Mini Review

Preneoplastic and Precancerous Lesions in Rodents: Morphologic and Molecular Characteristics

Jerrold M. Ward

Abstract: Cancer evolves through a sequential process from normal cells in many tissues of humans and animals. The natural history of tumor development can be seen histologically and by biochemical and molecular changes. There are two common basic pathways for the formation of malignant epithelial tumors; through preneoplastic foci and benign tumors (carcinoma developing in an adenoma) in parenchymal tissue or progression from intraepithelial neoplasia (IN) (atypical hyperplasia, noninvasive carcinoma, carcinoma in situ), a lesion in flat or lining epithelium. In epithelial-lining tissues of humans and rodents (e.g. cervix, mammary gland, prostate, skin), these lesions have been described as IN. In solid epithelial organs (liver, kidney, endocrine tissues) focal hyperplasia leads to adenomas. Adenomas develop foci of carcinoma, a process that is more common in rodents than in humans. These precancerous lesions in many rodent tissues often have multiple biochemical and molecular lesions which can be similar or different from those found in malignant tumors. The rodent molecular lesions include mutations in oncogenes (K-ras, H-ras) and tumor suppressor genes (p53, β-catenin, apc) or loss of heterozygosity (LOH) in tumor suppressor genes of mutant mouse models. This manuscript will review specific sequential morphologic and molecular lesions in the histopathogenesis of cancer in several rodent tissues. The significance of molecular lesions for diagnosis of rodent lesions will be discussed. (J Toxicol Pathol 2002; 15: 123–128)

Key words: pathology of cancer, molecular lesions, preneoplastic, precancerous

Introduction: The Histopathogenesis of Cancer

Cancer in both humans and rodents develops through a sequence of histogenetic and molecular changes or events. Histologically, the sequence of morphological lesions has been well described in humans and rodents in many tissues. The evolution of terminology and criteria for various lesions from hyperplasia to carcinoma has progressed from initial diagnoses by individual pathologists to classifications based on consensus diagnoses, and criteria have been developed by committees of expert pathologists under authority of various organizations including the World Health Organization and the United States Society of Toxicologic Pathologists (which included pathologists from the United States, Europe, Japan and other countries). While molecular lesions of various types have more recently been found in various stages of human cancer development, lesions have been more limited to mutations in specific genes in rodents. This paper will review the morphology, classification, and molecular pathology of preneoplastic and precancerous lesions in rodents.

Preneoplastic (Adenoma) Pathway (Fig. 1)

The first visible histological lesion in the evolution of cancer in a specific tissue in rats and mice can be classified as a preneoplastic lesion. Preneoplastic lesions have been described as a focus, preneoplastic focus, focus of cellular alteration (liver), focal hyperplasia, hyperplastic focus, atypical hyperplasia, nodular hyperplasia, hyperplastic nodule, atypia, and dysplasia (Table 1). These lesions are characteristically observed in parenchymatous epithelial tissues of, for example, lung and liver. Where studied in detail, such as in mouse and rat liver, these lesions appear to represent clonal expansion of single cells, presumed to be genetically altered. As the small preneoplastic lesions grow in size, they become nodular (show compression of adjacent tissue) and have been termed adenomas, hyperplastic nodules, nodular hyperplasia and even well differentiated carcinoma. These lesions, however, appear to represent benign tumors since they grow by expansion, are not invasive remain well differentiated, and do not have the morphologic characteristics of carcinoma. In mouse liver and lung adenomas, in skin papillomas, and, less commonly,
in rat liver and lung adenomas, focal areas of atypia, dysplasia or what appears as focal carcinoma can be found with increasing frequency within larger adenomas. Focal trabecular carcinoma can be seen in most mouse spontaneous and induced hepatocellular adenomas over 1 cm in diameter. As the carcinomatosus areas in the adenoma grow, the tumor becomes characteristically malignant and invasive and may metastatize to other tissues.

Adenomas may also simply enlarge and never progress to carcinoma. This situation is more common in humans even though some human cancers may develop within benign lesions including colon carcinomas developing from polyps (polypoid adenomas).

