# 抗癌生物活性肽对与肿瘤相关成纤维细胞共培养的食管癌细胞的增殖影响

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**摘要** 该研究旨在探讨肿瘤相关成纤维细胞(CAFs)对食管鳞状细胞癌(ESCC)细胞增殖的影响及抗癌生物活性肽(ACBP)对增殖的干预作用和可能的作用机制。收集5例手术切除患者的食管癌组织, 分离培养得到ESCC CAFs并进行特征指标鉴定; 建立CAFs与ESCC细胞共培养体系, CFSE染色和流式细胞术检测细胞增殖; 收集CAF-1的条件培养基, 加入ACBP进行联合培养KYSE140细胞, IncuCyte检测细胞增殖; qRT-PCR和Western blot检测Hedgehog信号通路相关基因的表达水平。该研究成功分离CAFs, Western blot结果显示CAFs均表达波形蛋白(Vimentin)及纤维连接蛋白(Fibronectin), 不表达E-钙黏蛋白(E-cadherin); 相比于单独培养, 与CAF-1共培养的KYSE140细胞的CFSE水平降低, 细胞融合率增加($P$<0.05), 细胞中的GLI1和PTCH1的mRNA和蛋白水平升高($P$<0.05); 相比于条件培养基组, ACBP加入可以使KYSE140细胞的融合率下降($P$<0.05); 相较于单独培养, ACBP加入后KYSE140细胞的GLI1和PTCH1的mRNA和蛋白水平显著下降($P$<0.05)。该研究表明, CAFs可以通过激活Hedgehog信号通路促进食管癌细胞增殖, ACBP可在与CAFs共培养条件下通过抑制Hedgehog信号通路抑制食管癌细胞的生长。

**关键词** 肿瘤相关成纤维细胞; 食管鳞状细胞癌细胞; 共培养; 抗癌生物活性肽; Hedgehog; 增殖

# Effect of Anticancer Bioactive Peptides on the Proliferation of Esophageal Cancer Cells Co-Cultured with Cancer-Associated Fibroblasts

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**Abstract** This work was to investigate the effect of CAFs (cancer-associated fibroblasts) on the proliferation of ESCC (esophageal squamous cell carcinoma) cells and the intervention effect of proliferation and possible mechanism of ACBP (anti-cancer biological active peptide). Five cases of esophageal cancer tissues were collected, from which ESCC CAFs were isolated and cultured, and then the characteristic indexes were identified; the co-culture system of CAFs and ESCC cells was established. CFSE staining and flow cytometry were used to detect cell prolifera-

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tion; the conditioned medium of CAF-1 was collected, and ACBP was added to co-cultured KYSE140 cells; cell proliferation was detected by IncuCyte, and the expression levels of Hedgehog signaling pathway-related genes were detected by qRT-PCR and Western blot. CAFs were successful separated. The results of Western blot showed that CAFs expressed Vimentin and Fibronectin without E-cadherin. Compared with the control group, the CFSE level of KYSE140 cells decreased and the fusion rate increased ($P$<0.05), and the mRNA and protein levels of GLI1 and PTCH1 were increased ($P$<0.05) after co-cultured with CAF-1; compared with the conditional medium group, the fusion rate of KYSE140 cells decreased ($P$<0.05) after ACBP was added; compared with the control group, the mRNA and protein levels of GLI1 and PTCH1 of KYSE140 cells decreased after ACBP was added ($P$<0.05). This study suggests that CAFs can promote the proliferation of esophageal cancer cells by activating the Hedgehog signaling pathway, and ACBP can inhibit the growth of esophageal cancer cells by inhibiting the Hedgehog signaling pathway under the co-culture with CAFs.

**Keywords** CAFs; ESCC cells; co-culture; ACBP; Hedgehoog; proliferation

食管癌是常见的消化道恶性肿瘤, 目前占全球恶性肿瘤死亡率第6位[1]。食管癌在组织学上主要分为食管鳞状细胞癌(esophageal squamous cell cancer, ESCC)和食管腺癌(esophageal adenocarcinoma, EAC)。食管癌目前的治疗措施以外科手术为主, 结合化疗及放射治疗。尽管靶向治疗和免疫治疗等新的治疗方法已取得长足进步, 但食管癌患者的5年生存率依旧徘徊在20%左右[2], 亟待寻找新的更有效的治疗方法改善治疗效果。肿瘤微环境(tumor microenvironment, TME)是癌细胞启动、进展和迁移的利基, 是肿瘤发生、免疫逃逸的关键[3-5]。其中肿瘤相关成纤维细胞(cancer-associated fibroblasts, CAFs)是TME的关键成分, 支持癌细胞的增殖和迁移, 同时也保护癌细胞免受抗癌药物的毒性, 其参与包括食管癌在内的癌症的发生、血管生成、增殖、侵袭、转移和复发等生理过程[6-11]。

