Molecular genetic analysis of chromosomal aberrations in DMBA-induced rat fibrosarcomas

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ABSTRACT

Cancer is a complex disease caused by alterations in several genes. It arises due to the stepwise accumulation of genetic changes in the descendants of tumor progenitor cells. During progression, the tumor cells acquire capabilities of enhanced growth and proliferation and ultimately they obtain the ability to invade adjacent tissues and to metastasize in other parts of the body. Genetic predisposition and environmental factors, such as diet, smoking, and exposure to carcinogenic compounds may promote initiation of tumors. Thus, the genetic events that ultimately lead to the formation of a malignant tumor are multi-facetted and complex. Consequently, some aspects of tumorigenesis may best be studied in a model system. In this study, first generation (F1; filial generation 1) animals from a cross of the inbred rat strains BN and LE were subcutaneously injected with the polycyclic aromatic hydrocarbon DMBA (7,12-dimethylbenz[a]anthracene). Using fluorescent PCR amplification of informative microsatellite markers, DNA from the resulting solid tumors and tumor cell cultures were screened for allelic imbalance. The microsatellite marker alleles were compared pair-wise between tumors and normal liver DNA from each animal. Regions of recurrent allelic imbalance were detected on several rat chromosomes. The findings were compared to karyotype data and CGH (comparative genome hybridization) data from the same tumors. Based on the detected genomic aberrations, rat chromosome 1 (RNO1) was selected for further studies. The chromosomal changes in RNO1 appeared mostly to correspond to deletions of genetic material, but in one of the tumor cell cultures a bimodal pattern of amplified regions was detected by CGH. A method for of quantitation of allelic imbalance was used to delineate the positions of the two amplicons in the rat linkage map. Based on a comparative mapping approach, ten candidate genes from the distal part of RNO1 were investigated. The *Jak2* oncogene was found to be both amplified and overexpressed. The two amplicons had probably originated through repeated cycles of break-fusion-bridges, a chromosomal rearrangement that requires an initial double-strand chromosome break, possibly at a so-called fragile site. Since loss of chromosomal parts leading to inactivation of tumor suppressor genes have been reported in the vicinity of double-strand DNA breaks, a detailed investigation of the region close to the initial break was undertaken. The *Pten* tumor-suppressor gene was shown to map close to the breakpoint. Analysis of a polymorphic *Pten* marker revealed heterozygous loss of one *Pten* allele in more than 60% of the tumors. This was a clear indication that *Pten* was selectively deleted in the fibrosarcomas, but the coding sequence of the remaining allele appeared to be normal. One plausible explanation for the absence of mutations may be that the tumor suppressive effect of *Pten* was haploinsufficient in our material. Thus, our tentative interpretation of the findings is that treatment with DMBA induces chromosomal breaks in RNO1, resulting in impaired *Pten* function and contributing to fibrosarcoma development.

Key words: cancer, rat models, microsatellite marker, allelic imbalance, DMBA-induced tumors, *Pten*

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