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Perspectives in Cancer Research

Paradox of Carcinogens as Cell Destroyers and Cell Stimulators. A Biochemical Hypothesis

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THE CARCINOGENESIS literature is replete with reports showing that while carcinogens often destroy cells, they also give rise to enhanced cell growth. Thus there seems to be a paradox: often the same compound, acting on the same type of cells, can act as a cell destroyer and later behave as an enhancer of these cells. I propose in this review to offer some reasons for this paradox: how can the same chemical substance serve as both a cell killer and as a cell enhancer? I propose to describe mechanistically both of these activities of carcinogens; cell proliferation inducer and cell killer.

The literature showing that carcinogens serve as stimulators of cells is sampled in Table 1 which presents a selection of 15 representative papers chosen from over 100 references known to me at this time, in which carcinogens are described as cell proliferation inducers (or as inducers of new DNA synthesis). Table 1 focuses on liver carcinogens and on nitroso type compounds. That this hyperplasia is the result of a direct effect of the carcinogen onto the target cells destined to give rise to neoplastic cells, rather than merely a regenerative response to carinogen-induced cell injury, is shown by Newberne [1], Hitachi (see Table 1) and others. The fact that the hyperplastic cells are largely abnormal, quite different from what would be expected simply on the basis of cell replacement, also illustrates this point. [2].

On the other hand, there is a body of literature showing that carcinogens, including those that cause cell proliferation, seem also to induce cell death or at least inhibition of growth under certain conditions, both *in vitro* [3] and *in vivo* [4]. There have been reports of carcinogens acting on the same cell system as both cell destroyers and as inducers of cell proliferation [4, 5].

The general picture I will present in this review is as follows: (1) it is the cells that are permeated by carcinogens that are those destroyed by the carcinogen, owing to a variety of destructive effects of carcinogens at many sites of the cells, especially those involved in DNA synthesis. The cells that are permeated are usually those involved in S phase of cell growth (rapidly dividing cells), which are usually more permeable to various solutes. (2) I propose that whenever resting cells are permeated (due perhaps to the nature of their membrane-associated c-GMP system), they too are destroyed. It is primarily the resting cells of a population which are transformed, in which carcinogens act like mitogens (hyperplastic agents), attacking primarily receptors at the cell membrane level. The carcinogen-modified receptors are, like mitogen-modified receptors, endocytosed and undergo lysosomal digestion. As with non-carcinogenic mitogens, the carcinogen-modified receptors yield, on such lysosomal digestion, small peptides which can very well serve to act as specific gene activators on entry into the nucleus. Since the carcinogen-

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Table 1.

References showing that liver carcinogens induce cell proliferation (hyperplasia) increased DNA synthesis of increased mitosis in target cells

S. GOLDFARB, A morphological and histochemical study of carcinogenesis of the liver of rats fed 3-methyl-4-dimethylaminoazobenzene. *Cancer Res.* **33,** 1119 (1973).

M. KITAGAWA and H. SUGAMBO, Combined enzyme histochemical and radioautographic studies on areas of hyperplasia in the livers of rats fed 2-fluorenylacetamide. *Cancer Res.* **33,** 2993 (1973).

H. D. RUEBER, Development of prencoplastic and neoplastic lesions of the liver of male rats given 0.25% *N*-2-fluorenyldiacetamide. *J. nat. Cancer Inst.* **34,** 697 (1965).

M. HITACHI, K. YAMADA and S. TAKAYAMA, Cytological changes induced in rat liver cells by short-term exposure to chemical substances. *J. nat. Cancer Inst.* **54,** 1245 (1975).

H. TSUKADA, Y. MICHIZUKI and M. GOTOH, Matrical inclusions induced by clofibrate in hepatic microbodies in rats fed 2-acetylaminofluorene. *J. nat. Cancer Inst.* **54,** 519 (1975).

K. OKITA, M. GRUENSTEIN, M. KLAIBER *et al.*, Localization of alpha-fetoprotein by immunofluorescene in hyperplastic nodules during hepatocarcinogenesis induced by 2-acetylaminofluorene. *Cancer Res.* **34,** 2758 (1974).

H. SIDRANSKY, M. ITO and E. VERNEY, Influence of alpha-naphthyl-isothiocyanate on liver tumorigenesis in rats ingesting ethionine and 2-fluorenylacetamide, *J. nat. Cancer Inst.* **37,** 677 (1976).

