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舒芬太尼调节MAPK/ERK信号通路对骨肉瘤细胞恶性生物学行为的影响

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摘要 该文主要探讨舒芬太尼(Suf)调节丝裂原活化蛋白激酶(MAPK)/细胞外信号调节激酶(ERK)信号通路对骨肉瘤细胞(OS)恶性生物学行为的影响。采用梯度浓度Suf处理人骨肉瘤细胞(143B细胞)和人成骨细胞系(hFOB1.19细胞), CCK-8筛选确定Suf最佳干预浓度; 将培养的143B细胞随机分为OS组、Suf低剂量组(Suf-L组)、Suf中剂量组(Suf-M组)、Suf高剂量组(Suf-H组)、Suf高剂量+ERK激活剂异丙肾上腺素组(Suf-H+ISO组)。通过EDU染色、克隆形成实验以及流式细胞术检测各组143B细胞增殖、凋亡能力; 通过Transwell和划痕实验检测各组143B细胞侵袭、迁移能力; Western blot检测各组细胞增殖、凋亡、侵袭和迁移以及MAPK/ERK信号通路相关蛋白表达情况; 裸鼠致瘤实验观察Suf对骨肉瘤生长状况的影响。该研究结果显示Suf对hFOB1.19细胞活力无统计学意义($P>0.05$); Suf对143B细胞具有细胞毒作用, 选取20、40、80 ng/mL为后续实验组Suf处理浓度。与OS组比较, Suf-L组、Suf-M组、Suf-H组143B细胞EDU阳性细胞率、克隆数、Ki67表达水平降低, 迁移率、侵袭数、VEGF和Vimentin表达水平降低, p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2降低, 以及存活素(Survivin)表达降低, 而细胞凋亡率、Bax表达水平升高($P<0.05$); 与Suf-H组比较, Suf-H+ISO组143B细胞EDU阳性细胞率、克隆数、Ki67表达水平升高, 迁移率、侵袭数、VEGF、Vimentin表达水平升高, p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2水平升高, 以及Survivin表达水平升高, 而细胞凋亡率、Bax表达水平降低($P<0.05$)。裸鼠致瘤实验结果显示, Suf组裸鼠移植瘤生长速度慢, 移植瘤体积、质量显著降低; 裸鼠移植瘤组织中p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2低于NC组, Ki67阳性率、VEGF阳性率均低于NC组, 而Bax阳性率高于NC组($P<0.05$)。总结得出Suf能够抑制骨肉瘤细胞恶性生物学行为发生, 可能与抑制MAPK/ERK信号通路密切相关。

关键词 舒芬太尼; 丝裂原活化蛋白激酶/细胞外信号调节激酶; 骨肉瘤细胞; 恶性生物学行为

The Effect of Sufentanil on the Malignant Biological Behaviors of Osteosarcoma Cells by Adjusting MAPK/ERK Signaling Pathway

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Abstract This article mainly discusses the effect of Suf (sufentanil) on malignant biological behaviors of OS (osteosarcoma) cells by regulating MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) signaling pathway. Human osteosarcoma cells (143B cells) and human osteoblast cell line (hFOB1.19 cells) were treated with gradient concentrations of Suf, and the optimal concentration of Suf was determined by

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CCK-8 screening. The cultured 143B cells were randomly separated into OS group, Suf low-dose group (Suf-L group), Suf medium-dose group (Suf-M group), Suf high-dose group (Suf-H group), and Suf high-dose + ERK activator isoproterenol group (isoproterenol group). EDU staining, colony formation assay and flow cytometry were used to detect the proliferation and apoptosis of 143B cells in each group. The invasion and migration abilities of 143B cells in each group were detected by Transwell and scratch test. Western blot was used to detect cell proliferation, apoptosis, invasion, migration, and the expression of MAPK/ERK signaling pathway related proteins in each group were detected. In addition, nude mouse tumorigenic experiment was used to observe the effect of Suf on the growth status of osteosarcoma. The results showed that Suf had no statistically significant effect on the viability of hFOB1.19 cells (P>0.05). Suf had cytotoxicity effect on 143B cells, and the concentrations of 20, 40, and 80 ng/mL were selected as the the Suf treatment concentrations in the subsequent experimental groups. Compared with the OS group, The EDU-positive cell rate, clone number and Ki67 expression of 143B cells in the Suf-L, Suf-M and Suf-H groups were decreased, and the migration rate, invasion number, VEGF and Vimentin expression were decreased, and p-MEK1/2/MEK1/2, p-ERK1/2/ERK1/2 were decreased. The expressions of survivin was decreased, while the apoptosis rate and Bax expression were increased (P<0.05). Compared with the Suf-H group, the EDU-positive cell rate, clone number and Ki67 expression of 143B cells in the Suf-H+ISO group were increased, and the migration rate, invasion number, VEGF and Vimentin expression were increased, and p-MEK1/2/MEK1/2, p-ERK1/2/ERK1/2 were increased. The expressions of Survivin was increased, while the apoptosis rate and Bax expression were significantly decreased (P<0.05). The results of the nude mice tumorigenesis experiment showed that the growth rate of transplanted tumor in nude mice in the Suf group was slow, and the volume and weight of transplanted tumor were significantly reduced. The expression of p-MEK1/2/MEK1/2 and p-ERK1/2/ERK1/2 in the tumor tissue of nude mice were lower than those in the NC group. The positive rate of Ki67 and VEGF were lower than those in the NC group, and the positive rate of Bax was higher than that in the NC group (P<0.05). In conclusion, Suf can inhibit the malignant biological behaviors of osteosarcoma cells, which may be closely related to the inhibition of the MAPK/ERK signaling pathway.

Keywords sufentanil; mitogen-activated protein kinase/extracellular regulated protein kinase; osteosarcoma cells; malignant biological behaviors

骨肉瘤(osteosarcoma, OS)作为原发性恶性骨肿瘤中具有侵袭性的类型，占据所有骨恶性肿瘤病例的20%~30%，大部分病例集中于青少年群体，是儿童和青少年癌症相关死亡的主要原因之一[1-2]。OS表现出显著组织异质性，肿瘤细胞能够异常增殖形成不成熟骨样组织，同时具备从原发部位向远处扩散的侵袭特性[3]。部分患者在初诊时已出现转移病灶，如肺部、骨骼系统以及中枢神经系统等。现有骨肉瘤的治疗方案包括新辅助化疗、根治性手术切除等，虽然将非转移性患者5年生存率大大提升，但对于转移性患者而言5年生存率仍然较低，且面临严重的相关并发症，包括继发性肿瘤、心脏毒性和生育功能障碍等，这一现状凸显开发新型治疗策略的迫切需求[4-5]。舒芬太尼(sufentanil, Suf)是一种高效合成的阿片类受体激动剂，具有镇痛和抗肿瘤作用[6]。研究表明，Suf能够抑制子宫内膜癌细胞增殖、迁移和侵袭等恶性生物学行为[7]。李进岚等[8]研究发现，Suf能够阻滞前列腺癌细胞G_0/G_1期，抑制细胞增殖，促进凋亡。虽然目前已有研究表明Suf可能参与调控多种肿瘤细胞的恶性表型转变，但其针对OS恶性生物学行为的研究却鲜有报道。研究表明，通过抑制丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)/细胞外信号调节激酶(extracellular signal-regulated kinase, ERK)信号通路活化，减少骨肉瘤细胞活性，进而抑制骨肉瘤进展[9]。任帅帅等[10]研究发现，抑制MAPK/ERK通路活化，能够抑制前列腺癌细胞增殖、迁移及侵袭。然而，关于Suf是否能够通过调节MAPK/ERK信号通路对OS细胞恶性生物学行为产生影响未有研究报道，因此，本研究以143B细胞为研究对象进行初步探讨。

