*J. theor. Biol.* (1996) **178,** 375–380

# Tumour Progression: Random Mutations or an Integrated Survival Response to Cellular Stress Conserved from Unicellular Organisms?

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(*Received on* 28 *June* 1995; *Accepted on* 15 *September* 1995)

The current paradigm states that cancer progression is caused by random independent mutations, each selected for its survival advantages. The accelerated rates of phenotypic changes, the pleiotropic effect of several genes involved in progression—which need not be necessarily mutated for inducing the observed changes in cancer cell behaviour—lead us to propose an alternative hypothesis. Malignant progression might be a result of the unveiling of a cell-survival program, induced by various aggressions in the same way as the SOS system is induced and regulated in bacteria. This hypothesis depends on the homology between several genes involved in cancer progression (such as bcl2, mdm2, the mismatch repair genes, the heat shock protein genes, the pleiotropic resistance genes, the telomerase gene . . .) and several genes involved in the survival of prokaryotes and eukaryotes under stress. The development of multicellular organisms could not take place without the building of a control program, exemplified by the so-called anti-oncogenes. However, this control program had to integrate some weaknesses, in order to allow for embryogenesis, growth, and wound healing. These weaknesses, neutral from an evolutionary point of view—since most cancers are sporadic and kill their hosts long after the birth of the offspring—are exploited by the survival program of individual cells, inherited from the genome of prokaryotes and unicellular eukaryotes, and repressed but not suppressed in animals. If this theory is true, it is probable that (i) no anti-oncogenes will be found in unicellular organisms, (ii) the sensitivity to mutations will be higher in genes involved in proliferation and in anti-oncogenes such as p53 and Rb, than in genes not involved in the cancer process, (iii) a process of transfer of genetic information exists in cancer cells as it exists in bacteria. The identification of the genes governing the survival program could lead to new therapeutic approaches.



## Introduction

Despite new drug modalities and strategies, the war against cancer has not improved significantly. Aggressive tumours kill their host after a more or less prolonged survival. The life expectancy of a patient presenting with one of the major epithelial cancers has increased over the years, and the disease progression may have been slowed down by various therapies, but the mortality remains relatively constant and the evolution under treatment is entirely predictable and implacable (Schipper *et al*., 1995). This is why the medical oncologist, after 35 years of therapeutic trials, is beginning to question the paradigm that cancer is a blind process, because of random mutations appearing at any time in random order and selected for the unpredictable advantage that some offer at the time of occurrence (Nowell, 1976; Cairns, 1975). The formidable survival efficiency, conferred in every case by the genetic and phenotypic changes that accumulate in cancer cells during their malignant progression, may be viewed as the result of combined and integrated genetic responses, induced by damaging or threatening environmental events which are in themselves not necessarily mutagenic. A fully malignant cell overcomes all these events, including those induced by the therapist. Malignant progression in this view is the process by which the progeny of

0022–5193/96/040375 + 06 $18.00/0

transformed cells will survive and proliferate despite any additional obstacle, in conformity with a program. Such a theory is compatible with Darwinian evolution: abnormal cells have inherited the genes of prokaryotes and unicellular eukaryotes (Margulis, 1970; Schwartz, 1978) and may have conserved the inducible pleiotropic responses reminiscent of such systems as the SOS regulon (Sedwick & Yarranton, 1982). The control program resulting in the possible development of multicellular organisms can be de-repressed for different reasons that will be reviewed below. Once this multilevel control has been de-repressed the transformed cells behave in the host as bacteria and unicellular eukaryotes faced with environmental aggressions, such as physical and chemical changes following a meteoritic impact. This behaviour, responsible for the success of unicellular organisms for 3.5 billion years, is what we call cancer in animals.

