RADIO-INDUCED CANCERS

Study on Cell Cultures

Like other physical or chemical agents, ionizing radiation can induce cancer. Research on cell in culture, as a complement to clinical, epidemiological and in vivo studies, helps us determine how and at what level an agent may intervene - directly or not - in the process of carcinogenesis, and so evaluate the risk incurred by exposure to that agent.

A. Gonin/CEA

Fluorescence microscope in use at CEA Fontenay-aux-Roses for the chromosome «painting» technique.

Epidemiological surveys show that certain substances (tobacco, asbestos, various chemicals, etc.) or physical agents (e.g., ultraviolet rays, **ionizing radiation**) are able to induce cancers. However, the data are most often incomplete, especially those concerning the evaluation of risk as a function of **dose** or exposure duration.

It is therefore necessary to rely on experimentation to support or complete the results of epidemiological surveys, varying the conditions of exposure to radiation (dose, **dose rate**, etc.), and associating other chemical or physical agents which can modify the response in order to evaluate the influence of the different factors on the incidence of cancer. Experiments can be carried out ***in vivo*** on animals exposed to a potentially carcinogenic agent, and then scoring the number of cancers occuring. However, such studies are rather costly and time consuming, because in principle the animals have to be monitored throughout their lifespan, which for rodents is two to three years. Easier experiments can be performed ***in vitro***, on **cell** cultures. They are the so-called tests for *cell transformation* (box).

Risk estimate can be achieved by a direct quantitative approach, but for small doses such an approach remains limited in scope owing to statistical difficulties, e.g., spontaneous incidence, population sizes submitted to each dose range, etc. A complementary approach, developed in parallel, focuses on the mechanisms of **carcinogenesis**, knowledge of which should enable us to determine whether a particular chemical or physical agent is able to participate in the process of cancer formation and at what level. The risk incurred by exposure to that agent can thereby be assessed. This research is conducted *in vivo* in animals and *in vitro* in cell cultures. An advantage of the *in vitro* models is that

they make it possible to study steps by steps the process without having to use a large number of animals.

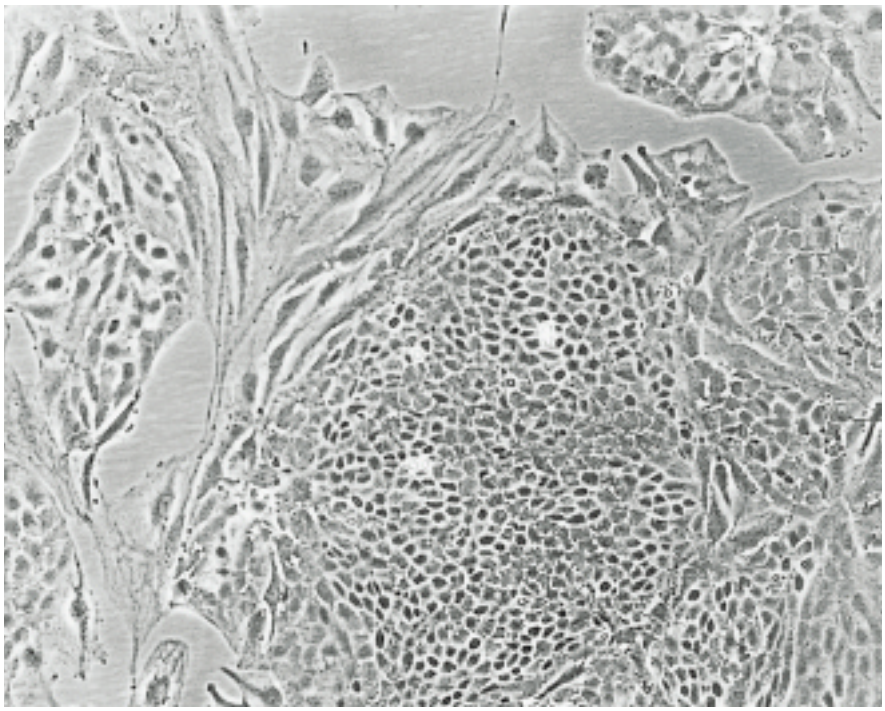
What is a cancerous cell?

In tumors, cells take on a different appearance from normal cells. They display a more or less complete loss of functional characteristics (secretion, absorption, etc.). Also, cancerous cells are able to accomplish a practically infinite number of cell divisions, whereas normal cells grow **senescent** after a limited number of. In addition, the time for a cancer cell population to double in size is shorter than for normal cells (box D, ***The cell, the essential link***, and box E, ***The cell cycle, duplication under control***). Finally, they can leave their original tissue, migrate and give rise to another tumor, a process called metastasis.

