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Biology of Cancer: Some questions to answer

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Abstract

Though great advances in cancer biology have taken place through these years, some fundamental questions are still to be explained. Some observations in this regard are discussed in the present paper.
In the course of experimental studies on hormonal stimulation of target cells, it was observed that goat granulosa cells showed differential proliferative response to sustained stimulation by oLH and hCG in culture. oLH caused cells to proliferate whereas hCG failed to stimulate the cells though both the gonadotropins have common receptors on the target cell. Further studies might throw some light on the mechanism of signal transduction in cell biology and neoplasia.
A question is also posed as to how to interpret thermodynamically the sustained growth of cancer vis-a-vis the host.

Introduction

In spite of vast knowledge and information on cancer, cancer biology has remained a riddle. We know many details about the neoplastic cells, yet we are in the dark. Several theories have been put forth to explain the disease. Nonetheless, they induced some thinking. Some of these reflections are touched upon in the present paper.

Carcinogenesis

Carcinogenesis is a process by which a normal cell is transformed to a cancer cell which grows into a population of cancer cells. It is a multistep process involving initiation, promotion and progression. In response to a carcinogen, a cell in a given cell population (a tissue) is "initiated". Initiation is undetectable by any physical means. It is sudden and irreversible. The initiated cell is stimulated to proliferate by either the same carcinogen or by a compound called pro-

moter which itself is not a carcinogen but promotes proliferation of the initiated cell. This is a promotion step. It is detectable, slow and reversible. If the treatment of the promoter or a carcinogen is withdrawn before its full course, the proliferated cells will regress back to the initiated cell. If the site is treated again even with a promoter, a tumor develops [1, 2, 3].

In this statement, there is a difficulty. The hyperplasia so formed will regress for want of adequate promoting stimulation. It regresses back to which cell generation? We do not know. But Mackenzie and Rous [2] claim that the regression goes till the initiated cell which if stimulated even after a lapse of a long time starts proliferating to grow into a tumor. As we know, to enter into the promotion stage, the initiated cell divides into two daughter cells which further divide by geometric progression. Yet it is a fact that a tumor develops out of the initiated cell after the regression, if it is stimulated again.

This suggests that the initiated cell remains there after complete regression. If this is so, the first division of the initiated cell should be asymmetric. Either of the two daughter cells acquires the capacity to multiply and the other remains quiescent.

Alternatively, we may assume that regression does not stop at the initiated cell, but leaves a few cells of the hyperplasia. But, why should regression be incomplete? Are these surviving cells (or cell) different from the regressed cells? If so, where does the difference lie? Ultimately it could be genetic. It therefore, supports the view that initiation alone is a result of mutation and therefore, it is irreversible. Promotion could be an epigenetic process.

It is now generally accepted that the development of cancer is by mutation. Mutation is alteration in the normal sequence of nucleotides in a gene. It may be caused by external factors or may have developed "spontaneously". External factors include chemicals, radiation and viruses. If cancer occurs in spite of the apparent absence of external agents, we say it has developed "spontaneously". However, the "spontaneous" development of cancer must have some causative factor which is not known to us.

When a given population of a people is uniformly exposed to a carcinogenic agent for a certain period of time, only a few are affected. Though the rate of incidence depends upon the carcinogen and the tissue involved, it is 0.01 to 0.1 percent. That means a relatively negligible fraction of the population develops cancer in response to the carcinogen. This is at the population level. At the individual level, generally it is supposed that only one cell (or a few cells) is transformed in an organ of the individual. An adult human has about 30 trillion cells in the body [4], out of these cells only few cells yield to the carcinogenic insult. Suppose when a population of 100,000 individuals is uniformly exposed to a carcinogen there are 100,000 x 30 trillion cells exposed to the carcinogen. Out of these many cells, a few cells or only one cell grows into a cancer.

