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PROSPECTS

Genetics of the Malignant Progression of Astrocytoma

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Abstract The tendency of human cancers to progress towards a more malignant state over time is a well described biological phenomenon. Recent investigations elucidating the genetic nature of malignancy and the possible mechanisms responsible for this evolution have suggested that a sequential pathway may exist whereby a cell population accumulates a nested set of genetic aberrations which endow it with the ability to overwhelm other populations and dominate the tumor. Human astrocytomas are a dramatic case in point, where specific genetic events of amplification and deletion are seemingly related to the stages of malignancy. The identification of these aberrations represents the first stage in the dissection of the temporal process of this cancer. Its augmentation with functional analyses will likely allow a fuller genetic description of in vivo transformation.

Key words: glioma, cytogenetics, molecular genetics, tumor progression, glioblastoma staging

Human cancer has as one of its most insidious features the predilection to continuously acquire more aggressive behaviors. Indeed, beyond the organismal level, there appears to be phenotypic evolution of the cells within the neoplasm, giving rise to one of the cardinal features of advanced malignancy: the wide regional heterogeneity of cell types. This cellular evolution is superimposed in a clonal fashion so that cells of a tumor have a common ancestry but are different by virtue of their acquired characteristics [1]. Statistical [reviewed in 2] and experimental results [reviewed in 3] have led to a reasonably robust appreciation of the extraordinarily limited number of genetic lesions required to allow entry of cells into the neoplastic pathway whereas less attention has been focused on the genetics of later stages of the progression of these initiates. Perhaps the most incisive analyses have been of the process of colorectal carcinogenesis in which the evolution of tumor cell populations appears to result from cascading molecular genetic changes involving positively and negatively acting oncogenes [reviewed in 4].

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Another example in support of this scheme are the cancers arising from neuroglial cells which make up the majority of primary central nervous system tumors. Approximately 60% of these neuroglial tumors are astrocytomas [5]. Astrocytic tumors can occur in the first instance in three clinically defined malignancy stages: low (termed astrocytoma), medium (termed anaplastic astrocytoma) or high (termed glioblastoma multiforme). Followup studies of patients with low or medium stage disease have shown that most will progress over time to a higher stage, suggesting malignant progression in vivo [6]. Risk factors for astrocytoma development include radiation and chemical exposure. In addition, possible genetic predisposition is suggested by the occurrence of familial cases, both in the absence or presence of systemic syndromes, such as types 1 and 2 neurofibromatosis, tuberous sclerosis, and Turcot's syndrome (colonic polyposis associated with glioma).

In this prospective, we develop a genetic model which encompasses many of the biological characteristics of the human astrocytic tumors. The model accounts for the clinical and histopathological evidence for tumor progression as well as the apparently ordered array of accumulated genetic defects identified to date.

