Cell Transformation and Radiation-induced Cancer

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Preface

The transformation of mammalian cells *in vitro* provides quantitative and qualitative information on the processes by which physical and chemical agents induce malignancy. Radiation-induced cancer is a major concern in radiological protection and cell transformation systems offer a complementary research pathway to epidemiological studies of exposed human populations and to animal experiments. Results from cell transformation research can provide information about the shape of dose-effect relationships, the role of dose rate and radiation quality, and the modifying effect of agents present during and after radiation exposure. Data can be obtained on initiation and promotion in the cancer process.

In 1985 a workshop was organised on *Cell Transformation in Radiobiology* which reviewed the then existing data on radiation-induced cell transformation but the proceedings were, unfortunately, never published. Since then considerable progress has been made especially on the development of new cell transformation systems more relevant to human cancer and on the role of genetic factors, such as oncogenes and suppressor or anti-oncogenes in cancer. In addition much more information is available on the effect of dose, dose rate, radiation quality and modifying factors on the frequency of transformation in the more 'classical' cell transformation systems.

The Radiation Protection Programme of DGXII of the Commission of the European Communities, the Office for Health and Environmental Research of the United States Department of Energy and the Nuclear Energy Board of Ireland organised a workshop on *Cell Transformation Systems relevant to Radiation-induced Cancer in Man* in Dublin from 4–7 April 1989 to provide a review of the current research and to encourage collaborative interactions between scientists working in the field. This book presents the proceedings of that workshop which should be of interest to cell biologists, oncologists, radiobiologists, biophysicists, cytologists, chemical toxicologists as well as others concerned with the scientific background to radiation-induced cancer.

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Chapter 1

An argument for using human cells in the study of the molecular genetic basis of human cancer

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Introduction

In the past several years we have undergone a revolutionary change in our understanding of the genetic basis of cancer and the molecular genetic analysis of human malignancies. These advances have included the identification of a large number of putative oncogenes with diverse functions and the beginnings of an understanding of the phenomenon of tumor suppression and the role that tumor suppressor genes play in the control of malignant expression.

Much of what we know of the role of oncogenes in neoplastic transformation has been derived from experimental model systems that utilize cultured rodent cell lines. The reason for this, in part, is because cultured normal human diploid cells are remarkably resistant to transformation by oncogenes and other carcinogenic agents.

In this presentation I wish to highlight the problems of relying solely on rodent experimental systems and discuss the pitfalls of extrapolating from rodent systems to the human situation in the absence of experimental data. I shall also indicate the existence of human cell systems that are now amenable to study neoplastic transformation.

Somatic Cell Hybrids and the Analysis of Malignancy

Two major advances have aided greatly in the analysis of the genetics of human malignancy, namely the development of somatic cell hybridization experiments and the discovery of human oncogenes. These experimental approaches have identified two seemingly different components of the genetic alterations that contribute to the cancerous state. First, the discovery of human genetic elements - termed oncogenes - that are capable, via DNA transfection, of transforming certain rodent cell populations into tumorigenic cells (Cooper, 1982; Weinberg, 1981) has led to an explosion of scientific inquiry into the role these genes play in the neoplastic progression leading to human tumors. The understandable excitement accompanying these studies has led more than one investigator to claim that cancer can be explained by the action of "dominantly-acting" oncogenes alone, and has even led to suggestions that the molecular details of carcinogenesis should be largely worked out within a few years. However, there is some evidence

which seemingly contradicts the dominant oncogene hypothesis. Somatic cell hybridization experiments, which preceded the discovery of oncogenes, revealed that the cancerous phenotype could be suppressed by the introduction of normal genetic information via whole-cell fusion (Harris, 1971; Stanbridge 1976).

Harris, Klein and their colleagues (Harris, 1971) in an extensive and critical series of experiments, demonstrated that when malignant mouse cells were fused with non-malignant mouse cells, the resulting hybrid cells were unable to form tumors, ie the tumorigenic phenotype was suppressed - a finding contrary to that of the concept of "dominantly-acting" oncogenes.

The early studies using intraspecies rodent cell hybrids and interspecies rodent x human cell hybrids were complicated by the rapid emergence of tumorigenic segregants. This was due to the chromosomal instability of the hybrid cells resulting in rapid chromosome loss presumably including those chromosomes containing "tumor-suppressor" genes that suppressed tumor formation.

This rapid chromosome loss, in addition to making the initial premise of suppression of malignancy hard to evaluate, renders the identification of specific chromosomes which possibly control the expression of the tumorigenic phenotype an extremely arduous task. Several years ago we re-examined the question of the genetic control of malignancy using intraspecies human cell hybrids. In this case, hybrid cells derived from the fusion of malignant HeLa cells with normal human diploid fibroblasts showed complete suppression of the tumorigenic phenotype (Stanbridge, 1976). The suppression of malignancy was extremely stable and tumorigenic segregants arose only rarely (Stanbridge et al., 1982). Thus, this approach, using intraspecies human cell hybrids, generated a stable genetic model with which one could look for the genetic factors which modulated malignant expression. The key to the stability of expression of the relevant phenotypic traits of these human somatic cell hybrids was their extreme chromosome stability.

