Neoplasia: A Somatic Mutation or a Heritable Change in Cytoplasmic Membranes?  

THERE ARE few fields in biology where the literature on the theoretical and hypothetical aspects of a problem exceed that written about cancer. Since the disease affects virtually all multicellular organisms while exhibiting numerous and diverse characteristics, the theoretical proposals expounded as mechanistic explanations of neoplasia have often been based on the author's experiences in a relatively narrow area of scientific knowledge. The statement that each of us studying the basic mechanisms of neoplasia is convinced that his own concepts and theories of the genesis of this disease are the correct ones is eminently reasonable, since few workers waste their time in areas seeming to lack credibility. Yet even the most unusual theory, if it suggests experiments and questions, is worthy of scientific consideration and scrutiny. As our knowledge of biology advances, we find that theories long ago discarded may pose scientific questions that are reasonable in light of present knowledge.

Cancer as a disease is a horrible enigma: horrible because of the pain and suffering it causes; an enigma because, though we know a great deal about its biology, its genesis, and even its control, we know little of the basic molecular mechanisms of neoplastic transformation. Chemical carcinogens or their metabolites react with DNA and are usually mutagenic (1–4). They also react with RNA, protein, and other cell constituents (2, 4, 5). DNA viruses transform cells after integration of the virus nucleic acid into the cell genome (6, 7), but revertant cells with integrated phenotype (12, 13). Thus, though the cancer phenotype is apparently heritable and thought by many to reflect a specific or at least altered genotype, the exceptions to any proposed generalization are quite significant; in fact, the crucial targets for oncogenes (chemical carcinogens, viruses, X-rays) are not known.

One of the more controversial facets of neoplastic disease (at least to some researchers) is the question of whether cancer is basically the result of an altered genotype or of a non-chromosome-heritable change in phenotype. It will probably be many years before this question can be satisfactorily answered for all neoplasms, but our present knowledge allows us to look critically at the riddle and to propose answers amenable to experimental verification.

GENETIC ORIGINS OF NEOPLASTIC TRANSFORMATION

Several lines of evidence support the concept that neoplasia is basically the result of a chemical alteration in the structure of one or more genes within the karyotype. Perhaps most outstanding is the obvious fact that when a cell exhibits biologic neoplastic properties, its progeny exhibit the same property to some degree. Such properties may be continued growth and replication, altered hormonal response, or excessive production of a cell product. However, some neoplasms may progress to a phenotype and genotype significantly different from those of the original neoplastic cell or tissue (14).

The genetics of neoplastic disease has been extensively studied in both rodents and man. Strains of mice specifically susceptible to certain types of neoplasms such as hepatomas (15, 16), mammary carcinomas (17), or lymphoma (18, 19) are well known. Recent studies have suggested that the latter disease in the AKR strain results from vertical transmission within the genome of the information contained in an oncogenic agent, probably viral (20). In man, several examples of neoplasms arising from a specific gene abnormality are known. Some retinoblastomas (21), familial polyposis of the colon (22), and bilateral medullary carcinoma of the thyroid (23), are all examples of neoplasms resulting from single, autosomal, dominant, genetic mutations. Several other genetic conditions in man predispose to specific types of neoplasia. Among these are xeroderma pigmen-
tosum, Bloom's syndrome, and Chediak-Higashi disease. In contrast to the dominant mutations predominantly associated with a single neoplasm, these conditions are autosomally recessive. One of them, xeroderma pigmentosum, results from a defect in the repair of DNA. Similar mechanisms after DNA damage by chemicals or radiation have been studied (24, 25). The proposal that neoplasia may result from damage to DNA and imperfect repair is a logical consequence of such experimentation (26), though at least one xeroderma patient showing normal repair still had numerous skin tumors (27).

