Carcinogenesis Studies in Human Cells and Tissues

Carcinogenesis researchers have striven for decades to interpret data obtained from animal studies in terms of their applicability to humans. The carcinogenic hazard of environmental chemicals has been determined primarily from studies with experimental animals and from retrospective epidemiological investigations. An experimental approach to the direct study of carcinogenesis in human tissues and cells was recently developed to assess the: (a) mechanisms of carcinogenesis in human cells, (b) variation of carcinogenic susceptibility among individuals, and (c) validity of the extrapolation of carcinogenesis data from experimental animals to the human situation. For stimulation of further progress in this new area of cancer research, a workshop sponsored by the Carcinogenesis Program, NCI, was held at the Given Institute of Pathobiology, Aspen, Colo., August 15 to 19, 1977, with 30 invited participants. The program consisted of lectures, group discussions, and laboratory demonstrations. The workshop focused on 5 major topics: (a) isolation and monolayer culture of human epithelial cells, (b) explant culture of human tissues, (c) xenotransplantation, (d) metabolism of chemical carcinogens, and (e) mutagenesis and neoplastic transformation of human cells.

In the first session of the workshop, James Rheinwald (Massachusetts Institute of Technology) described an in vitro system that permits cloning and serial cultivation and differentiation of diploid human keratinocytes with a feeder layer of irradiated mouse 3T3 cells. The addition of epidermal growth factor tripled the culture lifetime of epidermal keratinocytes from foreskins of newborn infants. The cultivation of both normal and neoplastic human prostatic epithelial cells was described by Edward Kaighn (Pasadena Foundation for Medical Research). The success of these studies was attributed in part to a new method of dispersing the prostatic epithelial cells by a 2-step procedure: (a) exposure of the cells to a hyposmolar solution containing a high concentration of potassium and (b) detachment of the cells with ethylene glycol bis(β-aminoethoxy) N,N′-tetraacetic acid i.e., EGTA.

Cultured human and rat mammary epithelial cells were shown to require near physiological levels of estradiol, progesterone, and prolactin for cell maintenance (Douglas H. Janss, Frederick Cancer Research Program). Gordon Sato (University of California, San Diego) presented evidence that the serum in cell culture medium can be replaced by a mixture of hormones, transferrin, and putative hormones or growth factors and that each epithelial cell type may require a different mixture of hormones.

Methods to isolate epithelial cells were discussed by Mortimer L. Mendelsohn (Lawrence Livermore Laboratory), Douglas H. Janss, and Gary D. Stoner (NCI). Flow cytometric and sorting methods, as well as density-gradient techniques, are now available and can supplement more traditional cell culture methods, e.g., cell cloning and selective culture media.

In the second session significant advances in the explant culture of adult human tissues were discussed. Bronchus, pancreatic duct, bladder, uterine cervix, breast, colon, esophagus, and aorta can all be cultured for weeks to months with maintenance of normal-appearing epithelium (Benjamin F. Trump, University of Maryland; Herman Autrup, NCI; and Sefton R. Welling, University of California, Davis). Chemically defined culture media have been developed for human bronchus, colon, and pancreatic duct, which eliminates the biological variability due to serum from studies of carcinogen interactions in these tissues.

Xenotransplantation, discussed in the third session, is required for the assessment of the neoplastic growth potential of cultured human cells exposed to chemical carcinogens, since these cells obviously should not be inoculated into humans. The congenitally athymic nude mouse accepts transplants of both fetal and adult human tissues. Maturation of xenotransplanted fetal tissues and the malignant growth of neoplastic cells were described by Carl Polvisen (Pathology and Anatomy Institute, Kommunehospitalet, Copenhagen). The growth of xenotransplanted human atypical mammary lobules, presumably preneoplastic lesions, was dependent upon treating the athymic nude mouse with estrogen and progesterone (S. R. Welling).

