COMMENTARY

Transgeneration transmission of carcinogenic risk

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Introduction

Molecular analysis of human tumors suggests that multiple genetic changes are required for a normal cell to become malignant (1). The multistage nature of carcinogenesis implies that cells which contain one or a few genetic mutations may behave normally; several other genetic insults are required to confer the tumor phenotype. It can be postulated that genetic damage may occur in germ cells but not be expressed until further genetic events occur in postnatal life. This sequence is potentially relevant for cancer prevention.

Transmission of carcinogenic risk is best demonstrated by cancer-prone families. The best-known cancer syndrome is hereditary retinoblastoma, for which germ cell alterations of the Rb gene have been identified (2). A recent study suggests that germ-line mutations of the p53 gene are responsible for the Li–Fraumeni syndrome, an association of tumors including breast cancer and soft tissue sarcomas (3). Transgenic mice that harbor activated oncogenes have been constructed (reviewed in 4). The appearance of multiple tumors in these mice suggests that transgenerational transmission of genetic alterations may lead to cancer.

Genetic alterations of germ cells predisposing to cancer may result from intrinsic genetic instability or from exposure to mutagens (5). It is our principal aim to consider the possible effect of mutagenic carcinogens on germ cells as the origin of genetic predisposition to cancer. The possibility of germ-line transmission of the effects of exogenous carcinogens has attracted renewed attention due to the recent report by Gardner et al. (6) of a significant increase in leukemia incidence among children whose fathers had worked at the Sellafield nuclear plant. Transgenic transmission of carcinogenic action of exogenous agents in experimental animals has been described by several authors (reviewed in 5). In epidemiological and experimental animal studies, exposure to carcinogens prior to conception is emphasized. Little is said about the possible interactions of pre- and postnatal exposures, in spite of the currently accepted hypothesis of multistage and multifactorial carcinogenesis. We review here the current evidence for multigeneration carcinogenesis. The proceedings of a recent meeting on this subject are available as an IARC Scientific Publication (7).

Multigeneration effect of carcinogens in experimental animals

Several animal experiments suggest that treatment of females with various types of carcinogens during pregnancy results in an increased incidence of tumors in the first and in subsequent generations of untreated descendants. An increased incidence of tumors has also been observed several generations following the exposure of males before mating (Table I). While most carcinogens are genotoxic, diethylstilbestrol (DES), which is known to induce gene mutations, has also been shown to be a transgeneration carcinogen in mice (8). Furthermore, female offspring of male descendants of DES-treated mothers mated with untreated females have a high incidence of uterine sarcomas and of benign tumors of the ovary (9). Molecular mechanisms of germ cell transmission of genetic damage may not be limited to mutation; mechanisms such as genomic imprinting may play an important role.

These animal studies suggest that a carcinogen can transmit its effect multigenerationally either when exposure occurs in utero, or when parents (most frequently fathers) are exposed before mating. Differential sensitivity of germ cells to carcinogens at different stages of spermatogenesis has been demonstrated through the induction of specific locus mutations and tumors. Nomura (10) reported that both male germ cells and oocytes of mice are sensitive to X-rays and urethane in inducing cancers in offspring. These results are in contrast to those of Russell (11), who reported a negligible risk for mutation in oocytes.

A clear effect of germ cell transmission on tumor 'initiation', expressed following postnatal exposure to promoting agents, was reported by Napalkov et al. (12) and confirmed by Loktionov et al. (13). They found a multigeneration effect of 7,12-dimethylbenz[a]anthracene (DMBA) on skin carcinogenesis only when a skin tumor promoting agent, 12-O-tetradecanoylphorbol-13-acetate (TPA), was also administered. When TPA was painted on the skin of second-generation mice, there was a clear increase in skin tumor incidence. Two-generation transmission of skin tumor initiation was recently extended to X-rays (14). Similarly, Nomura (15) and Vorobtsova and Kitayev (16) have found that large clusters of lung tumor nodules developed when the offspring from X-irradiated parents were treated postnatally with urethane.

