# 白鳍鲨*SBP1*基因的克隆、表达及抗癌活性分析

何曼文* 官昭瑛 余展旺 罗国生
(深圳技师学院应用生物系, 深圳 518116)

**摘要** 硒结合蛋白1(selenium-binding protein 1, SBP1)在多种癌症中起关键作用。目前对SBP1的研究大多集中在模式生物上, 对未知基因组物种的SBP1知之甚少。该研究前期基于白鳍鲨体内高硒含量特性及SBP1肽段信号, 首次克隆和测序分析了白鳍鲨*SBP1*的cDNA序列。白鳍鲨*SBP1*基因的cDNA全长为3 064 bp, 开放阅读框为1 419 bp, 编码472个氨基酸。白鳍鲨SBP1蛋白序列中组氨酸残基共15个, 占比3.2%; 其含有2个CxxC、2个HxD和3个HxxH保守区域。通过SWISS-MODEL构建白鳍鲨SBP1三维结构模型, 推测定位于β-折叠与连接各结构环交界处的第56位丝氨酸(Ser56)为硒结合位点。系统进化树分析表明, 白鳍鲨和象鲨的SBP1同源性最高。在人正常肝细胞HL-7702、人肝癌细胞HepG2和人宫颈癌细胞HeLa中分别转染重组子MYC-SBP1和空载体pcDNA3.1-MYC后, 使用Cell Counting Kit-8和CFDA SE细胞增殖示踪荧光探针研究鲨鱼SBP1的抗癌活性。研究发现, 无论培养基中是否添加$Na_2SeO_3$, 在HeLa和HepG2癌细胞中表达重组蛋白SBP1都会显著抑制细胞增殖。相反, 表达SBP1组将促进正常HL-7702细胞增殖。该研究推测硒的化学预防作用与SBP1的功能高度相关。在某种程度上, 可能是SBP1蛋白(而不是硒)在预防癌症中起着更关键的作用。

**关键词** 硒结合蛋白1; 白鳍鲨; cDNA克隆; 癌症

# Molecular Cloning, Expression, and Anti-Cancer Activity of *SBP1* Gene in Whitetip Reef Shark

HE Manwen*, GUAN Zhaoying, YU Zhanwang, LUO Guosheng
(*Department of Applied Biology, Shenzhen Institute of Technology, Shenzhen 518116, China*)

**Abstract** SBP1 (selenium-binding protein 1) plays a key role in a variety of cancers. At present, most researches have focused on model organisms, while few studies have explored the SBP1 of unknown genome species. In the early stage of this study, based on the discovery of the high selenium content and the SBP1 peptide signal in the whitetip shark, the *SBP1* cDNA sequence of the whitetip reef shark was first cloned and sequenced. The full-length cDNA of the whitetip reef shark *SBP1* gene is 3 064 bp and contains a 1 419 bp open reading frame that encodes 472 amino acids. There are 15 histidine residues within its protein sequence, accounting for 3.2%. It contains two CxxC, two HxD and three HxxH conserved regions. The three-dimensional structural model of SBP1 was constructed by SWISS-MODEL, and it was deduced that the 56th serine (Ser56) located at the junction between the β-sheet and structural loop was the selenium binding site. Phylogenetic tree analysis shows that whitetip shark and elephant shark have the highest SBP1 homology. After transfecting the recombinant MYC-SBP1 and the empty

收稿日期: 2021-11-17 接受日期: 2022-02-11
深圳技师学院校科研项目(批准号: 2011012)资助的课题
*通讯作者。Tel: 13510605390, E-mail: hmw.86@163.com
Reveived: November 17, 2021 Accepted: February 11, 2022
This work was supported by Research Project of Shenzhen Institute of Technology (Grant No.2011012)
*Corresponding author. Tel: +86-13510605390, E-mail: hmw.86@163.com

vector pcDNA3.1-MYC into HL-7702, HepG2 and HeLa cells respectively, Cell Counting Kit-8 and CFDA SE cell proliferation tracer fluorescent probe were used to investigate the anti-cancer function of shark SBP1. It was found that whether or not $Na_2SeO_3$ was added in medium, the expression of the recombinant protein SBP1 in HeLa and HepG2 cancer cells would significantly inhibit cell proliferation. In contrast, the SBP1 expression group promotes the proliferation of normal HL-7702 cells. This study speculates that the chemopreventive effect of selenium is highly related to the function of SBP1. To some extent, it may be SBP1 protein rather than selenium plays a more critical role in cancer prevention.

**Keywords** selenium-binding protein 1; whitetip reef shark; cDNA clone; cancer

硒结合蛋白1(selenium-binding protein 1, SELENBP1/SBP1/hSP56)属于含硒蛋白家族, 因其有直接结合硒的能力而得名。SBP1最早在小鼠肝脏中被发现[1]。人类56 kDa硒结合蛋白(*hSP56*)基因在1997年被克隆, 共编码472个氨基酸[2]。SBP1是一种高度保守的蛋白质, 预测的氨基酸序列在植物和哺乳动物中一致性为57%~60%[3]。硒可能通过硒硫键共价结合于SBP1[4]。SBP1被证明以硒依赖的方式在泛素化/去泛素化介导的蛋白质降解途径中发挥作用[5-6]。除了参与硒和硒蛋白的代谢外, SBP1还涉及在毒化/解毒过程、细胞生长调节、高尔基体内蛋白质转运、衰老和脂质代谢中发挥作用[7]。

相比于正常组织, SBP1蛋白在多数癌组织包括肝癌、肺癌、卵巢癌和子宫肌瘤等组织中表达下降[4]。癌变过程中SBP1的缺失通常预示着不良预后[8-9]。*SBP1*基因的敲除会加剧人肝癌、结直肠癌和宫颈癌细胞的增殖和迁移, 表明SBP1具有抑制癌细胞生长和增殖的屏障作用[10-11]。亚硒酸钠能诱导癌细胞中SBP1的表达, 并通过抑制Wnt/β-catenin信号通路来实现抗癌活性[12]。目前对SBP1的研究大多集中在小鼠、人类和拟南芥等模式生物上, 相比之下, 对未知基因组物种的SBP1知之甚少。对真核生物硒蛋白质组的进化动力学研究表明, 水生生物明显保留并扩大其硒蛋白质组, 而陆生生物相应减少, 这与适应陆地硒稀缺和水域硒丰富的脊椎动物相吻合, 也表明水生生物对硒的利用度高于陆生生物[13-14]。我们前期用原子荧光法测得白鳍鲨(whitetip reef shark, *Triaenodon obesus*)和其他鱼类(红三鱼、草鱼、鲤鱼和梭鱼)各组织的硒含量, 发现鲨鱼组织中硒含量显著高于实验中其他的鱼类(表1)。同时通过蛋白质组学方法检测到白鳍鲨肝组织中有多个SBP1的肽段信号。

目前白鳍鲨线粒体基因组已被测序[15], 而全基因组序列仍未知。为验证白鳍鲨高硒含量特性是否与SBP1相关以及增强对其SBP1结构和功能的认识, 本研究通过RT-PCR和cDNA末端的快速扩增(rapid amplification of cDNA ends, RACE)技术克隆了白鳍鲨*SBP1*的cDNA序列, 并在人正常肝细胞HL-7702、人肝癌细胞HepG2和人宫颈癌细胞HeLa中表达白鳍鲨SBP1蛋白, 研究其对硒的耐受作用和对癌细胞增殖的影响。

## 1 材料与方法

### 1.1 材料

白鳍鲨肝组织的总RNA和cDNA文库由深圳大学生命与海洋科学学院提供。RT-PCR反应试剂盒、5′-Full RACE试剂盒、pMD18-T载体和DNA Marker均购自日本TaKaRa公司; PCR引物由上海赛百胜生

**表1 不同鱼类中硒的含量**

**Table 1 The content of selenium in different fishes**

| 组织 Tissue | 鲨鱼肝脏 Shark liver | 鲨鱼软骨 Shark cartilage | 鲨鱼肉 Shark meat | 红三鱼肉 Red shirt fish meat | 草鱼肉 Grass carp meat | 鲤鱼肉 Carp meat | 草鱼骨 Grass carp bone | 鲤鱼骨 Carp bone | 梭鱼骨 Barracuda bone |
|---|---|---|---|---|---|---|---|---|---|
| Se / (μg/100 g) | 450.00±15.32 | 120.00±10.64 | 57.02±8.31 | 48.30±8.02 | 6.66±1.36 | 15.38±3.84 | / | / | / |

“/”表示未检测到硒元素。

“/” means selenium is not detected.