Further evidence supporting the genetic nature of neoplastic transformation is the finding that virtually all chemical carcinogens, most of which in themselves are unreactive with macromolecules including DNA, RNA, or protein, must be converted to "ultimate" carcinogens with high chemical reactivity before they exert an oncogenic effect (2, 5). Furthermore, most such agents in their active form are also mutagenic in several microbial and eukaryotic systems (2-4). Recent studies by Ames and his associates (28) used these metabolic conversions in a test system of liver microsomes to activate the potential carcinogen, and combined it with a strain of bacteria to show the mutagenic capability of the metabolic product(s).

Genetic information can now be experimentally introduced into cells to induce their neoplastic transformation. The nucleic acid from DNA oncogenic viruses is integrated into the host-cell DNA at an average of 5-60 molecules per cell genome (29). As a result of the discoveries of Temin and Mizutani (30) and Baltimore (31), integration of a DNA "provirus" into the host genome was demonstrated by Varmus and his associates (32) and Markham and Baluda (33). The oncogene hypothesis of Huebner and Todaro (34) proposes that one can find variants of cells transformed by either DNA or RNA oncogenic viruses, which are known to contain viral information but do not exhibit neoplastic properties. Under experimental conditions, suitable manipulation of several such revertants can bring out the neoplastic potential presumed inherent in the viral information inserted in the genome (35).

Certainly the most ubiquitous evidence that genome abnormality may be associated with the neoplastic transformation is seen in the numerous examples of abnormal karyotypes of neoplastic cells (36). Such abnormalities may be considered polygenic changes within the cell that are inherited by transmission from mother to daughter cell. At our present stage of knowledge, it appears that such karyotypic changes alter the dosage of those genes on the affected chromosomes. This may result in either an increase or decrease in the number of copies of a specific gene in the karyotype, though Ohno (37) has questioned the existence of viable neoplastic cells with less than the diploid content of DNA. As yet, except for possibly the Philadelphia chromosome seen in many patients with chronic myelogenous leukemia (38), no specific karyotypic pattern has been associated with neoplasia. More commonly, karyotype varies considerably in neoplasms arising from the same type of tissue within different individuals and sometimes even within the same individual (37, 39, 40). Recently Yamamoto et al. (41) demonstrated specific alterations in karyotypes of rodent cells transformed by polyoma virus. However, a significant number of neoplasms, especially in their early stages, do not exhibit any karyotypic anomalies when studied either by classical staining techniques (42, 43) or by the newer banding methods (44). Therefore, though relatively irreversible genetic changes, as evidenced by karyotypic anomalies, likely exist in most mammalian neoplasms, the pathway by which the genetic anomaly originated is probably quite different for each case, depending on the host as well as the carcinogenic stimulus (45).

NEOPLASIA AS A MANIFESTATION OF AN EXTRAGENOMIC ALTERATION

The concept that neoplasia is not necessarily a result of structural alteration(s) within the genome is not new or unique. Before the era of molecular biology and our understanding of genetic mechanisms, the descriptive biology of neoplasia, promoted primarily by pathologists, suggested alterations in differentiation as one primary etiologic factor in cancer (46). A recent review considered our knowledge in this area in light of recent advances (47). Theoretical concepts, from both this laboratory and others, using models derived from studies in basic molecular biology, purported to explain neoplasia as an alteration in metabolic regulatory circuits (48, 49).

Despite all these theoretical mechanisms, one must still accept the fact that whatever the result of the neoplastic transformation, it is clearly heritable. Therefore, any proposed mechanism of neoplasia must account for this phenomenon.

Differentiation as a Translational Function

To the pathology anatomist, the morphologic variation in human neoplasms, even in those derived from the same cell type, has been evident since the beginnings of cell pathology in the middle of the last century. With the advent of the electron microscope, the heterogeneity evident in the light-microscopic studies of neoplasms has been magnified. It is clear now that no single morphologic characteristic peculiar to all neoplastic cells has been, or probably ever will be, found.

