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Cancer Letters 171 (2001) 67–77

CANCER Letters

www.elsevier.com/locate/canlet

Expression of multiple larger-sized transcripts for several genes in oligodendrogliomas: potential markers for glioma subtype

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Received 4 April 2001; received in revised form 30 April 2001; accepted 2 May 2001

Abstract

Astrocytomas and oligodendrogliomas are two brain tumors that follow different clinical courses. Although many of these tumors can be identified based on standard histopathological criteria, a significant percentage present notable problems in diagnosis. To identify markers that might prove useful in distinguishing glioma subtypes, we prepared and analyzed cDNA libraries for differential expression of genes in an astrocytoma (grade II), an oligodendroglioma (grade II), and a meningioma (benign). The tumor libraries were compared by sequencing randomly selected clones and tabulating the expression frequency of each gene. In addition to identifying several genes previously reported or expected to be differentially expressed among these tumors, several potential new brain tumor markers were identified and confirmed by Northern blot analysis of a panel of brain tumors. A surprising result of this analysis was the observation that several larger-sized transcripts for various genes were predominantly expressed in the oligodendroglioma tumors, when compared to the other brain tumors or in non-tumor gray matter. These findings are consistent with different pre-mRNA splicing patterns observed between oligodendrogliomas and astrocytomas. In support of this hypothesis, our screen revealed significantly higher levels of two hnRNP A1 transcripts in oligodendrogliomas. hnRNP A1 is a component of the spliceosome whose expression levels affect splice site selection in vivo. The preferential expression of larger-sized transcripts for several genes in oligodendrogliomas may be useful for distinguishing astrocytic and oligodendroglial gliomas.

Keywords: Brain tumor; Oligodendroglioma; Astrocytoma; Meningioma; Gene expression; cDNA library

1. Introduction

Astrocytomas and oligodendrogliomas are two brain tumors that follow different clinical courses, but due to their similarities present significant problems in diagnosis. Astrocytomas often progress into glioblastoma multiforme and individuals possessing these tumors have an extremely poor prognosis, whereas patients with oligodendrogliomas generally have better clinical outcomes. Although many astrocytomas and oligodendrogliomas can be identified based on standard histopathological criteria, many do not satisfy all the distinguishing features as established by the World Health Organization [1]. The chromosomal deletions of 1p and 19q are now consid-

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0304-3835/01/$ - see front matter
PII: S0304-3835(01)00573-0

ered a marker for oligodendroglioma and these chromosomal anomalies are associated with better survival [2]; nevertheless, tumors with histopathological characteristics similar to both astrocytoma and oligodendroglioma are difficult to classify, and thus present a need for additional glioma markers.

Numerous approaches have been used in an attempt to identify glioma subtypes; in this study we have used electronic subtraction, a differential gene expression technique previously used to identify interferon γ-induced genes in HeLa cells [3]. Electronic subtraction is accomplished by sequencing randomly selected clones from the cDNA libraries to be compared and then tabulating the number of times each gene is observed in each library for frequency analysis. While electronic subtraction is more labor-intensive than other differential gene expression approaches, a comparison of several differential gene expression techniques showed that six of seven interferon γ-induced genes identified by electronic subtraction were not identified in the same experiment by differential display or subtractive hybridization methods [3]. This study demonstrated the utility of the electronic subtraction technique; furthermore, due to the shallow sequencing and the complexity of the mRNA profile in cells, the majority of genes identified by this approach are likely to be abundantly expressed. Such genes, in addition to providing good markers for brain tumors, might also yield insight into the mechanisms of brain neoplasia.

In this study, analysis of three brain tumors by electronic subtraction not only revealed several genes previously known to be expressed in these brain tumors subtypes, but also yielded a number of new potential brain tumor markers. A more detailed analysis of the mRNA expression of these genes in a brain tumor panel showed several genes with larger-sized transcripts associated uniquely with oligodendrogliomas. This may be an important characteristic of oligodendrogliomas and may be a general prognostic marker for these tumors.

2. Methods

2.1. Clinical samples

Brain tumors and non-tumor brain specimens were frozen at the time of surgery and stored at −80°C until further analysis. Tumors were from the Glioma Marker Network at The Johns Hopkins University Medical Institutions. Tumor sections were analyzed by standard histology and diagnosed by the institution (Johns Hopkins University) and reviewed by a neuropathologist (P.C.B.). The clinicopathological features of these tumors are representative of these tumor subtypes (Table 1). To validate the histological classification of the oligodendroglioma (T197) used for the electronic subtraction, comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analyses were found to show losses on chromosome 1p and 19q (data not shown).

