A Single-Hit Model of Embryonal Tumorigenesis

Louis Mandelbaum
Department of Pathology
Albert Einstein College of Medicine

Abstract

A single-hit model for dominantly heritable tumor syndromes is presented. This model suggests that: 1) During embryogenesis replication potential may normally be restricted by interaction of cell line-specific maturation factors with a finite number of surface receptors of each cell of the appropriately matched line. 2) In dominantly heritable multifocal tumor prone conditions, embryonic "rest" cells may result from chance insufficiencies of functional cell surface receptors at the time of peak concentration of the appropriate embryonic maturation factor. 3) Congenital unifocal non-heritable embryonic "rests" could arise as a consequence of rare chance failure of a normal, yet fallible, system. 4) Embryonal dominantly heritable tumors are of monoclonal derivation.

Introduction

Cancers were recently classified thus: Class I: Hereditary neoplastic conditions arising entirely independently of environmental factors; Class II: Those for which the pathogenesis can be accounted for on the basis of environmental influences alone; Class III: Those which occur as a result of a combination of environmental and genetic influences; and Class IV: "Background" cancers for which no role to either environment or genetics can be established (Knudson, 1980). Class I tumors inherited as Mendelian autosomal dominant disorders may also arise as Class IV sporadic, non-heritable, solitary primary neoplasms. For such tumors the dominantly inherited form is characterized by a high risk for a specific kind of tumor presenting as multiple primary tumors (Knudson, 1974). Examples of these disorders are: retinoblastoma, the phakomatoses, polyposis coli, nevoid basal cell carcinoma syndrome, malignant melanoma, multiple endocrine neoplasia syndromes, familial breast cancer, leukaemias, lymphomas, brain tumors, Wilms' tumor, neuroblastoma, pheochromocytoma, and chemodectoma (Knudson, 1973, 1978, 1980; Knudson and Strong, 1972).

Using retinoblastoma as a paradigm of such Class I/Class IV tumors, Knudson (1971, 1978) developed an hypothesis that the Class I neoplasias arise as a result of a germinal mutation followed by a single somatic mutation at each primary tumor site. Furthermore, according to Knudson the Class IV tumors resulted as a consequence of two separate, sequential somatic mutations, the first of these being a rare event which compensates for the absence of the germinal mutation. In these studies, data collected from retinoblastoma kindreds confirmed that carriers of the retinoblastoma gene are usually afflicted with one or more tumors in each eye but may escape with only a single unilateral tumor or sometimes even with no lesion at all. Knudson concluded that the precise number of primary tumors acquired by a gene carrier was purely a matter of chance. Hence his "two-hit" model for the origin of cancer rested, at least in part, on the implicit assumption that "chance" is synonymous with additional mutation (Knudson, 1971, 1978). Other models have also been proposed to account for the penetrance patterns of the retinoblastoma gene. For instance, Matsunaga (1978) proposed that retinoblastoma gene manifestation is a function of epigenetically inherited "host resistance," while Carlson and Desnick (1979) suggested multiple allelism and tissue mosaicism as the cause of variability of penetrance and expressivity in familial cases of retinoblastoma. But these latter hypotheses were not put forward by their authors to be universally applied and will not be discussed further here. Instead, a new model will be presented. This model potentially accounts for dominantly inherited tumors on the basis of single germinal mutations and may also provide insight into the pathogenesis of sporadic unifocal embryonal tumors. The proposed model is developed from the hypothesis that sequential cellular maturation (i.e., permanent restriction of replication potential) is directed at least in part by a complex network of embryonic inter-tissue coded chemical messengers which function as maturation hormones.

A Single Hit Model

Mintz and her coworkers (1977) have provided evidence in support of the concept that cellular maturation involves extrinsic signaling during embryogenesis. She took mouse malignant teratocarcinoma cells and found that upon their injection into early mouse embryos at the blastocyst stage, the cells became integrated into the developing embryo. In the mosaic mice that were formed, tumor-derived cells gave rise to the full range of normal functional somatic tissues and also to normal germ cells, implying some form of extrinsic regulation. If extrinsic maturation signals exist then cell receptors for those signals are likely to exist as well. Indeed Zelkowitz and Stambouly (1980) recently presented evidence that neurofibromatosis is associated with a membrane defect which is reflected by a fifty percent diminution of epidermal growth factor (EGF) binding by fibroblasts taken from affected individuals. They proposed that an embryonic membrane defect may underlie the pathogenesis of this disease. Moreover, as mentioned above, as each type of dominant embryonal tumor-prone disorder is associated with a specific kind of tumor, it may be anticipated that...
maturation signals include a system of cell line-specific hormones which interact with cell line-specific receptors. It is proposed that in each disease the underlying lesion is in a gene which encodes for a protein involved in some part of the maturation signal-receptor interaction. Furthermore, it is expected that on the average, during any growth cycle, a heterozygous cell’s pool of gene-product, which encodes receptor specificity, is about half normal and half mutant with respect to the altered locus. In addition, it is also likely that individual transcripts are selected randomly when removed from the nuclear pool and transported to the cytoplasm to serve as mRNA templates. Therefore, during any single embryonic cell growth-cycle of a heterozygote the numbers of normal functional receptors per cell would be distributed among the cells of the affected line according to a Gaussian distribution. As a result there would be a finite random number of variably located cells which utilize insufficient normal RNA to satisfy a minimum need for functional cell surface receptors during the period when maturation normally occurs. Thus, such temporarily “pseudohomozygous” cells would fail to mature and consequently would be left behind as embryonic “rests” - foci of potential neoplasia. Finally, neither the peak maturation hormone concentration nor the numbers and distribution of pseudohomozygous cells can be expected to be exactly reproduced from one individual embryo to another, hence chance variations of penetrance of single mutated genes.

