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Transgeneration Carcinogenesis: A Review of the Experimental and Epidemiological Evidence

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Introduction

In common language, the word 'environment' means the physical, chemical and biological world within which we live. In the context of research on disease etiology, the term environment means the whole complex of factors, including our behavior or lifestyle, that are not strictly inherent to the individual, and therefore not part of the genetic patrimony at the moment of conception. Although several pathological syndromes and diseases are clearly related to inherited conditions, and a genetic component of variable importance can be identified in many others, the role of environmental factors in the origin of human diseases appears to be predominant. It is generally accepted that individual differences in the risk of cancer are ultimately determined by the interaction between the environment and our genes, with additionally, a possible and at present imponderable participation of a purely random component.

The most visible genetic contribution to the origin of human cancer is the inheritance of a predisposition to certain tumors that, in the case of retinoblastoma, familial polyposis of the colon, neurofibromatosis and multiple endocrine neoplasia, is so high as to increase the risk of cancer up to several orders of magnitude over that of the general population.[1)] Because the risk is experienced at the same extremely high level in all environments studied so far, these cancers are called hereditary cancers, a term that could be regarded as a misnomer, in that what is inherited is a predisposition to cancer.[2)] In other instances, the risk is expressed in familial clustering of certain cancers (notably breast and colon) with a risk in the first-degree relatives of cases, that is generally one order of magnitude greater than in the general population and is inversely related to the age of onset of the tumor.[3)] Lastly, individual genetic constitution also participates in determining the wide spectrum of individual risks resulting from interaction with the environment. The distribution of a tumor, such as breast cancer, within the general population may include a relatively small proportion of genetic cases[3)] and a much larger proportion of cases to which genetic factors may contribute to some extent, although the role of other factors is predominant.

The hypothesis underlying this review is that some environmental factors can act on the germ cells of parents before conception to cause DNA alterations which affect the susceptibility of the progeny to cancer. Some of the cases of inherited predisposition to cancer may therefore be due to exposure to environmental factors in previous generations. Since damage to DNA in germ cells may occur either because of spontaneous errors of DNA replication and repair or as a response to a chemical or physical insult, if one looks at several generations the borderline between the contributions of environmental and genetic factors to the origin of human tumors may appear rather blurred. Seen from this angle, transgeneration carcinogenesis is possibly the best example of an integrated environmental-genetic interaction implicated in the causation of human cancer.

The suggestion that prezygotic exposure to a carcinogen or mutagen may lead to an increased risk of cancer in the progeny is derived from experimental and epidemiological observations.[4,5)] The variety of alterations which the genome may undergo following a DNA-damaging episode, the plasticity of the genome and the role that epigenetic events may play in transmitting different traits or characteristics all contribute to making this link between the genotype and phenotype unclear[6)] and certainly increase the difficulty of evaluating the relevance of both the experimental and the epidemiological results.

Certain human so-called hereditary cancers are similar to conditions seen in experimental animals. The closest similarity is between familial polyposis of the colon in humans and predisposition to multiple colon tumors in mice. The latter is brought about by a germ cell line mutation caused in a male mouse by treatment with ethylnitrosourea (ENU) before mating.[7)] Despite the biological plausibility of such an event occurring in humans, there has been no report yet of a human case comparable to these Moser mice. What is actually missing, therefore, is a convincing demonstration of an hereditary high predisposition to cancer in humans that is a direct consequence of damage to the germ cells caused by an environmental factor. It is a curious feature of our times that the ethical acceptability of induced germline modifications for disease prevention is being discussed[8)] in parallel with a strong resistance to acceptance that germline modifications can be induced by exogenous agents.

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In this paper, I briefly review the evidence for transgenerational transmission of an increase of cancer risk, beginning with the animal experiments.

