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INCREASED EXPRESSION OF RETROVIRAL SEQUENCES IN PROGRESSIONALLY ADVANCED RAT PROSTATIC TUMORS*

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Summary Differential hybridization analysis revealed two cDNA clones, pBUS19 and pBUS30, to be overexpressed in progressionally advanced rat prostatic tumors. Northern blot analysis suggested the clones to be related although no homology in nucleotide sequence could be shown. Isolation and characterization of a pBUS19-related clone, pJG116, and computer-assisted database comparison showed that all three clones could be mapped within a rat-specific endogenous retrovirus. The importance of overexpression of retroviral sequences in advanced prostatic cancer remains unclear.

In our studies to identify genes that are activated during the proces of prostate tumor progression, we applied the technique of differential hybridization analysis to compare the steady-state mRNA levels of two different tumor stages. Since no well defined human prostate cancer progression model system is available, two sublines of the established Dunning R-3327 rat prostatic cancer model system (1) were used. The serially transplantable Dunning R-3327 sublines represent the various stages of prostate tumor progression. Comparing the steady-state mRNA levels of the most benign Dunning R-3327-H tumor (hormone-dependent, well-differentiated, non-metastasizing), and the aggressive MatLyLu tumor (hormone-independent, anaplastic, metastasizing), three clones that are overexpressed in the metastatic tumors, were isolated (2). Two cDNA clones, pBUS19 and pBUS30, showed a striking homology

*Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. X62950 (R. rattus mRNA (pBUS19) with repetitive elements), X62951 (R. rattus mRNA (pBUS30) with repetitive elements), and X62952 (R. rattus mRNA (pJG116) with repetitive elements).

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in the mRNA expression patterns: a 7.0 kb transcript was highly expressed in the anaplastic, metastasizing (to lymph and lung) tumors AT3 and MatLyLu, but not in MatLu (metastasizes only to lung). pBUS30 showed the expression of additional transcripts in the anaplastic, non-metastasizing tumors and, at much lower levels, in the well- and moderately-differentiated tumors. Nucleotide sequence analysis and computer-assisted comparison revealed no homology between the clones, although both clones contained a poly-A-tail. Screening of computer databases showed no homology of pBUS30 to any of the known sequences whereas pBUS19 appeared to containsequences homologous to a retroviral LTR-like repeat, termed RAL-element (3). This RAL-element was reported to belong to a new family of LTR-like sequences abundantly expressed in rat tumors but rarely in normal tissues. In order to gain more information on the possible role of the repetitive RAL-elements in the progression of rat prostatic cancer, and to elucidate a possible relationship between pBUS19 and pBUS30, a MatLyLu cDNA library was screened using pBUS19 and pBUS30 as a probe to isolate additional cDNA clones that can be used for further characterization of the RAL-elements.

Materials and Methods

Dunning R-3327 sublines

The phylogeny and the characteristics of the Dunning R-3327 rat prostatic cancer model system have already been described extensively (1,2) and are summarized in Table 1.

Screening of cDNA library

Fifty thousand colonies of a cDNA library of the Dunning MatLyLu tumor (2) were screened according to Sambrook *et al.* (4), using either the 1.5 kb cDNA insert of pBUS19 or the 0.5 kb cDNA insert of pBUS30 as a probe.

Table 1 . In vivo biological characteristics of Dunning R-3327 rat prostatic cancer sublines

Subline	Histology	Doubling time (in days)	Androgen responsive	Metastatic ability*
H	Well-differentiated	22 ± 5	Yes	Low
HIS	Well-differentiated	24 ± 5	No	Low
HIM	Well-differentiated	9.0 ± 0.8	No	Low
HIF	Moderately-differentiated	4.8 ± 1.8	No	Low
G	Poorly-differentiated	4.0 ± 0.2	Yes	Low
AT-1	Anaplastic	2.5 ± 0.2	No	Low
AT-2	Anaplastic	2.5 ± 0.2	No	Low to moderate (lungs)**
AT-3	Anaplastic	1.8 ± 0.2	No	High (lymph nodes & lungs)
MATLu	Anaplastic	2.7 ± 0.2	No	High (lungs)
MATLyLu	Anaplastic	1.5 ± 0.1	No	High (lymph nodes & lungs)

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

** Organs in the parentheses are the site of the distant metastases for the individual sublines.

Northern blot analysis

Total RNA was isolated using the lithium chloride/urea method as described by Auffray and Rougeon (5). Ten microgram of total RNA was glyoxalated, separated on an agarose gel by electrophoreses and transferred to Hybond-N^+ (Amersham). Hybridizations were performed as described before (2).