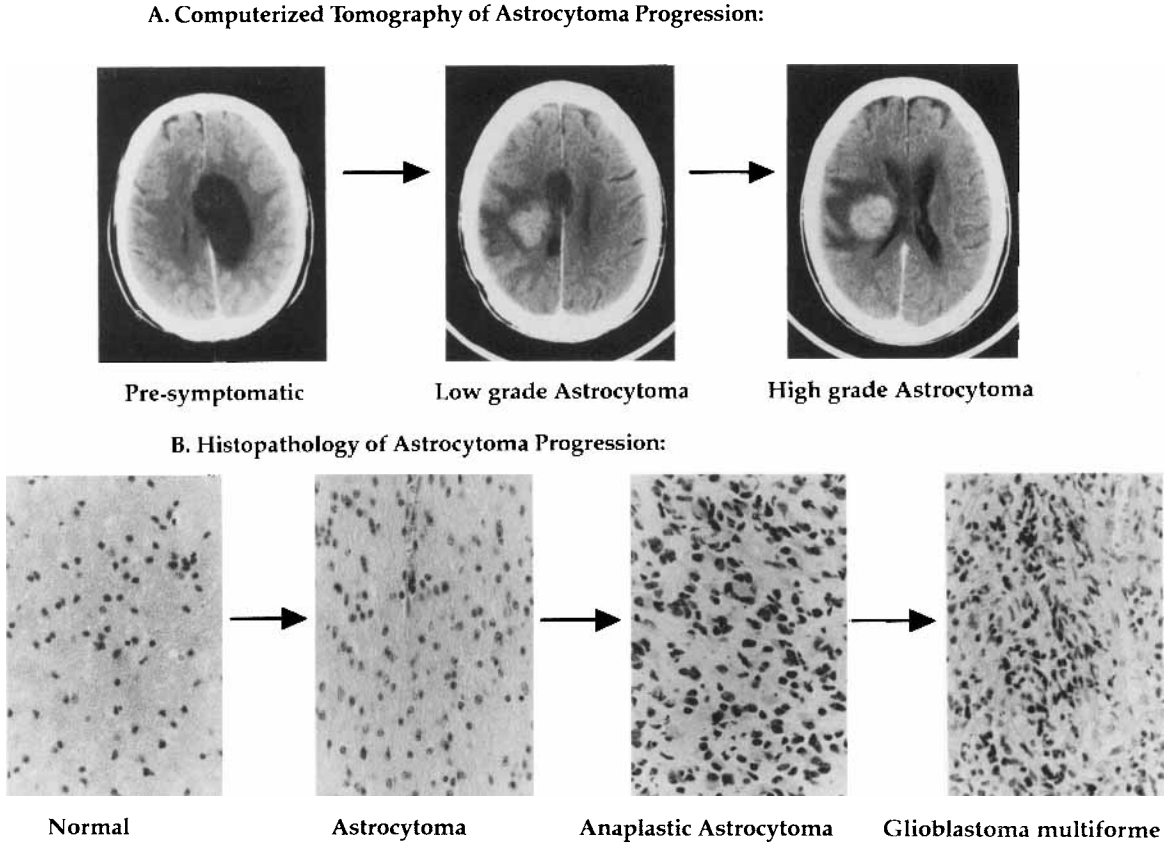

Fig. 1. Phenotypic consequences of malignant progression of astrocytoma. **A:** CT Scans of the same patient prior to disease (left), at the time of diagnosis with low grade tumor (middle), and upon recurrence, 11 months later with a high grade astrocytoma (right). **B:** Histologic features of the stages of astrocytoma progression—astrocytoma with relatively normal looking cells (left) anaplastic astrocytoma with prominent nuclear pleomorphism (middle), and glioblastoma (right) with more extensive pleomorphism, vascular proliferation, and necrosis.

BIOLOGY OF ASTROCYTOMA

The malignancy stages of astrocytic tumors are displayed graphically in Figure 1. Tumors of the cerebral hemispheres of young adults and on computerized tomographic (CT) scans (Fig. 1A) which appear as ill-defined, non-enhancing masses are diagnosed as low grade astrocytomas. At the microscopic level, tumors with these radiographic features show normal brain infiltrated with dispersed small astrocytic cells with somewhat enlarged and irregular nuclei, although the cell density may not be markedly different from normal tissue (Fig. 1B). Cells from some of these tumors have been shown to have some cytogenetic abnormalities, usually numerical changes of chromosomes 7, 10, 22, and Y. Some astrocytomas remain dormant, some enlarge slowly, and some progress to medium or high grade tumors. As can be seen in Fig. 1, these progressing tumors acquire distinct phenotypic features. For example, the higher stage recurrent tumor in the case shown in Fig. 1A became enlarged and enhanced on CT. As well, progression is marked morphologically by increased cellularity, nuclear pleomorphism, and endothelial proliferation; and, in glioblastoma, the most malignant stage of the disease, necrosis (Fig. 1B). Cells from tumors with either medium or high grade histology have shown some consistent cytogenetic aberrations including loss of chromosome 10, gains of chromosome 7, various aberrations of chromosomes 9 and 22, and double minute chromosomes [7].