E. RUBIN, K. MASUKO, S. GOLDFARB *et al.*, Role of cell proliferation in hepatic carcinogenesis. *Proc. Soc. exp. Biol. (N.Y.)* **115,** 381 (1964).

References showing that nitroso compounds induced hyperplasia as the initial (or very early) effects on target cells

D. P. CHOPRA and J. WILKOFF, Inhibition and reversal by beta-retinoic acid of hyperplasia induced in cultured mouse prostate tissue by 3-methylcholanthrene on *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, *J. nat. Cancer Inst.* **56,** 583 (1976).

P. POUR, J. ATHOFF and M. TAKAHASHI, Early lesions of pancreatic ductal carcinoma in the hamster model. *Amer. J. Path.* **88,** 291 (1977).

L. M. SHABAD, T. S. KOLESNICHENKO and N. I. GOLUB, The effect produced by some carcinogenic nitroso compounds on organ cultures from human embryonic lung and kidney tissues. *Int. J. Cancer* **16,** 768 (1975).

G. T. BRYAN, The pathenogenesis of experimental bladder cancer. *Cancer Res.* **37,** 2813 (1977).

E. KUNZE, A. SCHAUER and S. SCHATT, Stages of transformation in the development of *N*-butyl-*N*(4-hydroxybutyl)nitrosamine-induced transitional cell carcinomas in the urinary bladder of rats. *Z. Krebsforsch.* **87,** 139 (1976).

N. WITSCHI, The effects of diethylnitrosamine on RNA and protein synthesis in the liver and lung of the Syrian golden hamster. *Biochem. J.* **136,** 789 (1973).

N. SUZUKI, Histological studies on early changes of the hamster gingival epithelium by *N*-nitrosomethylurea. *Bull. Tokyo med. dent. Univ.* **23,** 115 (1976).

modified receptor is 'abnormal', there is found an abnormal pattern of gene activation on entry of these lysosomally-derived peptides into the nucleus. Thus I propose that carcinogens like non-carcinogenic hyperplastic agents, act on the nucleus by an indirect mechanism involving primary attack at the membrane level. That cancer cells in general arise from quiescent cells is pointed out by Gelfand [6].

LITERATURE SUPPORT FOR THIS HYPOTHESIS

Cytotoxic effects of carcinogens

While many studies have shown extensive carcinogenic transformation in cell systems that are rapidly dividing (in which there were many cells in mitosis), for the most part carcinogen-induced hyperplasia is observed in studies done *in vivo* in rather quiescent tissues or in organ culture, all of which are presumably rich in resting cells, contact inhibited from mitosis. On the other hand, Hooson and Grasso [3] observed extensive destruction of cells when treated with some 20 different carcinogens, an effect ascribed by these authors to extensive DNA damage by these agents. There is an abundance of additional evidence for the greater susceptibility of dividing cells to the cytotoxic effects of carcinogens compared with resting cells [7, 8].

Quite possibly the work of Hooson and

Grasso [3] might be explained by the possibility that cells during active proliferation might well be more permeable to carcinogens than are the cells not in cycle, with the exceptions (based on behavior of membrane-associated cyclic nucleotide systems) noted below. This possibility was enhanced by findings that at a dose of 40 mg/kg, *N*-hydroxy-2-acetylaminofluorene (*N*-hydroxy-2AAF) there was destruction of DNA and RNA synthesis in regenerating but not in normal liver [7]. That proliferating cells are, in fact, generally more permeable to a variety of molecules was shown in work reviewed by Pardee [9] and in the work of others. Whether this holds true of carcinogens entering fibroblasts is as yet unknown but the known information given by Pardee [9] strongly argues for the generally higher permeability of dividing cells to carcinogens, which might well explain the findings of cell death or arrest of DNA synthesis found in many populations containing high numbers of dividing cells [5, 7, 8].

It is clear that when a carcinogen enters a cell, it will not make a 'beeline' for the nucleus and ignore all other intracellular structures. It will attack just about every susceptible available structure in the cell. Thus in order to get to the nuclear DNA, the carcinogens must also encounter ribosomes, chromatin enzymes (synthesizing DNA and RNA), metabolic factors etc., on the way to the DNA, all with devasting effect. Furthermore, attack on DNA itself, in many cases, can result in loss of DNA synthesis rather than only subtle changes in the DNA that can be transmitted genetically. I will give only the highlights of the literature concerning destructive effects of carcinogens on some of these intracellular structures, space limitations forbidding a very much more extensive treatment.

