CARCINOGENESIS

Is imprinting to blame?

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THE report by Zhu and colleagues on page 312 of this issue[1], together with that recently published by Dryja *et al.*[2], suggests that new germ-line mutations to the heritable form of retinoblastoma are more likely to occur on the paternal rather than the maternal chromosome. These authors find no such bias, however, in tumours where the corresponding first mutation has occurred in a somatic cell. This differs from previous reports of Wilms tumour and osteosarcoma[3,4], which had indicated a strong preference for the first mutation to occur on the paternal chromosome in sporadic tumours, observations which led to speculation that differences in genomic imprinting of paternal and maternal alleles may be involved[4-6]. Why the discrepancy, and what do the new data mean?

The background has been reviewed in two recent News and Views articles[6,7]. Many familial tumours, among them retinoblastoma, osteosarcoma (which arises from mutation at the same locus as retinoblastoma) and Wilms tumour, arise by a 'two-hit' mechanism in which the tumour cells lose first one and then a second allele at a putative 'tumour-suppressor' gene locus. In familial cases, the first mutation is inherited in the germ line. The second mutation occurs in a somatic cell in the target tissue, which then gives rise to the tumour. Histologically similar tumours also commonly occur in a non-heritable form: in these cases, both mutations must occur in the same somatic cell. In either case, the second mutation is often associated with large-scale deletions or chromosomal loss from mitotic recombination or non-disjunction. Because of this, comparison of DNA polymorphisms in normal tissue and tumour from the same individual will often indicate which chromosome has been retained in the tumour and has, by inference, sustained the first mutational event.

Analysis of Wilms tumour and of osteosarcomas that were thought to be of non-heritable type (that is, where the first mutation arose within a somatic cell) showed a strong bias towards retention of the paternal allele. This implies that paternal and maternal alleles must therefore differ in the tissue in which the mutation occurred. The difference might be either in susceptibility to mutation or in expression. If the maternal tumour-suppressor allele was the less active, for example, the descendants of a cell in which the paternal allele had been inactivated as the first event would have relatively little suppressor activity, and would be more likely to develop into a tumour. These differences were thought to be the result of genomic imprinting[4-6].

The new data on retinoblastoma[1,2], by contrast, show no such bias towards paternal origin of the first mutation in non-heritable tumours. There is therefore no need to suppose any differences between paternal and maternal alleles in the target retinal tissue, and no need to invoke an influence of genomic imprinting on carcinogenesis. Consistent with this conclusion, there is no evidence in familial retinoblastoma for a difference in penetrance of the germ-line mutation, when it is transmitted through the male or female line. Such a difference would be expected if the expression or mutability of the allele differed in the target tissue.

Although the original report[3] on Wilms tumour was based on analysis of only five unilateral sporadic cases, there are unpublished data showing the same findings in many others. There would be no discrepancy between the Wilms and retinoblastoma results if many or most of the Wilms tumours had originated from a germ line rather than a somatic mutation. Lack of family history may not be much help here: very few individuals with Wilms tumour, whether unilateral or bilateral, have had affected children, and so germ-line mutations at Wilms loci on chromosome 11p may be poorly transmitted. Until the Wilms gene or genes are in hand, this question cannot be resolved, and it remains an assumption that most unilateral Wilms tumours originate from sporadic mutation. Allowing this assumption for now, if the tendency for the first somatic mutation in Wilms tumour and osteosarcoma to occur on the paternal chromosome is due to genomic imprinting, presumably the Wilms locus is imprinted in embryonic kidney, and the retinoblastoma (*Rb*-1) locus in bone but not in retina.

Chromosomal regions that are probably affected by imprinting have been mapped in the mouse[8]. Comparative mapping of the homologues of the Wilms and *Rb*-1 loci suggests that neither falls within regions for which there is any evidence for imprinting, but the homologous locus for Wilms tumour on mouse chromosome 2 lies between a region in which the effects of parental chromosome duplications suggest that imprinting does occur, and the homologue of the locus for the Prader–Willi and Angelman syndromes, which also show effects consistent with imprinting[8].

A piece of evidence previously cited as consistent with imprinting at the Wilms locus is that the age at onset of tumour in cases where the gene has been inherited from the mother is greater than when inheritence is paternal. This effect is not seen in retinoblastoma. But this evidence may no longer be relevant, because it is now known that the locus for the familial type of Wilms is not the same as the Wilms loci on 11p which have been analysed in the tumours. The difference between the results relating to somatic mutation in retinoblastoma tumours and osteosarcoma[4] is harder to explain, because the same gene is involved in each case. Zhu *et al.*[1] raise some questions about the interpretation of the osteosarcoma data, but if the interpretation is correct, the alleles at the *Rb*-1 locus in bone must differ in a way in which they do not in retinal cells.

The paternal origin of the new germ-line mutations in retinoblastoma also requires explanation. The observation is consistent with data from other inherited disorders, for example haemophilia, achondroplasia and Lesch–Nyhan syndrome. In these disorders, the incidence of new germ-line mutations rises steeply with paternal age[9]. This may be explained (at least in part[9]) by supposing that the mutations arise at DNA replication in a class of spermatogonial stem cells which are self-renewing, and which will therefore accumulate mutations with time. Retinoblastoma shows only a weak paternal age effect in comparison. Possibly, therefore, most germinal mutations at the *Rb*-1 locus are of a different type, for example involving deamination at 5-methylcytosine which may be independent of mitosis, or occur in cells in which mutations do not accumulate with time. Detailed study of the mutations at the DNA level, feasible now that the *Rb*-1 gene has been cloned, may provide some clues.

Perhaps the most important implication of the Wilms/osteosarcoma findings in relation to carcinogenesis is that alleles at suppressor gene loci may differ in expression or mutability within the tissue from which tumours arise. In these particular tumours, the differences may be ascribed to genomic imprinting: in other cases, perhaps differences in suppressor-gene activity could be due to polymorphisms at these loci in the general population. Such variation might contribute to inherited differences both in the risk of developing a tumour, and in the behaviour of tumours once they have developed, and might, for example, explain in part the very large variability in expression seen in many of the inherited cancer syndromes. □

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