[CANCER RESEARCH 60, 4526–4530, August 15, 2000]

Delineation of the 6p22 Amplification Unit in Urinary Bladder Carcinoma Cell Lines[1]

Jochen Bruch, Wolfgang A. Schulz, Jürgen Häussler, Ingo Melzner, Silke Brüderlein, Peter Möller, Ralf Kemmerling, Walther Vogel, and Horst Hameister[2]

Department of Medical Genetics [J. B., J. H., R. K., W. V., H. H.] and Department of Pathology [I. M., S. B., P. M., R. K.], University Ulm, Albert-Einstein-Allee 11, D-89069 Ulm, Germany; and Department of Urology, Heinrich-Heine-University Düsseldorf, Moorenstraβe 5, D-40225 Düsseldorf, Germany [W. A. S.]

ABSTRACT

Eight cell lines from transitional cell carcinoma of the urinary bladder were analyzed by comparative genomic hybridization. All tumor lines exhibited frequent chromosome gains (11.5/cell line) and losses (8.4/cell line). In six cell lines, gain of chromosome 5p was associated with gains of 6p and 20q. In five of these cell lines, amplification of parts of 6p was observed. Cytogenetic investigation combined with fluorescence *in situ* hybridization analysis revealed typical marker chromosomes with homogeneously staining regions (HSRs) containing material from 6p. By hybridizing individual yeast artificial chromosome probes from a chromosome 6p contig to these HSRs, a contig of three yeast artificial chromosomes common to all 6p HSRs was identified that spans less than 2 Mb. The genes *SOX4* and *PRL* were shown to map to this region and to be coamplified in the cell lines. However, *SOX4* was not overexpressed in any cell line and PRL was not expressed at all. Thus, the presumptive 6p oncogene remains to be conclusively identified.

INTRODUCTION

Cytogenetic investigations of tumors derived from epithelial tissues typically reveal a broad spectrum of chromosomal aberrations. This makes it difficult to detect those aberrations that are specific for a certain tumor type. To identify a tumor-specific pattern of chromosomal imbalances, cumulative cytogenetic data are evaluated (1). The next step is to indicate and isolate the respective oncogene or tumor-suppressor gene that maps to the site of the chromosomal imbalance. The strategy to isolate tumor-suppressor genes from deleted regions is well established. First, the smallest region of common deletion is defined followed by analysis of candidate genes from the deleted region for inactivating mutations (2, 3). Gains or amplifications of chromosomes or chromosomal regions point to sites of tumor-specific activated oncogenes. Considerable effort has been made to isolate oncogenes from amplified regions (4, 5). In a rather straightforward manner, the implication of the androgen receptor gene at Xq11–q12 in prostatic cancer (6), of the REL proto-oncogene at 2p14–p15 in non-Hodgkin lymphoma (7), and of the BCL2 gene at 18q21.3 in recurrent B-cell lymphoma (8) was shown. In these examples the identification of the respective oncogene was facilitated because the candidate gene approach was applied successfully. This means that the genes mapping to the amplified site in the respective tumor were already known from preexisting mapping data. However, when this approach turns out not to be successful, the search for the activated oncogene within an amplification unit has proven more tedious. For instance, amplification of 20q is observed in a variety of different cancer entities (9–12). This amplification unit was analyzed in detail in breast cancer (13) and only recently led to the description of the serine/threonine kinase BTAK, which is synonymous with STK15 (14). STK15 has been shown to interfere with centrosome function. Deregulation of STK15 causes chromosome mal-segregation and in this fashion supports tumor progression (14). Thus, the analysis of frequently recurring amplifications may lead to the identification of an important new class of tumor genes.

In this paper eight established transitional cell carcinoma (TCC)[3] lines derived from urinary bladder cancers were analyzed by comparative genomic hybridization. A specific association of gains and amplifications at chromosomes 5p, 6p, and 20q was observed in six TCC lines. The amplification unit at 6p was delineated in detail.

MATERIALS AND METHODS

Cell Lines. The TCC lines used are listed in Table 1. The cell lines 5637, HT1367, and T24 were kindly provided by Dr. Schmitz-Dräger (Düsseldorf, Germany). The cell lines 1HTB and 5HTB were purchased from American Type Culture Collection (Rockville, MD). The lines U-BLC1 (15), U-BLC2, and HIA were established at our institution. All cell lines were grown in DMEM medium supplemented with 10% FCS and antibiotics.

Cytogenetic Analysis. Chromosome preparation and GTG-banding were performed according to standard techniques. Whole chromosome paints (WCP) were used according to the instruction of the supplier (AGS, Heidelberg, Germany). yeast artificial chromosome (YAC)-DNA probes were ordered from CEPH (Paris, France) and were labeled by standard nick-translation with 16-biotin-dUTP. A total of 250 ng YAC-DNA was mixed with a 50-fold excess of Cot-1 DNA (Life Technologies, Inc., Eggenstein, Germany) and 20 μg herring-sperm DNA and was preannealed in hybridization mix for 35 min at 37°C. Hybridization and detection were performed as described (11). For microscopic evaluation, the Cytovision software package from Applied Imaging (Pittsburgh, PA) was used.

