Loss of cyclin-dependent kinase inhibitor genes and chromosome 9 karyotypic abnormalities in human bladder cancer cell lines

J Southgate1, J Profitt1*, P Roberts2, B Smith1 and P Selby1

1ICRF Cancer Medicine Research Unit and 2Cytogenetics Unit, St. James’s University Hospital, Leeds LS9 7TF, UK.

Summary Loss of cell cycle control through the structural or functional aberration of checkpoint genes and their products is a potentially important process in carcinogenesis. In this study, a panel of well-characterised established human bladder cancer cell lines was screened by the polymerase chain reaction for homozygous loss of the cyclin-dependent kinase inhibitor genes p15, p16 and p27. The results demonstrate that, whereas there was no genetic loss of p27, homozygous deletion of seven of 13 (54%) independent bladder cell lines tested. Differential loss of either the p15 or p16 gene was not seen. The p15 and p16 genes are known to be juxtaposed on chromosome 9p21 at the locus of a putative tumour-suppressor gene involved in the initiation of bladder cancer. Cytogenetic analysis of the cell lines revealed karyotypes ranging from near diploid to near pentaploid with complex rearrangements of some chromosomes and a high prevalence of chromosome 9p rearrangements, although all cell lines contained at least one cytogenetically normal 9p21 region. These observations support a role for p15/p16 gene inactivation in bladder carcinogenesis and/or the promotion of cell growth in vitro and lend support to the hypothesis that homozygous deletion centred on 9p21 is a mechanism by which both p15 and p16 genes are co-inactivated.

Keywords: cyclin-dependent kinase inhibitors; p15; p16; p27; bladder cancer; cell cycle; cytogenetics

Transitional cell carcinoma (TCC) is the most common cancer of the urinary bladder and originates from the normal stratified transitional epithelium, the urothelium. The natural history of TCC is unclear as it is currently not possible to distinguish the 3% subgroup of patients with superficial non-invasive papillary disease who will progress to muscle-invasive malignant disease from those patients in whom the disease is less aggressive. It is thought that the homozygous loss of a tumour-suppressor gene may be the initiating factor in bladder cancer and interest has centred on chromosome 9, which shows a high rate of allelic loss that is both stage and grade independent (Cairns et al., 1993; Miyao et al., 1993; Ruppert et al., 1993; Stadler et al., 1994). Deletion mapping studies of primary bladder tumours indicate the presence of two independent tumour-suppressor loci on chromosome 9, located proximally on 9p and 9q (Ruppert et al., 1993; Keen and Knowles, 1994). In two independent studies, the 9p locus has been mapped to the 9p21–22 region (Cairns et al., 1994a; Stadler et al., 1994b).

The 9p21–22 region is also implicated in tumour development of a wide spectrum of other human tumours, including familial and sporadic melanoma (Cannon-Albright et al., 1992; Fountain et al., 1992; Hussussian et al., 1994; Kamb et al., 1994a). The location of the p16 cyclin-dependent kinase 4 inhibitor (Cdk4i) gene at 9p21 has led to the hypothesis that inhibitors of G1/S cell cycle progression may show tumour-suppressor function and hence have oncogenic activity if homozygously lost through deletion and/or mutation (reviewed by Hartwell and Kastan, 1994; Marx, 1994a). In addition to p16 (Serrano et al., 1993), three other Cdk inhibitors have been identified: p15 (Hannon and Beach, 1994), p21 (El-Deiry et al., 1993; Harper et al., 1993; Xiong et al., 1993a) and p27 (Polyak et al., 1994a; Toyoshima and Hunter, 1994) and these too have the potential to act as tumour-suppressors or to confer growth advantage in vitro. Transcription of p27 is stimulated by p53 (El-Deiry et al., 1993) and by cell senescence (Noda et al., 1994) and expression of p21 protein is reduced in cells with null p53 (Xiong et al., 1993b). The p15 and p27 inhibitors are each implicated in TGF-β-mediated G1 phase arrest (Hannon and Beach, 1994; Polyak et al., 1994b). The potential role of the p27 gene in cancer is, as yet, undetermined.

