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Perspectives in Cancer Research

Human Tissues and Cells in Carcinogenesis Research[1]

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A central problem of cancer research is the extrapolation of carcinogenesis data and knowledge of carcinogenesis mechanisms from laboratory animals to humans and, within this heterogeneous population, extrapolation among individuals. An aspect of this problem is the difficulty associated with extrapolating from one level of biological organization to another, *i.e.*, from molecules to macromolecules to organelles to cells to tissues to intact organisms. Multiple experimental systems are needed to help investigators find solutions to these and other problems in carcinogenesis research. Animal models are obviously required for experimental *in vivo* carcinogenesis studies. They are also essential because the integral multisystemic interactions of the organism remain intact and because laboratory animals can be environmentally and genetically controlled. *In vitro* models using tissues, cells, and subcellular fractions are also useful. This approach can aid in the resolution of the central problem of extrapolation in that one can conduct comparative studies with tissues and cells from experimental animals and humans that are maintained in the same controlled *in vitro* experimental setting (Fig. 1). Carcinogenesis studies using human tissues and cells offer unique opportunities (1, 2). For example, some rare forms of human cancer reflect inherited, predisposing conditions, and their genetic basis and perhaps common pathways of carcinogenesis may be understood through the study of nontumorous cells from individuals with these specific types of cancer. In addition, because human cells *in vitro* are apparently genetically more stable and undergo less "spontaneous" neoplastic transformation than most rodent cells, they may be especially suitable for studying the multistage process of carcinogenesis.

Epithelial cells are of particular interest because most adult human cancers are carcinomas. Significant progress has been made in the past decade in developing methods for culturing human epithelial tissues and cells (Table 1). Chemically defined media have been developed for culturing normal human tissues and cells from organs with a high rate of cancer in humans. Serum-free media have several advantages in studies of cultured human cells, including: (*a*) less experimental variability compared to serum-containing media; (*b*) selective growth conditions of either normal cells of different types (*e.g.*, epithelial *versus* fibroblastic cells) or normal *versus* malignant cells; (*c*) identification of growth factors, inhibitors of growth, and inducers of differentiation; and (*d*) ease of isolating and analyzing secreted cellular products. Advances in cell biology, including the delineation of biochemical and morphological markers of specific cell types, have also facilitated the identification of cells *in vitro* (*e.g.*, keratins as markers for epithelial cells and collagen types I and III for identifying fibroblasts).

This perspective will focus on recent advances, derived from studies of *in vitro* models, that have fostered an increased understanding of the processes controlling growth, differentiation, and neoplastic transformation of human cells. Although the use of human tissues and cells has some unique advantages, it is also important, as indicated above, to conduct comparative *in vitro* studies of tissues and cells from laboratory animals and human donors. Therefore, selected comparative studies in the areas of carcinogen metabolism, DNA damage and repair, growth and differentiation processes, and oncogenes will be discussed. Because of space limitations, most of the discussion will be devoted to studies of epithelial cells.

Growth and Differentiation of Normal and Neoplastic Human Epithelial Cells

The balance between growth and terminal differentiation is strictly controlled in normal epithelial cells. Furthermore, carcinogenesis studies using murine epidermal cells suggest that defects in differentiation occur during tumor initiation and that selective clonal expansion of these initiated cells occurs during tumor promotion (3). Studies using human epithelial cells are producing results supporting this hypothetical sequence of aberrations in control of growth and differentiation.

Human epithelial cells respond to several chemical classes of growth factors. For example, EGF,[3] insulin, and hydrocortisone are mitogenic for most types of human epithelial cells (4). EGF is apparently a universal growth factor and binds to high-affinity epithelial membrane receptors. At high concentrations, insulin presumably exerts its mitogenic effects by binding to the membrane receptors for insulin-like growth factors.

Many epithelial cell growth factors, *e.g.*, the mitogenic factors found in pituitary extracts, have not as yet been identified. The growth of epithelial cells cultured in serum-containing media is enhanced by agents that elevate intracellular levels of cyclic AMP (5). Epinephrine, cholera toxin, and other cyclic AMP-elevating agents are not directly mitogenic in many types of epithelial cells but act indirectly by negating the growth-inhibitory effects of serum (6, 7). Epithelial and nonepithelial cells may respond differently to growth factors. For example, platelet-derived growth factor enhances the growth of fibroblasts, but epithelial cells are generally unresponsive.

The concept of autocrine production of growth factors has been proposed to explain the uncontrolled growth of some neoplastic cells (8). "Ectopic" hormones produced by carcinomas are candidates for autocrine growth factors. For example, gastrin-releasing peptide (the mammalian equivalent of bombesin) is secreted by most small cell carcinomas of the lung (9), and intracellular human chorionic gonadotropin is detected in many non-small cell carcinomas of the lung (10). A monoclonal antibody to bombesin blocks the binding of the hormone to cellular receptors and inhibits clonal growth of small cell carcinomas *in vitro* and their growth as xenografts *in vivo* (11). Both of these hormones enhance the growth of normal bronchial epithelial cells *in vitro* by binding to specific membrane receptors (12, 13).