Experimental Evidence

The first set of experimental data came from the work of Strong,[9, 10] who claimed to have developed sublines of mice in which the tumors for which they were selected following exposure to 3-methylchlolanthrene (MCA) occurred spontaneously, that is without any further exposure to the carcinogen. The unavoidable conclusion was that MCA had effects at two different levels: one on the genetic material of the somatic cells among which the first tumors arose, and one on the genetic material of the germ cells resulting in an increased susceptibility to neoplastic malignant changes in subsequent generations. To avoid all suspicion of Lamarckism, Strong emphasized that the concept of two different effects was quite different from claiming that somatic changes occur first and influence or produce later changes in germ cells. Strong's enormous efforts led to publication of many lengthy papers, but it is now difficult to verify the accuracy and completeness of his reports or even the experimental procedures he employed. He was certainly the first to present any data on the role of prezygotic events in determining cancer risks. The breeding manipulations and selections that Strong carried out for years have provided most of the mouse strains that have subsequently been used in cancer research.

Two decades later, Boutwell used an approach somewhat similar to that of Strong, to obtain a subline of Rockland-derived mice with a particular propensity for skin tumor formation.[11] He identified and selected for breeding mice that were responsive or non-responsive to a single application of 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by repeated painting with croton oil. A remarkable feature of Boutwell's experiments was the rapidity with which the selection between susceptible and resistant mice could be made. After four generations the percentage of responsive animals in the susceptible subline passed from 25% to 60%, reaching about 100% after eight generations, and in parallel the number of papillomas per mouse rose from 2.5 after 4 weeks to about 12 after eight weeks. Because of the small dose of DMBA used (75 μg/mouse applied to the skin), the possibility that the increased susceptibility might be the consequence of an effect of DMBA on the germ cells was discarded. An alternative explanation was that the selective breeding caused or enhanced a genetic instability and possibly favored the deregulation of normal development and cellular differentiation.

Effects of a comparable nature to those reported by Boutwell were obtained using fish of the genus *Xiphophorus*.[12–15] Forced hybridization between the spotted platyfish (*X. maculatus*) and the non-spotted swordtail fish (*X. helleri*) generated a sensitive genotype in which melanomas developed spontaneously, but which also acquired a susceptibility to chemical carcinogens that the parent strains did not have. These were probably the first results providing clear evidence that cellular genes that regulate the normal process of development and differentiation can be activated to direct the development of neoplasia. Interaction between a dominant tumor-formation gene and a repressor gene was demonstrated later by Anders.[15] As Friend has suggested, the *Xiphophorus* model may be particularly useful for the study of the complex interactions between multiple susceptibility genes and the several modifier genes that are probably involved in the development of melanomas.[16] Anders even advanced the hypothesis that "certain human cancers may be expected to occur "spontaneously" because of the combination of factors from both parents that, by themselves, like platyfish and swordtail, did not cause cancer in either parent."[15]

The results of Boutwell and of the various experiments using the *Xiphophorus* model provide evidence for the induction, not directly related to exposure to a carcinogen/mutagen, of a heritable enhanced susceptibility to cancer and to carcinogens, and for the role of modifier genes in determining the levels of this susceptibility. In the experiments described below, attempts were made to induce a heritable increase in susceptibility to cancer by deliberately exposing animals to a carcinogen or mutagen (Table I).

A first series of reports, concerning which it is today hardly possible to evaluate either the experimental procedures or the accuracy of reporting, indicated that exposure to a carcinogen, MCA, *o*-aminoazotoluene (O-AAT) and DMBA, before mating can result in an increased incidence of tumors in subsequent generations of untreated descendants.[17–20]

The first results in a subsequent set of experiments showed an increased incidence of tumors at various sites in two and three consecutive generations of mice descended from mothers treated with DMBA during pregnancy.[21, 22] Most of the tumors in the descendants occurred, as also noted in later experiments, at a relatively late age and this was seen as the expression of a low degree of inherited predisposition. Experiments in rats also showed an increased incidence of tumors in two or three generations of descendants from mothers exposed to either nitrosomethylurethane (NMUt), methylnitrosourea (NMU) or ENU during pregnancy.[23–26] In contrast, no increase in tumor incidence was observed in F2 and F3 hamsters descending from parents exposed *in utero* to N-nitrosodiethylamine (DEN).[27]