A number of cell lines have been established from bladder cancers, and these have been used extensively as in vitro models of neoplastic urothelial cell behaviour (reviewed Masters et al., 1986). Bladder cancer cell lines typically show complex karyotypes that reflect the genesis and evolution of the cancer and also the selection pressures imposed during adaptation to long-term survival in vitro. We have used the polymerase chain reaction (PCR) to screen a panel of well-characterised bladder cancer cell lines for homozygous loss of p15, p16 and p27 gene sequences.

Materials and methods

Cell lines

In total, 17 established human bladder cancer cell carcinoma cell lines were used: RT4, RT112, HT1157, HT1197, COLO232, KK47, VM-CUB-1, VM-CUB-3, 253J, EJ, HU456, HU961T, MGH-U2, T24, TCCSUP, HCV29 and ScaBER. These cell lines have been reviewed and referenced by Masters et al. (1986). The cell lines were maintained in a 1:1 mixture of RPMI-1640 and Dulbecco’s modified Eagle medium (DMEM) with 5% fetal bovine serum (FBS), as described previously (Trejdosiewicz et al., 1985).

Cytogenetic analysis

 Cultures of bladder cancer cell lines in exponential growth were exposed to 0.2 μl ml⁻¹ colcemid (Sigma) in growth medium for 17 h. Cells were removed from the substrate with a solution of 0.25% (w/v) trypsin and 0.02% (w/v) EDTA in phosphate-buffered saline (PBS) for 5 min and incubated for 15 min in 0.075 M potassium chloride before washing three times in Carnoy’s fixative. Slides were analysed by GTL banding and 3-6 metaphases were examined for each cell line.

DNA extraction

DNA extractions were performed using standard procedures involving digestion with proteinase K in the presence of sodium dodecyl sulphate (SDS) followed by phenol/chloroform extractions and ethanol precipitation. The integrity of DNA for each cell line was confirmed by PCR for an
unrelated gene (below) and the same DNA preparation was used in all subsequent analyses. The passage number of the cell line stocks used is shown in Table I.

Arbitrary primed PCR (AP-PCR)

AP-PCR was used to obtain genomic ‘fingerprints’ of the cell lines and was performed on 50 ng DNA in a 25 µl reaction volume containing 10 mM Tris pH 8.3, 50 mM potassium chloride, 0.125 mM each dNTP, 5 mM magnesium chloride, 1.6 µM oligonucleotide, 1 µg [F]PCR-dATP and 1 U AmpliTaq (Perkin Elmer). After incubation at 94°C for 4 min, five cycles of PCR were performed at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, followed by 30 cycles at 95°C for 1 min, 50°C for 1 min and 72°C for 1 min with a final extension at 72°C for 10 min. Samples were run on 5% polyacrylamide sequencing gels. Random oligonucleotide sequences of 20–28 base length were used to generate DNA banding fingerprints. The results described here refer specifically to oligonucleotide BO11 (Table II).

PCR

PCR was performed on 100 ng each of genomic DNA extracted from the human bladder cell lines and from human placental DNA (Sigma) included as positive control. Primer sequences are shown in Table II. For amplification of p15, primers p15(2)F and p15(2)R were used to amplify a 430 bp product from exon 2 of the p15 gene. As described by Jen et al. (1994). The reaction mix consisted of 67 mM Tris pH 8.8, 16.6 mM ammonium sulphate, 6.7 mM magnesium chloride, 10 mM β-mercaptoethanol, 6 % (v v) dimethyl sulphoxide (DMSO), 1.25 mM dNTPs, 1 µM of each oligonucleotide primer and 5 U of AmpliTaq. The PCR cycling conditions were denaturation for 2 min at 95°C followed by 35 cycles of denaturation at 95°C for 30 s, reannealing at 66°C for 1 min and extension at 70°C for 1 min, with a final extension at 70°C for 5 min.