These results suggest that, following the exposure of germ cells to a mutagen/carcinogen, an initiating event could be inherited by subsequent generations and revealed after postnatal exposure to mutagens/carcinogens or non-genotoxic agents. This has important ramifications for humans, since we are exposed throughout life to many environmental factors that may in various ways enhance the progression of cancer.

In multigeneration carcinogenesis, it is assumed that heritable changes induced in germ cells will be present in all somatic cells of offspring. We could therefore expect multiple tumors to occur more frequently in multigeneration cancer than in sporadic (non-genetic) tumors. Some studies suggest that tumor multiplicity is, in fact, a multigeneration effect of carcinogens (Table I), but the number of tumors present in a tissue is not invariably high. After postnatal exposure to tumor promoting agents, 10 or fewer skin papillomas were seen per mouse (12). These results suggest that the target genes may not necessarily be those that are directly responsible for carcinogenesis, but rather those that modify carcinogenic pathways. On the other hand, studies on transgenic mice that harbor oncogenes suggest that the multigeneration effect of carcinogens could involve these critical genes as well (4).
The most compelling evidence to date for human multi-
generational carcinogenesis comes from a study of radiation
workers near Sellafield in Great Britain (6). An increased rate
of leukemia was seen among local residents under age 25 whose
fathers were employed in the nuclear industry. When occupational
exposure histories of fathers of patients diagnosed between 1950
and 1985 were reviewed, four of 46 were found to have received
10 mSv or more of radiation in the 6 months prior to conception
(RR = 8.2; P < 0.05). In a similar group of children living
near the nuclear facility at Dounreay no association between
childhood leukemia and paternal occupational exposure to
radiation was found (24), but paternal preconceptional diagnostic
X-rays have been associated with childhood leukemia previously.

Graham et al. (25) found paternal preconceptional radiation to be
associated with a non-significant elevation of leukemia risk in
offspring (RR = 1.3; P = 0.16), and recently a relative risk
of 3.9 for all types of childhood leukemia was seen when fathers
experienced 11 or more X-rays, determined by household
interview (P < 0.01) (26). No association was found with
paternal occupational radiation exposure in a study of 126 children
with leukemia (27).

It is difficult to accept a germ-line mutagenic cause for the
observed association at Sellafield because of the very high
mutation rate that is implied (28). The minimum leukemia rate
at Sellafield consistent with the data is 1 in 1000 children of
exposed fathers. This rate is ~ 50 times greater than that expected
for the total rate of dominant genetic disease predicted from the
mouse dominant cataract studies for 10 mSv of exposure (29). In
contrast, the spontaneous mutation rate for hereditary
retinoblastoma, the most heritable of childhood cancers, is 6 per
100 000. Three cases of unilateral retinoblastoma have been
reported among grandchildren of workers at Sellafield (30). One
of these three carried a constitutional deletion of chromosome
13q12-21 that was not present in either parent. Although a formal
statistical analysis was not possible in this study, the authors note
that the three cases probably arose among a population of <5000
children resident at Seascale during this period. Based on this, the expected number of cases would be <1 ($P = 0.002$).

The results of the Sellafield study are in contrast to the negative results found for atomic bomb survivors. There were no excess leukemias among offspring of Japanese men who survived the atomic bomb blasts (and who received a mean radiation dose of 492 mSv) (31), in keeping with the results of other potential genetic effects of the atomic bomb (32). Yoshimoto et al. (33) analyzed the risk of cancer to children of atomic bomb survivors and found no excess. Several reasons have been offered to explain the apparent discrepancy of the studies of Sellafield and Hiroshima-Nagasaki. (i) Gardner's report suggests a high risk of childhood leukemia from paternal exposure in the 6 months before conception. In Hiroshima-Nagasaki, children born to survivors between 1946 and 1982 were studied; very few children were conceived during the first months following the atomic explosion. (ii) Gardner studied men who received chronic exposure to radiation, whereas the atomic bomb survivors received one high dose. (iii) Between 3 and 5% of tumors of childhood that were observed in control groups in Japan are estimated to be associated with a germ-line mutation, suggesting that only a small fraction of childhood tumors may be influenced by the multigeneration effect of radiation. It may have been that the statistical power was not adequate to detect a modestly increased rate of childhood cancer. (iv) It was proposed by Nomura (34) that there may be a different genetic susceptibility in English and Japanese; the multigeneration effect of radiation is strain specific in mice (10). (v) It may be important to account for postnatal exposure to other carcinogens. In spite of the uncertainties in the interpretation of the Sellafield study (6), these results do not lose their relevance and no etiological hypothesis, including the transmission of an increased leukemogenic risk via the germ cells, can be excluded.