Comparative Genomic Hybridization. DNA was prepared from the cell lines and human blood leukocytes as reference according to standard techniques. For comparative genomic hybridization (CGH), the protocol as described by Kallioniemi et al. (16) was followed with slight modifications as reported (11).

Semiquantitative PCR. The following polymorphic di- and tetranucleotide repeat markers from the region 6p21.3–p23 were selected from the Genéthon (17) and Marshfield Medical Research Foundation (18) maps: D6S1678, D6S938, D6S422, D6S507, D6S1665, D6S1588, D6S1029, D6S1660, D6S461, and D6S1050. PCR detection and semiquantitative PCR were performed as described previously (11).

Northern Blot Analysis. Total cellular RNA was isolated from six cell lines with the Qiagen (Valencia, CA) RNeasy purification kit as indicated by the manufacturer's instruction. Approximately 10 μg of each sample was loaded on a 1% denatured agarose/formaldehyde gel and run at 5 V/cm at 4°C. The RNA samples were blotted on a positively charged nylon membrane filter. The filter was probed in Express Hybridization solution (Clontech, Palo Alto, CA) with ^{32}P-labeled cDNA portions of *SOX4* and was reprobed with β-actin, omitting a stripping step. To achieve comparable hybridization conditions, both probes had roughly the same length and GC content. Hybridization conditions and washing procedures were followed as prescribed by Clontech. Autoradiographs were obtained by

Received 11/19/99; accepted 6/16/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Bausteinförderung P.0445 from the Universitätsklinikum Ulm and the Deutsche Forschungsgemeinschaft (Schm 782/3-1).

[2] To whom requests for reprints should be addressed, at Department of Medical Genetics, University Ulm, D-89070 Ulm, Germany. Phone: 49-731-5023436; Fax: 49-731-5023438; E-mail: horst.hameister@medizin.uni-ulm.de.

[3] The abbreviations used are: TCC, transitional cell carcinoma; WCP, whole chromosome painting; CGH, comparative genomic hybridization; HSR, homogeneously staining region; FISH, fluorescence *in situ* hybridization; YAC, yeast artificial chromosome.

Table 1 *Summary of the genetic imbalances detected by CGH in seven TCC cell lines of the bladder*

Cell Line	Loss	Gain
HIA	4q, 8p, 9p21-pter, 10p13-pter, 16p, Y	3q, **5**, **6p**, 7q32-qter, 8q, 9p13-qter, 10p12-qter, 11, 12, 14, 17, **20q**
U-BLC1	1p12-p21, 2q33-qter, 3pter-q13.2, 5q14-qter, 8p, 9q, 10q, 14q, 16p12-pter, 17p12-p13, 19p	1p31-p32, 1q21-q41 (>1.5: q22), 2cen-p22, 4p15.1-pter, **5p**, **6p21.2-p23** (>1.5: **p22**), 6q13-qter, 7cen-p14 (>1.5: p12), 8q22-qter, 9p21-pter (>1.5: p24), 10p12-pter, 13q, 15q21-qter, 17q12-q21, 17q23-qter, 18, **20**
U-BLC2	4p15-pter, 8p, 10q22-qter, 11p, 17p, 18q	1q21-qter, (>1.5: q21-q25), 2q11-qter, 7, 10q22, 12p11-pter, 15q11-q25, 17q11-q22, 18p, 19q, 20
5637	3cen-p23, 4, 6p21.2-q21, 8p22-pter, 9q13-q21, 10p, 11q23-qter, 16p, 17p, Y	3p24-pter (>1.5: p25-ter), 3cen-q27, **5p**, **6p21.3-p24 (>1.5: p22-p23)**, 6q22-qter, 9p23-pter, 9q22-qter, 11p13-q23, 12q24.1-qter, 13q22-qter, 17q, **20**, 21q
HT1376	2q14.3-qter, 4, 9pter-q21, 10q23-qter, 11q12-q13, 12pter-q13, 13q14, 14, 15q22-qter, 17, 19, Y	1p13-p34.1, 3q, **5 (>1,5: 5p)**, **6p21.3-pter (>1,5: p22-23)**, 7, 8q, 10p13-pter, 11p, 11q14-qter, 13q21-qter, 18q22-qter, **20**
T24	1q21-qter, 4, 6p12-p21.3, 8p, 9p21-pter, 10p12-pter, 10q24-qter, 13cen-q14, 17p, 21, Xp21-qter	7q32-qter, 8q, 9p21-qter, 11p, 12p, 14, 17q22-qter, 18, 20, 22
1HTB	2p, 2q34-qter, 4q, 8p12-pter, 10q, 11q22-qter, 21	3q24-qter, **5p**, **6p21.1-pter (>1,5: p21.3-pter)**, 8q21.1-qter, 9, 10p, 11pter-q22, 13q, **20q11.2-ter (>1,5: q13.1-13.2)**
5HTB	2, 4, 6q14-q25, 18q	1q, **5 (>1,5: 5p)**, **6cen-p23 (>1,5: p22)**, 7, 10q, 12q24.1-qter, 13q22-qter, 16, **20**

exposing the membrane for 14 h at −70°C, and the signals were quantified by ImageMaster VDS (Pharmacia Biotech, San Francisco, CA). The integrated optical densities of the signals were corrected for background, and the *SOX4*/*β*-actin ratios were calculated for each cell line.

