Association of Glyceraldehyde-3-phosphate Dehydrogenase Expression with Cell Motility and Metastatic Potential of Rat Prostatic Adenocarcinoma

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Abstract

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression is increased in Dunning R-3327 rat prostatic adenocarcinoma cell lines relative to normal rat ventral prostate tissue. GAPDH expression closely correlates with cell motility of Dunning prostate cancer cell lines and accurately distinguishes cell lines with high metastatic potential from those with low metastatic potential. Increased GAPDH expression in the cancer cell lines is not simply related to increased growth rate, since rapidly proliferating normal prostate tissue did not exhibit elevated GAPDH expression.

Introduction

In 1930, Otto Warburg demonstrated that cancer cells have increased levels of aerobic glycolysis and therefore produce lactate even in the presence of air (1). In recent years it has become apparent that several key glycolytic enzymes, including GAPDH, reversibly associate with cytoskeletal F actin (2, 3). The reversible association of glycolytic enzymes with the cytoskeleton affects cell structure and glycolytic activity and is thought to lead to the integration of cell motility and metabolism (2, 3).

This study was undertaken to clarify the role of glycolysis in prostate cancer cell motility and metastasis, two complex biological phenomena that are incompletely understood (4). We studied the expression of GAPDH, a key regulatory enzyme of glycolysis which generates the high-energy compound 1,3-bisphosphoglycerate, in the Dunning rat prostatic adenocarcinoma model. The Dunning prostate cancer model consists of several biologically diverse cell lines with a wide range of metastatic ability (Table 1; Refs. 5–7). We observed a close correlation between GAPDH expression and both cell motility and metastatic potential and believe that aerobic glycolysis may provide an energy source for cell motility, as has been suggested by others (8, 9).

Materials and Methods

Cell Culture. Ten sublines with a wide range of metastatic potential from the spontaneous Dunning R3327 tumor system of serially transplantable rat prostatic adenocarcinoma were studied. All sublines grew when inoculated into inbred Copenhagen rats (Harlan Sprague Dawley, Indianapolis, IN). The characteristics of 3 high metastatic (AT3, MAT-Lu, MAT-LyLu) and 7 low metastatic (G, H, HI-M, HI-S, HI-F, AT1, AT2) sublines are well established and are summarized in Table 1 (5–7). Except for the H tumor, all sublines were maintained as continuous in vitro cell lines as described previously (5).

Measurement of Motility. Time lapse videomicroscopic films of isolated cells from each of the 8 sublines were analyzed under similar conditions with a standard technique (10). In these studies, approximately 103 cells were plated in T-25 plastic tissue culture flasks (Falcon, Oxnard, CA) and equilibrated at 37°C in 3 ml of RPMI 1640 with 2% F mosquito l-glutamine (MA Bioproducts, Walkersville, MD) which contained 10% fetal calf serum (Hyclone Labs, Inc., Logan, UT), 250 ng dexamethasone (Sigma Chemical Co., St. Louis, MO), 100 units/ml of potassium penicillin G, and 100 units/ml of streptomycin sulfate under an atmosphere of 5% CO2:95% air. Twelve to 24 h later cells were viewed with an inverted microscope (IM35; Zeiss, Thornwood, NY) fitted with Hoffman optics. A programmable control box (Red Lion Controls, York, PA) coordinated illumination of the field with reference to focus and image capture. Images magnified 400 times were recorded every 15 s by a high-resolution black and white video camera (Model MTTI 66; Dage, Michigan City, IN) and time lapse videorecorder (TL AG-6050; Panasonic, Secaucus, NJ) on 0.5-inch vertical helical scan. The 720 frames were played at 24 frames/s which produced a 30-s film at 360 times normal speed.

Sixty-four films of single cells were graded for cell motility by two observers who had no knowledge of the identity of the specimens. Membrane ruffling, pseudopodal extension, and cellular translation were graded from 0 (no motility observed) to 5 (excessive motility) by each observer as described previously (10). The grades of both observers were summed to yield final scores from 0 to 10 for each motility parameter. The three types of motility grades were averaged to yield a combined motility index for each cell type.

Northern Analysis and Hybridization. Total cytoplasmic RNA was isolated from cell lines grown to confluence as described previously (11) with the following modifications. RNA was extracted once with 0.2 M sodium acetate (pH 4.0), 1 volume of acid phenol, and 0.2 volume of chloroform:isoamyl alcohol (24:1); reextracted with 1 volume of acid phenol and 0.2 volume of chloroform:isoamyl alcohol; precipitated with an equal volume of isopropanol; and resuspended in DEPC-treated water.

