Identification of High Mobility Group Protein I(Y) as Potential Progression Marker for Prostate Cancer by Differential Hybridization Analysis

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ABSTRACT

One of the major problems in the diagnosis of localized prostatic tumors is to predict the aggressiveness of an individual tumor, which is presumably associated with chance to progression. In an attempt to find molecular markers that are specific for aggressive prostatic cancer cells, we compared steady-state mRNA levels of progressionally related prostatic tumors. The Dunning R-3327-H subline, a relatively benign rat prostatic tumor, was compared to the therefrom derived highly aggressive MatLyLu tumor by differential hybridization analysis. The differential screening revealed 26 complementary DNA clones that detected transcripts overexpressed in MatLyLu. Upon further screening on the entire panel of Dunning R-3327 sublines, it appeared that three clones (pBUS1, pBUS19, and pBUS30), detected transcripts specifically expressed in metastatic rat prostatic tumors. The expression pattern of pBUS19 and pBUS30 suggested a relation between these complementary DNAs. Nucleotide sequence analysis, however, could not yet substantiate this. Computer-assisted comparison of the DNA sequences revealed the presence of rat long terminal repeat-like repetitive elements in pBUS19. The differential expression of repetitive elements in progressionally related tumors is interesting, yet similar findings have not been reported in human malignancies. Nucleotide sequence analysis of pBUS1 indicated that this clone is identical or related to high mobility group protein I(Y), a non-histonuclear protein. From recent studies it appeared that this protein might be implicated in replication and/or transcription processes. The overexpression of high mobility group protein I(Y) correlates rather with metastatic ability than with growth rate; hence it may serve as a valuable marker to identify progressionally advanced prostate cancer cells.

INTRODUCTION

Among cancer-related deaths in the American male population, prostate cancer is the second leading cause (i.e., 11%). Moreover, prostate cancer accounts for 21% of all newly diagnosed cancers in that group (highest incidence) (1). The majority of patients presents clinically with localized disease (Stage A-C). Patients with truly localized disease (i.e., no capsular penetration, Stage A-B) are amenable to curative radical surgery. However, a considerable fraction of this group clinically progress to a metastatic state. This can be explained only by the fact that at the time of surgery, dissemination of tumor cells had already occurred resulting in microscopic metastases. Identification of patients at risk for having such micrometastases is likely to be of great significance, since scientific therapeutic studies revealed that small disseminated lesions are still amenable to curative chemotherapy, whereas they are not later in their clinical progression (2). Clearly, adjuvant chemotherapy for all Stage A-B patients is unacceptable since the majority of patients would be overtreated.

Prediction of the aggressiveness of an individual tumor can be achieved by histological examination of the primary tumor. Whereas classical pathological grading according to the method of Gleason (3) is not able to discriminate tumors that do progress clinically from those that do not, analysis of nuclear morphometric characteristics seems to be more promising (4). Another approach is to identify molecular characteristics specific for aggressive tumor cells. Thus far no markers are available that meet the demands mentioned above.

A useful method for isolation and characterization of molecular markers for progressionally advanced cancer cells is comparison of steady-state mRNA levels by differential or subtraction hybridization analyses. Earlier studies using the well characterized rat prostatic cancer Dunning R-3327 model system revealed that fibronectin is down-modulated upon progression from anaplastic nonmetastasizing rat prostate tumors to metastasizing tumors (5). Thus far, there are no clinical implications of these findings for the early steps in the progression of prostate cancer, since well differentiated tumors also have a low expression of fibronectin. Furthermore, loss of a molecular characteristic is less suitable for diagnostic purposes. In the present study, we used the same technique to compare the most benign tumor from the Dunning R-3327 rat prostatic cancer model system, the H tumor, with the metastatic MatLyLu tumor by differential hybridization analysis, screening, in this case, for up-regulation of genes.

The cDNA clones that detected differentially expressed genes were evaluated for their relation to the aggressive phenotype by screening ten Dunning R-3327 sublines. Finally, the cDNA clones that met the selection criteria were sequenced and the resulting nucleotide sequences were compared to nucleotide databases to search for homology with known genes.

MATERIALS AND METHODS

Dunning R-3327 Rat Prostatic Tumors. The parental tumor from which all rat prostatic tumor sublines were derived is the original R-3327 tumor described by Dunning (6). To show differences in the history and characteristics of the various R-3327 tumors passaged at several institutes, the tumor lines are denoted by different letters. The G subline was developed by Dunning and is poorly differentiated but androgen responsive (7). The G subline was generously provided by Dr. Alice Claflin (University of Miami). The slow growing, well differentiated subline obtained from Dr. Arthur Bogden has been serially passaged at The Johns Hopkins University (Baltimore, MD) and was termed R-3327-H.

