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Use of Cell and Organ Cultures in the Identification and Characterization of Agents That Modify Carcinogenesis[1]

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Many dietary agents that modify carcinogenesis have been studied in *in vitro* systems, as has been reviewed elsewhere (1). These studies have included both agents that enhance transformation *in vitro* [*e.g.*, saccharin (2, 3), betal quid ingredients (4)] as well as agents that suppress transformation [*e.g.*, vitamin E (5, 6), selenium (6), carotenoids (7, 8), ascorbic acid (9, 10), etc.]. Conflicting reports exist for some agents; for example, vitamin D has been reported to enhance (11) and suppress (12) transformation *in vitro*; enhancing and suppressing effects have also been reported for vitamin D on carcinogenesis *in vivo* (reviewed in Ref. 12).

While there are many different types of cell and organ culture systems in which the effects of carcinogenesis modifying agents have been studied, the *in vitro* transformation systems utilizing rodent fibroblasts remain the most practical systems for studying the effects of agents on the carcinogenic process occurring in cell cultures. Several different types of compounds useful in cancer prevention studies have been studied in the rodent fibroblast systems. The most widely utilized system has been the C3H10T½ system developed by Reznikoff *et al.* (13, 14). This system has been extensively utilized by us for the characterization of anticarcinogenic protease inhibitors (*e.g.*, Ref. 15) and other agents which affect transformation (1) and by other laboratories for the characterization of retinoids, carotenoids (*e.g.*, Ref. 16), and many other agents that have the ability to modify transformation *in vitro*.

In general, there are two major types of rodent cell derived *in vitro* transformation systems that have been widely utilized. One involves the use of primary or secondary cultures of normal Syrian hamster embryo cells and the other involves the use of permanent cell lines, usually derived from mouse cells. The permanent cell lines that have been used the most as *in vitro* transformation systems are the C3H10T½ and BALB/3T3 cell lines. These different systems have been described in detail elsewhere (1). The advantages of the hamster embryo system are that the cells are normal in that they have a diploid karyotype, the test system takes only 7–10 days, and there is a very low rate of spontaneous transformation. The problems with the system are that the cloning efficiency of cells is very low, the cells have a very short life span in culture (so that large populations of cells cannot be produced for biochemical comparisons of normal and transformed cells, etc.), the cultures represent heterogeneous cell populations, and it has been difficult for many investigators to obtain reproducible results (1). The permanent cell lines have the disadvantage that the cells are aneuploid (thus, they cannot be considered normal cells) and the transformation assay system takes 4–6 weeks. The advantages of the permanent cell lines are that scoring is considerably less subjective and more quantitative than the hamster embryo system, the cells clone at high efficiency and represent a homogeneous population, and populations of nontransformed and transformed cells of these cell lines can be grown in large quantities and used for comparative studies. The cell lines are generally thought of as representing fibroblasts which are capable of producing fibrosarcomas when transformed, cloned, and injected into syngeneic hosts.

The *in vitro* transformation systems are not only useful as methods to characterize the effects of carcinogenesis modifying agents, they can also be utilized to identify previously unknown anticarcinogenic agents. As an example, the soybean derived Bowman-Birk protease inhibitor was initially identified as an anticarcinogenic agent in the C3H10T½ transformation system. The Bowman-Birk protease inhibitor was then shown to inhibit carcinogenesis *in vivo* in several different model systems (reviewed in Ref. 17). Studies to evaluate the Bowman-Birk protease inhibitor as an anticarcinogenic agent in human populations are now beginning. Many different *in vitro* systems are now being utilized to identify anticarcinogenic agents with the ability to suppress transformation *in vitro* or various markers associated with carcinogenesis. As examples of *in vitro* markers used in cancer chemoprevention research, the following biochemical assays have been developed by the National Cancer Institute Division of Cancer Prevention and Control: (*a*) inhibition of tyrosine kinase; (*b*) inhibition of ornithine decarboxylase; (*c*) free radical scavenging; (*d*) inhibition of carcinogen-DNA binding; (*e*) enhancement of glutathione; and (*f*) inhibition of poly(ADP-ribose) polymerase. These assay systems are being utilized to rapidly screen many potentially useful anticarcinogenic agents (18).

