lationship between raised serum VEGF levels and the extent of the disease. Further studies are warranted to assess the prognostic value of this peptide in lung cancer patients, as well as its ability, together with microvessel count [9], to predict the risk of metastasis.

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European Journal of Cancer Vol. 32A, No. 13, pp. 2369–2370, 1996

Printed in Great Britain
0959-8049/96 $15.00 + 0.00

PII: S0959-8049(96)00238-9

Abnormalities of the *P16^{INK4A}* Gene in Thyroid Cancer Cell Lines

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LOSS OF tumour suppressor gene activity is a key event in the genesis of most human tumour types. Predictably, some of these genes code for negative regulators of the cell cycle. Over the last 2 years, one of this group, the cyclin kinase inhibitor p16INK4a has been found to be abnormal in a wide range of primary cancers and tumour-derived cell lines [1]. Recently, we reported the first study of thyroid cancers in which four of a series of seven showed deletion of the *p16* gene locus [2]. In the light of recent reports pointing to the occurrence of point mutations [1] and differences in transcriptional regulation [3], we have now looked for more subtle lesions in the remaining cell lines that appeared to have a normal *p16* gene in our initial report, namely, FTC 133 (derived from follicular carcinoma), BCPAP and NPA (both from papillary cancers). Two types of analyses were performed; sequencing of genomic DNA and methylation status.

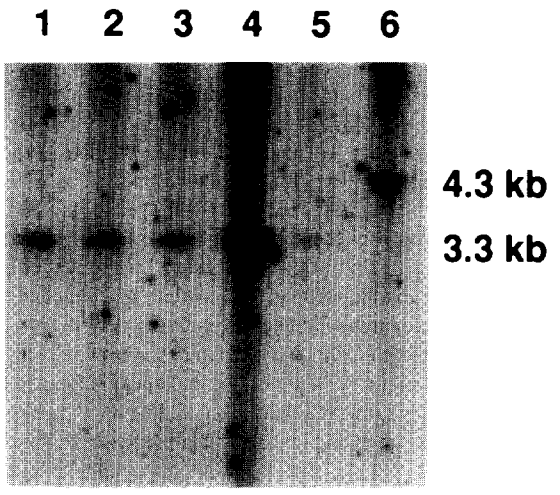

Figure 1. DNA digested with Sac II and EcoRI was probed with *p16* exon 1 after transfer to a Hybond N+ membrane. The probe hybridises to unmethylated and methylated exon 1 at 3.3 and 4.3 kb, respectively. Lanes: (1) placental tissue; (2) normal adult thyroid tissue; (3) thyroid cells prepared from Graves' disease (non-neoplastic); (4) ori 3 (an SV40 transformed epithelial cell line not expected to show *p16* abnormality); (5) FTC133 (follicular cancer cell line); (6) BCPAP (papillary cancer cell line).

In sequencing the genomic DNA, the two exons in which most of the *p16* gene (approximately 97%) lies, were isolated by PCR (polymerase chain reaction) using primers as follows: exon 1 GAAGAAAGAGGAGGGGCTG and GCGCTACCTGATTCCAATTC; exon 2 ACACAAGCTTCCTTTCCGTC and TCTGAGCTTTGGAAGCTC. Genomic DNA (100 ng) was amplified for 40 cycles (94°C, 40 s; 60°C, 40 s; 72°C, 90 s) under standard conditions with the addition of 3.6% formamide. Purified PCR products were subcloned using the TA system (Invitrogen). Both strands of three clones from each cell line were analysed by double-stranded DNA cycle sequencing on an ABI 373 system using the original PCR primers, ABI Prism dye terminators and Taq FS.

In the NPA cell line, a point mutation in the splice donor consensus site flanking exon 1, was identified (T → C at base 256, Genbank U12818). This would result in an incorrectly spliced mRNA and inappropriate translation, effectively abolishing *p16* function. This observation was confirmed by direct sequencing of another PCR product from NPA DNA. The other cell lines had wild type *p16* sequence.