Following in utero exposure to atomic bomb radiation, a higher incidence of adult-type cancers was seen (35). It is now important to know whether offspring of atomic bomb survivors exhibit a higher incidence of breast and other adult tumors, now that many of the second generation of survivors have entered the adult group.

**Effect of parental occupational exposure other than radiation**

Much literature exists to link the occupational exposure of parents to cancer in their children (36,37). One limitation of the study of maternal occupational exposure to carcinogens is that it is difficult to dissociate maternal exposure before and after conception, and therefore transplacental exposure to carcinogens may confound preconceptual exposure. Transplacental carcinoma-genesis in humans is well documented (notably in utero DES and vaginal adenocarcinoma) (38). Many animal experiments also suggest that in utero exposure to carcinogens increases cancer incidence. Experimental evidence supports the hypothesis that the major effect of transplacental carcinogens is through somatic rather than germ cells, though in certain studies transplacental exposure has led to a multigeneration effect (5). Therefore, to examine a possible transgeneration carcinogenic effect in humans, it may be prudent to concentrate on the father's exposure history (39).

More than 30 studies have been published in which an association is sought between childhood cancer and parental occupation. Sample sizes for individual studies are generally too small to reach a conclusion, and it is difficult to aggregate the studies because the investigators have categorized the exposures and occupations according to different schemes. Nevertheless, of the many associations reported, some appear more often than might be expected.

The most studied association is between motor vehicle exhaust fumes and childhood leukemia. In an early study by Fabia and Thuy (40), 7.3% of 218 fathers of children dying of childhood leukemia were working as motor vehicle mechanics or service station attendants at the time of the child's birth, versus 3.8% of 386 controls. In a second study (41), the odds ratio for fathers who were motor vehicle drivers was 1.50, not quite significant ($P < 0.10$). In a further study (42), 7 of 43 fathers of children under age 20 diagnosed with leukemia were employed as either a driver, motor vehicle mechanic, service station attendant, railroad worker or fireman, whereas 8 of 86 control fathers were employed in these occupations ($RR = 1.9; P = 0.25$).

Vienna et al. (43) studied 60 children diagnosed with acute leukemia during the first year of life because they felt that early onset disease was likely to be due to a germinal mutation or to reflect transplacental exposure. The odds ratio for high exposure to exhaust fumes (employed as either a gas station attendant, automobile or truck repairman, or an aircraft maintenance worker) was 2.43 ($P = 0.03$). The association was not seen among the fathers of children with neuroblastoma.

In 1989, Buckley et al. (44) reported on the occupational exposure of parents of 178 children with acute non-lymphocytic leukemia (ANLL). The authors focused on this subtype because ANLL may be induced by radiation or benzene and also occurs after chemotherapy with mutagenic drugs. Significant risks were found for exposure to petroleum products ($RR = 2.4$ for >1000 days exposure versus none; $P = 0.002$) and for solvents ($RR = 2.0; P = 0.003$). Although exposure to motor vehicle exhaust specifically was not reported, there is likely to be a large degree of overlap among these categories.

In the study by Lowengart et al. (45), there was a significantly elevated risk for manufacturers of machinery, motor vehicles, aircraft or boats ($RR = 2.0; P = 0.008$) and a non-significant elevation for other transportation workers ($RR = 1.4; P = 0.20$).