物公司合成; Gel Extraction Kit凝胶回收和Plasmid Mini Kit质粒提取试剂盒购自OMEGA BIO-TACH公司; pcDNA3.1-MYC真核表达载体、Lipofectamine 3000真核细胞转染试剂、PVDF膜和ECL化学发光底物购自Invitrogen公司; RIPA细胞裂解液、Cell Counting Kit-8和CFDA SE细胞增殖与示踪检测试剂盒购自上海碧云天生物技术有限公司; Myc标签抗体和羊抗鼠lgG-HRP分别购自SANTA CRUZ公司和生工生物工程(上海)股份有限公司。

### 1.2 RT-PCR

通过同源克隆技术, 在NCBI上找到同源物种*Salmo salar* (NM-001140173.1)、*Danio rerio* (NM-200570.1)、*Xenopus*(*Silurana*) *tropicalis*(NM-001016750.3)和*Rattus norvegicus*(NM-080892.1)的开放阅读框设计简并引物SBP1-F1和SBP1-R1(表2), 对白鲟鲨*SBP1*基因cDNA保守区进行扩增(图1)。PCR反应体系25 μL: 模板cDNA 1μL, 上下游引物各2 μL(10 μmol/L), 10×反应缓冲液2.5 μL, dNTP 1 μL(10 mmol/L), Ex Taq DNA聚合酶0.25 μL(5 U/μL)和dd$H_2O$ 16 μL。反应程序: 94 °C预变性1 min; 94 °C变性30 s, 50 °C退火30 s, 72 °C延伸1.5 min, 35个循环; 最后72 °C延伸10 min。用1%琼脂糖凝胶电泳检测并回收PCR产物, 连接至pMD18-T载体, 转化至大肠杆菌*E. coli* DH5α, 挑取经PCR检测的阳性单克隆送样测序。

### 1.3 RACE

根据获得的*SBP1*基因中间片段序列, 按照特异性引物设计原则, 分别设计上游SBP1-F2和下游引物GSP1/GSP2(表2), 采用RACE技术来获得*SBP1*基因的3′和5′末端序列(图1)。3′ RACE反应体系25 μL: 用接头引物反转录的cDNA模板1μL, SBP1-F2/3′ RACE引物对各1 μL(10 μmol/L), 10× 反应缓冲液2.5 μL, dNTP 1 μL(10 mmol/L), Ex Taq DNA聚合酶0.5 μL(5 U/μL)和dd$H_2O$ 18 μL。反应程序: 94 °C预变性1 min; 94 °C变性30 s, 58 °C退火30 s, 72 °C延伸1 min, 30个循环; 最后72 °C延伸10 min。用于5′ RACE的cDNA模板需要另外处理, 即在获得的总RNA中去除mRNA的5′帽子结构, 使用T4 RNA连接酶将5′ RACE接头连接到mRNA的5′端, 具体按照5′-Full RACE试剂盒说明书来操作。结合巢式PCR技术, 使用引物对5′ RACE outer primer/GSP1首先对靶cDNA进行第一步扩增, 然后从第一次反应产物中取出2 μL作为反应模板进行第二次扩增(使用引物对5′ RACE inner primer/GSP2)。两次PCR扩增反应程序如下: 94 °C预变性3 min; 94 °C变性30 s, 58 °C退火30 s, 72 °C延伸30 s, 20个循环(第二次扩增25个循环); 最后72 °C延伸10 min。用0.8%琼脂糖凝胶电泳检测并回收RACE产物, 连接至pMD18-T载体, 转化至大肠杆菌*E. coli* DH5α, 挑取经PCR检测的阳性单克隆送样测序。

### 1.4 序列分析

将扩增所得的*SBP1*基因的中间片段序列、3′和5′末端序列信息用DNAStar软件进行拼接分析, 获得白鲟鲨*SBP1*基因全长序列。开放阅读框通过在线程序ORF Finder(http://www.ncbi.nlm.nih.gov/orffinder/)进行分析; 蛋白质的分子量和等电点等理化参数通过在线程序ProtParam(https://web.expasy.org/prot-

**表2 本研究所用的引物**

**Table 2 Primer sequences used in this study**

| 引物<br>Primers | 引物序列(5′→3′)<br>Sequences (5′→3′) | 应用<br>Application |
|---|---|---|
| 3′ adaptor primer | GCT GTC AAC GAT ACG CTA CGT AAC GGC ATG ACA GTG TTT TTT TTT TTT TTT T | Reverse transcription |
| SBP1-F1 | AAR ACC CCH CTG GAY GCM ATG AA | Fragment amplification |
| SBP1-R1 | CCR TGM AGC CAG TTR CTR AAG T | Fragment amplification |
| SBP1-F2 | AGG TCA TCG CTG TGC CGA ACA AG | 3′ RACE |
| 3′ RACE primer | CGC TAC GTA ACG GCA TGA CAG TG | 3′ RACE |
| 5′ RACE outer primer | CAT GGC TAC ATG CTG ACA GCC TA | 5′ RACE |
| GSP1 | GTC GGT GAA TAA CCT GGG AGT AG | 5′ RACE |
| 5′ RACE inner primer | CGC GGA TCC ACA GCC TAC TGA TGA TCA GTC GAT G | 5′ RACE |
| GSP2 | CTG GCT TCT CAA TGT TGG TGT TC | 5′ RACE |
| MYC-F1 | CGC GGA TCC ATG GCG AAG TGT GGA GAA TGT GG | Constructing expression vector |
| MYC-R1 | GCT CTA GAC TAC AGC CAA ATA TCG GAA CTG C | Constructing expression vector |

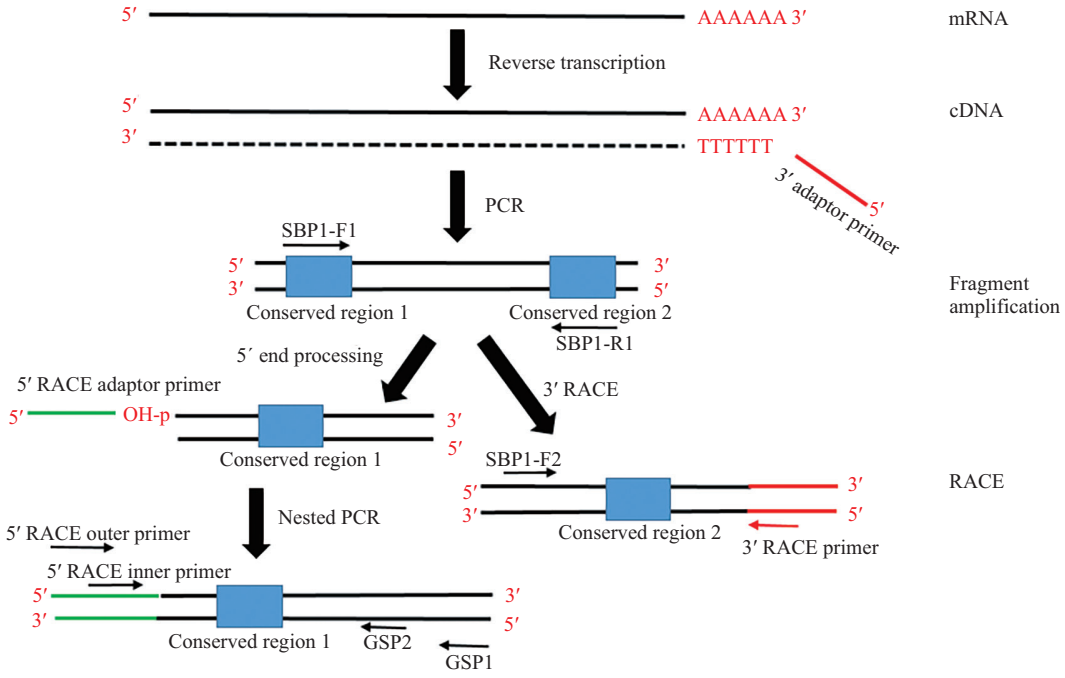



**图1 白鳍鲨*SBP1*基因全长cDNA克隆的示意图**

**Fig.1 Schematic diagram of full-length cDNA cloning of *SBP1* gene from whitetip reef shark**

param/)预测分析; 蛋白的二级结构通过在线分析软件SABLE(http://sable.cchmc.org/)进行预测; 蛋白的三级结构用同源建模服务器SWISS-MODEL(http://www.expasy.ch/swissmod/SWISS-MODEL.html/)进行预测; 序列同源比对使用ClustalX和GeneDoc软件进行分析; 系统进化树的构建使用MEGA7.0软件进行分析。