Protein synthesis has been shown to be essential for the maintenance of DNA synthesis by a number of authors, of which I will cite only two representative papers [10, 11]. Yet it was found that nitrosoamines do inhibit protein synthesis to a substantial extent [12]. Many other reports indicating severe effects of carcinogens on RNA synthesis, needed for protein synthesis, have been presented (in addition to a number of reports of carcinogen effects on protein synthesis in general) [12–14]. This protein synthesis inhibiting effect of carcinogens has been observed using a great many agents: alkylating agents, AAF, aflatoxin etc. Thus by inhibiting protein synthesis, which is required for continued DNA synthesis carcinogens can well cause loss of replication rather than simply subtle errors in replication.

More direct effects on DNA replication have been observed wherein carcinogens have been found to directly inhibit replication of DNA, possibly by mechanisms involving direct damage to DNA templates by the carcinogens [15–18]. It was found that treatment of bacterial transforming DNA with carcinogens causes extensive inactivation of the DNA [19, 20]. While only one marker was studied, it is not unreasonable to assume that a great many other loci are attacked as well, rendering carcinogen-attacked DNA incapable of further replication (especially in the absence of effective repair systems) via inactivation of DNA synthetase loci.

Another feature of the cytostatic or cytotoxic effects of carcinogens on entry into cells arises from the effects of alkylating carcinogens (i.e., DMN, methylnitrosourea etc.)—if in fact they act as primarily alkylating agents—in causing the formation of phosphotriesters of DNA (in addition to alkylation of base moieties) [21]. Such reactions, giving rise to phosphotriesters in high yields, also give rise to no specificity in position of attack on the DNA. These phosphotriesters resist the action of some nucleases [21] which could well be indicative of similar inhibitory effects of these phosphotriesters on some of the endonucleases required for replicon formation, a necessary first (initiation) step in DNA replication [22]. Thus again, direct reaction of carcinogens (such as alkylating agents) could rise to destruction of DNA synthesis rather than simple specific mutational changes in DNA synthesis. It might also be noted that some carcinogens decompose into highly cytotoxic compounds. MNU and enthylnitrosourea (ENU) decompose into the highly damaging cyanate ion [23] while DMN decomposes into cytoxic formaldehyde [24]. All of this argues that entry of these agents into cells, even if DNA is attacked, could well yield a dead rather than a transformed cell. [Another point which could well be investigated in future work is whether a carcinogen, on attacking DNA *in vivo*, attacks the DNA synthesizing enzyme regions of the DNA (i.e., the DNA polymerases genes) in such a way as to inactive these genes, and thus give rise to a mutation of the lethal or cytostatic variety].

What all of the above signifies is that when carcinogens encounter elements of the DNA synthesis system (including the protein synthesis system) there is significant elimination of DNA replication. If one supposes that

mutation involves errors in replication, it is seen that mechanisms involving direct entry of carcinogens into cells are highly unlikely since it is indeed very difficult to see just how there could be errors in replication, when, for the most part, there is very little or no replication (on direct attack by carcinogen onto the DNA synthesis system).

Yet it is known that even quiescent cells (such as those of intact non-regenerative liver) are destructively attacked by carcinogens. How does a carcinogen enter these resting cells? It was found that decreasing the intracellular levels of c-AMP and elevating the levels of c-GMP causes increased permeability of cells [25]. It was recently found that a number of carcinogens (but, to be sure, not all) increase the levels of c-GMP in cells, in a number of cell types including liver [26, 27]. Thus, this could be a partial explanation for the entry of some carcinogens into resting cells: they carry with them their own 'keys' to the intracellular space in the form of affecting the c-GMP system to raise the level of c-GMP and thus make the resting cell more permeable to the agents in a reaction involving the c-GMP cyclase at the cell membrane. Such entry of carcinogens, facilitated by carcinogen-induced elevation of c-GMP levels, could account for the findings of binding of carcinogens to the ribosomes and thus for the destructive effects of carcinogens on ribosomes of resting liver cells of normal animals, observed by a number of workers for example Plapp and Chiga [28]. Thus, carcinogens might well affect different cells of a heterogenous cell population differently depending on whether the particular cell has a sterically available (accessible) and susceptible c-GMP synthesizing system on its cell membrane.

