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# An unannotated α/β hydrolase superfamily member, ABHD6 differentially expressed among cancer cell lines

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**Abstract** Abhydrolase domain containing (*Abhd*) gene was a small group belongs to α/β hydrolase superfamily. Known members of this group are all found to be involved in important biochemical processes and related to various diseases. In this paper, we report the tissue distribution, subcellular location and differential distribution among cancer cell lines of *Abhd6*, one unannotated member of this group.

**Keywords** ABHD6 · Cancer · Hydrolase · Tumorigenesis

## Introduction

As one of the largest protein superfamilies, α/β hydrolase fold superfamily has gone through an interesting evolutionary process that seemly unrelated amino sequences can conform to structure with similarities. Ollis et al. named the same protein fold of 5 apparently unrelated hydrolases as α/β hydolase fold in the early 1990s. A canonical α/β hydrolase fold consists of an eightstranded parallel α/β structure [1].

Enzymes in this family may have unrelated sequences, various substrates, and different kinds of catalytic activities such as: carboxylic acid ester hydrolase, lipase, thioester hydrolase, peptide hydrolase, haloperoxidase, dehalogenase, epoxide hydrolase and C–C bond breaking enzymes [2]. The most distinguishing feature in common is that the enzymatic properties of the α/β hydrolases are based on the Nucleophile-His-Acid catalytic triad which is evolved to bind with different substrates in various biological contexts [2, 3]. The nucleophile is not fixed, it can be a serine, cysteine or an aspartate. Unlike fold size in other superfamilies, the fold of this family ranges widely from 197 to 583 residues [3]. The highly divergent evolution and relatively low structural similarity make α/β hydrolase fold family one of the most widespread and functionally diverse protein families in nature.

The α/β hydrolases involve in nearly all physiological and pathological processes and are always taken as drug targets when treating diseases like diabetes, Alzheimer's disease, obesity, and blood clotting disorders etc [4]. Given the significant role they played in drug design and protein engineering, over 50 α/β hydrolase fold structures have been solved so far [3].

Three genes containing α/β hydrolase fold were firstly cloned from murine lung by screening differentially expressed genes in emphysematous tissue. Then they were termed as abhydrolase domain containing (*Abhd*) genes, (*Abhd1*, *2*, *3*) with high sequence similarities with their respective human homologies. Up till now, 15 human α/β hydrolase domain containing genes have been reported, but most of them have not been functionally annotated. *Abhd1* was predicted to take part in metabolizing smoking xenobiotics [5]. Keishi et al.'s study illustrated that *Abhd*2 deficient mice showed enhanced migration of vascular smooth muscle cells. In their another paper, expression of ABHD2 in human varied with monocyte differentiating into macrophage, and this variation is critical for the development of atherosclerosis [6, 7]. *Abhd4* was proved to be involved in an alternative synthesis pathway of NAE [8]. Mutations in *Abhd5* (abhydrolase domain containing 5,also known as CGI-58) gene were found to contribute to

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Chanarin–Dorfman syndrome, an autosomal recessive disorder of neutral lipid metabolism [9, 10, 11].

Alignment of consensus active site motifs of members of mouse $\alpha/\beta$ hydrolase family performed by Gabriel M. Simon et al. showed that 12 of the 15 *mAbhd*s choose serine nucleophile to form G–X–S–X–G "nucleophile elbow" [11], the 12 genes are also grouped into serine hydrolases (SH) with serine residue in their active site. A novel strategy has just been developed for discovering inhibitors of SH, and *Abhd6*, like most SHs, was found to be inhibited by some specific carbamates [12]. They also suggested a role of this enzyme in nervous system metabolism and signaling.

Maier et al. identified *Abhd6* as a target gene for Epstein–Barr virus (EBV) nuclear antigen 2 (EBNA-2) who was a critical gene involved in pathogenesis of EBV-related disorders such as: endemic Burkitt's lymphoma, Hodgkin's lymphoma, and post-transplant lymphoma [13].

Fuelled by the remarkable number of uncharacterized members and potential roles in disease development of this enzyme group, we intend to start from the unannotated member *Abhd6*, whose function and cellular processes involved are basically unknown, and to explore its relation with diseases.

## Materials and methods

### Bioinformatics analysis

DNA and protein sequence comparisons were carried out using BLAST at NCBI (http://www.ncbi.nlm.nih.gov/blast).