In culture, beside the characteristics described above, cancer cells differ from normal ones in their marked autonomy with regard to external conditions. They tolerate a greater cell density and are less sensitive to the composition of the culture medium. They are independent of the support and do not need to anchor themselves to the bottom of the culture flask to proliferate. The tests for evaluating cell transformation are based on these criteria. Finally, if they are able to induce a tumor when injected into an animal, they can be considered as fully transformed : the cells are then said to be **tumorigenic**.

Cell culture

Cells when in culture flasks adhere to the bottom. The culture medium is changed regularly to avoid nutrient deficiencies and (or) toxic effects due to the accumulation of waste eliminated by the cells. The cells divide, and their numbers increase, spreading gradually to cover the whole of the bottom of the flask. They are then said to be *confluent*. At this moment they must be subdivided, i.e., released and reseeded at a lower concentration : this operation is called a **passage**. When cells are established in culture, they form a cell **line**.



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Cells undergoing transformation (foci in the center of the picture).

Cancer cells almost always have an abnormal **karyotype**. In some rare types of cancer specific rearrangements are observed, in particular a rearrangement of one **chromosome** with another. More generally, karyotype anomalies consist in both a modification of the total number of chromosomes, and imbalances, i.e., a reduction or an increase in the relative numbers of segments or whole chromosomes (see *Chromosomal Instability*). It is important to mention here that each type of tumor exhibits a characteristic chromosomal rearrangement profile, although not all the modifications are necessarily observed in any one patient, and tumors from different tissues can display varying degrees of analogy.

At the scale of the **gene**, cancer cells show various modifications, such as overexpression of **oncogenes**, i.e., genes that favor cell transformation, and loss or **mutations** of **anti-oncogenes**, i.e., genes that oppose it. These changes in the **genome** are accompanied by non-genetic modifications, in particular **metabolic** modifications, which certainly contribute to the carcinogenic process, although the mechanisms remain unknown.

Effect on the transformation of already immortalized cells

A great many studies have been conducted on immortalized mouse or hamster cells grown in cell lines. These cells are irradiated and then seeded at low density. After a certain time, set at six weeks to standardize the protocol, cell foci displaying certain morphological characteristics are scored.

A higher cell density reflects a proliferative advantage. The probability or incidence of transformation is calculated by dividing the number of foci either by the number of irradiated cells, or by the number of surviving cells. The incidence of transformation per irradiated cell increases with dose up to a maximum, and then falls off, as at high doses (greater than 2 Gy for gamma rays), a high proportion of cells fail to survive. In contrast the incidence of transformation per surviving cell increases regu-