Virtually all biochemical studies on mammalian neoplasms have in the final analysis supported the findings of the pathology anatomist on the extreme heterogeneity of both human and experimental neoplasia. Early investigations on the biochemistry of cancer demonstrated by glycolysis (50) and a number of other enzymatic capacities that cancer cells tend toward a single biochemical phenotype (51). However, data generated over the last two decades have shown that, when all experimental neoplasms of a specific class are studied, phenotypic heterogeneity is the rule rather than the exception. This includes data on hepatomas, mammary carcinomas, and myelomas (52). Furthermore, such phenotypic variability can be
traced to those conditions considered preneoplastic. The heterogeneity is seen not only in the activity and regulation of enzymes within neoplasms but also in the stability of messenger RNA templates (52) and in the turnover characteristics of membranes of the endoplasmic reticulum (53).

In multicellular organisms, examples of varied, heritable, phenotypic expression are well known as the process of differentiation. In such a phenomenon, the fertilized egg, a single cell with a specific genetic content, gives rise to multiple phenotypically different clones of cells which breed true both morphologically and biochemically in vivo. When explanted to tissue culture, “dedifferentiation” of normal tissues often may occur.

Considerable evidence indicates that differentiation is the result of differential and sequential gene activation (54-56) possibly mediated through interaction of specific segments of the DNA with nonhistone proteins (57) or modified histones (58).

On the other hand, another characteristic of differentiation having its counterpart in the neoplastic transformation is that of changes in messenger RNA template stability during the differentiation process. Numerous examples of the acquisition of template stability for messenger RNA coding for proteins involved in specific differentiated functions have been described (59). Changes in messenger RNA template stability have been demonstrated in adult tissue undergoing specific phenotypic changes such as regeneration or normal maturation (60). Dustin (47) and Markert (61) have suggested that malignant transformation results from an abnormality in cell differentiation. Whereas messenger RNA template stabilization may be only a single parameter in the major processes of developmental biology and neoplasia, such cytoplasmic events are apparently associated with changes in the phenotype of the developing or transformed cell. Kafatos (62) has argued that lifetimes and turned demonstrated rapid growth should possess extremely stable templates for those messenger RNA’s whose products are directly involved in cell replication.

**Reversion of Neoplastic Transformation**

While one popular dogma of oncology is that the neoplastic transformation is irreversible, a significant number of examples of reversion of the neoplastic process to a normal or quasi-normal state have been reported. One of the best known examples of such reversions or “dedifferentiation” is seen in the plant system fully described by Braun (63). However, recent evidence (64) has questioned the interpretation of those experiments. Furthermore, plant cells may undergo an extragenomic heritable cell change known as habituation, which can occur spontaneously and results in altered phenotypes in the cells undergoing the change (65). In the newt, epidermoid carcinomas produced by chemicals are reversible, once the carcinogenic stimulus is removed (66).

In the mammal, the mouse teratoma (13) is the best experimental example of redifferentiation of neoplastic cells. Here, as in the plant, single cloned neoplastic cells may exhibit a reversion of their biologically malignant potential to produce essentially normal tissues obeying the growth restraints and homeostatic mechanisms of the host in which they live. In the human, a classic example of such reversion to a fully differentiated state is seen in a small percentage of neuroblastomas, a highly malignant neoplasm of childhood which is almost always fatal (67). Sometimes, however, either in response to some therapeutic measure or unknown stimulus, a neuroblastoma undergoes differentiation to morphologically normal adult neurons, a process also occurring when the neuroblastoma cells are explanted in vitro.

Thus in a small but significant number of instances, a neoplastic transformation reversible in vivo demonstrates that all the biologic characteristics of neoplasia can occur in a cell and then be lost from the same cell at a rate exceeding by far the rate of any spontaneous mutations.