Information of mouse *Abhd6* was given by Mouse Genome Informatics (http://www.informatics.jax.org/).

SAGE analysis was performed by CGAP (http://www.cgap.nci.nih.gov/).

Prediction of signal peptide and transmembrane regions was done by InterProScan (http://www.ebi.ac.uk/InterProScan).

Alignment comparison between mouse and human *Abhd6* was done by using GeneDoc.

Semi quantification was performed by gel computer image system Quantity One (BioRad).

Phylogenetic tree of 15 known human $\alpha/\beta$ hydrolase domain containing genes was constructed with Mega 3.1.

### cDNA library

By using a SMART PCR cDNA library construction kit (Clontech), a high quality human fetal cDNA library was constructed from human fetal brain poly(A)+ mRNA. After SfiI digestion, cDNAs greater than 500 bp were ligated into the SfiIA and SfiIB sites of the modified pBluescript II SK (+) vector (two SfiI recognition sites, SfiIA and SfiIB, were introduced between the *Eco*RI and *Not*I sites of pBluescript II SK(+)(Stratagene)). Double-stranded cDNAs were synthesized using SMARTTM cDNA Library Construction Kit (Clontech). The cDNA inserts were sequenced with BigDye primer and BigDye terminator Cycle Sequencing Kit on an ABI377 sequencer (Perkin-Elmer) using M13 consensus primers. If necessary, primer walking was performed. Each part of the insert was sequenced at least three times bidirectionally. Full-length cDNA sequences were assembled by assembly program (Sanger Center).

### Plasmid construction

Complete open reading frame (ORF) of *Abhd6* gene was cloned from the fetal brain library using Pfu DNA polymerase. Primers were 5′-cggaattct GATCTTGATGTGGT TAACATGTTTGT-3′ (forward) and 5′-cgggatcc TCAGT CCAGCTTCTTGTTGTTGTC-3′ (reverse). The PCR products were then cloned into *Eco*RI and *Bam*HI restriction sites of pEGFP-C1 (Clontech) vector. Fusion plasmid has gone through high quality sequencing.

### Cell culture

AD293, Hela and U251 were incubated in DMEM supplemented with 10% newborn calf serum at 37°C, 5% $CO_2$.

PC-3, U2-OS and QGY-7703 were incubated in DMEM supplemented with 10% fetal bovine serum at 37°C, 5% $CO_2$.

HO-8910 and Jurkat was incubated in RPMI-1640 medium containing 10% fetal bovine serum at 37°C and 5% $CO_2$.

### Total RNA extraction and RT-PCR

Total RNA of the cells above (except AD293) were extracted by using Rneasy mini kit (Qiagen), and 1ug total RNA were immediately reversetranscripted in 20 μl RT-PCR reaction system using First Strand cDNA Synthesis kit (Toyobo). Template RNA was quantified before transcription by measuring O.D. A260 /A280 ratio in the range of 1.5–1.9.

### Subcellular location

When the growth density of AD293 cells reached 80%, 1.5 μg recombinant plasmid was transfected into with Lipofectamine 2000 (Invitrogen) according to the manufacture's instruction. After 36 h incubation, cell nucleuses were dyed with DAPI and green fluorescence

was viewed with Olympus inverted fluorescence microscopy.

### Expression profile

#### *Normal tissue panel*

Two human Multiple Tissue cDNA (MTC) panels (CLONTECH) were used to examine the expression pattern of *Abhd6* among tissues. The specific primers for *Abhd6* were 5′-GCTCAGTGTGGTCAAGTTCCTTCCA-3′ and 5′-TTCCATCACTACTGAGTGCCCACAG-3′. The primers for G3PDH were 5′-TGAAGGTCGGAGTCAA CGGATTTGGT-3′ and 5′-CATGTGGGCCATGAGGTCC ACCAC-3′. PCR conditions for *Abhd6* were 38 cycles at 94°C for denaturation (30 s), 60°C for annealing (45 s), and 72°C for extension (70 s); PCR conditions for G3PDH were 35 cycles of 94°C for denaturation(30 s), 58°C for annealing (40 s), 72°C for extension (60 s).

Each final extension step consisted of 72°C incubation for 5 min.

PCR products were then subjected to electrophoresis on a 1.5% agarose gel. The region between primers spans 673 bp in the cDNA from 622 to 1294 bp.

