论著·基础研究

BRCA1 R1325K突变对胆囊癌细胞增殖及凋亡的影响

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［摘要］**目的**·探究乳腺癌易感基因1（breast cancer susceptibility gene 1，*BRCA1*）第1325位精氨酸变为赖氨酸（R1325K突变）对胆囊癌细胞系GBC-SD和NOZ增殖和凋亡的影响。**方法**·使用*BRCA1*野生型过表达慢病毒、*BRCA1* R1325K突变过表达慢病毒以及阴性对照慢病毒载体构建胆囊癌细胞系GBC-SD和NOZ稳转株。细胞分为不含目的基因的对照组、*BRCA1*野生型组及*BRCA1*突变组，并通过Western blotting验证目的蛋白BRCA1的表达情况。选用针对*BRCA1*突变的抑制剂奥拉帕利（Olaparib）20 μmol/L处理*BRCA1*突变组胆囊癌细胞，并根据目的蛋白的表达情况和加药与否将胆囊癌细胞系分为对照组、*BRCA1*野生型组、*BRCA1*突变组和*BRCA1*突变+Olaparib组。通过CCK8实验和克隆形成实验观察*BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ增殖能力及克隆形成能力的影响，通过TUNEL实验观察*BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ凋亡情况的影响，并通过Western blotting检测凋亡相关蛋白cleaved PARP、Bcl-2和Bax的表达情况。使用抑制剂Olaparib处理*BRCA1* R1325K突变过表达胆囊癌细胞系GBC-SD和NOZ，并检测*BRCA1* R1325K突变引起的相应表型改变（促进增殖、增强克隆形成能力和抑制凋亡）是否能被抑制剂所逆转。**结果**·通过CCK8实验和克隆形成实验发现：相较于对照组及*BRCA1*野生型组，*BRCA1* R1325K突变能够促进胆囊癌细胞系GBC-SD和NOZ的增殖，并提高其克隆形成能力；抑制剂Olaparib处理则能够抑制*BRCA1*突变胆囊癌细胞系的增殖（均P<0.05）。通过TUNEL和Western blotting实验发现：相较于对照组，野生型*BRCA1*基因过表达能够诱导胆囊癌细胞系GBC-SD和NOZ的凋亡；*BRCA1*突变组相较于对照组和*BRCA1*野生型组，有抵抗凋亡的作用，且升高了凋亡抑制蛋白Bcl-2的表达并降低了促凋亡蛋白Bax的表达（P<0.05）。**结论**·*BRCA1* R1325K突变能够促进胆囊癌细胞系GBC-SD和NOZ的增殖并抑制其凋亡。

［关键词］胆囊癌；BRCA1；突变；增殖；凋亡
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Effect of *BRCA1* R1325K mutation on proliferation and apoptosis of gallbladder cancer cells

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[Abstract] **Objective**·To investigate the effects of breast cancer susceptibility gene 1 (*BRCA1*) R1325K mutation [arginine (R) to lysine (K) mutation at amino acid 1325] on the proliferation and apoptosis of gallbladder cancer cell lines GBC-SD and NOZ. **Methods**·*BRCA1* wild-type overexpression lentivirus, *BRCA1* R1325K mutation overexpression lentivirus, and negative control lentivirus were used to construct the stable transgenic strains of gallbladder carcinoma, cell lines GBC-SD and NOZ. The cells were divided into the control group without the target gene, the *BRCA1* wild-type group, and the *BRCA1* R1325K mutation group. The expression of target protein was verified by Western blotting. The *BRCA1* R1325K mutant gallbladder cancer cells were treated with