Some questions arise from this observation: One, are all cells of an individual exposed to the carcinogen? To answer this question, one may say, in most of the cases, yes, barring those carcinogens which come in direct contact with the skin, such as tar, and those which are inhaled like cigarette smoke, asbestos fibers. Carcinogenic chemicals which go into the intestine through food are absorbed in the blood and reach interior organs. Even if we consider the case of the tar which comes in contact with a part of the skin, that part of the skin contains several millions of cells out of which one or few cells develop into cancer. The question is: Why only one or few cells out of millions of cells are affected by the carcinogen? Let us take the lung as an example. A heavy smoker develops cancer of the lung. The organ has, say, 1^{07} cells. All these cells are uniformly exposed to the smoke. Yet only one cell of the organ grows into a tumor. The rest of the cells remain normal and healthy. Is the affected cell different from the other cells? Is it the most vulnerable cell and not fit to overcome the insult? We do not have answers to these questions. Every cell of the lung has the same genome. In a given tissue or organ, function and metabolism are the same in all the cells that constitute the tissue. Even if we assume that the DNA repair mechanism is at work in the cells, the question still remains why it fails in the initiated cell.

Do hormones have a role in carcinogenesis and growth of cancer?

Our premise is that to act on a body, environmental carcinogens should require a helper factor which should be intrinsic to the body. Such factor is essential because the carcinogenic substances in the environment are present in natural form and in very small quantity. At such concentration, the substances alone may not be effective as carcinogen. The factor being intrinsic to the body would explain the variations in the response of the people to the carcinogen. Since hormones have been shown to be involved in the development and growth of cancer, they can be the intrinsic factor for the environmental carcinogens to cause cancer in the body.

In view of this, our laboratory undertook studies to understand the role of hormones in the genesis and growth of cancer. Although there are several examples of hormonal induction of tumors [5, 6, 7, 8], we do not know how the tumorigenic action of hormones differs from their physiologic action. The same hormone that stimulates normal growth and differentiation in the target organ becomes tumorigenic to it.

What we know is that the target tissue develops into a tumor when there is hormonal imbalance resulting in a physiological stress (sustained stimulation) of the tropic hormone.

To understand this problem, it was conceived that the cell a culture system could be suitably used to translate this *in vivo* stimulation to study response of target cells to sustained hormonal stimulation at the cellular and molecular levels. The experimental model chosen for this was goat ovarian granulosa cells subjected to ovine luteinizing hormone (oLH, activity 1.19 units/mg) stress in cell culture, parameters being growth, function and receptor behavior. Granulosa cells in primary culture exposed once to LH are luteinized and therefore, differentiated. The cells may grow for a few generations, but they eventually die. However, if the hormonal stimulation is continued in the form of stress, the cultures acquire the capacity to multiply and grow into a cell line [9].

Several cell lines were developed by this system in our laboratory. The cultures received LH support up to passage 8 after which they became hormone independent. These cell lines were essentially contact inhibited primary cell lines with a finite life span. Biological and functional characterization of one of the cell lines (AIMS/GRXII) has been reported earlier [10]. However, three cell lines out of these attempts were spontaneously transformed. Two of them reverted. One of them, AIMS/GRXVIII, continued [11].

There are several tropic hormones for the granulosa cells besides LH. Human chorionic gonadotropin (hCG) is one of them. Though it is a human gonadotropin, it elicits response in non-human granulosa cells also [12]. Further, LH and hCG have common receptors on the target cells [13]. These considerations prompted us to find out if sustained stimulation by hCG also elicits the response in goat granulosa cells, similar to the one obtained by oLH.

The culture of goat granulosa cells is described earlier [9]. The cultures were treated with hCG at 25 iu/ml (activity 10,000 units/mg).The treatment schedule was followed exactly as for LH.

The hCG treated primary cultures attained confluency in 9 days and continued to grow up to passage 3. The cultures degenerated thereafter. The experiments were repeated several times and also were carried out by different workers at different times.

Thus, sustained treatment of the two gonadotropins, oLH and hCG elicit different proliferating responses in goat granulosa cells. As we have reported earlier and confirmed in the present studies, LH treated cultures grew into a primary cell line for 35 passages. hCG treated cultures did not grow beyond passage 3. This is interesting. Even though hCG and LH have common receptors, only LH could stimulate sustained cell proliferation.