#### *Expression pattern of tumor cell lines*

Semi-quantification PCR was performed among 7 tumor cell lines. Primers and PCR conditions for both *Abhd6* and G3PDH were the same as above. PCR products were sequenced to be correct. Each RNA quantity was normalized to its respective G3PDH control. Electrophoresis was performed on a 1.5% agarose gel.

## Results and discussion

### Bioinformatics

*Abhd6* gene is located on chromosome 3p14.3. The 2364 cDNA sequence (Accession No. NM_020676) has an ORF which encodes a 337 residue protein, from which 98-322 amino acid is an ABH domain.

The amino sequence of human ABHD6 was highly homologous to mouse ABHD6 protein (Fig. 1). Human ABHD6, similar to mouse ABHD6, was predicted to be a lipase involved in the process of aromatic compound metabolism. But so far, there is no direct evidence showing functional similarities between human and mouse *Abdh6* genes.

SAGE analysis showed that in tissues like bone marrow, brain, and liver, expression of *Abhd6* have relatively sharp differences between normal tissue and its corresponding cancer.

Phylogenetic trees of human (Fig. 2) and mouse [8] $\alpha/\beta$ hydrolase domain containing genes based on amino acid sequences were similar in topology. In both trees, *Abhd6* showed relatively close phylogenetic relationship with *Abhd4* and *Abhd5*, who were lipases involved in regulation of neutral lipid metabolism.

### Subcelluar location

As most of enzymes in this family, ABHD6-GFP fusion protein was detected in cytoplasm (Fig. 3), while pEGFP control was distributed throughout the whole cell as expected. Both human and mouse ABHD6 was bioinformatically predicted to have two transmembrane regions and a signal peptide at N-terminal by using InterProScan


**Fig. 1** Alignment of mouse and human ABHD6 protein sequences. Conserved active motif G–X–S–X–G is framed. Different amino acids are shown in black. Identity = 96%

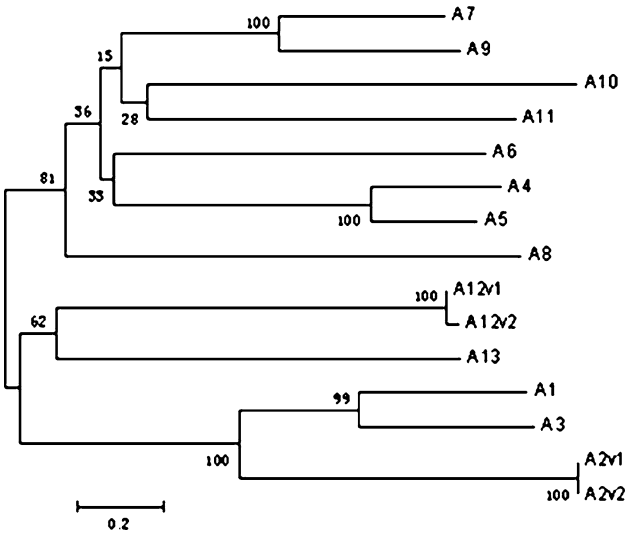


**Fig. 2** Phylogenetic tree of the human $\alpha/\beta$ hydrolase family based on amino acid sequence calculated by using the neighbor joining method. Branch lengths represent evolutionary distance. The analysis was supported by 1000 replicate bootstrap analysis. Numbers at nodes are percentages out of bootstrap analysis. A is short for ABHD

(http://www.ebi.ac.uk/InterProScan). We speculated that human ABHD6, similar to mouse ABHD6, which is an epoxide hydrolase, mainly distribute in cytosol, endoplasmic reticulum, mitochondria and peroxisomes. An interesting finding in Enayetallah et al.'s study [14, 15] was that subcellular localization of epoxide hydrolase were tissue dependent, so precise subcellular localization was needed for understanding the exact cellular process and function mechanism of this gene.

## ReverseTranscript-PCR

To examine tissue distribution of human ABHD6, RT-PCR analysis was carried out. The expected near 700 bp products were detected in most tissues. Expression level of ABHD6 was relatively high in liver, kidney, ovary (Fig. 4a). Such widespread tissue distribution of ABDH6 suggest that it might perform a vast spectrum of functions. And high expression in liver implied that ABHD6 might be necessary for lipid metabolism.