RESULTS

For analysis by CGH, DNA from eight TCC lines was available. In Table 1 the CGH data are listed separated according to losses and gains. A more detailed analysis of the cell line U-BLC1 is provided elsewhere (15), where a typical CGH profile is also given. In all cell lines a variety of losses (4–12, mean 8.4/cell line) and gains (10–17, mean 11.5/cell line) was observed. The most frequent loss was deletion of 8p, which was observed in six of eight lines. Deletion of 9p was evident in three lines and deletion of 9q in two lines. Analysis of gains revealed that in six of the cell lines gain of chromosomal material from 5p, 6p, and 20q occurred jointly. Only lines U-BLC2 and T24 failed to show this particular pattern of chromosomal gains. In five cell lines, the CGH data suggested that some material in 6p was amplified (Table 1).

Amplifications in tumors often manifest as homogeneously staining regions (HSRs). The cell lines U-BLC1, 5637, HT1376, and 5HTB were investigated cytogenetically for marker chromosomes with HSRs that contained the amplified material from 6p. Evident marker chromosomes were observed in cell lines U-BLC1 and 5637 (Fig. 1, *B* and *C*). In cell line 5HTB, two chromosomes 6 with an enlarged region 6p22 together with two chromosome 6 fragments, del(6)(pter-q14) were present (Fig. 1*A*). The 6p-derived marker chromosome in cell line HT1376 was detected by fluorescence *in situ* hybridization (FISH) with a WCP probe for chromosome 6 that labeled the proximal short arm and most of the long arm of a single submetacentric marker chromosome (Fig. 1*D*). In addition, four cytogenetically normal chromosomes 6 were painted in cell line HT 1376. FISH using WCP 6 as a probe confirmed the cytogenetic origin from chromosome 6 of all HSR-containing marker chromosomes shown in Fig. 1.

According to the CGH data presented in Table 1, the common region amplified in all four cell lines is 6p22. For this region, overlapping YAC contigs have been published (19–21). From these contigs, twenty YACs covering 6p21.3–p23 were selected and their localization confirmed by FISH on normal metaphase chromosomes (results not shown).

To analyze which YAC inserts are included in the amplification unit, FISH was performed on metaphase chromosomes prepared from the four TCC lines that contain HSRs derived from chromosome 6. The evaluation procedure is paradigmatically illustrated in Fig. 2 with metaphase chromosomes prepared from the cell line U-BLC1. In Fig. 2*A* FISH was performed with the YAC probe 753h12. Signals are detected at 6p21.3–p22 on two normal chro-

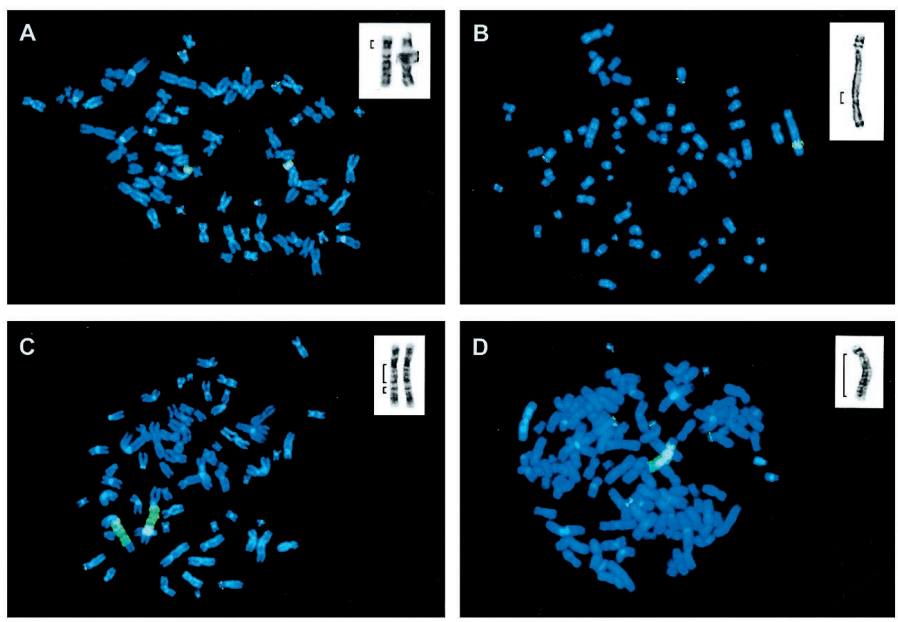

Fig. 1. Cytogenetic and FISH analysis of four TCC lines. The specific marker chromosomes bearing the 6p amplification are shown as inserts. *A*, Cell line 5HTB hybridized with YAC 958a10; *B*, cell line U-BLC1 hybridized with YAC 958h9; *C*, cell line 5637 hybridized with YAC 966e10; and *D*, cell line HT1376 again hybridized with YAC 966e10.

