British Journal of Cancer (1998) 77(5), 850–852

Letters to the Editor

p53 and angiogenesis in non-small-cell lung cancer

Sir

The prognostic role of p53 nuclear expression in non-small-cell lung cancer (NSCLC) remains contradictory. One of the first papers on early operable cancer failed to confirm an impact of p53 expression on survival (McLaren et al, 1992). In this study, five different antibodies were used in frozen material. Although some studies subsequently showed a positive correlation of mutant p53 expression with worse outcome, an equally high number of studies failed to confirm such an observation (Mitsudomi et al, 1993; Kashii et al, 1995; Nishio et al, 1996). Surprisingly, several studies correlated positive p53 expression with better prognosis (Lee et al, 1995; Passlick et al, 1995; Top et al, 1995).

The *p53* oncogene has recently been shown to inhibit angiogenesis through regulation of thrombospondin-1, an inhibitor of angiogenesis (Dameron et al, 1994). The first clinicopathological study that examined possible correlation of mutant p53 expression with angiogenesis was reported by Giatromanolaki et al (1996*a*), in which no correlation was found with vascular grade, assessed with JC70 MAb. JC70 (anti-CD31) is more specific than anti-Factor VIII antibody endothelial cell marker. In a comparative study, we observed that 22% of cases with high microvessel score on JC70 had a low score on anti-Factor VIII staining. Anti-CD31 reveals a 2.5 times higher number of microvessels and four times higher number of endothelial cells compared with anti-factor VIII staining (Giatromanolaki et al). Comparing vascular grade with the p53 score obtained by McLaren et al (1992) (five different antibodies), no correlation was found. In addition, using the results obtained with PAb248 (Pezzella et al, 1994) recognising the cytoplasmic wild-type p53 protein, no association was confirmed (F. Pezzella).

In a subsequent study on the angiogenic factor thymidine phosphorylase (PD-ECGF) expression in non-small-cell lung cancer, we observed that cancer cell overexpression associated with increased neoangiogenesis (Koukourakis et al, 1997). Again, p53 expression was not associated with TP expression. The interesting recent observation (Fontanini et al, 1997) that p53 nuclear expression correlates with angiogenesis and survival is not in accordance with our previously published results. The method of this paper considered microvessel counting in one focus of high (factor VIII assessed) vascularization while the mean score of p53-positive cells was assessed in five fields. It is well known that one angiogenic hot spot does not always define high vascularization. If loss of wild-type p53 does suppress thrombospondin-1 expression, increased angiogenesis in the stroma arround p53-stained areas would be expected. However, what the p53 status was within the area of high neovascularization or what the angiogenesis was within the fields of p53 positivity was not studied. The very low 'r-factor' (0.41) reported for linear regression analysis further suggests that p53 may have a more complicated role. An eventual angiogenesis regulating role of p53 or p21 alone or in cooperation with other oncogenes, such as *c-erbB*-2 (Giatromanolaki et al, 1996*b*) demands further investigation (Fig. 1).

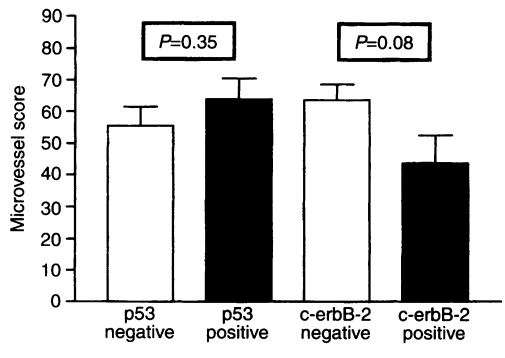

Figure 1 Microvessel score (MS), p53 and c-erbB-2 expression assessed with JC70, PAb1801 and NCL-CB11 antibodies, respectively, in 107 cases of non-small-cell lung cancer. The mean MS was 63 ± 49 and 55 ± 42 for p53-positive and p53-negative cases respectively ($P = 0.35$). The mean MS was 63 ± 47 and 43 ± 39 for c-erbB-2-positive and c-erbB-2-negative cases respectively ($P = 0.08$)

