ABSTRACT

Genetic alterations in experimental tumours with special reference to *Hgfr/Met* oncogene amplification

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Animal cancer models provide valuable systems for genetic studies of tumorigenesis. Molecular genetic analysis was undertaken in DMBA-induced rat sarcomas, and in rat endometrial carcinomas. In order to identify genetic changes associated with tumour development, the tumour materials were screened with cytogenetics, comparative genome hybridisation (CGH) and microsatellite allelotyping. Several regions of genetic alterations were observed in the tumours. Some of the most prominent changes detected with CGH were increases in DNA copy numbers on RNO4q11-q22 in several of the DMBA-induced sarcomas, whereas copy number increases often involved RNO4q11-q24, RNO6q13-q21, and RNO10q31-q32 in the endometrial carcinomas. In the latter tumours reductions in copy number were also frequently seen in RNO10.

Since the RNO4q11-q24 region was engaged in both models it was selected for further analysis. Based on our own studies of comparative mapping, we selected 12 cancer-related genes (*Cdk5, Hgf, Dmp1, Cyp51, Tac1, Cav1, Hgfr/Met, Wnt2, Cftr, Smoh, Braf, Tim*) that were predicted to be located in the region. Genomic clones were isolated for each of the genes and mapped by FISH to RNO4q11-q24 as predicted. The gene probes/clones were also used to study the occurrence of gene amplification in the two rat models.

Five out of 17 DMBA sarcomas exhibited amplification of the *Hgfr/Met* oncogene, and the same tumours showed overexpression of both *Hgfr/Met* mRNA and protein. The results from free chromatin analysis in one of the tumours suggested that the *Hgfr/Met* gene was the primary target for amplification in these sarcomas. These results prompted us to investigate the status of HGFR/MET amplification/expression in 102 human musculoskeletal tumours. We found that high level expression of HGFR/MET was associated with malignant potential, but that there was no amplification of the HGFR/MET gene.

Among ten rat endometrial carcinoma tissue cultures there was amplification of *Hgfr/Met* in four. The amplification was associated with mRNA and protein overexpression. A detailed analysis of the amplified region using all 12 gene probes revealed that the amplification of *Hgfr/Met* region was always the highest, but that several genes were coamplified in most amplicons. Furthermore, it was clear that the amplicon structure was quite different in the different tumours exhibiting amplification. Using two-colour FISH it could be shown that in one of the tumours the two amplified regions were situated in two different HSR, clearly showing that they had arisen from two separate amplification events. Thus, *Hgfr/Met* may be a primary target for amplification also in rat endometrial cancers, but a second more proximally located target also appears to be involved.

Keywords: cancer, rat, sarcoma, DMBA, endometrial adenocarcinoma, rat gene map, comparative mapping, zoo-FISH, comparative genome hybridisation (CGH), fluorescence *in situ* hybridisation (FISH), high resolution mapping, *Hgfr/Met* proto-oncogene, gene amplification.