For all other PCR reactions, each 50 µl reaction mix contained 10 mM Tris pH 8.3, 50 mM potassium chloride, 0.2 mM dNTPs, 2 mM magnesium chloride, 1 µM of each oligonucleotide primer (except for p16, where 0.15 µM of each oligonucleotide was used) and 1 U AmpliTaq. p16 primers were chosen that span an intron–exon boundary of the human Cdk4 (p16) gene and amplify a 167 bp product by PCR (Nobori et al., 1994). PCR cycling conditions were denaturation at 95°C for 1 min, reannealing at 62°C for 30 s and extension at 72°C for 30 s for a total of 40 cycles, with a final extension at 72°C for 10 min. In all cases where a negative results was obtained, the reaction was repeated.

PCR primers, p27AF and p27AR, were selected for the human p27 gene from cDNA sequence available from the Genbank database (accession number U10906). The PCR cycling profile for p27 was denaturation at 95°C for 1 min, reannealing at 60°C for 30 s and extension at 72°C for 30 s for a total of 40 cycles, with a final extension at 72°C for 10 min.

The integrity of DNA preparations was checked with primers HM1P1 and HM1P2 which amplify a 682 bp product from the 5' flanking region of the human LD78α gene located on chromosome 17q (Hirashima et al., 1992). The PCR cycling profile for the LD78α primers was denaturation at 95°C for 1 min, reannealing at 60°C for 1 min and extension at 72°C for 1 min for a total of 30 cycles, with a final extension at 72°C for 10 min.

PCR products from individual experiments were pooled and run on 2% agarose gels and visualised with UV light after staining with ethidium bromide. The 186 bp PCR product obtained with the p27 primers was confirmed as the correct sequence by blotting the gel onto Hybond N (Amerham International) and hybridising with oligonucleotide p27BF derived from the internal sequence.

Results

AP-PCR

Comparison of genomic ‘fingerprints’ generated by arbitrary primed PCR revealed a single band loss from the EJ, MGH-U2, HUCH11T, T24 and HU456 cells which was retained in the other cell lines (Figure 1). These results are consistent with earlier reports based respectively on isoenzyme analysis and minisatellite probes which suggested cross-contamination of these particular cell lines (O'Toole et al., 1983; Masters et al., 1988).

Cytogenetic analysis

Although the karyotypes of the cell lines have been previously reported, it was felt important to obtain specific karyotypes for the sublines used in this study in order to circumvent problems of genotypic drift. Cytogenetic analysis of the cell lines revealed karyotypes ranging from near di-

| Table I | PCR amplification of DNA from bladder cancer cell lines |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cell line passage no. | p15 | p16 | p27 | LD78α |
| COLO232 (5) | – | – | + | + |
| EJ 72a | + | + | + | + |
| HCV29 31 | – | – | + | + |
| HT1197 63 | + | + | + | + |
| HT1376 70 | + | + | + | + |
| KK47 24 | + | + | + | + |
| RT4 61 | – | – | + | + |
| RT112 77 | – | – | + | + |
| ScAbER (4) | + | + | + | + |
| VM-CUB-1 (3) | – | – | – | + |
| VM-CUB-3 27 | – | – | + | + |
| TCCSUP 70 | + | + | + | + |
| 253J 94 | – | – | + | + |

*Where absolute passage number is unknown, number of passages since receipt of cell line is shown in parentheses. 

| Table II | Oligonucleotide sequences |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gene | Primer | Position | Sequence |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| p15 | p15(2)F | Forward | 5'-CTTTAATGTCCTCCACCTG-3' |
| | p15(2)R | Reverse | 5'-CGTTGGAGCCTTATCG-3' |
| p16 | p16f | Forward | 5'-GGAAATTTGGAAACTGGAAGC-3' |
| | p16r | Reverse | 5'-CTGCCACTATCATGACCTG-3' |
| p27 | p27AF | Forward | 5'-CAAACTGTGGATGTCAAC-3' |
| | p27AR | Reverse | 5'-AATGCAAAAGGTCCATTGGG-3' |
| | p27BF | Internal | 5'-CAGAGACATGGAAGGCGA-3' |
| LD78α | HM1P1 | Forward | 5'-CTAGGCACTGTCAATTGAC-3' |
| | HM1P2 | Reverse | 5'-TTCTGAGCAGTTGACCCAGA-3' |
| – BO11 – | – | – | 5'-GTCAGTTAAGCAAGGGACTAAC-3' |
ploid to near pentaploid with complex rearrangements of some chromosomes. Chromosome 9p was targeted as a region of special interest and the results are summarised in Table III and illustrated in Figure 2.