An increased incidence of tumors of the genital tract was observed in the progeny of mice exposed *in utero* to

Table I. Transgeneration Effect of Carcinogens: Experimental Evidence

1. Early studies

Agent	Species (strain)	Treatment	Tumors observed in descendants	Reference
MCA	Mouse (NH)	Selected breeding of responsive animals	Gastric adenocarcinoma, various sites in subsequent generations	10
MCA	Rat (Wister)	Females before and shortly after mating	Various sites in F1-F2	17
MCA	Mouse (A, C3H)	Painting of ovary before mating	Lung, breast tumors in F1-F2	18
O-AAT	Mouse (C3HA)	Pregnant mothers	Liver tumors in F1-F2	19
DMBA	Mouse	Parents	Lung tumors in F1-F2	20

2. Treatment during pregnancy and follow-up for several generations

Agent	Species (strain)	Tumors observed in descendants	Reference
DMBA	Mouse (Swiss, MA)	Various sites in F1-F2	21
		Various sites in F1-F3	22
NMUt	Rat (WKA)	Tumors of nervous tissue in F1-F2	23
NMU	Rat (BD)	Tumors of kidney, CNS, mammary gland in F1-F3	24
ENU	Rat (BD)	Tumors of nervous tissue in F1-F3	25
DES	Mouse (CD-1)	Uterine and ovarian adenocarcinomas in F2f females	28
DEN	Hamster	Respiratory tract tumors in F1	27
		No effect in F2-F3	
BaP	Mouse (A)	Increased multiplicity in lung tumors in F1-F5	26
DES	Mouse (CBA)	Uterine sarcomas, ovarian tumors in F2m females	29

3. Treatment of males prior to mating with untreated females

Agent	Species (strain)	Tumors observed in descendants	Reference
X-rays urethane	Mouse (ICR)	Lung tumors in F1-F3 (ovarian tumors, leukemia)	30, 31, 32
ENU	Rat (BD)	Tumors of nervous tissue in F1	33, 34
ENU	Mouse (AKR/B6)	Multiple intestinal neoplasia	7
Neutron irradiation	Mouse (C3H)	Liver tumors in F1	35

4. Initiation and promotion

Agent	Species (strain)	Treatment	Tumors observed in descendants	Reference
X-rays	Mouse (ICR)	Male parent prior to mating + urethane in F1-F2	Multiplicity of lung tumors	36
DMBA	Mouse (SHR)	DMBA to pregnant mothers + TPA in F1-F2	Skin tumors	37
X-rays	Mouse (SHR)	Total body irradiation of males before mating + urethane to F1	Multiplicity of lung tumors	38
DMBA	Mouse (SHR)	DMBA in pregnant mothers + TPA in F1 and F2	Skin tumors	39

diethylstilbestrol (DES).[28,29] Walker found an increased incidence of uterine and ovarian adenocarcinoma in the progeny of female mice exposed *in utero* and mated with untreated males. Turusov *et al.* reported an increased incidence of uterine sarcoma and ovarian tumors in the progeny of male mice exposed *in utero* to DES and mated with untreated females. At the dose of DES used (1 μg/g body weight at the 17th day of pregnancy) the females exposed *in utero* were totally sterile. Preliminary results from experiments using lower doses of DES (0.1 μg/g

body weight) compatible with the maintenance of fertility of the females exposed *in utero* point to an increased incidence of tumors in both F2m and F2f descendants (V. Turusov, personal communication).

