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# Cellular and Molecular Aspects of Neoplastic Progression in the Mammary Gland

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BREAST CANCER appears to originate in focal mammary dysplasias [1, 2]. These premalignant lesions progress to malignancy and, beyond, to metastases in a multistep process similar to that which occurs in most neoplasms [1–6]. Thus, the process can be divided into at least three steps, (1) the transition from normal to abnormal but not malignant (dys- or protoneoplasia) [3], (2) the transition from protoneoplastic to malignant and (3) the transition from local to metastatic malignancy [5]. Each step ends in morphologically recognizable lesions.

The key to effective control of breast cancer is recognition and treatment of the earliest lesions [2]. In most cases, the malignant tumor and its metastatic progeny are readily identified by the clinical oncologist, pathologist and scientist. However, the ideal circumstance would be to identify and treat lesions before they become malignant. Unfortunately, the original, or protoneoplastic, lesions of the human breast are not well defined or understood [1]. Some forms of human mammary dysplasia are statistically related to the development of malignancy but direct proof of their malignant potential is not forthcoming [7]. Further, no significant antigenic or morphologic markers have been found in the earliest of the mammary lesions which can be used to reliably forecast their future biological behavior.

In recent years, a number of putative oncogenes have been found in association with rodent and human mammary cancer [6]. These observations provided hope that the genes involved would, in some manner, have either diagnostic or prognostic utility. The evidence that *HER*-2 gene is amplified in the DNAs of poor risk tumors fulfills these hopes [8]. It is now timely to examine the clinical implications of current oncogene research.

The themes of this essay are that (1) oncogenes are rearranged normal growth-related genes, (2) oncogene dosage and expression are both critical in the neoplastic progression and (3) oncogene expression is reflected in the morphological pattern of the neoplasm. The clinical implications of these premises are that (a) the prognosis of a given lesion will be related to the number and type of oncogenes activated, (b) the morphologic patterns will be used to predict oncogenes involved, (c) the clinical course of the neoplasm will be altered by controlling the oncogene expression or amplification and, thus, (d) oncogene-based treatment is possible. As is usually the case, most of the evidence to support these ideas is found in experimental animal models, but sufficient information is available from the observation of human tumors to be assured that the principles espoused here can be extrapolated to humans.

## ONCOGENES ARE REARRANGED NORMAL GENES

Once it was known that oncogenes had normal cellular precursors, or proto-oncogenes, the question became: what is the difference between tranforming and proto-oncogenes [9]? The initial findings appeared to satisfy Ockham's razor by being surprisingly and satisfyingly simple: a single point mutation could explain the difference between non-transforming and transforming genes [9, 10]. However, it was quickly pointed out that few, if any, of the viral oncogenes could be viewed as simple point mutations of host proto-oncogenes [10, 11]. Subsequently, the number of oncogenes known to be associated with tumors has increased and, with few exceptions, thorough analysis has revealed complex molecular additions, truncations and rearrangements of the transforming gene as compared to their proto-oncogene [9, 11, 12]. It is possible that a single mutation is sufficient to create a transforming gene; however, complex rearrange-

ments more commonly occur in nature [11].

The case of the *abl* gene associated with chronic myelogenous leukemia is illustrative. The translocation of the 22 chromosome onto chromosome 9 has placed the *abl* exons downstream from a new genetic element. This results in an elongated transcript and an enlarged gene product, so that the *abl* protein is 210 kD instead of the normal 145 kD [13]. The recently discovered *trk* gene appears to obey the same rules with a non-muscle tropomyosin exon placed in front of a protein kinase coding domain [14]. In breast cancer, Hynes and her coworkers have isolated a gene from a human breast cancer cell line which is homologous to *trk* but with a domain unrelated to tropomyosin at the 5 prime end [15]. The effects of these strange recombinants may be reflected in the rearrangements found in the viral oncogenes such as the exon −1 elements found in the viral H-*ras* [16].

Genes that can be related to oncogenesis are also related to the normal growth and development of the cell [17–19]. Hunter has classified the oncogenes into four functional groups and one function-unknown group [19]. Most of the known oncogenes fall into one of the functional groups. The transforming capacity of oncogenes related to two of the functional groups, growth factors and growth factor receptors or hormone receptors is readily envisaged. However, the protein kinase-related genes are a bit more perplexing because less is known about their role in growth control [17, 20]. However, the plethora of protein kinase-related oncogenes indicate that they have a very important role in growth and development. Ironically, we may learn important lessons about the normal functions by studying the abnormal genes.

It is important to note that the truncations and mutations which convert proto-oncogenes into oncogenic genetic elements may also change the function of the gene product [21]. For example, the *erb B*1 gene contains the protein kinase domain of the EGF receptor but does not have the external domain which binds EGF [22]. The various mutations of the *ras* gene create a protein which auto-phosphorylates and has limited ability to hydrolyze GTP [20]. The addition of the *bcr* encoded polypeptide to the normal *abl* polypeptide results in a dislocation of the polypeptide to the cytoplasm and a loss of membrane anchorage [21].

