^2) and incubated in 4 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in a humidified CO_2 incubator. After 2 days medium was renewed in all flasks. In the case of six flasks the medium was supplemented with 10 mM hydroxyurea. After 15 h cells were given a single gentle wash, and medium was again replaced and the flasks were left for a further 1 h. The medium was then poured off into a 50 ml tube. The cells were then washed gently 4 times with 10 ml of saline and the washings collected in the 50 ml tube. Care was taken to ensure that controls and hydroxyurea-treated cells were washed identically. The flasks were then trypsinized and the number of cells which had not been washed off with the saline washes were counted using a hemocytometer. Tubes containing the washings from each flask were centrifuged at 1000 *g* and the supernatant discarded. The pelleted cells were resuspended in 1 ml of saline and counted to give the number of cells in the washings.

Results and discussion

Figure 1 shows the number of cells attached as a percentage of total cells in the control (mean ± SD: 57 ± 4.8) and hydroxyurea-treated SW620 (84 ± 7.9) cells. The difference in the mean percentage of cells attached is highly significant (t=8.29, $p < 0.001$). It is apparent that treatment of these cells with hydroxy-

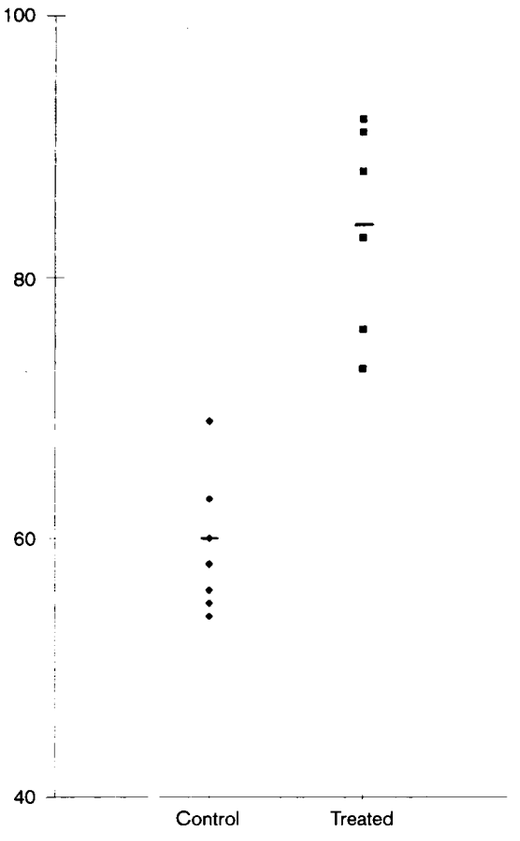

Figure 1. Percentage of untreated and hydroxyurea-treated SW620 cells adhering after saline washes.

urea results in them becoming more firmly attached to the tissue culture flask and presumably to each other. Hydroxyurea treatment results in a cell cycle block in late G_1/S, suggesting the possibility that cells at this stage of the cell cycle are more adhesive than those in other parts of the cycle. However, a further experiment showed that 3 h of hydroxyurea treatment resulted in 85% (±3.6) of cells adhering after washing compared with only 60% (±3.5) in control flasks (t=8.62, $p<0.001$). This would be too early for a significant cell cycle resdistribution to have occurred. Further, it has been shown that tumor cells still exhibit increased metastasis 6 h after release from a hydroxyurea block.[3] A recent[5] study which examined the expression of cell surface antigens on metastatic variants of a colonic tumor line found that higher metastatic acivity was associated with greater expression of the adhesive molecules, integrins. The up-regulation of these molecules can occur within 1.5 h.[6] It is therefore possible that the enhanced adhesiveness of SW620 cells after treatment with hydroxyurea observed in the present study is a consequence of increased cell surface integrin expression and that this enhancement contributes to increased metastatic potential.

References

1. Liotta LA. Gene products which play a role in cancer invasion and metastasis. *Breast Cancer Res Treat* 1988; **11**: 113–24.
2. Kerbel RS, Davies AJ. Facilitation of tumour progression by cancer therapy. *Lancet* 1982; **ii**: 977–8.
3. McMillan TJ, Rao J, Hart IR. Enhancement of experimental metastasis by pretreatment of tumour cells with hydroxyurea. *Int J Cancer* 1986; **38**: 61–5.
4. Iwasaki T, Shinkai K, Mukai M, *et al*. Cell cycle dependent invasion *in vitro* by rat ascites hepatoma cells. *Int J Cancer* 1995; **63**: 282–7.
5. Okazaki K, Nakayama Y, Shibao K, Hirata K, Nagata N, Itoh H. Enhancement of metastatic activity of colon cancer as influenced by expression of cell surface antigens. *J Surg Res* 1998; **78**: 78–84.
6. Quigley RL, Shafer SH, Williams CL. Regulation of integrin-mediated adhesion by muscarinic acetylcholine receptors and protein kinase C in small cell lung carcinoma. *Chest* 1998; **114**: 839–46.

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