A different approach was used in a third series of experiments, namely treatment of parents with a carcinogen before conception of offspring. In the largest experiment of this type, involving several thousands of mice, Nomura[30-32] exposed adult males and females to either X-rays or urethane before mating. He observed an increased incidence of malformations in the first generation of offspring, and tumors, in particular of the lungs, in the F1-F3 descendants. Nomura described the inheritance of the susceptibility to lung tumors as dominant with a 40% penetrance.[31] In an experiment in which ENU was given to male rats before mating with untreated females, an increase of borderline statistical significance of both central and peripheral nervous tissue tumors was observed in the progeny obtained from mating at 1 to 4 weeks after treatment. The increase in incidence was statistically significant in the group obtained by mating the male parents two weeks after treatment with ENU.[33] When this experiment was repeated several years later, the results again showed a slight increase in the incidence of brain tumors in the progeny of ENU-treated males, but did not confirm the previous finding of an increase in tumors of the peripheral nerves.[34] An increased frequency of liver tumors was observed in the progeny of neutron-irradiated male C3H mice before mating.[35]

Additional evidence for germline transmission of increased susceptibility was obtained in a series of experiments in which exposure to either X-rays or a chemical carcinogen was followed in subsequent generations by the application of a promoter. An increased susceptibility to lung tumors was observed in F1 and F2 descendants of X-ray-irradiated males following the application of urethane, while the application of 12-O-tetradecanoylphorbol-13-acetate (TPA) to F1 and F2 descendants of male and female mice exposed to DMBA *in utero* resulted in a significantly increased incidence of skin tumors.[36-39]

Somewhat analogous observations have been made with *in vitro* models of mutagenesis. When V-79 cells were exposed either to a single dose or to small repeated doses of X-radiation, their progeny showed hypersensitivity to the mutagenic effect of 8-methoxypsoralen plus UV light (PUVA), that persisted for many cell generations. This was particularly evident at low doses of PUVA that did not induce mutations in non-irradiated cells.[40] A known mutagen had therefore induced changes that persisted in dividing cells and predisposed them to the effects of subsequent exposure to a different agent.

The germ cell line mutation induced by the preconception treatment of a male mouse with ENU (120 μg/kg body weight)[7] has already been mentioned. The resulting predisposition to multiple intestinal tumors was dominantly inherited with almost complete penetrance. This finding possibly provides the best evidence that the exposure to a chemical carcinogen or mutagen can indeed produce a situation that is phenotypically and genotypically very similar to a human hereditary syndrome, in this case familial polyposis of the colon.[41]

Epidemiological Evidence — Occupational Exposures

The epidemiological evidence of a transgenerational effect of carcinogens is mainly derived from studies on childhood cancer, in relation to parental occupational exposures to chemicals, or occupational and non-occupational exposure to radiation. In spite of their rarity and the consequent difficulty of assembling sufficiently large numbers for a statistical analysis, childhood cancers provide a better chance of establishing an association with parental exposures before or at the time of conception than tumors occurring later in life. Paternal exposures are almost exclusively considered here, since it is often impossible to distinguish between pre- and post-conception maternal exposures. Some excellent reviews are available,[42, 43] and here I shall only try to highlight some of the key issues (Table II).

There have been six studies or case series on total childhood cancers. The first of these, and the one which attracted the most attention, was that of Fabia and Thuy[44] who reported in 1974 a significant association between the occupation of fathers as moter vehicle mechanic or service station attendant and leukemia in their children. Fabia and Thuy advanced the hypothesis that the agents responsible for the effects observed were polycyclic hydrocarbons. A possible association between hydrocarbon-related occupation of fathers and kidney tumors, but not leukemias, was also found by Sanders *et al.*[45] and by Kwa and Fine.[46] An association with paternal occupation as mechanic, machine repairer, smith or welder and kidney tumors was found by Olsen *et al.*[47] A study by Zack *et al.*[48] showed an inconclusive association of childhood cancer with a variety of hydrocarbon-related occupations, while one study in the Netherlands and one in the USA did not find an association between childhood leukemia and father's occupation.[49, 50] There have also been some reports on an apparent and unexplained increased risk of cancer in the progeny of parents of higher social class(es) or of those who gave academic degrees as an occupation.[45, 51]

Another type of investigation is case-control studies focused on a particular tumor type. Four studies on childhood leukemia have shown an association with a variety of occupations, including motor vehicle mechanic, manufacturer of machines and aircraft, service station attendant and those involving exposure to exhaust

