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ABSTRACT

Identification of the genes that are differentially expressed in brain tumor cells but not in normal brain cells is important for understanding the molecular basis of these neurological cancers and for defining possible targets for therapeutic intervention. In an effort to discover potentially antigenic proteins that may be involved in the malignant transformation and progression of human glioblastomas, a novel antibody-based approach was developed to identify and isolate gene products that are expressed in brain tumors versus normal brain tissue. Using this method, whereby tumor-specific antibodies were isolated and used to screen a glioblastoma cDNA expression library, 28 gene products were identified. Nine of these clones had homology to known gene products, and 19 were novel. The expression of these genes in multiple different human gliomas was then evaluated by cDNA microarray hybridization. One of the isolated clones had consistently higher levels of expression (3-30-fold) in brain tumors compared with normal brain. Northern blot analysis and in situ hybridization confirmed this differential overexpression. cDNA sequence analysis revealed that this gene was identical to a relatively new class of growth regulators known as granulins, which have tertiary structures resembling the epidermal growth factor-like proteins. The 2.1-kb granulin mRNA was expressed predominantly in glial tumors, with lower levels in spleen, kidney, and testes, whereas expression was not detected in non-tumor brain tissues. Functional assays using [3 H]thymidine incorporation showed that this protein product, not just the capacity to synthesize a protein. Furthermore, this endeavor has direct clinical relevance if combined with the development of innovative rational therapies that specifically target these differentially expressed gene products.

A variety of methods are currently used to isolate genes associated with particular differential phenotypes. Subtractive hybridization (6), differential display (7–10), representational difference analysis (11–14), serial analysis of gene expression (5, 15), and suppression subtractive hybridization (16, 17) all allow for the cloning and identification of differentially expressed sequences. Although all these techniques identify tissue-enriched mRNAs, none select for tissuespecific proteins. Because we initially were interested in identifying glioma-associated antigens that may be potential targets for brain tumor immunotherapy, we set out to devise a differential screening technique that provided actual confirmation of the presence of a protein product, not just the capacity to synthesize a protein. Furthermore, we wanted to select for proteins with antigenic determinants that may be potentially recognized by the immune system.

Here we report an approach to identifying differentially expressed gene products that are actually translated from mRNA species, using antibody-based screening of a cDNA expression library. We further show that this method, which we termed DIA,2 can be coupled to cDNA microarray screening and allowed the identification of a putative growth factor gene, granulin, which may play a role in the malignant progression of glioblastomas.

INTRODUCTION

Cancer is the result of cumulative multiple genetic mutations, which result in the activation of oncogenes and/or the inactivation of tumor suppressor genes. It is the differential expression of these critical genes and their downstream effectors that enables cells to override growth controls and undergo carcinogenesis (1, 2). The pathological changes that arise in cancer, whether caused by a single gene mutation or multiple genetic alterations, are essentially driven by changes in gene expression (1, 2). In the malignant progression of astrocytic cancers, it has been shown that accumulation of multiple genetic lesions underlies the neoplastic process. These lesions include mutations of the genes p53, p16, RB, and PTEN, as well as amplification of CDK4 and EGFR (3, 4). Although these known genetic abnormalities have been well documented in the formation of the most malignant brain tumor, glioblastoma, recent insight into the extent of gene expression differences underlying malignancy reveals that hundreds of gene transcripts may be expressed at significantly different levels between normal and neoplastic cells (5). Therefore, there is considerable room for the identification of novel genes that are differentially expressed in brain tumor cells to further our understanding of the complex molecular basis of these neurological cancers. Furthermore, this endeavor has direct clinical relevance if combined with the development of innovative rational therapies that specifically target these differentially expressed gene products.

MATERIALS AND METHODS

DIA. GBM tumor tissue was immediately snap frozen in liquid nitrogen at the time of surgery. Non-tumor brain was obtained from a surgical resection for trauma and similarly frozen. Both tissue specimens were homogenized in PBS (pH 7.0) with a glass mortar and pestle. The soluble material was aspirated, and the insoluble material was re-extracted into a second fraction, using 0.1% SDS. Both fractions were used for affinity purification and immunizations. Affinity chromatography was carried out using an aliquot of extracted material immobilized on CNBr-activated Sepharose 4B columns (Pharmacia) according to the manufacturer’s specifications. The immobilized GBM tumor tissue extract was loaded into a fritted column (Varian), blocked with 1 M glycine, preccyced with 0.1 M HCl, and neutralized with 0.1 M BBS (pH 8.4). The extract of non-tumor brain tissue was similarly immobilized.

