Expression of the Tumor Suppressor Gene DCC in Human Gliomas

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Abstract

Reduced expression and/or allelic loss of the putative tumor suppressor gene DCC has been demonstrated in colorectal, gastric, pancreatic, esophageal, breast, and hematological malignancies. We examined the expression of the DCC gene in 22 tissue samples from human gliomas (glioblastoma multiforme, oligodendroglioma, and mixed oligodendroglioma/astrocytoma). Seven of 8 glioblastomas multiforme (88%) had reduced or absent DCC expression, and 8 of the other 14 tumors underexpressed DCC when compared to normal brain tissue. These results demonstrate that reduced expression of DCC occurs in human malignant gliomas and may be part of a common genetic pathway leading to neoplastic transformation and/or tumor progression.

Introduction

Human glial neoplasms can arise from one or more cell types in the brain and are classified based on the presumed cell of origin. Principal tumors include astrocytomas, oligodendrogliomas, and oligoastrocytomas. The molecular mechanisms that underlie the cause of brain tumors have not been elucidated; however, regardless of the cell(s) of origin, these tumors all progress, eventually leading to the death of the patient. It is unclear whether there is a common genetic pathway leading to malignant transformation and/or progression in all tumors or whether different tumors each have a unique genetic evolution. Elegant studies by Cho and Vogelstein (1) have demonstrated that the progression of colorectal tumors from a benign to a malignant state is associated with the sequential activation of specific oncogenes and inactivation of tumor suppressor genes. Genes involved in these changes include activation of ras and deletion and/or inactivation of MCC, APC, p53, and DCC (Deleted in Colon Cancer) (2). Genetic changes involving these genes have also been implicated in the pathogenesis of other tumors including breast carcinomas (3), gastrointestinal cancers (3), ovarian adenocarcinomas (4), prostatic carcinomas (5), ductal pancreatic adenocarcinomas (3), and certain hematological malignancies (6); however, different frequencies of these abnormalities in various types of cancer suggest that the specific events leading to tumor progression may be tissue specific. We now report that reduced expression of the DCC gene can be found in human glioblastoma tumors, suggesting that this tumor suppressor gene may play a role in the pathogenesis of this disease.

Materials and Methods

Patient Selection and Tumor Histopathology. Tumor samples were obtained from patients undergoing craniotomy for resection of malignant brain tumors. One patient (LH) had a second operation for recurrent tumor (LHR). Histological grades were assigned using the Ringertz-Burger (7) and revised WHO (8) systems for the astrocytomas and the Kernohan system (9) for the oligodendrogliomas and oligoastrocytomas. Tumor tissue was frozen and stored in liquid nitrogen. To maintain patient confidentiality, patient samples were given a random letter code for identification. The clinical and histological data are shown in Table 1.

Isolation of Total Cellular RNA. Total cellular RNA was isolated from fresh or frozen tumor tissue and from paraffin-embedded tissue using RNAzol B (Cinna/Biotecx Laboratories, Inc., Houston, TX). RNA isolation from 80-200 mg of tissue was done using conditions specified by the manufacturer. RNA from 20-µm paraffin sections was obtained by mixing the tissue section with 400 µl RNAzol B and incubating it at 65°C for 3 h. Two hundred µl of chloroform were added; the samples were shaken well and placed on ice for 5 min. Samples were then centrifuged at 18,000 × g at 4°C for 15 min. RNA in the upper phase was precipitated with 0.5 ml of isopropanol and 10 µg glycogen at -20°C overnight.

