CONCEPTS FOR THE CURE OF CANCER BY EPIGENETICALLY INDUCING REDYSDIFFERENTIATION

RONALD LEE HANCOCK

ABSTRACT. The understanding of the biochemical mechanisms for a derivation of a cure for cancer requires guiding biological concepts. This paper is an attempt to create a generalized fundamental biological basis for such a cure. One of the major approaches stems from the idea that a neoplastic cell is in a dysdifferentiated state that involves derepressed embryonic type genes by processes of deheteochromatization. The reversal of this process by alterations in specific methylation patterns of embryonically active region of chromatin, hence epigenetic changes, in growth factor genes could be key in a curative approach to cancer treatment.

KEY WORDS. Cancer, epigenetic, methylation, dysdifferentiation, embryonic, heterochromatization, stem cells, biogradient, ethionine, carcinogens.

INTRODUCTION

The variety of treatment modes that have been tried on numerous types of cancer that exist many times have had limited success. There are anti-cancer treatments being used presently including chemotherapy, surgery and irradiation where the principle mechanism is to kill the neoplastic cell directly. Also one can include hormonal treatments such as anti-androgens to inhibit the growth of selected testosterone dependent tumors (prostatic carcinomas). A recent approach has been to use specific antibodies derived from the individual's own tumor. All of these methods have features which allow them to be of benefit.

The following is an attempt to create a generalized fundamental basis upon which treatment of any cancer can be devised successfully. In its present form this attempt necessarily relies upon several projected theories of molecular cell mechanisms where such mechanisms are, as yet, not understood. Thus, such a proposal as presented here will need to evolve.

Nevada Institute for Theoretical Medicine, 1225 Westfield Ave, Reno, NV, 89509, USA. / rhancock@unr.edu

BIOLOGICAL CONCEPTS

Reviewing some biological terminology by using the figure at the end of this text, one can follow embryonic cells (E) changing to adult cells (A), a process termed differentiation. The reverse process would be dedifferentiation where adult cells would become embryonic cells—a rare case scenario. Many tissues have had stem cells (S) identified in them and are considered to be quasi-differentiated cells. The writer is not aware of the reverse process (stem cells transforming to embryonic cells) as having been described. Stem cells are commonly believed to be the competent precursor to neoplastic (N) cells, created through a process one would call dysdifferentation (abnormal differentiation). The reverse process could be termed redysdifferentiation.

If the stem cell theory of the origin of cancer is incorrect, then the same labeling of processes could be applied towards adult to neoplastic transformations.

Note that there are subsets A and S of dysdifferentiation (and redysdifferentiation) referring to adult or stem cell origins of cancer, respectfully. Furthermore, as depicted in the figure, there are possibilities that some critical process could occur after some specific differentiating steps, thus the dashed lines connecting between the time progression arrows of dysdifferenitation and redysdifferentiation.

The bracketed sets of A, S or N of $P_{1,2,...n}$ refers to the potential or precursor (P) states at varying stages of development of adult, stem or neoplastic cells, respectively, as being the cell involved in the cellular transformation. Note that even varying states of neoplastic cells may theoretically transform towards normal states (See ahead under 'The Cure').

The writer knows of no example of an adult cell becoming an embryonic cell (dedifferentiation) [possibly in teratomas?].

IDEAS REGARDING THE NEOPLASTIC STATE

A malignant tumor mass of neoplastic cells probably does not contain a singular type of cell. Thus, in principle, every tumor cell in a tumor mass could be different biochemically or genetically, or the same genetically yet functionally different because of epigenetic changes.

If one induces normal liver cells to become neoplastic, the resulting tumor contains a great variety of neoplastic cells as easily demonstrated by simply staining for, say, glycogen. The amount of glycogen synthesis occurring in any one particular cell may be radically different from the adjacent cell. If one is to cause a redysdifferentiation of these various neoplastic cells to adult liver cells, then surely the resultant normal cells will include a great deal of variation also. In other words, normal liver

derive from hepatoma should be somehow different biochemically and gene expression-wise. Ethionine induces dysdifferentiation, hepatomas, in parenchymal liver cells believed to result in a variety of DNA methylation states of adult type genes, e.g., genes for glycogen synthesis may be deactivated. In principle, in each cell glycogen synthesis may remain present or be deactivated. Methionine antagonists via S-adenosyl-L-methionine might revert to such an epigenetic repression.