### 1.5 表达载体构建及细胞转染

设计引物(MYC-F1/MYC-R1)扩增*SBP1*基因的ORF片段, 并将其定向克隆入pcDNA3.1-MYC载体的*Bam*H I和*Xba* I酶切位点, 构建真核重组载体MYC-SBP1, 转化至大肠杆菌*E. coli* DH5α。阳性重组质粒用PCR、酶切及序列测定等方法鉴定。人宫颈癌细胞HeLa和肝癌细胞HepG2用含10%胎牛血清的DMEM完全培养基培养, 人正常肝细胞HL-7702用含10%胎牛血清的RPMI-1640完全培养基培养。培养皿放置于37 °C、5% $CO_2$培养箱内培养, 待细胞汇合度达到80%以上时进行转染实验。按照Lipofectamine 3000试剂使用说明书将MYC-SBP1重组质粒转染至细胞中, 48 h后收集备用。

### 1.6 免疫印迹分析

收集转染后的细胞, 加入RIPA细胞裂解液充分混匀, 冰上孵育30 min, 低温高速12 000 r/min离心30 min, 取上清即为总蛋白样品, 按Bradford法进行蛋白质浓度测定。制备10% SDS-PAGE凝胶, 上样量为50 μg, 电泳后将蛋白质转移至PVDF膜上, 采用含5%脱脂奶粉的TBST室温封闭2 h, 孵育一抗Myc(1:1 000)或者GAPDH(1:10 000) 4 °C过夜; 次日将PVDF膜用TBST洗涤3次, 二抗羊抗鼠IgG(1:10 000)室温孵育2 h, 再用TBST洗涤3次, 使用ECL化学发光显色, 以ChemiDoc™ Touch Imaging System (BIO-RAD)检测蛋白质条带, 用ImageLab软件进行灰度分析。

### 1.7 细胞增殖检测

培养癌细胞(HeLa、$HepG_2$)和正常细胞HL-7702, 各自单独加$Na_2SeO_3$溶液培养48 h; 或瞬时细胞转染MYC-SBP1重组质粒和MYC空载体24 h后, 再加硒继续培养24 h。加入10 μL CCK-8溶液在37 °C孵育2 h, 用酶标仪测定波长为450 nm处的吸光度(*D*)值, 统计结果; 再瞬时转染各株细胞, 先加入CFDA SE细胞标记液, 待转染24 h后, 从上述CCK-8实验结果中选取使细胞达到半致死率的$Na_2SeO_3$浓度继续培养24 h, 收集细胞, 用流式细胞仪检测和软件ModFit分析细胞的增殖情况。

## 2 结果

### 2.1 *SBP1*基因的克隆及序列分析

白鳍鲨*SBP1*基因cDNA的克隆是通过RT-PCR

方法, 并采用基于*SBP1*保守区域cDNA片段设计的简并引物完成的。利用RT-PCR方法成功扩增出长度为956 bp的特异性片段, 通过5′ RACE巢式PCR和3′ RACE将特异性产物克隆测序后分别得到298 bp和1 919 bp的片段(图2), 将所获得的这3个片段进行拼接, 最终获得全长3 064 bp的cDNA序列。利用ORF Finder分析该序列, 获得其开放阅读框长度为1 419 bp, 共编码472个氨基酸; 5′非翻译区(untranslated region, UTR)序列长度为155 bp; 含Ploy A尾巴的3′UTR序列长度为1 490 bp(图3)。白鳍鲨*SBP1*基因序列提交至GenBank数据库, 获得基因序列号为JN646104。

### 2.2 SBP1蛋白生物信息学分析

对白鳍鲨SBP1蛋白序列分析, 预测其相对分子量为52.56 kDa, 理论等电点(pI)为6.03, 分子式为$C_{2359}H_{3627}N_{635}O_{686}S_{22}$。使用SABLE工具分析其二级结构, 发现以无规则卷曲(68.43%)为主, 其次还有β折叠(28.81%)和α螺旋(2.75%)(图4); 跨膜区预测结果显示, 该蛋白质不具有跨膜区, 是一种胞内可溶性蛋白。通过SWISS-MODEL构建白鳍鲨SBP1三维结构模型, 推测定位于β-折叠与连接各结构环交界处的第56位丝氨酸(Ser56)为硒结合位点(图5)。

### 2.3 SBP1同源性和系统发育分析

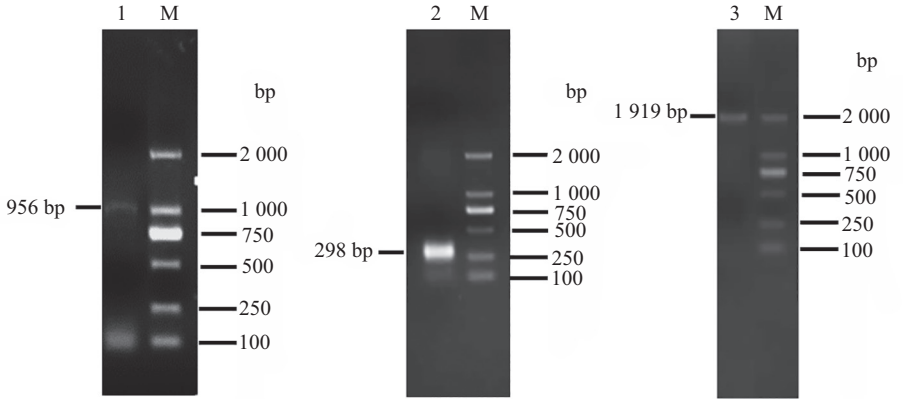
通过NCBI网站中Blast软件对序列进行同源检索, 发现白鳍鲨SBP1蛋白与其他物种的SBP1蛋白序列高度相似, 其中与金线鲃(*Sinocyclocheilus grahami*)、斑点雀鳝(*Lepisosteus oculatus*)、斑马鱼(*Danio rerio*)、亚洲龙鱼(*Scleropages formosus*)、双领鸻(*Charadrius vociferus*)和象鲨(*Callorhinchus milii*)的相似度分别为80.25%、79.83%、79.83%、79.24%、79.24%和78.39%(图6)。白鳍鲨SBP1蛋白序列中组氨酸残基共15个, 占比3.2%; 其含有2个CxxC、2个HxD和3个HxxH保守区域。

为了进一步表征白鳍鲨SBP1与其他物种同源蛋白之间的进化关系, 利用MEGA7.0软件采用NJ法构建了系统进化树(图7)。从进化树可以看出, 白鳍鲨与象鲨(*Callorhinchus milii*)的SBP1聚成一支, 该支进一步与尖吻鲈(*Lates calcarifer*)、亚洲龙鱼(*Scleropages formosus*)和喀麦隆壮象鼻鱼(Paramormyrops kingsleyae)聚成一支。

### 2.4 SBP1蛋白的表达及鉴定

从cDNA模板中扩增SBP1的ORF片段, 并在片段两端引入*Bam*H I和*Xba* I酶切位点, 如图8A所示, PCR结果在1 000 bp~2 000 bp之间获得一条亮带, 将条带切割回收后, 连接至以相同酶(*Bam*H I和*Xba* I)酶切的pcDNA3.1-MYC载体, 构建MYC-SBP1重组质粒。将获得的阳性克隆子经菌液PCR鉴定(图8B)后测序, 验证在双酶切位点序列之间为SBP1的ORF序列, 其大小正好为1 419 bp, 说明在成功构建重组质粒的同时, 也进一步确认了序列拼接的正确性。

在人正常肝细胞HL-7702、肝癌细胞HepG2和宫颈癌细胞HeLa三株细胞系中分别瞬时转染MYC-SBP1重组质粒, 48 h后收集细胞并提取总蛋白。利用Western blot检测出重组蛋白SBP1均能在三株细