The H subline is a well differentiated, androgen-responsive tumor. The H tumor was shown to be a heterogeneous tumor composed of both androgen-dependent and -independent cells (8). By growing the H tumor in mice, a nonmetastatic tumor was obtained, termed R-3327-M. The R-3327-M tumor was further passaged in vitro to obtain the R-3327-M subline. This R-3327-M subline is androgen responsive but not androgen dependent (9).
The Dunning R-3327-H tumor passaged at Johns Hopkins (7). All these sublines arose from the well differentiated tumor, HIM, and an even faster growing, moderately well differentiated subline, HIF. The sublines AT1 and AT2 arose from the AT tumor. AT3 arose from the HIF line. In rats, these last three sublines are suitably growing tumors with a low metastatic ability (7); i.e., less than 10% of the animals inoculated with tumor sublines develop distant metastases. MatLyLu (9) and MatLu (10) both arose from the AT1 tumor in castrated rats it appeared to be possible to select in vivo for hormone-independence growing tumors with a high metastatic ability (7); i.e., more than 90% of the animals inoculated with these tumor sublines develop distant metastases (7). The characteristics of the Dunning sublines used in this study are shown in Table 1.

Each of the tumor sublines described above is routinely passaged by inoculating male inbred Copenhagen (Cop) rats (Harlan Sprague-Dawley, Indianapolis, IN) s.c. in the flank with a 25-µg trocar piece of the respective tumor subline as described before (8). Tumor samples were harvested when the respective tumors were growing exponentially at a tumor volume of 1-2 cm³, frozen in liquid nitrogen, and stored at -80°C.

mRNA Isolation. Total RNA from the Dunning tumors was isolated using the lithium chloride/urea procedure as described by Auffray and Rougeon (11). Poly(A)⁺ RNA was purified by selection on an oligo(dT)-cellulose column (12).

Construction of cDNA Library. For the construction of the cDNA library, a strategy of adaptor ligation was used. According to the paper of Haymerle et al. (13) unphosphorylated adaptor oligonucleotides are ligated onto both vector and insert DNA. Upon removal of unligated adaptors and phosphorylation of the 5' hydroxystermini, the cDNA is ligated in the vector.

Briefly, 10 µg of poly(A)⁺ RNA isolated from the MatLyLu tumor were oligo(dT)₁₅₋₁₈ primed and cDNA synthesis was performed according to the method of Gubler and Hoffman (14). To the blunt-ended cDNA, a 3-fold molar excess of BamHI-cut polJCIS was added and the mixture was ethanol precipitated. After the vector and cDNA were dissolved, a 100-fold molar excess of unphosphorylated adaptor oligonucleotides [BamHI/blunt end (Boehringer)] was added. The sample was heated to 65°C for 5 min and then allowed to cool down to room temperature in 10 min. After overnight ligation (T4-DNA ligase) at 12°C, the sample was heated to 65°C for 5 min and loaded on a Bio-Gel A-50 column to remove the nonligated adaptors from the vector and cDNA. After the column was eluted with 10 mM Tris-HCl (pH 7.5-1 mM EDTA), the fractions containing the vector and cDNA were pooled, precipitated, and phosphorylated. Finally, the vector and the cDNA were ligated overnight and transformed to competent Escherichia coli (strain DH5α) cells.