Specific Chromosomes Associated with Control of Tumorigenic Expression

As mentioned above, the initial suppression of tumorigenicity following cell fusion is often followed by reexpression of this trait. Such changes are in consonance with the notion that genetic information from the normal parent initially represses tumorigenic expression. When this information is lost from the hybrid cell (perhaps as a consequence of chromosomal nondisjunction at mitosis), this genetic control is removed, thereby leading to the reexpression of tumorigenicity.

This provides an opportunity to associate the critical growth regulating information with karyotypically identifiable chromosomes, whose loss is repeatedly connected with reexpression of tumorigenicity. In this way, mouse chromosome 4 has been implicated in the control of malignant expression of a number of different mouse tumors (Evans et al., 1982), and human chromosome 11 in control of the neoplastic expression of hamster x human hybrid cells (Klinger, 1982). In intraspecies human cell hybrids, cytogenetic analyses implicate

chromosome 11 and chromosomes 1 and 4 in control of tumorigenic expression of HeLa x normal fibroblast and HT1080 fibrosarcoma x normal fibroblast hybrids, respectively (Stanbridge et al., 1981; Benedict et al., 1984). In certain cases the cytogenetic data have been reinforced by restriction fragment length polymorphism (RFLP) analyses using molecular probes that map to specific chromosomes. Thus, it would seem that multiple cancer-controlling genes exist which map to several different chromosomes.

Suppression by Single Chromosome Transfer

The suggestion that single chromosomes contain the genetic information required for control of neoplastic expression represented a welcome decrease in complexity from that which would prevail if genes on multiple chromosomes were required in concert. However, the evidence for such a role of single chromosomes was based only on indirect evidence correlating chromosome loss with reversion to tumorigenicity. A direct test of this model came from an ability to transfer single chromosomes individually from normal cells into cancer cells and to examine their effect on the tumor-forming properties of these recipients.

Such single chromosome transfer was made possible by the technique of microcell transfer (Fournier and Ruddle, 1977; Saxon et al., 1985). Briefly, proliferating cells are exposed to colcemid over a period of several days. The cells are initially blocked in metaphase but a significant proportion escape the block and enter G1. However, because there is no spindle formation, the chromosomes condense as individual units resulting in a multinucleate cell containing large numbers of micronuclei, each nucleus containing from one to a few chromosomes. Such cells are then enucleated with a combination of cytochalasin B and centrifugal force, resulting in a pellet of microcells each containing one or several chromosomes. The microcells are then fused to the recipient cell of interest. The obvious difference between this strategy and whole cell fusion is that only one or, at most, a few chromosomes are transferred. It is important to note that, as with whole cell fusions, the transferred chromosome is retained in succeeding generations as a complete structural unit. A further criterion for successful implementation of this technique is the requirement for dominant selectable markers which map to the chromosome interest. This is accomplished by the transfer and integration of such markers (eg the neomycin resistance genes) into individual chromosomes via DNA transfection or retroviral infection. Their presence makes it possible to select for recipient cells that have acquired the appropriate donor cell chromosome.

Using this technique it has been possible to show that transfer of normal fibroblast chromosome 11 into tumorigenic HeLa x fibroblast hybrids results in suppression of tumor-forming ability (Saxon et al., 1986). Transfer of the control chromosome X had no effect on tumorigenic behavior. Transfer of chromosome 11 into a Wilms' tumor cell line also resulted in suppression of tumorigenicity, whereas transfer of other chromosomes had no effect (Weissman et al., 1987). These results indicate that human chromosome 11 carries one or more genes expressing strong tumor-suppressing abilities.

Oncogene Expression and Suppression of Tumorigenicity in Hybrid Cells

When the first oncogenes were discovered by DNA transfection studies in mouse NIH3T3 cells (Krontiris and Cooper, 1981; Shin et al., 1981), there seemed to be a tacit assumption that a single, dominantly acting cellular gene was capable of transforming a cell into a cancerous state. This is a result that would be incompatible with both somatic cell hybridization data and epidemiological studies of human cancer. It was subsequently found that when primary or secondary cultures of rodent embryo cells were used in transfection assays, the cooperative action of two oncogenes was required to transform the cells (Land et al., 1983). This multigenic interaction most closely approximates the conditions necessary for the multistage progression of cancer that has been evident from epidemiologic and experimental systems (Foulds, 1975; Barrett and Ts'o, 1978). Regardless of the number of oncogenes required, these and many other studies indicate that the cellular oncogenes are "dominantly acting". Equally clearly, most of the evidence from somatic cell hybrid experiments suggests that the tumorigenic phenotype can be suppressed by the introduction of normal genetic information. Thus, it is important to resolve the paradox between "dominantly acting oncogenes" and "tumor-suppressor genes" capable of suppressing the tumorigenic phenotype. The extensive studies using HeLa as the malignant parental cell described above are uninformative in this regard, since no activated oncogene has been identified in this cell, and the DNA extracted from HeLa does not transform NIH3T3 cells in transfection assays.