Detection of cyclin-dependent kinase inhibitor gene sequences

These results are summarised in Table I and illustrated in Figure 3. Using the specific p16 primers, the expected 167 bp-sized single product was amplified by PCR from human placental DNA (included as a positive control) and with DNA from ten of the 17 bladder cell lines. Using the same optimised PCR conditions, no product could be amplified from any of the remaining seven cell lines (RT4, RT112, VM-CUB-1, VM-CUB-3, COLO232, 253J and HCV29). The ten p16-positive cell lines included all five of the cell lines suspected of cross-contamination. When only independent cell lines were considered, 6/13 were positive for p16.

The specific p15 primers amplified the expected single 430 bp product from the control human placental DNA and from the same ten bladder cell lines which were positive for p16 (above), including all five cell lines suspected of cross-contamination. All seven p16-negative cell lines failed to amplify any product with the p15-specific primers.

Table III. Chromosome 9 abnormalities in bladder cancer cell lines

| Cell line | Chromosome no. and ploidy | Expected no. of 9's | Observed no. of 9's | No. of normal 9's | Rearrangement affecting 9p | Other 9 rearrangements not affecting 9p | 9p status |
|-----------|---------------------------|---------------------|---------------------|---------------------|-----------------------------|----------------------------------------|----------|
| HCV29     | near diploid              | 47                  | 2                   | 1                   | –                           | –                                      | Monosomy |
| COLO232   | hyperdiploid              | 55                  | 2                   | 2                   | dic (7;9)(p13;p21-22)       | –                                      | Loss of 9p21-22→9pter |
| ScaBER*   | 106-110 near pentaploid   | 55                  | 6                   | 4                   | 2 copies of der (9;14)(q10;q10) | –                                      | Loss of all 9p in 2.6 9's |
| VM-CUB-3  | diploid                   | 46                  | 2                   | 2                   | –                           | –                                      | Unaffectedb |
| TCCSUP*   | 7q73 near triploid        | 58                  | 3                   | 3                   | –                           | –                                      | Unaffectedb |
| KK47*     | near triploid             | 58                  | 2                   | 1                   | 9p21-22 rearrangement       | –                                      | 9p21-22 terminal deletion + other possibilities |
| EJ*       | near tetraploid           | 91                  | 4                   | 2                   | 2 × 9's with del (9)(p12;22) marker with most of 9q but no 9p | –                                      | 9p21-22 terminal deletion in 2.4 9's |
| RT4       | 79-82 near tetraploid     | 91                  | 3                   | 2                   | 2 × 9's with der(9;7)(q10;q7) and marker chromosome with loss of all 9p | –                                      | Loss of 9p in 2.4 9's |
| HT1197*   | near triploid             | 91                  | 3                   | 1                   | –                           | –                                      | 9p status |
| 253J      | near triploid             | 59                  | 3                   | 3                   | –                           | –                                      | Unaffectedb |
| HT1376*   | near pentaploid           | 104-107             | 5                   | 4-6                 | i(9q)                       | Two copies 9q rearrangement           | Loss of 9p in 1.5 9's |
| RT112     | near diploidy             | 47                  | 2                   | 2                   | –                           | –                                      | Unaffectedb |
| VM-CUB-1  | near triploid             | 75                  | 3                   | 3                   | der (9;14)(q10;q10)         | –                                      | Loss of 9p in 1.3 9's |

*Indicates cell lines from which p16 was amplified by PCR. *No change in 9p.
Using the p27 primers, a single PCR product of 186 bp was amplified from control DNA and from all of the 13 independent bladder cell lines. The integrity of all the genomic DNA preparations was confirmed by successful amplification of a 682 bp product from the 5' flanking region of the human LD78a gene on chromosome 17q.