RT-PCR. RT followed by PCR was used to determine DCC expression in a semiquantitative manner. Total RNA (1 µg/20 µl of RT reaction) was reverse transcribed using the GeneAmp RNA PCR kit and random hexamer primers (Perkin-Elmer Corp., Norwalk, CT) with conditions specified by the manufacturer. Six µl aliquots of this reaction were used for PCR with primers from exons O (5'-TTCCCGCATGGTTTTAATA-3', sense) and P (5'-AGCTT-CATATTCCAGCCAAACA-3', antisense) of the DCC gene (10). Since these primers span an intron, they will amplify a 253-base pair fragment from complementary DNA only. The use of one RT reaction for multiple PCR reactions allowed us to use the "housekeeping" gene histone 3.3 (11) as a "loading" control. For analysis of DCC gene expression, reactions were run without an oil overlay in a Perkin-Elmer GeneAmp PCR System 9600 thermal cycler for 40 cycles consisting of 94°C for 1 min; 56°C for 2 min; and 72°C for 1 min. A 6-min incubation at 72°C was used at the end of the reaction to allow ample time for full extension of amplified DNA fragments. Histone was amplified using the same reaction conditions for 30–32 cycles. Samples were analyzed by gel electrophoresis in a 1.4% agarose gel, visualized using ethidium bromide, and a polaroid picture was taken of the resulting gel. Quantitation was done by densitometric analysis of the photographic negative (12). Negative controls without RNA were performed for each experiment.

Results

Of the eight GBM samples, only one tumor (KS) had a significant amount of admixed brain. This tumor had expression of DCC similar to that observed in normal brain tissue (Fig. 1; Table 2). The brain tissue in this tumor sample contributed to the DCC expression detected in this tissue; however, the relative amounts of benign brain cells and tumor cells suggest that the observed DCC expression is not due solely to the parenchyma. Seven of the GBM samples essentially consisted entirely of neoplastic cells and supporting vasculature with only minimal amounts of necrosis. Among these seven tumors, two had DCC expression approximately one-half that of normal tissue, two had barely detectable expression, and three of these samples had no detectable expression of DCC (Fig. 1; Table 2).

Patient LH is particularly interesting in that five months after an initial large resection for a typical highly pleomorphic GBM, a second large resection was performed and only diffusely infiltrative low grade astrocytoma was found. The primary tumor sample had no detectable expression of DCC; however, tissue from the recurrent tumor demonstrated expression approximately one-half that of normal brain.
possibly due to the included normal parenchyma or due to the character of the low grade astrocytoma (Fig. 2, a and b). This patient remains alive and well more than 3 years after the initial surgery.

Fourteen tumor samples from 13 patients diagnosed with oligodendroglioma or oligoastrocytomas were analyzed. Seven of these tumors were low grade (grade 1-2) and 6 were high grade (grade 3-4). Three of the seven (43%) lower grade tumors (PO, QK, and RT) and four of the six (67%) higher grade tumors (RW, QM, OG, and LI) expressed DCC at a level less than that of normal brain tissue. Five of the samples (LC, QK, OG, RV, and LI) had little residual parenchymal cells. The other tumor samples had significant numbers of nonneoplastic cells (Table 2). Expression of DCC varied in these tumors, ranging from absent to normal; thus, the DCC expression did not consistently parallel the estimates of residual parenchyma. This indicates that the variable expression of DCC was not simply due to the residual parenchyma and was rather a feature of the tumors themselves. The tumor sample RV, a grade 4 oligodendroglioma, demonstrated expression of DCC similar to that observed in normal brain (Fig. 1; Table 2); however, the sample that was available for analysis did not exhibit the high grade histological features seen elsewhere in the tumor.

Two samples were analyzed from case QK. On gross examination, one (QK) had the appearance of expanded white matter with an overly firm texture that was indicative of extensive tumor infiltration. The second specimen (QK-2) was soft and gray, with a somewhat translucent appearance indicative of a mucopolysaccharide-rich stroma. Microscopic examination confirmed the gross expectations. Examples can be seen in Fig. 2, c and d. QK had readily identifiable residual white matter, and the expression of DCC was approximately that of normal brain (Fig. 1; Table 2), while QK-2 had myxoid, microcystic stroma with little residual parenchyma (Fig. 2, c and d) and no detectable DCC expression (Table 2).

Discussion

Cancer is thought to be caused by the accumulation of specific genetic lesions; however, the changes leading to the initiation and
The progression of human glial tumors has not been fully elucidated. This may be due in part to the paucity of tumor tissue samples that are available from these tumors. Genetic aberrations to the progression of these tumors is, as yet, unknown.

Cho and Vogelstein (1) have demonstrated that there is a sequential accumulation of genetic defects as colorectal tumors progress from a benign to a malignant state. This suggests that an understanding of the genetic aberrations to the progression of these tumors is, as yet, unknown.