1980年在果蝇中发现的Hedgehog信号通路, 其是维持组织极性和干细胞数量的关键。Hedgehog信号通路的异常激活存在于各种类型的癌症中[12]。我们前期研究表明, 在癌前病变和食管癌中Hedgehog信号通路的表达被激活[13-14]。抗癌生物活性肽(anticancer biological active peptide, ACBP)是本实验室拥有自主知识产权的一种多肽, 前期研究显示, ACBP在体外和体内对胃癌和结直肠癌等肿瘤细胞具有显著的抑制作用[15-16]。本研究在建立ESCC细胞与原代培养CAFs共培养体系的基础上, 研究ACBP在CAFs存在的条件下是否影响食管癌细胞的增殖以及是否对Hedgehog信号通路产生影响。

## 1 材料与方法

### 1.1 细胞与主要试剂

人食管鳞状细胞癌细胞系KYSE140由内蒙古医科大学附属医院临床医学研究中心实验室保存。

PBS、DMEM/F12培养基、胎牛血清(fetal bovine serum, FBS)购自Gibco公司; Maxima Probe qPCR Master Mix购自Thermo Scientific公司; CFSE Fluorescent Cell Labeling Kit(货号ab113853)、PTCH1(货号ab53715)、山羊抗兔IgG(货号ab175781)购自Abcam公司; TRIzol试剂购自Invitrogen公司; ImPro-II™逆转录系统(货号A3800)购自Promega公司; 兔多克隆抗体E-cadherin(货号20874-1-AP)、Vimentin(货号10366-1-AP)、Fibronectin(货号15613-1-AP)、GAPDH(货号10494-1-AP)购自Proteintech公司; GLI1(货号2534)购自Cell Signaling公司; 微量总蛋白提取试剂盒购自Invent Biotechnologies公司; BCA蛋白质分析试剂盒购自北京索拉比奥科技有限公司。

### 1.2 方法

1.2.1 肿瘤相关成纤维细胞(CAFs)的分离与培养 本研究方案经内蒙古医科大学伦理委员会审核通过(编号: 2013026), 收集内蒙古医科大学附属医院手术患者的食管癌组织(所有患者均签订知情同意书)。将组织置于含有1%青霉素/链霉素双抗PBS中冲洗3次并用剪刀将其切碎成直径1 mm的组织块, 然后将其放入100 mm培养皿中并干燥以使其附着于培养皿上, 加入6 mL含有20% FBS的DMEM/F12培养基, 于37 °C、5% $CO_2$及饱和湿度的培养箱中培

表1 qRT-PCR引物与探针序列

Table 1 The primers and probes for qRT-PCR

| 基因 Gene | 序列(5′→3′) Sequence (5′→3′) |
|---|---|
| *PTCH1* forward primer | CGG CAG CCGCGATAAG |
| *PTCH1* reverse primer | TTA ATG ATG CCA TCT GCA TCC A |
| *PTCH1* probe | FAM-AGC CAG TTG ACT AAA CAG-BHQ1 |
| *GLI1* forward primer | GTT CAC ATG CGC AGA CAC ACT |
| *GLI1* reverse primer | TTC GAG GCG TGA GTA TGA CTT C |
| *GLI1* probe | FAM-CAC ACA AGT GCA CGT TT-BHQ1 |
| *GAPDH* forward primer | GAA GGT GAA GGT CGG AGT |
| *GAPDH* reverse primer | GAA GAT GGT GAT GGG ATT TC |
| *GAPDH* probe | FAM-CAA GCT TCC CGT TCT CAG CC-BHQ1 |

养。成纤维细胞在4或5天后从组织中生长出来。收集CAFs以备后续实验。实验中所用CAFs均在第6代以内。

常规培养CAFs, 当细胞汇合度达到80%, 弃去培养基, PBS清洗后加入无血清DMEM/F12培养基, 培养48 h后收集细胞培养液, 1 000 r/min离心5 min, 收集上清液作为CAFs条件培养基, 保存于–80 °C备用。

1.2.2 细胞培养 将食管癌细胞KYSE140于含有10% FBS的RPMI 1640培养基, 37°C、5% $CO_2$及饱和湿度的培养箱中培养。按CFSE Fluorescent Cell Labeling Kit厂家说明书将KYSE140用CFSE染色后, 再将其与CAFs通过Transwell共培养, 35 μg/mL ACBP处理48 h, 收集细胞进行流式细胞术检测。

1.2.3 ACBP对共培养条件下食管癌细胞KYSE140增殖的影响 将KYSE140细胞以5×$10^3$个/孔接种于96孔板中, 常规培养24 h后加入CAFs条件培养基, 设ACBP处理组、ACBP联合CAFs条件培养基处理组、CAFs条件培养基组、空白对照组, 每组至少设3个重复孔, 培养72 h, 使用IncuCyte® S3活细胞分析系统监测和分析细胞生长。实验重复3次。

1.2.4 荧光实时定量PCR(qRT-PCR) 用TRIzol试剂和ImPro-II™逆转录酶制备总RNA和cDNA第一链。用于检测*PTCH1*、*GLI1*和*GAPDH*的引物和探针序列见表1。用$2^{-\Delta\Delta Ct}$法分析目的基因表达水平, 以*GAPDH*为内参。