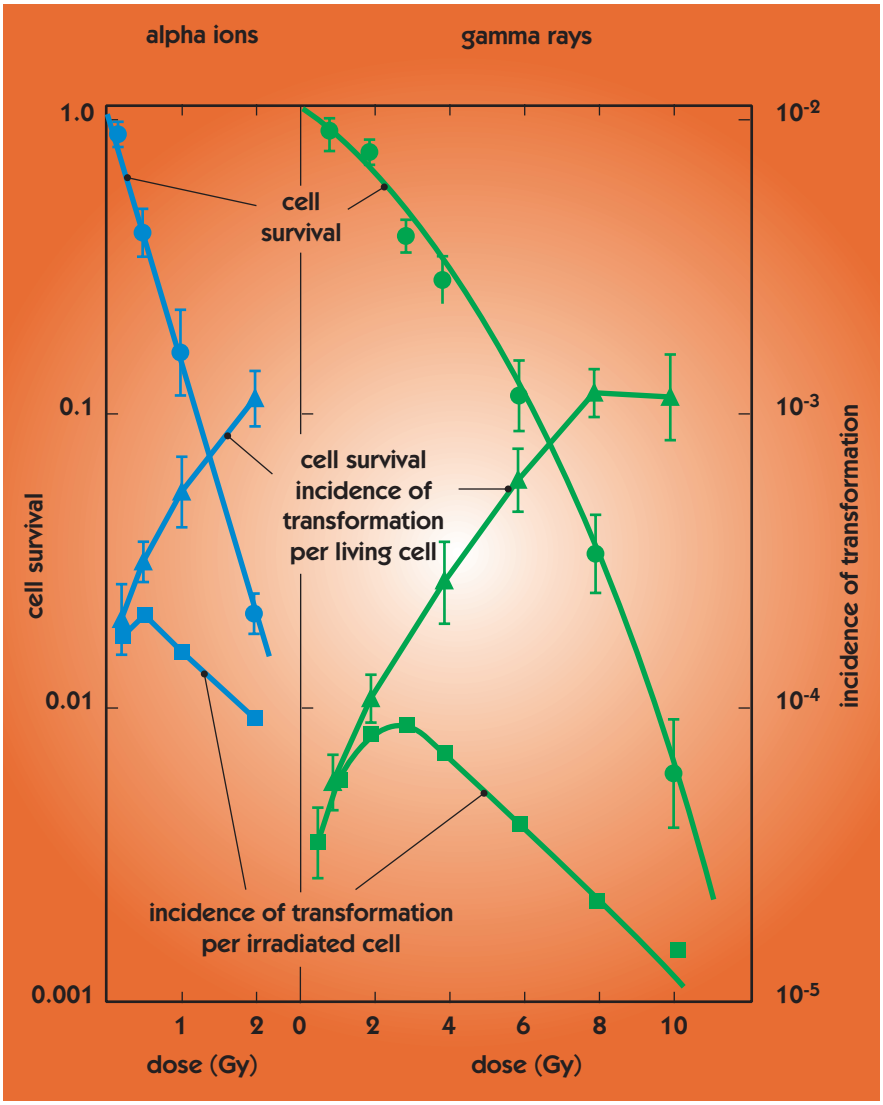


Figure 1. Influence of dose and type of radiation on the incidence of transformation of immortalized cells (from Hall E.J. and Hei T.K. 1987).

larly with the dose (Figure 1). These experiments have made it possible to evaluate the influence of various parameters on radio-induced cell transformation. Radiation with high **lineal energy transfer** (LET), such as neutrons or alpha particles, are more efficient than **X** or **gamma rays** (Figure 1). The influence of dose rate differs according to the type of radiation. A fall in dose rate lowers the probability of transformation for X and gamma rays, but raises it for high LET radiations, at least within certain limits. The time interval between irradiation and the transformation assay is very important. It allows the cells to repair part of the radio-induced damages, in part or in total, before dividing again, and therefore the probability of transformation decreases. Cell transformation requires cells to divide, which normally only occurs in certain tissues, or when radiation doses are high enough to cause massive cell death, of the order of several grays (Gy) for X or gamma rays. Exposure to various agents, e.g., chemical, can act in synergy with the irradiation in some cases, or antagonistically in others.

These experiments have thus made it possible to evaluate the risk of cell transformation after irradiation for different exposure conditions. However, the short period of time, few weeks between irradiation and *in vitro* transformation, is very different from the long latency, several years at least, observed *in vivo*. This suggests a single-step mechanism as if these cells, which already have a modified and unstable karyotype, had already done part of the way to transformation. This raises the question of what stage the ionization radiation acts at, and what it is that is actually being quantified.

A. Gonin/CEA

Changing medium in cell culture flasks under a laminar-flow hood at CEA Fontenay-aux-Roses.

The transformation of normal cells

As human cells do not spontaneously become immortalized in culture, compromise solutions are necessary, e.g., immortalization by genetic engineering, or the use of cells taken from animals that do not evidence this problem. For the models developed at the Cell Radiobiology Laboratory of the atomic energy commission (CEA) the choice made was to use cells from Sprague-Dawley rats, because a great many *in vivo* carcinogenesis experiments have already been carried out in this rat breed, in particular in CEA. One goal of this project was

to correlate the data obtained *in vitro* with observations made in human and animal tumors.

To study the transformation of normal cells into cancer cells, rats were irradiated during the last week of gestation using a cobalt 60 source (gamma radiation) with a dose rate of 0.022 Gy per hour, i.e., a dose of 0.5 Gy per day and a total dose of 3.5 Gy. At the end of radiation exposure, cells from fragments of brain and lung taken from the fetus were set in culture. The cells were maintained in culture for more than 100 passages (box). Various parameters such as cell population doubling time, **clonogenicity**, and **tumorigenicity**, as well as karyotype, were analyzed after an increasing number of cell culture passages (Figure 2).

During the first passages the lung cells were neither clonogenic nor tumorigenic, and the karyotypes were normal. There were therefore no chromosomal rearrangements induced by exposure to gamma radiation under these conditions of dose and dose rate. But at about 30 passages in cell culture, the cells began to display various chromosomal anomalies. One of these cells with anomalies had a proliferative advantage, because after a few more passages the whole population was derived from it.

