Who is at risk for developing a brain tumor? Exposure to some agents, such as vinyl chloride or radiation, may increase the risk. A family history of glioma can also be a risk factor, accounting for approximately 2% of glioma patients (1). However, the known causes of glioma represent only a small percentage of patients, with most cases arising “spontaneously.” Could a more subtle genetic predisposition underlie the etiology of gliomas?

In this issue of the Journal, Bondy et al. (2) have attempted to uncover differences between glioma patients and normal control subjects that may explain why some people develop gliomas. Using an agent known to induce gliomas in a small number of patients, namely γ-radiation, Bondy et al. measured the frequency of DNA double-strand breaks persisting 4 hours after irradiation as a surrogate for DNA repair capacity in lymphocytes from 219 glioma patients and 238 matched control subjects. Residual double-strand breaks after γ-radiation were estimated by using a standard technique of collecting mitoses (using Colcemid), staining chromosomes with Giemsa, and counting chromatid breaks. The key finding of the report by Bondy et al. is that the lymphocytes from glioma patients demonstrated 0.55 break per cell, which was significantly greater than the 0.44 break per cell observed in the control subjects. These findings suggest that a measurable DNA repair defect may underlie the formation of gliomas.

The lymphocyte-mutagen sensitivity assay has been investigated in other sites, though often not as rigorously as in the report by Bondy et al. (2). The core of this approach is to establish a lymphocyte culture from the individual to be studied, treat the lymphocytes with a DNA-damaging agent (typically, bleomycin or γ-radiation), and then assay for DNA damage. The DNA damage is typically quantified by counting chromosomal gaps or breaks. Berwick and Vineis (3) reviewed more than 30 studies that used the mutagen sensitivity assay as a measure of an individual’s repair capacity. Almost all of the studies showed a subtle but statistically significant repair deficiency (more chromatid breaks) in cancer patients than in control subjects. Tumors of the breast, colorectum, and certain head and neck sites and now glioma are among the tumors associated with diminished repair capacity. The repair deficiency as measured in this assay may even be a heritable trait. One study (4) reported a higher number of chromosome breaks in both patients with breast cancer and their cancer-free first-degree relatives compared with control subjects without cancer or a family history of cancer. Thus, these data support the use of the mutagen sensitivity assay as a very useful tool to understand cancer susceptibility.

The use of this assay to assess cancer predisposition raises some interesting, if yet unanswered, questions. First, is it reasonable to use lymphocytes to study the repair capacity of an arbitrary solid tumor? At first pass, the answer could be “yes.” Lymphocytes, colonic epithelium, and glial cells of an individual all share identical genetic information. However, DNA repair functions that should be operating in all cells, e.g., mismatch repair proteins hMSH2 or hMLH1 or double-stranded break repair proteins BRCA1 or BRCA2, lead to tissue-specific oncogenesis when absent or mutant. The environment or exposure of a cell and its repair capacity apparently interact to determine mutagenic risk in a tissue-specific fashion.

A second question concerns the choice of a DNA-damaging agent. Is bleomycin or even irradiation the correct insult to deliver to the lymphocyte culture? At least in the case of glioma, where there is some association between irradiation and glioma formation, the answer could be “yes.” However, ionizing radiation creates a plethora of lesions in DNA including double-strand breaks, single-strand breaks, and oxidative base damage. Double-strand breaks represent only a small minority of the lesions. Although the double-strand break is probably the key lesion leading to cell death, and therefore of enormous therapeutic importance, other lesions generated by ionizing radiation are likely more important in carcinogenesis. The fate of a cell with a double-strand break could be apoptosis (leading to no tumor), faithful repair using homologous DNA (no tumor), or nonhomologous repair (maybe mutation, maybe tumor). Chromatid breaks are likely lesions that are associated with the oncogenic event rather than the event itself. Miscoding oxidative base damage, such as 8-oxo-2′-deoxyguanosine, may actually play a more important role in generating the mutations necessary for tumor formation. In fact, p53 appears to be an important target for the development of low-grade gliomas, and most of these p53 mutations are point mutations (5). It would be interesting to know if the results of the study by Bondy et al. (2) would be different if a different genetic insult were used. A more challenging question is whether the assay might be improved by using mutagenesis as an end point instead of a double-strand break.

A third question posed by this assay concerns the multiple cellular functions involved in the appearance of a metaphase chromatid break. Specifically, breaks and other damage formed by ionizing radiation are detected by proteins, such as the ATM gene product and DNA-dependent protein kinase. Once sensed, the cellular response is signaled by proteins, such as Chk2, p53, and others, usually resulting in cell cycle arrest. The detection and response functions should determine not only the number of breaks seen in metaphase cells but also the number of cells in metaphase. An arrested cell should not appear in a metaphase spread, suggesting that at least some of the breaks being scored...
in this assay are in cells with altered detection or response functions. It will be very interesting to know how often a chromatid break appears in this assay as a result of a defect in a DNA repair enzyme versus a defect in a sensing, signaling, or response protein. Is this assay really a measure of altered response rather than repair?

As interesting as this study is mechanistically, it is important to point out that it is unlikely that this approach can be developed into a screening test for gliomas. First, approximately 15% of glioma patients could not have their lymphocytes cultured, whereas all control subjects could. This problem would diminish the sensitivity of the assay as a screening test. More importantly, the complexity of the assay, the small overall difference between glioma patients and normal control subjects, and the relative rarity of brain tumors in the general populations suggest that false positives would tend to overwhelm the number of true positives in a screening setting.

Carcinogenesis is clearly a very complex process. Molecular advances, such as sequencing the human genome, have led to an accelerated understanding of DNA repair. For example, Wood et al. (6) analyzed the human genome sequence to identify genes involved in DNA repair. They identified 130 genes either functionally shown to be involved in DNA repair or showing a high degree of homology to repair enzymes from other organisms. Additional repair functions and/or the lesions serving as substrates are likely to be discovered. Each of these functions is subject to variation, potentially leading to a carcinogenic phenotype. An assay such as the mutagen sensitivity assay may be a vital first step in measuring the overall repair capacity of an individual. Although we are still somewhat in the dark in the development of useful assays for predicting the risk of developing cancer, studies such as the one by Bondy et al. that focus on DNA repair suggest that at least we may be looking under the right lamppost.

REFERENCES


NOTE

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