Newer Insights Into the Pathogenesis of Liver Cancer

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A new hypothesis leading to a new model of liver carcinogenesis is described; it is based on the acquisition by carcinogen-altered hepatocytes during initiation of a new functional handle—resistance to the cytotoxicity of a carcinogen—and on the ability of such cells to proliferate in an environment that prevents proliferation of normal hepatocytes. The creation of such a differential environment now enables a quantitative analysis for initiation, the beginning synchronization of the putative premalignant hepatocytes for about 15 cell cycles, the study of the pattern of growth of such resistant cells to form nodules that have some resemblance to the organizational pattern of fetal liver, the analysis of the appearance of distinctive positive and negative markers for these cells, and the further investigation of the development of liver cancer from such cells. The remarkable similarity in overall pattern between the development of cancer in the skin and in the liver with chemicals and the possible role of both somatic mutation and neodifferentiation in carcinogenesis are briefly discussed. (Am J Pathol 89:477–482, 1977)

OUR LONG-TERM GOAL is the development of a sufficient scientific base for the diagnosis and eradication of preneoplastic lesions. We and others are of the opinion that the data already available indicate the feasibility of this objective, given sufficient basic research into the pathogenesis of cancer. The data in liver and in other organs, as is evident already from the presentations during this symposium, point clearly to the existence of distinctive properties of even the earliest recognizable preneoplastic lesion that conceivably might be exploited for diagnostic purposes.

Our approach is different than that in most studies on carcinogenesis, including our previous ones. These other studies for the most part use morphologic and/or biochemical criteria and depend upon the existence of correlations or lack thereof. In contrast, we are attempting to ask functional questions *in vivo* and then to study cellular structural and biochemical concomitants that are critically related, hopefully, to the hypothesis proposed.

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Presented at the Seventy-Fourth Annual Meeting of the American Association of Pathologists, Toronto, Ontario, Canada, March 15, 1977.

Supported by a development grant from the Connaught Fund of the University of Toronto; a research grant from the National Cancer Institute of Canada, Contract N01 CP-75879 from the National Cancer Institute, National Institutes of Health, US Public Health Service. Dr. Solt was supported by Training Grant DE-2268 from the National Institute of Dental Research to Temple University; Dr. Laishes was supported by a Fellowship from the National Cancer Institute of Canada.

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Working Hypothesis

Chemical carcinogens do not seem to induce cancer in the liver, but rather trigger a chain reaction which ultimately may eventuate in the development of cancer.[1] The links in this chain are different populations of hepatocytes that appear to undergo a process of cellular evolution to malignant neoplasia.

In beginning to analyze the essential nature of the links, we are impressed by at least three considerations:

1. Autonomous or semi-autonomous growth of initiated cells appears to be a property acquired late in carcinogenesis. For example, in the time scales usual for rats and mice, evidence of a relatively independent alteration in growth control does not usually appear until after 4 or 5 months, at a minimum, under conditions in which frank neoplasia is evident at 7 or 8 months. Thus, very roughly speaking, well over 50% of the time-span of most examples of carcinogenesis elapses before even semi-autonomous growth becomes manifest. This is true, not only *in vivo*, but also when initiated cells are removed from the constraints of the whole organism.[2] The data on carcinogenesis in skin, cervix, bronchi, breast, and other sites in the human are in accord with this notion.[3]

2. Virtually every chemical carcinogen is an inhibitor of cell proliferation. This "mitoinhibitory" effect is surprising and puzzling at first glance, since the disease induced by these same agents is characterized phenotypically as one of semi-autonomous or progressively autonomous growth.

3. The response of tissues or organs during carcinogenesis is *focal*, not general, even though many of the biochemical effects of carcinogens seem to affect most, if not all the cells in any tissue population.