Differential Screening of the Library. Approximately 10,000 colonies containing inserts were plated on nitrocellulose filters and 4 replicas were made. The replica filters were lysed according to the method of Sambrook et al. (15). Hybridization was performed according to the method of Hanahan and Meselson (16) in 40% formamide at 42°C for 6 h. For the differential screening, probes representative for the H tumor and the MatLyLu tumor mRNA populations were prepared as follows: 1.5 µg oligo(dT)₁₅₋₁₈ was annealed to 1 µg of poly(A)⁺ RNA by incubation at 68°C for 5 min and quenching on ice. First strand synthesis was then performed for 60 min at 37°C in 50 mM Tris-HCl (pH 8.3); 6 mM MgCl₂; 40 mM KCl; 1 mM dithiothreitol; 100 µg/ml BSA, 0.6 mM concentrations of unlabeled dATP, dGTP, and dTTP, 0.06 mM of unlabeled dCTP, and 10 µCi of [α³²P]dCTP (>3000 Ci/mmole) using 500 units of reverse transcriptase (BRL). Alkaline hydrolysis of the remaining RNA was performed for 30 min at 60°C in 10 mM EDTA, 0.3% SDS, and 160 mM NaOH. After addition of hydrcx yacetate and Tris-HCl (pH 7.5) to final concentrations of 140 and 60 mM, respectively, the sample is run on a Sephadex G-50 spin column to remove the excess of unincorporated nucleotides. The eluate is ethanol precipitated and this first strand cDNA was used as a template for a random prime labeling reaction (17) in the presence of 50 µCi [α³²P]dCTP (>3000 Ci/mmole). The specific activity of the cDNA probes thus obtained was 0.5-1.0 x 10⁶ dpm/µg template RNA which is at least 10-fold higher than can be achieved in a reverse transcriptase labeling. For the hybridization of the filters (2 replicas with probe derived from the H tumor, 2 replicas with probe derived from the MatLyLu tumor), a 1 x 10⁶ dpm/ml hybridization solution was used.

Northern Blot Analysis. Ten µg of total RNA were glyoxylated, size fractionated on 1% agarose gels, and transferred to Hybond-N (Amer sham). Probes were made of DNA, obtained from small scale plasmid isolations or of purified inserts of cDNA clones, by random prime labeling reactions (17).

Hybridizations were performed according to the method of Church and Gilbert (18); the membranes were preincubated in hybridization buffer (7% SDS; 1% BSA; 0.5 mM sodium phosphate buffer, pH 7.4; 1 mM EDTA; 100 µg/ml salmon sperm DNA) for 1-4 h at 65°C. The radioactively labeled probe was added to a maximum of 1 x 10⁶ dpm/ml and the membranes were hybridized overnight at 65°C. Filters were then washed to high stringency (i.e., buffers containing 1% SDS, 1 mM EDTA, and decreasing concentrations of sodium phosphate buffer; 0.5 M, 0.1 M, 0.05 M. Each wash step was performed at 65°C for 30 min.). Dehybridization was performed in 0.1 x Denhardt's solution, 5 mM Tris-HCl (pH 7.4), and 2 mM EDTA at 65°C.

DNA Sequencing and Computer Analysis. DNA fragments were ligated into the polylinker region of M13mp8-19. All of the DNA sequences were determined using the dideoxy sequencing method as described by Sanger et al. (19). The gel readings were recorded and edited using Intelligenetics computer software (release 5.35). Computer comparison studies were performed with the EMBL (release 22) and Genbank (release 60) nucleotide sequence databases (20).

RESULTS

Differential Hybridization Reveals 3 MatLyLu-specific cDNA Clones. A cDNA library was constructed from the anaplastic, hormone-independent, metastasizing tumor MatLyLu. The library had a complexity of 10,000 recombinant clones; i.e., with

<table>
<thead>
<tr>
<th>Subline</th>
<th>Histology</th>
<th>Doubling time (in days)</th>
<th>Androgen responsive</th>
<th>Metastatic ability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Well differentiated</td>
<td>22 ± 5</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>HIS</td>
<td>Well differentiated</td>
<td>24 ± 5</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>HIF</td>
<td>Well differentiated</td>
<td>90 ± 0.8</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>G</td>
<td>Moderately differentiated</td>
<td>4.8 ± 1.8</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>MATLu</td>
<td>Poorly differentiated</td>
<td>40 ± 0.2</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>AT-1</td>
<td>Anaplastic</td>
<td>2.7 ± 0.2</td>
<td>No</td>
<td>High (lungs)b</td>
</tr>
<tr>
<td>AT-2</td>
<td>Anaplastic</td>
<td>2.5 ± 0.2</td>
<td>No</td>
<td>Low to moderate (lungs)</td>
</tr>
<tr>
<td>AT-3</td>
<td>Anaplastic</td>
<td>1.8 ± 0.2</td>
<td>No</td>
<td>High (lymph nodes &amp; lungs)</td>
</tr>
<tr>
<td>MATLyLu</td>
<td>Anaplastic</td>
<td>1.5 ± 0.1</td>
<td>No</td>
<td>High (lymph nodes &amp; lungs)</td>
</tr>
</tbody>
</table>

* Low metastatic ability. <5% of s.c. inoculated rats develop distant metastases; moderate ability, >5%, <20%; high metastatic ability, >75% develop distant metastases.