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20 μmol/L Olaparib, a *BRCA1* mutation inhibitor. Gallbladder cancer cell lines were divided into the control group, the *BRCA1* wild-type group, the *BRCA1* R1325K mutation group, and the *BRCA1* R1325K mutation+Olaparib group according to the target gene expression and whether or not the inhibitor was added. The effect of *BRCA1* R1325K mutation on proliferation and clonogenesis ability of gallbladder cancer cell lines GBC-SD and NOZ was observed by CCK8 assay and clonogenesis assay, respectively. The effect of *BRCA1* R1325K mutation on apoptosis of gallbladder cancer cell lines GBC-SD and NOZ was observed by TUNEL assay. The expressions of apoptosis-related proteins, cleaved PARP, Bcl-2 and Bax, were detected by Western blotting. The inhibitor Olaparib was used to treat the *BRCA1* R1325K mutant gallbladder cancer cell lines GBC-SD and NOZ. The phenotypic changes (promoting proliferation, enhancing clonogenesis and inhibiting apoptosis) induced by *BRCA1* R1325K mutation were tested in the presence of Olaparib to determine whether the changes could be reversed by the inhibitor. **Results**·The results of CCK8 assay and clonogenesis assay showed that *BRCA1* R1325K mutation could promote the proliferation of gallbladder cancer cell lines GBC-SD and NOZ, and improve their clonal formation ability, compared with the control group and the *BRCA1* wild-type group. Olaparib inhibited the proliferation of gallbladder cancer cell lines overexpressing *BRCA1* R1325K mutation (P<0.05). Through TUNEL and Western blotting, it was found that overexpression of wild-type *BRCA1* could induce the apoptosis of gallbladder cancer cell lines GBC-SD and NOZ, compared with the control group. Compared with the control group and the *BRCA1* wild-type group, the *BRCA1* R1325K mutation group had anti-apoptotic effect, in which the expression of apoptosis-inhibiting protein Bcl-2 increased and the expression of pro-apoptotic protein Bax decreased (P<0.05). **Conclusion**·*BRCA1* R1325K mutation can promote the proliferation of GBC-SD and NOZ cell lines and inhibit their apoptosis.

[Key words] gallbladder cancer; BRCA1; mutation; proliferation; apoptosis

胆囊癌是一种常见的胆道恶性肿瘤，现已成为全球消化道肿瘤发病率排名第五的恶性肿瘤[1]。我国属胆囊癌高发地区，且近年来发病率持续上升[2-3]。由于胆囊癌发病隐匿，早期无特异性临床表现，但侵袭性强，进展迅速，诊断时往往已达晚期，手术切除率低，术后易出现复发和远处转移，预后较差，5年生存率仅5%~15%[4-6]。根据国际癌症研究机构（International Agency for Research on Cancer，IARC）2020年全球癌症数据库[7]，每年胆囊癌新发病例占所有肿瘤的0.6%，死亡病例则占所有癌症死亡人口的0.9%。因此，推进对胆囊癌的相关研究，寻找新的胆囊癌标志物和治疗靶点，对于提高胆囊癌诊疗水平显得愈发重要。

BRCA（breast cancer susceptibility gene）基因家族在DNA损伤修复中发挥着重要作用。其中，*BRCA1*参与激活双链断裂修复和同源重组，还可通过调控转录及细胞周期来影响DNA损伤修复过程[8]。*BRCA1*突变分为生殖系突变（体内所有细胞均存在突变）和体细胞突变（仅在肿瘤细胞中检测到*BRCA1*突变）。目前已有许多研究表明，*BRCA*基因家族生殖系突变与前列腺癌、乳腺癌、卵巢癌、胰腺癌等许多肿瘤的发生和发展密切相关[9-11]。其中*BRCA1*生殖系突变是已知的最有可能导致女性家族性乳腺癌和卵巢癌的遗传因素，且与不良预后相关[12-14]。*BRCA1*的体细胞突变发生率低于生殖系突变。也有研究[15-16]表明在卵巢癌等肿瘤中，体细胞突变患者发病年龄更晚，但在治疗敏感性和无进展生存时间上与生殖系突变患者无明显差异。还有的研究[17-18]发现，在其他一些肿瘤，*BRCA1*突变有促进增殖与抑制凋亡的作用。