1 材料与方法

1.1 OS细胞和裸鼠

人类骨肉瘤细胞(143B细胞)(货号: ZQ0455)购自上海中乔新舟生物科技有限公司; 人成骨细胞系hFOB1.19(货号: FY-K3921Z)购自上海富雨生物科技有限公司。BALB/c裸鼠均购自云南达生生物科技有限公司[实验动物使用许可证号: SCXK(滇)2022-0003]。所选实验动物均为6周龄雄性个体, 初始体质量20~25 g, 饲养于SPF级标准笼具, 自由获取灭菌饲料和纯净水。本研究所有动物实验经达州骨科医院伦理委员会审核并批准(批准号: 2025-01-11)。

1.2 主要试剂及相关仪器

Suf(批准文号: 国药准字H20054172)购自宜昌人福药业有限责任公司; ERK激活剂isoproterenol(货号: 15592)购自北京博奥派克生物有限公司; CCK-8试剂盒(货号: KTA1020)购自亚科因(武汉)生物技术有限公司; EDU细胞增殖检测试剂盒(货号: C10338)购自广州市锐博生物科技有限公司; 结晶紫染液(货号: 29524)购自上海艾研生物科技有限公司; 凋亡检测试剂盒(货号: BB-4125)购自上海贝博生物科技有限公司; 抗体Ki67、血管内皮生长因子(vascular endothelial growth factor, VEGF)、存活素(Survivin)、p-MEK1/2、MEK1/2、BCL-2相关X蛋白(BCL-2-associated X protein, Bax)(货号: NBAB-109279、NBL-247900、NBAB-101228、NBAB-108996、NBAB-108998、NBAB-101135)购自维百奥(北京)生物科技有限公司; Vimentin、p-ERK1/2、ERK1/2(货号: xy-3295、IC154377、XY-1051)购自上海信裕生物科技有限公司; 快速制胶试剂盒(货号: JA0203)购自百力格生物科技(上海)股份有限公司; 游标卡尺(货号: 300106)购自上海玉博生物科技有限公司。

1.3 方法

1.3.1 细胞培养 EMEM培养基作为143B细胞系基础培养体系, 培养基补充10%胎牛血清和1%青霉素–链霉素双抗, 培养于37 ℃恒温、5% CO_2浓度培养箱, 细胞需连续稳定传代至少3代后可用于后续实验。

1.3.2 Suf最适浓度筛选 选择处于对数生长期的143B细胞和hFOB1.19细胞, 以2×10^4/孔的密度均匀接种于96孔培养板中。实验设置6个不同浓度Suf处理组(10~160 ng/mL)及空白对照组(0 ng/mL)。药物浓度梯度设计采用2倍稀释法。药物处理24 h后, 每孔加入10 μL CCK-8溶液, 避光孵育2 h, 使用酶标仪测定*D*值。继续培养24 h, 分别计算24 h和48 h细胞存活率。

1.3.3 细胞分组 将143B细胞随机分为OS组, Suf低剂量组(Suf-L组)、Suf中剂量组(Suf-M组)、Suf高剂量组(Suf-H组)。OS组细胞使用正常EMEM培养基培养; Suf-L组、Suf-M组、Suf-H组细胞分别使用含20、40、80 ng/mL Suf的EMEM培养基进行培养; Suf高剂量+ERK激活剂异丙肾上腺素组(Suf-H+ISO组)在含高剂量Suf的EMEM培养基基础上加入1 μmol/L的isoproterenol[11]进行培养。所有实验组细胞均培养至对数生长期后, 统一进行后续检测和分析实验。

1.3.4 143B细胞增殖检测 EDU染色法: 各组143B细胞完成干预处理后, 将预先配制的EDU染色液以1 mL/孔的体积加入6孔板中, 置于细胞培养箱孵育2 h, 随后使用PBS洗涤3次, 加入0.3% TritonX-100试剂室温处理10 min, 按照试剂说明配制Apollo反应混合液, 每孔加入500 μL反应液, 避光孵育30 min, 使用DAPI溶液(1 μg/mL)进行细胞核避光染色10 min, 显微镜下进行图像采集, 软件分析计算EDU阳性细胞占比。

克隆形成实验: 将各组143B细胞以每孔5×10^5个接种于6孔板中进行集落形成实验, 每天通过倒置显微镜观察集落形成动态, 待集落形态发育完整且边界清晰可辨时, 使用预先冷却至4 ℃的甲醇溶液进行10 min固定处理, 然后选用结晶紫染色液在室温下进行细胞染色20 min, 显微镜下观察, 软件进行定量分析计算克隆形成数。

1.3.5 143B细胞凋亡检测 将各组143B细胞以每孔2.5×10^5个接种于12孔板中, 培养至对数生长期后, 采用0.25%胰酶-EDTA消化液37 ℃消化3 min。终止消化后, 使用PBS进行两次离心(1 500 r/min、5 min)洗涤。将细胞重悬于100 μL 1× 结合缓冲液中, 加入5 μL Annexin V-FITC, 同时加入5 μL碘化丙啶染液, 室温避光孵育10 min, 通过流式细胞仪立即在1 h内完成上机检测, 进行数据分析后, 获得各组143B细胞凋亡率。

1.3.6 143B细胞侵袭检测 将50 μL基质胶工作液均匀涂布于Transwell上室, 并置于恒温培养箱中固定30 min形成稳定基质层。取对数生长期各组143B细胞消化后, 收集细胞悬液并调整细胞至5×10^5/mL, 取200 μL接种于Transwell上室, 下室加入600 μL含

10%胎牛血清的完全培养基, 24 h后用PBS洗涤, 多聚甲醛4 °C固定15 min, 使用倒置显微镜进行图像采集, 并随机选取非重叠的5个视野, 应用ImageJ软件计算侵袭数量。

1.3.7 143B细胞迁移检测 将各组143B细胞以每孔5×10^{5}个接种于6孔板, 继续培养细胞至对数生长期, 选用200 μL无菌枪头保持与培养板呈90°的垂直角度, 施加恒定压力, 平行划痕后, 立即漂洗3次, 更换为无血清培养基培养, 分别在0 h和24 h进行图像采集, 软件进行分析计算各组细胞迁移情况。