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[1] As required by editorial policy, recent reviews and reports are frequently cited and are a source of a more extensive bibliography.

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[3] The abbreviations used are: EGF, epidermal growth factor; TGF-β, transforming growth factor type β; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; p21, M_r 21,000 protein.

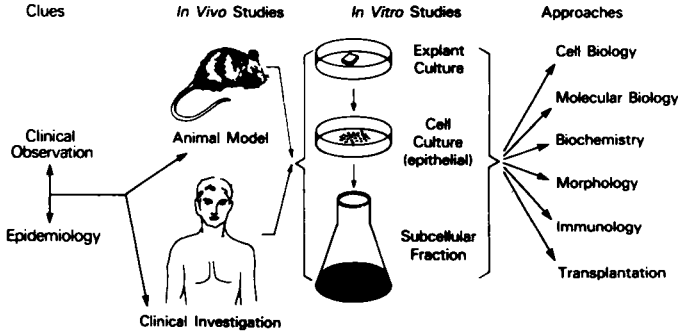

Fig. 1. Strategy for studying carcinogenesis.

Table 1 *Culture of normal human epithelial cells and tissues (2)*

Tissue type	Explant culture	Cell culture		
		Primary	Clonal growth	Serum-free media
Bronchus	+[a]	+	+	+
Breast	+	+	+	+
Esophagus	+	+	+	+
Epidermis	+	+	+	+
Bladder	+	+	+	+
Prostate	+	+	+	+
Colon	+	+	+	
Liver	+	+	+	
Endometrium	+	+		
Cervix	+	+		
Stomach	+	+		
Small intestine	+	+		
Kidney tubules	+	+		
Peripheral lung	+	+		
Exocrine pancreas	+	+		
Pancreas islet	+			

[a] +, techniques presently available.

Control of differentiation has been extensively studied with normal and neoplastic hemopoietic cells from experimental animals and humans (14, 15). Epithelial cells readily differentiate to squamous cells *in vitro*, and the recent success in culturing these cells is in part due to empirical observation of the conditions that inhibit terminal cellular differentiation. As noted above, cyclic AMP inducers act by neutralizing the growth-inhibitory and differentiating effects of TGF-β found in serum (7). Although TGF-β increases DNA synthesis in human fibroblastic and mesothelial cells (16), it inhibits growth of epidermal and bronchial epithelial cells (7, 17, 18). TGF-β binds to high-affinity membrane receptors and induces several markers of squamous differentiation in bronchial epithelial cells, including (*a*) inhibition of clonal cell growth, (*b*) irreversible inhibition of DNA synthesis, (*c*) an increase in extracellular plasminogen activator activity, and (*d*) an increase in cellular surface area (7). In contrast, growth inhibition is reversible in foreskin human epidermal cells (18). Differences in the culture media used to grow the two cell types, cell type differences, degree of cellular confluence, and/or the age of the donors may explain these apparently divergent results.

Serum induces terminal squamous differentiation of normal bronchial epithelial cells (19). Anti-TGF-β antibody neutralizes the inhibition of DNA synthesis by either TGF-β or serum in a dose-dependent fashion (7). These findings indicate that TGF-β is the serum factor primarily responsible for the growth inhibition and induction of squamous differentiation in normal human bronchial epithelial cells. Serum is a pathological fluid, and TGF-β most probably plays a role in the repair of normal tissues following wounding (20). Other endogenous molecules have been found to affect differentiation pathways in epithelial cells. 1α,25-Dihydroxyvitamin D_3 induces squamous differentiation of epidermal cells and has been proposed to be important in the maturation of skin (21). Diethylstilbestrol also inhibits growth and induces the formation of cross-linked envelopes in human cervical epithelial cells (22). Finally, because normal human epithelial cells undergo squamous differentiation after confluence *in vitro*, one may speculate that they secrete an inducer(s) of differentiation that reaches an effective concentration in this cell-packed microenvironment and/or that cell-cell contact is involved. Recent advances in molecular biology in microisolation and microsequencing of macromolecules now make the search for such inducers feasible.