2.2. RNA isolation and Northern blot analysis

Total RNA was prepared from rapidly frozen brain tumors using a triazol/phenol reagent, quantitated, and analyzed by Northern blotting as previously described [4]. For the preparation of probes, the cDNAs were amplified from brain tumor cDNA libraries by polymerase chain reaction (PCR) and the resulting cDNAs were radiolabeled by random priming with [^{32}P]α-dCTP as previously described [4]. PCR using high fidelity DNA polymerase was performed in 100 μl reactions as described by the manufacturer (Boehrin-

Table 1
Clinical data for brain tumor patients

Tissue/histology[a]	Age (years)	Sex	Survival (months)	Status
Gray matter	59	M	–	–
T58/astro II	44	F	59	Alive
T247/astro II	3	M	36	Alive
T391/astro III	44	M	18	Alive
T251/GBM	58	F	10	Dead
T256/GBM	47	F	7	Dead
T268/GBM	49	M	14	Dead
T269/GBM	72	F	13	Dead
T22/mening	43	F	21	Alive
T320/mening	70	M	3	Dead
T388/mening	45	F	16	Alive
T231/mening	72	F	9	Alive
T76/oligo II	26	M	25	Alive
T193/oligo III	43	M	51	Alive
T262/oligo II	29	F	26	Alive
T197/oligo II	44	M	37	Alive

[a] Astro, astrocytoma; GBM, glioblastoma multiforme/astrocytoma grade IV; mening, meningioma; oligo, oligodendroglioma.

ger Mannheim, Indianapolis, IN). The brain tumor libraries from which the original clones were isolated (1 μl) were used as templates for PCR. Primers and corresponding annealing temperatures were: creatine kinase B (CCATGCCCTTCTCCAACA and GCAGGAAGTCGGGGTTG, 56°C); cytochrome C oxidase subunit I (TTGAAACCAGCTTTGGGG and CTATCCGGAATGCACCGA, 54°C); heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1, TTGTAACCTGTAGCTTCCC and ATTTTGGACCCATGAAGGG, 56°C); mitochondrial malate dehydrogenase (GAGCCAGGGACAATTTGG and CTTCTCCCTTGTGGATGC, 56°C); NADH-ubiquinone oxidoreductase subunit I (ATTGTAATGGGTATGGAGAC and CCGAACTAGTCTCAGGCTT, 56°C); ribosomal protein S4 (ACTCTGGTGTGGGTCTTG and CTGTGCTGGTACGCCTC, 56°C); selenide dikinase (AGATTAAGAGGTGGCCCC and GCAGAGGAACGAGGTGT, 54°C); selenoprotein W (CTTCAGCTCAAGAAGAAGT and GAAGCGTCTGCTGAGAG, 54°C); translationally controlled tumor protein (TTAGACAACCTACATGACAT and ACATGAAATCAATCAAAGGG, 54°C); glial fibrillary acid protein (GFAP, GCCTCTGGCCAGGGC and GCCAGACGGGACCCATC, 50°C); prostaglandin D synthase (GCACGAGATCTCCTG and CACAGACTTGCACTA, 46°C); and insulin-like growth factor II (IGF-II; AACGCACGAAGCCAG and TACACACCTGCTAGC, 46°C). The amplified cDNAs corresponding to the sizes predicted from the nucleotide sequences were excised from low melting agarose gels and purified using Geneclean II according to the manufacturer's protocol (Bio 101, Carlbad, CA). A Northern blot was prepared by fractionating total RNA isolated from the brain tumors on 1% denaturing agarose gels by electrophoresis, and capillary transfer of RNA to Nytran membranes (Schleicher and Schuell, Keene, NH). After UV crosslinking the RNA to the membrane, the blot was probed, washed, and exposed to X-ray film as previously described [4]. The signals of genes on the Northern blots were also quantitated using a Phosphorimager (Molecular Dynamics Corp., Sunnyvale, CA).