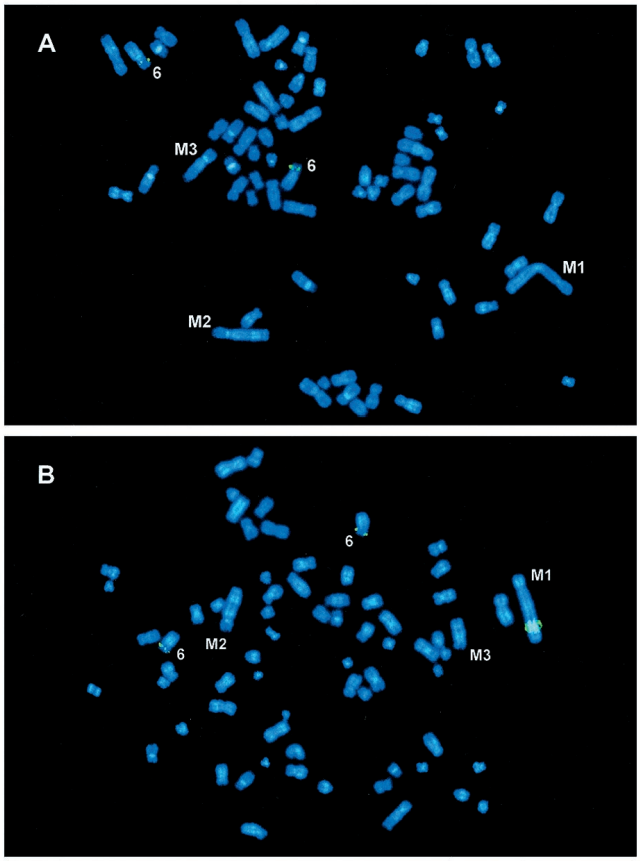

Fig. 2. YAC probes from 6p21.3–p23 hybridized to metaphases from cell line U-BLC1. *A*, Hybridization with YAC 753h12, which gives specific hybridization signals on both normal chromosomes 6 but not on marker M1. *B*, Hybridization of YAC 958h9. Again the two normal chromosomes 6 show signals and there is a rather bright signal on the distal long arm of marker M1. This YAC is included in the 6p amplification unit.

mosomes 6 but not on the HSR of marker chromosome M1. The YAC 753h12 insert therefore is not part of the 6p amplification unit in U-BLC1. In Fig. 2*B*, hybridization with YAC probe 958h9 is shown. With this probe, signals are present on the two cytogenetically normal chromosomes 6, however marker chromosome M1 is likewise highlighted. Therefore, this YAC is part of the 6p amplification unit in U-BLC1.

Twenty overlapping YACs were tested in this manner on metaphase chromosomes from all four cell lines. Representative FISH results are shown for YAC 958a10 applied to cell line 5HTB (Fig. 1*A*), YAC 958h9 applied to U-BLC1 (Fig. 1*B*), YAC 966e10 to 5637 (Fig. 1*C*), and the same YAC applied to HT1376 (Fig. 1*D*), respectively. The results are summarized in Fig. 3. The largest amplification unit was found in HT1376 and contained eight YACs from 755 g10 to 771h10. All four amplification units overlapped with each other. The smallest amplification unit was detected in cell line 5HTB, which included the YACs 958h9, 966e10, and 958a10. Therefore, these three YACs define the region of common amplification in the four TCC cell lines analyzed. In cell line 5637, hybridization intensity with these YACs was heterogeneous, with the highest signal intensity being observed for YAC 966e10 (not shown).

To determine the degree of amplification, 10 polymorphic microsatellite markers for chromosome 6p were selected from the Genéthon (17) and Marshfield Medical Research Foundation (18) genetic maps. For these markers the copy number was estimated by semiquantitative PCR. In cell line 5HTB the highest relative copy number (approximately 11 copies) was determined for D6S507 (not shown). By PCR this marker was located to the overlapping region of YACs 958h9 and 966e10 (Fig. 3).

To identify candidate genes, The Genome Database was searched for putative oncogenes mapping to 6p21.3–6p23. Eight genes (*TFAP2*, *E2F3*, *SOX4*, *HMGIY*, *CCND3*, *IRF4*, *HOX12*, and *PIM1*) were chosen for further investigation. Oligonucleotide primers were designed for these genes, and their presence on the three YACs was tested by PCR. From the above mentioned genes only the SRY-box 4 gene, *SOX4*, and the lactation hormone prolactin, *PRL*, map to YACs 958h9 and 966e10 (Fig. 3). The other six genes were excluded from this YAC contig.