Table II. Transgeneration Effect of Carcinogens: Epidemiological Evidence Occupational Exposure (Excluding Radiations) and Childhood Cancer

1. Studies on total cancers

Paternal occupations	Agent(s) involved	Tumor types	Odds ratio	Reference
Motor vehicle mechanics, machinists	Hydrocarbons Lead	Leukemia	1.2–2.5	44, 48
Metal workers		Kidney tumors	2.5–5.0	45, 51
Farmers	?			
Paper and pulp industry		Brain tumors	2.8–4.6	47,

2. Case-control studies

Paternal occupations	Agent(s) involved	Tumor types	Odds ratio	Reference
Motor vehicle mechanics				52, 53, 54, 55
Exposure to exhaust	?			
Fumes		All leukemias	2.0–2.4	
Service station attendants		Acute n.l. leukemia	2.0–2.4	
Manufacturers of machines, aircraft	Chlorinated solvents cutting oils			
Mechanics	Lead, hydrocarbons			56
Service station attendants	Aromatic hydrocarbons	Wilms' tumors	4.0–7.5	57, 58
Welders, machinists	Metals			
Aircraft industry	Solvents, paints			59, 60, 61, 62, 63,
Electricians	low frequency	Brain tumors	2.0–8.0	
Exposure to electro magnetic fields	fields			
Electrical repaires	?			
Agriculture, forestry				
Welders, machinists		Retinoblastoma	1.6	66
Aircraft production		Seminomas	2.0–5.3	67
Service station attendants				
Exposure to metals		Hepatoblastoma	3.0	68

3. Exposure to radiations

Preconception paternal exposure	Total dose	Tumors in progeny	Odds ratio	Reference
X-rays	1–>21 Diagnostic films	Leukemia	1.31 (RR)	78
Radiation-related occupations	Unknown	Bone tumors, Wilms' tumors	5.35 2.48	80
Industrial exposure to radiation	Unknown	Tumors of CNS	1.7–2.1	81
X-rays	Unknown	Leukemia	3.9	82
Nuclear plant	100 mSv	Childhood leukemia	8.4	83, 84
Various occupations	Exposure to radionuclides	All cancers leukemia	2.70 2.75	97
Occupational exposure of fathers to radiation	1–5 mSv	Leukemia and non-Hodgkin's lymphomas	9.0 (RR) (1.0–107.8)	99
Occupational exposure of fathers to radiation	>0.1 mSv	No association found	—	95
Occupational exposure of fathers to radiation	0.1–10 mSv	No association found	—	94
Preconception maternal exposure to X-rays	1–>21 Diagnostic films	Leukemia	1.7 (RR)	78
Preconception maternal exposure to X-rays	Unknown	Childhood cancers	2.6	79
Preconception exposure of both parents (atomic bomb)	0.37–3.8 Sv	No increase in incidence of cancer in the first 2 decades of life	—	85

fumes. The odds ratios for these associations were 2.0–2.4.[53-56] Three studies focused on Wilms' tumors and found a positive association with occupation as mechanic, service station attendant, welder or machinist, with odds ratios of 4.0–7.5.[56-58] Five studies were focused on brain tumors and a positive association was found with occupation in the aircraft industry, electrical manufacturing, electrical repairs, forestry and agriculture, with odds ratios of 2.0–8.0.[59-63] Brain tumors, as well as rhabdomyosarcoma, have also been reported to be associated with paternal smoking.[64, 65] Finally, a study on retinoblastoma showed an association with occupation as welder or machinist, with an odds ratio of 1.6,[66] a study on seminomas pointed to an association with occupation in aircraft production or as a service station attendant[67] and a study on hepatoblastoma showed an association with occupation involving an exposure to metals with an odds ratio of 3.0.[68]