Combined with the results of SAGE analysis (not shown), we selected seven tumor cell lines which may have differential expression with normal tissues: Hela (cervical carcinoma), QGY-7703 (liver cancer), Jurkat (T-lymphocyte leukemia), PC-3 (prostate cancer), HO8910(ovary cancer), U20S(osteosarcoma), U251(human glioblastoma) to detect expression level of *Abhd6* gene.

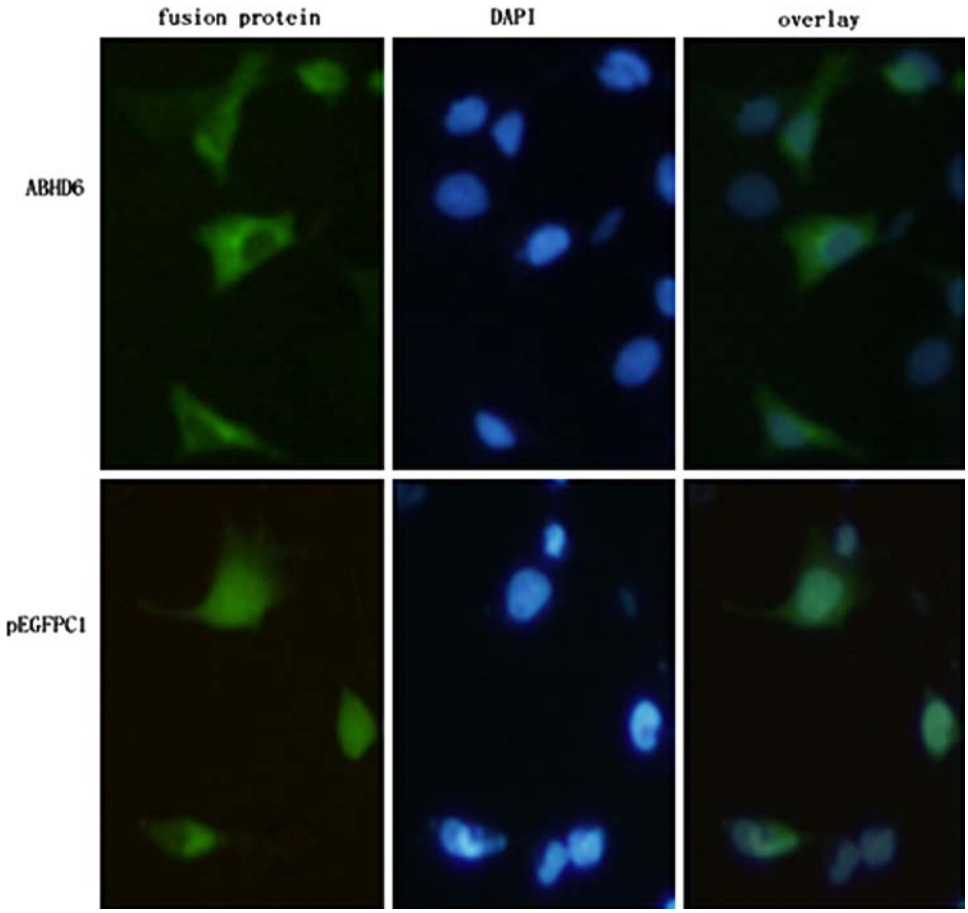


**Fig. 3** Subcellular localization of ABHD6 fusion protein in AD293. Upper panel from left to right are: ABHD6-GFP fusion protein, nucleuses stained by DAPI, overlay produced by merging two signals. Lower panel from left to right are: GFP protein, nucleuses stained by DAPI, and overlay produced by merging two signals