Other examples of these types of malfunctional behavior can be documented and will no doubt be a topic of future research. At the present time, it is not clear how these displacements of space and function affect the normal growth and development of the cell. The effects of a simple mutation are much easier to envisage than the complex rearrangements that are present in most transforming DNAs. The general assertion that the oncogenes are rearranged normal growth related genes may be true, but they may not function in the same manner in the growth cycle.

## ONCOGENE DOSAGE AND EXPRESSION

Many studies have demonstrated that the mere presence of a mutated oncogene is insufficient for malignant transformation [12, 18]. The oncogene must have a mutation which is critical to the function of the gene product [18]. Recent studies of clinical material have demonstrated that amplification of certain oncogenes is associated with a poor clinical prognosis [23]. The *myc* oncogene is amplified in high grade small cell carcinomas of the lung [24] and in some human breast cancers [25]. N-*myc* is amplified in the more highly malignant types of neuroblastomas [26]. In a rather extensive study of a variety of tumors, Cline's group concluded that certain oncogenes are associated with specific types of human tumors [27]. In particular, this group found that 20% of breast cancers had perturbations in the DNAs of at least one of nine proto-oncogenes.

Even more provocative than the perturbations found in the breast cancer DNA was the recognition that the amplification of the *HER*-2 gene is associated with high risk-poor prognosis breast cancer [8]. Slamon and associates found that the *HER*-2 gene was amplified in 30% of 189 breast cancer DNAs. They also found that the tumor DNAs with amplified *HER*-2 generally came from patients with regional or distant metastases [8]. Since this observation conforms to the trend established with *myc*, amplification of other genes in other neoplasms can be expected.

The amplification theme opens another type of possibility that should be considered and explored. A variety of studies in animal models have demonstrated that the activation of a single oncogene is neither sufficient nor necessary for malignancy [7, 10, 12, 18]. Examples from mammary models have included the observation of activation of the *int* gene in protoneoplastic mouse mammary hyperplasias [28]. Although murine mammary tumorigenesis is frequently associated with insertion–activation by the mouse mammary tumor virus, the protoneoplastic precursor lesion rarely has *int* gene activation. This suggests that *int* activation is associated with tumor progression and not with initiation [7, 28]. On the other hand, rearranged *int*-1 was found in one transplantable hyperplasia with a low tumor potential implying that activation of this gene was neither sufficient nor necessary for tumor progression. Similar examples can be found with *ras* in association with skin papillomas [29].

The now well-known experiments by Land *et al.* [30] and Ruley [31] demonstrated that a single oncogene by itself cannot transform fibroblasts.

Several groups have now developed transgenic mice which carry one or two known oncogenes as a part of their genotype [31–34]. These animals exhibit normal growth and development but have a high mammary tumor incidence. These types of observation support the hypothesis that the activation of a single oncogene does not cause malignancy. Since the pattern of *ras* expression and tumor development appears to be dependent upon which tissue-specific enhancer is injected with the oncogene, yet another level of control may determine the malignant potential of a given gene: namely non-transcribed regulatory elements [12].

If, as hypothesized here, neoplastic progression is a multistep process requiring many genetic events, simple gene amplification may be a convenient source of many of those required events. However, it is likely that single mutations in many genes can also cause cancer. Both possibilities would add up to the same terminal event: malignancy.

## ONCOGENES AND PATTERN OF GROWTH

The last premise is based on personal experience and is speculative. Surgical pathologists are trained to search for patterns. Pattern recognition becomes the basis for classification of tumors. Having examined a number of murine tumors in my laboratory and the laboratories of other investigators, certain associations begin to emerge. These associations are discussed here with the hope that other observers will search even more thoroughly for such morphological associations and that those of us involved in human pathology will be stimulated to ask the same questions of human breast cancer.

Three types of mammary dysplasia have been well documented in the mouse breast: (1) hyperplastic alveolar nodules (HANs) [35], (2) hormone-dependent plaques [36] and (3) ductal dysplasias [37]. The HANs have lobulo-alveolar development which is hard to distinguish from prelactating mammary gland [35]. In contrast, hormone-dependent plaques have a solid ductal development, while the ductal dysplasias induced by chemical carcinogens typically develop squamous metaplasia [37, 38].

As might be expected, the tumors emerging from these protoneoplasias retain features characteristic of the original lesion. The tumors from ductal dysplasias are usually adenoacanthomas with extensive squamous differentiation [38]. The early mammary tumors from plaques are more likely to be classified as type P tumors [39], while those from the HAN will have extensive lobular–alveolar development and be classified as type A tumors [40]. In short, the pattern reflects the origin of the tumor.