Although some late stage tumors have clearly evolved from less malignant precursors, a large number seem to arise de novo. Whether de novo glioblastomas have evolved differently, or just more rapidly and subclinically, is debatable. Further, there is a curious relationship between age, tumor stage, and clinical behavior of astrocytic tumors. As a general rule, astrocytic tumors increase in frequency with advancing age,

the proportion of malignant to benign tumors increases, and tumors of any stage tend to behave more aggressively in older patients. The basis for this relationship is unclear [8].

ASTROCYTOMA GENETICS

The assumption that astrocytomas arise from astrocytes is supported by two observations. First, astrocytoma cells have the morphologic characteristics of astrocytes and express the astrocyte marker, glial fibrillary acidic protein (GFAP). Second, mature astrocytes appear to retain the ability to divide, a feature characteristic of cells capable of neoplastic changes. On the other hand, these correlations have limitations especially when one considers mixed gliomas, tumors with more than one differentiated element [9]. One hypothesis is that astrocytomas arise from initiated periventricular stem cells and that further genetic damage to these pluripotent precursors gives the tumors their individual peculiarities [10]. This argument is particularly relevant when considering the relationship of the relatively less differentiated glioblastomas to other members of the pathway.

To date, evidence for the progressive nature of astrocytic malignancy has relied solely on the clinical and morphological analyses exemplified in Figure 1. One of the cardinal features of advanced malignancy, the wide regional heterogeneity of cell types (both in terms of differentiation of the cells and their degrees of anaplasia) seems to suggest an ongoing evolution of cellular populations. Though apparently clonal, tumors appear to acquire divergent genetic characteristics through this evolution, resulting in genotypically and phenotypically diverse populations in the same tumor.

In order to address some of these aspects of astrocytoma biology and to determine the underlying basis for progression of these tumors, we have undertaken a genetic lineage analysis. We have taken advantage of the wealth of information indicating the involvement of two classes of genetic aberrations in growth dysregulation and other phenotypes in the malignant cell. On one hand, the overexpression and genetic rearrangement of cellular or viral alleles of proto-oncogenes has been shown to occur with genetically dominant effects on the acquisition of malignant characteristics [11]. On the other hand, the loss of alleles of genes presumably responsible for the maintainence of the normal growth state has also been shown to occur in tumors, relative to constitutional genotypes [12]. Thus, the malignant phenotype appears to be the result of a combination and accumulation of events which act at loci controlling both the positive and negative regulation of cell growth.

The clues we have followed in mapping these loci have come, in the main, from cytogenetics. For example, the previously mentioned presence of double minute chromosomes and polysomy of chromosome 7 accomplishes genomic amplification of the epidermal growth factor receptor (EGFR) gene which is located on chromosome 7q [7]. The incidence of EGFR amplification in gliomas is in the range of 40% [13], and is exclusive to the more malignant stages. The aforementioned aberrations of chromosome 9p appear to result in deletions of the interferon (IFN) α and β loci [14,15] and these aberrations include most of the malignancy stages. The observation of numerical changes of chromosomes 10 and 17 in glioblastoma has been refined and extended through the comparison of alleles at loci on these chromosomes in normal and tumor tissues of the various stages [14,16]. These latter analyses showed that loss of alleles caused mainly by mitotic recombination of chromosome 17 homologues occurred in the low, medium, and high grade tumors. Point mutations of the p53 gene (located on chromosome 17p) have been detected in glioblastomas [17] and are now being analyzed in lower grade tumors as well. Allelic comparisons of chromosome 10 loci have shown a nearly obligate loss of one entire homologue in glioblastomas but not in lower stage tumors. Examples of the data supporting each of these inferences of genetic change are shown in Figure 2.

Although the functional influence of these changes on the attainment of malignancy is yet subject to experimental testing, they can be used to make several inferences about the process. Excepting alterations of chromosome 17p, these abnormalities have been restricted to glioblastoma and anaplastic astrocytoma, representatives of the malignant end of the spectrum. These data when combined with the biological evidence for malignant progression of fibrillary astrocytomas suggest that losses of genetic information for chromosome 10, deletion of the IFN loci, and other less frequent events are likely to be more closely related to tumor progression than initiation.