## About the Control Program

Biologists have unveiled some of the mechanisms by which tissue size and function are maintained through embryogenesis and growth and through an adult life where various injuries including large wounds, are repaired and healed. A primary level of control of cell proliferation already exists in unicellular eukaryotes. It includes the complex machinery of cyclins, cyclin-dependent kinases and cdk inhibitors needed to regulate proliferation in a changing world (Murray & Hunt, 1993; Nurse, 1990; Norbury & Nurse, 1992; Nobori *et al*., 1994). However, in higher organisms, evolution has added several levels of regulation and control, including so-called tumour suppressor genes (Weinberg, 1991; Hollingsworth *et al*., 1993; Friedberg *et al*., 1995; Levin *et al*., 1991) with the paradigmatic examples of p53 and Rb. In addition, nm23 (Steeg *et al*., 1988) and signals from the surrounding cells and cell adhesion molecules (Trosko, 1987; Barritt, 1992; Paulsson, 1992; Burridge *et al*., 1988; Bates *et al*., 1994) should also be included, with the significant example of teratocarcinoma cells participating in the development of a normal adult when inserted into a normal mouse blastula (Mintz & Illmensee, 1975). The third level of control includes endocrine signals, hormones and negative factors such as TGF$\beta$ (Slingerland *et al*., 1994; Koff *et al*., 1993). The integrated way in which all these signals come into play, depending on local situations and environmental changes, in order to maintain tissue homeostatic conditions, deserves the name of a program. Such a program, however, had to have built-in weaknesses, in order to allow for embryogenesis, growth, wound healing etc. Allowing temporary and controlled derepression, was compatible with repression, but not suppression of the cellular response to stress inherited from unicellular organisms.

## Phenotypic Changes and Consequences of Malignant Progression

The phenotypic changes observed in a tumour cell that has reached full malignancy and has become capable of surviving multiple combined threats to its proliferative integrity are summarized here. Tumour cells escape differentiation induced by paracrine and endocrine signals (Sporn & Roberts, 1983). They also escape apoptosis normally induced when DNA damage cannot be properly repaired (Reed, 1993). They acquire motility (Liotta *et al*., 1986) and invasiveness (Liotta & Stetler-Stevenson, 1991) which allow them to reach capillaries and metastasize (Sastre-Garau, 1994; Israel, 1994) and they find their way in blood vessels, acquire deformability which allows survival despite their journey through the lungs and survive changes in acidity and oxygen partial pressure (Alexander, 1985). Attacks by natural killer cells and monocytes are resisted by various mechanisms (Israel *et al*., 1980, 1982, 1984; Samak *et al*., 1982) and tumour cells can induce suppressor T cells. They are able to aggregate platelets (Blood & Zelter, 1990) and to retract endothelial cells in their way towards other organs. They express autocrine growth factors and receptors to hormones and growth factors which are unexpressed by their normal counterparts (Israel, 1984; Aaronson, 1991). By several mechanisms they are, or rapidly become, resistant to various toxic and cytostatic drugs as well as to free radical and ionizing radiations (Fojo *et al*., 1987; Chin *et al*., 1993; Hall, 1986; Haimovitz-Friedman, 1991; Kramer *et al*., 1990). In addition, they induce surrounding fibroblasts and macrophages to cooperate in the tumour growth process by secreting proteases (Zucker & Biwas, 1994), growth factors (Adam *et al*., 1994), prostaglandins that facilitate proliferation (Israel, 1990) and angiogenic agents (Folkman *et al*., 1989). The tumour cells reach the vessels by the 20th doubling (1 mm in diameter, $10^6$ cells) and start to metastasize. By the 30th doubling (time of detection, 1 cm in diameter, $10^9$ cells in the primary) thousands or millions of microscopic metastases already exist in normally aggressive tumours and will exhibit resistance to diverse therapeutic modalities. As previously discussed these tumour cell characteristics are entirely predictable. They take place in a relatively short time, 10–20

doublings, the doubling time being on the average 100 days at the time of detection and much shorter before, according to the Gompertzian equation that defines the growth curve (Israel & Chahinian, 1976). Taking into account the lengthening of the cell generation time from the beginning, where it is around 24 hr, it can be calculated (personal estimation) that all the changes mentioned above will take place in 500–1000 generations.

## Genetic Events of Malignant Progression are Compatible with a Non-Random Process

The time in which the genetic events that govern all the phenotypic changes that accumulate in a fully malignant cancer cell is short and requires a high genetic instability (Israel, 1990; Usmani, 1993). The mutations registered in the genes responsible for mismatch repair (Loeb, 1994; Palombo *et al*., 1994; Friedberg *et al*., 1995) which may occur not only in hereditary cancers but also in some sporadic tumours, may allow for such an instability and the shortness of time may also be compensated by inherited mutations or deletions in the tumour suppressor genes (Weinberg, 1994). However, even if mutations are, in a majority of cases, the first transforming events, it does not follow that all the ensuing genetic events are random or that they necessarily consist of mutations.

