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CULTURE SYSTEMS FOR STUDYING MALIGNANCY

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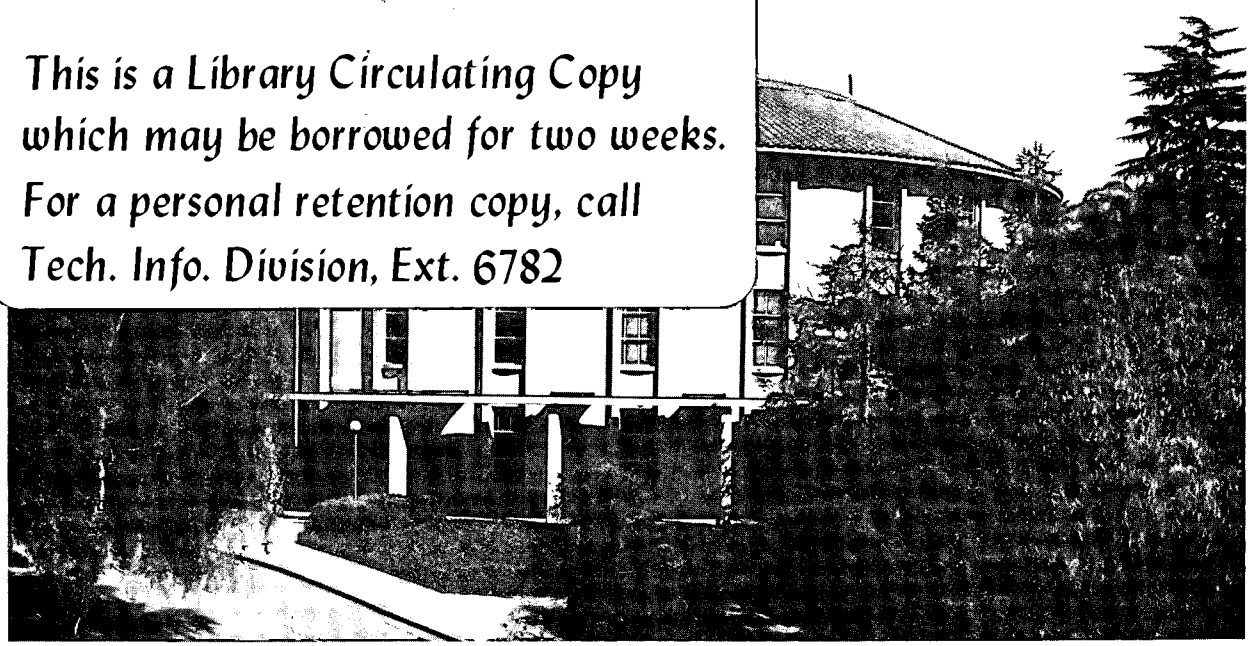

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CULTURE SYSTEMS FOR STUDYING MALIGNANCY

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MEETING REPORT

Culture Systems for Studying Malignancy[1]

The above was the theme for an international symposium held at LBL[2], University of California, Berkeley, on April 5-7, 1979. The symposium was organized to celebrate the opening of PCRI, a joint venture of the Peralta Hospital (Oakland) and LBL. PCRI was established to conduct research into the nature of cancer by focusing on development and characterization of human culture systems.

The subject matter of the symposium reflected the organizer's conviction that a critical look at the systems which are used in cancer research may help identify the areas where more emphasis is needed. They felt that a dialogue to define the relationships and differences between cultured systems and in vivo models on the one hand, and the animal cell systems and the rapidly emerging human culture systems on the other, may be beneficial to the field in general. The meeting was attended by 100 invited scientists from the U.S. and abroad.

Properties of Transformed Cells (chaired by Helene S. Smith, PCRI and LBL)

Ruth Sager (Sidney Farber Cancer Institute, Boston) presented results with hybrids, cybrids, micro-cell hybrids and reconstituted cells using a new Chinese hamster embryo fibroblast line (CHEF/18) which has all the normal characteristics, and its tumorogenic subclones. The results

implicate both nuclear and cytoplasmic determinants in the expression and the supression of tumorigenicity.

Garth Nicholson (UC, Irvine) reviewed the animal tumor models which have been developed for studying blood born metastasis after passaging the cells through cycles of growth in vivo and in culture. Experiments indicate that metastatic tumor spread is non-random and is due to highly malignant cell subpopulation that possesses unique cell surface properties. The relationship of tumor cell surfaces to blood born arrest and invasion was discussed.

Isaiah Fidler (Frederick Cancer Research Center, Maryland) spoke on the heterogenious nature of murine neoplasms of recent origin in invasion and metastasis. Different clones of murine fibrosarcoma which demonstrate varied metastatic behavior in vivo, were similar in chromosome mode and range, susceptibility to host hymphocytes or macrophages or in their growth properties in culture. However, anchorage independence growth rate in culture (measured in 0.6% Nobel agar) seemed to distinguish between clones with high and low metastatic capability.