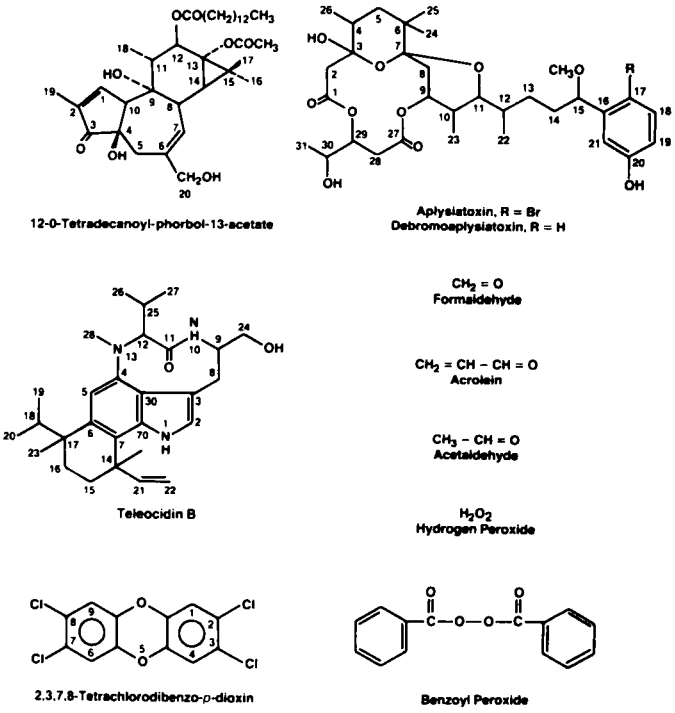

Fig. 2. Tumor promoters, aldehydes, peroxides, and dioxin induce terminal squamous differentiation of normal human bronchial epithelial cells.

Exogenous agents can also induce squamous differentiation of epithelial cells (13) (Fig. 2). These agents appear to mediate their effects by activating protein kinase C and/or by increasing intracellular concentrations of calcium ions. Their effects may result from direct interaction between the agent and the cell (*e.g.*, TPA activation of protein kinase C) and/or indirect mechanisms mediated by membrane lipid peroxidation and generation of active oxygen species that modify mitochondrial membranes, leading to release of calcium ions into the cytosol (23). In addition, aldehydes, including those found in tobacco smoke (*i.e.*, formaldehyde, acrolein and acetaldehyde) peroxides, and the neutral fraction of tobacco smoke condensate, produce many of the same changes (24).[4] It is noteworthy that growth inhibition, produced by either depleting the culture medium of mitogenic factors or exposing cells to cytotoxic agents, does not necessarily induce terminal squamous differentiation of human epithelial cells (7).[4] Thus, induction of squamous differentiation is not simply a response to nonspecific growth inhibition and/or cytotoxicity. The data are consistent with the model proposed by Scott and Maercklein (25) that implicates a distinct arrest point in the cell cycle for the differentiation pathway.

Normal epithelial cells may differ from preneoplastic cells in

[4] J. C. Willey, R. C. Grafstrom, C. E. Moser, Jr., C. Ozanne, and C. C. Harris. The effects of cigarette smoke condensate and cigarette smoke condensate fractions in normal human bronchial epithelial cells, submitted for publication.

their response to tumor promoters. For example, TPA inhibits the growth of normal human colonic epithelial cells and is mitogenic in cultures of epithelial cells from adenomatous polyps (26). Therefore, an imbalance between the pathways of growth and differentiation (Fig. 3) could provide a selective clonal expansion advantage for preneoplastic and neoplastic human cells, whereas normal epithelial cells respond by terminally differentiating.

Other hypotheses of selective clonal expansion can be envisioned, including defective control of cellular growth and differential response to exogenous as well as endogenous toxic agents (Table 2). As noted above, autocrine production of growth factors could provide a selective clonal expansion advantage for preneoplastic and neoplastic cells. A heightened sensitivity to nominal concentrations of systemic and/or locally elaborated mitogens could also provide a selective expansion advantage. This increased sensitivity could arise from an increase in (*a*) the number of receptors, (*b*) their binding affinity for growth factors, and/or (*c*) the efficiency of signal transduction. Although squamous cell carcinomas tend to have an increased number of EGF receptors per cell, the functional significance of these cell membrane changes is unknown; in many carcinomas the receptor number is either unchanged or decreased (27). Stimulation of mitogenesis by a constitutively switched-on receptor that does not require binding by the ligand is another hypothetical possibility and has been proposed for the action of a truncated version of the EGF receptor, the oncogene *erb*-B product (28). This and other connections between oncogenes and mediators in the cellular growth pathways was the subject of a recent article in the *Perspectives in Cancer Research* series (29).

Preneoplastic and neoplastic cells could also gain a clonal expansion advantage by being relatively resistant to cytotoxic agents. Evidence consistent with this hypothesis has been discussed in terms of (*a*) the role of hepatitis B virus in human liver carcinogenesis (30), (*b*) the differential response of preneoplastic cells in chemical carcinogenesis studies involving animal models (31), and (*c*) the relative resistance of some human cancer cell lines to oxidative stress (32).

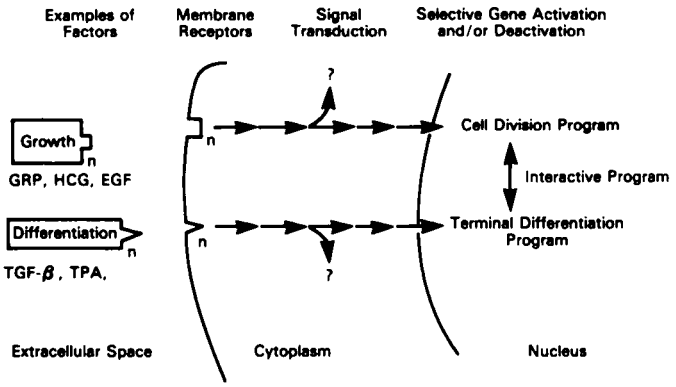

Fig. 3. Growth and differentiation pathways in normal human bronchial epithelial cells mediated by endogenous and exogenous factors. *GRP*, gastrin-releasing peptide; *HCG*, human chorionic gonadotropin.