Nucleotide sequence analysis

DNA fragments were ligated into the polylinker region of M13mp8-19. All of the DNA sequences were determined on both strands using the dideoxy sequencing method as described by Sanger *et al.* (6). The gel readings were recorded and edited using IntelliGenetics computer software (release 5.35). Computer comparison studies were performed with the EMBL (release 28) and Genbank (release 68) nucleotide sequence databases (7).

Results

Screening of the cDNA library

About fifty thousand clones of the MatLyLu cDNA library were screened using either the 1.5 kb *Bam*HI cDNA insert of pBUS19 or the 0.5 kb *Bam*HI cDNA insert of pBUS30 as a probe. pBUS30 did not detect any related cDNA clones. Of 132 pBUS19-positive clones, 24 were randomly chosen and DNA was isolated. *Bam*HI and *Eco*RI/*Hin*dIII restriction digestions revealed that we had isolated 13 different cDNA clones. pJG116 was selected for further characterization since it contained the largest insert (2.7 kb), possibly containing an overlap with pBUS30. To ascertain that pJG116 was indeed related to pBUS19, the insert of pJG116 was used for Northern blot analysis. Interestingly, a hybridization pattern similar to the one found for pBUS30 was seen (see Fig. 1); in addition to the 7.0 kb transcript in the anaplastic, metastasizing (to lymph and lung) tumors AT3 and MatLyLu (as found for pBUS19), a 6.0 kb transcript was seen in the anaplastic, non-metastasizing tumors AT1 and AT2, whereas in the more benign Dunning sublines (G, H, HIS, HIM) a low expression of a 6.7 kb transcript is seen. The poorly-differentiated, hormone-independent HIF tumor expresses transcripts of 6.0 and 7.0 kb. This suggested that indeed pJG116 may contain sequences also present in pBUS30 and thus may help unravel the relation between the different clones. When pJG116 was used for a Southern blot analysis, a smear identical to that seen for pBUS19 and pBUS30 was found in the Dunning sublines whereas no signal was detected in human liver (data not shown), confirming the species specificity of the repetitive sequences.

Nucleotide sequence analysis

In order to determine the nucleotide sequence of pJG116, a restriction map was constructed. The map of the 3' end of pJG116 was almost identical to that

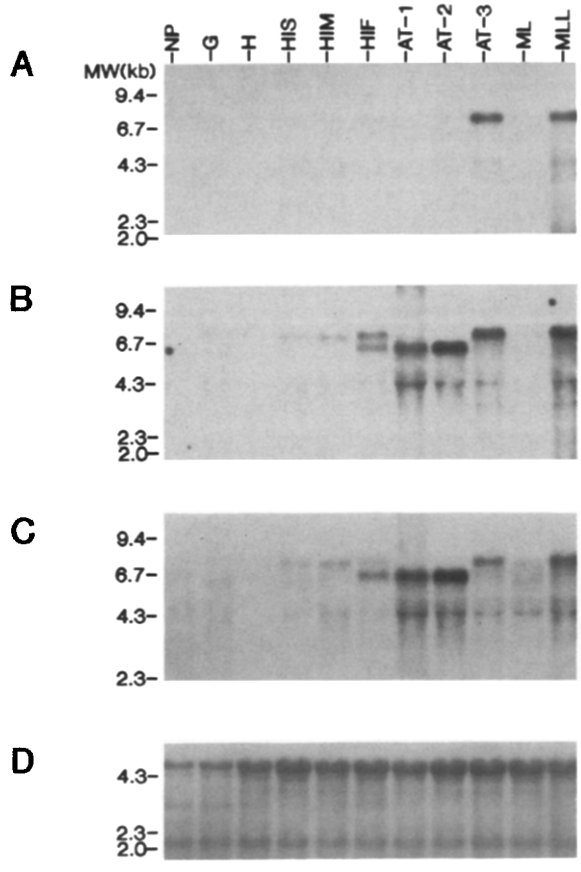

Figure 1. Northern blot analysis of pBUS19 (A), pBUS30 (B) and pJG116 (C). Ten μg of total RNA of normal prostate and 10 Dunning tumors were loaded per lane. rRNA was used as an internal control for the amount of RNA loaded (D).

determined for pBUS19, confirming the expected correlation with pBUS19 and showing that pJG116 was a 5'-end extended clone of pBUS19. Next, the nucleotide sequence of pJG116 was determined. Computer assisted analysis of the nucleotide sequences of pBUS19, pBUS30 and pJG116 showed that pBUS19 and pJG116 shared a high homology (94 %). An overlap between pJG116 and pBUS30 could not be found. In order to find out what was contained in the additional sequences of pJG116 and to make an update search for pBUS19 and pBUS30, nucleotide sequences of the three clones were used for computer-assisted database comparison with all known sequences. This revealed the relationship between all clones: pBUS19 and pJG116 showed homology with the RAL-elements but new retroviral sequences had become available (8). As shown in figure 2, pBUS19 and pJG116 are located at the 3' end of a 7.3 kb rat endogenous retrovirus which