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p53 and angiogenesis in non-small-cell lung cancer Reply to the letter from Giatromanolaki and Koukourakis

Sir

We completely agree with Dr Giatromanolaki about the controversial prognostic role of p53 expression in human cancers and, in particular, in lung cancer. There are in fact several studies that fail to evidence any statistical association between p53 alterations and poor prognosis in NSCLC (McLaren et al, 1992; Kashii et al, 1995; Kwa et al, 1996; Nishio et al, 1996) and others that find an association between p53 alterations and a favourable behaviour. However, several other studies, which evaluated both p53 mutations and p53 nuclear overexpression, have underlined a strong statistical association between p53 alterations and poor prognosis in this type of cancer (Quinlan et al, 1992; Horio et al, 1993; Mitsudomi et al, 1993; Fontanini et al, 1995*a*; Harpole et al, 1995; Dalquen et al, 1996; Irie et al, 1996; Dosaka-Akita et al, 1997; Fontanini et al, 1997, Fukuyama et al, 1997), stimulating further investigation in this field. As regards the discrepancies that arise between these studies, we have identified some possible causes: (1) the cohorts of patients analysed (prospective or retrospective); (2) the different stages of the tumours; (3) the histological types investigated; (4) the methodologies used. We believe that the most correct analyses tending towards a prognostic evaluation of a specific factor should use prospective and consecutive series of patients, because retrospective cohorts more often lead to the introduction of potential biases.

With regard to the evaluation of the relationship between angiogenesis, oncogenes and tumour-suppressor genes, we believe that in this field also there is a long way to go. In fact, very few studies have been performed in this respect, and it is too restrictive to draw final conclusions on the basis of only two investigations, mainly because these analyses disagree on some points. We are not completely sure that anti-FVIII antibodies are less specific than other types of endothelium-related antibodies as the anti-FVIII antibody has been defined as being the most specific marker for endothelial cells (Weidner, 1995; Folkman and Weidner, 1996), although it may be a little less sensitive in non-expert hands. Moreover, it has been reported by Folkman and Weidner (1996) that, although apparently more sensitive, CD31 strongly cross-reacts with plasma cells (DeYoung et al, 1993; Longacre et al, 1994), and this complication can markedly obscure the microvessels in tumours with a prominent plasmacellular inflammatory background. Weidner (1995) reports that a valid alternative to FVIII antibodies may be represented by the anti-CD34 antibody and, recently, Tomisaki et al (1996) demonstrated a very strong correlation between FVIII and CD34 immunoreactivity in colorectal cancer ($r = 0.956$, $P = 0.01$). Like antibodies to FVIII, anti-CD31 and anti-CD34 do not immunostain all intratumoral microvessels, and it would be useful to dispose of new antibodies raised against proliferating or activated endothelial cells. However, we would like to underline that anti-FVIII antibodies have been used in most of the analyses on vascular count performed in NSCLC (five out of seven), and a significant association between vascular count and poor prognosis has been found in all of these series (Macchiarini et al, 1992; Yamasaki et al, 1994; Fontanini et al, 1995*b*; Angeletti et al, 1996; Giatromanolaki et al, 1996; Harpole et al, 1996).

According to Weidner (1995) and Folkman (1995), we evaluated the vascular count in our tumours in the areas with a greater number of microvessels ('hot spot') after scanning more than one section of the tumour. The 'hot spot', so defined, is considered as being representative of tumoral angiogenesis by these authors, despite the many discussions held in this field so far. In general, we agree with the concept that tumour cells have different angiogenic potentials and that the 'hot spot', if carefully identified by an expert pathologist, may provide reliable information on the angiogenic pattern of a tumour. From this point of view, we believe that the association that we found between p53 and vascular count in our series of patients (Fontanini et al, 1997) should be considered exciting, although it is not in agreement with the data by Giatromanolaki et al (1996) – it represents, on the one hand, a further contribution to the discussion on the relationship between tumour-suppressor genes and angiogenesis and, on the other hand, it prompts us to perform further studies as suggested by Dr Giatromanolaki.

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