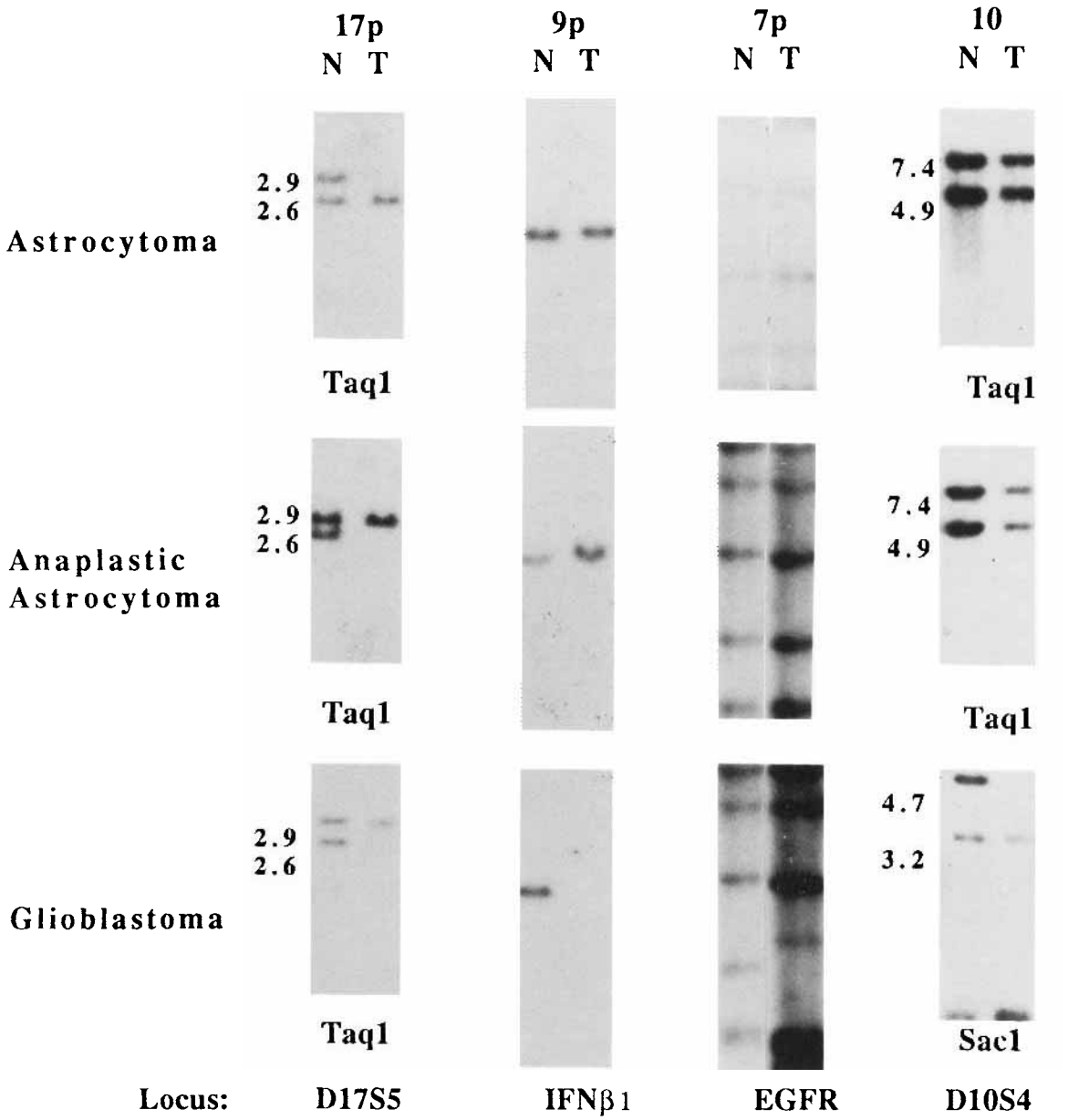

Fig. 2. Genetic alterations in the malignancy stages of astrocytoma—alleles present at loci on chromosome 17 (D17S5), 9 (IFNβ1), 7 (EGFR), and 10 (D10S5). DNA was extracted from normal tissue (peripheral blood; lanes N) and from primary tumors (lanes T) from patients with astrocytoma, anaplastic astrocytoma, and glioblastoma. Allelic sizes and designations are shown, as are probe names and restriction enzymes used.