1.3.8 143B细胞增殖、凋亡、侵袭和迁移以及MAPK/ERK信号通路相关蛋白表达检测 收集各组143B细胞, 加入裂解缓冲液进行冰上裂解30 min后, 12 000 ×*g*离心15 min, 收集上清蛋白液, BCA法测定蛋白浓度, 并调整为统一浓度。配制10%分离胶和浓缩胶后, 每孔上样20 μg蛋白, 依次进行电泳, 转膜, 封闭, 抗体Ki67、VEGF、Survivin、p-MEK1/2、MEK1/2、Bax、Vimentin、p-ERK1/2、ERK1/2(1:2 000)孵育(4 °C过夜), 二抗(1:5 000)室温孵育, 曝光显影, 应用ImageJ软件灰度值测定, 并以GAPDH为内参计算相对表达量。

1.3.9 裸鼠致瘤实验 选取健康状况良好BALB/c裸鼠, 在SPF环境中预饲养7天以稳定生理状态。143B细胞培养至对数生长期, 制备成浓度1×10^7/mL的细胞悬液, 将细胞悬液注射至小鼠右侧前肢腋下皮下组织(消毒处理后操作), 注射体积10 μL, 待肿瘤体积达到30 mm^{3}随机分组, 即NC组和Suf组, 各6只, 其中Suf组尾静脉注射5 μg/kg Suf(溶于生理盐水)[12], 隔日给药, 固定时段; Control组给予等量生理盐水, 每周固定时间点测量肿瘤生长状况(采用游标卡尺测量)。28天后安乐死小鼠, 分离肿瘤组织并称量湿重, 检测MAPK/ERK信号通路相关蛋白表达。

1.3.10 肿瘤组织Ki67、Bax、VEGF表达检测 肿瘤组织经过石蜡包埋后, 采用切片机制备切片, 置于60 °C恒温烘箱进行烘干, 然后将切片脱水、脱蜡, 并使用柠檬酸钠缓冲液进行微波热诱导抗原修复, 阻断内源性过氧化物酶, 使用正常山羊血清室温封闭30 min, 提前配制Ki67、Bax、VEGF(1:1 000)一抗工作液, 均匀覆盖组织后, 置于湿盒4 °C孵育过夜, 二抗(1:5 000)继续室温孵育1 h, DAB显色后, 苏木精进行细胞核复染, 显微镜观察Ki67、Bax、VEGF表达变化。

1.4 统计学分析

SPSS 25.0统计软件进行数据分析处理, 针对实验获得定量数据, 采用均值±标准差形式进行数据呈现, 预先进行方差齐性和正态性检验, 单因素方差分析进行多组间比较, 进一步使用SNK-*q*检验进行两组间比较。当P<0.05时, 判定组间差异具有统计学意义。

2 结果

2.1 Suf对143B细胞和hFOB1.19细胞活力影响

10、20、40、80、160 ng/mL与0 ng/mL相比, Suf对hFOB1.19细胞活力无明显变化, 但能够降低143B细胞存活率, 说明Suf对正常成骨细胞无细胞毒性。其中20、40、80、160 ng/mL Suf能够显著降低143B细胞存活率(P<0.05)。与80 ng/mL相比, 160 ng/mL Suf对143B细胞存活率的影响无显著变化, 因此, 选取20、40、80 ng/mL Suf作为后续实验组Suf-L组、Suf-M组、Suf-H组的处理浓度, 见表1和表2。

2.2 Suf对143B细胞增殖影响

Suf-L组、Suf-M组、Suf-H组与OS组比较, 143B细胞EDU阳性细胞率、克隆数、Ki67表达降低(P<0.05); Suf-H+ISO组与Suf-H组比较, 143B细胞EDU阳性细胞率、克隆数、Ki67表达水平增加(P<0.05), 见图1~图3和表3。

2.3 Suf对143B细胞侵袭、迁移影响

Suf-L组、Suf-M组、Suf-H组143B细胞迁移率、侵袭数、VEGF、Vimentin表达水平比OS组低(P<0.05); Suf-H+ISO组143B细胞迁移率、侵袭数、VEGF和Vimentin表达水平比Suf-H组高(P<0.05), 见图4~图6和表4。

2.4 Suf对143B细胞凋亡影响

Suf-L组、Suf-M组、Suf-H组143B细胞凋亡率、Bax表达高于OS组, Survivin表达水平低于OS组(P<0.05); Suf-H+ISO组143B细胞凋亡率、Bax表达水平低于Suf-H组, Survivin表达水平高于Suf-H组(P<0.05), 见图7、图8和表5。

2.5 Suf对143B细胞MAPK/ERK信号通路相关蛋白表达影响

Suf-L组、Suf-M组、Suf-H组与OS组比较, 143B细胞p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2水平降低(P<0.05); Suf-H+ISO组与Suf-H组比较, 143B细胞p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2水平升高, 见图9和表6。

表1 Suf对hFOB1.19细胞存活率影响

Table 1 Effect of Suf on hFOB1.19 cell viability

Suf浓度/ng·mL^{-1} Suf concentration /ng·mL^{-1}	细胞存活率/% Cell survival rate /%	
	24 h	48 h
0	98.25±1.75	98.39±1.61
10	97.68±2.32	98.46±1.54
20	99.12±0.88	98.45±1.55
40	99.75±0.25	99.87±0.13
80	97.61±2.39	98.51±1.49
160	99.52±0.48	99.31±0.69

n=6, $\bar{x}\pm s$; *P<0.05, 与0 ng/mL组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with 0 ng/mL group.

表2 Suf对143B细胞存活率影响

Table 2 Effect of Suf on 143B cell viability

Suf浓度/ng·mL^{-1} Suf concentration /ng·mL^{-1}	细胞存活率/% Cell survival rate /%	
	24 h	48 h
0	99.16±0.84	99.28±0.72
10	96.89±3.11	94.53±2.41
20	86.25±9.15*	82.37±8.76*
40	75.94±8.03*	70.65±7.29*
80	62.72±6.38*	58.49±6.43*
160	60.15±6.26*	57.86±5.95*

n=6, $\bar{x}\pm s$; *P<0.05, 与0 ng/mL组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with 0 ng/mL group.

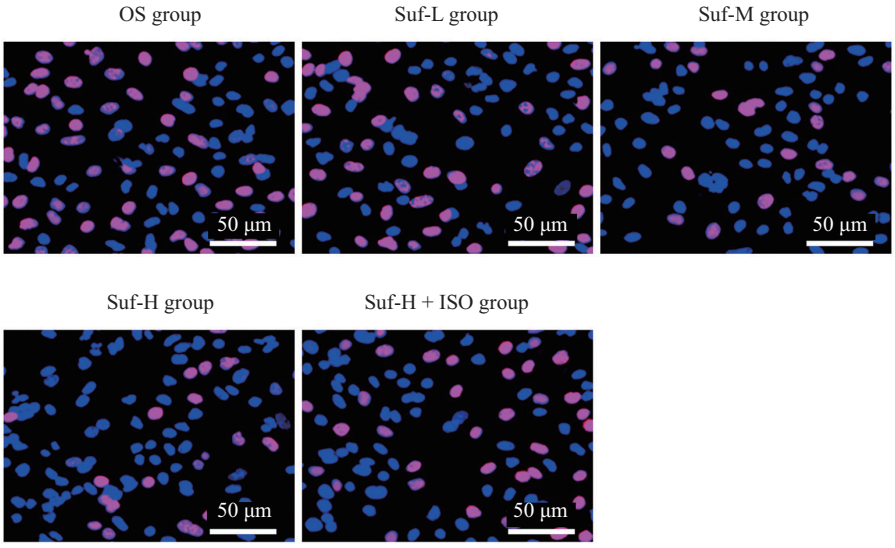

图1 EDU染色检测143B细胞增殖情况

Fig.1 Measurement of 143B cell proliferation by EDU staining

2.6 Suf对裸鼠移植瘤影响

Suf组裸鼠移植瘤生长速度慢, 移植瘤体积、质量、p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2水平均低于NC组(P<0.05), 见图10~图12、表7和表8。