Transforming and hyperplastic effects of carcinogens

Since, as I have shown above, entry of carcinogens into cells probably leads to cell damage or death, we can see that if carcinogens do attack target cells directly at all, in their transforming activity, they must do so by action at the cell periphery of resting cells. Yet cancer is most probably the result of some hereditary change in the target cell genome. How can agents acting primarily at the cell periphery affect nuclear DNA? We can see from above, that carcinogens act in the first instance as hyperplasia inducers in a process of carcinogenesis going in an ordered progression, from normal to hyperplastic to neoplastic cells [1]. We also know that most hyperplastic agents (cell specific growth factors such as the epidermal, fibroblast, etc. growth factors, growth hormone, lectins and antigens acting on lymphocytes) are postulated to act at the cell membrane level. That is they seem to have dramatic effects on the nucleus but react primarily with membrane receptors (space does not permit me to review here the entire literature showing that these mitogens do in fact react with membrane receptors). Carcinogens, being mitogens (initially) might well act the same way as do other mitogens, primarily at the cell membrane level.

Concerning time frame of action, we know that lymphocyte mitogens cause extensive T- and B-cell hyperplasia *in vivo* (in spleen) [29], an effect which seems very similar to the effects of carcinogens reviewed in Table 1. This effect of lymphocyte mitogens seems to require very few days to produce hyperplasia [29]. This seems to be what is also observed for some carcinogens wherein there have been reports of carcinogen-induced mitogenesis within time periods of immediately to 7 days after treatment [30, 31]. Often, of course, carcinogen-induced hyperplasia takes much longer to develop.

I want to stress this point: I fully agree with the traditional concept of DNA being the ultimate target in chemical carcinogenesis. What is new about my hypothesis is that I propose that DNA is changed by carcinogens indirectly rather than by direct carcinogen–DNA interactions. Rather, I suggest that they behave initially like the mitogens (non-carcinogenic) they so much resemble in initial effect, acting primarily at the cell membrane. In the course of proposing my mechanism for carcinogenesis, I hope to suggest what makes one mitogen a carcinogen and another non-carcinogenic.

As a background for my hypothesis, the following is known: (1) many mitogens such as as the epidermal growth factor (EGF) cause, on reaction with their receptors, the mitogen-modified receptor to undergo endocytosis and lysosomal digestion [32]; (2) it was also found that carcinogens give rise to increase in lysosome amounts and enyzme activities [33, 34]. While endocytosis and lysosomal increase caused by carcinogens might reflect simple autophagy, as proposed by many authors [35, for example], in light of the more current work on the EGF receptor [32], another possibilty is open and forms the basis of my hypothesis. I submit that the involvement of lysosomes (increase in lysosomes, endocytosis of mitogens and their re-

ceptors, on carcinogen and mitogen attack on cells) might very well have a fundamental function significance (in non-necrotic cells) to the process of mitogenesis in general (i.e., by agents such as EGF, lectins etc.) and carcinogenesis in particular.

MY HYPOTHESIS OF CHEMICAL CARCINOGENESIS AND CELL GROWTH

On reacting with mitogens (carcinogenic and non-carcinogenic), the cell membrane receptor, modified by this reaction, is thus caused to endocytose and undergo digestion in the resulting lysosomes. The receptor might still be attached to the mitogen upon entry into the lysosome. In the lysosome the proteases act on the receptor (modified by carcinogen or non-carcinogenic mitogen) in a slow, step-wise fashion to cause the formation of sets of oligopeptides, some not larger than di- or tripeptides. Upon their formation, again, in a step-wise fashion, these sets of peptides leave the lysosome, perhaps in some manner facilitated by cyclic nucleotides affecting lysosomal membrane permeability. Among other activities, these peptides will enter the nucleus, where they can well serve as very specific gene activators. It was proposed [36] that repressor proteins are bound to specific regions of DNA by way of specific sequences of amino acids in the repressor protein molecule. Quite possibly therefore, small peptides (derived from lysosomal digestion of carcinogen or growth factor modified membrane receptors) containing the same specific sequences of amino acids (that bind repressor to its specific region of DNA) could very favorably compete with repressor at this region for the DNA and thus dislodge the large repressor molecule from the DNA. This would expose very large, highly specific, regions of the DNA to the transcription process. This then is the mechanism whereby the lysosomally derived peptides described above, can serve as specific gene activators.