2.3. Preparation and sequencing of phage cDNA libraries

Tumors 58, 197, and 231 (see Table 1) were used to prepare λZAP2 phage cDNA libraries as described by the manufacturer (Stratagene Corp., La Jolla, CA) and modified as described [5]. The amount of tissue used, total RNA isolated, amount of mRNA used to prepare the libraries, and resulting number of primary clones are shown in Table 2. Clones from the libraries were rescued into Bluescript plasmids (Stratagene Corp., La Jolla, CA) by mass excision, arrayed by the I.M.A.G.E. Consortium [6], and analyzed by automated DNA sequencing using the –40 universal primer (Life Technologies Inc., Rockville, MD) to obtain sequence from the 3′ ends of the clones. The homology of clones to known genes was assessed using BLAST [7]. A profile of the sequencing and homology search results are shown in Table 2.

2.4. Statistical analysis

The band intensities on Northern blots were quantitated using a Phosphorimager (Molecular Dynamics). The gene expression levels were normalized to the

Table 2
Data for preparation and sequencing of brain tumor libraries

	Astrocytoma	Meningioma	Oligodendroglioma
Tissue (g)	2.5	1.5	3.1
Total RNA (mg)	1.3	2.4	1.3
Poly A^+ RNA (μg)	5.5	8.9	5.9
Primary phage	1.5×10^5	7.0×10^6	8.3×10^5
Sequencing results			
Clones sequenced	550	1142	1139
Clones with good sequence	459	864	753
Previously known genes	288	604	456
Previously unknown cDNAs	171	260	297

corresponding 28S ribosomal RNAs, which were measured by densitometry of ethidium-stained gels using Brain Image software. Statistical comparison of gene expression levels in brain tumor groups was analyzed by ANOVA regression and a Tukey multiple comparison test using statistical software (http://faculty.vassar.edu/~lowry/VassarStats.html) to determine significant differences ($P < 0.05$).

3. Results

3.1. Identification of candidate genes differentially expressed in brain tumors by electronic subtraction

To identify candidate genes that are differentially expressed in gliomas, a representative astrocytoma (grade II) and oligodendroglioma (grade II) were selected for analysis. A benign meningioma was analyzed in parallel as a control because these tumors should have distinct gene expression patterns. Histopathological analysis of these tumors revealed features typical of these lesions and analysis of the oligodendroglioma using both CGH and FISH demonstrated losses on chromosomes 1p and 19q, which are characteristics of this tumor [2,8,9].

To perform electronic subtraction, oligo-dT primed cDNA libraries for the astrocytoma, oligodendroglioma, and meningioma tumors were prepared. The amounts of tumors used ranged from 1.5 to 2.5 g and yielded 4.6–7.9 μg of poly A^+ RNA, and 0.8–7.0×10^6 primary recombinant phage (Table 2). After mass excision of the λZAP2 phage cDNA libraries, individual clones were arrayed, recombinant pBS plasmids were isolated from each clone, and the cDNA inserts were sequenced from the $3'$ end using the –40 universal primer (Life Technologies Inc., Rockville, MD). A summary of the sequencing results is shown in Table 2. The sequences for these clones can be retrieved from the NCBI dbEST nucleotide database using 'Schiller' and the tumor type as queries.

To identify genes that might be differentially expressed in the three brain tumors, BLASTN was used to search the non-redundant nucleotide database for genes homologous to each cDNA [7]. The number of novel genes ranged from 30 to 39% at the time of sequencing. For the genes homologous to previously known genes, a frequency analysis was performed by tabulating the number of times each gene occurred in each tumor; the frequencies were normalized to the total number of clones sequenced in each library. We chose to further examine genes occurring at least twice in one of the tumor samples since genes observed only once are more likely to reflect chance observations given the small percentage of the total transcripts that were sequenced (assuming 200,000 transcripts/cell, approximately 0.2–0.4% were sequenced). There were 119 previously known genes that were observed at least two times in one of the tumors. Table 3 shows a subset of these genes that were either very abundant or predicted to be differentially expressed.

The electronic subtraction analysis was validated by the correct identification of several genes that were known to be differentially expressed in the types of brain tumors examined. These included brevican core precursor and complement C4 in astrocytic tumors [10,11]. More sequences for glial fibrillary acidic protein transcripts were observed in astrocytomas than in oligodendrogliomas and none were found in meningiomas [12]; prostaglandin D synthase, IGF-II, procollagen, and vimentin were more prevalent in meningiomas as previously reported [13–16]. One pattern that emerged from the electronic subtraction analysis was the differential expression of genes involved in metabolism between the astrocytoma and oligodendroglioma. The number of sequences for creatine kinase and fructose bisphosphate aldolase were higher in the astrocytoma while cytochrome C oxidase subunits I, II and VIIc, isocitrate dehydrogenase, malate dehydrogenase, and NADH ubiquinone oxidoreductase were higher in the oligodendroglioma.