**Discussion**

Recent advances in understanding how regulation of the cell cycle is achieved through the coordinated activity of cyclin-dependent kinases and checkpoint controls has revealed groups of genes with hitherto unforeseen oncogenic and tumour-suppressor potential (Hartwell and Kastan, 1994; Marx, 1994a). The Cdk4 (p16) gene is one such candidate and resides within the critical deleted region 9p21–22 implicated in tumour development progression of a wide spectrum of human tumours. In particular, the p16 gene is homozygously lost with high frequency in cell lines derived from a large range of human tumours, including bladder (Kamb et al., 1994b; Nobori et al., 1994). This has important implications for bladder cancer as the grade and stage independence of the chromosome 9p changes would suggest that, if p16 is the putative tumour suppressor, deregulation of cell cycle control is an important initiating event in human bladder cancer. However, the apparently higher frequency of p16 loss in bladder cell lines compared with the primary tumour counterparts (Cairns et al., 1994b; Spruck et al., 1994) has thrown the role of p16 as a major multiple tumour-suppressor gene into some dispute (Bonetta, 1994; Marx, 1994b; Wainwright, 1994). An alternative hypothesis is that loss of p16 confers a selective growth advantage during adaptation of tumour cells to culture and hence is important in the generation of bladder cancer cell lines (Cairns et al., 1994b; Spruck et al., 1994; Yeager et al., 1995). A definitive answer to this debate is hindered by the problems of isolating tumour cell DNA uncontaminated by normal stromal cell DNA.

In this study, homozygous loss of the p15 and p16 genes was associated with 54% (7/13) bladder cancer cell lines. Kamb et al. (1994b) have previously reported homozygous p16 gene deletions from 33% (5/15) bladder cancer cell lines but did not name the cell lines used. The potential problems of long term cross-contamination of cell lines underlines the importance of individual cell line pedigree in such studies. The cell lines MGHU2, HU961, T24, HU456 and EJ, although received originally (c. 1978) from different sources, were revealed to have identical DNA banding patterns on AP-PCR-generated fingerprints and furthermore, have previously been reported to derive from one originating cell line (O'Toole et al., 1983; Masters et al., 1988). Had these five cell lines been independently included in the p16 gene analysis, the results would have suggested p16 gene loss from only 41% (7/17) of lines.

Our findings agree well with Spruck et al. (1994), who also found p16 loss in 54% bladder cancer cell lines. Of the 13 cell lines studied by Spruck et al. (1994) five were duplicated in our study (RT4, HT11376, TCCSUP, SCaBER, T24EJ and of these, the SCaBER line was revealed to contain a mutated p16 sequence. When combined, the data from these two studies suggest that from a total of 21 cell lines, p16 was homozygously lost from 62% (13) bladder cancer cell lines. It has recently been suggested that the high incidence of homozygous deletions around the 9p21 region may be a mechanism by which both p16 and the neighbouring p15 gene are efficiently and simultaneously inactivated (Jen et al., 1994) and our results support this hypothesis.

Cytogenetic analysis has confirmed the frequent involvement of chromosome 9p in bladder cancer cell lines, reminiscent of the involvement of chromosome 11p rearrangements in Wilms' tumours (reviewed by Junien and Henry, 1994). Nevertheless, the seven p16-negative bladder cancer cell lines all contained at least one cytogenetically normal 9p21 region, demonstrating that loss of the p16 gene was too small to be detected cytogenetically. This is consistent with the data of Nobori et al. (1994) who found that deletions at 9p21 in tumour cell lines were centred on the p16 gene and were often small. In terms of the likely succession of genotypic changes, it seems probable that many of the chromosome 9p rearrangements preceded changes in ploidy, as three cell lines (SCaBER, EJ and HT1197) revealed more than one copy of the same derivative 9p chromosome and three p16-negative cell lines (VM-CUB-1, RT4 and 2531) contained more than one cytogenetically normal copy of chromosome 9. It is also worth noting that chromosome 9p rearrangements were detected in five of the six cell lines from which a p16 PCR product was amplified: further studies will be needed to determine whether these cell lines actually transcribe and translate a functional p16 gene product before this can be taken as prima facie evidence for alternative tumour-suppressor genes residing at 9p21–22 in bladder cancer (Bonetta, 1994; Spruck et al., 1994).