Chromatin created during differentiation that occurs on embryonic growth genes and other genes expressed during the embryonic phase [1], may be differently constructed. Especially different should be the methylation pattern since maintenance methylases that maintain a, e.g., liver cell, to divide into two liver cells and not into other types of cells, must now, during differentiation of a given embryonic cell, be perturbed to create a differently progressed chromatin as a biogradient resulting in a more differentiated state.

This embryonic type chromatin, i.e., different in structure, needs to be suggested because of the differential response between the effect of some carcinogens that will not effect thousands of genes and yet will effect the relatively rare number of embryonic genes. That difference is also revealed when one gives ethionine to a rat and a small group of hepatomas arise after a given time. What is happening at the molecular level must be a very rare event, since all liver parenchymal cells do not become neoplastic. Such a rare event must relate to the specific array of embryonic (and other?) genes that become activated. Yet, even this process needs a specific differentially-induced aspect of a hyper-sensitive alteration of an embryonic type chromatin. This can be thought of in the following terms. Let us say that embryonic genes j, k and p are required to be derepressed for an expression of the neoplastic phenotype. Yet, there are a... z genes present. Then the simple conclusion of the variety of producing clones of transformed cells can be reduced to those, say six, liver cells growing to produce six hepatomas because they were the ones containing the rare combination of active genes necessary. Incidentally, this can be considered to be a typical experimental situation; six months or so are required for the clones to develop into visible sizes. Thus j, k and p genes were derepressed in the original liver cells destined to become cancerous.

This final process would also relate to such the carcinogen as ethionine that the writer studied over decades (e.g., references 3 - 8). But what about other carcinogens such as asbestos, which is an entirely different kind of chemical? It would seem as a generalization that the broad spectrum of carcinogens will require a variety of mechanisms. Asbestos could be inducing a production of mesenchymal inflammatory cells with a gene expression closer to the embryonic phenotype, thus allowing further factors (carcinogens from smoking, etc.) to more easily induce the cancer

phenotype. Other carcinogens such as radiation, etc., have been presented elsewhere [8] and need only to be mentioned briefly here. Radiation can cause chromosome breakage allowing relocation and anomalous locations of heterochromatic segments. If this occurs near normal repressed embryonic growth factor genes, then a process of deheterochromatization can occur causing the embryonic gene to become activated. Many such scenarios can be created that reduce to a generalized scheme of derepression of embryonic genes causing the resultant dysdifferentiation state.

THE CURE

Now, how can we correct the situation, that is, if a neoplastic cell is a dysdifferentiated cell? Of course, dedysdifferentiate the cell.

To visualize such a process let us examine the ethionine case again. In principle one should simply be able to treat, say, the hepatoma cell with some biologically suitable methylating agent such as S-adenosyl-L-methionine. But S-adenosyl-L-methionine is present normally in most cell states. So what is it that keeps a cancer cell from correcting itself? If hypomethylated embryonic chromatin due to ethionine becomes remethylated, then the reverse process that of rerepression could occur. (The writer knows of no such results with S-adenosyl-L-methionine treatment of hepatoma cells). But such agents are the sort of approaches to the possibility of the neoplastic phenotype reversing—if not to its original cell phenotype, at least, it may redesign itself to a non-neoplastic form.

Since the details of such changes in embryonic chromatin [2], a theory in itself, is unknown, it will suffice to say here that this mechanism is preferentially hypersensitive to specific carcinogens, e.g., tRNA methylase-induction with ethionine. These carcinogens can be directly involved such as methylation interference or indirectly affected such as 'microheterochromatization' [10].

If there are a variety of inducing agents for the induction of cancer, how can there be a general curative treatment? Thus we have proposed a generalized carcinogenic process, involving the derepression of a set of unspecified embryonic growth genes. If this holds, then, although there are a wide variety of agents/carcinogens that created this cellular situation the final situation, cancer, is a single set of derepressed genes, at least, that are essential for the induction of the phenotype.