Received 10/1/99; accepted 1/5/00.

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2 Supported in part by grants from the National Cancer Institute (Grant NIH CA 82666-01), the American Cancer Society (Grant IN-131), the Howard Hughes Medical Institute (Grant 76296-549701), and the STOP Cancer Foundation (awarded to L. M. L.). We also thank the generous support of the Henry E. Singleton Brain Cancer Research Institute (Grant 76296-549701), and the STOP Cancer Foundation (awarded to L. M. L.).

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2 The abbreviations used are: DIA, differential immuno-absorption; GBM, glioblastoma multiforme; BBS, borate-buffered saline; PBS, fetal bovine serum; EGFR, epidermal growth factor; EGFR, EGFR receptor.
Antisera were raised against the GBM tumor homogenate by s.c. and i.m. immunization of New Zealand White rabbits, using complete and incomplete Freund’s adjuvants. Several bleedings were collected from two animals, pooled, and diluted 1:2 with 0.1 M BBS (pH 8.4). The diluted antisera was passed over the GBM affinity column, and unbound material was washed off with BBS. Bound material was eluted off using glycine buffers adjusted to pH 3, pH 2, and then pH 1. The effluent and eluate were monitored at 280 nm (LKB), and the antiserum was passed repeatedly through the column until depleted. The eluate was then collected into BBS, checked for neutral pH, and cross-absorbed repeatedly (until depleted of cross-reactive antibodies) against the column of non-tumor brain to select out antibodies that may bind normal brain antigens. The unbound material was further cross-absorbed against normal human plasma to select out nonspecific antibodies. The final product was concentrated and dialyzed against 0.1 M Tris (pH 9.0), and then 20% ethanol was added to precipitate the protein. After centrifugation, the precipitate was collected and washed twice with 95% ethanol, and then air-dried.

Construction and Screening of cDNA Expression Library. For construction of the glioblastoma cDNA expression library, a human GBM tumor was snap frozen in liquid nitrogen at the time of surgery and stored at −80°C. Total RNA was extracted from 500 mg of fresh frozen tumor tissue, using Trizol reagent according to the manufacturer’s protocol (Life Technologies, Inc.). mRNA from a total of 30 μg of RNA was isolated using double chromatography on oligo-dT cellulose columns (Life Technologies, Inc.). Double-stranded cDNA was synthesized from this mRNA, using a Superscript II cDNA synthesis kit (Life Technologies, Inc.), and the cDNAs were ligated into a λ ZAP-cDNA phagemid vector (Life Technologies, Inc.). We obtained a library titer estimated at 5.0 × 10⁹ plaque-forming units. Approximately 2.0 × 10⁹ plaque-forming units were plated and grown in the presence of isopropyl-1-thio-beta-β-D-galactoside, lifted onto nitrocellulose membranes, and incubated with biotinylated anti-GBM antibodies (1:10,000 dilution). The membranes were then incubated with streptavidin-horseradish peroxidase and dialyzed against PBS containing 0.1% SDS at 37°C for 20 min, followed by 0.2 M SSC containing 0.1% SDS at 61°C for 20 min, and exposed to X-ray film (Kodak) at 80°C. Blots were then stripped with 0.1% SSC at 105°C for 15 min and reprobed with ³²P-labeled ribosomal 18S cDNA to control for gel loading and RNA integrity.

In Situ Hybridization of Granulin mRNA Expression. In situ hybridization was performed using ³³P-labeled riboprobes following previously published protocols (22). Briefly, surgically resected human brain tissues (tumor and non-tumor) were rapidly frozen in isopentane directly from the operating room. Frozen tissues were sectioned on a cryostat at 20-μm thickness, post-fixed in 4% paraformaldehyde, washed, and stored at −75°C. Sections were washed, acetylated, defatted, and incubated with ³³P-labeled sense or antisense granulin cRNA probe (10⁷ cpm/ml) at 60°C overnight (18–24 h). Following hybridization, membranes were washed (2× SSC containing 0.1% SDS at 37°C for 20 min, followed by 0.2× SSC containing 0.1% SDS at 61°C for 20 min), and exposed to X-ray film (Kodak) at 80°C. Blots were then stripped with 0.1% SSC at 105°C for 15 min and reprobed with ³²P-labeled ribosomal 18S cDNA to control for gel loading and RNA integrity.