(i) All carcinogens are not mutagens. Normal cells in culture may undergo transformation if submitted to various constraints, such as preventing their confluence, thus suppressing cell–cell communications (Parodi & Brambilla, 1977; Parshad & Sandford, 1968; Rubin, 1980; Chowo *et al*., 1994). However, it must be acknowledged that the human genome is more resistant to transformation than the murine genome. It would be most interesting to investigate the spontaneous transformation of normal human cells under the same conditions.

(ii) Cells undergoing mutations by contact with an initiating agent, may not display any phenotypic change for long periods of time before a promoter agent, without mutagenic power in general, is repeatedly applied (Delclos *et al*., 1980; Parson *et al*., 1995).

(iii) Genetic instability, responsible for random mutations, should induce a number of neutral mutations, a fact that has not been substantiated. Almost all paraneoplastic syndromes observed in human tumours are caused by biologic products that play a role in tumour growth (Israel, 1991).

(iv) As mentioned before it is well established that mutations in genes which are involved in proliferation (Weinberg, 1982), or in anti-oncogenes, induce transformation and malignant progression. However, it is also well established that unmutated genes, when activated and amplified or overexpressed, may largely contribute to cancerous behaviour in cells through a network of pleiotropic effects. For example, bcl2, when activated by an oxidative stress, inhibits apoptosis of damaged cells (Hocken-Bery *et al*., 1993), whereas the same aggression induces a loss of adhesion of the cells to the basement membrane and enhances experimental metastases (Kundu, 1995), a process that requires the activation of a number of other genes. Another example concerns the heat shock proteins, inducible by different kinds of physical and chemical aggressions, and whose activation induces, for example, mdr, one of the genes involved in pleiotropic resistance against toxic agents (Osterreich *et al*., 1993; Fuller *et al*., 1994; Lindquist & Craig, 1988). Along the same lines, the amplification of mdm2, a gene overexpressed in several human tumours, induces the inhibition of p53 (Momand *et al*., 1992). Amplification of mdm2 takes place with the amplification of GLI (a gene which has transforming activity), and CD4 (a specific kinase for D cyclins, Khatib *et al*., 1993). It is also interesting to mention that cancer cells express the telomerase gene which is repressed in almost all normal animal cells but expressed in all unicellular organisms, and which ensures an unlimited posterity (Kim *et al*., 1994; Takoda *et al*., 1992). In addition, a recent study of progressive DNA alterations in apparently normal breast reveals that changes leading to a premalignant phenotype progress in a non-random fashion (Malins *et al*., 1995).

These facts taken together favour the hypothesis that damaging events, or events which are a threat to cell survival and function, may, whether mutagenic or not, induce a cascade of genetic responses which overcome the consequences of the initial damage and render the cell capable of dealing with several kinds of unrelated threats. The cancer phenotype is very similar to a survival phenotype. This behaviour is certainly facilitated by mutations in a few genes, but the monotonous and identical, rapidly instaured pleiotropic set of events that leads to this phenotype, seems to be of some other nature than random.

## Similarity of the Genetic Events Observed in Cancer Cells, Bacteria and Unicellular Eukaryotes Under Stress

It was indicated above that multicellular organisms had to inherit the genome of their unicellular

ancestors. The genes that participate in malignant behaviour such as bcl2, heat shock genes, mdm2, genes of the mdr family, telomerase, genes involved in mismatch repair and the mutator phenotype etc, all have homologues in bacteria and mostly in yeasts (Langridge, 1991; Fojo, 1987; Chin, 1993; Friedberg, 1995).

