Chromosomal abnormalities subdivide neuroepithelial tumors into clinically relevant groups

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Abstract. Gliomas are the most common primary brain tumor, and are histopathologically classified according to their cell type and the degree of malignancy. However, sometimes diagnosis can be controversial, and tumors of the same entity possibly have a wide range of survival. Genetic analysis of these tumors is considered to have great importance in terms that it can provide clinically relevant classification of the tumors and compensate for the limitation of the histological classification. Previous studies using comparative genomic hybridization (CGH) demonstrated that copy number aberrations (CNAs) were frequently recognized in these tumors, and revealed that a gain on chromosomal arm 7q was the most common CNA in diffuse astrocytomas, whereas a small population of the tumor showed losses on 1p/19q which characterizes oligodendrogliomas with good responsiveness to chemotherapy using procarbazine, nitrosourea and vincristine. High grade (malignant) gliomas (i.e. anaplastic astrocytomas, anaplastic oligodendrogliomas and glioblastomas) have been reported to have a gain on 7p and losses on 9p and 10q. In case of ependymomas, frequent chromosomal aberrations in intracranial tumors were a gain on 1q and losses on 6q, and, on the other hand, a gain on chromosome 7 was recognized almost exclusively in spinal cord tumors. These data suggest that intracranial and spinal cord ependymomas are different genetic diseases and comprise different subgroups within one histological entity. In conclusion, genetic analysis of gliomas may help to classify these tumors and provide leads concerning their initiation and progression. The relationship of these aberrations to patient outcome needs to be addressed. (Keio J Med 55 (2): 52–58, June 2006)

Key words: comparative genomic hybridization, astrocytoma, oligodendroglioma, ependymoma

Introduction

Gliomas, tumors of neuroepithelial cell origin, comprise the most frequent primary brain neoplasm. These tumors are classified according to their histopathological finding based on determination of the cellular type (i.e. astrocytomas, oligodendrogliomas and ependymomas are considered to consist of astrocytic, oligodendroglial and ependymal cells, respectively). Furthermore, histological grades are defined by the malignant features of the tumor and, in general, tumor response to therapy varies with histological grade. Histological evaluation has been the most important diagnostic examination in making a decision on the most appropriate treatment strategy for glioma patients to date. However, there are limitations in the systems presently used to define these tumors since interpreting cellularity, anaplasia, or even cell type is not always easy because of the histological heterogeneity when the tumor is of histologically high grade or mixed type (i.e. oligo-astrocytoma) and the lack of any tumor-specific marker when the tumor’s histologic grade is low. Thus, there are wide variations in the frequency of the diagnoses “oligodendroglioma” and “astrocytoma”, and even practicing neuropathologists who examine cases together regularly with the object of producing consensus diagnosis sometimes fail to reach agreement. Distinct diagnostic criteria therefore may help to explain why the clinical outcome varies widely within the same histologic grade, for example, median survival...
for diffuse astrocytoma is 5–7 years but some patients progress quickly to a higher grade.\textsuperscript{7–9} Chromosome aberrations, mutations, and amplifications occur frequently in gliomas. Malignant progression has been associated both with particular genetic abnormalities and an increase in the number of aberrations.\textsuperscript{10,11} The molecular events that trigger the development of low grade gliomas are unknown. However, it is believed that alterations of particular genes are responsible for the way a tumor behaves. A better understanding of glioma initiation may help to identify groups at risk for early progression, and specific genetic aberrations may serve as reliable predictors of clinical outcome. For example, Cairncross \textit{et al.} reported that allelic loss (or loss of heterozygosity) of chromosome 1p was a statistically significant predictor of chemosensitivity for anaplastic (malignant) oligodendrogliomas, and that combined loss involving chromosomes 1p and 19q was statistically significantly associated with both chemosensitivity and longer recurrence-free survival after chemotherapy although no clinical or pathologic feature of these tumors had previously allowed accurate prediction of their response to chemotherapy.\textsuperscript{12} Thus, the importance of genetic analysis on gliomas has been widely acknowledged recently, whereas the histological examination still provides information indispensable to treatment of gliomas. In this article, the authors review reports on genetic analysis of gliomas and clues to establish clinically relevant diagnostic criteria in combination with histological subtyping of these tumors.

