

ARE THERE FACTORS PREVENTING CANCER DEVELOPMENT DURING EMBRYONIC LIFE?

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(Accepted 6 September, 1982)

On the basis of the following literature observations, a hypothesis is advanced that the development of cancer is actively inhibited during embryonic life. Although the processes of cell differentiation and proliferation are - without comparison - most pronounced during embryonic life, cancer is rarely found in the newborn and is seldom a cause of neonatal death or spontaneous abortion. Attempts to induce cancer in early-stage animal embryos by irradiation or by transplacental chemical carcinogenesis have been unsuccessful, even when exposed animals have been observed throughout their lifetime. After the period of major organogenesis, however, the embryos become susceptible to carcinogenesis. In humans, the most common embryonic tumors arise in tissues which have an unusually late ongoing development and are still partly immature at or shortly before birth. For many human embryonic tumors the survival rates are higher, and spontaneous regression more frequent, in younger children, i.e. prognosis is age-dependent. Thus, although cancer generally appears in tissues capable of proliferation and differentiation, induction of malignancy in the developmentally most active tissues seems to be beset with difficulty. One possible explanation for this paradox could be that cancer is controlled by the regulators influencing development, regulators that are most active during embryonic life.

Carcinogenesis

Embryonic tumor

INTRODUCT.

The susceptibility of a tissue to cancer is related to its proliferative capacity [1]. Most human tumors arise in continuously regenerating tissues. In tissues with little or no capacity for renewal, such as adult neural and striated muscle tissue, cancer is rare. Sensitivity to carcinogens also seems to be heightened during active tissue renewal [1]. The period of life in which the processes of cellular differentiation and proliferation are most intense is during the embryonic stage of intra-uterine life. The embryonic period might, therefore, be expected to be active in the production of malignant tumors.

In this paper, I wish to put forward the view that cancer development is inhibited during this period of life. The hypothesis is based mainly on data culled from the literature.

Low incidence of cancer in neonates

Only 36.5 per million live-born infants are found to have cancer at birth or within the first 28 days of life [2] (Swedish Cancer Registry data for 1960-74, unpublished data). This should be compared to the 1-4% of live-born who have a diagnosable malformation at birth [3]. The death rate from cancer within the first 28 days of life has been reported to be 7.6 per million live births [2]. In 3 000 neonatal autopsies, Wells [4] found only four tumors; all were neuroblastomas and three of them, small nodules in the adrenal glands, were clearly not the cause of death.

A possible explanation for the low incidence of malignant tumors in neonates and infants is spontaneous abortion of the affected fetus. The literature contains few studies in which aborted embryos and fetuses have been examined thoroughly. However, in three reports of abnormalities in a total of approximately 1,700 spontaneously aborted intact embryos and fetuses [5-7] only one possibly malignant tumor was mentioned, a sacrococcygeal teratoma in a relatively mature fetus. Also, mothers of children developing retinoblastoma (a hereditary or spontaneous embryonic tumor) do not have a higher than normal rate of miscarriage [83]. According to pedigrees shown in the same report, this is true also of mothers with several retinoblastoma children. The precursor cells of rods and cones, from which retinoblastomas arise, are present from early in gestation [8], and would thus have been available for transformation.

These circumstances argue against a high rate of malignant tumors arising in utero but disguised by spontaneous abortion of the fetus.

Carcinogenesis in animal embryos

Another possible explanation for a low incidence of neonatal cancer is that cancer may be initiated in the embryo but become manifest only later in life. The results of attempts to induce cancer in animal embryos, however, do not support such an assumption.

In various experiments chemical carcinogens have been administered to pregnant rats and mice, and the offspring have been observed throughout their lifespan. The results appear to be uniform. When carcinogens have been administered before or during the period of major organogenesis (days 6-12 in mouse and rat), the offspring have developed a high rate of malformations but very rarely tumors. However, after the completion of organogenesis, susceptibility to malformations declines and the fetuses rapidly become more at risk of tumor-induction with chemical carcinogens (for reviews, see Refs. 9-12).

Attempts to induce tumors in embryos by ionizing radiation have shown a similar time-response relationship. Mouse embryos were exposed to radiation (10-80 r) on days 12-18 of gestation and were killed 90 weeks later [13]. In mice irradiated on day 16 or day 18 there was a heightened rate of lung, liver and kidney tumors. This increase was not seen in mice irradiated on day 12 or day 14. In another study [14], mouse

embryos were irradiated (200 r) on day 12 or days 16-18 of gestation and the offspring were observed throughout their lifespan. Mice irradiated between days 16 and 18 of gestation had a small but significant increase of lung, pituitary and ovarian tumors. Irradiation on day 12 was associated with a significant increase of congenital malformations, but with a decrease in tumor incidence, as compared to non-irradiated controls. This decrease could not be attributed to earlier death in non-neoplastic diseases in the irradiated group.