These observations raised a few questions: Are the mechanisms of LH action to stimulate normal growth and differentiation, and to stimulate sustained cell proliferation different? Is stimulation of sustained cell proliferation not via known LH receptors? Why does hCG not stimulate cell proliferation? Extending a point a little, it may be said that LH may be preferentially activating an oncogene which hCG does not.

We have also observed in the course of our earlier studies that the LH receptors on the granulosa cells in culture were fully occupied in first or second passage of the cultures (Nishi Sharma, personal communication). Still the cells required LH support for proliferation till passage 8. If the hormone is withdrawn short of schedule, cultures cease to grow. This is suggestive of a possibility that the mechanism of LH action for cell proliferation does not involve known LH receptors. What is that mechanism? Are there more than one kind of receptor for LH and does hCG not have such double receptors?

These questions are basic and important to understand hormonal etiology of neoplasia. Further studies will contribute to elucidate some of the fundamental phenomena of signal transduction in cell biology or neoplasia.

Mutation

Though mutation is a theory of carcinogenesis, it is not yet possible to detect the mutation by relatively a direct method. To identify the mutated gene is a relatively difficult task [14, 15]. If we accept mutation as a biological method of carcinogenesis, we should get its mutated protein. Altered proteins are not always found in cancers [14]. Deletions are sometimes noticed. In breast cancer [16], retinoblastoma [15, 17, 18] and colon cancer [15, 17, 18], certain genes are deleted. Many laboratories are engaged in finding out if there is a new protein in the cancer cells, which does not exist in their normal counter part or in other cells of the body. Since altered or mutated proteins may not have an exclusive epitope, an altogether new protein is a proper candidate for an immunotherapeutic approach. A new protein means a new gene. It is not possible that a new gene will be added up to the existing genome unless the genes of any infecting organisms are incorporated into the genome. Such genes are already there in the form of protoncogenes, which are present in cancer as well as in the normal cells of the body. Therefore, there is no qualitative difference between cancer cells and normal cells. The differences are mostly quantitative. Many workers tried the approach of antibody–based therapies and claimed to identify cancer-specific anti-

gens. Unfortunately, none of these claims held up to careful scrutiny. The problem is that there is no common denominator for all cancers [19].

Immortalization

Cancer cells are said to be immortal. They are immortal because they do not age. Instead, they multiply unrestrictedly by geometric progression, provided the cells get minimum essential nutrients either from the host body when the tumor is in the organism or from the culture medium if the cells are grown in tissue culture *in vitro*. One cell divides producing two daughter cells in its place. And both the daughter cells retain the capacity to further divide.

Non-ageing of cells and unrestricted cell proliferation should be considered as two distinct phenomena. In adult individuals, cells of many organs do not multiply. But they do not age either as a consequence of non-multiplication. Then why are cancer cells called immortalized cells? They are called so, simply because the DNA of the parent cell is passed on to daughter cells with the assurance of heritability of the neoplastic character.

Thermodynamically, immortalization of cells is untenable even for an open system. Organisms, although in a dynamic state of equilibrium with their environment, go into senescence. Cancer is an open system exchanging matter and energy with the host. Like any other multicellular organism, it must die. But it shows no senescence. Unlike its host, it has an ability to continuously utilize precursors and energy from the host for its growth. Only when the supply of the requirement is stopped (by way of the death of the host) cancer cells die. How can the sustained growth or immortality of cancer be thermodynamically explained. Is it that its free energy does not decrease? If disorganized cell proliferation is considered to be disordered, then we may say entropy is increased. But if we take cell generation as ordered, then free energy should increase, and entropy decrease.

Entropy in the host is caused by the sustained growth of cancer. If cancer and its host are considered as one system, the cancer growth may be said to be obeying the thermodynamic principle. Yet the question remains unanswered why organisms go into senescence and cancer does not. Is perhaps genetic control above physical laws?

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