b Organs in parentheses are the site of the distant metastases for the individual sublines.
a probability of 99%, a mRNA expressed at a relative abundance of 0.05% will be represented in this library (21). After amplification of the library, approximately 10,000 colonies were plated and 4 replica filters were prepared for in situ colony hybridization. Sets of two replica filters were hybridized with cDNA probes, using as template poly(A)+ RNA of the MatLyLu tumor and the more benign H tumor (well differentiated, hormone sensitive, nonmetastasizing). After 60 h of hybridization, the filters were washed and exposed for autoradiography for 3 days (see Fig. 1). Extensive comparison of the resulting autoradiographs revealed 18 clones that seemed to be differentially expressed; i.e., a signal was evident on both replicas hybridized with the cDNA probe derived from the MatLyLu tumor and no or a very weak signal was detected on any of the replicas hybridized with the probe derived from the H tumor. Upon long exposure (14 days, 2 intensifying screens; Kodak XAR5) 8 additional cDNA clones appeared to detect differentially expressed mRNAs.

The secondary screening of these 26 cDNA clones was a Northern assay on a panel of three Dunning tumors, in which cDNA-containing plasmids were radiolabeled and hybridized on blots containing 10 µg of total RNA of the H, AT2 (anaplastic, hormone independent, nonmetastasizing), and MatLyLu tumors. The expression patterns of the most significant clones are shown in Fig. 2. From this secondary screening it appeared that the hybridization patterns could be divided into three groups: no significant difference in the three tumors (i.e., false positives); increase in expression towards MatLyLu, whereas the increase in expression was less than 3-fold (e.g., Fig. 2; pBUS4, 6, 8, 17, 22); and increase in expression towards MatLyLu more than 5-fold (e.g., Fig. 2; pBUS1, 10, 14, 19, 24, 25, 30). The cDNA clones from the last group were analyzed on Northern blots containing 10 µg of total RNA of normal rat prostate and ten Dunning sublines representing the different stages of tumor progression (see Table 1). pBUS10, 14, 24, and 25 showed no consistent relation between the mRNA levels and progression of prostate cancer whereas the expression patterns detected with pBUS1, 19, and 30 did correlate with progression-related parameters (i.e., growth rate, hormone dependency, histology, and metastatic ability).

Expression Pattern of pBUS1. pBUS1 detects a single transcript of 1.8 kilobases (Fig. 3A). The expression level is high in all metastatic Dunning sublines tested; i.e., pBUS1 transcripts are at least 10-fold more abundant in the metastasizing tumors AT3, MatLu, and MatLyLu than in the anaplastic, nonmetastasizing tumors AT1 and AT2 (concluded from densitometric scanings of longer exposures of the same autoradiograph as shown in Fig. 3A). Moreover, in the hormone-responsive G and H sublines, as well as in the lines HIS, HIM, and HIF that arose from the H tumor through castration-induced selection, no detectable levels of pBUS1 transcripts were found. Also in normal prostate tissue no pBUS1 mRNA was detectable. (Even upon longer exposure no signals were detected in normal prostate, G, H, HIS, HIM, or HIF.)

Thus it appears that pBUS1 expression is highly correlated with the metastatic phenotype, rather than with growth rate [e.g., MatLu has a doubling time of 2.7 days, while AT1 and AT2, which show a much lower expression, have a doubling time of 2.5 days (see Table 1)].

Expression Patterns of pBUS19 and pBUS30. The Northern analyses using pBUS19 and pBUS30 as a molecular probe revealed expression patterns that at some points are strikingly similar yet at other aspects differ significantly (Fig. 3, B and C). Both probes detect a 7.0-kilobase transcript, abundantly expressed in the metastasizing AT3 and MatLyLu tumors while MatLu, a tumor line that metastasizes exclusively to lungs, contains no detectable transcripts. The 7.0-kilobase pBUS19/30 mRNA is expressed at much lower levels in G, HIS, HIM, and HIF (longer exposure, not shown in Fig. 3B, revealed a very low expression of the transcript). It should be noted that
in G, HIS, and HIM the transcript might be slightly smaller than 7.0 kilobases (see Fig. 3C). The relative intensities of the transcript using either probe were similar (based on densitometric scanning; data not shown). However, pBUS30 detected an additional transcript of 6.0 kilobases exclusively found in AT1, AT2, and HIF. The expression of this transcript is higher in AT1 and AT2 than in the HIF tumor. Considering the data on the 7.0-kilobase transcript we conclude that pBUS19 and pBUS30 might be related cDNA clones. (For both Fig. 3A and Fig. 3C, the band seen at approximately 4.3 kilobases is probably due to background hybridization.)