Rugh et al. [15] X-ray-irradiated (100 r) mouse embryos at different timepoints of gestation and observed the mice throughout their lifespan. Although the authors report no significant increase of tumors in prenatally-irradiated mice as compared to nonirradiated mice, their table shows interesting changes in the susceptibility to oncogenesis during gestation. Mice that had been exposed to irradiation up to day 13 of gestation developed tumors to a lesser extent than non-irradiated controls (4.8 vs. 6%), whereas mice irradiated thereafter showed a marked increase in their susceptibility to oncogenesis (10.4%). Brent [81] described different effects of irradiation on rat and mouse embryos at various stages of gestation. No increase in tumor induction was observed with irradiation on days 0-8 of gestation (rat). A questionable effect was seen with irradiation on days 8-13. An effect on tumor induction was demonstrated with irradiation on days 13-22 of gestation.

In another study, X-ray-irradiation (25-200 r) of rat embryos on day 9 of gestation [16] was followed within 3 days by the appearance of tumor-like growths in and around the brains of all embryos irradiated with 100-200 r. Progressive atrophy of the tumors occurred thereafter. At birth most of the tumors had disappeared and the few nodules that remained were small, with no evidence of proliferative activity. Neither prenatal nor postnatal death could be attributed to the presence of tumors.

Tumor transplantation in animal embryos has mostly been done to analyse the concept of tolerance. Donors and recipients, therefore, have usually been of different species. In one study a homologous tumor was transplanted into rat embryos at different stages of gestation [82]. The tumor, a sarcoma, grew if the embryo had reached the last third of gestation. When the sarcoma was transplanted to younger embryos, the rats were born without tumors. It should be noted, however, that a high percentage of the embryos were aborted.

A study by Dawe et al. [17] indicates that virus-induced tumors in some cases also follow this pattern. They infected submandibular salivary glands from mouse embryos with polyomavirus at different days of gestation. Subsequently they transplanted the glands to adult mice. Glands infected on day 12 of gestation did not develop tumors. Glands infected on day 13 and later developed an increasing number of tumors. However, at all stages 60~ 70% of the recipient adult mice developed tumors in their salivary glands, which shows that the early non-tumorous glands also harbored the virus.

Jaenisch and Mintz [18] microinjected SV40 DNA into mouse blastocysts. 40% developed to term and became healthy adults without apparent tumors, although many carried the SV40 genome. However, mouse embryos microinjected with Moloney leukemia virus

at day 8 or 9 of gestation [19] developed a high rate of leukemia at 4-6 months of age.

Prenatal carcinogenesis in man

Time response relationships in possible prenatal carcinogenesis in man are difficult to elucidate. Some retrospective studies on the relationship between childhood leukemia and prenatal exposure to irradiation indicate that children with leukemia have a 25-75% higher rate of such exposure than do healthy controls [20-23]. Prospective studies [24, 25] - among those a 10-year follow-up study of 1 292 children exposed in utero to irradiation from the atomic bomb explosions in Hiroshima and Nagasaki [26] - have not supported this connection, possibly because of the paucity of leukemia cases in these studies. Diamond et al. [25] found a higher incidence of leukemia among children prenatally exposed to irradiation in the white population, but a lower incidence among prenatally exposed in the black population.

Human organogenesis takes place mainly during the first 2 months of gestation. In a retrospective study based on interviews with mothers of leukemic children [27], a higher incidence of X-ray examinations was found particularly during the first trimester of pregnancy. Another retrospective study [23] failed to confirm this observation, although it confirmed a generally higher prenatal exposure to irradiation among leukemic children. The hospitals in the latter study were selected for their accurate X-ray data, and the author, therefore, did not have to rely on interviews. It has been argued that the mother of a leukemic child is more likely than the mother of a healthy child to recall an X-ray examination during pregnancy [28]. In both of these retrospective studies, however, the numbers of leukemic children were small. Graham et al. [29] found a similar excess of leukemia in children whose mothers reported diagnostic X-ray exposures as long as 10 years before conception of the child.