2.7 Suf对肿瘤组织Ki67、Bax、VEGF表达影响

Suf组与NC组比较, 肿瘤组织Ki67阳性率、VEGF阳

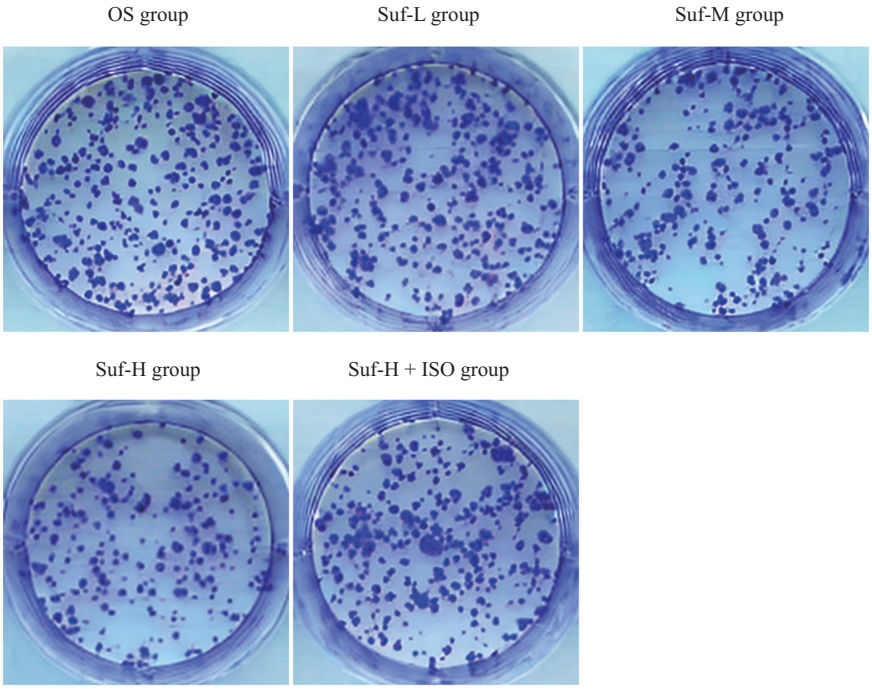

图2　平板克隆形成实验检测143B细胞增殖情况

Fig.2　Measurement of 143B cell proliferation by plate clone formation assay

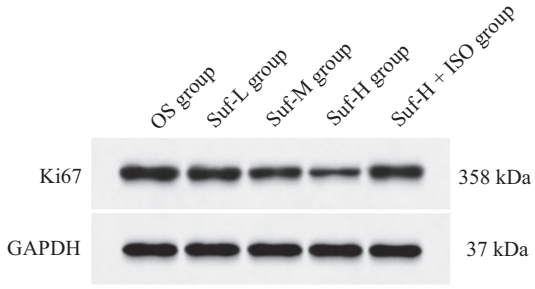

图3　Western blot检测143B细胞Ki67表达情况

Fig.3　Ki67 expression in 143B cells measured by Western blot

表3　143B细胞EDU阳性细胞率、克隆数、Ki67表达比较

Table 3　Comparison of EDU-positive cell rate, clone number, and Ki67 expression in 143B cells

组别 Groups	EDU阳性细胞率/% EDU-positive cell rate /%	克隆数 Clone number	Ki67
OS group	53.27±5.64	109.52±12.38	0.86±0.12
Suf-L group	41.56±4.38*	91.34±9.82*	0.72±0.09*
Suf-M group	29.39±3.25*#	73.26±7.53*#	0.51±0.06*#
Suf-H group	18.62±2.69*#&	57.49±6.75*#&	0.37±0.05*#&
Suf-H + ISO group	37.98±3.96@	85.73±8.61@	0.68±0.08@

n=6, $\bar{x}\pm s$; *P<0.05, 与OS组相比; #P<0.05, 与Suf-L组相比; &P<0.05, 与Suf-M组相比; @P<0.05, 与Suf-H组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with OS group; #P<0.05 compared with Suf-L group; &P<0.05 compared with Suf-M group; @P<0.05 compared with Suf-H group.

性率均降低, Bax阳性率升高(P<0.05), 图13~图15和表9。

3　讨论

OS多发于青少年群体, 常见于长骨干骺端, 具有显著的局部浸润能力和高转移倾向, 是骨科及肿瘤学领域极具挑战性的恶性疾病[13]。尽管目前医学已通过根治性手术联合系统性化疗显著提升患者生存率, 但对于转移性或复发性OS患者临床治疗效果仍然不容