Next the genes *PRL* and *SOX4* were tested in a multiplex PCR reaction on DNA from the cell lines with 6p amplification using the ubiquitously expressed transcription factor *SP1* as a control. Calculation of the absorbance of the fluorescence image of the PCR products confirmed that *SOX4* is indeed amplified in all cell lines with 6p amplification, whereas *PRL* was not amplified in cell line 5HTB (results not shown). Expression of both genes was analyzed by RT-PCR and Northern blot analysis. *PRL* was not expressed at all. Variable expression of *SOX4* was observed. A respective Northern blot is shown in Fig. 4*A*, and in Fig. 4*B* the quantitative calculation when compared with β-actin as control is added. Only in cell line HT1376 is *SOX4* expression enhanced. In cell line U-BLC2, no 6p amplification was observed during the preceding CGH analysis (compare Table 1), but U-BLC2 shows the same *SOX4* expression level as the four further TCC cell lines with 6p amplification. This renders *SOX4* very unlikely to be a tumor-specific gene in TCC of the urinary bladder.

DISCUSSION

The cytogenetic and molecular investigations reported here may contribute to the delineation of a pathogenetic cascade of genetic changes during progression of TCC of the urinary bladder. Different studies agree that deletions affecting chromosome 9 represent the most important initiating event in TCC (22, 23). The subsequent changes leading to progression are less well defined. Losses of chromosomes 11p, 13q, 17p, 8p, and others are found in many advanced urinary bladder carcinomas (24). Comparative genomic hybridization studies suggest a dynamic pattern of genetic changes with no common pathway, because no single aberration exceeds a frequency of 50% across all tumors (11, 12, 25, 26). In early stage tumors deletions are more frequent, whereas in later stage tumors chromosomal gains predominate (11, 12). Gene amplifications are prevalent only in advanced stages of TCC of the bladder.

In this study we have focused on established TCC cell lines by comparative genomic hybridization. The cell lines analyzed here are highly aneuploid with a variable chromosome number ranging from 63 to 118 chromosomes. Several marker chromosomes were observed. These marker chromosomes contribute to the high rate of chromosomal aberrations determined by CGH analysis (Table 1). More gains than losses (11.5 and 8.4/cell line, respectively) were determined by CGH in the cell lines analyzed here. This is similar to the aberration pattern observed in locally advanced invasive ($\geq$pT2) tumors *in vivo* (11, 12). A major concern with tumor cell lines is whether they retain an entity-specific pattern of chromosomal imbalances. As shown by CGH (Table 1), the established cell lines of the bladder analyzed here present many of the changes seen in primary tumors. For instance, three of the seven cell lines have deletions of 9p, and two lines have deletions of 9q. Both deletions are frequently observed in early-stage tumors (2, 3, 22–24). Likewise 8p, the most frequent deletion in the cell lines (six of eight cell lines), is an early change observed during tumor progression *in vivo* (24, 25). The 8p

