Gross Rearrangements and Deletions of the Retinoblastoma Gene Are Rare in Malignant Melanoma

Sir,

Retinoblastoma (RB) is a common malignant disease of childhood, with an incidence of approximately 1 in 14,000 live births (1). Clinically it occurs in two forms, familial and sporadic. The RB gene has been identified and cloned (2) and is located on chromosome 13q14.1 spanning over 200 kbp (3). cDNA clones have been derived and used to identify mutations in both alleles of the RB gene, using Southern blot analysis. No specific mutation hot spots have been identified, although exons 13–17 are more commonly involved than others (3).

It is well established that survivors of hereditary RB and their relatives (but not of the sporadic form) have a significantly increased risk of developing other secondary non-ocular tumours, in particular soft tissue sarcomas, osteosarcomas and melanoma (4–6).

In the tumours shown to have increased incidences, the role of RB gene mutations and deletions has been investigated with the exception of malignant melanoma. In this study we attempted to identify gross rearrangements and homoxygous deletions of the RB gene in melanoma tumour samples.

DNA was extracted from 20 primary (10 superficial spreading melanomas, SSM, and 10 nodular melanomas, NM) and 13 secondary melanomas (7 inguinal nodes, 4 auxiliary nodes and 2 subcutaneous nodules) removed from patients undergoing surgery at the University Hospital of Wales and 2 secondary melanomas from the Royal Marsden Hospital. In addition, DNA was also extracted from 10 established melanoma cell lines: MEWO, RPM1 5966, Mel Swift, SK 6005, Mel 28, NK 14, Mel 19, SK Mel 64, Mel 57, Mel 2 A.

DNA (10 μg) was digested with the restriction enzyme Hind III (Gibco BRL). Equal amounts of Hind III-digested control (normal surrounding skin) and tumour DNA were transferred to nylon membranes (Hybond N, Amersham) using a standard Southern blot transfer, fixed using ultra-violet light and hybridized with a 4.7 kb RB1 cDNA clone divided into 0.9 and 3.8 kb fragments by an internal EcoR1 site (7). Probes were labelled with alpha-^{32}P dCTP by the random primer method (Boerhinger). Autoradiography revealed the characteristic product bands for the RB gene obtained with Hind III digestion.

Of the 20 primary and 15 secondary melanomas analysed, all showed a strong hybridization signal indicating no bilateral loss of the RB gene. A novel fragment of approximately 2 kb was detected using the 0.9 kb probe in one patient, in a secondary subcutaneous melanoma nodule. The rearrangement was absent from the control DNA derived from normal surrounding skin. A second rearrangement was identified using the 3.8 kb probe in a second patient with primary melanoma. This time the tumour was an *in situ* primary melanoma of the thigh, and again the novel fragment was not present in the control DNA. The presence of a normal band pattern in addition to the rearrangement indicates a heterozygous rearrangement in both cases. None of the 10 melanoma cell lines showed any loss of hybridization signal or abnormality in the restricted fragments obtained.

In our series we found an overall incidence of gross rearrangements in the RB gene of 4.5% and no homozygous gene deletions. This figure is close to the frequency of 6% of individuals treated for familial RB who develop maglignant melanoma (8).

The implication of these studies is that gross rearrangements, while easily identifiable, are not numerically important causes of RB gene mutation. Point mutations detected by other methods are important not only in RB itself but also in other associated tumours, including bladder and small cell lung cancers. This study has found RB gene rearrangements to be present in a small subset of malignant melanomas.

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REFERENCES

1. Vogel F. Genetics of retinoblastoma. Hum Genet 1979; 52: 1–7.
2. Lee W-H, Bookstein R, Hong F, et al. Human retinoblastoma susceptibility gene: cloning, identification and sequence. Science 1987; 235: 1394–1399.
3. T'Ang A, Wu K-J, Hashimoto T, et al. Genomic organisation of the human retinoblastoma gene. Oncogene 1989; 4: 401–440.
4. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with hereditary retinoblastoma. Br J Cancer 1986; 53: 661–671.
5. Traboulsi EI, Zimmerman LE, Manz HJ. Cutaneous malignant melanoma in survivors of heritable retinoblastoma. Arch Ophthalmol 1988; 106: 1059–1061.
6. Sanders BM, Jay M, Draper GJ, et al. Non ocular cancer in relatives of retinoblastoma patients. Br J Cancer 1983; 60: 358–365.
7. Fung YKT, Murpheree AL, T'Ang A, et al. Structural evidence for the authenticity of the human retinoblastoma gene. Science 197; 236: 1657–1661.
8. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986; 323: 643–646.

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