Such *in vitro* biochemical markers are widely used in studies aimed at determining the mechanism of action of modifying agents for carcinogenesis. The general theories concerning mechanisms of action of tumor promoting agents (reviewed in Ref. 1) and cancer chemopreventive agents (19, 20) are discussed in detail elsewhere. The mechanisms of action of many of the carcinogenesis modifying agents are not known primarily because the mechanism by which a normal cell is transformed into a cancer cell is still unknown. Until the mechanism of cancer causation is known with certainty, the mechanism of action of many of the carcinogenesis modifying agents cannot be determined.

The mechanisms of action of the carcinogenesis modifying agent which are thought to be "known" are through their specific effects on carcinogen metabolism, biochemical pathways thought to be important in carcinogenesis, or effects on the reasonably well-characterized initiation, promotion, or progression stages of carcinogenesis. As an example of a known mechanism, certain compounds interfere with carcinogen metabolism by preventing the formation of a carcinogen from a precursor substance. By preventing the formation of a cancer causing substance, carcinogenesis will not occur. Many of the cancer chemopreventive agents have suppressive effects on carcinogenesis by inhibiting the interaction of a carcinogen with critical sites in the target cell. Once the active carcinogenic agent has interacted with the cell, presumably the DNA, the potential mechanisms involved in the initiation, promotion, and progression stages of carcinogenesis are more numerous and controversial.

Many agents have been classified as promoters or antipromoters, and a number of systems exist for the study of promotion *in vitro*. The rodent fibroblast systems can be used to study multistage carcinogenesis *in vitro* (1) as can rodent epidermal cell cultures such as mouse JB6 cells (21–23). Because two-stage and later multistage carcinogenesis *in vivo* was initially characterized in mouse skin, a mouse epidermal cell transformation system is particularly appropriate for studying mechanisms of promotion or antipromotion. The JB6 family

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of cell lines has the advantages and disadvantages associated with being immortalized, clonal, and nondiploid. JB6 cells do not offer utility for studying keratinocyte differentiation. Unique advantages are that JB6 promotion sensitive (P^+) cells are postinitiated, preneoplastic, and stable with respect to spontaneous transformation. They respond to various classes of tumor promoters with a high frequency neoplastic transformation response. Several classes of antipromoters block the transformation response. There are promotion resistant (P^-) variants of JB6, which offer the possibility of identifying events that may inhibit transformation and therefore might be exploited in designing prevention strategies. Critical molecular events that occur preferentially in P^+ variants also offer molecular targets for prevention. One such potential target is tumor promoter induced AP-1 transcriptional activation (23).

The development of an *in vitro* transformation system that closely mimics human carcinogenesis has proved to be a challenging problem. Transforming human cells *in vitro* with chemicals or radiation is not as easily accomplished as is the *in vitro* transformation of rodent cells. The *in vitro* transformation of human epithelial cells is particularly problematic. Some relatively new systems of human epithelial cell *in vitro* transformation have been developed (*e.g.*, Ref. 24) and appear highly promising as systems in which mechanisms of human carcinogenesis can be studied. These systems are very time consuming, inasmuch as morphological transformation does not occur as it does in the rodent fibroblast transformation systems. To determine whether cells have been transformed, they must be injected into animals and *in vivo* tumor formation monitored. Thus, these systems are not practical ones for the screening of potential dietary agents that would affect carcinogenesis.

Cell cultures can be utilized to study many processes related to carcinogenesis in a highly controlled fashion. As examples, *in vitro* studies using cells of the rodent fibroblast *in vitro* transformation assay systems were among the first to suggest that oncogenes could play important roles in radiation and chemical carcinogenesis (25) and that cooperation between oncogenes could result in cellular transformation (26). Various different *in vitro* systems have been used to study the role of proliferation in carcinogenesis (*e.g.*, Refs. 27–29), the effects of carcinogenesis modifying agents on differentiation (*e.g.*, Refs. 30–32) and gene expression (*e.g.*, Refs. 23, 31 and 33–36), biochemical pathways important in carcinogenesis (*e.g.*, Refs. 37 and 38), etc., and numerous other end points related to carcinogenesis.

A number of different organ culture systems have been developed recently for the study of mechanisms involved in carcinogenesis (*e.g.*, Refs. 39–43). While these studies have been very useful for studying short-term end points, such as carcinogen/DNA adduct formation, DNA repair processes, carcinogen metabolism, oncogene expression, etc., they have not been developed to the point of serving as a useful screening system for the detection of modifying agents for carcinogenesis.

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