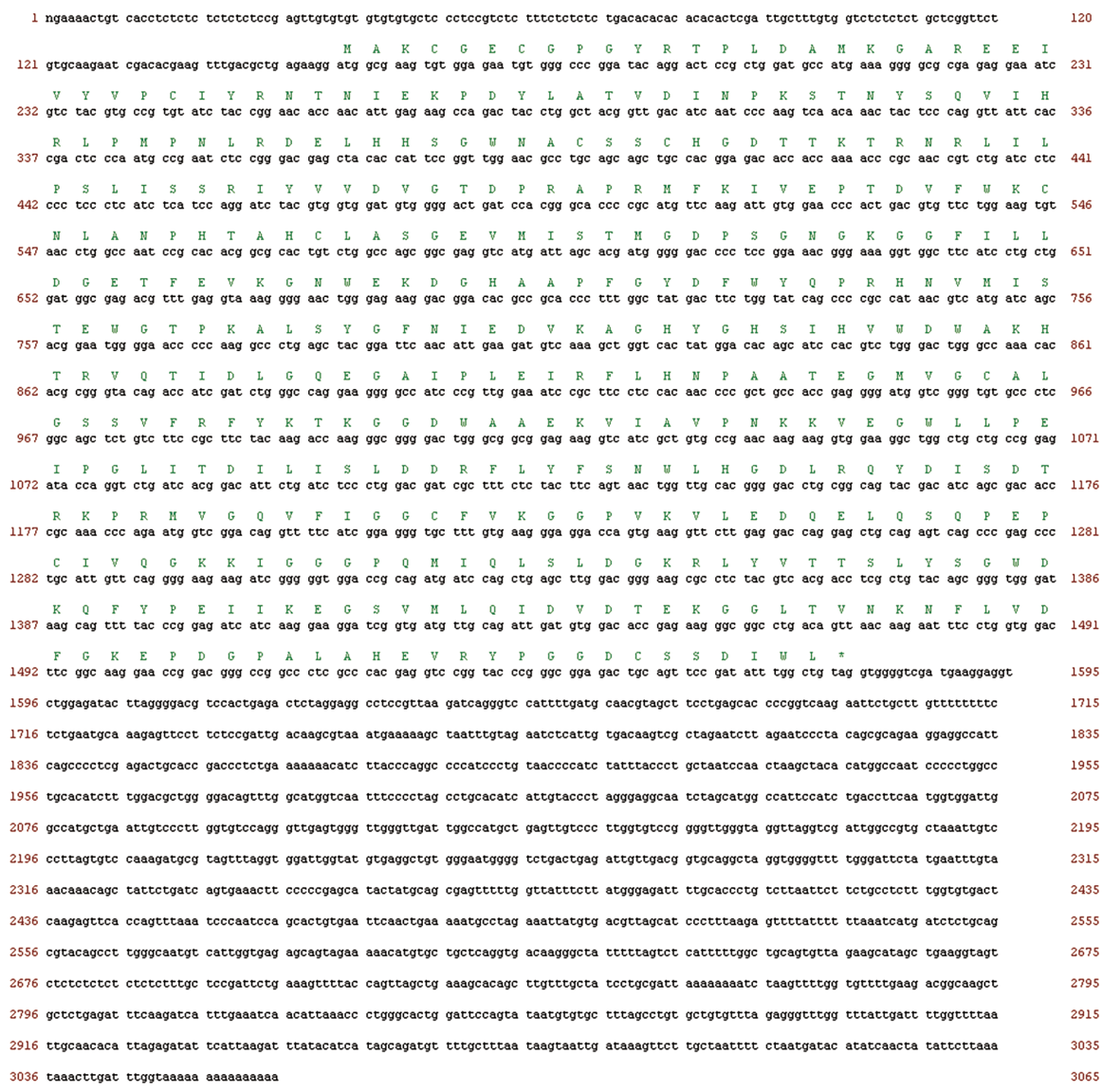



泳道1: *SBP1*基因的保守区域扩增, 泳道2: 5′ RACE扩增产物, 泳道3: 3′ RACE扩增产物, 泳道M: DNA标准品DL2000。

Lane 1: conserved region amplification of *SBP1* gene, lane 2: product of 5′ RACE PCR, lane 3: product of 3′ RACE PCR, lane M: DNA marker DL2000.

**图2 白鳍鲨*SBP1*基因全长cDNA序列克隆结果**

**Fig.2 Cloning results of full-length cDNA sequences of *SBP1* gene from whitetip reef shark**




序列以5′→3′方向显示, 推导的氨基酸序列显示在核苷酸序列上方。TAG终止密码子以星号突出显示。

The sequences were displayed in the 5′→3′ direction and the deduced amino acid sequence was shown above the nucleotide sequence. The TAG stop codon was highlighted by an asterisk.

**图3 白鳍鲨*SBP1*全长cDNA核苷酸及推导的氨基酸序列(GenBank收录号: JN646104)**

**Fig.3 Full-length cDNA nucleotide and the deduced amino acid sequence of *SBP1* from whitetip reef shark (GenBank accession number: JN646104)**

胞中表达(图8C), 灰度分析表明蛋白表达量在各株细胞中无显著性差异(图8D, $P>0.05$)。

### 2.5 SBP1的抗癌活性分析

单独加硒在较低浓度(≤1 μmol/L)下对正常人肝细胞HL-7702的生长没有显著影响, 而在≥2 μmol/L浓度下会降低正常细胞的存活率($P<0.05$, $P<0.01$, 与未加硒组比较, 图9A)。单独加硒在高浓度(≥15 μmol/L或≥60 μmol/L)下对肝癌细胞HepG2和宫颈癌细胞HeLa的生长具有抑制增殖作用(图9B和图9C)。在正常的人肝细胞HL-7702中表达重组蛋白SBP1, 可使细胞的存活率明显提高(图9D, 未加硒组, 152.7% *vs* 100%, $P<0.01$, $n=6$)。加0.25 μmol/L硒, 表达SBP1组的细胞存活率也高于对照组(118.4% *vs* 93.5%, $P<0.05$, $n=6$)。而在人肝癌细胞HepG2中表达重组蛋白SBP1, 可使细胞的存活率急剧下降(图9E, 未加硒组, 65.3% *vs* 100%, $P<0.01$, $n=6$)。分别加0.25、0.5和1 μmol/L硒, 表达SBP1组的细胞存活率仍显著低于对照组(67.4% *vs* 96.5%, 54.7% *vs* 93.2%, 62.1% *vs* 91.5%, $P<0.01$或$P<0.05$, $n=6$)。在人宫颈癌细胞HeLa中表达重组蛋白SBP1, 细胞存活率与对照组无明显变化(图9F, 未

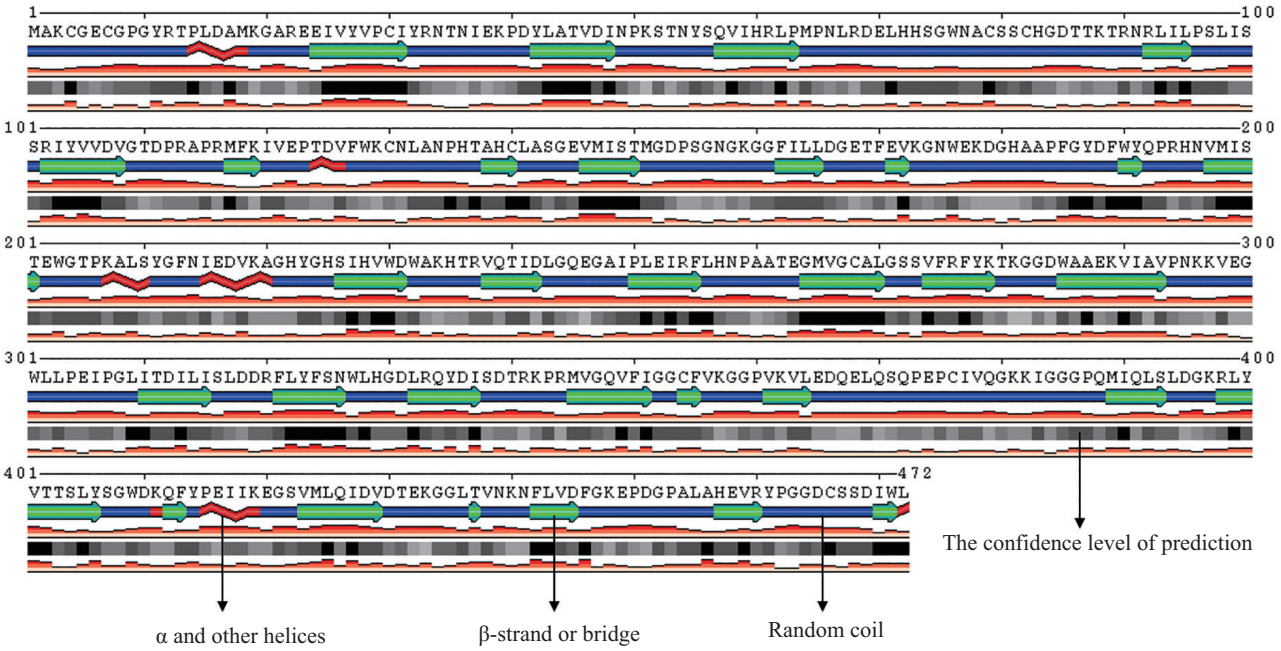
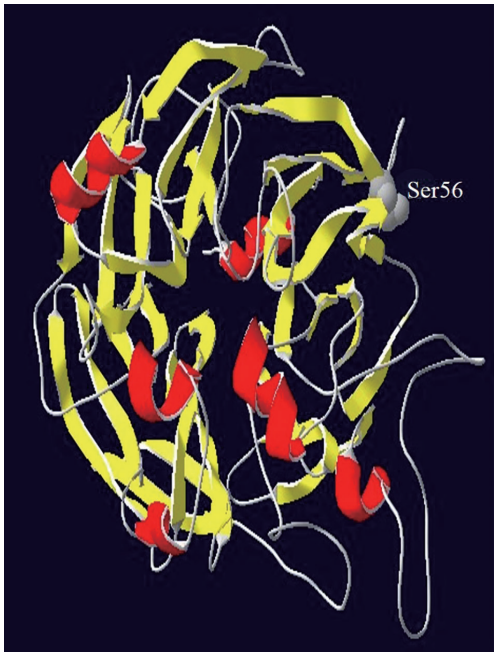