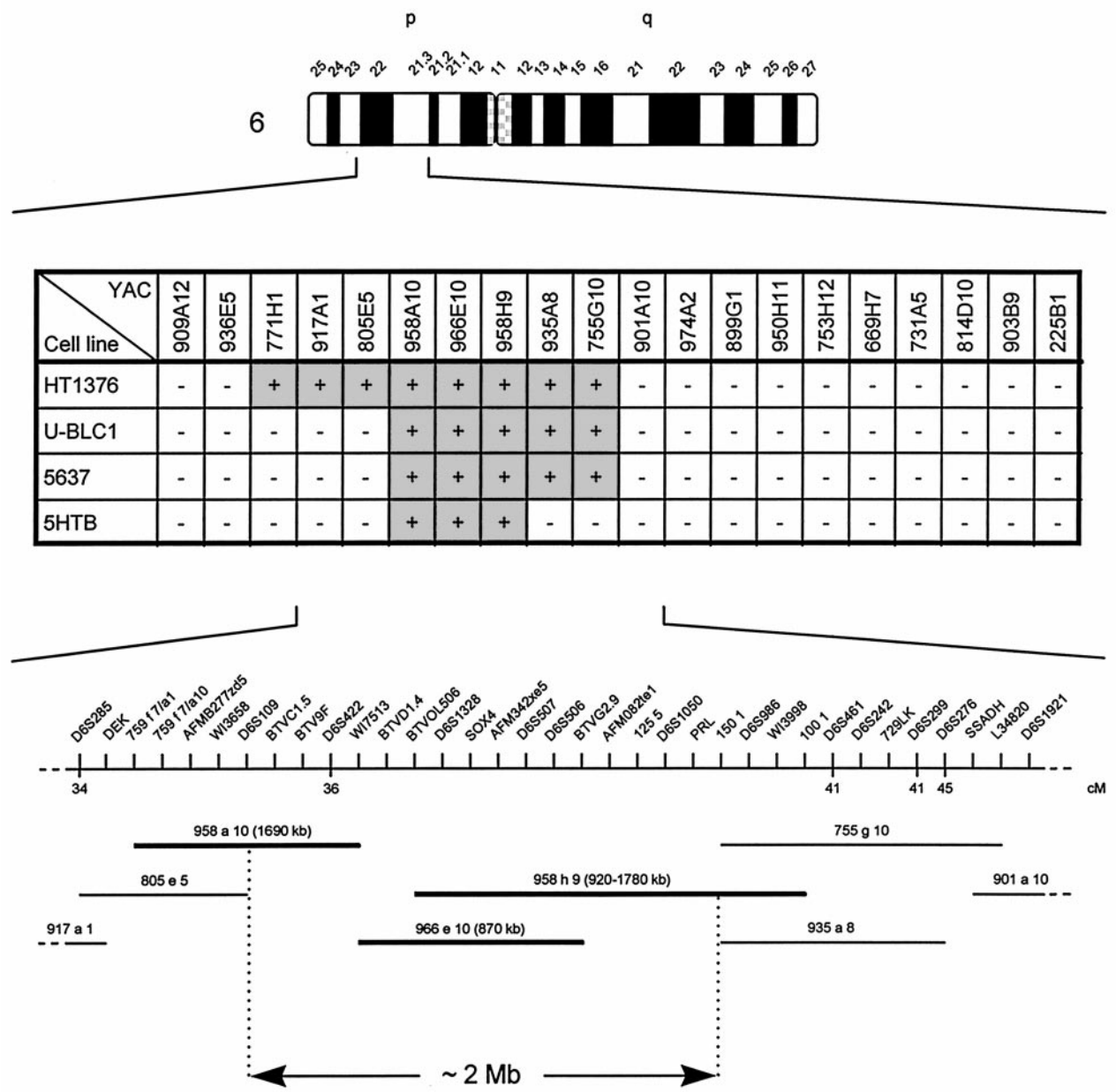

Fig. 3. Graph summarizing the hybridization results with YAC probes from 6p21.3–p23 hybridized to marker chromosomes from the cell lines HT1376, U-BLC1, 5637, and 5HTB. At the top, an ideogram of chromosome 6 is shown. The YAC probes tested are listed in the second row, and positive hybridization signals are marked by shadowing. In the next row, a genetic map (adopted from Refs. 19 and 20) is shown (not drawn to scale). At the bottom a schematic drawing of the YAC contig is added. YACs drawn with bold lines are contained within the 6p amplification unit of cell line 5HTB.

deletions are not only common in primary TCC of the urinary bladder (>33%) but also in cancers of the breast, kidney, and prostate (26).

As in advanced stages of TCCs *in vivo* (11, 12, 27, 28), gains of 1q, 5p, 6p, 8q, and 20q were frequent findings in TCC lines (Table 1). Whereas gains of 1q, 8q, and 20q are also common in advanced cancers originating from other tissues (26), gains of 5p and 6p seem to be relatively tissue specific. The association of gains at 5p and 20q was observed in 25% of locally advanced invasive tumors *in vivo* (11). In TCC lines this association was retained and was found to include gain at 6p. Analysis of *in vivo* tumors revealed gain of 6p in 29% of ≥pT2 urinary bladder cancers (11, 12, 25). It is conceivable that the association of gains at 5p, 6p, and 20q is specific for TCC lines of the urinary bladder. On an anecdotal note, probably the most famous bladder carcinoma cell line, T24, in which the first ras mutation was observed (29), proved to be an exception of this rule (Table 1). From this study we may conclude that during establishment of TCC lines *in vitro* a particular tissue-specific aberration pattern in primary tumors is retained or even becomes more pronounced. Thus, TCC cell lines are a valuable resource whose molecular and cytogenetic aberrations and biological properties reflect a special subset of urinary bladder carcinoma.

From this analysis, gain at 6p emerges as one prominent tumor-specific aberration present *in vivo* and selected for *in vitro*. In primary tumors, 7 of 24 locally advanced invasive T2 tumors (29%) exhibited gain at 6p, and in only one tumor was a 6p amplification present (11). Among TCC lines, six from eight lines displayed gain of 6p, and five lines contained even amplifications of 6p. The CGH data allow fine localization of the common region of amplification to 6p21.3–p23 (Table 1). Because no obvious oncogene candidates are known to map in this region, the 6p amplification consensus region was delineated (Fig. 3). This commonly amplified region encompasses the YACs 958h9, 966e10, and 958a10.