Table 2 *Possible selective clonal expansion advantages of preneoplastic and neoplastic cells*

A. Defect in control of differentiation, *e.g.*, resistance to induction of terminal differentiation by endogenous and exogenous factors
B. Defect in control of growth
1. Autocrine production of growth factors
2. Increased sensitivity to growth factors produced by other cells
3. Decreased sensitivity to inhibitors of growth
C. Differential response to cytotoxic agents
1. Inhibition of viral cytopathological response
2. Resistance to damage by electrophils
3. Resistance to oxidative stress
D. Other
1. Escape from intercellular control mediated by cell-to-cell communication
2. Increased capacity to repair DNA damage

Carcinogen Metabolism, DNA Damage, and DNA Repair

One important use of cultured human epithelial tissues and cells is in the study of activation and deactivation of chemical procarcinogens. There are several reasons for pursuing these investigations: (*a*) many environmental chemicals must be enzymatically activated to exert their carcinogenic effects; (*b*) the activation:deactivation ratio of a carcinogen may in part determine an individual's susceptibility to that carcinogen; and (*c*) if the metabolism of a carcinogen in a human tissue is identical to that in experimental animals, then the extrapolation of carcinogenesis data from these animal species to humans is more likely to be valid than if the metabolic pathways differ.

Metabolism of carcinogens from several chemical classes, including *N*-nitrosamines, polycyclic aromatic hydrocarbons, hydrazines, mycotoxins, and aromatic amines, has been studied in human tissues and cells (33–37). The enzymes responsible for the activation and deactivation of procarcinogens, the metabolites produced, and the carcinogen-DNA adducts formed by cultured human tissues and cells are generally qualitatively similar among donors and tissue types. The DNA adducts and carcinogen metabolites are also very similar to those found in most laboratory animals, an observation that supports the qualitative extrapolation of carcinogenesis data from the laboratory animal to the human situation. Some notable differences among animal species have been reported, including metabolism of aromatic amines in the guinea pig (38), benzo(a)pyrene in the rat (39), and aflatoxin B_1 in the Syrian golden hamster (40).

Table 3 lists examples of procarcinogens activated by cultured human tissues into metabolites that bind covalently to DNA. Although the major DNA adducts are qualitatively similar for every chemical thus far studied, quantitative differences have been found among individuals and their various tissues and in outbred animals. These differences in enzymatic activities and number of DNA adducts generally range from 10- to 150-fold among humans and are of the same order of magnitude found in pharmacogenic studies of drug metabolism (33–37, 41, 42).

Table 3 *Variation among three human tissues in the activation of chemical carcinogens to form DNA adducts*

Chemical class and examples of carcinogens	Carcinogen-DNA adduct formation		
	Bronchus	Colon	Esophagus
Polynuclear aromatic hydrocarbon			
Benzo(*a*)pyrene (1.5 μM)	100[a]	20	81
7,12-Dimethylbenz(*a*)anthracene (1.5 μM)	100	7	21
N-Nitrosamine			
Nitrosodimethylamine (100 μM)	100	6	67
Nitrosodiethylamine (100 μM)	78	8	100
Nitrosopyrrolidine (100 μM)	100	52	ND[b]
Mycotoxin			
Aflatoxin B_1 (1.5 μM)	61	8	100
Hydrazine			
1,2-Dimethylhydrazine (100 μM)	72	100	81

[a] The tissue type with the highest number of DNA adducts is indicated by the value of 100%. Normal tissue explants from immediate autopsy donors were exposed to a nontoxic dose of each chemical carcinogen for 24 h (45).[6]
[b] ND, not done.

Because studies using experimental animals generally indicate that their cancer risk is influenced by the capacity for metabolic activation of procarcinogens, it is likely that a similar relationship exists for humans.

Carcinogens such as aflatoxin B_1 can be activated to carcinogen-DNA adducts by cultured human tissues (*e.g.*, bronchus, esophagus, colon, and bladder) even though epidemiological studies have not convincingly implicated aflatoxin B_1 as an etiological agent in cancer at these tissue sites. Several plausible explanations have been proposed. Epidemiological methods may be too insensitive to detect any associations. Alternatively, cocarcinogens and tumor enhancers may have a major influence in determining the tissue site of cancer, as in the induction of liver cancer by hepatitis B virus and aflatoxin B_1. The hormone-dependent status of human tissue has been shown to affect metabolism of carcinogens (37) and is a well-documented determinant in experimental carcinogenesis (43). Biodistribution of carcinogens and variation in DNA repair rates and/or fidelity may also determine the tissue site of cancer development. Finally, DNA damage is obviously only one aspect of the complex process of carcinogenesis.