There have been several negative case-control studies as well. Only one of 158 fathers of leukemia cases had been a motor vehicle mechanic or service station attendant in a Texas study (46). There was no association between paternal hydrocarbon exposure in general, or for motor vehicle drivers in particular for fathers of 339 leukemia in a Finnish study (47). The same proportion of leukemia case fathers and controls were employed as mechanics or gas station attendants in the study of Kwa and Fine (48). There were no elevated occupational risks in studies in California (49) or Shanghai (26); in the California study mechanics and service station attendants actually had a significantly decreased risk ($OR = 0.37; P < 0.03$). Significantly increased risks were also observed for children of fathers employed in the manufacture of iron and metal structures, in machine repair workshops, as machinists and as smiths (50).

Although none of these reports is evidence for causation, a pattern is suggested of an increased risk of leukemia associated with paternal exposure to the products of combustion engines. However, because of the small magnitude of the risks proposed, it would require a very large multicenter study to clarify the situation.

The other association that demands the greatest attention is the appearance of Wilm's tumor in the children of welders (51–54). If the four studies that reported specifically on this occupation are combined, 14 fathers of Wilms' tumor cases were employed as welders (3.2% of the total) versus 3 control fathers (0.7%). The relative risk of 4.9 for the aggregated studies is highly
Several antineoplastic drugs are mutagenic in experimental systems and it is therefore reasonable to enquire if they may act as germ cell mutagens in humans as well. Cancer survivors are an ideal study group because genotoxic agents in high doses are widely used in treatment. There is little possibility of misclassification of exposure between cases and controls, and drug doses can usually be extracted from treatment records. Cancers among a total of 4298 offspring of survivors of childhood and adult cancer have recently been reviewed (55). Of the 32 cancers in offspring, 22 were retinoblastoma. Because one-half of retinoblastoma is hereditary (20), the malignancy is probably the most relevant genetic cancer endpoint. However, in the series summarized the recurrent cases of retinoblastoma were invariably familial and consistent with the transmission of dominant mutation; retinoblastoma was not seen in a child of a parent treated for another type of cancer. Of four other tumors in offspring of treated parents there were three leukemias, which probably represent examples of the Li–Fraumeni syndrome. These results do not support the hypothesis that the risk of childhood cancer is elevated in offspring of parents with cancer in the absence of known genetic syndromes.

**Effect of other exposures**

Several studies have shown an association between paternal smoking and childhood cancer, e.g. with childhood brain tumors (56) and with rhabdomyosarcoma (57). However, since passive smoking appears to increase cancer incidence, it is difficult to rule out the effect of in utero exposure and postnatal exposure to such cigarette side-stream. In utero exposure to cigarette smoke was associated with increased risk of all cancers, and especially of reticuloendothelial cancers and of acute lymphoblastic leukemia (58).

**Molecular mechanisms of multigeneration carcinogenesis**

Cellular oncogenes and tumor suppressor genes are critically involved in carcinogenesis (59). Analysis of induced animal tumors suggests that certain oncogenes are the specific targets of chemical carcinogens (60,61). Direct induction of specific mutations of cellular ras genes by a carcinogen, DMBA, and their causal role in cell transformation were recently demonstrated (62). Since cellular oncogenes are involved in control of cell differentiation and proliferation, germ cell mutations of such genes may not be compatible with normal development. However, exogenous oncogenes with specific gene promoters have been successfully introduced into germ cells without disturbing development; such transgenic mice are prone to tumors (4).

There is no clear evidence that chemicals cause the activation of endogenous oncogenes in germ cells. However, Moser et al. (63) reported a pedigree of mice exposed to ethylnitrosourea which became predisposed to multiple intestinal neoplasia. The mutant gene (named min for multiple intestinal neoplasia) was found to be dominantly expressed and fully penetrant. Affected mice developed multiple adenomas throughout the entire intestinal tract at an early age. Dreyfuss and Gross (64) reported on the spontaneous conversion of a family of rats with a low incidence of leukemia to a high incidence of leukemia. These results suggest that cancer susceptibility genes may undergo alterations in the germ-line, either spontaneously or induced by external factors, and that the resulting cancer predisposition may be transmitted to subsequent generations.