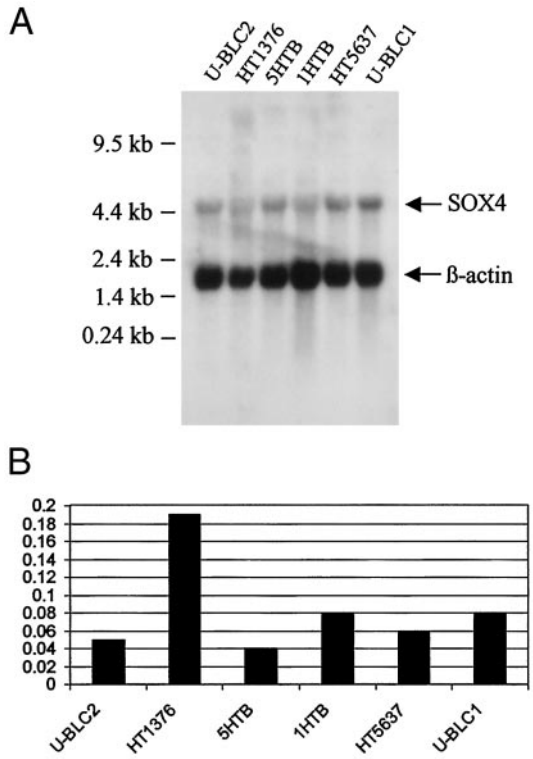

Fig. 4. Northern blot analysis of *SOX4* and β-actin as control of six TCC cell lines of the urinary bladder with (HT1376, 5HTB, 1HTB, HT5637, and U-BLC1) and without (U-BLC2) chromosome 6p amplification. In *B*, the ratio *SOX4*/β-actin of the integrated optical densities in *A* is added.

The known STS markers, microsatellites and genes, mapping to this YAC contig are additionally indicated in Fig. 3. Among eight examined putative candidate oncogenes, only the SRY-box 4 gene, *SOX4*, and the gene encoding prolactin, *PRL*, were found to be present in the contig, namely in the YACs 958h9 and 966e10. *SOX4* is an HMG-box-containing transcription factor that recently was suggested to be engaged in transcriptional activation of tissue-specific protein kinases with oncogenic potential (30). As shown by multiplex PCR analysis, *SOX4* is located in the segment showing the strongest extent of amplification, whereas *PRL* is not amplified in cell line 5HTB, which bears the smallest amplification unit (Fig. 3).

Whereas these data suggest *SOX4* as a possible oncogene on chromosome 6p, the expression analysis (Fig. 4) is not compatible with this idea. Whereas *PRL* was not expressed at all, *SOX4* was expressed at enhanced level only in cell line HT1376, but at a seemingly normal level in the other TCC cell lines with and without 6p amplification. Emanating from CGH results we were able to narrow down the common region of amplification in TCC cell lines to a segment of 2 Mb in 6p22. Although our candidate gene approach was not successful, the delineation of this region will be a helpful starting point once the entire sequence of 6p becomes available.