Therefore, it is proposed to rerepress this particular necessary set with a variety of processes, that incidentally may derepress other repressed genes that would be considered non-consequential to the changing of the cancer phenotype. In order to accomplish this, one needs, say, not only to be able to replace a methylation pattern on some set of genes but if the induction is, for instance, induced by a new positioning of a heterochro-

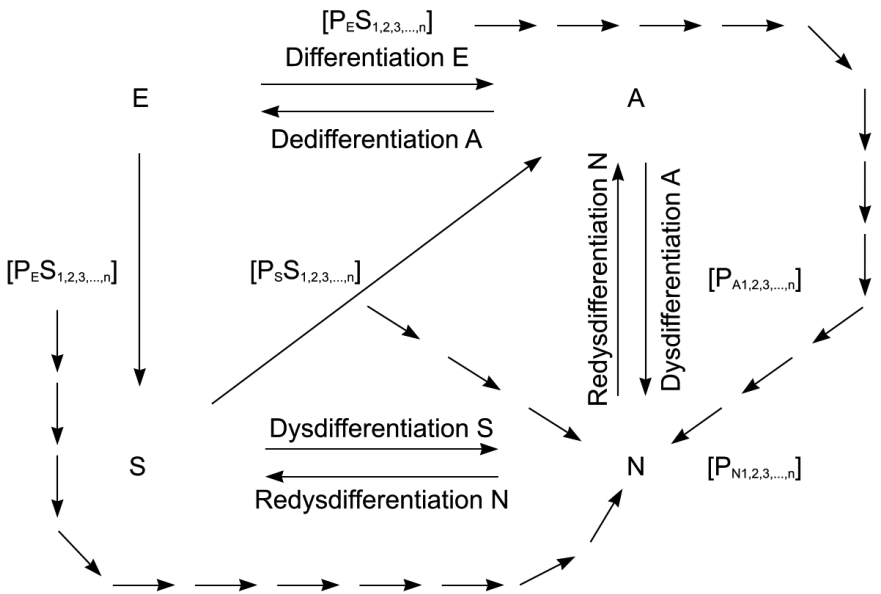
matic block, then we will need to know how to reheterochromatize this segment of chromatin.

It should be mentioned that cell signaling is not discussed in this paper although important achievements have been made for individual types of tumors [11]. Recall that this paper is devoted to finding a generalized feature adaptable for any cancer type to base a cure upon. Signal factors are themselves a second phase in the processes of cellular responses in that the stable heritable feature of cancer cells demonstrate that the fundamental aspect of the cancer phenotype is genetic or perhaps epigenetic.

Of course, such basic concepts of curing cancer, even if they are correct, leave the bigger problem of the practical treatment of the human organism. For instance, say a specific redifferentiation agent was found to effectively repress embryonic gene activity *in vitro*, how then might it affect the remaining normal cells of the cancer patient? Or how could such specificity be accomplished at the biomolecular level? Might not such a drug deactivate stem cell activity required for 'every day' maintenance of a tissue? This example could be multiplied many fold making the approach untenable. At this point, we should, at least, develop such agents and explore their potentials and possibly some new insight might be achieved giving the desired result according to the scope and type of cancer.

In summary, cancer may be cured by reversing the process of dysdifferentiation that occurs during carcinogenesis. To reverse the dysdifferentiated state, one must rerepress embryonic type growth genes that have become activated by a variety of carcinogens. This process is theorized to be controlled probably through epigenetic mechanisms on a special embryonic type chromatin and may also involve heterochromatization.

Differentiation is a 'powerfully' regulated process. If it were not strictly programmed, organisms would have a great deal of imperfections and defects. This highly guarded sequence of molecular changes that allows a complicated organism, such as a human, to exist is due to this protected biomolecular process*. Yet, having said how conserved and stable the mechanisms are, dysdifferentation can occur rarely—cancer.


[PES1,2,3,...,n]
Differentiation E
E
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[PSS1,2,3,...,n]
[PA1,2,3,...,n]
Dysdifferentiation S
S
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Redysdifferentiation N

* The writer did an unpublished experiment with ethionine, an antagonist of methyl metabolism. Hundreds of fertile chicken eggs were injected with varying amounts of ethionine at varying incubation times. Almost all of the eggs hatched and produced normal chickens as revealed by gross autopsy. The directed process of differentiation had been highly protected against epigenetic gene expression at least under the terms of the criteria of the experiment, i.e., no observable gross anomalies.

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