A GENETIC MODEL OF THE DISEASE

We have taken the aforementioned biological and genetic description of astrocytoma into account in the model shown in Figure 3, which consists of an orderly sequence of events of genomic loss and overexpression or mutation, whereby initiated cells traverse the pathway through accumulating sets of alterations. The implication of a lineage relationship between the stages of the disease (Fig. 3A) has been substantiated in several instances through the commonality of the genetic changes (Fig. 3B) with respect to alleles lost at the various genetic marker loci or amplified at the EGFR locus in temporally related cases. Although we have presented the model as if there is an absolute order required for progression, this is not demanded by the model. In fact, it is reasonable and provocative to consider the order shown as preferred and that the biological consequences of inver-

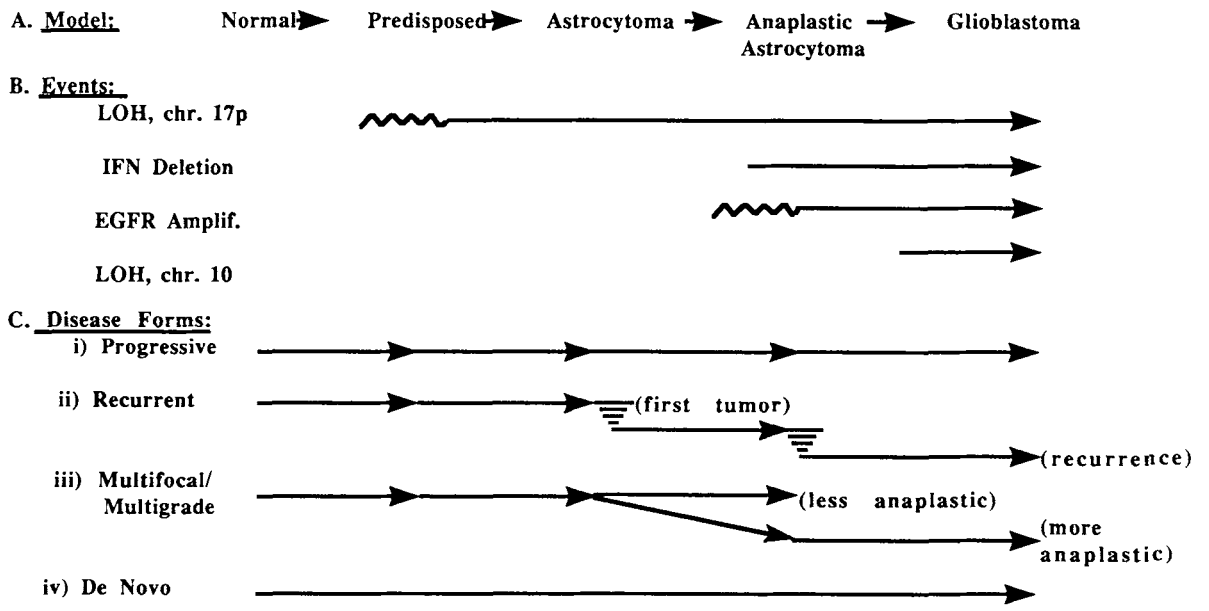

Fig. 3. Predicted genetic alterations in astrocytoma with relevance to malignant progression. The accumulation of genetic events is schematically related to the progression of malignancy. The initial mutations (LOH 17p) are clinically subthreshold related to tumor initiation, whereas the subsequent events (IFNβ deletion, EGFR amplification, and chromosome 10 LOH) are present in the progressive phases of astrocytoma in various clinical/pathologic situations. The ~~ refers to the flexible timing of recurrence, whereas the ⏚ reflects the resection of the tumor at its initial occurrence and the subsequent passage of time.

