LETTERS TO THE EDITOR

INFLUENCE OF INTRATUMOUR HETEROGENEITY IN THE INTERPRETATION OF MARKER RESULTS IN PHAEOCHROMOCYTOMAS

In a recently published paper, Krijger et al. try to predict the clinical behaviour of a phaeochromocytomas (PCCs) on the basis of the immunoreactivity of three markers (p53, bcl-2, and c-erbB-2). They conclude that the co-expression of p53 and bcl-2 proteins may assist that prediction. Our own preliminary results mainly agree with theirs and point towards two interrelated biological issues: firstly, the clonal evolution of neoplasms and intratumour heterogeneity of markers; and secondly, the importance of apoptosis in tumour initiation and progression.

The results of Krijger et al. and other previous findings indicate that several genes must be involved in PCC pathogenesis, regardless of the genetic background (sporadic or familial). Although the authors do not mention variability of the immunostaining, this must be assumed, because most PCCs are scored 1+ (10–50 per cent positive cells). These relatively low values must be associated with heterogeneous expression of the protein. Our preliminary results with p53 and pRB (both immuno-expression and the genetic evaluation of polymorphic DNA regions) support this point.

Tumour cell selection will determine progression, cellular heterogeneity, and clonal expansion. Loss of heterozygosity (LOH) analyses have showed random and non-tumour-related DNA deletions in 4–20 per cent of normal tissues, confirming cellular heterogeneity, which should be considered a limiting threshold in the interpretation of any tumour marker. Intratumour heterogeneity for a given marker can represent either the expression of selective tumour evolution, or a simple passive by-product of other mechanisms, such as genetic instability. In any case, the association of multiple genetic alterations would become statistically less probable as the number of molecular markers increased and would explain why the combination of p53 and bcl-2 was strongly correlated with malignant PCC.

The mechanism of selection of cell clones would allow us to use genetic markers to define tumour progression, but these genetic changes are unpredictable and their heterogeneity precludes their extensive clinical use for diagnostic and prognostic purposes. This heterogeneity also calls for caution in evaluating markers of malignancy; they must be extensively screened and validated in order to avoid misinterpretations leading to false-positive and false-negative cases.

The results of Krijger et al. also stress the importance of p53 in the pathogenesis of a subgroup of PCCs. Our own experimental results (manuscript in preparation) support this point of view, based on the analysis of five polymorphic DNA regions (microsatellites) located on introns of four tumour suppressor genes (p53, RB1, WT1, and NF1). The comparative study of peripheral and internal tumour areas confirmed that there was an increased accumulation of genetic deletions in the peripheral tumour compartment, probably reflecting both tumour progression and multistep tumourigenesis (≥ two loci showed LOH).

In addition, tumour cell selection is the expression of cellular kinetics. Any genetic alteration leading to cellular ageing, differentiation, or activation of the apoptotic pathway will be non-tumour productive. The presence of a given marker points to an extensive kinetic advantage provided by the marker itself, or linked to it; it also represents the basic mechanism of cell selection and tumour progression. In this framework, Krijger et al. report the expression of bcl-2 in malignant PCC. bcl-2-expressing cells would be protected from apoptosis, thus contributing to clonal expansion, and the absence of normal p53 would protect genetically damaged cells from apoptosis.

The associated expression of abnormal p53 in malignant PCC would also maintain the proliferation of transformed cells. Krijger et al. clearly indicate the importance of p53 and bcl-2 in the pathogenesis of a subgroup of PCCs. However, the variability of marker expression and the potential relationship with kinetic features are also helpful in understanding the pathogenesis of PCCs.

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AUTHORS’ REPLY

We would like to thank Dr Diaz-Cano and Dr Blanes for their comments on our paper,\(^1\) which they seem to use as an alibi for extensive reflections on tumour heterogeneity.

Human tumours are frequently genetically heterogeneous, as has recently been discussed in an excellent review article.\(^2\) The differential acquisition of genetic abnormalities will lead to the formation of tumour cell clones with selective growth advantage, which will determine tumour behaviour and thus patient prognosis. Research into the pathogenesis of several major cancer types has benefitted from the comparative analysis of lesions in various stages of development, which has led to the discovery of genetic abnormalities that are essential and/or specific for the development of particular tumours. As regards PCCs, however, precursor lesions have not (yet) been defined. Potentially, adrenal medullary hyperplasia may serve as some kind of precursor lesion, although the difference with PCC is only quantitative, not qualitative.

As is the case in other human cancers, PCCs have (considerable) genetic heterogeneity. In previous work, we have shown that 4/27 (15 per cent) benign and 1/29 (3 per cent) malignant sporadic PCCs have somatic RET missense mutations, thus implying that there are other genes involved in the pathogenesis of PCCs.\(^3\) At present, we are addressing VHL somatic mutations in benign and malignant PCCs.

In our paper, we reported on p53 and bcl-2 immunoreactivity. As Diaz-Cano and Blanes noticed, the number of p53- and bcl-2-immunoreactive cells varied from one tumour to another, amounting to between 10 and 50 per cent of the tumour cells. The immunoreactive cells had a somewhat patchy distribution in several cases, which might be taken to reflect the presence of distinct clone cells. However, without microdissection and clonal analysis, this is entirely speculative. Diaz-Cano and Blanes point out that potential markers for adverse tumour behaviour must be extensively evaluated and validated. Our agreement with this comment is manifest in the discussion, where we have explicitly stated the practical implications of our analysis.

RE. SV40-LIKE DNA SEQUENCES IN PLEURAL MESOTHELIOMA, BRONCHOPULMONARY CARCINOMA AND NON-MALIGNANT PULMONARY DISEASE

We read with interest the paper of Galateau-Salle et al. describing the presence of SV40 or SV40-like viral DNA in neoplastic, non-neoplastic mesothelium, and bronchial carcinoma.\(^1\) It is indeed a recognized phenomenon that some SV40 primer sets result in a higher percentage of positive PCR than others.\(^2\) Preliminary data suggest that PCR primers considered specific for SV40 would, under certain conditions, amplify what appeared to be host DNA sequences, indicating that PCR-based assays must be interpreted with caution and correlated with viral antigen expression.\(^3\)

The authors used one monoclonal antibody, Pab 419 (Ab-1 Oncogene Science), to investigate the presence of viral SV40 antigens, as confirmation for their PCR-based assay. This antibody detects an epitope common for both the p94 large T- and the p21 small t-antigen. Its main applications have been described for immuno-