**图4　使用SABLE软件预测SBP1蛋白的二级结构**

**Fig.4　Prediction of amino acid secondary structure of SBP1 with SABLE**




α螺旋用红色表示, β折叠片用黄色表示, 与其他结构相连的环用灰色表示。第56位丝氨酸(Ser56)可能位于灰色实心球处。

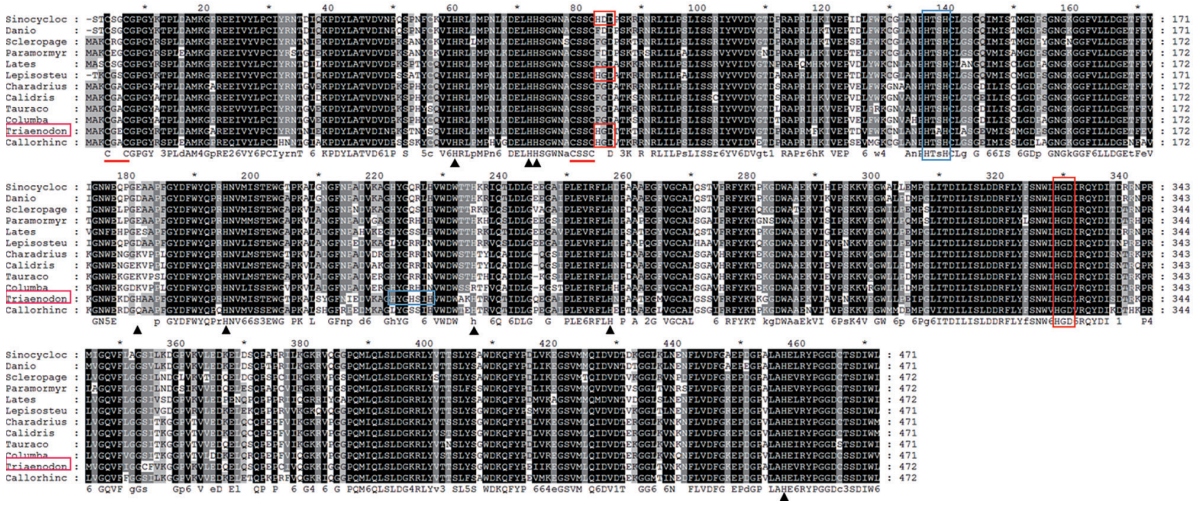
The α helices were shown in red, the β-pleated sheets were shown in yellow, the loops connecting with other structure were shown in gray. The Ser56 might be located at the gray solid balls.

**图5　使用SWISS-MODEL软件构建SBP1蛋白的3D模型**

**Fig.5　Construction of amino acid 3D models of SBP1 with SWISS-MODEL**

加硒组)。而加高浓度硒(100 μmol/L和150 μmol/L), 表达SBP1组的细胞存活率显著低于对照组(24.4% *vs* 82.5%, 13.5% *vs* 42.8%, $P<0.05$和$P<0.01$, $n=6$)。

根据上述结果, 分别选择8和100 μmol/L硒浓度来进一步验证白鲟鲨SBP1的抗癌功能。用亚硒酸钠处理的表达SBP1蛋白的HL-7702、HepG2和HeLa细

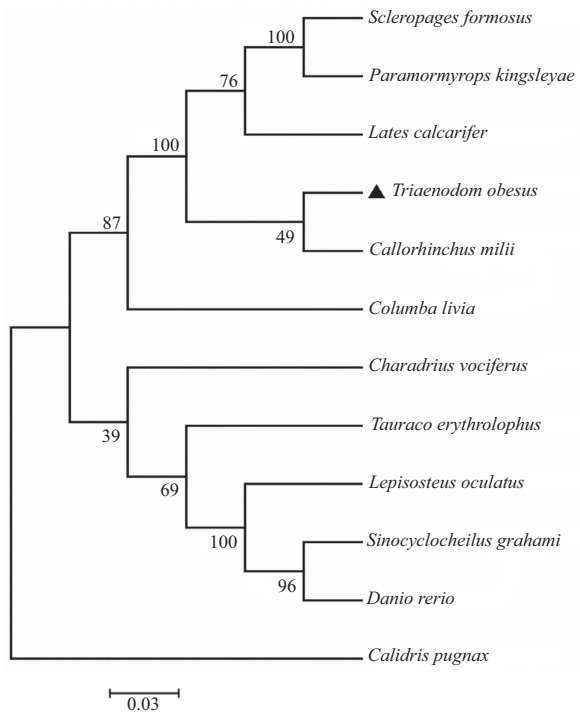


所有氨基酸序列均来自GenBank数据库。各序列登记号如下: 金钱鲃(XP_016132063.1)、斑马鱼(XP_005158264.1)、亚洲龙鱼(KPP73163.1)、喀麦隆壮象鼻鱼(XP_023657255.1)、尖吻鲈(XP_018534299.1)、斑点雀鳝(XP_006641819.2)、双领鸻(XP_009894086.1)、流苏鹬(XP_014816335.1)、红朱蕉鹃(XP_009976395.1)、原鸽(XP_021136064.1)、白鳍鲨(AEQ33586.1)、象鲨(XP_007883260.1)。多序列比对中黑色框区域为保守区。红色下划线区代表CxxC结构域, 红框和蓝框分别为推测的金属结合位点HxD和HxxH。白鳍鲨SBP1序列中其他组氨酸用黑色三角形(▲)标出。

All amino acid sequences were obtained from the GenBank database. The accession numbers were listed as follows: *Sinocyclocheilus grahami* (XP_016132063.1), *Danio rerio* (XP_005158264.1), *Scleropages formosus* (KPP73163.1), *Paramormyrops kingsleyae* (XP_023657255.1), *Lates calcarifer* (XP_018534299.1), *Lepisosteus oculatus* (XP_006641819.2), *Charadrius vociferous* (XP_009894086.1), *Calidris pugnax* (XP_014816335.1), *Tauraco erythrolophus* (XP_009976395.1), *Columba livia* (XP_021136064.1), *Triaenodon obesus* (AEQ33586.1), *Callorhinchus milii* (XP_007883260.1). Black shaded boxes represented the conserved amino acids of SBP1. The putative CxxC sequence motifs were underlined and the putative metal-binding sites HxD and HxxH were boxed in red and blue respectively. Additional H residues in the SBP1 sequence of *Triaenodon obesus* were marked with arrowheads (▲).