sion of the order of some events to be evocative of the biological heterogeneity of the disease. This may suggest that it is the aggregate accumulation of genetic aberration that is ultimately important for the disease occurrence and progression. This consideration may be especially important in the earliest stages of the disease, predisposition and initiation. The data obtained to date [16] are consistent with the notion that, at least in a considerable proportion of cases, these two steps may comprise a loss-of-function mutation at a locus on chromosome 17p (perhaps the p53 locus) compounded by loss of its corresponding wild type allele. By analogy with retinoblastoma [18], such mutations could occur somatically in the precursor astrocyte or be transmitted through the germline. In the latter instance, it may be that the consequence of mutations in one predisposing gene is the occurrence of glioma only whereas mutations in another might result in gliomas in association with the syndromes mentioned above. In either case, the model predicts the somatic accumulation of the required subset of events listed in Figure 3B, a prediction consonant with experimental data derived from glioblastomas of either type. These considerations require careful linkage mapping in families with simplex glioma and others with glioma in combination with other organismal abnormalities. Further, the assumption of the foregoing discussion is that the loss of constitutional heterozygosity in early stage disease represents the unmasking of a recessive mutation which is predisposing. This is also not required by the model as such events might also produce gene dosage alterations and, as well, single allele mutations might have dominant loss-of-function characteristics. These alternate possibilities are rendered less likely by the finding [16] that homozygosity is achieved through mitotic recombination resulting in two identical alleles for loci on chromosome 17p. Nonetheless, they remain open to formal testing.

As previously mentioned, one of the hallmark features of astrocytoma is its propensity to recur in a more aggressive form. Such phenomenology is easily encompassed within the model (Fig. 3Cii) by assuming that astrocytoma cells, like astrocytes, are somewhat motile, leading to difficulty in surgical removal of the total tumor. Any cell which remained and suffered the additional genetic events would then recur in a more advanced state of malignancy.

In fact, such in situ progression may be exemplified by regional anaplastic heterogeneity within tumors (Fig. 3Ciii). Such local grade dif-

ferences might be the result of the accumulation of a greater number of the requisite defects in a portion of the tumor which could be expected to ultimately outgrow its less anaplastic sister clone with time. We have tested such predictions in some cases by careful dissection and analysis and the grade-specific events described above for unrelated tumors appear to occur as predicted. Finally, a significant porportion of glioblastomas are diagnosed with no evidence of antecedent astrocytoma (i.e., de novo glioblastoma). Although this can be easily ascribed to a lack of symptomatology of the low stages of these particular cases, it seems unlikely that this is the explanation in all. Several possibilities exist for this interesting class of tumors. They may be an entirely different tumor than the progressive glioblastomas. This seems unlikely because the same genetic lesions are shared between the two types [14,16]. It is possible that a precursor cell by chance suffers two or more of the requisite events, akin to a mutational "jackpot," and is thus endowed at initiation with highly aggressive properties. Finally, it may be that the mutation which initiates these tumors is not in one of the genetic targets described in Figure 3B but, rather, in a gene required for orderly mitosis. Such a mutation could give rise to disordered chromosome segregation and, perhaps, an enhanced likelihood of genetic selection of the necessary defects. The experimental elucidation of any of these mechanisms would be of obvious significance in understanding the process.

CONCLUSION

The identification of specific genetic alterations in malignancy grades of astrocytoma has allowed the beginning of a lineage analysis of the process through their use as markers of subsets of cells and clonal aberrations which may be critical in the events of malignant progression. Obviously, the next phase of the analysis is the testing of the importance of these alterations by functional analyses of their influence on the malignant phenotype. Genetic reconstitution of malignant tumor cells with microcell-derived whole human chromosomes, chromosome subsegments, and specific gene sequences will likely be critical in this substantiation.

Strategies to clone the specific genes involved in the progression are also required. One can easily imagine the use of fine structure mapping of genetic lesions in these tumors as a prelude to their isolation by "reverse genetic" approaches. Once these genes are in hand, genetic reconstitution and ablation experiments in glial cells and cells derived from tumor stages will provide an opportunity to influence the pathways of malignant evolution in experimental animals and may in the end provide a detailed genetic description of the intricate process of transformation of this cell type.

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