Medulloblastoma in childhood: a clinical and biological study

Michiels, E.M.C.

Publication date
2000

ADDENDUM
TO CHAPTER 5

Introduction

The results, obtained by analysis of the SAGE libraries of medulloblastoma and fetal brain, were used as a basis for more detailed analysis of some of the tags which showed a significant difference in expression between the two tissues. It is obvious that the number of differentially expressed and therefore interesting tags is enormous, so a selection has to be made to identify tags that will be analyzed in more detail. Selection criteria will differ according to the goal pursued. Looking for genes that are specific for the tumor studied and thus can potentially be used as a diagnostic tool will require analysis of tags that are highly expressed in the tumor in comparison to the normal tissue. On the other hand, one can concentrate on analysis of known genes and investigate their functional role in the tumor, or look for new genes and thus start further analysis with tags that show homology with Expressed Sequence Tags (EST) of unknown function or tags that show no homology with any known sequence at all (no matches).

Materials and Methods

As we were interested in the first place in novel genes that are specific for medulloblastoma and thus can a/o be used as a diagnostic tool, we used the following selection criteria : 1) high expression in medulloblastoma in comparison to fetal brain and 2) no homology with a known gene of general function. Next steps were designed to 1) identify a longer sequence of the gene corresponding to the tag analyzed, 2) examine the expression level of that transcript in other medulloblastomas in order to focus on transcripts that are highly expressed in a majority of medulloblastomas and 3) examine the expression level in different brain regions and other tissues in order to find out if the transcript is specific for brain tissue or some specific region in the brain.
Step 1 was done by examination of the EST database in case of matches with multiple ESTs, or by RACE-PCR as described by Frohman *et al.* [1]. "No match" tags were first converted into sequence by performing a 3'-RACE-PCR with the tag as a forward primer. Examination of the expression level in other tumors and other tissues was done by Northern blot analysis.

Results

TAG	FB (tags)	M1 (tags)	FB	M1	MB1	MB2	MB 3	MB 4	MB 5	MB 6	MTB	mRNA size*
1	2	35	-	++	+	+	+	-	-	-	#	1,6 kb
4	0	21	#	#	#	#	#	#	#	#	#	
17	1	13	-	++	+	+	++	+	++	+	brain	3,5 kb
25	0	9	-	+	-	-	-	-	-	-	#	1.5 kb
27	1	11	+/-	+	++	-	-	+	-	+/-	-	> 5 kb
OTX2	0	10	-	++	-	++	-	+	++	++	-	2,6 kb
Zic	0	17	-	++	+	+	+	+	++	++	#	3,0 kb
y-actin	23	26	++	++	++	++	++	++	++	++		2,5 kb

Table 1.
Results of SAGE and Northern blot analysis of the tags studied.
Column 2 and 3 : number of times the tag was found in fetal brain and medulloblastoma respectively by SAGE analysis.
Column 4 and 5 : Northern blot analysis of the same tissues of which the SAGE library was constructed.
Column 6 to 12 : Northern blot analysis of 6 other medulloblastomas.
Column 13 : Northern blot analysis of a multiple tissue blot.
Column 14 : size of the mRNA as estimated on Northern blot.
FB : fetal brain, M1 : medulloblastoma of which the SAGE library was constructed, MB : medulloblastoma, MTB : multiple tissue blot, * as estimated on the Northern blot, # : not tested
++ very intense signal on Northern blot
+ medium intensity of Northern blot signal
+/- low intensity of Northern blot signal
- no signal visible on Northern blot

Seven tags were analyzed in more detail. Results are shown in Table 1. Of these 7 tags, 5 showed homology to ESTs of which the position of the last CATG in the EST respective to the poly(A) tail was unknown. Two of them showed no homology to any known sequence and were classified as "no match".
Tag number 1 showed high expression in 4/7 medulloblastomas tested and hybridized to a mRNA of about 1.6 kb.
Tag number 4 turned out to match with mitochondrial mRNA, and was not tested further. Although the sequence of mitochondrial genes is known, their sequence is joint in GenBank into one long nucleotide sequence of about 16 kb. Screening of GenBank with tags of these genes will not result in any hits because only the tag of the last gene in the sequence is screened by the SAGE software. Another problem to be kept in mind when screening GenBank is the fact that of many known genes, the complete 3'UTR with poly(A) tail is not shown. Thus, screening with tags of these

genes will not reveal matches and more thorough examination of GenBank is necessary.
Of two tags that matched with multiple ESTs (numbers 17 and 27), clones that were found in the database were retrieved from the I.M.A.G.E. Consortium and used for the Northern blot analysis. In case of tag number 17, expression was seen in all medulloblastomas tested, and also in brain tissue on a multiple tissue blot. The blots showed a signal at around 3.5 kb. Tag number 27 only showed a clear signal in 3 of 7 tumors tested, and a weak signal in a 4th tumor. This probe detects a mRNA which is very large (> 5 kb).
One tag (number 25) was not tested further because only the medulloblastoma of which the SAGE library was constructed, proved to express this mRNA. The 6 other medulloblastomas tested were negative on Northern blot analysis
Tag numbers 13 and 33 showed no homology with any known sequences. A RACE-PCR procedure is currently being done to reveal more of the nucleotide sequence.

Conclusion

Results created by the SAGE technique can be used for further analysis of transcripts of interest. Depending on the aim of the study, other tags will be selected to be examined in detail e.g. tags that are expressed at a higher level in the tumor if the goal is to look for genes specific for that tumor. First step in further analysis must be the analysis of the expression level in other tumors, to be able to exclude from further analysis transcripts that only show a high expression in a small minority of tumors tested.

References

1. Frohman MA, Dush MK, Martin GR. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proceedings of the National Academy of Sciences of the United States of America 1988; 85:8998-9002.