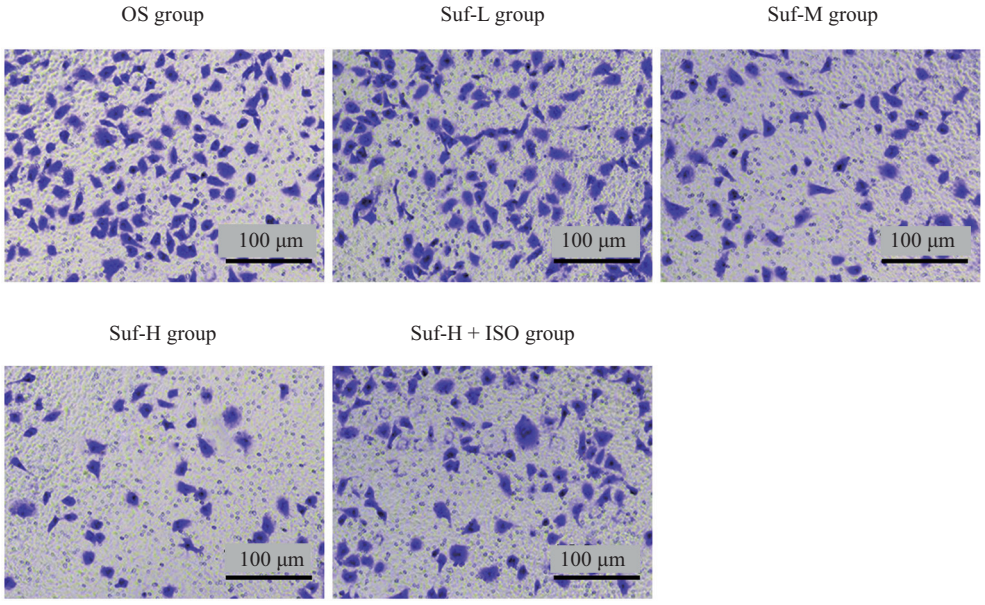

图4 Transwell实验检测143B细胞侵袭情况

Fig.4 Detection of 143B cell invasion by Transwell assay

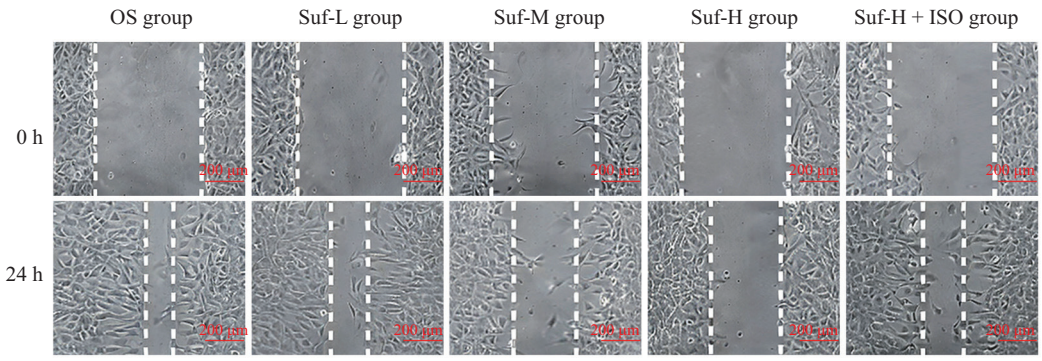

图5 划痕实验检测143B细胞迁移情况

Fig.5 Detection of 143B cell migration by scratch assay

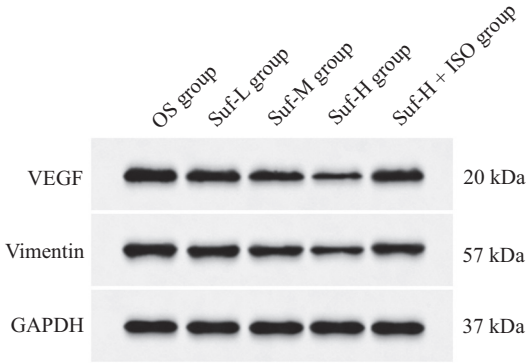

图6 Western blot检测143B细胞VEGF、Vimentin表达

Fig.6 Expression of VEGF and Vimentin in 143B cells detected by Western blot

乐观[14]。面对这一困境，探究OS发生发展的分子机制和有效药物用于改善患者预后成为迫切需求。

Suf作为一种强效μ-阿片受体激动剂，在临床麻醉和镇痛方面具有重要地位[15]。近年来，越来越多研究表明，Suf能够影响肿瘤细胞的生物学行为，在肿瘤治疗中具有潜在作用。LI等[16]研究发现，Suf通

表4 143B细胞迁移率、侵袭数、VEGF、Vimentin表达比较

Table 4 Comparison of 143B cell migration rate, number of invasion, VEGF and Vimentin expression

组别 Groups	侵袭数 Number of invasion	迁移率/% Rate of migration /%	VEGF	Vimentin
OS group	118.51±13.26	69.58±7.05	1.06±0.12	0.92±0.11
Suf-L group	91.68±10.34*	56.27±6.71*	0.84±0.11*	0.76±0.08*
Suf-M group	72.42±8.59*#	42.91±5.16*#	0.62±0.08*#	0.53±0.06*#
Suf-H group	49.83±6.72*#&	31.85±3.58*#&	0.37±0.04*#&	0.41±0.05*#&
Suf-H + ISO group	85.27±9.15@	52.64±5.63@	0.78±0.09@	0.69±0.07@

n=6, $\bar{x}\pm s$; *P<0.05, 与OS组相比; #P<0.05, 与Suf-L组相比; &P<0.05, 与Suf-M组相比; @P<0.05, 与Suf-H组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with OS group; #P<0.05 compared with Suf-L group; &P<0.05 compared with Suf-M group; @P<0.05 compared with Suf-H group.

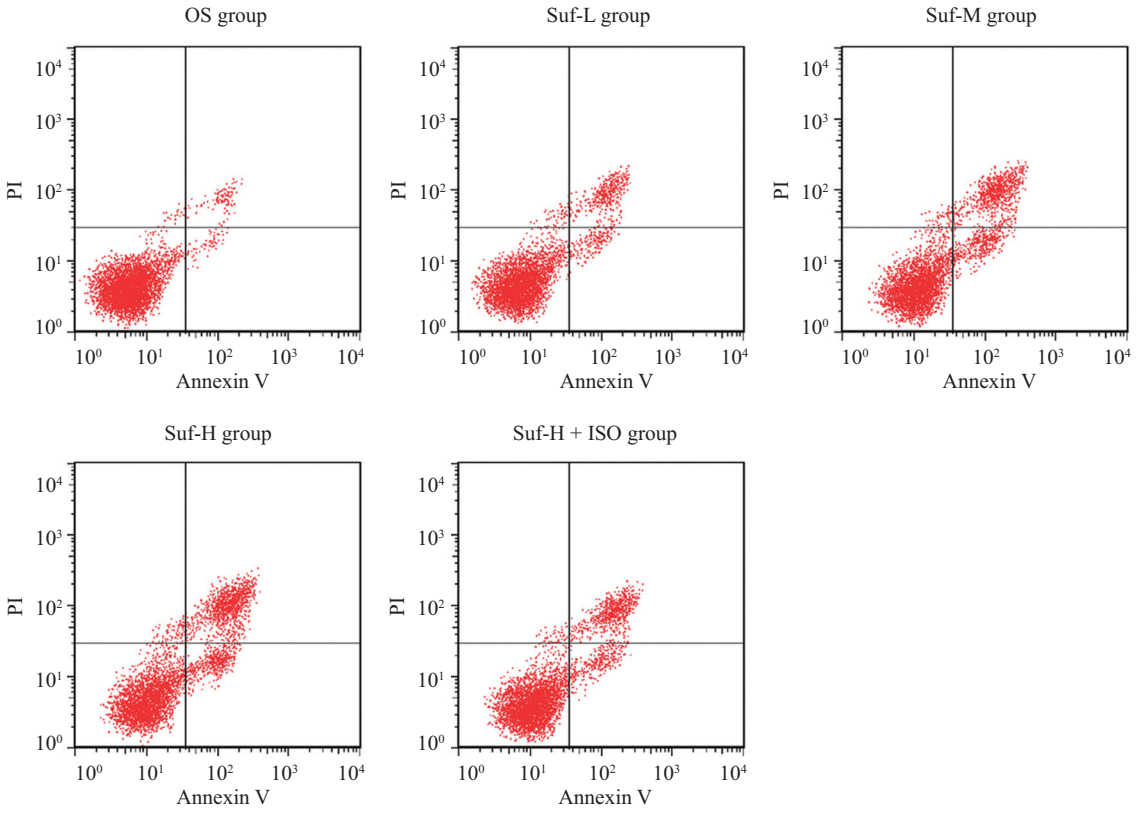

图7 流式细胞术检测143B细胞凋亡情况

Fig.7 Apoptosis of 143B cells detected by flow cytometry

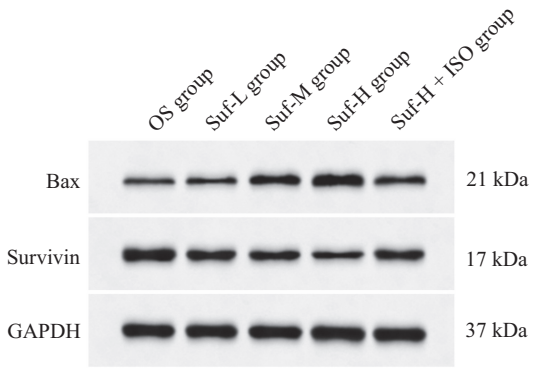

图8 Western blot检测143B细胞Bax、Survivin表达变化情况

Fig.8 Expression changes of Bax and Survivin in 143B cells detected by Western blot