**Precancerous Pathway (Fig. 2)**

In humans, many epithelial cancers evolve from noninvasive lesions developing within flat lining epithelium. These noninvasive lesions have been classified as focal hyperplasia, atypical hyperplasia, atypia, dysplasia, carcinoma in situ, and most recently, intraepithelial neoplasia (IN) (Table 1). Adenomas and papillomas are also by definition precancerous lesions. The precancerous pathway reviewed here refers primarily to invasive carcinoma arising from flat hyperplastic lesions in flat epithelium as opposed to foci in benign tumors in solid tissues. In humans, this pathway is the most common pathway for cancer development in many tissues including breast (DIN), prostate (PIN), stomach, esophagus and pancreas (PANIN). In rodents, a precancerous pathway is common in chemically induced epithelial tumors (e.g. skin, stomach, prostate) and in the prostate and mammary gland of transgenic and knockout mice. IN is a relative new concept first used for prostate lesions in humans. It is a hyperplastic precancerous/preinvasive state in a specific epithelial lining tissue. IN is morphologically defined for each tissue/species/animal model and proven progression to invasive carcinoma is required. These preinvasive lesions often have molecular changes (as described below) similar to or different from those found in invasive carcinomas in the same tissue.

**Molecular Lesions in Preneoplastic and Precancerous Lesions**

A long list of various types of molecular lesions have been found in all stages of human cancer progression (Table 2). These lesions include chromosomal changes...
precancerous lesions or benign tumors. Changes in rodent carcinogenesis since they are often found in transgenic mice. These molecular events may occur as early amplification is common in tissues of many types of heterozygous for heterozygosity (LOH) is often found in mutant mice have been found in the rodents, many induced by chemical carcinogens, mutations have been found in the mammary gland. However, less information is available on ras mutations in the preneoplastic or precancerous lesions of rodents having been reported only in mouse liver foci, mammary hyperplastic alveolar nodules, and mouse lung hyperplasias, and in rat aberrant colonic crypts. The incidence and type of mutations can vary as a function of the chemical carcinogen, the dose and the animal model system used.

Table 2. Classification of Molecular Changes in Preneoplastic, Precancerous Lesions, and Tumors

| 1. Chromosomal changes (instability, translocations, insertions, deletions, amplification) |
| 2. DNA changes (microsatellite instability, genomic instability) |
| 3. Specific gene changes (mutations, deletions, methylation, loss of imprinting, loss of heterozygosity, amplification) |
| 4. Mitochondrial DNA changes |

(Mitochondrial DNA changes, DNA changes (microsatellite instability, genomic instability), specific gene changes (mutations, deletions, methylation, loss of imprinting, LOH, amplification) and mitochondrial DNA changes.

The most thoroughly studied model has been colon cancer. In this disease, sequential changes involving at least 5 molecular events have been described: 5q loss/APC mutation in colon dysplasia, DNA methylation changes in early adenoma, K-ras activation in intermediate adenoma, 18q LOH in late adenoma, and p53 mutation in adenocarcinoma. Much less is known about rodent tumors where usually only a single mutation or other molecular change has been found in benign or malignant tumors.

The significance of molecular changes is potentially immense. Depending on the gene in question, even a single mutation can have large physiological consequences and each subsequent molecular change can have additive effects on the disruption of normal cell cycle control, growth and tissue properties. The change should result in permanent lesions in genes (leading to abnormal gene expression) which has consequences for function of the gene and its protein. Molecular lesions producing a growth advantage to a cell or tissue can result in clonal expansion of the mutated cell clone and lead to hyperplasia, benign neoplasia, malignant conversion, invasive lesions and metastatic disease.

Molecular Lesions in Preneoplastic and Precancerous Lesions of Rodents

In preneoplastic and precancerous lesions and tumors of rodents, many induced by chemical carcinogens, mutations have been found in the ras oncogenes, and in the tumor suppressor genes p53, apc, and β-catenin. Also, loss of heterozygosity (LOH) is often found in mutant mice heterozygous for p53, men1, Rb, or Pten and gene amplification is common in tissues of many types of transgenic mice. These molecular events may occur as early changes in rodent carcinogenesis since they are often found in preneoplastic or precancerous lesions or benign tumors. With the exception of some genetically engineered mouse models, a sequence of molecular changes has not yet been described in rodent carcinogenesis as it has been in several human cancers. We may find that, because experimental models are less genetically diverse than is the human population, single molecular lesions may be sufficient for carcinogenesis in mice and rats.