3.2. Evaluation of candidate genes by Northern blot

To further validate the findings of our electronic subtraction, several of the genes were evaluated by Northern analysis [3]. We chose to examine genes with the potential for distinguishing astrocytic and oligodendroglial tumors; the two most abundant genes in the meningiomas were also evaluated. Since a gene that is elevated in one tumor type may not be elevated in more tumors of the same histological grade and class, we examined a tumor panel

Table 3
Results for electronic subtraction of brain tumors

Accession no.	Gene name	Astrocytoma[a]	Meningioma[a]	Oligodendroglioma[a]
AI459799	Alcohol dehydrogenase	0	0	2
AI460011	Annexins 2, 4, 5, and 6[b]	0	6	1
AI366118	Apolipoprotein E precursor[b]	7.5 (4)	7	3.5 (3)
AI460384	Brain protein H5	0	0	3.5 (3)
AI214076	Breast basic conserved protein 1[c]	9 (5)	3	1
AI214132	Brevican core precursor[b]	11 (6)	0	8 (7)
AI366500	Casein kinase ε	0	1	2
AI366490	Clusterin	15 (8)	19	9 (8)
AI366224	Complement C4[b]	5.5 (3)	1	0
AI366155	Creatine kinase, B chain[b]	5.5 (3)	1	0
AI272311	Cystatin C precursor[c]	0	2	2
AI205186	Cytochrome C oxidase subunit I	2 (1)	3	9 (8)
AI365973	Cytochrome C oxidase subunit III[c]	0	1	3.5 (3)
AI272373	Cytochrome C oxidase subunit VIIc	0	0	1
AI366131	Fructose bisphosphate aldolase A[b]	9 (5)	1	0
AI272154	G/T mismatch-specific thymine DNA glycosylase[c]	0	0	2
AI366117	G1/S-specific cyclin D1[b]	0	3	0
AI214180	Glial fibrillary acidic protein[b]	47 (25)	0	10.5 (9)
AI366130	Growth arrest specific protein (GAS-???)[c]	0	3	0
AI366478	Guanine nucleotide binding protein β-subunit	0	4	2
AI365956	Guanine nucleotide binding protein G(S)α	0	2	2
AI366077	HAX-1	0	3	0
AI207809	HSP-90β[b]	0	2	3.5 (3)
AI366487	hnRNP A1[c]	7.5 (4)	3	1
AI459496	HU-K4	0	0	2
AI581684	Hypothetical 109.6 KD protein	0	0	2
AI366138	Isocitrate dehydrogenase	0	1	2
AI207804	Initiation factor 4A-1[c]	0	2	3.5 (3)
AI366226	IGF-II[b]	0	22	0
AI366069	MAC-2 binding protein[c]	0	3	0
AI272205	Malate dehydrogenase	0	0	4.5 (5)
AI880156	MCM3 homolog[c]	4 (2)	0	0
AI272194	MEL-18 DNA binding protein	0	0	2
AI366538	NADH-ubiquinone oxidoreductase subunit 1	0	1	4.5 (4)
AI459723	Neuromodulin[b]	0	0	2
AI207938	Polycystic kidney disease associated protein[c]	0	0	2
AI214179	Polycystwin	4 (2)	0	0
AI366503	Prostaglandin D synthase[b]	2 (1)	48	3.5 (3)
AI366489[c]	Procollagen α1(1,3), α2(1,4),-chain[b]	0	11	0
AI459385	Procollagen C-proteinase enhancer protein	0	4	0
AI460366	Protein β	0	0	2
AI581679	RAB 31 (Ras-related protein)	0	0	2
AI207762	Ribosomal protein L3[c]	4 (2)	1	0
AI459506	Ribosomal protein S4[c]	0	1	7 (6)
AI272197	Ring finger protein	0	0	2
AI460241	RTP	0	0	2
AI459726	Selenide dikinase	0	0	1
AI207941	Selenoprotein W	0	0	2
AI205173	Seven transmembrane-domain receptor	0	0	3.5 (3)
AI581676	SH2-B (FCERIγ interacting protein)	0	0	2
AI880059	Smg p25A GDP dissociation inhibitor	0	0	3.5 (3)
AI272383	Thiol-specific antioxidant protein	0	0	2