Results from *in vitro* studies serve as a basis for investigations in biochemical and molecular epidemiology. For example, the observation that the carcinogen-DNA adducts formed in cultured human tissues are generally the same as those found in experimental animals for which these chemicals induce cancer has encouraged investigators to search for these DNA adducts in biological specimens obtained from people exposed to specific carcinogens, *e.g.*, benzo(a)pyrene or chemotherapeutic agents. The recent development of highly sensitive methods for detecting carcinogen-DNA adducts has made this search possible. Methods currently used include ^{32}P-nucleotide postlabeling and chromatography (44), synchronous scanning fluorescence spectrophotometry (45, 46), and enzyme immunoassays (47–49). These methods are being used to measure carcinogen-DNA adducts in cells from people exposed to carcinogenic chemicals (50–56) and cancer chemotherapeutic agents (57). Although these techniques measure DNA lesions considered to be important in carcinogenesis, it is unlikely that they will be quantitative predictors of cancer risk.

DNA repair enzymes modify DNA damage caused by carcinogens. Studies of cells from donors with xeroderma pigmentosum have been particularly important in expanding our understanding of DNA excision repair and its possible relationship to risk of cancer (58). The rate but not the fidelity of DNA repair can be determined by measuring unscheduled DNA synthesis and removal of DNA adducts, and interindividual variations in DNA repair rates have been observed (59–62). In addition to finding excision repair rates severely depressed in xeroderma pigmentosum cells (*e.g.*, complimentation group A), an approximately 5-fold variation among individuals in unscheduled DNA synthesis induced by UV exposure of lymphocytes *in vitro* has been found in the general population (59). Greater interindividual variation has been noted in the activity of O^6-alkylguanine-DNA alkyltransferase, the enzyme that repairs *N*-nitroso compound-induced damage to O^6-deoxyguanines found in DNA (60–62). In addition to these person-to-person differences, wide variations in DNA repair activities have been observed in different types of tissues, and fetal tissues exhibit 2- to 5-fold weaker activities than the corresponding adult tissues. The influence of these variations in DNA repair rates in determining tissue site and risk of cancer in the general population remains to be determined.

Oncogenes and Chromosomal Abnormalities

Oncogenes have become the touchstone for scientists in molecular, cellular, and developmental biology; cytogenetics; and cancer research. The convergence of these scientific fields is fostering cross-fertilization of ideas and experimental approaches. Studies of protooncogenes and oncogenes in tumorous and nontumorous cells from cancer patients continue to play a critical role in this rapidly evolving field and have been reviewed in detail recently (63–67). Therefore, only a few of the contemporary issues will be discussed.

Chromosomal abnormalities are exceedingly common in human cancers and specific abnormalities are associated with certain types of cancers, *e.g.*, translocations in Burkitt's lymphoma and chronic myelogenous leukemia and deletions in Wilm's tumor, small cell carcinoma, and retinoblastoma (68, 69). Translocations involving the immunoglobulin genes may be the result of mistakes mediated by V-D-J joining enzyme(s) of similar signal sequences on the translocated chromosomes. Combinations of certain chromosomal breakpoints (at least 83 have been enumerated to date) are associated with various types of cancer (70). It is of interest that 19 of the 26 oncogenes mapped to human chromosomes are located near these cancer-specific chromosomal rearrangements (71). The genetic elements at the sites of these breaks and their possible role in activating neighboring oncogenes and growth-related genes and deactivating tumor suppressor genes are areas for future investigation.

Base substitutions are not of course visible in cytogenetic preparations of human chromosomes, but they can be revealed in DNA by restriction enzyme analysis, differential binding of synthetic polynucleotide probes, or nuclease digestion of mismatched nucleotides in the nucleic acid hybrids. Such base substitutions are well-known mechanisms by which *ras* protooncogenes are activated (72–74). However, overexpression of the *ras* protooncogene appears to be much more common in human cancers than mutation by base substitution (75–78). Studies using experimental animal cells suggest several mechanisms. A quantitative change in c-Ha-*ras* gene expression can be caused by either an upstream insertion mutation (79) or truncation of a 5′ exon (exon-1) (80). The variable tandem repeat region that is 3′ to the Ha-*ras* structural gene may have enhancing activity (156).[5] Whether any of these mechanisms occur in human cancers is unknown.

Studies using animal models and cultured rodent cells suggest that the activation of *ras* by base substitution may be concomitant with tumor initiation and may also play a role in tumor promotion and progression (66). Although the mechanism by which the mutated *ras* p21 proteins cause neoplastic transformation is still uncertain, their possible involvement in dysregulation of G-protein circuitry is an active area of investigation (81). The balance between the expression of normal and mutated *ras* alleles could influence this circuitry and the transformation process. Several studies (see, *e.g.*, Ref. 82) have shown a reduction of GTPase activity in p21 proteins with amino acid substitutions at position 12 that correlated with their transforming potential. However, recent studies have demonstrated that activated p21 proteins with normal levels of GTPase activity can also transform mammalian cells (83); therefore, alternative mechanisms [*e.g.*, changes in GDP-GTP exchange rates due to structural differences in p21 proteins (84)] must be considered. Decreased expression of the normal N-*ras* allele due to deletion has been observed in mouse lymphoma cells transformed by

[5] A. D. Levinson, personal communication.

chemical carcinogens (85) and in 6 of 36 human cancers (75); the deletion was twice as frequent in metastases (29%) as in primary human tumors.