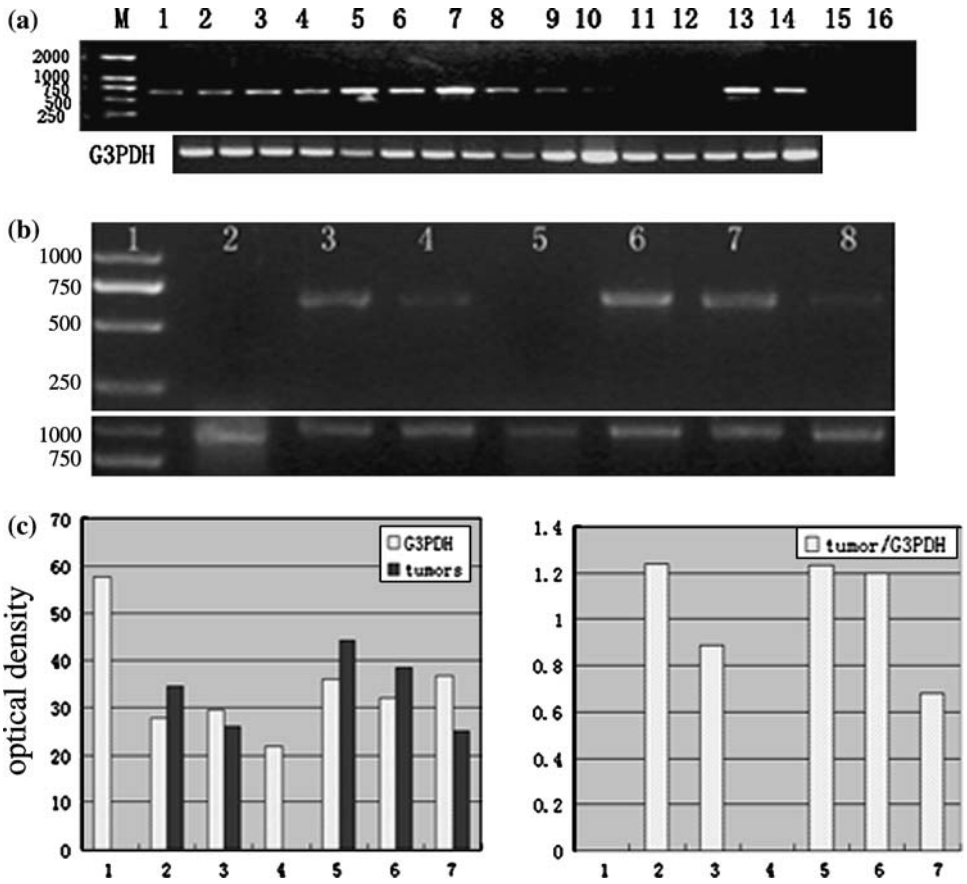


**Fig. 4** (**a**) ABHD6 expression panel in normal tissues with G3PDH as external control. The tissues from left to right are: (1) heart, (2) Brain, (3) Placenta, (4) lung, (5) liver, (6) Muscle, (7) Kidney, (8) Pancreas, (9) Spleen, (10) Thymus, (11) Prostate, (12) Testis, (13) Ovary, (14) Small Intestine, (15) Colon, (16) Leukocyte. (**b**) ABHD6 expression by cancer cell lines with corresponding G3PDH. Lane 1: DNA ladder, Lane 2: Hela, Lane 3: Jurkat, Lane 4: QGY-7703, Lane 5: U251, Lane 6: U2OS, Lane 7: PC-3, Lane 8: HO-8910. Lower panel are corresponding G3PDH. (**c**) Densitometric analysis of the levels of ABHD6, G3PDH in tumors and their relative Densitometry (tumor/G3PDH ratio). Cell types are (1) Hela, (2) Jurkat, (3) QGY-7703, (4) U251, (5): U2OS, (6) PC-3, (7) HO-8910

ABHD6 was found differentially expressed among 7 tumor cell lines. The expression level was extremely high in U2OS (bone), PC-3 (prostate) and Jurkat (leukocyte) but relatively low in QGY-7703 (liver) and HO8910 (ovary). But we didn't detect any expression in Hela (cervical) and U251 (brain). G3PDH was used as an external control.

Noticeably, the expression profiles in leukocyte and prostate greatly differed from the results in MTC, where *Abhd6* expression was not detected (Fig. 4b). And densitometric analysis showed variable expression level of ABHD6 among tumors (Fig. 4c), which ratio is rather higher in prostate cancer, leukemia and brain tumor than in other tumor cell lines studied. This will attract us to see more clearly with the roles *Abhd6* might play in these changes.

The high identity between human ABHD6 and mouse ABHD6 implied that they might implement similar functions in two species. Mouse ABHD6 was known as an epoxide hydrolase which is involved in the metabolism of carcinogenic chemicals, and polymorphisms of epoxide hydrolase, in many papers have previously been linked to increases in risk for colorectal [16], colon [17], orolaryngeal cancer [18, 19], ovarian [20] and lung [21] cancers. There is no direct study of the relation between *Abhd6* polymorphisms and cancer susceptibilities so far. Metabolism of lipid and lipoprotein in liver, which is closely related to liver diseases [22], is also regulated by various $\alpha/\beta$ hydrolases.