Total cytoplasmic RNA was isolated from 50-mg pieces of tissue which were placed in ice cold phosphate buffered saline immediately following removal. Animals were sacrificed by CO2 inhalation. The tissue pieces were homogenized in 0.5 ml of an ice cold solution of 0.74 M guanidinium thiocyanate, 39 mM sodium citrate (pH 7), 0.78% n-laurysarcosine heated to 65°C prior to addition, and 0.1 M 2-mercaptoethanol added on the day of use in a pellet pestle with a tube (Kontes Scientific Glassware/Instruments, Vineyard, NJ). RNA was electrophoresed in a formaldehyde-agarose gel as described previously (12) and blotted onto nylon filters (Zetaprobe GT; Bio-Rad Laboratories, Richmond, CA). Radioactive probes were prepared from purified cDNA inserts by the random prime method as described by Feinberg and Vogelstein (13) (Amersham multiprime labeling kit). The 0.6-kilobase C cDNA sequence which corresponds to the major prostate secretory protein was the kind gift of Dr. Malcolm Parker (14). The 1.0-kilobase GAPDH cDNA sequence used as probe was purchased from Clontech, Palo Alto, CA. Filters were prehybridized overnight at 55°C in 50% deionized formamide, 2× saline-sodium phosphate-EDTA, 7% SDS, 0.5% nonfat dry milk, and 200 μg/ml salmon testis DNA which was boiled for 5 min and chilled on ice prior to being added to the prehybridization mix. Filters were hybridized overnight at 55°C with 32P radioactive probe added to a freshly prepared aliquot of the same solution which was used for prehybridization. Filters were then washed in 2× standard saline-citrate-0.1% SDS for 15 min at room temperature and in 0.2× standard saline-citrate-0.1% SDS for 15 min at room temperature. Filters were exposed to Kodak XAR-5 film at −70°C with intensifying screens.

Androgen Withdrawal and Rechallenge. Adult male Sprague-Dawley rats (250-300 g) were castrated via the scrotal route at staggered times to ensure that all animals would be sacrificed and all tissues harvested on the
pseudopodal extension, and ruffling, did not discriminate between high and low metastatic cell lines in normal prostate tissue. The expression was determined as described previously (5-7).

Cancer cell lines and normal prostate was performed as described above using equal quantities of total cytoplasmic RNA per lane. Bands corresponding to GAPDH mRNA were quantitated by densitometry and normalized for rRNA content. GAPDH expression for each prostate cancer cell line was then compared to that for normal ventral prostate tissue and expressed as fold induction.

Northern analysis. The filters were probed with C3 as an internal control to demonstrate the glycolytic enzyme, GAPDH. Increased expression of GAPDH mRNA in Dunning prostatic adenocarcinoma cell lines relative to normal and regenerating prostate is depicted in Fig. 1. GAPDH expression accurately differentiated cell lines with high metastatic potential from those with low metastatic potential and was more effective that motility index in differentiating the two groups (Fig. 1).

Table 1. Biological characteristics of ten sublines of the Dunning R3327 rat prostate adenocarcinoma

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Histology</th>
<th>Growth rate (days)*</th>
<th>Androgen sensitivity</th>
<th>Metastatic potentialb</th>
<th>Site of metastases</th>
<th>Host survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low metastatic potential</td>
<td>G tumor</td>
<td>Poorly differentiated</td>
<td>4.0 ± 0.2c</td>
<td>Yes</td>
<td>Low (50)c</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>H tumor</td>
<td>Well differentiated</td>
<td>21 ± 6.0</td>
<td>Yes</td>
<td>Low (50)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>HIF tumor</td>
<td>Poorly differentiated</td>
<td>4.8 ± 1.8</td>
<td>No</td>
<td>Low (25)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>HI-S</td>
<td>Well differentiated</td>
<td>2.4 ± 5</td>
<td>No</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>HI-M</td>
<td>Moderately differentiated</td>
<td>9.0 ± 0.8</td>
<td>No</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>AT-1 tumor</td>
<td>Anaplastic</td>
<td>2.5 ± 0.2</td>
<td>No</td>
<td>Low (25)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>AT-2 tumor</td>
<td>Anaplastic</td>
<td>2.5 ± 0.2</td>
<td>No</td>
<td>Low (50)</td>
<td>None</td>
</tr>
<tr>
<td>High metastatic potential</td>
<td>MAT-LyLu tumor</td>
<td>Anaplastic</td>
<td>2.7 ± 0.3</td>
<td>No</td>
<td>High (50)</td>
<td>Lungs (50-350)b</td>
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<td></td>
<td>MAT-LyLu tumor</td>
<td>Anaplastic</td>
<td>1.7 ± 0.3</td>
<td>No</td>
<td>High (50)</td>
<td>Lympnodes and lung (25-250)</td>
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<tr>
<td></td>
<td>AT-3 tumor</td>
<td>Anaplastic</td>
<td>1.8 ± 0.2</td>
<td>No</td>
<td>High (50)</td>
<td>Lympnodes and lung (20-200)</td>
</tr>
</tbody>
</table>