In humans, studies on hereditary and sporadic retinoblastomas suggest that the target genes in germ cells and in somatic cells may be the same. Recently, Loktionov et al. (13) investigated whether the oncogene altered in mice exposed to carcinogens was the same as that activated in tumors found in their offspring. Pregnant mice were exposed to DMBA and their offspring were painted with the tumor-promoting agent TPA. A high incidence of skin tumors was seen in animals exposed in utero only when TPA was applied. When these exposed mice were mated, and their offspring painted with TPA in turn, the increased frequency of skin papilloma persisted, confirming the transgeneration ‘initiating’ effect of DMBA. Since DMBA-induced tumors often contain an A → T transversion mutation of the ras gene (65,66), skin tumors were examined for H-ras mutations. Tumors produced through in utero exposure contained this mutation, but tumors in second-generation mice did not. These results suggest that this specific oncogene mutation, which is frequent in DMBA-induced tumors, is not directly involved in the transgenerational component of the carcinogenic effect. Because even after TPA treatment in the F1 mice of DMBA exposed parents the skin tumors were sporadic and discrete (rather than numerous and contiguous), it is likely that DMBA damaged a gene indirectly involved in cancer formation.

While both cellular oncogenes and tumor suppressor genes may be the critical targets of carcinogens in somatic cells, it may be that recessive tumor suppressor genes are the preferred targets in germ cells. The possibility that mutant tumor suppressor genes can be transmitted through germ cells has been shown in humans. It is proposed that the Li–Fraumeni syndrome is due to a germinal mutation of the recessively acting p53 gene and that the hereditary form of retinoblastoma is due to a point mutation of retinoblastoma gene. Since many environmental carcinogens are known to cause somatic mutations, induced transgeneration effects involving tumor suppressor genes are plausible. Currently there is no direct evidence for this hypothesis.

It is important to note that if a particular mutation were induced in both somatic and germ cells at the same rate, the expected effect will depend on whether the mutation is dominant or recessively acting. For example, if a purely dominant mutation were induced in both germinal and somatic cells in exposed fathers at the rate of $1 \times 10^{-6}$ per cell, there would likely be no measurable increase in cancer incidence in their offspring. Even if the mutation were fully penetrant, only one in a million children would be at risk. On the other hand, many mutant somatic cells would result. Should the mutation incur a selective growth advantage, then clonal expansion and neoplasia could result.

For recessively acting cancer mutations two events are required to initiate a cell. In this situation the contribution of the germinal mutation to cancer incidence may be substantial. With a germ-line mutation present, the entire target cell population is at risk to develop the second mutation. The rate of cancer induced would be proportional to the product of the rates of germinal mutation and somatic (second) mutation.

The sensitivity of germ cells to mutagens varies, depending on the stage of development. The stage-dependent induction of germ cell mutations in the mouse-specific locus test by various chemicals has been reviewed (67). The results of the specific locus test for 14 chemicals have shown that the post-spermatogonial stages of male germ cells are generally more vulnerable to chemical insults than spermatogonial stem cells.
Nomura (10) compared the effect of X-rays on germ cell-mediated carcinogenesis at spermatogonial and post-meiotic stages in mice. While both stages showed effect, he concluded that the spermatogonial stage is the more sensitive period in producing tumors in F1 mice. The varying sensitivity to carcinogens of germ cells at different stages of their maturation may be specific for the expression of particular target genes. During spermatogenesis, the protooncogenes raf-1, Ki-ras and fos are predominantly expressed before or during meiosis (i.e. spermatogonia), whereas mos, abl, int-1, pim-1 and N-ras are expressed in post-meiotic haploid spermatids (68).