In order to investigate this apparent paradox the human bladder carcinoma cell line EJ, which contains an activated c-Ha-ras gene was fused with normal human diploid fibroblasts. The DNA extracted from EJ cells is capable of transforming mouse NIH3T3 cells in a transfection assay. In these experiments, all of the EJ x fibroblast hybrid cells studied failed to form tumors in athymic mice, although the cells behaved like transformed cells in culture (Geiser et al., 1986). Tumorigenic segregants were isolated from the suppressed hybrids that had regained full tumorigenic potential. These hybrid cells were examined for expression of the product of the c-Ha-ras gene (a p21 protein), both at the messenger RNA level via Northern blot analysis and by immunoprecipitation of the p21 protein. A similar result was found, namely, that the nontumorigenic and tumorigenic segregant hybrid cells express the same level of p21 protein. In this case, because the p21 protein product of the activated c-Ha-ras migrates more slowly than that of the normal p21 protein in polyacrylamide gels, it was possible to confirm that the high expression of p21 was that of the activated oncogene product. Further gene copies of the activated c-Ha-ras were transfected into the nontumorigenic EJ x fibroblast cells to see whether an enhanced expression of the activated p21 protein would lead to tumorigenic expression. Again, all the hybrids containing multiple copies of the activated c-Ha-ras gene remained nontumorigenic in spite of the elevated expression of p21. Thus, suppression of the tumorigenic phenotype was accomplished in the presence of continued expression of the activated c-Ha-ras oncogene.

There could obviously be a number of explanations for these results; however, a very likely explanation is that although the activated ras oncogenes seem to be late acting and their products crucial for

neoplastic expression of transfected mouse cells, their expression in human somatic cell hybrids, although dominant, is insufficient to endow the cells with tumor-forming ability. This is not to suggest that the activated ras oncogenes play no role in the progression to tumorigenicity of the EJ bladder carcinoma. It should be remembered that the nontumorigenic hybrid cells still behave like transformed cells in culture. Thus, in the cancer cells, where these activated oncogenes originate, they may play a role in some earlier stage of neoplastic progression.

Disparities between Rodent and Human Cell Experimental Models

Reliance on data from experimental models that utilize cultured rodent cells for extrapolation to the human situation of cancer without experimental support has led to serious errors of interpretation. Two examples will serve to illustrate this point.

Several years ago the correlate between anchorage independent (AI) growth and the tumorigenic phenotype was established in rodent cells (Freedman and Shin, 1974; Shin et al., 1975). Although there were certainly exceptions to this rule the correlate in general held up and allowed for selection of neoplastically transformed cells by growth in soft agar. However, the situation is not the same with cultured human cells. It was initially shown with human somatic cell hybrids that there was no correlation between AI growth and the tumorigenic phenotype (Stanbridge and Wilkinson, 1978). Later, it was also shown that normal human diploid fibroblasts are capable of AI growth when cultured in the presence of high levels of bovine serum (Peehl and Stanbridge, 1981). In spite of these reports there was a flurry of studies claiming neoplastic transformation of human fibroblasts by ionizing radiation, chemical carcinogens and oncogenes using AI growth as the selective measure (Milo and DiPaolo, 1978; Maher et al., 1982; Stevens et al., 1988; and Sutherland et al., 1980). It was later shown that not only were fibroblasts treated in this manner not neoplastically transformed they also were not immortalized! More recently, careful control of the parameters associated with AI growth of human fibroblasts suggests that it may be useful for indicating certain parameters of transformation in human cells (McCormick et al., 1985; McCormick and Maher, 1988). However, it is an experimental measure that is to be used with great caution in evaluating neoplastic transformation of human cells.

A second, more egregious and flawed extrapolation is that dealing with the role of activated oncogenes in human cancer. The earliest studies with human activated oncogenes were performed using cultured mouse 3T3 cells and rat embryo fibroblasts transfected with activated oncogenes derived from human cancer cells. The interpretations of these experiments began with the hypothesis that a single dominantly-acting oncogene was capable of causing cancer, later modified to two or more cooperating oncogenes (Land et al., 1983). The direct test of such an hypothesis is, of course, to transform normal human cells with such activated oncogenes. Here the extrapolation falls down. Despite intensive efforts to transform normal human diploid fibroblasts or epithelial cells with varying combinations of activated cellular oncogenes the efforts have been uniformly unsuccessful (Sager et al.,