Donald Glaser (UC, Berkeley) discussed the finding that cell colonies growing on top of agar develop into a wide variety of shapes due to complex cell-cell interaction. Colony morphogenesis is found to be characteristic of cell strains, but is also a sensitive and selective indicator of hormone action. The use of automated instruments in measuring the size

and shape of large numbers of colonies is permitting the isolation of cell mutants and the study of hormone action.

Judah Folkman (Harvard Medical School, Boston) discussed the relation of cell shape to growth control. He described an ingenious new technique for quantitatively reducing the adhesivity of tissue culture plastic and thus altering cell shape upon plating. This led to the finding that cell conformation is coupled to cell proliferation. For non-transformed cells, stepwise increase in cell spreading permits DNA synthesis and entry into the cell cycle.

Mechanisms of Carcinogenesis I and II (chaired by James. C. Bartholomew, LCB, and Donald Glaser, UC, Berkeley)

The talks in these two sessions fell into two groups: those dealing with initiation of carcinogenesis and those dealing with tumor promotion. Dr. Katherine Sanford (NIH) shed some light on the mechanism of "spontaneous" transformation of rodent cells. She reported that repeated short (3 hr) or long (24 hr) exposure of mouse cells to visible light and atmospheric oxygen enhanced both the chromosomal instability and malignant transformation. This process could be prevented by exogenous catalase, thus implicating hydrogen peroxide as a causative agent. Susceptibility to light-induced chromosome damage increased after prolonged culture, and appeared to be associated with or requisite for malignant transformation. Peter Cerutti (Swiss Institute for Experimental Cancer Res., Lusanne) and Veronica Maher (Michigan State Univ., East Lansing) addressed the question of mechanism of repair of

carcinogen or UV induced damage. Cerruti reported that the repairability by excision of the covalent guanine adducts of BP was limited in human epithelial lung cells, a sizeable fraction of lesions remaining unexcised over a prolonged period. This is also the case for the covalent guanine adducts formed by the liver carcinogen aflatoxin B_1 in human lung and fetal human hepatocytes where lesion modification appears to precede lesion excision. In contrast, Maher reported that diploid human fibroblasts are capable of rapid excision of DNA damage caused by UV irradiation or BP. Following exposure, there is a critical time in which a cell must manage to remove potentially cytotoxic and/or mutagenic damage from its DNA and so its survival is dependent upon the rate of excision. If normal cells or xerodermal pigmentosis cells with measurable but decreased rates of excision are prevented from replicating by being held in a density inhibited state but allowed to repair, they can gradually remove all the damage and reach 100% survival. Margaret Terzaghi-Howe (Oak Ridge National Laboratory, Tenn.) and Ellen Borenfreund (Sloan-Kettering Institute, N.Y.) discussed two novel cell systems that are derived after in vivo exposure to carcinogens. Terzaghi reported on rat tracheas which were derived from DMBA treated animals. Using this system, the dynamics of development of altered cell compartments endowed with different proliferative and neoplastic potential may be studied. Borenfreund discussed the characteristics of a liver-derived transformed cell population which was obtained after oral administration of

diethyl-nitrosamine. The cells contain a lesion (consisting of 100 Å filaments) which resembles Mallory's hyalin in the liver of alcoholics with advanced cirrhosis. The lesion could be repaired reversibly with sodium butyrate.

I. Bernard Weinstein (Columbia University, N.Y.) summarized the current state of knowledge on the mechanism of action of tumor promoting agents. In contrast to initiating carcinogens, the action of the tumor promoting phorbol esters (exemplified by TPA) does not appear to involve covalent binding to cellular DNA, or mutagensis. In cell culture TPA induces several reversible changes that resemble those seen in cells transformed by chemical carcinogens or tumor viruses. When transformed cells are exposed to TPA, the expression of these features is further accentuated. The phorbol esters also reversibly inhibit a variety of terminal differentiation programs. Weinstein suggested that the primary target of TPA may be the cell surface receptors for epidermal growth factor. The model of "two stage" carcinogenesis encompassing the known molecular and cellular effects of initiating carcinogens and tumor promoters was described: initiating carcinogens induce stable alterations in the cell genome but these are not manifest until tumor promoters modulate gene expression and induce the clonal proliferation of the initial cell. James Bartholomew (LCB) discussed another action of tumor promoters: initiators of carcinogenesis act by inhibiting the initiation and/or termination of DNA synthesis, while promoters drive the DNA synthetic machinery through the block, possibly by calling