Table 3 (*continued*)

Accession no.	Gene name	Astrocytoma[a]	Meningioma[a]	Oligodendroglioma[a]
AI365950	Translationally controlled tumor protein[b]	4 (2)	2	0
AI460047	Ubiquinol cytochrome C reductase Fe-S subunit	4 (2)	0	0
AI459737	Ubiquitin	9 (5)	2	8 (7)
AI205250	Vimentin	1 (2)	11	2
AI366499	Zinc Finger protein 84	0	4	0

[a] Numbers indicate the number of times each sequence was observed in sequencing of the specific tumor library.
[b] Genes that were previously shown to be associated with brain tumors.
[c] Genes that were previously shown to be associated with cancer of other organs.

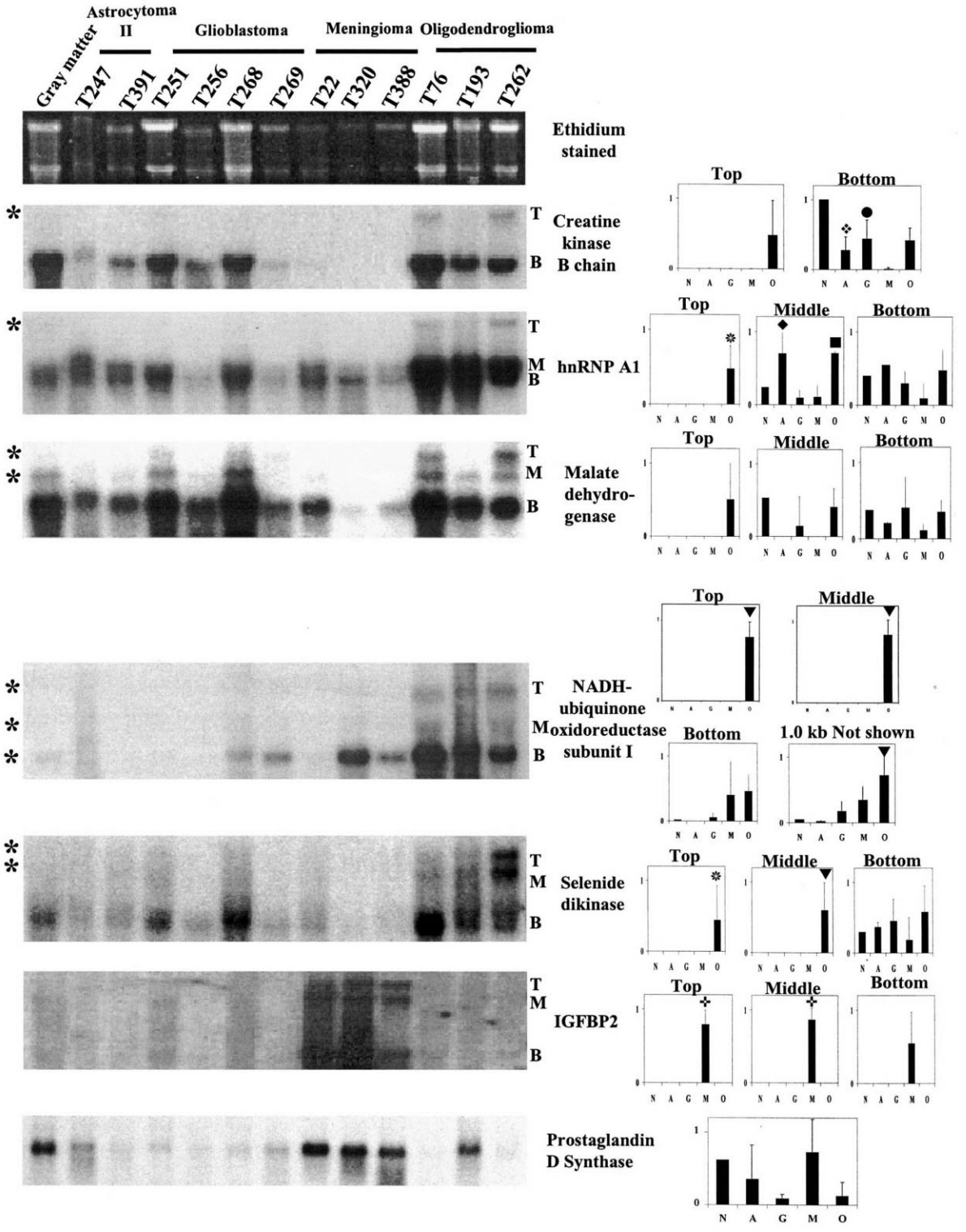
consisting of non-tumor gray matter and several grade II astrocytomas, grade IV glioblastoma, benign meningiomas, and oligodendrogliomas (Fig. 1). The average signals and standard deviations for the expression levels of select genes within brain tumor types were used to determine significant differences (Fig. 1).