The appearance of chromosomal modifications corresponds to the time when the cells become clonogenic and tumorigenic. The chromosomal rearrangements observed must therefore be related to the radio-induced transformation process and not directly to the irradiation. In addition to the chromosomal rearrangements, in particular **gene amplification**, that characterize the initial predominant clone, other anomalies occur. Overall, after a large number of passages, the karyotype is considerably modified. Besides, the time for the cell population to double decreases at the time the predominant clone is selected, and then remains stable.

A study was conducted in parallel on brain cells from rats irradiated in the same conditions. The process was practically the same, the cells remaining apparently normal during the first passages in culture, before chromosomal rearrangements, clonogenicity and tumorigenicity evolved synchronously as in the lung cells. However, there was no gene amplification and the number of chromosomes evolved in a pattern already described for human tumors, i.e.

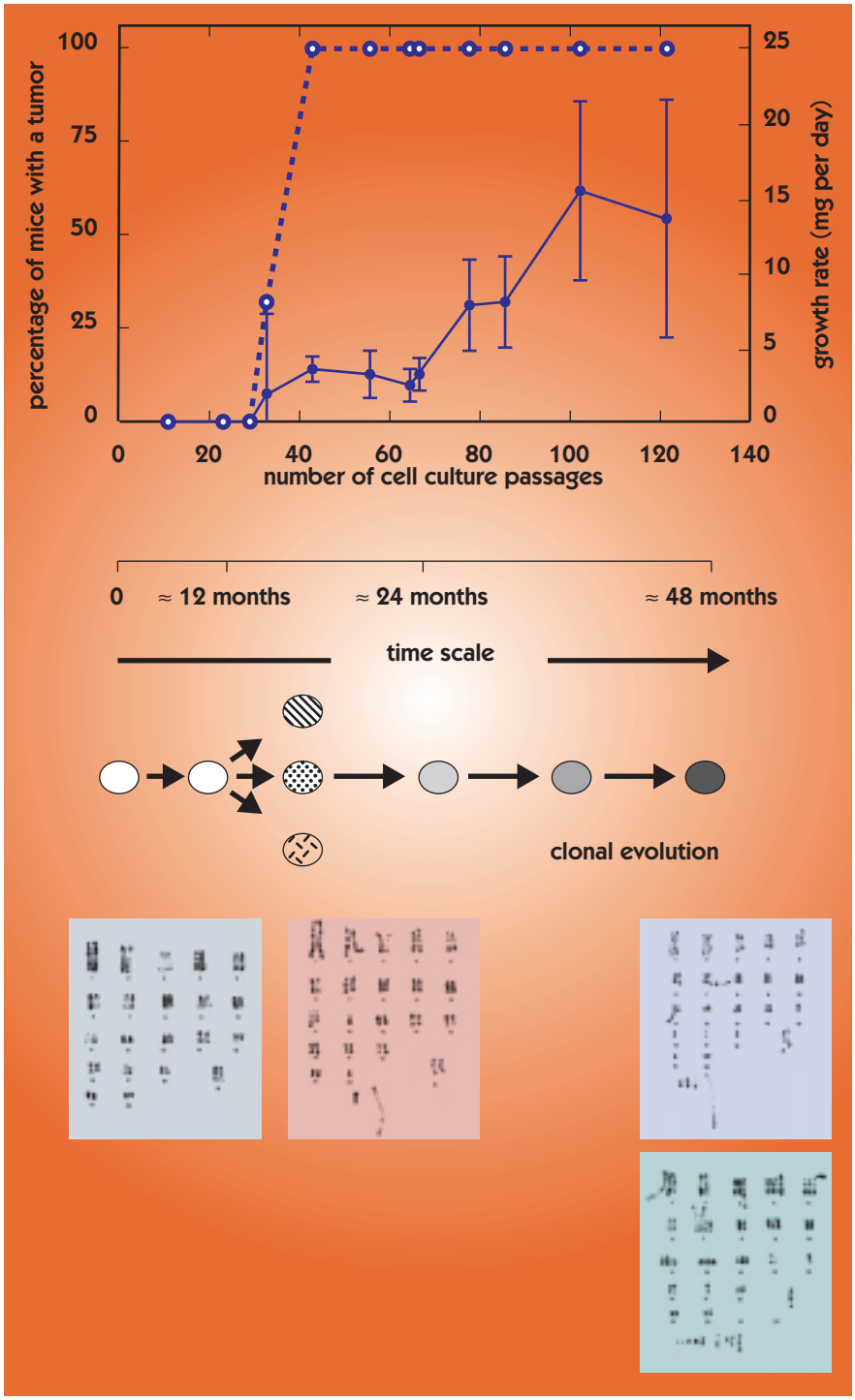
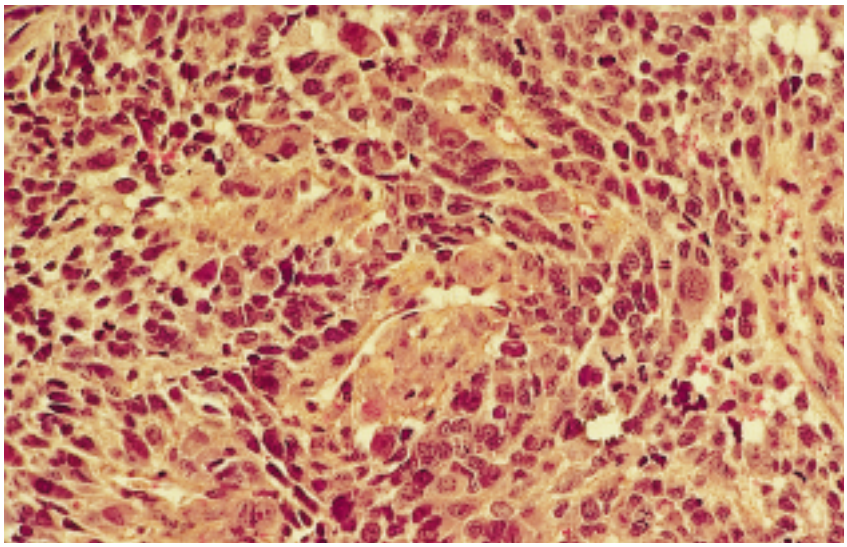