* Tumor volume doubling time (days).
b Low metastatic potential, <10% of s.c.-inoculated rats develop distant metastases; high metastatic potential, >90% develop distant metastases.
Mean ± SD.
Numbers in parentheses, the number of animals/group upon which these data are based.

Measurement of Relative GAPDH Expression for Dunning Prostate Cancer Cell Lines. GAPDH expression of each of the Dunning prostate cancer cell lines and normal ventral prostate was performed as described above using equal quantities of total cytoplasmic RNA per lane. Bands corresponding to GAPDH were quantitated by densitometry and normalized for rRNA content. GAPDH expression for each prostate cancer cell line was then compared to that for normal ventral prostate tissue.

GAPDH Expression Differentiates Dunning Prostate Cancer Cell Lines with High Metastasic Potential from Those with Low Metastatic Potential. GAPDH expression of each of the Dunning prostate cancer cell lines relative to normal ventral prostate is depicted in Fig. 1. GAPDH expression accurately differentiated cell lines with high metastatic potential from those with low metastatic potential and was more effective than the motility index in differentiating the two groups (Fig. 1).

Increased GAPDH Expression in Dunning Prostate Cancer Cell Lines Is Not Simply Due to Rapid Growth. Results of Northern analysis of GAPDH and C3 mRNA expression of Dunning prostate cancer cell lines and normal prostate tissue at baseline and following androgen withdrawal and rechallenge are shown in Fig. 2. C3 is the principal androgen regulated secretory protein in prostatic fluid (14). The filters were probed with C3 as an internal control to demonstrate that prostate regeneration with return of function occurred following androgen rechallenge, but not following injection of vehicle alone. We showed previously that DNA synthesis in rat ventral prostate is maximal at 3 days following androgen treatment of castrates (15). It is apparent from the data that GAPDH expression relative to total RNA content is increased in the high metastatic AT3 Dunning cell line as compared to normal and regenerating prostate. Increased GAPDH expression is therefore not simply due to cellular proliferation but is instead associated with metastatic potential.

GAPDH Expression Correlates with Motility of Dunning Prostate Cancer Cell Lines. Scores for ruffling, pseudopodal extension, translation, and motility index of 8 Dunning cell lines plotted versus GAPDH expression are presented in Fig. 3. There is a statistically significant correlation between GAPDH expression and each of the motility features except ruffling.

Discussion

We show by Northern analysis that mRNA abundance for a key glycolytic enzyme, GAPDH, is increased in Dunning prostatic adenocarcinoma cell lines as compared to normal prostate tissue. GAPDH expression, which is increased in other types of cancer (16-19), was not increased in regenerating prostate tissue following androgen withdrawal and rechallenge and is therefore not simply a marker of prostate cell proliferation, but a reflection of metastatic potential.

Increased GAPDH expression in the Dunning rat prostate adenocarcinoma model is probably a reflection of increased aerobic glycolysis, which has been observed for many types of cancer since Otto Warburg first described the phenomenon over 60 years ago (1, 20). However, GAPDH is also known to have functions which are seemingly independent of glycolysis, such as sequence specific binding to tRNA (21).

The results of this study are consistent with the well documented reversible association of glycolytic enzymes with cytoskeletal F actin.
and the hypothesis that aerobic glycolysis is an important energy source for cell motility and metastatic ability. If GAPDH expression is found to closely correlate with metastatic potential of human prostate cancer specimens as it does with metastatic potential of the well characterized Dunning rat prostate cancer model, GAPDH expression may be a candidate as a prognostic and diagnostic index for patients with prostate cancer.

Acknowledgments

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References

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