表5 143B细胞凋亡率、Bax、Survivin表达比较

Table 5 Comparison of apoptosis rate, Bax and Survivin expression in 143B cells

组别 Groups	凋亡率/% Apoptosis rate /%	Bax	Survivin
OS group	1.24±0.15	0.24±0.03	0.86±0.09
Suf-L group	13.56±1.62*	0.35±0.05*	0.71±0.08*
Suf-M group	25.72±3.28*#	0.56±0.08*#	0.54±0.06*#
Suf-H group	36.85±4.05*#&	0.84±0.09*#&	0.39±0.05*#&
Suf-H + ISO group	15.49±1.76@	0.38±0.04@	0.63±0.08@

n=6, $\bar{x}\pm s$; *P<0.05, 与OS组相比; #P<0.05, 与Suf-L组相比; &P<0.05, 与Suf-M组相比; @P<0.05, 与Suf-H组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with OS group; #P<0.05 compared with Suf-L group; &P<0.05 compared with Suf-M group; @P<0.05 compared with Suf-H group.

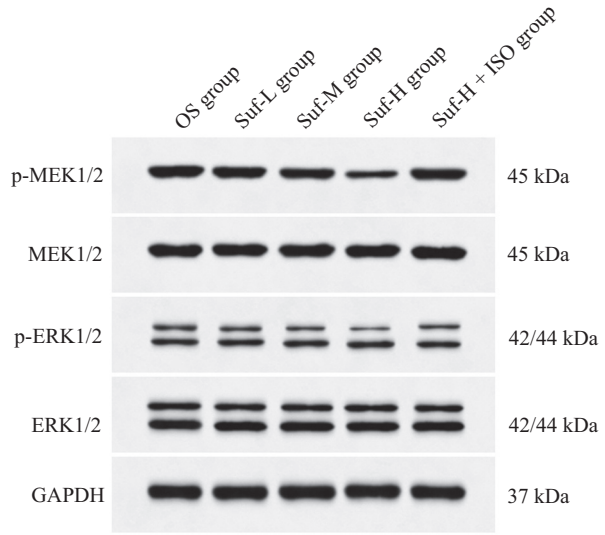

图9 Western blot检测143B细胞MAPK/ERK信号通路相关蛋白表达变化

Fig.9 Expression changes of MAPK/ERK signaling pathway-related proteins in 143B cells detected by Western blot

表6 143B细胞p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2表达比较

Table 6 Comparison of p-MEK1/2/MEK1/2 and p-ERK1/2/ERK1/2 expression in 143B cells

组别 Groups	p-MEK1/2/MEK1/2	p-ERK1/2/ERK1/2
OS group	0.89±0.09	0.92±0.11
Suf-L group	0.73±0.08*	0.79±0.09*
Suf-M group	0.58±0.06*#	0.61±0.07*#
Suf-H group	0.35±0.05*#&	0.43±0.06*#&
Suf-H + ISO group	0.67±0.07@	0.75±0.09@

n=6, $\bar{x}\pm s$; *P<0.05, 与OS组相比; #P<0.05, 与Suf-L组相比; &P<0.05, 与Suf-M组相比; @P<0.05, 与Suf-H组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with OS group; #P<0.05 compared with Suf-L group; &P<0.05 compared with Suf-M group; @P<0.05 compared with Suf-H group.

过抑制乳腺癌细胞增殖、侵袭、上皮–间质转化和炎症, 诱导细胞凋亡, 进而延缓乳腺癌进展。ZHAO等[17]研究表明, Suf呈剂量依赖性抑制卵巢癌细胞增殖、迁移和侵袭, 促进细胞凋亡。辛曙光等[18]研究说明, Suf可显著抑制宫颈癌细胞恶性生物学行为, 对宫颈癌具有潜在疗效。本研究结果显示, 通过不同浓度Suf处理143B细胞, 细胞活力呈现显著下降趋势, 提示Suf对143B细胞具有杀伤作用。Ki67作为细胞周期调控的核心蛋白, 是评价骨肉瘤细胞增殖的指标。本研究结果显示, 通过Suf处理后, 143B细胞

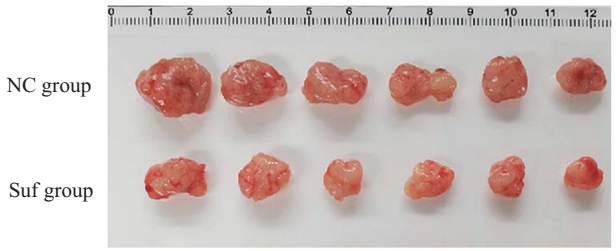

图10 移植瘤大体外观图

Fig.10 Gross appearance of the transplanted tumor

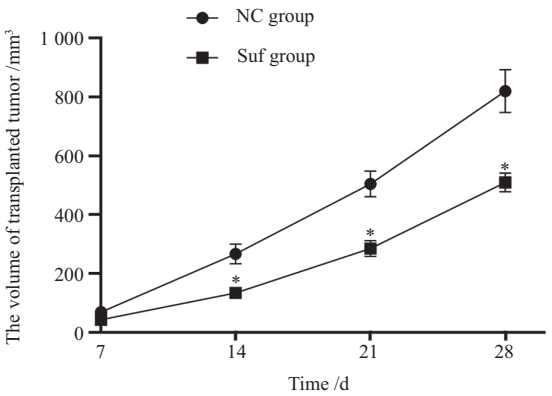

图11 移植瘤体积变化曲线

Fig.11 Curve of transplanted tumor volume change

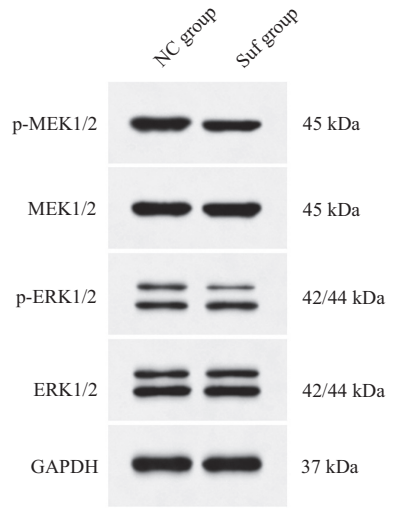

图12 Western blot检测移植瘤MAPK/ERK信号通路相关蛋白表达变化

Fig.12 Expression changes of MAPK/ERK signaling pathway-related proteins in transplanted tumors detected by Western blot

表7 移植瘤p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2表达比较

Table 7 Comparison of p-MEK1/2/MEK1/2 and p-ERK1/2/ERK1/2 expression in transplanted tumors

组别 Groups	p-MEK1/2/MEK1/2	p-ERK1/2/ERK1/2
NC group	0.91±0.13	0.86±0.09
Suf group	0.56±0.06*	0.41±0.05*

n=6, $\bar{x}\pm s$; *P<0.05, 与NC组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with NC group.