The adaptation of tumours to growth in vitro places rigorous selection pressures on the individual tumour cells. Indeed, compared with normal bladder epithelium (Southgate et al., 1994), the majority of primary bladder tumours do not readily adapt to in vitro growth (Niell and Soloway, 1983, and unpublished observations). Derangement of cell cycle control through loss of function of a Cdk inhibitor protein is an adaptive growth advantage in vitro. Expression of the p53-associated Cdk inhibitor protein, p21, has been shown to be reduced in cells with p53 mutations (Xiong et al., 1993). Thus, whereas the p21 gene is not a direct target, loss of expression through p53 mutation can result in a phenotypic loss of suppressor function. Spruck et al. (1994) showed that, of the six bladder cancer cell lines that were p16 positive,
only one cell line contained a wild-type p53 gene. Homozygous loss of p53 is a relatively late event in the progression of bladder cancer (Sidransky et al., 1991) and, as yet, it is unknown whether loss of p16 or p21 function could offer differential survival benefits in vitro. Our studies suggest that the p27 gene is not commonly homozygously deleted in human bladder cancers. However, further studies will be needed to determine whether loss of p27 protein function is important either in bladder tumour progression and or in the evolution of bladder tumour cell lines.

Acknowledgements

We are grateful to the Imperial Cancer Research Fund and the Yorkshire Cancer Research Campaign for financial support. We thank P Horsfield, S Morris and N Telford at the Cytogenetics Unit for expert assistance in cytogenetics.

References

BONETTA L. (1994). Open questions on p16. Nature, 370, 180.
CAIRNS P. SHAW ME AND KNOWLES MA. (1993). Initiation of bladder cancer may involve deletion of a tumour-suppressor gene on chromosome 9. Oncogene, 8, 1083–1085.
CAIRNS P. TOKINO K. EBY Y AND SIDRANSKY D. (1994a). Homozygous deletions of 9p21 in primary human bladder tumours detected by comparative multiplex polymerase chain reaction. Cancer Res., 54, 3653–3658.
CAIRNS P. MAO L. MERLO A. LEE DJ. SWABB D. EBY Y. TOKINO K. VAN DER RIEP P. BLAUGRUND JE AND SIDRANSKY D. (1994b). Rates of p16 (MTSI) mutations in primary tumours with 9p loss. Science, 265, 415–416.
CANNING-ALBRIGHT PA. GOLDGAR DE. MEYER LJ. LEWIS CM. ANDERSON DE. FOUNTAIN JW. HEGE MI. WISEMAN RW. PETTY EM. BALE AE. ULMINILAYO OI. DIAZ MO. KWIATKOW-SKI DJ. PIEPKORN MW. ZONE JJ. AND SKOLNICK MH. (1992). A deletion distal to the cyclin-dependent kinase-4-inhibitor gene in human bladder cancer. Nature, 358, 753–756.
CASADESUS J. KATZ Y. SOLOMON MJ. SJER CJ. MASSAGUE J. ROBERTS JM AND KOFF A. (1994a). The p27kip1 gene encodes a cell cycle regulator which inhibits p21CDK4. Science, 260, 103–112.
CDK1 in bladder cancer J Southgate et al.