DNA Sequence Analyses of pBUS1, pBUS19, and pBUS30. To obtain further information on the cDNA clones, the cDNA inserts were subcloned in M13mp8-19 and the nucleotide sequence was determined using the dideoxy sequencing method. Computer-assisted comparison of the resulting nucleotide sequences with the EMBL and Genbank nucleotide sequence databases should reveal homology with known sequences.

To investigate whether pBUS19 and pBUS30 are indeed related, we first compared the nucleotide sequences of these two cDNA clones. No homology was found between pBUS19 and pBUS30. Furthermore, upon screening the nucleotide sequence databases, no homology to any of the known sequences could be found for pBUS30 (cDNA insert 0.5 kbp compared to a mRNA size of 6.0 and 7.0 kilobases). For pBUS19 (cDNA insert 1.4 kbp), however, the computer comparison showed the presence of parts of two rat-specific repetitive elements, RAL6 and RAL10 (22). As outlined in Fig. 4, pBUS19 contains 289 base pairs of the RAL6 element (89% homology) and 91 base pairs of the RAL10 element.

Fig. 2. Northern blot analysis of 12 cDNA clones isolated upon differential hybridization. Ten µg of total RNA from the H tumor (Lane 1), the AT-2 tumor (Lane 2), and the MatLyLu tumor (Lane 3) were loaded per lane. 32P-labeled DNA probes were derived from small scale plasmid isolations of the cDNA clones. kb, kilobase; MW, molecular weight.

Fig. 3. Northern blot analysis of pBUS1 (A), pBUS19 (B), and pBUS30 (C). Ten µg of total RNA of normal prostate (NP) and 10 Dunning tumors were loaded per lane. 32P-labeled DNA probes were derived from purified inserts of the cDNA clones. rRNA was used as an internal control for the amounts of RNA loaded (D). kb, kilobase; MW, molecular weight.

Fig. 4. Schematic representation of the alignment between pBUS19 and the RAL elements. Bottom, sequence alignments. bp, base pair.
pairs of the RAL10 element (92% homology). The RAL elements are very homologous to one another and belong to a family of long terminal repeat-like sequences. It should be noted that pBUS19 and pBUS30 contain only small portions of the entire mRNAs of 6.0 and 7.0 kilobases. Since both cDNA clones contain poly(A) tails, it is very likely that they represent the 3′ noncoding (and probably less conserved) sequences of the transcripts. Hence evidence for the putative relation between pBUS19 and pBUS30 might come from sequence analysis of the upstream regions, not yet cloned.

For pBUS1 (cDNA insert 0.9 kbp) a high homology to the mouse (83%) (23) and human (71%) (24) HMG-I(Y) cDNA sequence was found (HMG-Y is an isoform). A schematic representation of the alignment of the sequence data is shown in Fig. 5. pBUS1 contains 0.9 kbp of the 3′-end of the murine HMG-I(Y) cDNA which is 1.7 kbp. As illustrated in Fig. 5, only a small part of the HMG-I(Y) cDNA is coding. pBUS1 contains only noncoding sequences (3′-UTR) in which, nevertheless, a high homology to mouse and human sequences is found; hence it is very likely that pBUS1 is the rat homologue of HMG-I(Y).

**DISCUSSION**

Many molecular alterations appear to be associated with the process of tumor progression. Molecular studies on the development and progression of cancer have indicated several groups of genes that might be implicated in this process. These groups comprise oncogenes, tumor suppressor genes, genes encoding growth factors, growth factor receptors, transcription factors, extracellular matrix proteins, and cell adhesion molecules. The expression of oncogenes has been studied in both human primary prostate tumors (25, 26), as well as in Dunning sublines (27). Even though the studies on primary tumors suggested a correlation with ras and myc expression and tumor progression, studies on the Dunning system did not corroborate these findings. Our own studies (data not published) confirm these last results.

The objective of the study described here was to identify genes that are specifically expressed or overexpressed in metastasizing prostate tumors, using the technique of differential hybridization. In a previous experiment, this technique was successfully applied to compare the steady-state mRNA populations of the AT1 and MatLyLu Dunning sublines (5). In order to be able to identify transcripts that are expressed at lower abundance and to increase the specificity, we improved the technique of differential hybridization. Screening of 4 replicates decreased the number of “false positives” and the alternative strategy to label the mRNA populations increased the sensitivity of the experiment: the threshold level for abundance of mRNAs that can be detected was decreased from 0.1–0.5% to 0.05% (estimation). Thus, comparison of the mRNA populations of the most benign Dunning tumor, the H tumor, and the most progressionally advanced Dunning subline MatLyLu, revealed 26 cDNA clones that are overexpressed in the MatLyLu tumor. After selection by Northern blot screening, three clones appeared to detect mRNAs the expressions of which correlate well with the metastatic phenotype. Other clones showed only a slight increase in expression during tumor progression.