An additional association may now be introduced. A high frequency of type A tumors is associated with rearrangements of *int*-1 [7]. Type P tumors are associated with activation of *int*-2 [6]. Finally, tumors induced by chemical carcinogens usually have activation of the H-*ras* gene [41]. It is unlikely that all tumors with any one of these oncogenes activated will have the same pattern. The fact that malignancy is not caused by the activation of a single oncogene suggests that the oncogenes will be activated in various combinations, perhaps each combination leading to a unique pattern. However, at the present time, we must be content with the trends described above. Personal observation of lesions created by the deliberate introduction of recombinant oncogenes suggests that each oncogene will create morphologically distinct lesions. This suggests that the morphological patterns associated with each combination of oncogenes will be more clearly understood when such prospective studies are completed.

## CLINICAL IMPLICATIONS

The demonstrable molecular relationship between animal and human tumors is one of the most exciting aspects of modern tumor biology. The experimental evidence discussed above can no longer be ignored as abstractions limited to animal models. However, principles must be aggressively applied to the clinical situation.

Slamon *et al.*'s landmark paper indicates that the *HER*-2 gene is very important in breast cancer and that amplification of *HER*-2 is a sign of very poor prognosis [8]. Other genes have been implicated in human and murine mammary cancer [6]. If malignancy is, as postulated here, gene dose dependent, it is very important to identify the numbers and types of oncogenes activated in each tumor. One has to suspect that relatively few poor-risk tumors will be defined by the amplication of a single gene. It seems much more likely from the current evidence that the majority of tumors will have combinations of activated oncogenes. The problem for the clinician will then become to identify which oncogenes are activated. The technologies and the reagents for such an effort are becoming available to the scientific community but a more extensive coordinated effort must be made to make these reagents available to the physician-scientists and their patients. At the moment, the next step will have to be the development of panels of antibodies and probes for a more thorough examination of oncogene patterns.

A number of morphologically based classifications have been developed for the purpose of predicting the biological behavior of a given breast lesion. The mammary dysplasias have been particularly difficult to classify. However, most pathologists recognize certain types of dysplasia as having a high tumor risk. A number of prospective and retrospective studies have provided statistical evidence to support the contention that mammary

dysplasia associated with nuclear pleomorphism is associated with breast cancer [42]. These efforts have done little to alter our clinical approach to the patient. We pathologists cannot predict when a given lesion will become malignant.

Attempts to provide a morphological classification of invasive breast cancer which has prognostic significance have been much more promising. Whether the pathologist uses a subjective nuclear grading system such as taught by Black and Chabon [43] or uses an image analyzer, most agree that the DNA content of the tumor cells is an important prognostic feature [42]. The limitation of this approach is seen in the high proportion of women with primary breast cancer who have demonstrable cancer cells in the bone marrow at the time of presentation [44]. Who are these women and what is the nature of their tumors?

Based on our studies of murine mammary cancer, there is reason to believe that the pattern of activated oncogenes will influence the morphogenesis of the tumor. The current problem is that there are virtually no studies to support this hypothesis and the reagents are just now becoming available. The medical community must organize its resources so that the appropriate studies can be done quickly and accurately as the reagents become available. Just as important, the pathologists must ask the correct questions as they go about their routine examination of these breast cancer specimens. Perhaps, the perplexing minor variations that pathologists routinely detect in common forms of breast cancers will soon have a recognized molecular significance. More likely, the pathologist will be using the pathological pattern of staining with a variety of antibodies or nucleic acid probes against oncogenes or other growth factors as an adjunct to classifying breast cancers. Eventually, some astute pathologist will be using the histological pattern to predict which oncogenes will be discovered in a given cancer. This will culminate in a combined classification for breast cancer very similar to those currently used to classify lymphomas and leukemias.

It is now quite obvious that the introduction of a single mutation into a cell can have a major effect on the biological behavior of that cell. The insertion of a single mutated *ras* gene into an initiated cell can cause a malignant transformation of that cell [12]. The insertion of the same gene into a transformed cell under the appropriate experimental conditions can force the cell to become metastatic [44, 45]. If we look at the other side of this issue, another question is raised. What if we could take that gene away from the cell?

Since the oncogene must be expressed to maintain the transformed phenotype, modulation or down regulation of its expression should have a profound effect on the biological behavior of the cell. In other words, if we can shut off the oncogene will the tumor regress? Direct experimental support for this hypothesis can be found in the utilization of temperature-sensitive mutants to modulate phenotype [46]. If the oncogene is temperature sensitive, the cells will have a normal phenotype at the higher temperatures [46]. A second type of experiment demonstrating the same point involves the injection of antibodies against mutant *ras* protein [47]. The antibody injection resulted in a temporary reversion to a normal phenotype [47]. Recombinant DNA that makes RNA complementary to mRNA (antisense) has been used to successfully 'cure' cells with virus infections and genetic abnormalities [48]. Thus, expression of growth-related elements can be controlled or modulated using exogenous messages [17]. Unfortunately, these approaches to the control of the expression of undesirable genes all currently have technical limitations. However, these examples indicate that the control of gene expression is more than just theoretically possible. It has been done.

These considerations lead to the last postulate. If all of the above is true, oncogene-based therapy is possible.

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