**Comparative Genomic Hybridization (CGH)**

Comparative genomic hybridization (CGH) is a genetic analytical method to determine gains and losses of genetic material in tumors and cell lines.\textsuperscript{13–15} CGH detects and maps copy number aberrations (CNAs) as a function of the position on normal chromosomes. Tumor and normal DNAs are labeled with different fluorophores, usually green and red, and simultaneously hybridized to normal metaphase chromosomes (Fig. 1). If DNA is amplified in the green-labeled tumor DNA, more green-label than red-label will hybridize to homologous sites in the normal metaphase. If DNA is deleted in tumor DNA, less green signal will hybridize. Thus, the ratio of fluorescence intensities along the normal metaphase chromosomes measures and maps alterations in the DNA sequence copy number throughout the genome. In case of infiltrating tumors such as diffuse astrocytomas, such investigations can be difficult, since samples are often intermixed with normal brain because of the tumor’s infiltrative nature.\textsuperscript{16} Therefore, methods to exclude normal tissue and utilize small amounts of DNA for CGH are needed. A previous study validated a CGH method that uses DNA prepared from microdissected fixed tissues using a degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR).\textsuperscript{17} This technique uses specific PCR primers to make a global representation of small amounts of DNA.\textsuperscript{18–20} Consecutive sections from a formalin fixed paraffin embedded block are cut and mounted. One slide is stained with hematoxylin and eosin (HE) and the rest with methyl green (MG). After the HE slide is marked to define areas for microdissection, a small piece of tissue (<5 × 5 mm) from the MG slide within the area corresponding to the marked area on the HE slide is microdissected. DNA is extracted from these pieces and subjected to DOP-PCR. Labeling of test (tumor) DNA and reference (normal) DNA are accomplished with another DOP-PCR reaction. Metaphase spreads are prepared from phytohemaglutinin-stimulated human peripheral blood lymphocytes from a normal healthy male. The probes
are hybridized to metaphase spreads, and, after washing away unhybridized probes, the metaphase spread is observed under fluorescence microscope and images are acquired with an image processing system. The ratios of fluorescence intensity along the chromosomes are quantified.

**Diffuse Astrocytomas (low grade astrocytomas)**

Hirose *et al.* studied 30 cases of diffuse astrocytoma using tissue microdissection and DOP-PCR as described above. CNAs were recognized in more than 80% of the cases analyzed. The most frequent aberration was a gain on chromosome arm 7q. The finding agreed with the results published by Schröck *et al.*, who saw similar gains in five of 10 grade II cases. A previously reported limited karyotypic analysis of a small number of grade II cases suggested the involvement of chromosome 7. Furthermore, rat astrocytomas induced by ethynitrosourea frequently had a gain on rat chromosome 4, which shares considerable homology with human chromosome 7. Since the involvement of chromosome 7 in low grade tumors agreed with the results showing that a gain on chromosome 7 was the most frequent aberration in malignant (high grade) astrocytomas as mentioned in the next section, this gain on 7q appeared to be a key early event in a subgroup of astrocytomas (Fig. 2), and appeared to be most frequent in tumors arising in adults.

Candidate oncogenes on 7q include *MET*, a gene at 7q31 which encodes the receptor for hepatocyte growth factor/scatter factor, and its transcript is abundant in these tumors. However, *MET* is only one of many possible amplified sequences on 7q. Since CGH can detect highly amplified sequences >2–5 megabases in length, a more sensitive assay system, for example DNA microarray CGH or normalized expression arrays targeted to chromosome 7 are possible methods that may help to narrow the region of interest.

**Malignant (high grade) Astrocytomas (anaplastic astrocytomas and glioblastomas)**

Concerning high grade astrocytomas, anaplastic astrocytomas and glioblastomas, several CGH studies have been published. Mohapatra *et al.* reported that there were fewer CNAs in anaplastic astrocytomas (median = 4) than in glioblastomas (median = 7), consistent with the idea that lower grade tumors are more genetically stable with fewer genetic aberrations than higher grade tumors. These tumors differed in both the quality and the quantity of their CNAs. Glioblastomas had more amplifications present than anaplastic astrocytomas, and the most frequent was at 7p12, the location of *EGFR*.