While the experimental studies suffered from the limitations of the small number of animals involved and in some instances from inadequate reporting, two different limitations affect the epidemiological studies. The first is related to information on exposure, that in most studies was restricted to a gross definition of occupation, with no details on the type, extent and intensity of exposures. This shortcoming may explain the incongruities and at least part of the contradictory findings between studies. The second is the scarcity of the events investigated (namely childhood cancer) and the consequent difficulty in assembling a large enough number of cases to allow a satisfactory statistical detection of anything but extremely large differences in mortality or incidence; even where the odds ratio or relative risk (RR) appears high, it rarely reaches statistical significance. Due to the scarcity of specific exposure information and the presence of confounders, even in the rare cases where statistical significance is reached, it is impossible to establish firmly a causal relationship of the association or to be sure that the association pathway does involve the germ cells.

The only situation where information on exposure is quite accurate is that of cancer patients successfully treated with radiation and/or chemotherapeutic agents. An excess of cancer cases in childhood has been observed in progeny of patients surviving childhood and adolescent cancer, but this appeared to a large extent related to an excess of retinoblastoma. These data were based on an average period of follow-up of the offspring of about ten years, a duration that would favor the detection of cancers with an important genetic component.[69] No excess has been reported in the offspring of surviving adult cancer cases, but the period of follow-up has been relatively short (for a review see ref. 69). In patients with retinoblastoma, radiotherapy appears to increase the inborn susceptibility to develop a second primary neoplasm,[70] with a certain similarity to the increased susceptibility to chemical and radiation carcinogenesis seen in Eker rats,[71, 72] which are carriers of a germline mutation.[73, 74] In these Eker rats, the inherited genetic susceptibility appears to be strictly cell-specific. When the carcinogen DEN, which induces epithelial and mesenchymal tumors of the rat kidney, is given to Eker rats, the incidence of only the kidney carcinomas, but not of mesenchymal tumors, is increased.[73]

The excess of deaths from second primary cancer has now been seen to continue beyond the first 40 years of follow-up of the retinoblastoma cases, who are approaching the age when the cancer incidence sharply increases among the general population.[70] An increased risk of second malignant tumors has also been observed in survivors of childhood cancer other than retinoblastoma.[75-77]

Exposure to Radiation

An increased risk for leukemia has been reported by Graham *et al.*[78] following preconception exposure of fathers (RR: 1.3) as well as of mothers to diagnostic X-rays (RR: 1.7). An increased risk for childhood malignant tumors was reported following preconception maternal exposure to X-rays.[79] A positive association was found between bone and Wilms' tumors in children and father's exposure to radiation-related occupation.[80] An association was reported between central nervous system tumors and paternal occupational exposure to ionizing radiation that was not, however, confirmed when instead of industrial codes, occupation titles were used as an indirect definition of exposure.[81] A significant positive association was found between paternal preconception exposure to X-rays and childhood leukemia, with an odds radio of 3.9.[82] All these studies, while pointing to the possible existence of a causal relationship, suffered from many inadequacies, not the least of which is the unclear definition of the intensity and duration of exposure.

The study of Gardner and colleagues attracted and polarized attention and instigated a number of investigations to prove or disprove the proposed hypothesis.[83, 84] This hypothesis was that external ionizing radiation of fathers prior to conception increases leukemia risk in the offspring, in particular when exposure was close to the time of conception. This latter detail is of importance as it could help to explain the absence of a similar effect in the progeny of parents exposed to atomic bomb radiation[85] and, more generally, in some of the other "negative" studies of parental exposure. Gardner's data incited an unusual amount of reaction, possibly because they could be seen as providing the first demonstration in humans of the transmission via the germ cells of a cancer risk related to exposure to an exogenous agent, but perhaps also because they cast a further shadow on the nuclear industry, already much feared by the public.