1.2.5 Western blot 使用动物培养细胞和组织的微量总蛋白提取试剂盒获取细胞总蛋白。使用BCA蛋白质分析试剂盒对蛋白质进行定量。总蛋白(30 μg)在100 °C下加热5 min, 进行SDS-PAGE电泳, 转膜至PVDF膜上。在5%牛奶(*w/V*)的TBS-T中室温封闭2 h, 分别与5%牛奶TBS-T中的一抗(稀释比例均为1:500) E-cadherin、Vimentin、Fibronectin、GLI1和PTCH1在4 °C孵育过夜。以Alexa Fluor 790标记的山羊抗兔IgG为二抗(稀释比例为1:10 000)。使用红外成像系统(奥德赛LI-COR)采集荧光信号。以GAPDH为内参, 结果使用ImageJ软件进行分析。

1.2.6 数据分析 所有实验数据显示为3个独立实验的均数±标准差($\bar{x}\pm s$)。所有统计分析均采用SPSS 13.0统计软件。*t*检验用于两组之间的比较。采用单因素方差分析和Tukey检验进行多重比较, 以确定多组的统计显著性。$P<0.05$具有统计学意义。

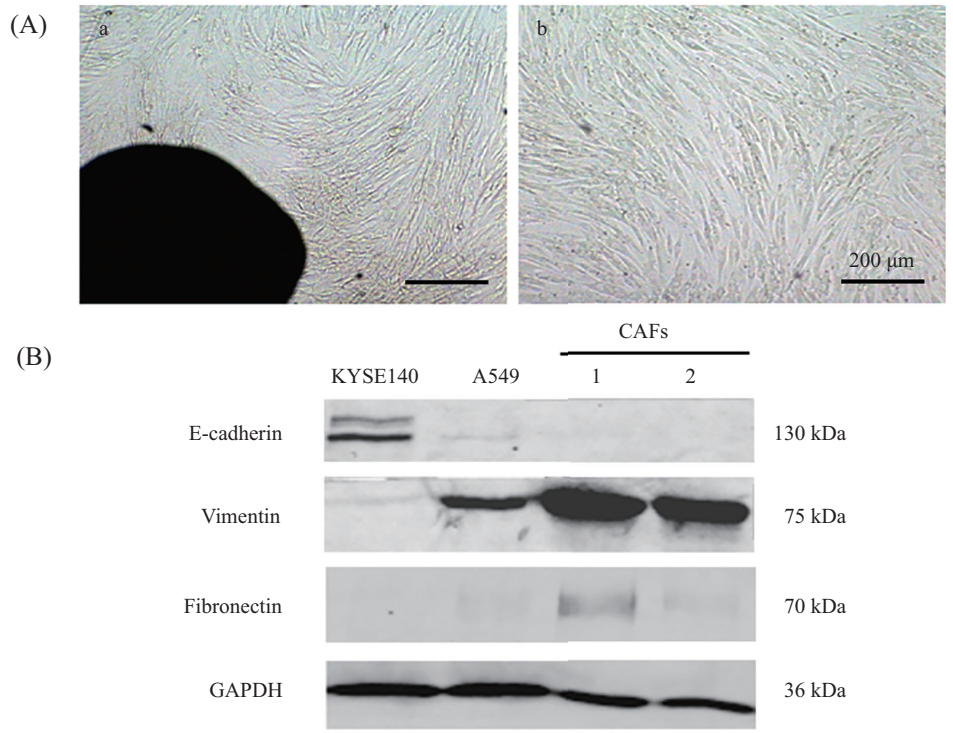
## 2 结果

### 2.1 肿瘤相关成纤维细胞的培养与鉴定

ESCC组织培养4~5天后, 显微镜下显示贴壁的ESCC组织块周围有细胞长出。从5个ESCC标本中分离培养了5个ESCC CAFs(图1A, 结果展示2个CAFs), 传代后细胞形态均呈长梭形成纤维样细胞。在分离的CAFs中通过Western blot检测上皮细胞标志物E-钙黏蛋白(E-cadherin)以及间质细胞标志物波形蛋白(Vimentin)和纤维连接蛋白(Fibronectin)的表达。5个CAFs均显著表达Vimentin, 表达Fibronectin, 不表达E-cadherin(图1B), 说明我们成功分离ESCC CAFs, 并且其中未混有上皮细胞。

### 2.2 ESCC CAFs对共培养条件下ESCC细胞增殖的影响

为了探讨ESCC CAFs是否能促进ESCC细胞KYSE140的增殖, 我们用CFSE染色KYSE140细胞, 单独培养或通过Transwell与1号CAF(CAF-1)共培养,

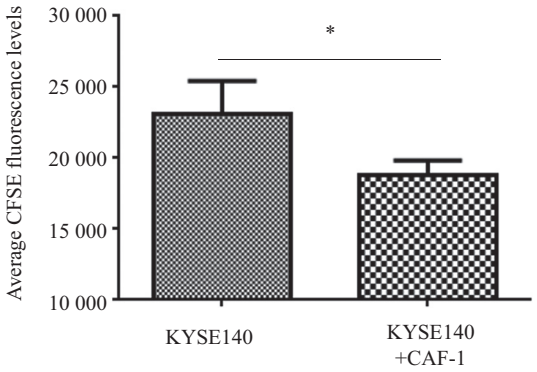



A: 分离培养的ESCC CAFs, 细胞从组织块中爬出(a), 消化后的传代细胞(b); B: Western blot鉴定CAFs标记蛋白的表达; KYSE140和A549为对照细胞。

A: ESCC CAFs were isolated and cultured; cells grow out from tissue blocks (a), passage cells after digestion (b); B: the expression of CAFs protein measured by Western blot; KYSE140 and A549 were control cells.