Measurement of Cell Proliferation. The effect of granulin D peptide and granulin antibody on the proliferation of primary rat astrocytes and three early-passage human glioblastoma cell lines were examined. Synthetic peptide, consisting of the 55-amino acid sequence of granulin D (26), was provided by Research Genetics. For the antibody studies, a polyclonal antibody was raised against this 55-amino acid synthetic peptide conjugated to keyhole limpet hemocyanin. The IgG fraction was isolated from sera, using protein A-Sepharose (Zymed), concentrated using a Centri-cell concentrator (Amicon), and then 10 μg/ml of each antibody was added to the cultures. The cultures were incubated for 72 h, and then the number of cells per well was determined by a microtiter reader (Bio-Rad) at 570 nm (human astrocytes) or 540 nm (rat astrocytes).
and stored in borate buffer consisting of 25 mM sodium borate, 100 mM boric acid, 75 mM NaCl, and 5 mM EDTA.

The biological effects of increasing concentrations of granulin D peptide and antibody on *in vitro* cell growth rates were assayed using [3H]thymidine incorporation. Cells were grown to 60–70% confluence in T75 flasks (Costar) and then plated in 12-well plates (Corning) at a density of 10^4 cells/well in 1 ml of DMEM supplemented with 10% FBS. One day after plating, the medium was removed and replaced with medium containing increasing concentrations of either synthetic granulin D peptide (0–1000 ng/ml) or granulin D antibody (1:1000 to 1:100) in triplicate. Three days later, the medium was again cross-absorbed against a normal brain affinity column (D) and anti-GBM antibodies that did not bind were collected. This final antibody preparation was neutralized, concentrated, dialyzed, and biotinylated. These biotinylated antibodies were then used to screen a GBM cDNA expression library transferred to nitrocellulose filter replicas (E).

**RESULTS**

Isolation of Glioblastoma-associated Gene Products by DIA. We hypothesized that tumor-specific antibodies could be generated and used to screen a glioblastoma cDNA expression library to identify gene products specifically expressed in brain tumors. To test this possibility, we used human GBM tumor homogenate to immunize rabbits to obtain anti-GBM antiserum. This antiserum was passed over a GBM affinity column, and anti-GBM antibodies were eluted off. Antibodies that were not specific for tumor antigens were absorbed out by subsequent passage through a normal brain affinity column and cross-absorption against normal human plasma. The tumor-associated antibodies were then biotinylated and used to screen a glioblastoma phagemid cDNA expression library (Fig. 1). Using this approach, which we term DIA, positive reactions were found in 28 plaques. Positive clones were isolated, subcloned into the pZL1 plasmid vector by *in vivo* excision, and partially sequenced.

BLAST analysis of these sequences revealed 19 novel clones and 9 genes contained in the GenBank database. Of the nine known clones that were identified by our DIA technique, two are known to regulate growth, one codes for a chemotherapy resistance protein, and two regulate gene expression (Table 1). Of the 19 novel sequences that were identified, Northern blot analyses were performed on 9 of these cDNAs and differential expression was confirmed in tumor tissues versus normal brain in all nine of the clones tested (data not shown).

**cDNA Microarray Analysis of DIA Products.** To perform high-capacity screening of our DIA clones in multiple tumor and non-tumor brain tissues, we coupled our novel DIA approach with cDNA microarray analysis (18, 20, 21). Twenty-six of the 28 plasmid inserts isolated were successfully PCR-amplified using vector-specific primers that amplify the inserted fragment from the pZL1 vector. Each PCR product was then arrayed onto glass slides and hybridized with two-color fluorophore-labeled probes in a manner similar to that already published (19). The cDNA made from each sample of tumor or normal brain mRNA was labeled with the fluorescent dye Cy3 (green) and mixed with a common reference probe labeled with a second fluorescent dye, Cy5 (red). Using this high throughput arrayer, we determined the relative abundance of each of our 26 DIA clones in eight different brain tumor samples compared with non-tumor brain tissue (Fig. 2). As seen in Fig. 2, for the majority of the 26 genes tested, the three glioblastoma samples analyzed appeared to have higher levels of differential expression than the other tumor types. Furthermore, analysis of the expression of these candidate tumor-specific genes revealed that one of our isolated clones, *L5*, consistently had much higher levels of expression in gliomas compared with normal brain (3–30-fold).