But it is also known that bacteria possess an integrated and inducible defence system, the SOS regulon (Friedberg *et al*., 1995) which works like a real program of response to stress situations. It is made of several genes and though activated by different kinds of threats or damage, it depends on the activation of a single protein which inhibits a repressor shared by all the genes involved, so that any threatening event taking place in the environment will induce the whole system (Horri *et al*., 1981). Its activation leads to several kinds of responses, involving mitotic delay, decrease in cellular metabolism and reinitiation of DNA replication even if aberrant. This "error prone" part of the system, that introduces errors during mismatch repair rather than letting damaged cells die, has ensured the maintenance of unicellular life through oxygen changes in the atmosphere, volcanic eruptions, meteoritic impacts and climatic upheavals for 3.5 billion years. The similarity of the genes involved in cancer and in the defence program of bacteria and eukaryotes against unfavourable changes, and the similarity of their integrated functioning, again lends support to the hypothesis presenting cancer as a set of responses to danger, inherited from our unicellular ancestors. In addition, it seems in order to question the applicability to cancer of the well-established Luria theory (Luria & Delbrück, 1943) according to which resistance in bacteria is a result of the selection of random mutations. This may be true for micro-organisms at rest in a non-hostile environment, a situation in which random-point mutations may explain the selection of a few individuals. But we have seen that under stress, bacteria put at work an inducible system. Similarly cancer cells already under stress because of the first transforming events, do not respond like naive bacteria but rather like a colony facing severe and sustained threats, by unveiling their defence program of coordinated phenotypic changes.

## About the Place of Cancer in Animal Evolution

From the point of view of a species, a disease that kills its host after his or her offspring are able to survive without the parents is neutral. In that sense, the building of a control program which is able to stand a few decades against destabilization and damage is a good compromise between the inheritance of a unicellular defence program, and the necessity of allowing for evolution on the one hand, and the necessity of maintaining homeostatic conditions and a predictable behaviour on the other. Even in the case of inherited genetic predisposition to cancer, a large majority of the cases meet the conditions. Cancer and leukaemia in childhood are, fortunately, rare but they constitute a failure of the system. The longer life expectancy now enjoyed by our species is recent; a few millenia ago, most people would die before any cancer became apparent.

## Predictions and Questions

If the theory proposed in the present paper is correct it can be predicted that no homologue of anti-oncogenes will ever be found in unicellular eukaryotes (with the exception of cyclin inhibitors). It is also predictable that some genes found to be expressed in several animal cancers, such as clusterin (French *et al*., 1994) and pleiotrophin (Fang *et al*., 1992) have homologues in unicellular eukaryotes. However, although no inducible system such as the SOS has been observed in animal cells to date, it seems probable that some integrated genetic system exists. A question that should be addressed, is, what is the exact status of the gene USP in *E. coli* (Nystrom & Neidhardt, 1992, 1994) and does a homologue of this gene exist in higher organisms.

Now a few questions directly related to this present theory. If cancer is indeed the inevitable consequence of a mechanism of unicellular survival, built by trial and error through the times by Darwinian evolution, would it be possible that a particular genetic instability in certain genes plays a part in this mechanism? In other words, would it be possible that such genes as Rb, and p53 with its "hot spots" and its slow repair (Service, 1994) be more prone than others to mutations, thus unveiling without delay the defence program? Could the susceptibility to sporadic cancer be related to such a hypermutability in some individuals? Probing the question of genetic instability in cancer cannot avoid studying these problems unorthodox as they may seem. Another unorthodox question that needs to be asked: could it be that cancer cells may regain the property of several prokaryotes of exchanging DNA, as it has been sometimes advocated (Cadman *et al*., 1986)? Could this be a mechanism which is able to reinforce the cooperation between cancer cells and to accelerate progression? If the answer to these questions is yes, the present hypothesis would received strong support.

## A Word on Cancer Therapy

In the future, it may be that some measures like anti-angiogenesis or gene therapy will prove effective. In the meantime, therapists should give their attention to the dark side of our current paradigm, according to which an oncologist has to kill a maximum of cancer cells again and again, with second and third line cytostatic combinations when the first one has failed, and measure the duration of response. A response that gets shorter and shorter with time because of the fact that cancer cells surviving a given therapy become more resistant, more aggressive and more malignant. In convergence with Schipper (1995), one is led to advocate controlled studies of different measures less aggressive to cancer cells (such as differentiating agents, growth factor deprivation, inhibition of oxidative stress, metabolic intervention through omega 3 fatty acids etc.) with the objective of rendering a tumour compatible with the prolongation of a relatively normal life. Killing the last cancer cell without killing the host is an objective that has not yet been reached. Cancer cells are endowed with the same resistance and survival abilities shown by unicellular life under stress through geological times and catastrophes.

I wish to express my thanks to François Jacob, Bernard Kerdelhue, Gérard Milhaud, Ruth Sager and Guy de The for their helpful criticisms.

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