**图6 白鳍鲨SBP1蛋白序列与其他物种的多序列比对**

**Fig.6 Alignment of multiple sequences of whitetip reef shark (*Triaenodon obesus*) SBP1 protein and other species**

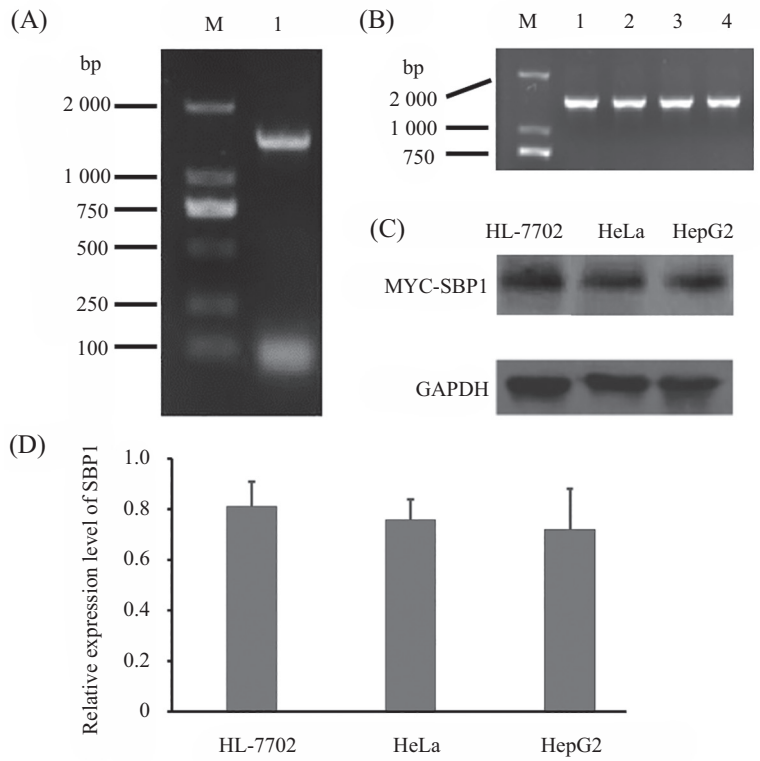



系统发育树中白鳍鲨所在位置用黑色三角形标出。

The location of *Triaenodon obesus* in the phylogenetic tree is marked with a arrowhead.

**图7 基于MEGA软件(7.0版本)构建白鳍鲨SBP1序列与其他物种SBP1的系统发育树**

**Fig.7 Phylogenetic analysis of whitetip reef shark (*Triaenodon obesus*) SBP1 with SBP1 of other species constructed by MEGA (version 7.0)**




A: 从cDNA模板扩增*SBP1*的ORF片段。泳道1: 使用特异性引物对MYC-F1/R1获得的PCR产物。泳道M: DNA标准品DL2000。B: 重组质粒MYC-SBP1的菌落PCR鉴定。泳道1~4: 插入片段的鉴定。泳道M: DNA标准品DL2000。C: Western blot检测重组蛋白MYC-SBP1在正常肝细胞系HL-7702、宫颈癌细胞系HeLa和肝癌细胞系HepG2中的表达。GAPDH作为内参。D: 定量分析MYC-SBP1重组蛋白在HL-7702、HeLa和HepG2细胞中的相对表达水平。数值从三次独立实验的平均值±标准偏差来表示。在三株细胞系之间没有观察到重组蛋白表达的显著性差异($P$>0.05)。

A: amplification of *SBP1* ORF fragment from cDNA template. Lane 1: PCR product using specific primers of MYC-F1 and MYC-R1. Lane M: DNA marker DL2000. B: colony PCR identification of recombinant plasmid MYC-SBP1. Lane 1-4: identification of inserted fragments. Lane M: DNA marker DL2000. C: Western blot identification of recombinant MYC-SBP1 protein expressed in HL-7702, Hela, and HepG2 cells. GAPDH serves as a loading control. D: quantitative analysis of relative MYC-SBP1 protein expression in HL-7702, HeLa, and HepG2 cells. Values are presented as $\bar{x}\pm s$ from three independent experiments. No significant differences were observed among the three cell lines ($P$>0.05).

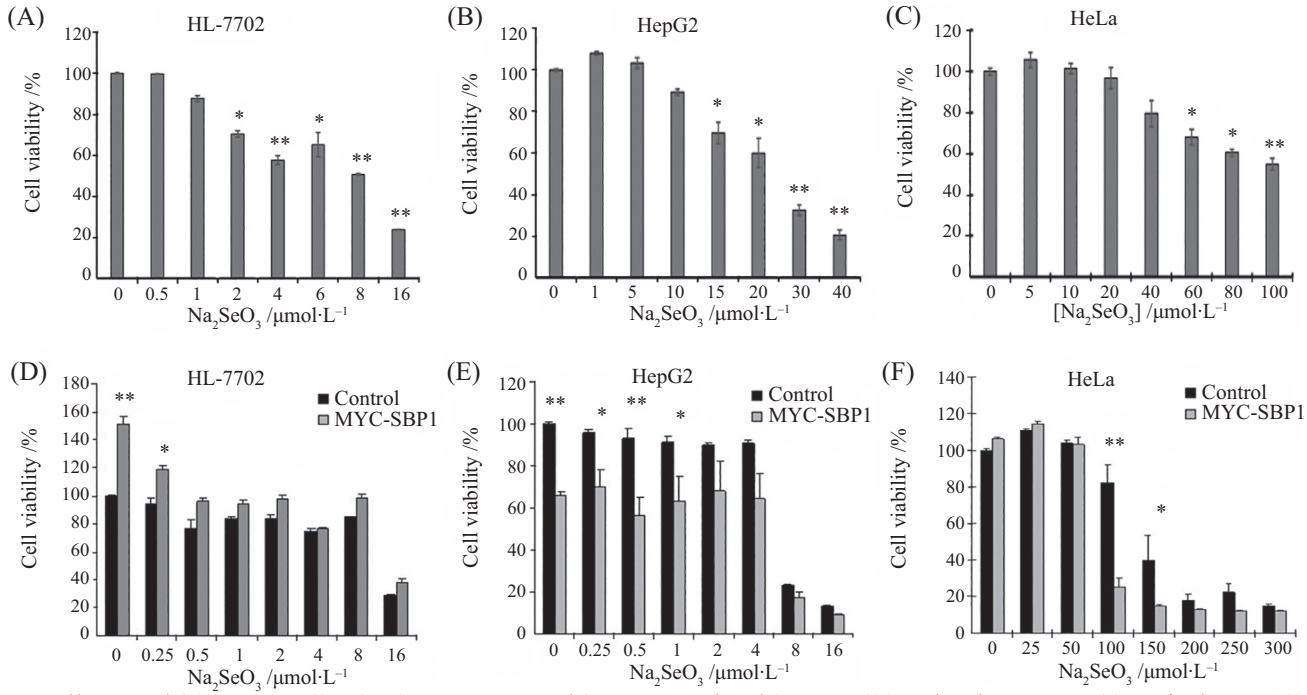
**图8 重组质粒MYC-SBP1的构建及SBP1蛋白在HL-7702、HeLa和HepG2细胞中的表达**

**Fig.8 Construction of recombinant plasmid MYC-SBP1 and analysis of SBP1 expression in HL-7702, HeLa and HepG2 cells**

胞, 通过CFDA SE细胞增殖实验分析细胞的增殖情况(表3)。相比于对照组, 在正常HL-7702细胞中表达SBP1组在0和8 μmol/L硒浓度下细胞增殖指数分别增加了0.66和1.37(7.41 *vs* 8.07, 3.82 *vs* 5.19, $P$<0.05), 而肝癌细胞HepG2中表达SBP1组在0和8 μmol/L硒浓度下细胞增殖指数分别下降了0.37和0.17(4.48 *vs* 4.11, 3.43 *vs* 3.26)。宫颈癌细胞HeLa在100 μmol/L高硒浓度下也使细胞增殖指数下降(4.00 *vs* 3.34, 5.55 *vs* 4.26, $P$<0.05)。观察各细胞分裂代数情况, 表达SBP1组在正常HL-7702细胞中促进细胞增殖, 有20.0%细胞已进入第六代分裂(0 μmol/L硒), 而对照组只有3.84%细胞进入第六代分裂。当添加8 μmol/L 硒时, 表达SBP1组有17.5%细胞进入第五代, 而对照组为5.77%; 大多数对照组细胞停留在第二代(84.9% *vs* 48.6%)。表达SBP1组在癌细胞中抑制细胞增殖, 只有11.3%的HeLa细胞进入第四代(0 μmol/L硒), 而对照组有27.2%。在100 μmol/L高硒浓度下, 表达SBP1组只有12.1%的HeLa细胞进入第四代, 而对照组有40.5%。这些结果与CCK-8结果一致。