Two of the differentially expressed cDNA clones that met all selection criteria, pBUS19 and pBUS30, showed RNA expression patterns that share some characteristics: a high expression of a 7.0-kilobase transcript in the metastasizing tumors AT3 and MatLyLu, while MatLu, which exclusively metastasizes to lungs, shows no transcript, and the appearance after longer exposure of the autoradiographies of this transcript in the more benign G, HIS, HIM, and HIF tumors (the size of the transcript in the G, HIS and HIM sublines seems to be slightly smaller than 7.0 kilobases). However, the cDNA clones differ by the fact that pBUS30 detects an additional transcript of 6.0 kilobases that is expressed in HIF and at a much higher level in the anaplastic tumors AT1 and AT2. To show a possible relationship between pBUS19 and pBUS30, the nucleotide sequences of the cDNA clones were determined and compared. Even though both clones contain a poly(A) tail, no homology could be found. (Further studies are necessary to establish or exclude an overlap of the two clones, possibly due to alternative splicing.) The DNA sequences were also used for computer-assisted screening of nucleotide sequence databases. For pBUS30 no homology was found with any of the known sequences. Computer comparison of pBUS19, however, revealed the presence of parts of repetitive RAL elements. Although it is reported that these elements can be specifically expressed in rat tumors (22), in our rat tumor model system we see that only two of ten tumors show expression of this transcript. Southern blot analysis of human chromosomal DNA revealed that no RAL homologous elements are present in the human genome (data not shown). Furthermore, to our knowledge, specific expression of repetitive elements in human malignancies has not been shown yet.

The expression of pBUS1 correlates well with metastatic behavior: a transcript of 1.8 kilobases is expressed at very high levels in the three tested metastasizing tumors; the expression in the anaplastic, nonmetastasizing tumors is at least 10-fold lower; while in the more benign tumors no transcripts are detected. DNA sequence and computer analysis revealed that pBUS1 has a high homology with human (71%) and murine (83%) HMG-I(Y) cDNA. HMG-I belongs to the high mobility group proteins which are non-histone, chromatin binding (23). HMG-Y is an isoform of HMG-I, lacking 33 nt in the coding sequences. The HMG-I and -Y proteins appear to be members of an isoform family of proteins (all of the members of which have not yet been fully characterized) that are probably derived by alternative splicing of a common precursor mRNA (28). Nucleotide sequence data of HMG-I(Y) reported thus far indicate that the 3′-UTR of all of the potential isoforms is on a single exon, *i.e.*, transcribed from one gene. Thus from the nucleotide sequence analysis of only the 3′-UTR it is not possible to determine which of the isoforms of the HMG-I family pBUS1 represents. Previous studies report on the possible involvement of HMG-I(Y) in metaphase chromatin condensation (29), in heterochromatin nucleosome phasing (30), in nuclear matrix-DNA interactions (31) or in the 3′-end processing of genes (32, 33). p16, which is most likely a member of the HMG-I isoform family, is implicated in the regulation of the rRNA gene expression (34). Initial reports mention that...
HMG-I(Y) transcripts are most abundant in fast proliferating, undifferentiated cells (23). Moreover, Giancotti et al. (35, 36) suggest that HMG-I(Y) expression is rather related to a highly malignant phenotype than to neoplastic transformation. Comparative analysis of HMG-I(Y) expression in the Dunning system, in which degree of differentiation, growth rate, and metastatic capacity are represented in the various lines, revealed that, whereas the correlation between expression of HMG-I(Y) and growth rate/differentiation was low, there is a clear correlation with metastatic capacity, i.e., the highly malignant phenotype. Likewise, AT1, AT2, AT3, MatLu, and MatLyLu are all anaplastic tumors; nonetheless, the more malignant sublines (i.e., metastatic) have significantly higher levels of HMG-I(Y)-related transcripts. Hence our data support those of Giancotti et al. (35, 36). To substantiate whether there is a functional relation between the expression of HMG-I(Y) and acquisition of the metastatic phenotype, the effects of modulation of the HMG-I expression in the Dunning lines (by cDNA-mediated transfection) will be studied.

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REFERENCES

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