forward post-replication or SOS-like repair pathways. Using mouse liver epithelial cells and flow cytometry, he found that BP slowed the progress of cells through the DNA synthetic period. When TPA was added at the same time as BP the inhibitory effects of the initator on DNA synthesis was abolished and the cells moved through S at a rate equivalent to control cells. Bartholomew also discussed studies on the effect of carcinogens on SV40 DNA replication. The diolepoxide derivative of BP inhibits DNA synthesis by blocking initiation and termination processes. The SV40 DNA molecules that are synthesized in the presence of diol-epoxide contain randomly distributed gaps in the newly synthesized strand. Charles Heidleberger (Univ. Southern Calif., L.A.) reviewed the use of the C3H/10T 1/2 mouse embryo cell system that was developed in his laboratory to study different aspects of carcinogenesis. The cells undergo oncogenic transformation following treatment with alkylating agents and polycyclic aromatic hydrocarbons, which induce aryl hydrocarbon hydroxylase activity. The level of activity is highly dependent on the growth state of the cells. Microsomes from these cells metabolize BP at the 7,8,-and 9,10 positions, but not at the 4,5 (K-region). The cells do not activate aromatic amines, nitrosamines or aflatoxin B_1. The cells lend themselves to mutant selection (e.g., Ouabian resistance or temperature sensitive for transformation) and tumor promotion studies.

Nancy Colburn (NCI) reported on a mouse epidermal culture system which may be a good model for promoter-dependent late

preneoplastic progression. She has isolated cell lines which will respond to tumor promoting (but not non-promoting) phorbol esters by irreversibly gaining the ability for anchorage independent growth. The change occurs by induction of new variants rather than by selection of preexisting ones. The system holds promise for detection and the study of mechanism of ation of tumor promotion.

Dr. Eli Huberman described his studies with some myeloid human leukemia and melanoma cells which indicated that tumor promoters can, at extremely low doses, induce terminal differentiation. In the melanoma cells these agents inhibit cell growth, stimulate melanin synthesis and induce the formation of dendrite-like structures characteristic of normal melanocytes. He suggested that phorbol diester-like compounds may offer a means by which some human tumors may be controlled by converting them into differentiating cells and not merely by killing rapidly dividing cells as is usually the basic principal in cancer chemotherapy. However, at this stage these experiments can only be performed in culture with some myeloid leukemia and melanoma cells.

Human Cell System (chaired by Martha Stampfer, PCRI, LBL)

Curtis Harris (NCI) summarized his groups studies using long term explant cultures of bronchus, esophagus, peripheral lung, pancreatic duct and colon from adult humans and experimental animals to study the metabolism of chemical carcinogens. They have found that pathways of BP metabolism and position of carcinogen-DNA adducts are similar in humans

and other animals; however, substantial quantitative differences between animals and humans and among humans exist. Harris also discussed recent progress in developing culture conditions for the selctive growth of human bronchial epithelial cells as well as morphologic, cytochemical, and immunological criteria for distinguishing the epithelial cells from fibroblasts and endothelial cells. In addition, he showed that asbestos causes hyperplastic lesions with cellular atypia in the cultured human bronchial epithelium.

Herbert Lazarus (Sidney Farber Cancer Institute, Boston) reported that human leukemias can now be more precisely categorized on the basis of immunological and biochemical criteria. Using established cell lines derived from various categories of human leukemia, important differences in response to clinically effective chemotherapeutic agents may be demonstrated. Furthermore, the response to several of these agents can be predicted on the basis of the leukemic subset to which the individual cell line belongs.

Jan Ponten (Univ. of Uppsala, Sweden) discussed the use of human glial cell lines as model systems, drawing attention to the wide range of phenotypes characterizing the transformed state. Lines derived from normal and tumor tissue differed from each other in their growth regulatory properties but had no other detectable properties that distinguished them consistently. An examination of actin filament organization, fibronectin levels, plasminogen activator production and mucopolysaccharide composition failed to demonstrate a

correlation between these properties and malignancy. Martha Stampfer (PCRI, LBL) described recent advances in culturing normal human mammary epithelial cells. She found that conditioned media from specific human epithelial cell lines as well as certain hormones allowed active epithelial cell proliferation for 1-3 months and could generate approximately $4\text{-}7\text{x}10^7$ cells in secondary culture from one small flask. The identity of these cells was verified by the presence on their surface of the human mammary specific milk fat globule antigen. The cells also demonstrate other epithelial properties including junctional complexes and secretory dome formation. The increased proliferative ability of these cells will facilitate their use for studies of human epithelial cell differentiation and carcinogenesis.

Helene Smith (PCRI, LBL) discussed the use of human epithelial cell substrates derived from tissues of varying pathology to study the expression of malignant progression in culture. She compared these cultures for properties known to correlate with malignancy while there was partial correlation for the properties of growth on contact inhibited monolayers or anchorage independence. Absence of fibronectin was consistently noted in the cultures derived from agressive metastatic lesions. Smith also discussed the use of mammary epithelial cells in studies of chemical carcinogenesis since addition of BP led to growth inhibition of the epithelial but not fibroblastic cells.