Figure 2. Evolution of tumorigenicity and chromosomal rearrangements in parallel with clonal evolution in cells from irradiated rat lung. The curves indicate the percentage of nude mice in which the injected cells gave rise to tumors (dashed line) and the rate of growth of these tumors in milligrams per day (continuous line). Below, from left to right, karyotypes of lung cells in early passages (16, normal karyotype), intermediate passages (49, visible rearrangements) and late passages (113 numerous rearrangements). Bottom: karyotype of a brain cell at passage 110.



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Histological section of a tumor obtained on a nude mouse after injection of transformed cells. The nude mouse is devoid of immune defenses, and so does not reject grafts of cells from other species. This breed is used to test cell tumorigenicity, and also to increase amounts of human tumor material, as samples, obtained by therapeutic exeresis or biopsy and made available for fundamental research are often small.

duplication of the number of chromosomes followed by the gradual loss of some of them. This process took place repeatedly.

The two models share some common features. Clonogenicity, tumorigenicity and cell karyotype are normal during early passages. Then, during an intermediate period of apparent genetic instability cells with abnormal but different karyotypes are observed. One of them displays a proliferative advantage, so that after a few further passages all the surviving cells are derived from it. In addition, tumorigenicity evolves in stages, and the number of chromosomal anomalies increases during transformation. The study of these cells at different transformation stages will help identify the genes implicated in the transformation of normal cells into cancer cells. Finally, by studying in parallel spontaneous transformation or those induced by various carcinogens, one should be able to identify any specificities or else analogies between the different processes.

Other research is currently directed toward the study of the transformation of human cells immortalized by genetic engineering, but a question arises: is the immortalization process the same as the one occuring during cell transformation?

Need for *in vivo* studies

Understanding processes as complex as those involved in carcinogenesis requires complementary approaches. Studies carried out on cell cultures do not take in account normal physiological processes such as hormone levels and the organization of cells in tissue with a specific architecture and the presence of cells with different origins. Besides, the reaction of the organism, and in particular the role of its immune defenses, is not taken into account. Moreover, when regularly subdivided as in culture, cells have to multiply permanently, which happens normally only in **stem cells** of certain tissues. *In vivo* carcinogenesis experiments in animals are thus required, especially now that it is possible to study the genetic modifications in tumor cells and so identify the genes involved. Furthermore, the results obtained from current experimental models can be interpreted only in the light of those from the analysis of human cancers themselves, although this is difficult because of individual variations and because the tumor, removed at the time of therapeutic **exeresis**, represents only a sort of photograph taken at a particular moment after a period of evolution that may have been long.

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