**图1 ESCC CAFs的生长特性与特异标记鉴定**

**Fig.1 The growth characteristics and special molecular marker identification of ESCC CAFs**




*$P$<0.05.

**图2 CAF-1与KYSE140细胞共培养后CFSE的平均荧光水平**

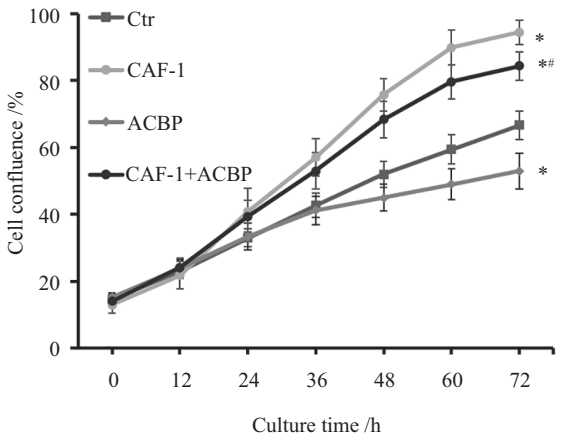
**Fig.2 The average fluorescence level of CFSE in KYSE140 after co-cultured with CAF-1**

然后采用流式细胞仪分析荧光强度。我们发现, 与CAF-1共培养的KYSE140细胞的CFSE荧光强度较低, 表明与CAF-1共培养的KYSE140细胞比单独培养的条件下有更多的增殖细胞(图2)。

将CAF-1收集的条件培养基与KYSE140细胞共培养。结果显示, 培养72 h, 在CAF-1条件培养基中培养的KYSE140细胞的融合率高于对照组(图3)。这说明与ESCC CAF-1联合培养KYSE140具有更高的增殖活性, ESCC CAF-1可以促进ESCC细胞的增殖。

### 2.3 ESCC CAFs对共培养条件下KYSE140细胞Hedgehog信号通路的影响

为了明确CAF-1是否通过激活Hedgehog信号通路促进KYSE140细胞的增殖, 我们使用Transwell将KYSE140细胞与CAF-1共培养, 与单独

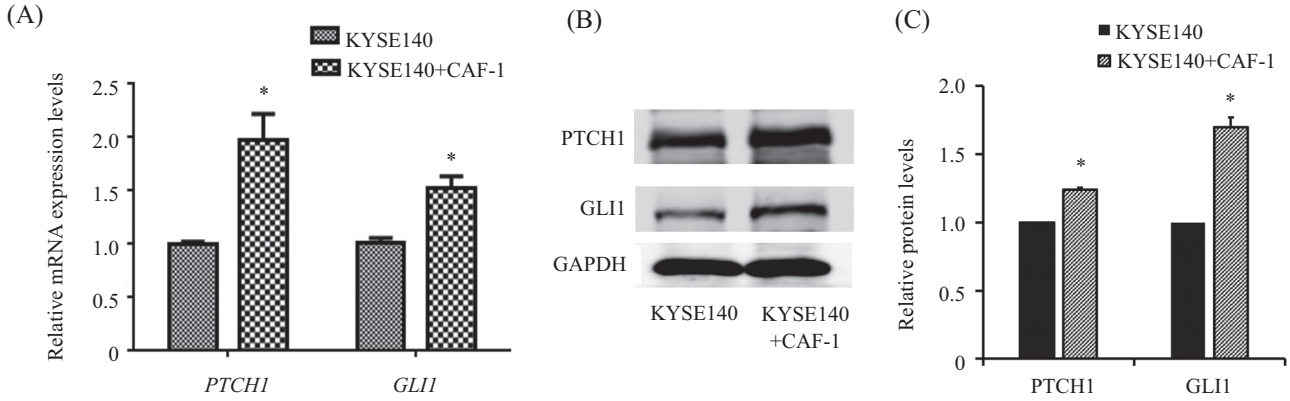



Ctr: 对照组; CAF-1: CAF-1条件培养基处理组; ACBP: ACBP处理组; CAF-1+ACBP: CAF-1条件培养基联合ACBP处理组; *$P$<0.05, 72 h时与对照组相比; #$P$<0.05, 72 h时与CAF-1组相比。

Ctr: the control group; CAF-1: CAF-1 conditional medium treatment group; ACBP: ACBP treatment group; CAF-1+ACBP: CAF-1 conditional medium combined with ACBP treatment group; *$P$<0.05 *vs* control group at 72 h; #$P$<0.05 *vs* CAF-1 group at 72 h.