Studies from the laboratory of Sachs (68), Heidelberger (69), and DiPaolo et al. (70) demonstrated that neoplastic transformation in cultured cells can be induced by chemicals, viruses, or high-energy radiation. Sachs showed that a highly significant number of neoplastic transformations can be reversed and the cells can lose some or even all the phenotypic characteristics of the transformed state. Extending these studies, he and his co-workers also demonstrated that most of these revertants show polyploid or near polyploid karyotypes (71). From this, they proposed that through such additional genetic information, reversion suppresses the transformation characteristics (68).

The recent descriptions of nuclear transplantation from renal tumors of the frog into ova with subsequent development of tadpoles, exhibiting no evidence of neoplasia (72), also argue that the phenotypic changes in this neoplasm are extragenomic, despite the addition by a herpesvirus of new genetic information into the original neoplasm (73).

**Template Stability and Membrane Configuration**

Accepting the initial premise of this section that neoplasia is a result of heritable extragenomic changes in the cell, we are still confronted with the question of their mechanism. Theoretical considerations from this laboratory proposed that messenger RNA stabilization results from the interaction of the messenger RNA template with intracellular membrane systems (47, 74). This membrane-RNA interaction is thought to involve an association between those proteins closely associated with messenger RNA and proteins of the surface of the endoplasmic reticulum, the outer nuclear membrane, or the internal surface of the plasma membrane. The theoretical requirements for such an association were extensively treated in a recent review (75). The mosaic characteristics of cell membranes are well documented (76). The relationship of the polysome to the macromolecular mosaic
pattern of the membrane forms a structure that has been termed the membron (75).

The principal question involves the heritability of this membrane mosaic. From the model of the inheritance of the external membrane pattern of certain protozoa (77), specific membrane mosaic patterns may act as their own templates in further membrane synthesis (74, 75). Altered mosaic patterns induced either by chemicals, virus synthesis, or radiation damage would then be inherited in subsequent generations. Since new membrane synthesis occurs during specific times of the cell cycle (78), one would expect that the altered pattern would be "fixed" into the new membrane of daughter cells during a single cell cycle. The phenomenon of fixation in the induction of neoplastic transformation and its probable need for a complete cell cycle are now well documented from both in vivo and in vitro studies (79).

Theoretically, a heritable membrane change could give rise to a heritable phenotypic change based on messenger RNA template stabilization. Such a model could explain several phenomena such as differentiation of embryonic antigens by neoplastic cells (75). The principal difficulty in such a concept in relation to cancer is that it does not account for the obvious genetic changes occurring in established, growing neoplasms. The final section of this paper will attempt to resolve the 2 viewpoints into a single picture of the mechanism of neoplastic transformation.

MEMBRANES AND CHROMOSOMES IN THE NATURAL HISTORY OF NEOPLASIA

Our knowledge of the biologic development of cancer in vivo stems from the original investigations and subsequent proposals of Berenblum (80), Boutwell (81), and Salaman and Roe (82) on the initiation and promotion of neoplasms in their normal biologic environment. These concepts have not changed, though in more recent studies on in vitro neoplastic transformation it is not so easy to distinguish the two or more stages recognizable in the whole animal.

The direct evidence for genomic alteration(s) or somatic mutation(s) associated with, if not causally related to, neoplastic transformation is supported principally by the abnormal karyotypes characteristic of most established neoplasms (37) and the addition of viral information during transformation by such agents. That most chemical carcinogens are mutagens supports more indirectly a genetic basis for the malignant transformation. On the other hand, many neoplasms expressing a high degree of differentiation, exhibiting relatively slow growth rates, and generally being in the class biochemically characterized by Potter as minimal deviation tumors (83) appear, by even the most modern techniques, to have a normal chromosomal constitution (44). The evidence for reversal of the neoplastic to the normal or quasi-normal state has also been obtained with well-differentiated neoplasms both in vivo and in vitro (66, 67). The studies on the alteration of messenger RNA template stability and the membron concept were formulated primarily from experiments done with minimal deviation hepatomas.