## 3 讨论

鲨鱼是现存最古老的有颌脊椎动物之一, 其基因组是所有脊椎动物中进化最慢的基因组[16-17]。SBP1在脊椎动物进化过程中高度保守[18]。本研究成功克隆并测定了白鲟鲨*SBP1*的mRNA序列。白鲟鲨SBP1一级结构序列与其他鱼类(金线鲃、斑点雀鳝和斑马鱼等)高度相似。曾报道斑马鱼SBP1序列与哺乳动物人、鼠、牛等SBP1序列高度相似。斑马




CCK-8检测硒和过表达SBP1处理的正常肝细胞HL-7702、肝癌细胞HepG2和宫颈癌细胞HeLa的细胞存活率。A~C: 三种细胞系只加硒处理的细胞存活率, 以未加硒处理的细胞存活率为参比(100%); D~F: 三种细胞系只过表达SBP1以及既表达SBP1又加硒处理的细胞存活率。对照组和MYC-SBP1组是指分别转染了空载质粒pcDNA3.1-MYC和重组质粒MYC-SBP1的细胞。以未加硒处理的对照组细胞存活率为参比(100%)。数值由三次重复实验的平均值±标准偏差来表示。*$P$<0.05, **$P$<0.01, 与对照组比较。

CCK-8 analysis of the cell viability in Se-treated and SBP1-overexpressing normal hepatocytes HL-7702, hepatoma cells HepG2 and cervical cancer cells HeLa. A-C: the cell viability of the three cell lines treated with only selenium. The cell viability of Se-untreated group was set as 100%; D-F: the cell viability of the three cell lines treated with only SBP1-overexpressing and both expressing SBP1 and adding selenium. Control *vs* MYC-SBP1 groups were the cells which were transfected with empty pcDNA3.1-MYC plasmid and recombinant plasmid MYC-SBP1, respectively. The cell viability of Se-untreated control group was set as 100% and the other groups compared to this criterion. All data are shown as the $\bar{x}\pm s$. *$P$<0.05, **$P$<0.01 *vs* control group. The experiment was repeated three times.

**图9 硒应激条件下表达重组蛋白SBP1对细胞存活率的影响**

**Fig.9 Influence of recombinant SBP1 expression to cell viability under Se stress**

鱼SBP1的表达与年龄相关, 是一种潜在的生物标志物[19]。SBP1序列中保守的CSSC基序(图6)是蛋白参与氧化还原调控的一个重要特征。含有CSSC基序的蛋白质对硒具有很强的亲和力。CSSC序列附近保守的组氨酸残基与金属结合结构域HxD和HxxH上保守的组氨酸残基都可能指向硒潜在的结合位点[3]。白鳍鲨SBP1序列上特有的连续HxxH区域(His222、His225和His228)可能有其独特的功能。系统进化树分析表明, 白鳍鲨与象鲨的SBP1同源性最高, 均具有编码472个氨基酸的开放阅读框。象鲨基因组保守序列与人基因组保守序列的相似度高于硬骨鱼与人的相似度[20]。曾推测人类SBP1(hSP56)蛋白序列上第57位的半胱氨酸(Cys57)能与硒共价结合并且对蛋白功能具有重要作用[21]。对白鳍鲨SBP1的氨基酸序列分析表明, 其Ser56-Gln57-Val58-Ile59-His60序列对应于人类SBP1的Cys57-Gln58-Val59-Ile60-His61序列, 同源物种均具有Ser-Gln-Val-Ile-His/Cys-Gln-Val-Ile-His保守序列。Ser与Cys化学结构相似, 对白鳍鲨SBP1蛋白的三维结构分析, 推测定位于β折叠与连接各结构环交界处的Ser56为硒结合位点。

微量元素硒对人类健康至关重要。膳食中添加硒(250~300 μg/天)具有抗癌效果[22]。硒是调节人体肝细胞氧化还原状态的重要组分[23]。硒化合物在体内作为化学预防剂能有效调节细胞周期和刺激细胞凋亡, 并能抑制体外肿瘤细胞的迁移和侵袭[24]。单独加硒处理各株细胞的实验表明, 亚硒酸钠呈剂量依赖性造成肝癌细胞HepG2和宫颈癌细胞HeLa的存活率下降。这与前人用MTT法检测的结果相似, 亚硒酸钠诱导的HepG2和HeLa癌细胞凋亡是由活性氧(reactive oxygen species, ROS)介导的线粒体途径诱导[25-27]。常规培养的细胞含有血清, 已补充微量元

表3 表达SBP1实验组和对照组细胞在硒应激下的增殖指数

Table 3 Cell proliferation index of SBP1 expression group and control group under Se stress

| 类别 Types | 细胞系 Cell line | HL-7702 | | | | HepG2 | | | | HeLa | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 μmol/L | | 8 μmol/L | | 0 μmol/L | | 8 μmol/L | | 0 μmol/L | | 100 μmol/L | |
| | | Ctrl | Exp | Ctrl | Exp | Ctrl | Exp | Ctrl | Exp | Ctrl | Exp | Ctrl | Exp |
| Parent | | 1.43% | 1.69% | 4.10% | 1.16% | 0.98% | 0.67% | 0 | 0.87% | 1.08% | 2.66% | 0.25% | 0.62% |
| Generation 2 | | 0.72% | 0 | 0 | 2.40% | 8.11% | 12.10% | 17.00% | 20.90% | 10.90% | 17.40% | 2.22% | 0 |
| Generation 3 | | 14.60% | 19.90% | 84.90% | 48.60% | 47.40% | 53.60% | 82.40% | 76.60% | 60.20% | 68.40% | 43.30% | 85.20% |
| Generation 4 | | 30.60% | 42.70% | 3.63% | 28.90% | 43.30% | 33.50% | 0 | 1.19% | 27.20% | 11.30% | 40.50% | 12.10% |
| Generation 5 | | 47.90% | 13.80% | 5.77% | 17.50% | 0 | 0 | 0 | 0 | 0.18% | 0 | 11.65% | 0.32% |
| Generation 6 | | 3.84% | 20.00% | 1.22% | 0.95% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proliferation index | | 7.41 | 8.07 | 3.82 | 5.19 | 4.48 | 4.11 | 3.43 | 3.26 | 4.00 | 3.34 | 5.55 | 4.26 |

“0 μmol/L”表示未在培养基中添加亚硒酸钠, “8 μmol/L”表示在培养基中添加8 μmol/L的亚硒酸钠。*n*=3。

“0 μmol/L” means sodium selenite is not added to the medium, “8 μmol/L” means 8 μmol/L of sodium selenite is added to the medium. *n*=3.

素, 额外加硒对正常肝细胞HL-7702的存活率影响不大, 反而过量补充(≥2 μmol/L)会造成细胞毒性。SBP1作为一个肿瘤抑制因子, 其表达能抑制癌症细胞的血管生成, 影响肿瘤微环境[28]。在各株细胞过表达SBP1的实验表明, 白鲟鲨SBP1的表达造成人肝癌细胞HepG2的存活率下降, 而明显提高正常人肝细胞HL-7702的存活率。SBP1的过表达能减少癌细胞的耗氧量并增加AMP活化的蛋白激酶活性, 进而减弱癌细胞的生长与迁移[29]。曾报道HBV病毒对SBP1表达的抑制可能是HBV感染引起的肝癌发生的原因之一[30]。而健康细胞中SBP1通过产生$H_2O_2$、$H_2S$和对AMP活化的蛋白激酶的激活来调节细胞内的氧化磷酸化。表达SBP1及加硒同时进行的实验表明, 细胞存活率与硒剂量无明显关联。在癌细胞中, 表达SBP1组的细胞存活率会比对照组的细胞存活率低。单独亚硒酸钠诱导的HepG2和HeLa癌细胞凋亡所需的浓度IC50(分别约20 μmol/L和100 μmol/L)均比表达SBP1及加硒同时处理的浓度要高(<8 μmol/L和<100 μmol/L), 说明是SBP1在发挥抗癌的作用, 也可能是表达的SBP1与游离的硒结合在发挥抗癌作用。宫颈癌细胞HeLa比肝癌细胞HepG2和正常肝细胞HL-7702具有更高的硒耐受性。CCK-8实验发现在100 μmol/L高硒浓度下大部分HeLa细胞已凋亡, 而CFDA SE分析显示在该浓度下HeLa细胞的增殖指数却比未加硒组高。通过显微镜观察癌细胞形态, 发现表达SBP1组癌细胞在100 μmol/L高硒浓度下已变圆且不少已漂浮凋亡。因此, 我们推测过度增殖可能是硒胁迫下HeLa细胞的特征。本研究发现HeLa细胞能耐受高浓度硒(100 μmol/L), 并且表达的白鲟鲨SBP1蛋白加剧了癌细胞的凋亡。