表8 各组裸鼠移植瘤体积、质量比较

Table 8 Comparison of transplanted tumor volume and mass in nude mice of each group

组别 Groups	体积/mm^3 Volume /mm^3	质量/g Mass /g
NC group	819.65±72.56	0.69±0.08
Suf group	509.51±31.72*	0.35±0.04*

n=6, $\bar{x}\pm s$; *P<0.05, 与NC组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with NC group.

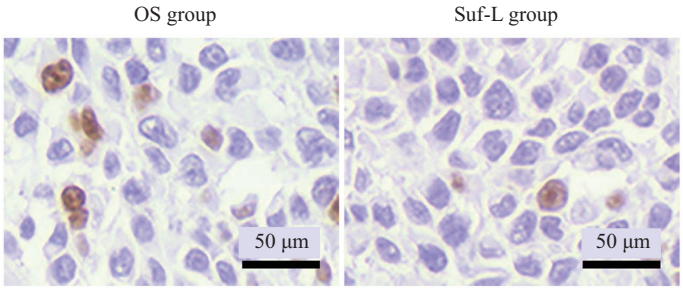

图13 免疫组化检测肿瘤组织Ki67表达情况

Fig.13 Immunohistochemical detection of Ki67 expression in tumor tissues

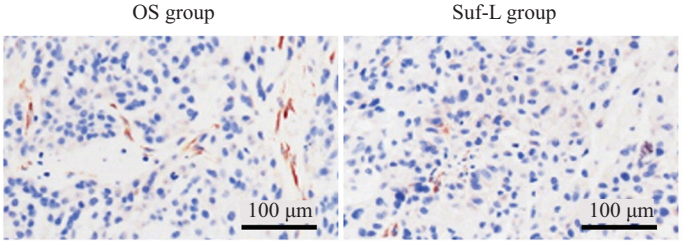

图14 免疫组化检测肿瘤组织VEGF表达情况

Fig.14 Immunohistochemical detection of VEGF expression in tumor tissues

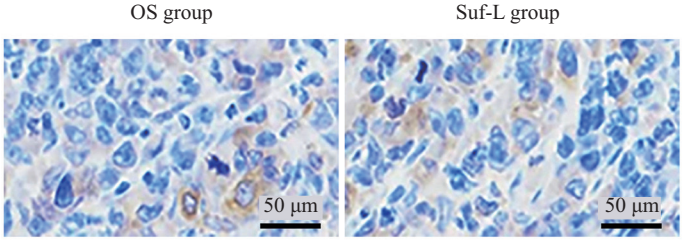

图15 免疫组化检测肿瘤组织Bax表达情况

Fig.15 Immunohistochemical detection of Bax expression in tumor tissues

表9 各组肿瘤组织Ki67、Bax、VEGF表达比较

Table 9 Comparison of Ki67, Bax and VEGF expression in tumor tissues of each group

组别 Groups	Ki67阳性率/% Ki67 positive rate /%	Bax阳性率/% Bax positive rate /%	VEGF阳性率/% VEGF positive rate /%
NC group	15.24±2.62	4.38±0.57	18.96±3.15
Suf group	5.69±0.86*	13.95±1.53*	6.87±0.91*

n=6, $\bar{x}\pm s$; *P<0.05, 与NC组相比。

n=6, $\bar{x}\pm s$; *P<0.05 compared with NC group.

EDU阳性细胞率、克隆形成数、Ki67蛋白表达减少，与李济宇等[19]研究结果中143B细胞经过药物处理后Ki67表达变化结果一致，提示Suf能够抑制OS细胞增殖。VEGF在骨肉瘤病理生理过程中具有调控作用。Vimentin表达水平升高是上皮间质转化的重要标志，在骨肉瘤侵袭转移中发挥重要作用[20]。本研究结果显示，Suf处理后，143B细胞侵袭数量、迁移率以及VEGF、Vimentin蛋白表达水平降低，与前人研究结果中143B细胞经过药物干预后VEGF、Vimentin蛋白表达变化一致，提示Suf能够抑制OS细胞迁移和侵袭[21]。Survivin是凋亡抑制蛋白，参与骨肉瘤发生发展过程。本研究结果显示，通过Suf处理，143B细胞凋亡率、Bax表达水平增加，Survivin表达水平降低，与XIE等[22]研究结果中143B细胞经过药物干预后Bax、Survivin表达变化一致，提示Suf显著促进OS细胞凋亡。这些研究结果说明Suf能够抑制143B细胞恶性生物学行为，但其分子机制尚未明确。

MAPK信号转导通路作为细胞内外信息传递的关键枢纽，在肿瘤发生和发展中扮演关键性角色[23]。SONG等[24]研究发现，抑制MAPK/ERK信号通路激活，能够抑制膀胱癌细胞生长、迁移和侵袭。LI等[25]研究表明，激活MAPK/ERK信号通路，能够促进p-MEK/MEK、p-ERK/ERK比值增加，诱导上皮间充质转化，促进乳腺癌迁移和侵袭。本研究结果显示，143B细胞经过Suf处理后，p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2比值降低，推测MAPK/ERK信号通路可能参与Suf抑制OS恶性生物学行为过程。进一步通过高剂量Suf和ERK激活剂isoproterenol同时处理143B细胞，结果显示Suf对143B细胞恶性生物学行为的抑制作用被显著削弱，证实上述推测。本研究继续通过构建裸鼠移植瘤实验发现，Suf组裸鼠移植瘤生长减慢，且移植瘤质量和体积低于NC组，p-MEK1/2/MEK1/2、p-ERK1/2/ERK1/2比值、Ki67阳性率、VEGF阳性率降低，Bax阳性率升高，进一步说明Suf可能通过抑制MAPK/ERK信号通路，抑制OS细胞恶性生物学行为，促进其凋亡。

综上所述，Suf能够抑制OS细胞增殖、侵袭和迁移，促进细胞凋亡，可能与抑制MAPK/ERK信号通路有关，表明其抗肿瘤特性。但本研究仅从一株细胞系展开研究较为局限，后续将进一步选择U2OS、MG63等其他OS细胞系和建立动物模型探讨Suf治疗OS的作用机制。

参考文献 (References)

[1] LI S, ZHANG H, LIU J, et al. Targeted therapy for osteosarcoma: a review [J]. J Cancer Res Clin Oncol, 2023, 149(9): 6785-97.

[2] BELAYNEH R, FOURMAN M S, BHOGAL S, et al. Update on osteosarcoma [J]. Curr Oncol Rep, 2021, 23(6): 71-82.

[3] YANG Y, HUANG Z, YUAN M, et al. Genomic and transcriptomic characterization of pre-operative chemotherapy response in patients with osteosarcoma [J]. Sci Rep, 2023, 13(1): 20914-23.