In a recent review, MacMahon [30] discussed the different reports concerning childhood cancer and prenatal irradiation and arrived at the conclusion that the question of an association is still largely unresolved. Herbst et al. [31] noted that a very rare clear cell adenocarcinoma of the vagina was found mostly in adolescent girls whose mothers had been treated with stilbestrol during pregnancy. Clear cell adenocarcinoma of the cervix was also associated with this exposure [32]. Although most cancers were found in girls whose mothers had been treated with stilbestrol throughout pregnancy, it is noteworthy that in no case was treatment known to have been initiated after the 18th week of gestation [33]. The cancer incidence in the daughters of stilbestrol-treated mothers was very low, estimated at 0.14-1.4/1 000 [33,34], whereas the incidence of other vaginal epithelial changes, such as adenosis, was as high as 34% [35]. Such changes were usually also found in association with the cancers. Furthermore, since nearly all cancer cases were girls in the age group 14-23, with a steep rise in incidence in early puberty, Herbst et al. [33] suggested that stilbestrol in itself is not a complete carcinogen, but that some other factor present during puberty acts to modify the pathological changes initiated by stilbestrol.

In this context it is interesting to note that although maternal cancer occurs in one out of 1 000 pregnancies, there are, in the world literature, only 30-40 reported cases of maternal cancer metastasizing to the product of conception [36-38].

Human embryonic tumors and maturity of affected organs at birth

Embryonic tumors form a special group of tumors that in humans arise mainly during the first 4 years of life, but occasionally also later in life. They are comprised of immature tissues normally seen only during embryonic development. Although most human organogenesis occurs during the first 2 months of gestation, some organs are still in a process of additive genesis at the end of fetal life, and even for some time after birth. Willis [39] noted that the commonest embryonic tumors in humans - Wilms' tumor, neuroblastoma, medulloblastoma and retinoblastoma - arise in organs with an unusually late ongoing development*.

This suggests that embryonic tumors in humans arise preferably at the end of gestation or after birth. If the embryonic environment is important for counteracting carcinogenesis, processes of additive genesis occurring late in fetal life may be less strictly controlled than those occurring during the period of major organogenesis.

Spontaneous regression and prognosis of embryonic tumors at different ages

A hypothetical mechanism preventing tumor development in the embryo may, as indicated, become increasingly less effective in the growing fetus and after birth. At autopsies of children dead of other causes at ages younger than 3 months, several investigators found a high rate of what they called 'neuroblastoma in situ' [40-42]. Adrenal lesions of this type were reported from autopsies in approximately 1 in 250 children aged less than 3 months [40]. They were not found in older children, which suggests spontaneous regression. The overall incidence of neuroblastoma in childhood is only about 1 per 10 000. Similar findings of nephroblastoma in situ have been reported [41]. There

* Wilms' tumor is a malignant tumor of the kidney derived from the metanephric blastema. In the normal kidney, new nephrons are formed as late as the last month of gestation, or possibly even after birth [39, 80]. Neuroblastoma arises from primitive neuroblasts derived from the neural crest. The tumors are more or less restricted to sympathetic ganglia and the adrenal medulla. In normal development, neuroblasts from the neural crest mature into sympathetic ganglia from the second month of gestation until after birth, possibly as late as the tenth year of life [39]. Remaining nodules of neuroblasts are often found in the adrenals up to 3 months of age (see neuroblastoma in situ) [43]. Medulloblastoma is a poorly differentiated tumor of the brain. Multiplying medulloblasts of the brain persist up to 2 years after birth [39]. Retinoblastoma is formed from the precursor cells of rods and cones, arising in the nuclear layers of the retina. Rods and cones are the last cells to develop in the retina, around the seventh month of gestation. In the macular and fetal fissure areas, retinal development proceeds until the fourth postnatal month [8].

is, however, some controversy as to what extent these lesions can be regarded as true tumors [43]. They may simply represent embryonal rests.

In adults, spontaneous regression of cancer is extremely rare. However, it is relatively common in at least some types of embryonic tumors. Numerous cases of spontaneous regression have been reported for neuroblastoma. Of 29 cases of spontaneous regression of neuroblastoma most were infants, and the mean age was 3 months [44]; spontaneous regression of neuroblastoma after the age of 2 years has seldom, if ever, been observed [45]. Also for retinoblastoma there have been several reports of spontaneous regression [46-49].