The presence of normal cells increases the apparent expression of DCC mRNA found in seven of our tumor samples. In addition, there was variable expression (ranging from absent to normal) of DCC among tumors with similar amounts of normal brain. Since the results obtained from RT-PCR are an average of the expression found in all of the cells in the tumor sample, a reduction in the apparent expression of DCC may be more significant than a finding of normal or near-normal DCC expression.

Another factor which may contribute to the variation seen in DCC expression is the heterogeneity inherent in human glial brain tumors (13). Significant heterogeneity has been described in these tumors with respect to tumor cell morphology, phenotype, flow cytometry, Ki-67 labeling indices, genetics, and molecular biology (20-25). This heterogeneity often complicates precise diagnosis and thus estimates of patient prognosis. Not surprisingly, the expression of DCC demonstrated intratumor heterogeneity in the single tumor in which two separate samples (QK and QK-2) were analyzed. The availability of molecular markers of tumor progression could greatly facilitate diagnostic and prognostic determinations. The underexpression of DCC in 7 of 8 GBMs suggests that underexpression of this gene may be one such marker. Furthermore, the variable expression observed in the lower grades of oligodendrogliomas and oligo/astro tumors suggests that loss of DCC expression may not be an early event in the progression of this disease.

The results of this study demonstrate that a significant number of human malignant brain tumors show reduced expression of the tumor suppressor gene DCC. The addition of gliomas to the list of tumors with reduced expression of DCC suggests that the pathways leading to neoplastic transformation and/or tumor progression in different tissues may have some genetic changes in common. Furthermore, the identification of specific genetic abnormalities in different stages of neoplasia (as has been demonstrated in the progression of colorectal carcinoma from dysplasia to invasive carcinoma) may provide molecular markers of tumor progression. The loss of DCC expression evident in most of the GBMs and some of the oligodendrogliomas and oligo/astros suggests that reduced expression of DCC may be useful as

### Table 2 Expression of the DCC gene in gliomas and mixed gliomas

<table>
<thead>
<tr>
<th>Tissue code</th>
<th>Diagnosis</th>
<th>Grade</th>
<th>% non neoplastic cells</th>
<th>Survival (days)</th>
<th>Status</th>
<th>DCC expression</th>
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<tr>
<td>KE</td>
<td>GBM</td>
<td>4</td>
<td>+/-</td>
<td>408</td>
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<td>+/-</td>
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<tr>
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<td>GBM</td>
<td>4</td>
<td>+/-</td>
<td>237</td>
<td>D</td>
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<td>KV</td>
<td>GBM</td>
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<td>+/-</td>
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<tr>
<td>OF</td>
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<td>+/-</td>
<td>158</td>
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<td>Oligo/Astro</td>
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<td>+/-</td>
<td>953</td>
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<td>+</td>
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<td>170</td>
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<td>+</td>
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<tr>
<td>QK-2</td>
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<td>+/-</td>
<td>170</td>
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<td>-</td>
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<tr>
<td>RW</td>
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<tr>
<td>LF</td>
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<td>RV</td>
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<td>+/-</td>
<td>857</td>
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<td>+</td>
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<td>LI</td>
<td>Oligo</td>
<td>3</td>
<td>+/-</td>
<td>498</td>
<td>D</td>
<td>-</td>
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</tbody>
</table>

a Tumor code is a random 2 letter code assigned to the tumor. Recurrent tumor from the same patient is given the same 2 letter code followed by an “R.”
b GBM, glioblastoma multiforme; LGA, low grade astrocytoma; Oligo/Astro, oligoastrocytoma; Oligo, oligodendroglioma.
c Grading systems used are described in “Materials and Methods.”
d + +, 25-50%; +, 12.5-25%; +/-, minimal.
e Survival is in days postsurgery.
f GBM, glioblastoma; LGA, low grade astrocytoma; Oligo/Astro, oligoastrocytoma; Oligo, oligodendroglioma.
g NA, not available.

DCC EXPRESSION IN HUMAN GLIOMAS
a diagnostic and/or prognostic marker in gliomas. A more detailed evaluation of DCC gene expression in gliomas with regard to its role in glioma development and/or progression and its potential diagnostic usefulness is warranted.

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References

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