Genetic events that are not classical mutations, such as amplification and hypomethylation of genes, may also be implicated in the carcinogenic process. It is relevant to ask whether epigenetic phenomenon may be transmissible via the germ-line. Prody et al. (69) found amplification of the cholinesterase gene in two generations in a family subjected to a prolonged exposure to organophosphorus insecticides. Although amplification of the gene may have occurred independently in two generations, the extent of amplification was similar, and suggests the possibility of germ-line transmission. Genomic imprinting during gametogenesis has been confirmed in mice, possibly through DNA methylation. It has been suggested that most testis-specific genes are unmethylated during various stages of spermatogenesis (70). Agents that alter DNA methylation in germ cells may induce heritable changes, though these may be limited to a single generation. Henry et al. (71) have shown that in patients with the Beckwith-Weidemann syndrome, both copies of chromosome 11p may originate in a single parent (uniparental disomy). This overgrowth syndrome carries a 10% risk of childhood cancer, one-half of which are Wilms' tumor. This experiment supports the possible role for erroneous genetic imprinting in Wilms' tumor, a mechanism that has been proposed because of the observation that the maternally derived copy of chromosome 11p13 is preferentially lost in tumor tissue (72). It has been hypothesized that a separate mutation of a gene that directs the imprinting of chromosome 11p may confer familial predisposition to Wilms' tumor (73). Interestingly, of all the tumors studied for possible association with paternal occupations, the data regarding Wilms' tumor (and fathers employed as welders) are the most consistent.

Conclusions

In the last decades of cancer research the hypothesis of the multistage and multifactorial nature of the carcinogenic process has been accepted, yet risk assessments are carried out in most cases as if the carcinogen or risk factor considered were acting in isolation. This discrepancy between our understanding of the mechanisms underlying the effects leading to malignancy and our application of such knowledge may be due to the slow adjustment of the scientific establishment, as well as to the difficulty of applying biological knowledge to quantifiable risk assessment.

For instance it is still rather logical that, having to test a chemical for its carcinogenicity, the test begins by testing the chemical by itself alone. It is perhaps less logical today, though possibly dictated by overwhelming budgetary restrictions, that an expensive and long-lasting test is run without serious consideration given to possible mechanisms of action.

The evidence discussed above of the possible consequences of preconceptual exposure to carcinogens and of the potential for heritable damage in germinal cells adds complexity to accurate risk evaluation. Prezygotic exposure to a carcinogen may be at the origin of a so-called 'initiating' event that is one of many in the sequence of events leading to neoplasia. Postnatal exposure to other promoters or enhancers may therefore be necessary for the manifestation of the disease (66).

The transgeneration transmission of carcinogenic risks has been reviewed regarding possible germ cell mutations induced by environmental carcinogens. It is possible that multiple mutations may accumulate within a few generations, and result in an increased cancer risk in the descendants. It is equally possible that germ cells with multiple mutations will not function as proper gametes and are inviable. It should also be considered that damage to germ cells may not necessarily be expressed as a mutation, but as an associated heritable change such as gene amplification or DNA hypomethylation (18).

Genetic aspects of carcinogenesis are often studied in isolation from the effect of environmental carcinogens. Yet it is widely accepted that a large proportion of human cancers are related to exposure to various environmental carcinogens (74). Exposure to particular environmental agents may be sufficiently high or prolonged that they alone may be sufficient to cause tumors; but in most instances the effect of a particular environmental agent probably adds to the effects of other external as well as endogenous factors, and are modulated by the individually different genetic predisposition to cancer.

The transgeneration studies reviewed here suggest the possibility that an increased genetic predisposition to cancer may in certain instances be the consequence of a prenatal/prezygotic exposure to a carcinogen, and emphasize the importance of an interaction between genetic and environmental factors in the origin of human cancer (19).

The importance of avoiding exposure to carcinogens in our environment is therefore increased by the fact that such exposures may have an adverse effect not only on the individuals directly exposed, but also on their descendants. The awareness of the possibility of the transgeneration transmission of cancer risk may lead to more efficient primary prevention and to an increase in the proportion of avoidable cancer cases.

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