**图3 CAF-1与ACBP对KYSE140细胞的增殖的影响**

**Fig.3 The effects of CAF-1 and ACBP on the proliferation of KYSE140 cells**




A: qRT-PCR检测KYSE140与CAF-1共培养后细胞中*PTCH1*和*GLI1* mRNA表达水平; B、C: Western blot检测KYSE140与CAF-1共培养后细胞中PTCH1和GLI1蛋白表达水平。*$P$<0.05, 与KYSE140相比。

A: the mRNA levels of *PTCH1* and *GLI1* detected by qRT-PCR in KYSE140 cells after co-cultured with CAF-1; B,C: the protein levels of PTCH1 and GLI1 detected by Western blot in KYSE140 cells after co-cultured with CAF-1. *$P$<0.05 *vs* KYSE140 group.

**图4 KYSE140细胞与CAF-1共培养后对Hedgehog信号通路的影响**

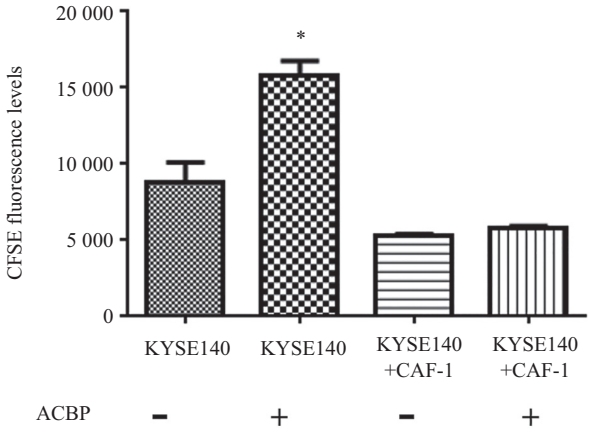
**Fig.4 Effects of CAF-1 on Hedgehog signaling pathway of KYSE140 cells under co-culture conditions**

培养KYSE140细胞相比, 与CAF-1共培养的KYSE140细胞中可检测到*PTCH1*和*GLI1*的mRNA表达水平显著增加(*PTCH1*: $P$=0.016 5; *GLI1*: $P$=0.012 9)(图4A)。Western blot检测显示, CAF-1作用后PTCH1和GLI1的蛋白相对表达量较对照组明显上调(PTCH1: $P$=0.000; GLI1: $P$=0.005), 差异具有显著性(图4B和图4C), 说明共培养条件下ESCC来源CAFs通过激活Hedgehog信号通路促进了KYSE140细胞增殖。

### 2.4 ACBP对共培养条件下KYSE140细胞增殖的影响

如图5所示, 与对照组相比, ACBP作用48 h后KYSE140细胞中的CFSE水平显著升高, 说明ACBP对KYSE140细胞的增殖具有明显的抑制作用; 在与CAF-1共培养体系中, ACBP对KYSE140细胞的增殖具有一定的抑制增殖作用 。

IncuCyte结果(图3)显示, 单独培养条件下, ACBP作用后KYSE140细胞增殖速率明显降低($P$=0.03), 共培养条件下, ACBP的加入对KYSE140细胞增殖有显著抑制作用(与CAF-1组相比$P$=0.042, 与对照组相比$P$<0.001), 结果表明不论在CAF-1是否存在下, ACBP都可以对食管癌KYSE140细胞的生长产生抑制作用。