Reduction of gene expression, caused by *de novo* methylation of CpG islands within exon 1 of *p16*, has been identified in both primary tumours and tumour-derived cell lines [3].

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Received 20 May 1996; accepted 3 Jun. 1996.

Methylation of exon 1 can be detected by the failure of the restriction enzyme Sac II to cleave a 4.3 kb EcoRI fragment. The methylation state of *p16* in the thyroid cell lines was examined on Southern blots of 5 μg genomic DNA digested with 15 IU Sac II and EcoRI and probed with exon 1 (conditions as previously reported [2]). In five of six samples, signals at 3.3 kb were detected indicating unmethylated exon 1 (see Figure 1). However, in the papillary cancer cell line BCPAP, a signal at 4.3 kb resistant to cutting by Sac II was seen, indicating methylation of exon 1 on both alleles which, from previous studies [3], would be predicted to downregulate *p16* expression.

We are now examining DNA from primary thyroid tumour samples using these methods in order to determine the importance of *p16* abnormalities *in vivo*. The data reported here confirm the necessity of studying multiple aspects of *p16* structure and function when assessing its role in tumorigenesis.

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Acknowledgements—The authors thank Joyce Hoy, University of Wales College of Medicine Molecular Biology Facility for DNA sequencing. The work was supported by the Cancer Research Campaign, U.K.

Table 1. Patient and treatment characteristics

	HD	NHL	Total number of patients
Number of patients	15	17	32
Median age (range) in years	31(16–50)	62(38–79)	43(16–79)
Number who received previous treatment			
1	3	9	12
2	3	4	7
3	2	3	5
4	2	1	3
>4	5	—	5
Disease status			
Refractory	8	4	12
Relapsed	7	13	20
Disease extension			
≤3 sites	8	8	16
>3	7	9	16
CY dose calculated by			
BSA			15
GFR			17
Number of courses of CY			
1	4	8	12
2	3	6	9
3	4	1	5
4	2	1	4
>4	2	1	2
Median (range)			2(1–8)
Delayed CY	7	8	10
Median delay time (days)			7
Range			4–15
Dose reduction	4	1	2

HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; BSA, carboplatin dose based on body surface area; GFR, carboplatin dose based on glomerular filtration rate; CY, carboplatin.

European Journal of Cancer Vol. 32A, No. 13, pp. 2370–2371, 1996

Printed in Great Britain
0959-8049/96 $15.00 + 0.00

PII: S0959-8049(96)00285-7

Lack of Carboplatin Activity in Malignant Lymphomas

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THE ACTIVITY of cisplatin as a single agent in haematological neoplasms [1] has led to the development of association regimens such as DHAP (dexamethosone, high dose cytarabine and cisplatin) [2] and ESHAP [3] (etoposide, methylprednisolone, high dose cytarabine and cisplatin) for salvage treatment in both Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL). These regimens produce an overall response rate of 50–60%, and long-term survival rates slightly in excess of 10%. Thus, these are often used as conditioning regimens before high-dose chemotherapy programmes [4]. In spite of the fact that carboplatin activity at conventional doses has only been tested in a single study with overall negative results [5], it has sometimes been included at higher doses in the induction phase of autologous bone marrow transplantation (ABMT) programmes [6, 7].

In an attempt to verify carboplatin activity at conventional doses in lymphomatous malignancies, a phase II trial was started in patients with relapsed or refractory HD or intermediate/high grade NHL. Eligible patients were required to have a good performance status (Karnofsky > 60), adequate bone marrow reserve (unless impaired due to bone marrow infiltration), normal organ function (heart, liver and kidney), and measurable and/or evaluable disease. Written informed consent was required, and the trial was conducted according to good clinical practice recommendations.

When feasible, the carboplatin dose was calculated according to glomerular filtration rate in order to provide an

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Received 13 Jun. 1996; accepted 24 Jun. 1996.