Metabolism of chemical carcinogens by human cells and tissues was the third major topic of the workshop. Elliot S. Vesell (Hershey Medical Center) reviewed the interindividual variation in metabolism of drugs and carcinogens. Genetic factors, disease states, and the environment may all alter the metabolism of xenobiotics. The metabolism of a carcinogenic polynuclear aromatic hydrocarbon, BP, and its interindividual variation have been extensively studied in human tissues and cells. The activity of BP 3-mono-oxygenase in liver biopsy specimens varied 30-fold (Olavi Pelkonen, National Institute of Child Health and Human Development). The interindividual variation in binding levels of BP to DNA in cultured human colon and bronchus was even higher, i.e., 60- to 70-fold (H. Autrup and Curtis C. Harris, NCI). Within an individual, binding levels of BP to cellular DNA also vary among human tissues, e.g., bronchus, pancreatic duct, and colon. This variation among tissues may in part be due to different types of cytochrome P-450's, as well as to the balance between activation and deactivation pathways of carcinogen metabolism (O. Pelkonen).

The many metabolic pathways of BP and the divergent pathways between microsomal preparations and intact cells were described by James K. Selkirk (Oak Ridge National Laboratory). The pathway of BP metabolism, leading to the major adduct formed with DNA in cultured human bronchus, is similar to that found in cells of experimental animals (Shen Yang, Uniformed Services University of the
Health Sciences). In addition, the major BP-DNA adduct formed in both cultured human bronchus and colon has been identified as resulting from the trans addition of the 2-amino group of guanine to position 10 of BP diol-epoxide I (Alan Jeffrey, Columbia University). BP can also be metabolized by human arteries. Earl P. Benditt (University of Washington) reviewed these and other data that suggest the monoclonal and mutagenic origin of atherosclerotic plaques that may be considered benign tumors of the arterial wall. The metabolism of another polynuclear aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene, has been studied in rat and human mammary epithelial cells in culture; the major metabolite has been identified as 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene (D. H. Janss).

In addition to polynuclear aromatic hydrocarbons, other chemical classes of carcinogens found in the environment and/or tobacco smoke, e.g., N-nitrosamines, mycotoxins, and hydrazines, can be metabolized by cultured human bronchus and colon (H. Autrup and C. C. Harris). One of these chemicals, aflatoxin B1, may be the most potent carcinogen known in experimental animals. Its major adduct, formed with DNA incubated in vitro with either rat or human liver microsomes, has been identified as 2,3-dihydro-2-(N7-guanyl)-3-hydroxyaflatoxin B2 (Gerald N. Wogan, Massachusetts Institute of Technology).

The final group of presentations focused on studies of mutagenicity and neoplastic transformation. Helmut Bartsch (International Agency for Research on Cancer) observed a marked variation in mutation frequencies with N-nitrosamines (Ames' Salmonella test) when liver surgical specimens from different individuals were used for metabolic activation. He also reported that N-nitroso-N'-methylpiperazine was 3 to 40 times more mutagenic with microsomes versus cell versus tissue versus organism. In addition, people may differ markedly in their ability to activate and deactivate chemical carcinogens metabolically because of variation of host biological factors. This variation should be considered in efforts to develop systems for the screening of chemicals for carcinogenic and/or mutagenic activity. Test systems for screening may also not be suitable as model systems for the study of molecular mechanisms of carcinogenesis. Both approaches are needed. Finally, studies of neoplastic transformation, especially with epithelial cells, are hampered by the lack of sensitive and early occurring markers for transformed cells.

Although this field is in an early stage of development, it was evident from this meeting that many human cells and tissues can now be maintained in a controlled experimental setting and that mechanism studies in carcinogenesis are already being conducted in human cells.

Curtis C. Harris
Umberto Saffiotti
Experimental Pathology Branch
Carcinogenesis Research Program
National Cancer Institute
Bethesda, Md. 20014

Benjamin F. Trump
Department of Pathology
University of Maryland
School of Medicine
Baltimore, Md. 21201
Carcinogenesis Studies in Human Cells and Tissues

Curtis C. Harris, Umberto Saffiotti and Benjamin F. Trump


Updated version

Access the most recent version of this article at:

http://cancerres.aacrjournals.org/content/38/2/474.citation

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.