Detection of transforming genes in human tumors has generally been accomplished by transfecting tumor DNA into the aneuploid mouse NIH3T3 cells. In this immortalized recipient cell line, *ras* oncogenes act as "dominant" transforming genes. However, studies of hybrids between normal and tumor cells indicate that the malignant phenotype is "recessive" (86–88); in normal human fibroblast × HeLa cell hybrids, the location of the tumor suppressor gene(s) has been tentatively assigned to chromosome 11 (86, 88). This apparent paradox can be resolved by (*a*) fusing tumor cells containing an activated *ras* oncogene with their normal progenitor cells or (*b*) transfecting tumor DNA into the appropriate normal human progenitor cell. These latter experiments will necessitate the development of a high-frequency transfection method for gene transfer in normal human epithelial cells.

Xenotransplantation of human tissues into athymic nude mice (89–91) provides an opportunity to study the effects of tumor promoters and carcinogens *in vivo*. For example, TPA causes hyperplasia and hyperkeratosis of xenotransplanted human skin (92–94). Although exposing human skin xenotransplanted onto the backs of athymic nude mice to either carcinogenic polycyclic aromatic hydrocarbons or UV did not lead to human carcinomas, these studies were prematurely terminated because the mice developed multiple murine tumors at the margins of the xenografts (92, 94). Exposing human xenografts of skin or bronchus to carcinogenic polycyclic aromatic hydrocarbons has led to dysplastic (95) and morphologically neoplastic (91, 94) lesions.

Neoplastic Transformation of Human Cells *in Vitro*

In vitro transformation of normal human cells has proved to be more difficult than transformation of rodent cells (96, 97). This difficulty may relate to our inability to easily culture preneoplastic and neoplastic human cells. However, the increasing success in culturing normal and malignant cells makes this a less likely explanation. A more plausible hypothesis is that human cells, like primate cells, may be intrinsically different than rodent cells, especially murine cells. Perhaps the relatively greater karyotypic stability of human cells is associated with their lack of "spontaneous" neoplastic transformation *in vitro*. Another hypothesis, which was recently discussed by Sager (98), is that the minority population of emerging preneoplastic cells is suppressed by the majority population of untransformed human cells in the culture. This suppression could be caused by the secretion of growth inhibitors by the normal cells and/or intercellular transport of inhibitors via junctional complexes. Interestingly, normal rodent cells will suppress the growth of transformed cells, and this suppression is correlated with the occurrence of communication via gap junctions between the cells (99). Because only low molecular weight compounds (M_r <2000) can pass through these intercellular junctions, ions, nucleotides, and amino acids might produce this growth inhibition.

Studies of human cell carcinogenesis *in vitro* are hampered by the perplexing problem of identifying preneoplastic and neoplastic cells. Tumorigenicity with invasion and metastasis is the major criterion for malignancy. Since it is ethically and morally impossible to test the malignancy of *in vitro*-transformed cells by transplanting them into humans, animals such as athymic nude mice are used as surrogates. This assay has a relatively low level of sensitivity, because many cancers isolated from patients at the time of surgery either fail to produce tumors or produce regressing tumors in these mice. This weak sensitivity has led many investigators to use less stringent criteria to identify putative neoplastic cells, such as (*a*) formation of expansile intracranial tumors that kill the mouse host, (*b*) formation of invasive s.c. tumors containing histologically malignant-appearing cells that either regress or do not progressively grow in the athymic nude mouse, (*c*) invasion of the human cells into the chicken amnion or another type of membrane *in vitro*, or (*d*) "anchorage-independent growth" in semisolid media. The latter criterion probably reflects an altered response to growth factors, because normal human fibroblasts will grow in semisolid media if the serum and hydrocortisone concentrations are increased (100). In addition, transformed human cells selected by growth in semisolid media usually senesce with continued culturing and fail to produce progressively growing tumors in athymic nude mice. We have termed these cells "phenotypically altered" (101), while Kakunaga *et al.* (96) have called them "partially transformed."

With less stringent criteria, transformation of human cells by chemical, physical, or microbial agents has been reported (Table 4). In most cases, fibroblasts have been studied. Cell lines immortalized by SV40 (102) and X-irradiation (103) have been established. These lines rarely produce progressively growing tumors when xenotransplanted into the mouse host (102).

