Commentary

A new mutator phenotype in breast cancer?

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Ever since the discovery of oncogenes and tumor suppressor genes, it has been recognized that mutations in these genes could provide the initiation events for cancer. This discovery, therefore, linked mutagenesis to control of the cell cycle and to carcinogenesis. We now understand that the development of a cell from normal to tumorigenic involves a sequence of mutational and chromosomal events. In 1990, Fearon and Vogelstein (1) reported that at least nine chromosomal alterations were found in up to 50% of colon adenocarcinomas in humans. Subsequently, Loeb (2) suggested that many other smaller and harder to detect mutations would be present in these cells; he postulated the theory of a tumor mutator phenotype to account for the accumulation of these mutations. A mutator phenotype may arise when repair of DNA lesions is compromised through a defective DNA repair gene. Several genes involved in DNA repair in humans have been identified that result in increased mutation when defective (3). Defects in the *hMSH2* and *hMLH1* mismatch repair genes, homologues of the bacterial *mutS* and *mutL* genes, respectively, are associated with spontaneous frameshift mutation and microsatellite instability in hereditary nonpolyposis colorectal cancer. Liu *et al.* (4) in this issue of PNAS report on a gene in a new category, a receptor for cellular growth factors, whose overexpression can result in a mutator phenotype in mammary tumors. The Liu group used mice that overexpress the *erbB*-2 gene in mammary tissues.

The *erbB-2* gene codes for the ErbB-2 protein, a member of the ErbB family of growth factor receptors. The ErbB family consists of the ErbB-1 (epidermal growth factor receptor), ErbB-2 (HER-2/neu), ErbB-3 (HER-3), and ErbB-4 (HER-4). The members of this family are part of an extensive network of cellular signals that control several processes, including embryogenesis and carcinogenesis (5, 6). Slamon *et al.* (7) and King *et al.* (8) reported that in 20–30% of human adenocarcinomas of the breast, endometrium, ovary, stomach, and lung an overexpression of the *erbB-2* gene is found. The ErbB-2 protein is a transmembrane receptor tyrosine kinase. Whereas the other ErbB receptors bind certain groups of ligands, the ErbB-2 protein appears to interact and form heterodimer complexes with other ErbB family members to alter their affinities and increase affinity for members of the heregulin family (9). In the 10–30% of human breast cancers with abnormal ErbB-2, it is found that overexpression by increase of transcription or amplification of the gene is involved, rather than activation by mutations. The mouse strain used by Liu *et al.* in this study (4), therefore, uses the mouse mammary tumor virus long terminal repeat sequences to drive expression of the *erbB-2* gene. This results in overexpression of the gene specifically in the mammary glands and has been shown to result in mammary tumors in mice (10, 11). It may, therefore, be a good model for human mammary cancer.

The importance of the article by Liu *et al.* (4) is the finding that a gene that is not thought to be involved in repair of DNA damage or maintenance of its integrity affects the occurrence of mutations. In particular, the type of mutations recovered in the mammary tumor tissues when ErbB-2 is overexpressed bears no resemblance to those found when mismatch repair is altered. Transversion mutations are relatively rare events in spontaneous background mutation and are not elevated in mismatch repair deficient cells. The tumors in the ErbB-2 mice, however, have a 6- to 7-fold increase in transversions, mainly GC → TA, whereas transitions are increased less than 2-fold, and frameshifts are not affected. GC → TA transversions are more common after DNA damage from genotoxins such as benzo[*a*]pyrene (to which these animals are not exposed) and in particular as a result of oxidative DNA damage (12). Repair of oxidative damage to DNA, in particular guanine bases, has been documented in various organisms. The MutM (or *fpg*) and MutY gene products in *Escherichia coli* prevent transversions by removing oxidized guanine residues or preventing base pair formation with adenine, respectively, and defects in these genes result in strong mutator phenotypes (13). The Rad6/Rad18 proteins play a similar role in the yeast *Saccharomyces cerevisiae*, whereas the human *hOGG1* gene is homologous to the bacterial *MutM* gene and the yeast *OGG1* gene (14). A deficiency in, or interference with, the human hOGG1 gene product would be expected to result in transversion mutation, as has been observed in *E. coli* and *S. cerevisiae* (15, 16).

The increased frequency of transversions in the breast tumors of the mice may be caused by a mechanism other than oxidative damage.

Reanalysis of the transversion mutator phenotype phenomenon with c-ErbB2 overexpression in *cII*-bearing fibroblasts indicated, however, that although ErbB-2 expression may be required, it is not sufficient for increasing genetic instability. The reason for this is unknown. Normal mammary tissues of the same animals also have an increased mutation frequency compared with wild-type animals, although not as high as in the tumors. It is conceivable that additional alterations take place in the tumor cells or that the increased cell number in the growing tumor provides a larger target for additional genomic instabilities (17). In this regard it is interesting to note that OB2–1, the transcriptional activator for the *erbB-2* gene, is a complex of the products of three *AP-2*-related genes (18). The gene encoding the human DNA repair enzyme *N*-methylpurine DNA glycosylase, which is also overexpressed in breast cancer (resulting in resistance to chemotherapy), has two overlapping consensus binding sites for the AP-2 factor in its regulatory region. Bessho *et al.* (19) reported that the mouse *N*-methylpurine DNA glycosylase removes 8-hydroxyguanine from DNA in *E. coli*, both *in vivo* and *in vitro*. This finding may indicate that the increased frequency

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of transversions in the breast tumors of the mice may be caused by a mechanism other than oxidative damage. On the other hand, it must be kept in mind that overexpression of *N*-methylpurine DNA glycosylase in mammalian cell lines has been found to contribute to sister chromatin exchanges, chromosomal aberrations, and gene mutations (20).

This is not the first paper in which an analysis of mutation in tumors has been reported. The effect of a deficiency in DNA mismatch repair on mutational levels in thymic lymphomas has been demonstrated by Baross-Francis *et al.* (21, 22) and Zhang *et al.* (23). Defects in the *MSH2* gene result in large increases in the frequency of frameshifts and complex alterations, as well as transitions at A:T base pairs in lymphomas compared with those in normal thymi of the same animal (23), confirming the genomic instability caused by a mutator phenotype in the tumor. The recovered frequencies of mutation, however, varied widely between tumors, whereas nonthymic *MSH2*-deficient tumors did not show an increase in *lacI* mutant frequency. Clonal relation between the mutants was found in some, but not all, tumors (23), in agreement with comments by Loeb and Christians (17). Also, different parts of a tumor may have different clonally related mutations (23), indicating the expansion of several mutations during the development of a tumor.

The findings by Liu *et al.* (4) promise the elucidation of new, interesting, and unexpected pathways involved in maintaining genetic stability and offer the potential for discovering new ways of preventing the onset of cancer.

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