Thus, the natural history and pathogenesis of neoplasia in vivo may well relate to genetic constitution. Although the genetic and the epigenetic theories of neoplasia initially appeared to be at odds, the two can now be reconciled in relation to our knowledge of the natural history of cancer.

Initiation and Promotion: Membrane Alteration and Genomic Multiplicity, Respectively

Berenblum's original concepts (80) argued that the phenomenon of tumor initiation was probably irreversible, whereas tumor promotion could be modulated. Roe et al. (84) recently showed, however, that, in the mouse skin system, initiation by hydrocarbons is not totally irreversible (84). The fact that certain neoplasms early in their natural history may undergo a reversion or differentiation both in vivo and in vitro affirms that the initiation of neoplasia is an immutable process. However, modulation of the neoplastic phenotype and genotype during the phase of promotion is the rule of essentially all established neoplasms.

The scheme shown in text-figure 1 relates the classical phases of neoplastic progression, those of initiation and promotion, to the genetic and epigenetic concepts discussed thus far. In this hypothesis, a class exists of neoplasms that are the direct progeny of the theoretically initiated cell (85). To this class

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**Text-figure 1.**—Pathogenesis or natural history of neoplasia.
of transformed cells belong those neoplasms exhibiting no gross karyotypic abnormalities but having heritable phenotypes altered from those of their normal precursor cells, with such change resulting from modification in the stability of messenger RNA templates. Alteration of the templates would result from changes in the membrane mosaic structure of the cell, inherited by the mechanisms discussed earlier (76, 77). In these cells, the membrane alterations producing heritable phenotypic changes may not lead to the enhancement of growth potential seen in grossly visible neoplasms. However, these changes may permanently alter the phenotype of the cell such as has been described in relation to the feeding of hepatocarcinogens to rats (86). The existence of non-neoplastic aneuploid cells has been known for some time (87, 88).

In the natural history of neoplasia, most highly differentiated neoplasms exhibiting enhanced growth potential would be expected to progress to the promoted state. The promoted cell, as defined in text-figure I, is biologically malignant and has karyotypic changes resulting from gene multiplicities or deletions, predominantly the former (37). In accord with this is the fact that metastases, the sine qua non of the malignant as opposed to the benign neoplasm, are almost always aneuploid (39, 89). The importance of gene dosage effects in biologically malignant neoplasms was discussed earlier in this paper in reference to the work of Yamamoto et al. (41) and Sachs (68). Recently, Gandin (90) also proposed theoretical concepts related to neoplastic transformation and gene dosage. Many neoplasms produced by carcinogenic agents apparently can exhibit karyotypic abnormalities in their earliest definable cell populations. This fact demonstrates that the initiated state as defined in text-figure 1 may be bypassed, depending on such factors as the dosage of the inducing agent and its general effects on the cell.

What is the relationship between changes in the macromolecular mosaic of intracellular membranes in the initiated state and the aneuploid or gene multiplicities and/or deletions characteristic of the promoted cell? The association between membrane and cell replication, though fairly clearly delineated in prokaryotes, is still unclear in the eukaryotic cell (91, 92). However, evidence supports a relationship between the nuclear membrane and interphase chromosome structures (93, 94). Therefore, it seems reasonable to argue that the transition from the initiated to promoted state may be related to heritable changes in membrane structure which can in turn alter the normal sequences of chromosome DNA synthesis and mitosis within the replicating cell.

CONCLUSIONS

With our present knowledge, no attempt to generalize a mechanistic explanation for a biologic phenomenon is satisfactory. The theses presented here attempt to relate apparently divergent concepts of neoplastic transformation to a single picture correlating both the natural biologic history of the pathogenesis of neoplastic development in vivo with the presumed critical biochemical or molecular biologic changes associated with this pathogenesis. Until we fully understand in molecular terms the biology of neoplasia as expressed by the disease in the living animal, we will not have accomplished the principal goal of experimental oncology, and even then we may still be unable to control completely the manifestations of this disease in the human.

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