Detailed analyses of a small subset of genes identified by the screen also validated the electronic subtraction method. In good agreement with the frequency analysis of the identified genes (Table 3), Northern analysis showed that prostaglandin D synthase and IGF-II transcript levels were elevated nearly exclusively in meningiomas. Unlike non-tumor tissue or other brain tumors that express mainly low levels of a single 1.8 kb IGF-II transcript, significantly higher levels of 1.8, 4.8 and 6.0 kb IGF-II transcripts were identified in meningiomas. Elevated expression of both IGF-II and prostaglandin D synthase in meningioma have been reported previously [17,18]; the current data corroborated with those results.

Only a few genes identified in our screen appeared to discriminate between astrocytoma and oligodendroglioma. However, in the subset we examined by Northern blotting, we identified unique transcripts for NADH ubiquinone oxidoreductase subunit I, selenide dikinase, and hnRNP A1, three genes previously not associated with brain tumors, to be elevated in oligodendrogliomas. Among non-tumor brain, astrocytoma, glioblastoma, and meningioma, the 3.4, 4.8, and 6.3 kb NADH ubiquinone oxidoreductase subunit I transcripts were elevated several fold in oligodendroglioma ($P < 0.01$). Unique 4.8 and 6.3 kb transcripts were present only in the oligodendroglioma tumors ($P < 0.01$). High levels of 3.4 and 4.3 kb selenide dikinase transcripts were found in the oligodendroglioma, and these transcripts were not detected in astrocytoma, meningioma, glioblastoma, and non-tumor gray matter. A unique 4.3 kb hnRNP A1 transcript was detected only in oligodendroglioma tumors. Hence, the expression of these three genes may be useful for distinguishing oligodendrogliomas from other brain tumors.

A similar analysis revealed two additional brain tumor markers that may warrant further study. Translationally controlled tumor protein, a protein associated with oncogenesis in several tumor types [19], was significantly higher in the oligodendroglioma than in the astrocytoma and glioblastoma tumors ($P < 0.05$, data not shown). Cytochome C oxidase subunit I, a protein involved in oxidative metabolism, was higher in astrocytomas than in glioblastomas ($P < 0.05$) and meningiomas ($P < 0.01$) (data not shown). The lower expression of cytochrome C oxidase with astrocytoma grade may be consistent with decreased oxidative metabolism in higher grade tumors.

Perhaps of equal importance is the observation that oligodendrogliomas demonstrate preferential expression of multiple larger-sized transcripts for several genes. Five of the 13 genes that were analyzed by Northern blotting showed larger-sized transcripts that were preferentially expressed in the oligodendroglioma (Fig. 1, asterisk, some data not shown). Two larger selenide dikinase transcripts of 3.4 and 4.3 kb appeared to be uniquely associated with oligodendrogliomas ($P < 0.01$). Three NADH ubiquinone oxidoreductase subunit I transcripts of 1.0 (data not shown), 4.8, and 6.3 kb were highly expressed at significantly higher levels in oligodendrogliomas; lower levels of the 1.0 (data not shown) and 3.4 kb transcripts were


Astrocytoma II
Glioblastoma
Meningioma
Oligodendroglioma
Gray matter
T247
T391
T251
T256
T268
T269
T22
T320
T388
T76
T193
T262
Ethidium stained
Creatine kinase B chain
hnRNP A1
Malate dehydro-genase
NADH-ubiquinone oxidoreductase subunit I
Selenide dikinase
IGFBP2
Prostaglandin D Synthase
Top
Middle
Bottom
1.0 kb Not shown
T
M
B
N A G M O

present in meningioma and glioblastoma, but absent from lower grade astrocytoma, whereas the 4.8 and 6.3 kb transcripts were detected only in oligodendrogliomas. Similar results were observed when creatine kinase, hnRNP A1, and malate dehydrogenase were examined (Fig. 1). In addition to the preferential expression of selenide dikinase, NADH ubiquinone oxidoreductase subunit I and hnRNP A1 transcripts in oligodendrogliomas, larger-sized mRNA transcripts may be a general feature of oligodendrogliomas associated with altered gene product expression, changes in mRNA stability, and/or changes in translation efficiency when compared to other brain tumors.