对于*BRCA1*突变的肿瘤，存在同源重组修复异常，而使用聚ADP-核糖聚合酶（poly ADP-ribose polymerase，PARP）抑制剂能够抑制DNA损伤修复过程，造成DNA双链断裂，继而引起修复混乱，通过“合成致死”效应达到促进肿瘤细胞凋亡的目的[19-20]。在此前的临床工作里，我们收治了1例*BRCA1* R1325K（第1325位精氨酸变为赖氨酸）生殖系突变胆囊癌患者；该患者使用PARP抑制剂奥拉帕利（Olaparib）后肿瘤缩小降期，达到R_0切除，成功实现了转化治疗。目前，在胆囊癌中，尚无*BRCA1* R1325K突变及其作用的相关报道。基于上述基础，本研究拟进一步探索*BRCA1*及其R1325K突变对于胆囊癌细胞增殖及凋亡的影响。

1 材料与方法

1.1 主要实验材料及仪器

胆囊癌细胞系GBC-SD和NOZ均为本实验室保存。使用GV705载体构建添加Flag标签及嘌呤霉素抗性基因但不含有目的基因的对照过表达慢病毒载体（Vector）、*BRCA1*野生型过表达慢病毒载体及*BRCA1* R1325K突变过表达慢病毒载体，并包装慢病毒（由上海吉凯生物科技有限公司完成）。

抗甘油醛-3-磷酸脱氢酶（GAPDH）抗体、α微管

蛋白（α-tubulin）抗体、Flag抗体、cleaved PARP抗体、Bcl-2抗体、Bax抗体（美国Cell Signaling Technology公司），BRCA1抗体（武汉三鹰生物技术有限公司），CCK8试剂盒（翊圣生物科技上海有限公司），一步法TUNEL细胞凋亡检测试剂盒（绿色荧光）、苯甲基磺酰氟（PMSF）（上海碧云天生物技术有限公司），Olaparib（美国MedChemExpress公司），中强度RIPA裂解液、5×上样缓冲液（上海雅酶生物科技有限公司）。

光学倒置显微镜（日本Nikon公司）；Leica倒置荧光显微镜DM2500［徕卡显微系统（上海）贸易有限公司］；美国MD SpectraMax 190全波长酶标仪［美谷分子仪器（上海）有限公司］；5424R型高速冷冻离心机（德国Eppendorf公司）；ChemiDoc XRS+凝胶成像系统（美国Bio-Rad公司）。

1.2　实验方法

1.2.1　*BRCA1*野生型和突变型胆囊癌稳转细胞系的构建　选用GBC-SD和NOZ胆囊癌细胞系，分别用*BRCA1*野生型及*BRCA1* R1325K突变过表达慢病毒颗粒感染，构建相应的胆囊癌稳转细胞系，并使用相应的阴性对照慢病毒构建对照胆囊癌稳转细胞系。具体如下：在六孔板中，每孔接种约1×10^5个细胞，待细胞生长至约70%融合度后，按慢病毒感染复数（MOI）为10加入*BRCA1*野生型或*BRCA1* R1325K突变过表达慢病毒载体感染，48 h后更换为含5 μg/mL嘌呤霉素的培养基（DMEM培养基+10%胎牛血清）进行抗性筛选，每日观察细胞生长情况，7 d后通过Western blotting验证目的蛋白BRCA1的表达情况。

刮取筛选后的细胞，使用含1% PMSF的RIPA裂解液和超声破碎仪充分裂解细胞。4 ℃、14 000×*g*离心15 min后吸取上清液，加入上样缓冲液，100 ℃加热10 min。使用7.5%凝胶，上样10 μL进行电泳（130 V，80 min），电泳结束后，冰浴下转至PVDF膜（转膜条件：100 V，200 min）。转膜结束后使用5%脱脂奶粉封闭1 h，洗净脱脂奶后分别滴加BRCA1一抗和α-tubulin一抗（均为1∶1 000稀释），4 ℃孵育过夜，次日PBST洗膜3次，每次10 min；加入辣根过氧化物酶标记二抗室温孵育1 h，PBST洗膜3次，每次10 min；滴加显影液于PVDF膜上，ChemiDoc XRS+凝胶成像系统曝光并观察条带。