Development of carcinoma and other diseases are related with complicated pathways involving numerous enzymes working synergistically. In order to better understand the complicated network, it is important to make clear which enzymes involved in the process and how they interact. The possibility that *Abhd6* relate to tumorigenesis will draw more attention pays on it. Since most enzymes in this family still lack experimentally verified endogenous substrates and knowledge of metabolic and cellular functions so far, further investigation is required to elucidate the functional mechanism of these genes in more details, which will be helpful for pharmacological development and mechanism study.

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## References

1. Ollis DL, Cheah E, Cygler M et al (1992) The alpha/beta hydrolase fold. Protein Eng 5:197–211

2. Holmquist M (2000) Alpha/beta-hydrolase fold enzymes: structures, functions and mechanisms. Curr Protein Pept Sci 1:209–235
3. Heikinheimo P et al (1999) Of barn owls and bankers: a lush variety of a/b hydrolases. Structure 7:R141–R146
4. Thornberry NA et al (2007) Add added discovery of JANUVIA? (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type2 diabetes. Curr Top Med Chem 7(6):557–568
5. Edgar AJ, Polak JM (2002) Cloning and tissue distribution of three murine a/b hydrolase fold protein cDNAs. Biochem Biophys Res Commun 292:617–625
6. Keishi M, Yuichi O, Takayuki H et al (2005) Increase of smooth muscle cell migration and of intimal hyperplasia in mice lacking the a/b hydrolase domain containing 2 gene. Biochem Biophys Res Commun 329:296–304
7. Miyata K et al (2007) Elevated mature macrophage expression of human ABHD2 gene in vulnerable plaque. Biochem Biophys Res Commun. doi:10.1016/j.bbrc.2007.10.127
8. Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. J Biol Chem 281:26465–26472
9. Doganci T, Gurakar F, Karaduman A et al (2005) Hepatobiliary and pancreatic: Dorfman–Chanarin syndrome. J Gastroenterol Hepatol 20:156. doi:10.1111/j.1400-1746.2004.03752.x
10. Bruno C, Bertini E, DiRocco M et al (2007) Molecular analysis of the ABHD5 gene in Italian patients with multisystem triglyceride storage disease (Chanarin–Dorfman syndrome). Neuromuscul Disord 17(9–10):862–863
11. Lefevre C et al (2001) Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin–Dorfman syndrome. Am J Hum Genet 69:1002–1012
12. Weiwei Li, Jacqueline LB, Benjamin FC (2007) A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J Am Chem Soc 129(31):9594–9595
13. Maier S, Staffler G, Hartmann A et al (2006) Cellular target genes of Epstein–Barr virus nuclear antigen 2. J Virol 80(19):9761–9771
14. Pahan K, Smith BT, Singh I (1996) Epoxide hydrolase in human and rat peroxisomes: implication for disorders of peroxisomal biogenesis. J Lipid Res 37:159–167
15. Enayetallah AE, French RA, Barber M et al (2006) Cell-specific subcellular localization of soluble epoxide hydrolase in human tissues. J Histochem Cytochem 54(3):329–335
16. Gregory JT, Andrew TC, Edward G et al (2005) Epoxide hydrolase and CYP2C9 polymorphisms, cigarette smoking, and risk of colorectal carcinoma in the nurses' health study and the physicians' health study. Mol Carcinog 44(1):21–30. doi:10.1002/mc.20112
17. Harrison DJ, Hubbard AL, MacMillan J et al (1999) Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. Br J Cancer 79:168–171
18. Park JY, Schantz SP, Lazarus P (2003) Epoxide hydrolase genotype and orolaryngeal cancer risk: interaction with GSTM1 genotype. Oral Oncol 39(5):483
19. Jourenkova-Mironova N, Mitrunen K, Bouchardy C et al (2000) High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. Cancer Res 60:534–536
20. Lancaster JM, Brownlee HA, Bell DA et al (1996) Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. Mol Carcinog 17:160–162
21. Gsur A, Zidek T, Schnattinger K et al (2003) Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. Br J Cancer 89:702–706. doi:10.1038/sj.bjc.6601142
22. Jiang J-T et al (2007) Lipids changes in liver cancer. J Zhejiang Univ Sci B 8(6):398–409