4. Discussion

Unlike other cDNA-based subtraction methods, the shallow sequencing of brain tumor libraries using the electronic subtraction method has the promise of identifying differentially, but abundantly expressed genes among the different brain tumor subtypes. Many of these genes may not demonstrate practical utility because of the prevalence of the corresponding transcripts in normal and tumor tissues, but the identification of those genes that do demonstrate tumor specificity would be extremely advantageous in generating robust signals for tumor classification.

The resulting electronic subtraction data we obtained from one such experiment examining differential gene expression in astrocytoma, oligodendroglioma, and meningioma (Table 3) have validated the methodology based on several important criteria. The electronic subtraction identified the presence of some genes known to be prevalent in tumors, including apolipoprotein E in the three brain tumors types tested [20] and GFAP in astrocytomas [16]. Sequences for IGF-II and prostaglandin D synthase were the most abundant transcripts found in the meningioma by frequency analysis [17,21] and were confirmed by Northern blot analysis.

Both IGF-II and prostaglandin D synthase are expressed normally in the meningies and the levels of these genes are elevated in meningiomas [17,21]. While meningiomas express abundant IGF-II, we have found that the astrocytomas, glioblastoma, and oligodendrogliomas do not express detectable IGF-II message, which is consistent with other studies [13,21]. A low IGF-II/IGFBP2 ratio is an indicator of good prognosis for patients with meningiomas [18]. Also in good agreement with previous studies, we found high levels of prostaglandin D synthase (also known as β-trace) in meningiomas [17] and diminished transcript levels in gliomas when compared to normal gray matter. The lower levels observed in gliomas are consistent with the observation that the levels of prostaglandin D synthase in cerebrospinal fluid are reduced in patients with gliomas [22].

In the course of our analyses, we noted a preponderance of larger-sized transcripts for several genes associated mostly with oligodendrogliomas. Although some of these larger-sized transcripts can be detected in meningiomas and glioblastoma, the appearance of these variant transcripts was most prevalent in oligodendrogliomas. Since multiple sizes of transcripts have not been reported for astrocytomas, we suggest that this may be an important feature for distinguishing between astrocytoma and oligodendroglioma. While this hypothesis remains to be rigorously tested

Fig. 1. Analysis of gene expression in brain tumors by Northern Blot. The ethidium-stained gel and autoradiograms of a tumor panel probed with specific genes are indicated. Tumor subtypes and tumor numbers are indicated above each lane of the blot. Abbreviations for genes are heteronuclear ribonuclear protein A1 (hnRNP A1) and insulin like growth factor II (IGF-II). When more than one band was observed with a particular cDNA probe all bands are shown and indicated by T for top, M for middle, and B for bottom; quantitation of these bands is shown on the bar graphs with each band indicated. An * indicates larger-sized transcripts that are expressed in oligodendroglioma tumors. For the graphs of gene expression in tumor subtypes, the abbreviations are non-tumor gray matter (N), astrocytoma (A), glioblastoma (G), meningioma (M), and oligodendroglioma (O). Values are a percentage of the highest signal measured using a Phosphorimager. Error bars reflect the standard deviation of the expression level of individual tumors within a tumor subtype. Symbols for statistical significance are (❖) astrocytoma > meningioma ($P < 0.05$), (●) glioblastoma > meningioma ($P < 0.01$), (❁) oligodendroglioma > astrocytoma, glioblastoma, and meningioma ($P < 0.01$), (◆) astrocytoma > glioblastoma and meningioma ($P < 0.01$), (■) oligodendroglioma > glioblastoma and meningioma ($P < 0.01$), (▼) oligodendroglioma > astrocytoma and glioblastoma ($P < 0.05$), and (✣) meningioma > glioblastoma, astrocytoma, and meningioma ($P < 0.01$).

with a larger sample of genes and tumors, this finding may represent a fundamental difference in oligodendroglioma tumorigenesis.