Masters JW. BEDFORD P. KEARNY A. POVEY S AND FRANKS LM. (1988). Bladder cancer cell line cross-contamination: identification using a locus-specific minisatellite probe. Br. J. Cancer, 57, 284–286.
MIYAO N. TSAI YC. LERNER SP. OLUMI AF. SPRUCK III CH. GONZALEZ-ZULUETA M. NICHOLS PW. SKINNER DG AND JONES PA. (1993). Role of chromosome 9 in human bladder tumours. Cancer Res., 53, 2060–2063.
NIELL HB AND SOLOWAY MS. (1983). Use of the tumour colony assay in the evaluation of patients with bladder cancer. Br. J. Urol., 55, 271–274.
NOBORI T. MIURA K. WU DJ. LOIS A. TAKABAYASHI K AND CARLSON DA. (1994). Deletions of the cyclin-dependent kinase-4-inhibitor gene in human bladder cancers. Nature, 368, 753–756.
NODA A. NING Y. VENABLE SF et al. (1994). Cloning of senescence cell-derived inhibitors of DNA synthesis using an expression screen. Exp. Cell Res., 211, 90–98.
POLYAK K. LEE M-H. ERDUMULT-JROMAGE K. KOFF A. ROBERTS JM. TEMPT P AND MASSAGUE J. (1994a). Cloning of p27kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular anti-mitogenic signals. Cell, 78, 59–66.
POOCHAI K. KATO J-Y. SOLOMON MJ. SJER CJ. MASSAGUE J. ROBERTS JM AND KOFF A. (1994b). p27kip1, a cyclin-dependent kinase inhibitor, links transforming growth factor-β and contact inhibition to cell cycle arrest. Genes Dev., 8, 9–22.
OTTOLE CM. POVEY S. HEBURN P AND FRANKS LM. (1993). Identity of some human bladder cancer cell lines. Nature, 301, 429–430.
RUPPERT JM. TOKINO K AND SIDRANSKY D. (1993). Evidence for two bladder cancer suppressor loci on human chromosome 9. Cancer Res., 53, 5093–5095.
SEKARNO M. HANNON GI AND BEACH D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D CKDK4. Nature, 366, 704–707.
SIDRANSKY D. VON ESCHENBACH A. TSAI YC. JONES P. SUMMER-HAYES I. MARSHALL F. PAUL M. GREEN P. HAMILTON SR. FST P AND VOGELSTEIN B. (1991). Identification of p53 mutations in bladder cancers and urine samples. Science, 252, 706–709.
SOUTHGATE J. HUTTON KAR. THOMAS FDM AND TREJDOVI-CE WJ. (1994). Normal human urothelial cells in vitro: proliferation and induction of stratification. Lab. Invest., 71, 583–594.
SPRUCK III CH. GONZALEZ-ZULUETA M. SHIIBATA A. SIMONEAU AR. LIN MF. GONZALEZ F. TSAI YC AND JONES PA. (1994). p16 gene in uncultured tumours. Nature, 370, 183–184.
STADLER WM. SHERMAN J. M. SCHLINDER SK. ROULSTON D. DREYLING M. RUKSTAS D AND OLOPADE O. (1994). Homozygous deletions within chromosome bands 9p21–22 in bladder cancer. Cancer Res., 54, 2060–2063.
TOSHIHIMA H. AND HUNTER T. (1994). p21, a novel inhibitor of G1 cyclin/CDK protein kinase activity, is required to p21. Cell, 78, 67–74.
TREJDOSEWICZ LK. SOUTHGATE J. DONALD JA. MASTERS JW. HEBURN PJ AND HODGES GM. (1985). Monoclonal antibodies to human urothelium and hybrids: production and characterization. J. Urol., 133, 533–538.
WINwright B. (1994). Familial melanoma and p16. A hng jury. Nature Genet., 8, 3–5.
XIONG Y. HANNON GW. HANG H. CASSO D. KOBAYASHI R AND BEACH D. (1993). p27 is a universal inhibitor of cyclin kinases. Nature, 366, 701–704.
XIONG Y. ZHANG H. AND BEACH D. (1993). Subunit rearrangement of the cyclin-dependent kinases is associated with cellular transformation. Genes Dev., 7, 1572–1583.
YEAGER T. STADLER W. BELAIR C. PUTHENVEETTI J. OLOPADE O AND REZNIKOFF C. (1995). Increased p16 levels correlate with pRb alterations in human urothelial cell lines. Cancer Res., 55, 493–497.