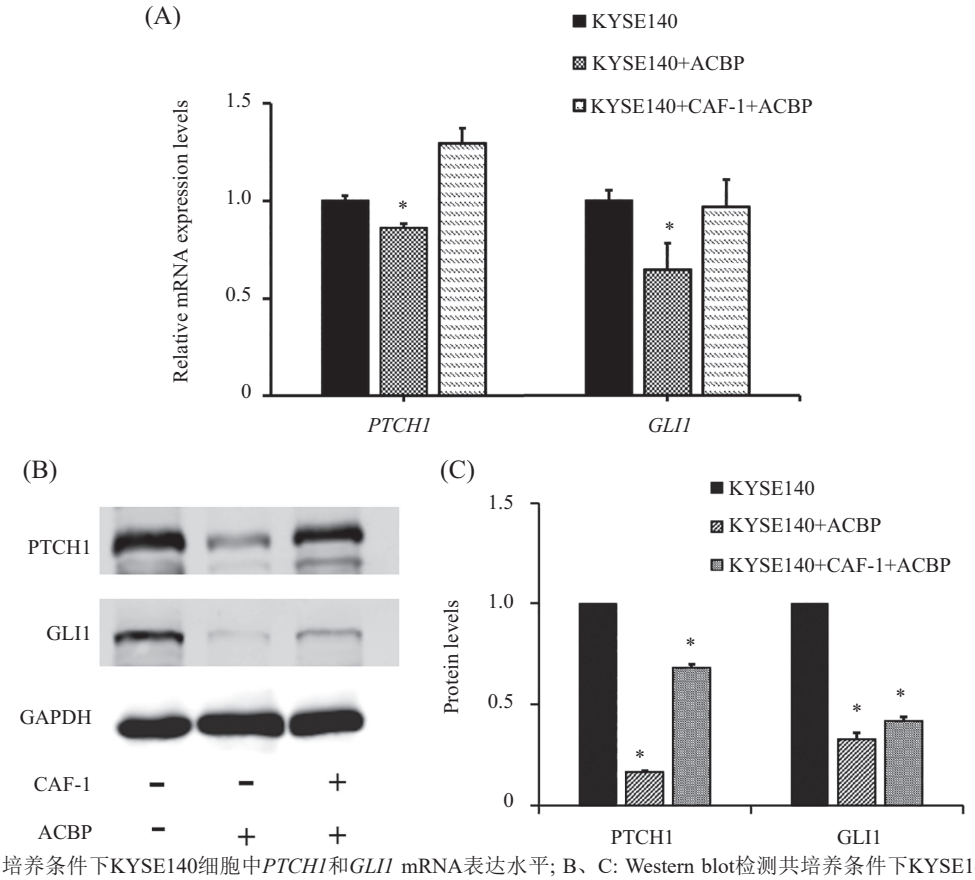



ACBP作用48 h后流式细胞术检测KYSE140细胞的CFSE水平。*$P$<0.05, 与KYSE140组相比。

After 48 h of ACBP treatment, the CFSE level of KYSE140 cells was detected by flow cytometry. *$P$<0.05 *vs* KYSE140 group.

**图5 ACBP在共培养条件下对KYSE140增殖的影响**

**Fig.5 Effects of ACBP on the proliferation of KYSE140 under co-culture conditions**




A: qRT-PCR检测共培养条件下KYSE140细胞中*PTCH1*和*GLI1* mRNA表达水平; B、C: Western blot检测共培养条件下KYSE140细胞中PTCH1和GLI1蛋白表达水平。*$P$<0.05, 与KYSE140相比。

A: qRT-PCR detected the mRNA levels of *PTCH1* and *GLI1* in KYSE140 cells under co-cultured conditions; B,C: Western blot detected the protein levels of PTCH1 and GLI1 in KYSE140 cells under co-cultured conditions. *$P$<0.05 *vs* KYSE140 group.

**图6 ACBP对共培养条件下KYSE140细胞的Hedgehog信号通路的影响**

**Fig.6 Effects of ACBP on Hedgehog signaling pathway of KYSE140 cells under co-culture conditions**

## 2.5 ACBP对共培养条件下KYSE140细胞中Hedgehog信号通路的影响

为了研究ACBP是否影响KYSE140细胞的Hedgehog信号通路, 通过qRT-PCR结果显示, 与对照组相比, ACBP可以下调KYSE140细胞中*PTCH1* ($P$=0.005 1)和*GLI1*($P$=0.026 8)的mRNA水平(图6A); Western blot

检测结果显示, ACBP单独作用后, KYSE140细胞的PTCH1($P$=0.000)和GLI1($P$=0.001)的蛋白相对表达量较对照组明显下降, 差异具有统计学意义($P$<0.05)(图6B和图6C)。在共培养条件下, ACBP作用可以抑制细胞的PTCH1和GLI1蛋白表达($P$<0.01), 差异具有统计学意义。

## 3 讨论

肿瘤微环境中含有多种成分, 包括CAFs、免疫细胞、内皮细胞、神经元、脂肪细胞和细胞外基质等, 其中CAFs被认为参与了包括食管癌发展在内的所有过程。CAFs分泌多种因子, 包括生长因子、趋化因子和细胞因子来调节肿瘤和TME中的其他成分。如ESCC来源的CAFs分泌的IL-6不仅支持肿瘤细胞生长, 而且促进成纤维细胞活化[17], 调节肿瘤浸润淋巴细胞(tumor infiltrating lymphocytes, TILs)及其在肿瘤免疫抑制中的作用[18], 同时促进肿瘤细胞和巨噬细胞的迁移[19]。CAFs对IL-6的表达上调了STAT3/NF-κB对CXCR7的表达, 在化疗耐药中起着重要作用[20]。CAFs可以通过分泌TGFβ1[21]和PAI-1[22], 高表达CXCL1[23], 从而提高食管癌的放化疗抵抗。另外, CAFs能够促进食管鳞状细胞癌淋巴结转移[24]。研究显示, 在ESCC CAFs的裂解液、条件培养基和外泌体中都有SHH(Sonic Hedgehog)的高表达, 而且CAFs可以促进食管癌细胞株的增殖和迁移[25]。但是CAFs对ESCC细胞株本身的Hedgehog信号通路的影响鲜有报道。本文首先研究了CAFs对食管癌细胞增殖的影响, 研究结果显示, 食管癌细胞KYSE140在与CAFs共培养条件下或者CAFs条件培养基中的增殖速度均高于单独培养KYSE140组, 说明CAFs能够促进食管癌细胞KYSE140的增殖。