Kunwar *et al.* reported that most gains on chromosome 7 in glioblastomas involved the whole chromosome, whereas most gains on chromosome 7 in primary anaplastic astrocytomas, as well as low grade astrocytomas, involved pieces of the chromosome. These differences suggest that mechanisms of genetic damage leading to CNAs on chromosome 7 differ in anaplastic astrocytomas and glioblastomas, although they have the common effect of increasing the copy number on parts of chromosome 7 (Fig. 2). They also indicated that CNAs involving chromosome 7 are key determinants of clinical outcome, such as those with longer survival (normal 7) and those with extremely poor survival (+7p), and concluded that a gain on 7p represented a poor prognostic marker. Patients with tumors that contained no aberrations had greater than 5 years follow-up, and this trend is similar to that observed for low grade astrocytomas with no CNAs.

![Genetic model of progression of “astrocytic” and “oligodendrogial” tumors. Published data suggest that tumors with a gain on 7q and losses on 1p/19q are of astrocytic and oligodendrogial lineage, respectively, regardless of their morphological (histological) aspects. Low grade (WHO grade 2) “astrocytic” tumors develop from normal cells (or tumor precursor cells) with a gain on 7q, and as the tumor grade progresses to grade 3, a gain on 7p and/or a loss on 19q are added. In grade 4 tumors (glioblastomas), the CNAs described above involve the common genetic events even though a gain on 7q (most frequent in astrocytomas) and a loss on 1p/19q (most frequent in oligodendroglial tumors) were mutually exclusive aberrations when tumors were at a histologically low grade (WHO grade 2).](image-url)
Burton et al. tried to identify CNAs likely related to the clinical outcome of glioblastomas, and revealed that tumors from long-term survivors (>3 years) exhibited fewer genetic aberrations, on average, than tumors from short-time survivors. They concluded that aberrations previously implicated in the molecular pathogenesis of glioblastomas (gain on 7, loss on 9p, and loss on 10q) were, in general, less frequent in long-time survivors and additional aberrations not previously emphasized as potentially prognostic (6q loss and 19q gain) were associated with the short-time survivor group. Conversely, a loss on 19q was restricted to long-time surviving patients and was considered to represent a marker of improved outcome in glioblastoma patients.

Oligodendrogliomas

Since Cairncross et al. reported that anaplastic oligodendrogliomas with losses of heterozygosity (LOH) on chromosomal arm 1p and 19q had shown remarkably good response to chemotherapy using procarbazine, nitrosourea and vincristine, various genetic analyses on 1p/19q have been widely undertaken. In every method, DNA copy number losses on 1p and 19q were the most frequent aberration in oligodendrogial tumors. Bigner et al. reported that, although LOH analysis was regarded as the gold standard for detecting losses on 1p and 19q in oligodendrogial tumors, CGH and LOH showed an excellent correlation between losses on these chromosomal arms undoubtedly because the regions of loss were large.

In studies using CGH, which offers the advantage in LOH analysis of being able to conduct an investigation on the whole genome at a single analysis, low grade oligodendrogliomas had losses on 1p and 19q, either alone or with additional gains or losses (with no consistent pattern). Anaplastic (high grade) oligodendrogioma had many molecular features in common with the well-differentiated oligodendrogliomas including losses on 1p and 19q, but also had additional deviations of a gain on chromosome 7 and losses on 4, 9p and 10. A CGH study on recurrent anaplastic oligodendrogliomas also confirmed that a gain on 7 and a loss on 10 could occur during malignant progression of typical oligodendrogial tumors that contain losses involving chromosomes 1p and 19q. It is noteworthy that high grade (malignant) oligodendrogliomas share the common genetic events even though losses on 1p/19q and a gain on 7q were mutually exclusive when tumors were at a histologically low grade. These data suggest that oncogenesis of these tumors occurs in different genetic pathways, and that they progress through the common pathway consisting of a gain on 7 (7p) and a loss on 10 (Fig. 2). However, even when these aberrations are associated, losses on 1p/19q still characterize oligodendrogliomas as having a good response to chemotherapy, which differentiates anaplastic oligodendrogliomas from high grade astrocytomas, especially glioblastomas, the most malignant astrocytomas. Several studies have reported that expression of DNA repair enzyme O6-methylguanine-DNA methyltransferase, which renders the tumor resistant to DNA-alkylating chemotherapeutic agents, was low in the tumors with 1p/19q losses. However, the mechanism of the silencing of this enzyme in association with losses on 1p/19q is still unclear.