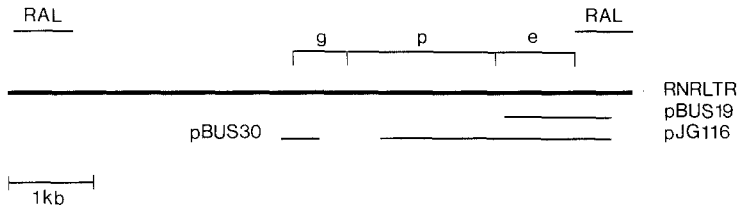

Figure 2 . Schematic presentation of the location of pBUS19, pJG116 and pBUS30 compared to the endogenous retrovirus (RNRLTR) as described by Nakamuta et al. (8). Also the gag (g), pol (p) and env (e) homologous regions (as indicated by Nakamuta et al. (8)) are shown as are the positions of the RAL elements.

contains the RAL-elements: pBUS19 has a homology of 90.7 % over 1010 nt (of 1303 nt determined), pJG116 has a homology of 91.3 % over 2300 nt (of 2684 nt determined). The computer showed that pBUS30 is also contained within the endogenous retrovirus: 87 % homology over 466 nt (of the 466 nt determined). This comparison also showed that the assumed poly-A-tail of pBUS30 is due to an internal A-stretch in the retrovirus, probably allowing oligo-dT-priming. This explains why no evident poly-A-addition-signal could be found in pBUS30. The fact that pBUS30 is not located at the 3'-end but is primed at an internal A-stretch may also explain why no additional cDNA clones were isolated.

Discussion

The isolation of pJG116 and an update database screening revealed the relation of the three cDNA clones pBUS19, pBUS30 and pJG116. All three cDNA clones contain parts of a rat endogenous retrovirus of 7.3 kb (RNRLTR, (8)), which was shown to be abundantly expressed in rat hepatic tumors whereas no expression was detected in normal liver. In rat prostatic tumors, we also see an overexpression of the retroviral sequences when compared to normal prostate, but instead of a smear pattern on Northern blot as found by Suzuki *et al.* (3), we detect transcripts of discrete sizes. The transcript in the anaplastic, metastasizing tumors AT3 and MatLyLu are approximately 7.0 kb and may represent a putative full-length transcript of the retroviral sequences (7.3 kb). The fact that in the anaplastic, non-metastasizing tumors AT1 and AT2 a transcript of 6.0 kb and in the more benign Dunning tumors a transcript of 6.7 kb is found, raises the question as to whether the transcripts are derived from different retroviral sequences, whether there is a change of transcription-start-point during tumor progression or if we are dealing with specific

splice-products? Since pBUS19 and pJG116 are not 100 % identical this suggest that there are several, highly homologous, retroviral sequences in the genome of the rat, and they might give rise to different transcripts in different tumors or tumor stages.

The role of the induced expression of the endogenous retroviral sequences in rat prostatic tumors remains to be established. Endogenous retroviral-like sequences in eukaryotic cells have been extensively studied (9) and it was shown that usually the expression of the retroviral sequences is restricted to specific stages of embryonic development or to specific tissues (10). However, also the specific enhancement of expression of repetitive sequences has been reported: the repetitive sequences are activated during several processes including differentiation (11), tumorigenesis (12) or due to induction by exogenous factors (13). On the other hand, also loss of expression of endogenous proviruses in tumors has been described (14). The involvement of deregulation of the expression of endogenous retroviral sequences has been shown in a variety of tumors and transformed cells (15-20), and the altered pattern of expression of retroviral transcripts may provide markers for the detection of neoplastic disease (20). On a possible functional /structural relation between the expression of endogenous retroviral sequences and prostate tumor progression, one can only speculate. It is very well possible that we are dealing with an epiphenomenon in that the endogenous retroviral-like sequences are activated non-specifically. In cancer research the activation of cellular genes by promoter/enhancer insertion of retroviral sequences is a known mechanism but although the correlation is striking, i.e. in the anaplastic lines an overexpression is found, we did not find evidence for fusion of RAL-elements with endogenous genes. In contrast, Liu and Abraham (21), studying differential gene expression in human prostatic cancer cell lines, identified a cDNA containing human endogenous retroviral sequences spliced to human calbindin. The long terminal repeat (LTR) of this retroviral sequence was suggested to possibly activate the calbindin gene. The relation between overexpression of retroviral sequences and progression of rat prostatic cancer, however, remains elusive.

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