Hedgehog信号通路最初在果蝇中被发现, 是一种进化上保守的信号机制, 在胚胎发生、生长和模式形成中起着重要作用。该通路的重要组成部分包括Hedgehog配体[包括SHH、IHH(indian Hedgehog)和DHH(desert Hedgehog)]、Patched受体(PTCH1和PTCH2)、融合抑制因子(suppressor of fused, SuFu)和GLI转录因子(包括GLI1、GLI2和GLI3)。在大多数研究中, SHH与PTCH1的结合导致SMO的去表达, 从而激活SHH信号, 导致GLI1向细胞核的移位[26-27]。核GLI1可以调节包括PTCH1和Gli1在内的许多基因的表达。我们对共培养条件下CAFs对KYSE140细胞的Hedgehog信号通路标志进行检测, 结果显示, CAFs上调KYSE140细胞中PTCH1和GLI1的mRNA和蛋白水平, 说明CAFs能够激活食管癌细胞KYSE140的Hedgehog信号通路, 并且CAFs可能参与对KYSE140细胞的促增殖作用。

ACBP是本实验室从山羊脏器中分离得到的一种多肽[28]。前期研究表明, ACBP通过激活半胱氨酸天冬氨酸蛋白酶-3(Caspase-3)诱导细胞凋亡, 从而在体内外抑制胃癌细胞和胃癌干细胞的增殖[15-16]; 在结直肠癌模型中, ACBP通过调节PARP-p53-Mcl-1信号通路, 抑制结直肠癌细胞生长, 诱导细胞凋亡[29]。此外, ACBP与顺铂联合应用, 能够发挥减毒增敏的作用, 提高荷瘤小鼠的生活质量[15]。在我们的研究中, ACBP单独培养食管癌细胞KYSE140可以抑制其增殖, 由于CAFs通过激活Hedgehog信号通路促进KYSE140增殖, 我们研究了ACBP是否也干预Hedgehog信号通路, 发现ACBP单独作用KYSE140可以降低细胞的PTCH1和GLI1的转录和蛋白水平。在与CAFs共培养条件下, ACBP同样可以抑制PTCH1和GLI1的蛋白水平, 具有抑制Hedgehog信号通路的作用, 说明ACBP可以通过调节Hedgehog信号通路干预CAFs对食管癌细胞的增殖的促进作用。

综上所述, 本研究发现从食管癌组织中分离的CAFs可以通过Hedgehog信号通路的激活促进食管癌细胞的增殖, ACBP可以抑制食管癌细胞的增殖, 并通过抑制Hedgehog信号通路来干预CAFs对共培养条件下食管癌细胞增殖的促进作用。这些结果为我们理解ACBP的抗癌活性的分子机制提供了新的见解, 也将为ACBP作为生物活性剂参与治疗食管癌提供参考依据。

## 参考文献 (References)

[1] SUNG H, FERLAY J, SIEGEL R, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. CA Cancer J Clin, 2021, 71: 209-49.

[2] 赵霄瀚, 王潞, 朱益平. 食管癌的分子靶向治疗进展[J]. 河南医学研究(ZHAO X H, WANG L, ZHU Y P. Research progress on molecular targeted therapy of esophageal cancer [J]. Henan Medical Research), 2018, 27(5): 68-72.

[3] WAGNER M, KOYASU S. Cancer immunoediting by innate lymphoid cells [J]. Trends Immunol, 2019, 40(5): 415-30.

[4] SCHNEIDER G, SCHMIDT-SUPPRIAN M, RAD R, et al. Tissue-specific tumorigenesis: context matters [J]. Nat Rev Cancer, 2017, 17(4): 239-53.

[5] LINARES J, MAR N-JIM NEZ J, BADIA-RAMENTOL J, et al. Determinants and functions of cafs secretome during cancer progression and therapy [J]. Front Cell Dev Biol, 2020, 8: 621070.

[6] RAHMAN F, KADOWAKI Y, ISHIHARA S, et al. Fibroblast-derived HB-EGF promotes Cdx2 expression in esophageal squamous cells [J]. Lab Invest, 2010, 90(7): 1033-48.

[7] NOMA K, SMALLEY K, LIONI M, et al. The essential role of fibroblasts in esophageal squamous cell carcinoma-induced angiogenesis [J]. Gastroenterology, 2008, 134(7): 1981-93.

[8] ZHANG C, FU L, FU J, et al. Fibroblast growth factor receptor 2-positive fibroblasts provide a suitable microenvironment for tumor development and progression in esophageal carcinoma [J]. Clin Cancer Res, 2009, 15(12): 4017-27.

[9] OKAWA T, MICHAYLIRA C, KALABIS J, et al. The functional interplay between EGFR overexpression, hTERT activation, and p53 mutation in esophageal epithelial cells with activation of stromal fibroblasts induces tumor development, invasion, and differentiation [J]. Genes Dev, 2007, 21(21): 2788-803.