The prognosis for at least some types of embryonic tumors is most favorable in very young children. This applies to neuroblastoma [50-53], in which the survival rate in children less than 1 year old has been stated as 35%, but of children diagnosed after 2 years of age only 5-6% survive [53]. Similar observations are found for sacrococcygeal teratoma [54, 55]. Donnellan and Swenson [54] found that of teratomas discovered before 2 months of age 10% were malignant, whereas of those discovered after 2 months of age 92% were malignant. They also found that teratomas discovered at birth, but not operated on for more than 4 months, in most cases remained benign. Some [56-58] but not all [52] investigators have found the same age specific prognostic differences for Wilms' tumor. Paradoxically, the histologically differentiated forms of nephroblastomas are most often found in the youngest patients [59]. The reason for the contradictory findings concerning age-specific prognosis may be the controversy as to whether most of the kidney tumors observed in children below 1 year of age should be regarded as Wilms' tumors at all [59-62]. In general, kidney tumors known to be present at birth or detected during the first 3 months of life pursue a more benign course than most later-appearing tumors [62-64].

Knudson's two-mutational hypothesis

Children carrying a germ-line mutation predisposing for retinoblastoma can be born with a normal retina and not necessarily develop retinoblastoma (80-90% penetrance) [65,83], although all retina cells carry the mutation. Knudson and co-workers [65-67] therefore suggested that an extra, somatic mutation, beside the germ-line mutation, is required for the development of cancer. Another explanation would be that the development of retinoblastoma is actively inhibited during embryonic, fetal and possibly even during post-natal life and, if embryonic precursor cells still remain, malignancy develops when this control is overtaken, rather than through an extra mutation*.

* Knudson further argues that the development of the spontaneous, non-germ line-transmitted form of retinoblastoma likewise requires two mutations. Most cases of bilateral hereditary retinoblastoma are diagnosed during the first year of life, whereas spontaneous, non-hereditary cases of retinoblastoma are evenly distributed during the first 3 years of life. This delay for non-hereditary cases Knudson

Development vs. neoplasia - inseparable or incompatible?

We thus end up with the curious picture, that, although cancer only arises in tissues capable of developmental processes, it seems that during the most developmentally active period in life, the embryonic period, cancer induction is unlikely. One possible explanation for this paradox could be that cancer is a developmental deviation and as such is controlled by those regulators influencing development, regulators that are most active during embryonic life.

The possible role of induction

One of the most thoroughly investigated - and one of the most elusive - processes in embryonic development is the process of induction, the process by which one tissue induces another to differentiate in a certain direction. Since Spemann and Mangold [68] showed that the blastopore lip of the amphibian gastrula induces formation of the embryonic axis, secondary inductive tissues have been detected throughout fetal development (for reviews, see Refs. 69-71). Induction usually requires fairly close apposition between the inducing and the reactive tissues, but since, in some cases, induction has been shown to depend on diffusible substances [72-74], inductor signals are probably biochemical. All the same, attempts to isolate and characterize inductors biochemically have as of yet been largely unsuccessful. The reason may be that many non-specifically acting compounds can mimic the action of some inductive tissues. Inductors are interesting in this context, since they are the initiators and probably controllers of development and cancer most likely arises as a result of uncontrolled developmental deviations. The modes of inductor action appear to have some similarities with the few known modes of spontaneous regression of tumors. Cell death, but also cytodifferentiation to benign ganglioneuroma cells, can be seen during regression of neuroblastoma [44]. Necrotic or calcified tumor cells are found following spontaneous regression of retinoblastoma [47,49]. Tumors in amphibia, likewise, can regress either by cell differentiation or by cell death [75-77]. Death or differentiation of cells seem, therefore, to be the known modes of spontaneous regression of malignant tumors. Inductors induce differentiation, but can probably also cause cell death [78, 79]. Although the similarities may be coincidental, a closer investigation of the effects of inductive tissues on tumor cells might be rewarding.

attributes to the necessity for two somatic mutations, i.e. one more somatic mutation than for hereditary cases. However, these data do not express the same thing in both populations. The age-distribution of spontaneous cases is a true expression of the risk of developing a tumor at different ages in the general population. The age-distribution of hereditary germ-line transmitted cases is an expression of the earliest diagnosis of tumors in a group of people of which the majority will continue developing multiple tumors if untreated.

ACKNOWLEDGEMENTS

I would like to thank Drs. Eduardo Mitrani and Fanny Doljanski, Jerusalem, and Drs. Britta Wahren and Jerzy Einhorn, Stockholm, for helpful advice.

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