Various types of mutations in K-ras or H-ras are very common in both spontaneous and induced mouse tumors including those in liver, lung, skin, blood vessels, forestomach, and mammary gland. ras mutations are also common in rat tumors including those of the liver and mammary gland. However, less information is available on ras mutations in the preneoplastic or precancerous lesions of rodents having been reported only in mouse liver foci, mammary hyperplastic alveolar nodules, and mouse lung hyperplasias, and in rat aberrant colonic crypts. The incidence and type of mutations can vary as a function of the chemical carcinogen, the dose and the animal model system used.

p53 mutations have been found in induced tumors of rat liver, lung, bladder, and colon, and in mouse tumors of the small intestine, lung, bladder, and skin. The reported incidences of these mutations are often low in one study and high in another. Few preneoplastic or precancerous lesions have been shown to have these mutations consistently although mouse skin papillomas showed a high frequency of p53 mutations in some studies but low incidences were found in rat liver foci and other lesions.

Recent studies have been performed for determination of mutations in β-catenin and apc genes. β-catenin mutations have been found in mouse liver, colon, and bladder tumors, and in rat aberrant colon crypts, further, at least one hepatic preneoplastic focus was immunoreactive for abundant β-catenin protein. Apc mutations have been detected in the intestinal tumors of min mice and in rat colon tumors. In mouse Apc mutants, additional Smad4 mutations appeared to contribute to invasive colon carcinogenesis.

Abnormal Expression of Antigens as Evidence of Molecular Lesions

By immunohistochemistry, one can show expression of proteins in normal tissues, in preneoplastic and precancerous lesions and in both benign and malignant tumors. In the case of oncogenes, tumor suppressor genes, and some growth factors, any detectable expression strongly suggest molecular lesions are present in the tissues; for example, over-expression of TGFα is localized to the cell membranes of preneoplastic and neoplastic liver lesions in TGFα transgenic mice (Fig. 3). Expression of nuclear p53 in tumors is often the result of a more stable mutant protein immunoreacting with antibodies to p53 and ras 21 immunoreactivity on cell membranes often indicates mutations in ras oncogenes. Likewise, β-catenin expression on cell membranes is highly suggestive of a mutation.
Loss of Heterozygosity (LOH) in Mouse and Human Mutants

LOH is used to describe loss of specific chromosomes or chromosome segments, chromosome deletions, chromosome rearrangements, loss of one parental copy of a gene (i.e. allelic loss) and reduction to homozygosity. Loss of one parental copy of a gene frequently occurs in humans with mutations in tumor suppressor genes such as \( VHL \), \( MEN1 \), and \( p53 \) since their germline DNA is already heterozygous for the mutated gene. A homozygous mutant state has not been described in humans for these genes and is assumed to be embryonic lethal; mice homozygous (\(-/-\)) often show embryonic lethality, while heterozygous (\(+/-\)) mice develop tumors showing LOH as they age. This phenomenon has been found for induced mouse mutants of \( p53 \), \( Men1 \), \( Pten \), \( APC \), \( Tsc2 \), and \( Tsc1 \). LOH in tumors in wild-type mice or rats have been found in mouse liver tumors \( \beta \)-catenin liver section, in mouse thymic lymphomas, and in mouse mammary tumors, often as a result of exposure to chemical carcinogens.

Conclusion

Molecular changes are common in tumors and in the preneoplastic and precancerous lesions of both rodents and humans. They are usually permanent, but may not be conclusively indicative of early neoplasia, early cancer or even preneoplastic or precancerous lesions in humans and possibly in rodents. However, the specific molecular changes described in this review have always been found in preneoplastic or precancerous lesions and in benign or malignant tumors of rodents and should be considered diagnostic of carcinogenic progression for the present time.

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