Fewer investigators have studied human epithelial tissues and cells. This is partly the result of difficulties encountered in culturing these cell types. "Phenotypically altered" epithelial cells have been produced by chemical carcinogens (104–106), SV40 (102), and nickel sulfate (107). Although our group and others have obtained only hyperplastic and preneoplastic lesions in human tissue explants exposed to chemical and physical carcinogens (108–112),[6] Parsa *et al.* (113) have reported that explants of fetal human pancreas exposed to chemical carcinogens became malignant and produced progressively growing carcinomas when xenotransplanted into athymic nude mice. Human cells have also been transformed to malignant cells by oncogenic viruses (114) or transfected genetic elements of oncogenic DNA and RNA viruses (115, 116). In these cases, the transformed cells are apparently immortal, are aneuploid, and produce progressively growing carcinomas in the athymic nude mouse assay. Interestingly, a single transfected oncogene, v-Ha-*ras*, can cause a cascade of events leading to neoplastic transformation of human bronchial epithelial cells (116). This cascade may be due to enhanced genetic instability mediated by the transfected *ras* (13)[6] and is consistent with the hypothesis of clonal evolution in neoplastic populations recently discussed by Nowell (117). Our results are also consistent with the *in vitro* transformation by *ras* of fibroblastic cells from various types of rodents (118); however, others have not observed neoplastic transformation of rodent or human fibroblasts by the transfected *ras* oncogene (119, 120). The human bronchial epithelial cells transformed by v-Ha-*ras* are highly invasive and metastatic from the primary s.c. injection site to multiple organs, including liver, spleen, kidney, and lung (116).[6] The transformed cells are also relatively resistant *in vitro* to inducers of terminal squamous differentiation, *e.g.*, TPA, and produce an "ectopic" hormone and growth factor (human chorionic gonadotropin); these findings are consistent with the hypothesis that neoplastic cells have an imbalance in the control of their growth and differentiation pathways.

[6] C. C. Harris et al., unpublished results.

Table 4 *In vitro transformation of human cells*

Cell and tissue type	Examples of agents	Extended *in vitro* life span	"Immortalization"	Karyotype	Transformation assay: Anchorage-independent growth *in vitro*	Transformation assay: Progressively growing s.c. tumors in athymic nude mice	Examples of reports and reviews
Epithelial							
Skin	SV40	+	+	A[a]	+	–	102, 136
	SV40-adeno-12 and Kirsten sarcoma virus	+	+	A	+	+	114
	SV40-adeno-12 and MNNG or 4-NQO	+	+	A	+	+	137
	AFB, MNNG, PS, PL, UV	+	–	NR	+	–	105
Lung	v-Ha-*ras*[b]	+	+	A	+	+	116
	DEN	+	–	D	+	–	106
Mammary	BP	+	+	NR	+	–	104
	SV40	+	+	A	+	NR	138
Pancreas	NMU	+	+	NR	NR	+	113
Prostate	SV40	+	–	A	+	–	139
Kidney	Adeno-5[b]	+	+	A	+	+	115
Colon	SV40 and azoxymethane	+	NR	NR	NR	NR	140
Retina	Adeno-12	+	+	A	NR	+	141
Amnion	SV40 and Kirsten sarcoma virus	+	+	A	+	+	142
Bladder	SV40 and MCA	+	+	A	+	+	143
Esophagus	DEN	+	–	A	+	–	144
Mesenchymal							
Foreskin fibroblast	UV	+	–	NR	+	–	145
	4-NQO	+	–	D	NR	NR	146
	SV40	+	+	A	+	–	147
Lip fibroblast	4-NQO	+	+	A	+	+	148
Embryo fibroblast	X-ray	+	NR	D	+	–	149
	^{60}Co	+	+	A	+	+[c]	103
	^{60}Co + Harvey sarcoma virus	+	+	A	+	+	150
	UV	+	–	NR	+	NR	151
Endometrium	MNNG	+	–	NR	+	–	152
Mesothelium	Asbestos	+	–	A	–	–	153
Lymphoid							
B-cell	EBV	+	+	NR	+	–	154
	EBV + 4-NQO	+	+	NR	+	+	128,[d]
T-cell	HTLV-I	+	+	NR	NR	NR	155

[a] Adeno, adenovirus type 5 or 12; AFB, aflatoxin B_1; MNNG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; PS, propanesultone; PL, β-propriolactone; DEN, *N*-nitrosodiethylamine; BP, benzo(*a*)pyrene; NMU, *N*-methyl-*N*-nitrosourea; MCA, 3-methylcholanthrene; 4-NQO, 4-nitroquinoline 1-oxide; EBV, Epstein-Barr virus; HTLV-I, human T-cell leukemia virus type I; A, aneuploid; NR, not reported; D, diploid.

[b] Transfected DNA.

[c] Tumor growth in the cheek pouch of the hamster.

[d] D. J. Kessler, C. A. Heilman, J. Cossman, R. T. Maguire, and S. S. Thorgeirsson. Transformation of EBV immortalized human B cells by chemical carcinogens, submitted for publication.