These considerations suggest that a property of the focal responsive population is a relative resistance to the inhibitory effects of carcinogens or perhaps other hepatotoxic agents. Thus an attractive hypothesis can be formulated as follows: a hepatocarcinogen, as one of its earliest effects, induces a change in some hepatocytes such that these altered cells can grow in an environment that inhibits the growth of the surrounding "initiated" hepatocytes. If the carcinogen or other xenobiotic agents create a local selection pressure of this nature, the initiated resistant cells would be at an advantage over the surrounding original cells.

The concept that resistance to some of the cytotoxic properties of chemical carcinogens may play a role in cancer development is an old one.[4] However, it has not been subjected to critical testing in studies in carcinogenesis. The majority of the studies until recently concentrated on comparisons between extremes—malignant neoplastic cells and normal

cells—without any focus on the cell populations involved in between, i.e. the new preneoplastic cells that are, or contain, the presumptive precursor for cancer.

Several studies in liver carcinogenesis during the past few years have begun to focus on the putative preneoplastic hepatocytes induced by one of several different chemical carcinogens.[3,5] This research has clearly shown that hyperplastic liver nodules, highly probable precursor lesions for liver cancer,[3,5] are resistant to several cytotoxic properties of hepatocarcinogens, including the inhibition of proliferation of normal hepatocytes. The basis for this resistance may well be a large decrease in the ability of the nodules to take up and/or to suitably activate chemical carcinogens.[5-12]

The results of these studies led naturally to the hypothesis that a very early step in carcinogenesis, if not the first step, is the induction of a resistant hepatocyte which would grow and thrive in an environment unfavorable for normal hepatocyte proliferation [13] and that such cell populations are the precursors for cancer.

In order to begin subjecting such a hypothesis to critical scrutiny, it became important to devise an assay that would allow the identification and quantitation and the beginning characterization of such a hypothetical altered hepatocyte. We have been able to devise such an approach based on the creation of a selection pressure following exposure to an initiating dose of a carcinogen.[13] Using mainly diethylnitrosamine (DEN) but also dimethylnitrosamine (DMN), aflatoxin B_1, methyl azoxymethanol (MAM), *N*-hydroxy-2-acetylaminofluorene (*N*-OH-2-AAF) and 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) as initiators, a non-initiating exposure to 2-AAF to inhibit normal hepatocyte proliferation, and a strong stimulus for cell proliferation (partial hepatectomy [PH]), one can induce the rapid emergency of initiated hepatocytes and follow such cells for about twelve to fifteen cell cycles as they continuously grow into foci and nodules.[13,14] In addition, one can now pose many specific questions concerning carcinogenesis, including the role of cell proliferation, the possible role of DNA synthesis and specific DNA repair, the persistence of initiated cell populations, the sequential properties acquired by initiated cells, and the effect of endogenous and exogenous factors and how they could influence qualitatively or quantitatively the evolution of cancer from initiated cells.

Some Early Results With the Model

Although this model of liver carcinogenesis is still largely unexplored, it has led already to the following set of facts: [13-15]

Assuming an origin of each focus from a single cell, approximately 1×10^8 resistant cells are induced by 200 mg/kg DEN in a 5-g liver, or one hepatocyte in 10^6 original cells has been so altered. With most of the other carcinogens mentioned above, the efficiency is 1 : 10 or 1 : 100, i.e., one cell per 10^7 or one cell per 10^8.

If the resistant cells are not subjected to the selection pressure exerted by the assay procedure, they persist in latent form for up to 17 months without any loss of their number.

If the resistant cells are selected to proliferate rapidly by the assay procedure, a sequence of foci and nodules develops in the liver that is indistinguishable from the various putative preneoplastic and premalignant proliferative hepatocyte lesions seen with continuous or intermittent multiple exposures to many different hepatocarcinogens.[14] Foci and hyperplastic nodules appear with the same histologic, histochemical, and biochemical features as in the conventional models.[14]

With a single 200 mg/kg dose of DEN, no hepatocellular carcinomas develop in the Fischer rat by 9 months. With the selection pressure devised, 70% of animals develop liver cancer, some with metastases to the lung.