Alternative hypotheses to explain Gardner's findings have been proposed,[86-91] and several studies have been conducted in which his findings have not been confirmed.[92-95] Evidence in favor of an association between an increased risk of leukemia and the Sellafield radioactive discharges has also been put forward[96] and the results of two studies seem to be consistent with Gardner's hypothesis, even though different routes of exposure to radiations are involved. In the first of these two studies carried out using the data of the Oxford Survey of Childhood Cancer, an increased risk of childhood cancer was found to be associated with preconception exposure to radionuclides, and not to external ionizing radiation.[97] Similarly, in the second study an increased risk for leukemia and non-Hodgkin lymphoma was found to be associated with preconception paternal exposure to radiation around nuclear facilities that was interpreted to be more likely to be due to internal contamination than to external exposure.[98]

The evaluation of the possible genetic effects of ionizing radiation and mutagenic chemicals on humans is recognized as one of the most intractable epidemiological endeavors — but at the same time there has been a certain convergence of opinion on the fact that humans are more resistant than mice to the genetic effects of radiation.[99] The difficulty should not be underestimated, however, that while in the experimental setting it is possible to exploit the selection of many appropriate endpoints to evaluate the effects of radiation, in the human situation the observations are generally limited to the monitoring of sentinel phenotypes.[100, 101] There is at least one important built-in weakness in such a system for mutation surveillance, namely that only germinal mutations of the dominant type can be detected. Since there is a continuum in the degree of dominance of both spontaneous and induced mutations, the estimation of the heterozygous impact of recessive mutations remains at present one of the most vexing problems in understanding the impact of mutations on populations.[99]

Another important limitation of epidemiological investigations carried out up to now with the aim of detecting the genetic contribution to the human cancer burden is that they have usually been limited to the occurrence of tumors in childhood or in the first two decades of life. The establishment of cohorts that could be followed for life has been possible for the offspring of atomic bomb survivors and for the offspring of surviving childhood cancer patients, the follow-up being at present of about twenty years in the first case and of about ten years in the second.[69, 85] Nothing can yet be said about the cancer experience of these two cohorts in later life.

In addition, the frequency of diachronous second primary cancer is expected to increase, given the improved and extended survival of childhood and adolescent cancer patients.[102] The surveillance of trends in the occurrence of second primary tumors could be of great relevance for a better understanding of the interaction between genetic and environmental factors.[103]

Conclusions

In the light of this review of the available experimental and epidemiological data, the question must be raised whether they provide convincing evidence for a transgeneration transmission of cancer risks related to preconception exposure to exogenous agents. If they do, we should also ask whether a satisfactory mechanism to explain it can be proposed.

A series of experimental results in mice and rats indicates that preconception treatment with a chemical carcinogen or radiation is at the origin of an increased cancer risk in the progeny. Although most of the experimental results are open to criticism, in particular because of the small number of animals used and the consequent lack of statistical power of the data, there is an impressive convergence of evidence in favor of the induction of an alteration in the germ cell followed by an increase in cancer risk in the progeny. The strongest evidence is possibly the Moser mice, in which a dominantly inherited predisposition to multiple intestinal tumors was induced by the preconception treatment of males with ENU.[7]

There is still no direct evidence in humans for the induction of a germinal mutation by a chemical mutagen that increases the risk of cancer in the progeny which could be compared with the germinal mutation induced by ENU in the Moser mice.[7] The epidemiological data, almost exclusively derived from the investigation of occupational exposures and indicating an association between a preconception paternal exposure to a variety of chemicals or chemical mixtures, are, like the experimental data, open to criticism, in particular for the lack of precise information on type, duration and intensity of exposures. This is certainly one reason for the contradictory findings between some studies.

The situation is rather different with regard to radiation. The experimental data are derived from a very large study with numbers such that, in spite of a certain lack of details in the reporting of the data, the evidence for an increased risk of cancer in the progeny of parents exposed to X-rays before conception is rather convincing.[30, 31] Three years ago, for the first time, it was claimed that exposure to external radiation of male parents prior to conception was associated in humans with an increased risk of leukemia in their children. The implication is that radiations can induce a heritable change (mutation) in the germ cells that is directly or indirectly leukemogenic.[83, 84] Alternative explanations have been offered, and a few later investigations have failed to con-

firm the observations of Gardner and colleagues. Nevertheless, Gardner's conclusion has not really been refuted and two other studies seem instead to confirm his hypothesis. Although rather fragile, being based on a small number of cases, this remains the only reasonably well documented human evidence of a transgenerational transmission of cancer risks. As such a phenomenon is biologically plausible and cancer families and hereditary cancer syndromes do exist, the doubt concerns not so much the hereditary transmission of a predisposition to cancer in humans, but rather the possibility that an exogenous agent, and in particular radiation, can induce it.