Suppression of Neoplastic Transformation

Despite the large number of progenitor cells, clinically evident cancer is a pathobiological event of exceedingly low probability. Although systemic host factors such as the immune system may largely account for its rarity, the lack of convincing reports of "spontaneous" transformation of human cells *in vitro* and the difficulty in inducing their *in vitro* neoplastic transformation with chemical, physical, and viral oncogenic agents attest to the presence of inherent suppressing factors at the biological level of the progenitor cells. Evidence for these presumed dominant-acting "cancer suppressor" genes has arisen primarily in epidemiological studies (121), molecular analysis of polymorphic DNA restriction fragments showing a reduction to homozygosity of chromosome 13 found in retinoblastoma and osteosarcoma (122) and of chromosome 11 in Wilm's tumor (123–126) and bladder cancer (127), and in studies with human cell hybrids in the field of somatic cell genetics (86–88). Considering the epidemiological data indicating that heterozygotic individuals are at increased risk of developing retinoblastoma or Wilm's tumor, one would predict that retinoblasts, renal cells, and perhaps other cell types from these people would be more easily transformed *in vitro* than normal cells from the unaffected population. Epidermal cells from patients with xeroderma pigmentosum may have an increased susceptibility to transformation by UV light *in vitro*. Furthermore, cells from people with inherited chromosomal instability syndromes who are predisposed to cancer may also be candidates worthy of investigation in *in vitro* carcinogenesis studies. For example, B-lymphoblastoid cell lines immortalized by Epstein-Barr virus from patients with Bloom's syndrome may be more easily transformed to malignant lymphoma cells by chemical carcinogens than lymphoblastoid cell lines from normal donors (128).

Conclusions

In vitro studies using human cells and tissues are making important contributions to our understanding of carcinogenesis. The findings from these studies complement and validate the results of laboratory animal studies, from which a much larger body of information is derived. The need for more investigations comparing normal and abnormal cellular processes in various animal species, including humans, is obvious.

Recent methodological advances have led to the successful culture in serum-free media of many types of normal human tissues and cells, including epithelial cells from the major tissue sites at which human cancers originate. These *in vitro* models are being used to investigate the molecular circuitry controlling

normal cellular growth and differentiation and its dysregulation during carcinogenesis. Carcinogen metabolism, DNA damage, and DNA repair have also been extensively investigated. Although 5- to 150-fold person-to-person quantitative differences have been observed in both the activities of enzymes responsible for carcinogen metabolism and the formation of carcinogen-DNA adducts by human tissues and cells *in vitro*, the metabolic pathways of carcinogen activation and their DNA adducts are generally qualitatively similar to those found in experimental animals. These observations strengthen confidence in the extrapolation of carcinogenesis data from animal models to the human situation and have led to the detection of carcinogen-DNA adducts in biological specimens from people exposed to chemical carcinogens.

Interactions among normal cells of a common type as well as different types (*e.g.*, epithelial and stromal cells) can be investigated *in vitro*. The identification of intercellular signals affecting their growth and differentiation should be a fruitful area of future research. Aberrations in cellular responsiveness to these signals may be involved in the process of carcinogenesis. Interactions between cells could also contribute to their transformation. For example, human phagocytes can release free radicals (129, 130), activated metabolites from procarcinogens (131), and growth factors (132) into their extracellular microenvironment, and human epithelial cells can activate procarcinogens and mediate mutagenesis in cocultivated Chinese hamster V-79 cells (133–135). It is now feasible to study the pathobiological effects of these genotoxic agents and growth factors released into the microenvironment using cocultivated human cells as targets.

In vitro transformation of normal human epithelial, lymphoid, and fibroblastic cells to malignancy has proved difficult but was recently achieved. With the advent of DNA transfection methods suitable to various types of human cells, it is now possible to directly assess in progenitor cells the role of oncogenes isolated from human carcinomas and oncogenic viruses in carcinogenesis. As candidate "cancer-suppressing" genes are isolated, they can also be tested for biological activity by transfecting them into malignant human cells *in vitro*.

In conclusion, the relative resistance of human cells to *in vitro* transformation provides an opportunity to dissect the multistage process of carcinogenesis and to discover "dominant"-acting genes that control the malignant phenotype. These genes are likely to include those that regulate expression of protooncogenes and control the balance between growth and differentiation pathways in normal human cells.

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Note Added in Proof

Oncogenes and growth factors have been identified and extensively studied during the last decade. As noted above, negative growth regulators, inducers of terminal differentiation and cancer-suppressor genes are also being actively investigated. Recently, a DNA with properties of the Rb gene, whose loss correlates with the development of retinoblastoma and osteosarcoma, has been isolated (157). Stanbridge and coworkers (158) have also reported suppression of tumorigenicity with continued expression of the c-Ha-*ras* oncogene in EJ bladder carcinoma-human fibroblast hybrid cells. This finding suggests that, even in the presence of an activated oncogene, cancer suppressor genes are "dominant-acting." Growth inhibition of murine thymocytes and human endothelial cells by TGF-β has recently been described.

In addition to those reports cited in the text, more reports of the effects of oncogenes in human cells are appearing. Microinjection of c-Ha-*ras* DNA induced DNA synthesis in nonproliferating quiescent human fibroblasts (161). Anchorage-independent growth of human embryonic kidney cells transfected with BK virus DNA and EJ c-Ha-*ras* (162) and human fibroblasts transfected with v-Ha-*ras* has been reported (163, 164). These transfected cells were, however, not immortalized. Transfected v-*myc* and v-Ha-*ras* had no detectable effects on cellular DNA synthesis or lifespan of normal human lymphocytes (165). Finally, malignant transformation of 2 human epidermal cell lines by Ha-*ras* has been recently described (166).

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