The variant transcripts may arise from a number of posttranscriptional processing mechanisms including alternative splicing, usage of different 5′-promoters, selection of alternative 3′-polyadenylation signals, and variations in the length of the poly A^+ tail. These modifications may alter the translation product, regulate mRNA stability, or alter the translation of the specific mRNAs. We are intrigued by the possibility of different alternative splicing in oligodendrogliomas. This hypothesis is supported by our finding of the increased expression levels of a unique larger hnRNP A1 transcript in the oligodendroglioma tumors and not in the other brain tumors or non-tumor gray matter examined. hnRNP A1 is a component of the spliceosome that affects pre-mRNA splice site selection in vivo [23], suggesting that its increased expression in oligodendrogliomas may cause different processing of pre-mRNAs when compared to other brain tumors. Furthermore, overexpression of hnRNP A1 in cells silences splice sites in CD44 pre-mRNA processing [23] in vivo, which is consistent with our observation of larger-sized transcripts for several genes. This splicing is regulated by oncogenic forms of Cdc42 and Ras, suggesting an association with neoplasia. All but one of the nine larger-sized transcripts observed in oligodendrogliomas (Fig. 1, asterisk) were not present in the non-tumor brain sample, suggesting that the larger transcripts may be specifically formed in the oligodendroglioma tumors and may contribute to the transformation mechanism, although it is just as possible that this is an epiphenomenon.

Our electronic subtraction analysis suggested that selenium metabolism may be unique in oligodendrogliomas when compared to other gliomas. Sequences for two genes involved in selenium metabolism, selenide dikinase [24] and selenoprotein W [25], were found in the oligodendroglioma and not in the other brain tumors examined. This is an intriguing finding because the maturation of oligodendrocyte precursors and expression of proteolipid protein and myelin basic protein in oligodendrocytes require selenium [26], suggesting that expression of these genes involved in selenium metabolism may be a specific property of oligodendroglioma tumors. We further examined the expression of these two genes in brain tumors by Northern blot and found that expression of selenide dikinase transcripts not only appeared higher in oligodendrogliomas, but also demonstrated larger-sized transcripts that were not present in the other tumors examined (Fig. 1); the expression of selenoprotein W was variable in the tumors (data not shown). Selenium has been investigated as a treatment for brain tumors due to its antioxidant properties. Although treatment of human gliomas with sodium selenite shows no significant effect as a therapy [27], a case study treating a patient with sodium selenite after rescission of a recurrent oligodendroglioma showed no recurrence of the tumor and improvement of physical and mental condition 15 months later, at the last reported follow-up [28]. These experiments warrant further study of selenium metabolism in oligodendrogliomas.

Our frequency analysis revealed at least one sequence for S3, S4, S11, S12, S13, S15, S18, S26, S28, L14, L17, L23A, L29, L7 and L8 ribosomal proteins in the oligodendrogliomas and meningiomas, but not in the astrocytoma, which is consistent with our previous findings of differential expression of ribosomal proteins in gliomas (unpublished data). Sequences for other proteins that play a role in translation such as elongation factors 1-α-1, 1-β, and 1γ and initiation factor 4A-1 were also more prevalent in the oligodendroglioma than in the astrocytoma. Whether these transcripts, as well as the many others identified in our screen, will prove useful in discriminating oligodendroglioma from astrocytoma remains to be established in future experiments.

In conclusion, we have used electronic subtraction to identify new candidate markers for oligodendroglioma, astrocytoma, and meningioma. This experiment identified several genes that are highly expressed in some of these tumors, which may demonstrate utility as selective tumor markers. Further evaluation revealed the preferential expression of multiple larger-sized transcripts for several genes in the oligodendroglioma tumors and not in the other brain tumors or in non-tumor gray matter. On the basis of these findings and the differential expression of unique larger hnRNP A1 transcript, a component of the spliceosome that affects splice site selection, we propose that oligodendrogliomas may process pre-mRNAs in a way that is different from other gliomas and normal brain, but this hypothesis will need to be rigorously tested.

Acknowledgements

We wish to thank Drs Burt G. Feuerstein and Yuri Minn for cytogenetic analysis of tumor samples. We appreciate the advise of Drs Victor May and Wallace Deckel in the preparation of this manuscript. We thank Patricia Goldwaithe for management of the Glioma Marker Network Tumor Bank, and Rosemary Rogers, Candice Berman, and Lydia Burnett for secretarial assistance. This study was supported by the National Cancer Institute, U01 CA64925-0, and the U.S. Department of Energy under contract W-7405-Eng-43.

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