The existence of such a system of cell line-specific growth maturation hormones and receptors would also account for, at least, a portion of the subgroup of Class IV “background” tumors which are of embryonic “rest” origin. This follows from the fact that interactions between hormones and their receptors are random chance events. Therefore, the probability of the occurrence of any one such interaction is a function of finitely limited variables - the hormone concentration, the number of receptors per embryonic cell, and the reaction time available. Thus, this probability is in itself finite, and where a minimum finite value as it helps to further reduce the incidence of such catastrophes.

This one-hit model, in addition to accounting for variation in penetrance of cancer pre-disposing states, also gives a theoretical basis for the wide variation of the expression of these conditions. Retention of the embryonic state as a result of failure to interact with maturation factors - may be viewed as a necessary but probably insufficient condition for neoplastic replication of cells to occur. Additional factors such as growth space and non-readily available specific nutrients and hormones may be auxiliary requirements for their development into symptomatic neoplasias. For instance, in neurofibromatosis the principal lesions are tumors of supporting elements of the nervous system such as neurofibromas, Schwannomas, and meningiomas. This condition is characterized by long periods of slow progression or even total quiescence and exacerbations during or following puberty or pregnancy (Condle and Bebin, 1972; Crowe et al., 1956). The progression of this disease is believed to depend on special hormonal conditions (Mobley et al., 1977). On the other hand, “rest” cell neoplasias which are malignant may be seen either as arising from a cell-line which normally undergoes maturation in a relatively undifferentiated state or else as resulting from dedifferentiation of a pre-existing benign differentiated “rest” tumor. Retinoblastoma (Apt and Gaffney, 1977) and neurofibrosarcoma are examples of the former and latter, respectively.

Are Embryonal Tumors of Monoclonal or Polyclonal Origin?

According to the single-hit model, individual embryonal tumors are derived from single cells, i.e., are of monoclonal origin. The major approach to determining whether embryonal tumors are of monoclonal or polyclonal origin
has been through studies of tumors taken from affected females who are coincidentally heterozygous for an X-chromosomal marker. Since in each of any female's somatic cells one of the two X-chromosomes is randomly selected for inactivation during the process of Lyonization, monoclonal origin of a tumor can be demonstrated by showing that all the tumor cells have either one or the other of the X-chromosomal markers. However, the prevalent notion that the converse (presence of both markers implies polyclonal origin) is also true may be misleading. Thus, for example, hereditary neurofibromas were subjected to this kind of test by Fialkow and colleagues (1971). They found that both X-chromosomal markers were expressed in neurofibromas from their test subjects and suggested that neurofibromas are of polyclonal origin. However, it is questionable to apply this test to neurofibromas as both light and electron microscopy have shown that neurofibromas contain both Schwann cells and fibroblasts (Escourolle and Poirier, 1978). In addition, Fialkow and his collaborators add the caveat that the validity of the polyclonal interpretation may be incorrect if an initially affected monoclonal cell is selected from the tumor mass. Thus, it should also be noted that normal mature Schwann cells adjacent to a site of nerve injury are known to undergo limited reactive replication in attempts to repair the injury (Hall, 1978). Consequently, even in tumors which are pure Schwann cell outgrowths (Schwannomas), X-chromosomal marker analysis is liable to yield misleading results since inappropriate replication of an original embryonic "rest" cell may lead to limited replication of neighboring normal Schwann cells. Indeed, presence of two intermixed tissue types, dense fibrillary (Antonini A) and loose reticulated (Antonini B) (Escourelle and Poirier, 1978), hints that such a mechanism may in fact be operative in Schwannomas.

The present model predicts that one of the two tissue types of a Schwannoma should be of monoclonal origin. In fact, certain dominantly inherited embryonal tumors have already been shown to be of monoclonal origin. For instance, Baylin and his coworkers (1976) found that inherited pheochromocytomas and medullary thyroid carcinomas have single X-chromosomes in appropriate heterogeneous test subjects. In addition, where an individual has multiple primary medullary thyroid carcinomas, they are generated as separate monoclonal outgrowths (Baylin et al., 1978) as predicted by the single-hit model.

**Differences Between the Single Hit Model and Knudson's Two Hit Model**

The phenomenon of spontaneous regression of disseminated neoplasia can be explained in terms of the single-hit model by an unscheduled, but timely, burst of synthesis of maturation factor. However, because of the obvious difficulty in reconciling spontaneous regression of disseminated malignancy with the tandem two mutation scheme, Knudson and Meadows (1980) recently suggested that certain embryonal tumors may be products of single rather than double "hits". They then proceeded to assign various embryonal tumors to either one or the other of these two categories. Their "one-hit" list included regressing neuroblastoma and neurofibromas while nonregressing neuroblastoma and neurofibrosarcoma were placed on the "two-hit" list. However, as mentioned above, since Knudson visualized a need for a second mutation in order to account for variation in penetrance of a major tumor-promoting gene, it remains unclear how Knudson and Meadows, having placed neurofibromas on a one-hit list, propose to account for the divergence of penetrance of neurofibromas in affected monozygous siblings (Vaughn et al., 1981).

**Acknowledgments**

I am grateful to Drs. Harold M. Nitowsky and Julius Marmur for stimulating discussions and helpful suggestions. Without the kind, dedicated, and patient secretarial assistance of Ms. Mimi Lanzano this manuscript would not exist—my sincerest thanks to her and also to Drs. Jean L. Cook, Sasha Englard, Henry Hoberman, Anna Kadish, Richard Lent, Sam Seitter, and many others for their support during the research and writing of this paper.

**References**