This differentially expressed clone, *L5*, was therefore chosen for further characterization. The 590-bp cDNA of clone *L5* was manually

<table>
<thead>
<tr>
<th>Clone</th>
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<th>Function</th>
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<tbody>
<tr>
<td>L2, L6</td>
<td>Human GFAP</td>
<td>Glial fibrillary acidic protein; expressed in astrocytes; used as marker for gliomas</td>
</tr>
<tr>
<td>L5</td>
<td>Human granulin</td>
<td>Peptide isolated from human granulocytes; autocrine growth factor for teratoma-derived PC cells; mitogen for 3T3 fibroblasts</td>
</tr>
<tr>
<td>L9</td>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase; glycolytic enzyme; increased in lung cancer; putative role in apoptosis and neurodegenerative diseases</td>
</tr>
<tr>
<td>L10</td>
<td>Carbonic anhydrase</td>
<td>Enzyme involved in chemotheraphy resistance and free radical modulation</td>
</tr>
<tr>
<td>L19</td>
<td>DNA-dependent ATPase (putative) &amp; X-linked nuclear protein</td>
<td>Helicase involved in transcription initiation and brain differentiation (putative)</td>
</tr>
<tr>
<td>L20</td>
<td>Homologous with tyrosine kinases</td>
<td></td>
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<tr>
<td>L24</td>
<td>Ho2, mitochondrion cytochrome oxidase</td>
<td></td>
</tr>
<tr>
<td>L25</td>
<td>Osteopontin</td>
<td>Ligand for integrin; involved in adhesion, migration, and osteoclastogenesis</td>
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*Function not known.*
sequenced and found to be identical to the human granulin/epithelin precursor. Granulins (also known as epithelins) are cysteine-rich polypeptides that have growth factor-like activity. They represent a relatively new class of growth regulators, first described in 1990, with possible roles in inflammation and tumorigenesis (27, 28). The granulin gene exists as a single copy in the human genome on chromosome 17 (29). It is widely expressed in epithelial and tumorigenic cell lines in vitro, many of which respond to the gene product by enhanced cell proliferation, suggesting an autocrine or paracrine role for these factors (30–32). Several of the known structural and biological properties of the granulins resemble those of the EGF-like proteins (33–35). It is unclear whether this is physiologically significant because the in vivo expression and function of granulin have not yet been well defined.

**Granulin mRNA Expression in Human Gliomas.** The differential expression of granulin in human gliomas was confirmed by Northern blot analysis, which showed a transcript of 2.1-kb expressed in 86% (18 of 21) of human gliomas and 0% (0 of 3) of the non-tumor brain tissues analyzed (Fig. 3A). Interestingly, of the three gliomas that had absence of any granulin signal, one was from a patient who had received previous radiation therapy and one was from a low-grade oligodendroglioma. These data would suggest that granulin expression may be mitigated by radiation and/or may be related to higher malignancy and tumor progression. Further studies with greater numbers of irradiated or low-grade glioma samples would be necessary to confirm this hypothesis.

To better appreciate the potential role of this gene product, it would be essential to know the extent to which it is expressed within other human tissues. To determine this, radiolabeled granulin cDNA was used to probe Northern blots of a variety of peripheral organs. The probe hybridized predominantly to a 2.1-kb transcript in human testes, spleen, and kidney after 2 weeks of exposure, but not to all other human tissues tested, including normal brain, lung, heart, skeletal muscle, pancreas, liver, and adrenal gland (Fig. 3B).

Differential expression of granulin mRNA was also seen in tumor versus non-tumor tissues, using in situ hybridization. Granulin antisense riboprobe hybridized predominantly to hypercellular areas of tumor tissue (Fig. 4, A and B). The identity of these cells labeled by in situ hybridization was suggested by counterstaining the tissue sections with H&E, which revealed that the majority of the RNA was...
within tumor cells and not in the tissue stroma (Fig. 4, C and D). Sense strand riboprobe cDNA was used as a control and showed no specific labeling (data not shown), indicating that the cellular hybridization obtained with the antisense probe was specific for the granulin mRNA. Quantitation of granulin hybridization densities was measured from the in situ slides, using image analysis software. This analysis revealed significantly greater numbers of silver grains within cells of the most malignant brain tumors (e.g., anaplastic astrocytomas and GBM) compared with non-tumor glial cells (P = 0.006), confirming that elevated levels of granulin mRNA are expressed in high-grade primary brain tumors (Table 2).