[10] UNDERWOOD T, HAYDEN A, DEROUET M, et al. Cancer-associated fibroblasts predict poor outcome and promote periostin-dependent invasion in oesophageal adenocarcinoma [J]. J Pathol, 2015, 235(3): 466-77.

[11] STANISAVLJEVIC J, LOUBAT-CASANOVAS J, HERRERA M, et al. Snail1-expressing fibroblasts in the tumor microenvironment display mechanical properties that support metastasis [J]. Cancer Res, 2015, 75(2): 284-95.

[12] 孙佳鑫, 苏依拉其木格, 苏秀兰. Hedgehog通路抑制剂靶向治疗肿瘤的研究进展[J]. 生命的化学(SUN J X, SUYILA Q M G, SU X L. Research progress on targeted therapy of tumor with Hedgehog pathway inhibitors [J]. Chemistry of Life), 2020, 40(6): 819-26.

[13] YANG L, BIAN Y, HUANG S, et al. Identification of signature genes for detecting hedgehog pathway activation in esophageal cancer [J]. Pathol Oncol Res, 2011, 17(2): 387-91.

[14] YANG L, WANG L, CHEN X, et al. Hedgehog signaling activation in the development of squamous cell carcinoma and adenocarcinoma of esophagus [J]. Int J Biochem Mol Biol, 2012, 3(1): 46-57.

[15] SU X, DONG C, ZHANG J, et al. Combination therapy of anticancer bioactive peptide with Cisplatin decreases chemotherapy dosing and toxicity to improve the quality of life in xenograft nude mice bearing human gastric cancer [J]. Cell Biosci, 2014, 4(1): 7.

[16] YU L, YANG L, AN W, et al. Anticancer bioactive peptide-3 inhibits human gastric cancer growth by suppressing gastric cancer stem cells [J]. J Cell Biochem, 2014, 115(4): 697-711.

[17] KARAKASHEVA T, LIN E, TANG Q, et al. IL-6 mediates cross-talk between tumor cells and activated fibroblasts in the tumor microenvironment [J]. Cancer Res, 2018, 78(17): 4957-70.

[18] KATO T, NOMA K, OHARA T, et al. Cancer-associated fibroblasts affect intratumoral CD8 and FoxP3 T cells via IL6 in the tumor microenvironment [J]. Clin Cancer Res, 2018, 24(19): 4820-33.

[19] HIGASHINO N, KOMA Y, HOSONO M, et al. Fibroblast activation protein-positive fibroblasts promote tumor progression through secretion of CCL2 and interleukin-6 in esophageal squamous cell carcinoma [J]. Lab Invest, 2019, 99(6): 777-92.

[20] QIAO Y, ZHANG C, LI A, et al. IL6 derived from cancer-associated fibroblasts promotes chemoresistance via CXCR7 in esophageal squamous cell carcinoma [J]. Oncogene, 2018, 37(7): 873-83.

[21] ZHANG H, XIE C, YUE J, et al. Cancer-associated fibroblasts mediated chemoresistance by a FOXO1/TGFβ1 signaling loop in esophageal squamous cell carcinoma [J]. Mol Carcinog, 2017, 56(3): 1150-63.

[22] CHE Y, WANG J, LI Y, et al. Cisplatin-activated PAI-1 secretion in the cancer-associated fibroblasts with paracrine effects promoting esophageal squamous cell carcinoma progression and causing chemoresistance [J]. Cell Death Dis, 2018, 9(7): 759.

[23] ZHANG H, YUE J, JIANG Z, et al. CAF-secreted CXCL1 conferred radioresistance by regulating DNA damage response in a ROS-dependent manner in esophageal squamous cell carcinoma [J]. Cell Death Dis, 2017, 8(5): e2790.

[24] KASHIMA H, NOMA K, OHARA T, et al. Cancer-associated fibroblasts (CAFs) promote the lymph node metastasis of esophageal squamous cell carcinoma [J]. Int J Cancer, 2019, 144(4): 828-40.

[25] ZHAO G, LI H, GUO Q, et al. Exosomal Sonic Hedgehog derived from cancer-associated fibroblasts promotes proliferation and migration of esophageal squamous cell carcinoma [J]. Cancer Med, 2020, 9(7): 2500-13.

[26] LI S, LI S, WANG B, et al. Hedgehog reciprocally controls trafficking of Smo and Ptc through the Smurf family of E3 ubiquitin ligases [J]. Sci Signal, 2018, 11(516): eaan8660.

[27] NIYAZ M, KHAN M, MUDASSAR S. Hedgehog signaling: an Achilles' heel in cancer [J]. Transl Oncol, 2019, 12(10): 1334-44.

[28] SU L, XU G, SHEN J, et al. Anticancer bioactive peptide suppresses human gastric cancer growth through modulation of apoptosis and the cell cycle [J]. Oncol Rep, 2010, 23(1): 3-9.

[29] SU L, SHI Y, YAN M, et al. Anticancer bioactive peptides suppress human colorectal tumor cell growth and induce apoptosis via modulating the PARP-p53-Mcl-1 signaling pathway [J]. Acta Pharmacol Sin, 2015, 36(12): 1514-9.