REFERENCES

1. Mitelman, F., Mertens, F., and Johansson, B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. Nat. Genet., *15:* 417–474, 1997.
2. Habuchi, T., Luscombe, M., Elder, P. A., Knowles, M. A. Structure and methylation-based silencing of a gene (DBCCR1) within a candidate bladder cancer tumor suppressor region at 9q32–q33. Genomics, *48:* 277–288, 1998.
3. Hornigold, N., Devlin, J., Davies, A. M., Aveyard, J. S., Habuchi, T. Knowles, M. A. Mutation of the 9q34 gene TSC12 in sporadic bladder cancer. Oncogene, *18:* 2657–2661, 1999.
4. Guan, X. Y., Meltzer, P. S., Dalton, W. S., and Trent, J. M. Identification of cryptic sites of DNA sequence amplification in human breast cancer by chromosome microdissection. Nat. Genet., *8:* 155–161, 1994.
5. Fischer, U., Heckel, D., Michel, A., Janka, M., Hulsebos, T., and Meese, E. Cloning of a novel transcription factor-like gene amplified in human glioma including astrocytoma grade I. Hum. Mol. Genet., *11:* 1817–1822, 1997.
6. Visakorpi, T., Kallioniemi, A. H., Syvanen, A. C., Hyytinen, E. R., Karhu, R., Tammela, T., Isola, J. J., and Kallioniemi, O. P. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridisation. Cancer Res., *55:* 342–347, 1995.
7. Joos, S., Otano-Joos, M. I., Ziegler, S., Brüderlein, S., du Manoir, S., Bentz, M., Möller, P., and Lichter, P. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. Blood, *87:* 1571–1578, 1996.
8. Monni, O., Joensuu, H., Franssila, K., and Knuutila, S. DNA copy number changes in diffuse large B-cell lymphoma: comparative genomic hybridisation study. Blood, *87:* 5269–5278, 1996.
9. Kallioniemi, A., Kallioniemi, O-P., Piper, J., Tanner, M., Stokke, T., Chen, L., Smith, H., Pinkel, D., Gray, J-W., and Waldman, F. M. Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc. Natl. Acad. Sci. USA, *91:* 2156–2160, 1994.
10. Solinas-Toldo, S., Walrapp, C., Müller-Pillasch, F., Bentz, M., Gress, T., and Lichter, P. Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization. Cancer Res., *56:* 3803–3807, 1996.
11. Bruch, J., Wöhr, G., Hautmann, R., Mattfeldt, T., Brüderlein, S., Möller, P., Sauter, S., Hameister, H., Vogel, W., and Paiss, T. Chromosomal changes during progression of transitional cell carcinoma of the bladder and delineation of the amplified interval on chromosome 8q. Genes Chromosomes Cancer, *23:* 167–174, 1998.
12. Richter, J., Lorenza, B., Wagner, U., Schraml, P., Gasser, T. C., Moch, H., Mihatsch, M. J., and Sauter, G. Patterns of chromosomal imbalances in advanced urinary bladder cancer detected by comparative genomic hybridization. Am. J. Pathol., *153:* 1615–1621, 1998.
13. Anzick, S. L, Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science (Washington DC), *277:* 965–968, 1997.
14. Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R., and Sen, S. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat. Genet., *20:* 189–193, 1998.
15. Bruch, J., Wöhr, G., Brüderlein, S., Barbi, G., Wolter, H., Dixkens, C., Mattfeldt, T., Möller, P., Paiss, T., Hautmann, R., Vogel, W., and Hameister, H. Detailed marker chromosome analysis in cell line U-BLC1, established from transitional-cell carcinoma of the bladder. Int. J. Cancer, *80:* 903–910, 1999.
16. Kallioniemi, A., Kallioniemi, O. P., Sudar, D., Rutovitz, D., Gray, J. W., Waldman, F., and Pinkel, D. Comparative genomic hybridisation for molecular cytogenetic analysis of solid tumors. Science (Washington DC), *258:* 818–821, 1992.
17. Dib, C., Faure, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J., and Weissenbach, J. A. Comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature, *380:* A58–A63, 1996.
18. Yuan, B., Vaske, D., Weber, J. L., Beck, J., and Sheffield, V. C. Improved set of short-tandem-repeat polymorphisms for screening the human genome. Am. J. Hum. Genet., *60:* 459–460, 1997.
19. Olavesen, M. G., Davies, A. F., Broxholme, S. J., Wixon, J. L., Senger, G., Nizetic, D., Campbell, R. D., and Ragoussis, J. An integrated map of human chromosome 6p23. Genome Res., *5:* 342–358, 1995.
20. Malaspina, P., Roetto, A., Trettel, F., Jodice, C., Blasi, P., Frontali, M., Carella, M., Franco, B., Camaschella, C., and Novelletto, A. Construction of a YAC contig covering human chromosome 6p22. Genomics, *36:* 399–407, 1996.
21. Lauer, P., Meyer, N. C., Prass, C. E., Starnes, S. M., Wolff, R. K., and Gnirke, A. Clone-contig and STS maps of the hereditary hemochromatosis region on human chromosome 6p21.3–p22. Genome Res., *7:* 457–470, 1997.
22. Spruck, C. H., III, Ohneseit, P. F., Gonzalez-Zulueta, M., Esrig, D., Miyao, N., Tsai, Y. C., Lerner, S. P., Schmütte, C., Yang, A. S., Cote, R., Dubeau, L., Nichols, P. W., Hermann, G. G., Steven, K., Horn, T., Skinner, D. G., and Jones, P. A. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res., *54:* 784–788, 1994.
23. Reznikoff, C. A., Belair, C. D., Yeager, T. R., Savelieva, E., Blelloch, R. H., Puthenveettil, J. A., and Cuthill, S. A. Molecular genetic model of human bladder cancer pathogenesis. Semin. Oncol., *23:* 571–584, 1996.
24. Sandberg, A. A., and Berger, C. S. Review of chromosome studies in urological tumors. II. Cytogenetics and molecular genetics of bladder cancer. J. Urol., *151:* 545–560, 1994.
25. Richter, J., Jiang, F., Görög, J. P., Sartorius, G., Egenter, C., Gasser, T. C., Moch, H., Mihatsch, M. J., and Sauter, G. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. Cancer Res., *57:* 2860–2864, 1997.
26. Koo, S. H., Kwon, K. C., Ihm, C. H., Jeon, Y. M., Park, J. W., and Sul, C. K. Detection of genetic alterations in bladder tumors by comparative genomic hybridization and cytogenetic analysis. Cancer Genet. Cytogenet., *110:* 87–93, 1999.
27. Eydmann, M. E., and Knowles, M. A. Mutation analysis of 8p genes POLB and PPP2CB in bladder cancer. Cancer Genet. Cytogenet., *93:* 167–171, 1997.
28. Forozan, F., Karhu, R., Kononen, J., Kallioniemi, A., and Kallioniemi, O. P. Genome screening by comparative genomic hybridisation. Trends Genet., *10:* 405–409, 1997.
29. Goldfarb, M., Shimizu, K., Perucho, M. M., and Wigler, M. Isolation and preliminary characterisation of a human transforming gene from T24 bladder carcinoma cells. Nature (Lond.), *296:* 404–409, 1982.
30. McCracken, S., Kim, C. S., Xu, Y., Minden, M., and Miyamoto, N. G. An alternative pathway for expression of p56lck from type I promoter transcripts in colon carcinoma. Oncogene, *15:* 2929–2937, 1997.

Delineation of the 6p22 Amplification Unit in Urinary Bladder Carcinoma Cell Lines

Jochen Bruch, Wolfgang A. Schulz, Jürgen Häussler, et al.

Cancer Res 2000;60:4526-4530.