IN SILICO EXPRESSION ANALYSIS OF HUMAN NOVEL GENE UBAP1 IN MULTIPLE CANCERS

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ABSTRACT

Objective: To identify the differential expression profile of human novel gene UBAP1, a putative nasopharyngeal neoplasms (NPC) relate gene, in multiple cancers. Methods: We first present an EST approach for electronic Northern in silico to analyse expression patterns of UBAP1 in tumor and normal tissues. Full length cDNA of UBAP1 gene was taken as a “probe” sequence, and a blastn search was performed against human EST Database. The Blastn report can be used to determine the fold differences between the pedigree ESTs in different libraries. Especially, the ESTs corresponding to UBAP1 present in fifteen tumor-derived libraries were compared against their normal counterpart to produce an electronic differential expression profile. Second, the distinct down-regulation of UBAP1 in meningioma, glioma, and colorectal tumor was confirmed by differentially RT-PCR analysis. Results: Database surveys indicated that UBAP1 gene was not only ubiquitously expressed in many normal tissues with various levels but also differentially expressed in different tumor tissues, especially down-regulated in multiple neoplastic tissues such as brain, breast, skin, colon, testis and uterus. Furthermore, differential RT-PCR analysis demonstrated that expression of UBAP1 was down-regulated or absent in 7 of 12 (58%) meningioma samples, 6 of 9 (66%) glioma and 7 of 11 (63%) colorectal tumor tissues respectively. Conclusion: we described a data mining procedure in silico that proved to be useful for the identification of differential expression patterns of UBAP1. These findings could be valuable for the investigation of the mechanism the differential expression of UBAP1 gene and its significance in the progression of multiple cancers.

Key words: UBAP1, tissue expression, expressed sequence tag

Loss of heterozygosity (LOH) on human chromosome 9p21-22 is one of the most frequent genetic alterations in many common sporadic cancers, including breast cancer, lung cancer, bladder cancer, melanomas, gliomas and nasopharyngeal carcinoma. So it was postulated that there might be some other unknown tumor susceptibility or suppressor genes, which are correlated with the occurrence of these cancers[1-5]. In order to obtain the novel genes associated with human nasopharyngeal carcinoma (NPC) on chromosome 9p21-22, we have delineated a new common region of deletion flanked by D9S161 and D9S1853 on 9p21.1 in NPC in the previous study. And a novel human gene, UBAP1, was isolated from this region. As a novel member of UBA domain protein family, UBAP1 was found to be down-regulated in NPC, suggesting that UBAP1 may be involved in the pathogenesis of NPC[5, 6]. Here our analysis of expressed sequence tags corresponding to UBAP1 gene further demonstrated it may be a multiple cancer-relate candidate gene, which has a distinct differential expression patterns in normal and tumor tissues.
MATERIALS AND METHODS

Database Searches and Digital Expression Analysis

In our electronic Northern blot, complete cDNA of UABP1 gene was taken as a “probe” sequence, and human EST Database search was carried out using BLASTn programs (www.ncbi.nlm.nih.gov). Our choice for the stringency parameters defining sequence homology were 10^-4 for the E value and 95% sequence identity. In addition, the analysis of every hit of BLAST search was combined with that of every EST in UniGene cluster HS.75425 corresponding to UBAPI gene, which is assembled and updated automatically by NCBI (National Center for Biotechnology Information) in USA. We eventually used all-versus-all comparison to eliminate redundant ESTs corresponding to the same cDNA clone in libraries. For each type of DNA library, expression ratio of UBAPI ESTs was calculated as the fraction of the number of ESTs matching the UBAP1 gene over the total number of ESTs. cDNA libraries containing UBAPI ESTs were subsequently grouped per tissue and disease status (normal and neoplastic). Expression ratio was averaged to overcome cDNA library size differences, again per tissue and disease status. In order to assess the distribution of hits between normal and affected tissues observed in a BLAST search, we applied Fisher’s exact test, which is a conservative test as compared to other statistical tests [17], therefore selection of expression patterns for further investigation based upon the criterion of small P values can be considered restrictive.

RNA Isolation and Differential RT-PCR

Total RNA of biopsy samples from twelve meningiomas, nine gliomas, fifteen colorectal tumors and nine gastric tumors were extracted using RNA isolation kit (Gibco BRL, USA). The experimental details of differential RT-PCR was described in the reference 6[6].

RESULTS

Digital Expression Patterns of UABP1 in Normal Tissues

Our previous report has indicated that UABP1 expression is down-regulated in NPC[6]. Taking its map location and ubiquitous expression patterns in human normal tissues into consideration, we have therefore addressed the question of whether this down-regulation is observed in other types of cancer. In order to answer this question, we first performed a UBAP1 expression analysis by a human EST database searching as described in Materials and Methods. On the basis of the complete cDNA sequence of UBAP1 gene, we identified 245 matches of human ESTs. We reviewed the identified ESTs and their alignment with the query sequence. As part of our quality control measures, we restricted the retrieved ESTs to the 192 matches which were isolated from 106 cDNA libraries from a wide range of tissues in the public human EST database. Of 192 ESTs, there were 119 ESTs derived from 51 different normal cDNA libraries representing 42 types of characterized tissues (data not shown). This proportion of tissues exhibiting ubiquitous expression of UABAPI gene far exceeded that seen in previous analysis by multiple-tissue Northern blot[6].

Digital Differential Expression Profile of UABP1 in Multiple Tumors

Differential expression analysis of UABP1 in normal and tumor tissues was performed with the ESTs that were generated from a variety of non-normalized cDNA libraries. Like its ubiquitous expression patterns in normal tissues, UABP1 gene was found to have 73 identical ESTs derived from 47 different cDNA libraries representing 34 types of characterized nepotistic tissues with different expression ratio. As shown in Figure 1, down-regulation of UBAP1 expression could be found ubiquitously in 73% tumor tissues (11/15). More interestingly, as for the tissues such as brain, breast, skin, colon, testis and uterus, UBAPI has a distinct down-regulated expression in these tumor tissues by comparison with those of normal counterparts (P<0.05), whereas the ratio of UBAPI ESTs derived from kidney or pancreas tumor tissues is significantly higher than in nontumor tissues (P<0.05).

![Fig. 1. Differential expression analysis in 16 human normal and tumor tissues based on EST data. The UBAP1 expression is estimated from ratio (y-axis) of human ESTs in DNA libraries grouped according to tissue types and disease status (x-axis). Bars represent normal (open) and neoplastic (filled) tissues. Expression ratio is defined as in Materials and Methods.](image-url)