Here is the core of the explanation of why some mitogens are carcinogenic while others are not. Since the cell membrane receptors of these agents might differ and since the product of the reaction of the receptor (even if it is the same receptor in all cases) would be different, the result is different sets of peptides on lysosomal digestion. These different sets of peptides might well derepress formation of different sets of mRNA. Thus both non-carcinogenic and carcinogenic mitogens might very well activate the same genes that code for the DNA synthesis enzymes, explaining the mitogenic effects of both types of mitogens (qualifying the carcinogens as mitogens, hyperplastic agents). However, those peptides formed from lysosomal digestion of receptors to carcinogenic mitogens can differ from those from digestion of non-carcinogenic mitogen receptors in the following ways: (1) Lysosomal digestion of endocytosed carcinogen-modified receptors might well yield gene activators giving rise to abnormal error prone DNA polymerases as suggested in the work of Chan and Becker [37]. This can cause permanent changes in DNA as would any direct carcinogen–DNA reaction. Indeed some enzymes such as the DNA ligase might be deficient or absent in such nuclei due to the lack of the proper gene activators supplied by lysosomal digestion of the carcinogen-modified membrane receptor. This could result in the production of abnormally short DNA molecules (and thus chromosome aberrations) in this, case since it was found that DNA varies in size distributions in different mixtures of DNA polymerase and ligase [38]; (2) One set of peptides formed from lysosomal digestion of carcinogen-modified receptors might serve to activate genes coding for the c-type oncogenic viruses. Sets of peptides formed from lysosomal digestion of receptors to non-oncogenic mitogens may lack this activity.

The effects of co-carcinogens (promoters) can be accommodated to this hypothesis. For one possibility, it is known that co-carcinogens enhance the production of phospholipid, thus perhaps providing more lipid membrane material needed for endocytosis of carcinogen-modified receptors, which precedes the onset of DNA synthesis in mouse skin [39, 40]. Thus co-carcinogens can increase levels of carcinogen-altered receptor endocytosis and lysosomal digestion by providing more material (phospholipids) needed to form the structures of pinosomes and lysosomes, which may well be the key structures in the transforming effects of carcinogens (i.e., their initial hyperplastic activities) and in induced cell growth in general.

The literature provides much more support for the ideas presented here than there was space to describe in detail. In sum, I attempt to show in this hypothesis just how a carcinogen can cause changes in target cell DNA without ever coming in direct contact with DNA, but rather by acting, like the mitogens it so much resembles in gross hyperplastic activity, at the cell membrane level with

transmission of effect by way of specific unique sets of peptides formed from lysosomal digestion of the endocytosed carcinogen-modified receptors. The carrier, the second messenger, therefore, are these sets of peptides acting at the nuclear level as specific (but abnormal) gene activators.

Notes—(1) In the mechanism provided above, it is proposed that the stimulation of DNA synthesis by carcinogenic and non-carcinogenic mitogens entails the endocytosis and lysosomal breakdown of carcinogen- or mitogen-modified membrane receptors to products that serve as nuclear gene activators. This concept would seem at first glance to be undermined by a paper which appeared after the acceptance of this Perspective. LeCam *et al.* [41] first treated liver cells with rhodamine fluorescently labeled insulin and then found that methylamine($MeNH_2$) treatment of the cells caused a total loss of the internalization (lysosomal uptake) of the insulin which is found to occur in the absence of $MeNH_2$. They also report that treatment with $MeNH_2$, rather than causing a loss of DNA synthesis stimulated by EGF, in fact caused an enhancement of such EGF-induced DNA synthesis. On the basis of this they postulate that clustering and internalization of EGF or insulin or their receptor complexes are not required for DNA stimulation activity of the hormones. This obviously is at variance with the mechanism presented in this Perspective.

It is not unreasonable to assume that EGF and insulin and that NH_3 and $MeNH_2$ operate in rather similar manners with respect to DNA stimulation in most types of epithelial and fibroblast cells, as implied by LeCam *et al.* [41]. In view of these considerations, it should be pointed out that the observations of LeCam [41] seem to be at variance with earlier work. Turkington [42] showed that rather than potentiating the DNA stimulator effect of insulin on mammary epithelial cells (as would be expected on the basis of the reported potentiating effect of $MeNH_2$ on EGF-induced DNA synthesis [41], NH_3 considerably reduced the effect of insulin. The LeCam work [41] seems also inconsistent with two other reports showing either no effect or suppressive effects of NH_3 and amines on DNA synthesis, assuming that growth factors were present in the systems reported [43, 44]. Thus until these discrepancies can be resolved, even in the light of the LeCam work [41], the mechanism of induced cell growth (normal and abnormal) presented in this Perspective remains consistent with the reports in the literature. (To evaluate these reports, reactions of RNH_2 with chromatin should be studied.)

(2) The "recovery" of protein synthesis reported [12] 8–24 hr after nitrosamine treatment might reflect some replacement of carcinogen-destroyed cells by other liver cells derived from cells unaffected by carcinogen.

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