If the sequence is reversed, i.e., if the low level of 2-AAF plus hepatectomy (PH) precedes the injection of DEN, much smaller foci develop, and the number is no more than with DEN alone without the 2-AAF plus PH. Long-term studies are under way to observe the cancer incidence under these conditions.

The addition of phenobarbital (PB) or 3-methylcholanthrene (MC) to the diet containing 2-AAF abolished the ability of the 2-AAF to select for resistant cells; PB and MC are well known as agents which inhibit liver cancer induction by 2-AAF.

The pattern of growth of hepatocytes in foci and nodules is quite different than the pattern of the hepatocytes in normal mature liver. The liver cells are arranged in plates more than one cell thick and in tubules and resemble fetal liver more than normal liver. The arrangement has a resemblance to many hepatocellular carcinomas.[16]

Several histochemical and biochemical positive markers (γ-glutamyl transpeptidase, the β-subunit of human chorionic gonadotropin, and PN-antigen) appear very early in initiated hepatocytes and persist in foci, nodules, and cancer but not in surrounding hepatocytes.

Carcinogen-induced resistant cells can be stimulated to undergo at least fifteen cycles of cell proliferation without expressing any evidence of autonomous growth.

The resistant cells seem to be synchronous for about fifteen cell cycles.

Increasingly autonomous growth of resistant initiated hepatocytes is a late phenomenon in their evolution to cancer.

This, with the new model, it now becomes possible for the first time to study the intimate cellular and subcellular changes in initiated cells as they undergo gradual evolution to malignant neoplasia.

Tentative Conclusions

Although the model is new, it is beginning to give us new insights into the carcinogenic process in the liver.

Cells initiated for carcinogenesis are not recognized by the host in a manner that destroys them or otherwise causes their biologic inactivation.

The fate of cells altered by exposure to an initiating dose of a carcinogen is largely dependent on the environment. If this is appropriate for selective growth, cancer develops. If this is not, no cancer may develop in the lifetime of that organism, even though the initiated cells may persist for a major segment of its life-span. Carcinogens or probably non-carcinogenic promoting agents play a major role in creating this selection environment.

A functional property other than acquisition of the ability to grow autonomously or semi-autonomously characterizes the early cell populations in liver carcinogenesis. In this organ a general resistance to certain cytotoxic effects of many carcinogens may be the functional property that allows for selective growth of the initiated cells vis-à-vis the surrounding noninitiated hepatocytes.

Carcinogenesis in liver consists of two segments—a short initial segment resembling a mutation and a prolonged subsequent one resembling a differentiation process. The initial one consists of a rapidly induced, essentially irreversible change in a very small minority of hepatocytes that could very well be a somatic mutation. The subsequent phase of evolution consists of an apparently programmed series of changes with options that has some similarities to the process of differentiation. Thus the induction of cancer does not seem to consist of either somatic mutation or altered differentiation, but rather of both types of changes.

The second phase, differentiation, seems to begin with a totally novel cell—one that has many functional and structural characteristics of a mature liver cell and some characteristics of embryonic and fetal cells. This cell undergoes a series of changes that have at least two options—maturation to apparently mature liver and evolution to cancer. Since this pattern seems not to be simply a repetition of a previous one but rather a new one, we tentatively consider this as a process of *neodifferentiation*.

Interruption of maturation seems to be a constant characteristic of the pathway of differentiation leading to neoplasia.

A major impression gained so far is that the pattern of development of cancer in the liver with chemicals is remarkably similar to that seen during skin carcinogenesis. This is impressively reassuring for those of us who are working in one model and have the strong feeling that the similarities between models are far greater in principle than are the differences. Thus, as has been the experience to date, intensive study of cancer development in the liver may offer new insights into mechanisms of carcinogenesis in many other organs.

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