Most epidemiological studies on the possible role of prezygotic events in the origin of human cancer have concentrated on childhood or adolescent cancers. There are obvious difficulties in extending such investigations over most of the lifespan. Nevertheless, there is evidence that in experimental animals preconception exposures may be related to an increase in cancer risk appearing late in life, and studies in survivors of childhood cancer (mainly retinoblastoma) indicate that the risk of second primary cancers continues to be high even beyond 40 years of observation.[70] Germ-line mutations may therefore play a role in the origin of both early- and late-appearing human tumors.

Germline p53 mutations have been observed in members of families with the Li-Fraumeni syndrome[104] but have also been reported in a proportion of children and young adults with second primary malignant tumors.[104, 105] As second primary cancers in individuals who were later shown to be carriers of a germline mutation occurred up to 30 years after the first primary, it would appear worthwhile to check for the presence of germline mutations also in individuals in whom the first tumor occurred later than during childhood. In addition, while it is assumed that the carrier rates of germline p53 mutations in the general population are low, it is very likely that further cancer susceptibility genes will be identified in the near future.[106]

The development of more precise and efficient methods for detecting changes in the rate of germline mutations would be helpful in elucidating the possible role of prezygotic exposures in their causation.[107] Evidence for the induction of germ-line mutations in mice has already been obtained on a small population sample and at relatively low radiation doses, by measuring minisatellite mutation rates using multilocus satellite probes. Preconception irradiation of male mice with 0.5 G of γ rays, followed by mating six weeks later with non-irradiated females, was shown to cause a significant increase of mutations in the offspring.[108] It this method could be applied to humans the investigation of a relatively small population sample would be sufficient to detect germline mutations and also possibly to distinguish between spontaneous and induced mutations. A different problem to be resolved would then be to establish their actual significance in determining or contributing to cancer risks.

To what extent preconception exposures may contribute to DNA changes other than mutations, such as abnormal genomic imprinting[109] that may also be involved in determining individual cancer risks, is not clear. Altered DNA methylation, which is the proposed mechanism for abnormal imprinting, has been described as being possibly the most common and frequent DNA change in cancer,[110] associated with changes in transcription, subject to fluctuations during gametogenesis and development and heritable in a chromosome-specific manner, but also as being reversible.[111]

In conclusion, review of the experimental and epidemiological data on transgenerational transmission of cancer risk as a whole supports the hypothesis that exposure to exogenous agents before conception can cause alterations of the germ cells that are variably expressed in the progeny. These may be rarely expressed as acute extreme situations, such as a high familial predisposition to cancer; more frequently, they may modulate the consequences of the interaction with the postnatal environment and may in this way contribute to the lifelong risk of cancer. The public health relevance of this hypothesis is that, by taking into account the possible risk for future generations, it may contribute to a reinforcement of the criteria for primary prevention.

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Note added in proof: After this review was accepted for publication, a commentary has been published in which the hypothesis that the increased number of childhood leukemia cases near a nuclear plant in England were related to the preconception exposure of fathers to radiation, is denied.[1] While ample space in the commentary is given to the conclusions of a judge involved in a legal case, the mechanistic argument against the possible role of preconception radiation in increasing the risk of leukemia in the progeny, is almost entirely built on just one set of radiation studies on cultured somatic cells. The authors may have overlooked other studies which support the biological plausibility of the hypothesis on the role of a preconception parental exposure to a carcinogen/mutagen in increasing the risk of cancer in the progeny[2,3] (and see also refs. 40, 107 and 108 in this review).

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