Differentiation and Malignancy (chaired by Mina J. Bissell, LCB)

Beatrice Mintz (Institute for Cancer Research, Fox Chase Cancer Center, Phila.) described the loss of malignancy in mouse teratocarcinoma stem cells after injection into early embryos, through normal differentiation into somatic and germinal tissues. Before the stem cells are placed in embryos, they can be grown in culture to obtain clones with specific mutation of interest. Thus, it is possible to produce laboratory animals with predetermined genetic changes, including some that are models of human genetic diseases. The awesome potential of these studies and their important implication in understanding development and cancer was reflected in the discussion which followed this talk. Peter Jones (Children's Hosptial, Los Angeles) presented experiments showing that the nucleoside analog, 5-azacytidine, was capable of inducing the formation of functional and biochemically differentiated striated muscle cells, adipocytes and chondrocytes from 10T 1/2 and mouse embryo cell lines. The effect seem to require the incorporation of 5-azacytidine into DNA and considerable cell division after treatment. Thus, this analog may be of particular value in investigations of the nature of the differentiated state.

Mina Bissell (LCB, UC Berkeley) presented metabolite "fingerprints" data indicating that patterns of carbon flow and pool sizes are dramatically different not only in different tissues but also in cultured cells. Thus, the separation of a

cell's functions into "luxury" vs "housekeeping" molecules would seem to be unnecessary and at times misleading. Bissell also discussed the use of cultured avian tendon cells as a model for studying gene regulation and cell-virus interaction. These cells are capable of synthesizing an *in ovo* level (30%) of collagen in culture and the level may be modulated by positive effectors such as ascorbic acid and cell density and negative effectors such as tumor viruses, TPA and serum. Howard Green (Massachusetts Institute of Technology) described the serial cultivation of human epidermal cells and their two principal differentiated properties--the synthesis of keratins, and synthesis and assembly of a cross-linked submembraneous protein envelope. The possibility of utilizing this elegant system for the scoring of neoplastic transformation in culture, whether by chemicals or by viruses, deserves intensive investigation. Finally, Cesar Milstein summarized the hybrid myeloma technique for the preparation of monoclonal antibodies. This exciting new development has been very successful in defining and characterizing cell-surface differentiation antigens and their pattern of expression in normal and malignant tissues. Individual specificities were found in several cases to be expressed in cell populations belonging to different differentiation pathways ("jumping" specificities). Such results indicate the danger of using individual markers to come to conclusions about the relationships and origins of tumor cells. But the day is near when a multiplicity of reagents will be available for studying patterns of antigenic

expression. This will not only be more reliable but will also permit the study of the dynamics of the expression of developing cell surfaces.

General Conclusions

1) Both mutational events and changes in gene expression by themselves or in combination could be involved in cancer induction and progression.

2) The carcinogenic process is multifactor in its causation and multistep in its evolution. There is as yet no single characteristic of transformed cultured cells that is universally associated with the malignant state.

3) It is important to define the "normal" differentiated state of cells in culture and to have an *in vivo* reference point in order to distinguish between the artifacts arising from culturing the cells and the genuine markers for the transformed cells. The role of substrata, matrix and cell shape in growth regulation and tumor progression deserves further study.

4) Physical and chemical agents induce mutation and gross morphological damage, but replication and repair play a crucial role in the final expression of transformation.

5) The mechanism of carcinogen and UV induced damage (initiation) seems to be similar in animal and human cell systems, although there may be a difference in the rate of repair.

6) While the mechanism(s) of tumor promotion is under intense investigation, additional model systems to study different facets of carcinogenesis, especially in human cells, are urgently needed.

7) There is an almost universal requirement that a tumor be heterogeneous to succeed in the host. Additional model systems are needed also for scoring tumor progression and metastasis.

8) The availability of differentiated cell systems, the capability of making mosaic mice from mutant cultured cells and the availability of monoclonal antibodies should contribute greatly to our understanding of both differentiation and malignancy.

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Foot Notes

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2. Abbreviations used: LBL, Lawrence Berkeley Laboratory, University of California, Berkeley; LCB, Laboratory of Chemical Biodynamics, LBL, U.C. Berkeley; PCRI, Peralta Cancer Research Institute; BP, benzo(a)pyrene; DMBA, dimethylbenz(a)anthracene; TPA, 12-O-tetradecanoylphorbol 13-acetate.

3. This work was supported in part by the Biomedical and Environmental Research Division of the U.S. Depatment of Energy under contract No. W-705-ENG-48, and in part by the National Science Foundation grant No. PCM 77-14982.

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