Ependymomas

A CGH study showed clear and more remarkable cytogenetic differences between tumors that occurred in intracranial and spinal cord ependymomas. First, there were far more CNAs in spinal cord than in intracranial tumors. Secondly, the CNAs in these two groups were different. Spinal cord tumors featured a gain on chromosome 7. Other frequent CNAs seen in the spinal cord cases included gains on 2, 5, 9, 12, 15, 18, 20q and X; and losses on 13q and 22q; these CNAs were far less frequent in the intracranial cases. On the other hand, cases of intracranial tumors, especially those of grade 3, had frequent gains on 1q and losses on 9; these CNAs were nearly absent in the spinal cord tumors. Carter et al. confirmed this finding by analyzing 86 ependymomas from children and adults. It is well known that intracranial tumors frequently relapse and that spinal cord tumors rarely relapse after gross total resection. These data suggest that intracranial and spinal cord ependymomas progress along substantially different pathways although they comprise one histologic entity. CNAs frequently seen in other intracranial neuroepithelial tumors, gain on 7, losses on 1p/19q, and a loss on 10q were rare in intracranial ependymomas, which suggests that ependymomas develop through unique genetic modifications compared with astrocytomas and oligodendrogliomas. The relationship of intracranial ependymoma grade to outcome is controversial. Nonetheless, there were indications that a gain on 1q and a loss on 9 were preferentially associated with histologic grade 3 among intracranial tumors, therefore these CNAs might be indicators of outcome. The data also suggested that intramedullary spinal cord ependymomas and myxopapillary ependymomas were different genetic subgroups although both shared the common genetic characteristic of chromosome 7 gain. Loss on 22q, gains on 15q and 12 did not occur in myxopapillary tumors, while losses on chromosomes 1, 2, and 10 occurred solely in the myxopapillary group. Even though myxopapillary tumors grow slowly, they do have a greater potential for dissemination through the central canal than other spinal
ependymomas. Thus, different CNAs in these two groups of spinal ependymomas may underlie differences in their clinical behavior.

In the case of ependymomas, CNAs are well correlated to the histology in the case of spinal cord ependymomas, but, intracranial and spinal (intramedullary) tumors, which are histologically classified into one entity as “ependymoma”, were clearly differentiated by genetic analysis. This is an example which supports the idea that genetic analysis can provide information on oncogenesis of the tumors, which has not been achieved by histological examination.

**Conclusion**

Although genetic assay systems that are more sensitive than CGH, for example DNA microarray CGH, have been developed recently, CGH has the advantage of utilizing archival paraffin-embedded tissue, and can provide genetic data which have profound implications for understanding and predicting the behavior of neuroepithelial tumors. At present, the loss of 1p/19q alone is the only genetic marker that helps in the decision regarding therapy for gliomas, and this fact limits the clinical usefulness of genetic analysis. However, the authors believe that genetic profiles can supplement current historical criteria to improve the accuracy of survival predictions and eventually to provide a more objective method than histology for classifying tumors. Information on chromosomal aberrations in gliomas will provide a clue to develop a reliable and more simplified method to detect much smaller genetic abnormalities in the tumor. On the basis of the associations between clinical and genetic characteristics, further investigation is needed to identify genes in the chromosomal regions implicated in tumor development and behavior.

**Table 1 Comparison of Genetic Characteristics between Intracranial and Spinal Cord Ependymomas**

<table>
<thead>
<tr>
<th>Location</th>
<th>Intracranial</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent aberration</td>
<td>grade 2</td>
<td>grade 3</td>
</tr>
<tr>
<td></td>
<td>no consistent pattern</td>
<td>+1q, –6q, –9p</td>
</tr>
<tr>
<td>Range of aberration</td>
<td>partial chromosome</td>
<td>whole chromosome</td>
</tr>
<tr>
<td>Aberrations per case</td>
<td>few</td>
<td>many</td>
</tr>
</tbody>
</table>


Hirose Y and Yoshida K: Chromosomal aberrations in gliomas