[4] GILL J, GORLICK R. Advancing therapy for osteosarcoma [J]. Nat Rev Clin Oncol, 2021, 18(10): 609-24.

[5] YUAN P, MIN Y, ZHAO Z. Multifunctional nanoparticles for the treatment and diagnosis of osteosarcoma [J]. Biomater Adv, 2023, 151(8): 213466-78.

[6] LIU D, HUANG Y, SHANG Y. Sufentanil suppresses cell carcinogenesis via targeting miR-186-5p/HMGB1 axis and Wnt/β-Catenin pathway in non-small-cell lung cancer [J]. Mol Biotechnol, 2025, 67(3): 1054-64.

[7] 李特, 张君, 周睦川, 等. 舒芬太尼调节CAV-1/NF-κB信号通路对子宫内膜癌细胞增殖、迁移和侵袭的影响[J]. 中国优生与遗传杂志(LI T, ZHANG J, ZHOU M C, et al. Effects of sufentanil on proliferation, migration and invasion of endometrial cancer cells by regulating CAV-1/NF-κB signaling pathway [J]. Chinese Journal of Eugenics and Genetics), 2025, 33(3): 499-505.

[8] 李进岚, 方晓, 黄银芳, 等. 舒芬太尼通过Notch信号通路对前列腺癌细胞的影响[J]. 中国临床药理学杂志(LI J L, FANG X, HUANG Y F, et al. Effect of sufentanil on prostate cancer cells through Notch signaling pathway [J]. Chinese Journal of Clinical Pharmacology), 2021, 38(12): 1344-8.

[9] REN Z, XIAO W, HE M, et al. Chitosan targets PI3K/Akt/FoxO3a axis to up-regulate FAM172A and suppress MAPK/ERK pathway to exert anti-tumor effect in osteosarcoma [J]. Chem Biol Interact, 2023, 373(3): 110354-93.

[10] 任帅帅, 孙倩, 刘春华. 瑞芬太尼对前列腺癌细胞增殖,迁移及MAPK/ERK信号通路的影响[J]. 中国老年学杂志(REN S S, SUN Q, LIU C H. Effects of remifentanil on proliferation, migration and MAPK/ERK signaling pathway in prostate cancer cells [J]. Chin J Gerontology), 2023, 43(12): 3036-9.

[11] QIN Q, RAMESH S, GOMEZ-SALAZAR M, et al. CNTNAP4 signaling regulates osteosarcoma disease progression [J]. NPJ Precis Oncol, 2023, 7(1): 2-14.

[12] 甄磊, 张懿兰, 王晓娜, 等. 舒芬太尼调节cAMP/PKA/CREB信号通路对卵巢癌细胞增殖、凋亡和侵袭的影响[J]. 中国细胞生物学学报(ZHEN L, ZHANG Y L, WANG X N, et al. Effects of sufentanil on proliferation, apoptosis and invasion of ovarian cancer cells by regulating cAMP/PKA/CREB signaling pathway [J]. Chinese Journal of Cell Biology), 2024, 46(3): 445-55.

[13] CHEN C, XIE L, REN T, et al. Immunotherapy for osteosarcoma: fundamental mechanism, rationale, and recent breakthroughs [J]. Cancer Lett, 2021, 500(3): 1-10.

[14] BISHOP M W. Osteosarcoma: diagnosis, treatment, and emerging opportunities [J]. Hematol Oncol Clin North Am, 2025, 39(4): 749-60.

[15] TANG H, LI C, WANG Y, et al. Sufentanil inhibits the proliferation and metastasis of esophageal cancer by inhibiting the NF-κB and snail signaling pathways [J]. J Oncol, 2021, 2021(12): 7586100-9.

[16] LI M, GU K, KONG Q, et al. Sufentanil inhibits the metastasis and immune response of breast cancer via mediating the NF-κB pathway [J]. Immunopharmacol Immunotoxicol, 2023, 45(6): 663-71.

[17] ZHAO F, LIU G, XIONG L, et al. Effect of sufentanil on the proliferation, apoptosis, and epithelial-mesenchymal transition of ovarian cancer cells by regulating the SMAD3/SNAIL signaling pathway [J]. J Mol Histol, 2025, 56(2): 99-105.

[18] 辛曙光, 李高翔, 崔吉正. 舒芬太尼通过circCAMSAP1/miR-409-3p对宫颈癌细胞增殖和迁移及凋亡的影响[J]. 热带医学杂志(XIN S G, LI G X, CUI J Z. Effects of sufentanil on proliferation, migration and apoptosis of cervical cancer cells through circCAMSAP1/miR-409-3p pathway [J]. J Tropical Med), 2023, 23(1): 16-20,40.

[19] 李济宇, 冯敏, 吴哲宇, 等. 姜黄素通过靶向FoxO3A在骨肉瘤中发挥抗癌作用[J]. 海南医科大学学报(LI J Y, FENG M, WU Z Y, et al. Curcumin plays an anti-cancer role in osteosarcoma by targeting FoxO3A [J]. Journal of Hainan Medical University), 2025, 31(8): 561-9.

[20] 刘德国, 徐天波, 张颉鸿, 等. 知母皂苷AIII通过调节Notch信号通路调控骨肉瘤细胞增殖、凋亡和上皮间质转化的机制[J]. 浙江中西医结合杂志(LIU D G, XU T B, ZHANG J H, et al. Mechanism of asphodeloside AIII regulating the proliferation, apoptosis and epithelial-mesenchymal transition of osteosarcoma cells by regulating Notch signaling pathway [J]. Zhejiang Journal of Integrated Traditional Chinese and Western Medicine), 2023, 33(8): 692-7,709.

[21] 袁道通, 张志猛, 巩瑞, 等. 黄芪–莪术药对通过PI3K/Akt/HIF-1α通路调节细胞黏附及血管再生抑制骨肉瘤生长进展研究[J]. 中国中药杂志(YUAN D T, ZHANG Z M, GONG R, et al. Effect of astragalus and zedoary turmeric on cell adhesion and angiogenesis through PI3K/Akt/HIF-1α pathway in osteosarcoma [J]. Chin J Materia Medica), 2025, 50(8): 2217-28.

[22] XIE X, LI Y, ZHU H, et al. Melittin inhibits growth of human osteosarcoma 143B cells through induction of apoptosis via suppressing the wnt/β-catenin signaling pathway [J]. Anticancer Agents Med Chem, 2022, 22(18): 3172-81.

[23] PASHIRZAD M, KHORASANIAN R, FARD M M, et al. The therapeutic potential of MAPK/ERK inhibitors in the treatment of colorectal cancer [J]. Curr Cancer Drug Targets, 2021, 21(11): 932-43.

[24] SONG F, KOTOLLOSHI R, GAJDA M, et al. Reduced IQGAP2 promotes bladder cancer through regulation of MAPK/ERK pathway and cytokines [J]. Int J Mol Sci, 2022, 23(21): 13508-22.

[25] LI F, WANG J, YAN Y Q, et al. CD147 promotes breast cancer migration and invasion by inducing epithelial-mesenchymal transition via the MAPK/ERK signaling pathway [J]. BMC Cancer, 2023, 23(1): 1214-24.