In Vitro Growth Regulation of Glial Cells by Granulin D. Four granulins, A, B, C, and D, were previously isolated from human inflammatory cell exudates (28, 33, 36, 37). Each is a small protein of ~6 kDa that is derived from a larger precursor of 593 amino acids, known as acrogranin (38–40). The acrogranin cDNA (clone L5) that we isolated from human glioblastomas contained the entire sequence for granulin D (bp 1254–2099; Ref. 26).

The implication of granulin molecules in growth regulation with a tertiary structure reminiscent of that of EGF suggested a potentially important role for our L5/granulin D clone as a putative growth factor. To determine whether granulin D may modulate glial cell proliferation, we synthesized a 55-amino acid peptide corresponding to the known sequence of granulin D (26). We then studied the effect of this synthetic peptide on proliferation rates of rat astrocytes in culture, using a standard [3H]thymidine incorporation assay. As shown in Fig. 5, addition of synthetic granulin D peptide stimulated DNA synthesis of rat astrocytes in vitro up to 300% in a dose-dependent manner (Fig. 5, A and B). Statistically significant increases in cell proliferation (up to 150% of controls) were seen with the addition of as little as 1 ng/ml (169 pm) of granulin D to cell culture (P = 0.025). Interestingly, this synthetic peptide had a much more modest effect on the proliferation of primary human glioblastoma cells in culture, showing only a 120–150% increase (P = 0.068) in growth with the addition of >1000 ng/ml (169 nm) granulin D (Fig. 5C). This may be explained by the fact that these human cells were tumorous and already expressed high levels of granulin (as shown by Northern blot and in situ hybridization). Thus, the putative receptors of this potential autocrine growth factor may be saturated by endogenous granulin and thereby preclude further growth stimulation by the addition of exogenous peptide. To further evaluate the growth regulatory role of granulin D on human tumor cells in vitro, a polyclonal antibody was raised against the 55-amino acid granulin D peptide and assayed for its ability to inhibit cell proliferation in three primary human glioblastoma cultures. As shown in Fig. 5D, the addition of increasing concentrations of purified granulin D antibody to early-passage human brain tumor cell cultures significantly inhibited cell growth in vitro. [3H]Thymidine incorporation was suppressed down to only 18.6% of controls with the highest concentration of antibody tested (1:100 dilution; P = 0.035).

DISCUSSION

The detection and characterization of a putative growth factor differentially expressed in brain tumors versus non-tumor brain tissue demonstrates the usefulness of our novel DIA technique for identification of subtractive tissue-specific gene products that may have significant biological activity. The DIA method described here is an alternative to currently established methods for the purposes of identifying differences in gene expression. It has the advantage of selecting for gene products that are actually translated from mRNA species, which can readily be cloned and synthesized for use in functional assays as we have described here. Furthermore, because this technique is based on the generation of subtractive antibodies used to screen cDNA expression libraries, antibodies to clones of interest can be generated for antibody-based studies. Because it is still speculative whether the tumor-associated antigens we identified using this approach can actually induce effective humoral or cytotoxic antitumor immune responses, further studies are needed before such conclusions can be made. Nevertheless, the results reported in this study demonstrate the potential of this technique to identify candidate glioma-associated peptides that readily allow study of expression patterns and biological function.

With current microarray technology, it is feasible to screen relatively large numbers of tumor samples for the expression of subtractive products. This allows easy discrimination of redundant clones and rapid confirmation of truly differentially expressed genes. Although we isolated and screened only 26 clones from our DIA method, it is
conceivable that thousands of differentially expressed gene products could be identified among the ~15,000 individual mRNA species in a pair of human cell populations (i.e., tumor versus normal). This is based on the assumption that perhaps 15% of the estimated 100,000 genes in the human genome are expressed in any individual cell type at a particular time (41). Even if thousands of differential clones are generated from our subtractive approach, current robotic microarray technology allows for the fabrication of arrays containing up to 20,000 distinct cDNA targets (18). The expression of these thousands of targets can be monitored in multiple tissue samples, just as we measured the relative expression of our 26 clones in various brain tumor tissues. Thus, microarrays in concert with subtractive gene hunting methods could serve as useful tools for the identification of biologically intriguing and clinically relevant human gene sequences.