## 4 结论

本文首次对白鲟鲨*SBP1*的cDNA全长进行了克隆分析, 初步研究了白鲟鲨SBP1蛋白的结构和抗癌活性。白鲟鲨SBP1蛋白与其他物种SBP1蛋白一级结构的相似性表明SBP1蛋白在生物进化上是保守的。SBP1在正常细胞和癌细胞的生长与增殖过程中起着相反的作用, 这为进一步深入研究SBP1的功能及其参与的肿瘤抑制调控机制奠定了基础。我们推测硒的化学预防作用与SBP1的功能高度相关。在某种程度上, 可能是SBP1蛋白(而不是硒)在预防癌症中起着更关键的作用。

## 参考文献 (References)


[1] BANSAL M P, MUKHOPADHYAY T, SCOTT J, et al. DNA sequencing of a mouse liver protein that binds selenium: implications for selenium’s mechanism of action in cancer prevention [J]. Carcinogenesis, 1990, 11(11): 2071-3.

[2] CHANG P W, TSUI S K, LIEW C, et al. Isolation, characterization, and chromosomal mapping of a novel cDNA clone encoding human selenium binding protein [J]. J Cell Biochem, 1997, 64(2): 217-24.

[3] FLEMETAKIS E, AGALOU A, KAVROULAKIS N, et al. Lotus japonicus gene Ljsbp is highly conserved among plants and animals and encodes a homologue to the mammalian selenium-binding proteins [J]. Mol Plant Microbe Interact, 2002, 15(4): 313-22.

[4] ELHODAKY M, DIAMOND A M. Selenium-binding protein 1 in human health and disease [J]. Int J Mol Sci, 2018, 19(11): 3437.

[5] JEONG J Y, WANG Y, SYTKOWSKI A J. Human selenium binding protein-1 (hSP56) interacts with VDU1 in a selenium-dependent manner [J]. Biochem Biophys Res Commun, 2009, 379(2): 583-8.

[6] JEONG J Y, ZHOU J R, GAO C, et al. Human selenium binding protein-1 (hSP56) is a negative regulator of HIF-1alpha and suppresses the malignant characteristics of prostate cancer cells [J]. BMB Rep, 2014, 47(7): 411-6.

[7] PAPP L V, LU J, HOLMGREN A, et al. From selenium to selenoproteins: synthesis, identity, and their role in human health [J]. Antioxid Redox Signal, 2007, 9(7): 775-806.

[8] CHEN F, CHEN C, QU Y, et al. Selenium-binding protein 1 in head and neck cancer is low-expression and associates with the prognosis of nasopharyngeal carcinoma [J]. Medicine, 2016, 95(35): e4592.

[9] ZENG H, ZHAO X, TANG C. Downregulation of SELENBP1 enhances oral squamous cell carcinoma chemoresistance through KEAP1-NRF2 signaling [J]. Cancer Chemother Pharmacol, 2021, 88(2): 223-33.

[10] ZHAO C, ZENG H, WU R T, et al. Loss of selenium-binding protein 1 decreases sensitivity to clastogens and intracellular selenium content in HeLa cells [J]. PLoS One, 2016, 11(7): e0158650.

[11] HUANG C, DING G, GU C, et al. Decreased selenium-binding protein 1 enhances glutathione peroxidase 1 activity and downregulates HIF-1alpha to promote hepatocellular carcinoma invasiveness [J]. Clin Cancer Res, 2012, 18(11): 3042-53.

[12] GONG J, LI L. Sodium selenite inhibits proliferation of gastric cancer cells by inducing SBP1 expression [J]. Tohoku J Exp Med, 2016, 239(4): 279-85.

[13] LOBANOV A V, FOMENKO D E, ZHANG Y, et al. Evolutionary dynamics of eukaryotic selenoproteomes: large selenoproteomes may associate with aquatic life and small with terrestrial life [J]. Genome Biol, 2007, 8(9): R198.

[14] SARANGI G K, ROMAGNE F, CASTELLANO S. Distinct patterns of selection in selenium-dependent genes between land and aquatic vertebrates [J]. Mol Biol Evol, 2018, 35(7): 1744-56

[15] JOHRI S, CHAPPLE T K, SCHALLERT R, et al. Complete mitochondrial genome of the whitetip reef shark Triaenodon obesus from the british indian ocean territory marine protected area [J]. Mitochondrial DNA B Resour, 2020, 5(3): 2347-9.

[16] TAN Y Y, KODZIUS R, TAY B H, et al. Sequencing and analysis of full-length cDNAs, 5'-ESTs and 3'-ESTs from a cartilaginous fish, the elephant shark (Callorhinchus milii) [J]. PLoS One, 2012, 7(10): e47174.

[17] VENKATESH B, LEE A P, RAVI V, et al. Elephant shark genome provides unique insights into gnathostome evolution [J]. Nature, 2014, 505(7482): 174-9.

[18] WU C L, ZHANG W B, MAI K S, et al. Molecular cloning, characterization and mRNA expression of selenium-binding protein in abalone (Haliotis discus hannai Ino): response to dietary selenium, iron and zinc [J]. Fish Shellfish Immunol, 2010, 29(1): 117-25.

[19] FENG S, WANG S, WANG Y, et al. Identification and expression of carbonic anhydrase 2, myosin regulatory light chain 2 and selenium-binding protein 1 in zebrafish Danio rerio: implication for age-related biomarkers [J]. Gene Expr Patterns, 2018, 29: 47-58.

[20] VENKATESH B, KIRKNESS E F, LOH Y H, et al, Survey sequencing and comparative analysis of the elephant shark (Callorhinchus milii) genome [J]. PLoS Biol, 2007, 5(4): e101.

[21] RAUCCI R, COLONNA G, GUERRIERO E, et al, Structural and functional studies of the human selenium binding protein-1 and its involvement in hepatocellular carcinoma [J]. Biochim Biophys Acta, 2011, 1814(4): 513-22.

[22] RADOMSKA D, CZARNOMYSY R, RADOMSKI D, et al. Selenium as a bioactive micronutrient in the human diet and its cancer chemopreventive activity [J]. Nutrients, 2021, 13(5): 1649.

[23] LI D, WU K, HOWIE A F, et al. Synergy between broccoli sprout extract and selenium in the upregulation of thioredoxin reductase in human hepatocytes [J]. Food Chem, 2008, 110(1): 193-8.

[24] ZENG H, COMBS G F Jr. Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion [J]. J Nutr Biochem, 2008, 19(1): 1-7.

[25] ZOU Y, NIU P, YANG J, et al. The JNK signaling pathway is involved in sodium-selenite-induced apoptosis mediated by reactive oxygen in HepG2 cells [J]. Cancer Biol Ther, 2008, 7(5): 689-96.

[26] FU L, LIU Q, SHEN L, et al. Proteomic study on sodium selenite-induced apoptosis of human cervical cancer HeLa cells [J]. J Trace Elem Med Biol, 2011, 25(3): 130-7.

[27] JABLONSKA E, LI Q, RESZKA E, et al. Therapeutic potential of selenium and selenium compounds in cervical cancer [J]. Cancer Control, 2021, doi: 10.1177/10732748211001808.

[28] SCHOTT M, DE JEL M M, ENGELMANN J C, et al. Selenium-binding protein 1 is down-regulated in malignant melanoma [J]. Oncotarget, 2018, 9(12): 10445-56.

[29] ELHODAKY M, HONG L K, KADKOL S, et al. Selenium-binding protein 1 alters energy metabolism in prostate cancer cells [J]. Prostate, 2020, 80(12): 962-76.

[30] LEE Y M, KIM S, PARK R Y, et al. Hepatitis B virus-X downregulates expression of selenium binding protein 1 [J]. Viruses, 2020, 12(5): 565.