Using the combination of DIA and microarray hybridization, we have identified a potentially interesting candidate oncogene, granulin D, with a likely function in glial cell proliferation. This granulin peptide belongs to a family of putative growth factors that previously have been characterized by a unique structural motif and implicated in growth regulation (28, 30, 31, 33–35). Structurally, granulins consist of 12 cysteines with four cysteine pairs flanked by two single cysteines at both the NH2 and the COOH termini (26, 42). The predicted protein architecture consists of four stacked b-hairpins, each connected to the next with two parallel disulfide bridges, and a peptide backbone arranged as two ladders in a left-handed superhelix (34). Interestingly, this tertiary structure is partially homologous to that of

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Table 2  Relative quantitation of in situ hybridization of granulin mRNA in normal brain and primary brain tumors

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of silver grains/mm²</th>
<th>Average no. of silver grains/cell</th>
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<tr>
<td>Normal brain (n = 2)</td>
<td>50</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>5.3</td>
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<tr>
<td>Pilocytic astrocytoma (n = 3)</td>
<td>3,800</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>3,900</td>
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<tr>
<td></td>
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<td></td>
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Fig. 4. Granulin mRNA in situ hybridization in normal and tumorigenic brain tissues. Top panels, dark-field photomicrographs of representative sections through normal white matter (A) and glioblastoma tissues (B) processed for in situ hybridization using [35S]-labeled granulin cRNA. Note the significantly greater hybridization densities (white silver grains) in the glioblastoma compared with the normal brain section. Original magnification, ×40. Bottom panel, high-powered bright-field image of in situ hybridization of [35S]-labeled granulin cRNA in sections of normal brain (C) and tumor (D) counterstained with H&E. Note that the density and distribution of the intensely hybridizing areas (arrows) appear to be within tumor cells and not in the surrounding tissue. Also note the relative lack of labeling in the non-tumor glial cells. Original magnification, ×200.
EGF. Functionally, several independent investigators have found granulin peptides to be regulators of cellular proliferation, with biological activities reminiscent of the actions of other polypeptide growth factors such as EGF. Granulin proteins have been shown to have mitogenic activity in murine embryonic 3T3 cells, in the tumor-igenic teratoma-derived PC cell line, in human epithelial and fibroblastic cells, and in murine keratinocytes (27, 30, 33, 35). We now report similar growth regulatory effects of this peptide in primary rat astrocytes and in early-passage human glioblastoma cell lines.

The surprising parallels between the granulin and EGF systems are of interest. Given that amplification of the EGFR gene is one of the most common findings in glioblastomas and malignant astrocytomas (1, 43), it is intriguing that one of the glioblastoma-associated clones identified via our novel DIA technique may be related to the EGF/EGFR system. Nevertheless, there are many molecules that have EGF-like domains, and other investigators have found that granulin does not bind to wild-type EGFR (also called erbB-1; Ref. 44). Furthermore, Western blot analysis of EGFR expression in the human glioblastomas used in our bioassay revealed EGFR overexpression in only one of the three tumors tested, with no direct correlation between EGFR overexpression and granulin-induced growth regulation. Interestingly, however, all three of the tumors we studied had overexpression of the closely related EGFR-like transmembrane receptor tyrosine kinase erbB-2 (also called HER-2 or neu; data not shown). The significance of this coincident overexpression of granulin and erbB-2 is unknown at present.

Also intriguing in this context is the fact that both granulin and erbB-2 are genes located on chromosome 17 (29, 45). Previous reports in the literature have found that high-grade gliomas have overrepresentation of chromosome 7 and gain of chromosome 17q at the cytogenetic level (46, 47), which presumably relates to amplification of EGF and erbB-2 at the gene expression level (48–50). Although overexpression of erbB-2 has been found in a subset of primary brain tumors, its putative ligand in brain cancers is not yet known. It would be interesting to determine whether tumors with amplification of chromosome 17q have coordinate overexpression of erbB-2 and granulin, which may support the idea of granulin being a ligand for the erbB-2 proto-oncogene autocrine/paracrine loop. Additional studies of granulin binding data and signal transduction pathways are warranted and may aid in our understanding of the oncogenesis of brain neoplasms. Given the tissue-specificity of granulin for tumor versus normal brain, its EGF-like domains, its location on chromosome 17, and its implicated role in glial cell growth regulation, it is conceivable that this gene product may be a useful target for the development of new therapeutics for malignant brain tumors.

ACKNOWLEDGMENTS

We thank Drs. Donald Becker and Timothy Cloughesy for helpful discussions and encouragement. We thank Trung Vu and Michael Soung for technical assistance. Barry Merriman for help with quantification of microarray data, Jim Pretorius for help with analysis of in situ hybridization data, and Melissa Dickey for administrative assistance.

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