1.2.2　CCK8实验　细胞分为对照组（Vector组）、*BRCA1*野生型组（*BRCA1* wt组）和*BRCA1*突变组（*BRCA1* mut组）和*BRCA1* mut+Olaparib组。将细胞消化后进行计数，吹打均匀后按1 200个/孔均匀铺于96孔板中，每孔培养基（DMEM培养基+10%胎牛血清）总量100 μL。待细胞贴壁后，*BRCA1* mut+Olaparib组更换为含20 μmol/L Olaparib的新鲜全培养基，其他组更换为新鲜全培养基，并分别在细胞贴壁后0、24、48、72、96 h时，用CCK8试剂盒进行检测。具体方法为：吸净待测孔的培养基，每孔加入100 μL无血清培养基和10 μL CCK8检测液，37 ℃避光孵育2 h，用酶标仪检测450 nm波长处的吸光度值。每组设置5个复孔。实验数据用Graphpad Prism 9软件绘制折线图。

1.2.3　克隆形成实验　将Vector组、*BRCA1* wt组、*BRCA1* mut组和*BRCA1* mut+Olaparib组细胞消化后进行计数，轻柔吹打均匀使之分散成单个细胞，并按600个/皿铺于35 mm培养皿中。待细胞贴壁后，*BRCA1* mut+Olaparib组更换为含20 μmol/L Olaparib的新鲜全培养基，其他组更换为新鲜全培养基。每3日更换1次对应的新鲜全培养基，每日光学倒置显微镜下观察细胞的增殖情况。当培养皿中出现肉眼可见的克隆时，吸净培养基，每皿用PBS洗2遍后加入4%多聚甲醛，固定30 min后用结晶紫染色，洗净结晶紫后拍照，光学倒置显微镜下计数大于10个细胞的克隆数并用Graphpad Prism 9软件绘制柱状图。

1.2.4　TUNEL实验检测细胞凋亡　使用Vector组、*BRCA1* wt组、*BRCA1* mut组和*BRCA1* mut+Olaparib组细胞制作细胞爬片。制作完成后，用PBS清洗1次，加入4%多聚甲醛固定30 min后，PBS清洗1次，加入含0.3% Triton-X的PBS室温孵育5 min，PBS清洗2次。按照试剂盒所示比例配置检测液（TdT酶：荧光检测液=1∶9），每片细胞爬片滴加25 μL检测液，4 ℃摇床避光孵育过夜，次日PBS清洗3次，用含DAPI的抗荧光淬灭封片剂进行封片。封片后37 ℃避光孵育45 min，于荧光显微镜下观察，分别拍摄DAPI染色图像（曝光时间20 ms）和TUNEL染色图像（曝光时间400 ms），并使用Image J软件进行合并。计算凋亡细胞比例并用Graphpad Prism 9软件绘制柱状图。

1.2.5　Western blotting检测凋亡相关蛋白的表达　刮取细胞，分别置于1.5 mL离心管中，洗净残余培养基，加入100 μL含1% PMSF的RIPA裂解液，吹匀后，用超声破碎仪充分裂解细胞。4 ℃、14 000×*g*离

心15 min后吸取上清液，加入上样缓冲液，100 ℃加热10 min。使用10%电泳凝胶，上样10 μL进行电泳（130 V，65 min）。电泳结束后，冰浴下转至PVDF膜（转膜条件：100 V，100 min），转膜结束后使用5%脱脂牛奶封闭1 h。洗净后分别滴加cleaved PARP一抗、Bcl-2一抗、Bax一抗和GAPDH一抗（均为1∶1 000稀释），4 ℃孵育过夜，次日PBST洗膜3次，每次10 min；之后加入辣根过氧化物酶标记的二抗室温孵育1 h，再次PBST洗膜3次，每次10 min；之后滴加显影液于PVDF膜上，ChemiDoc XRS+凝胶成像系统曝光并观察条带。再用Image J软件进行灰度分析，并用Graphpad Prism 9软件对相对蛋白表达量进行统计作图。

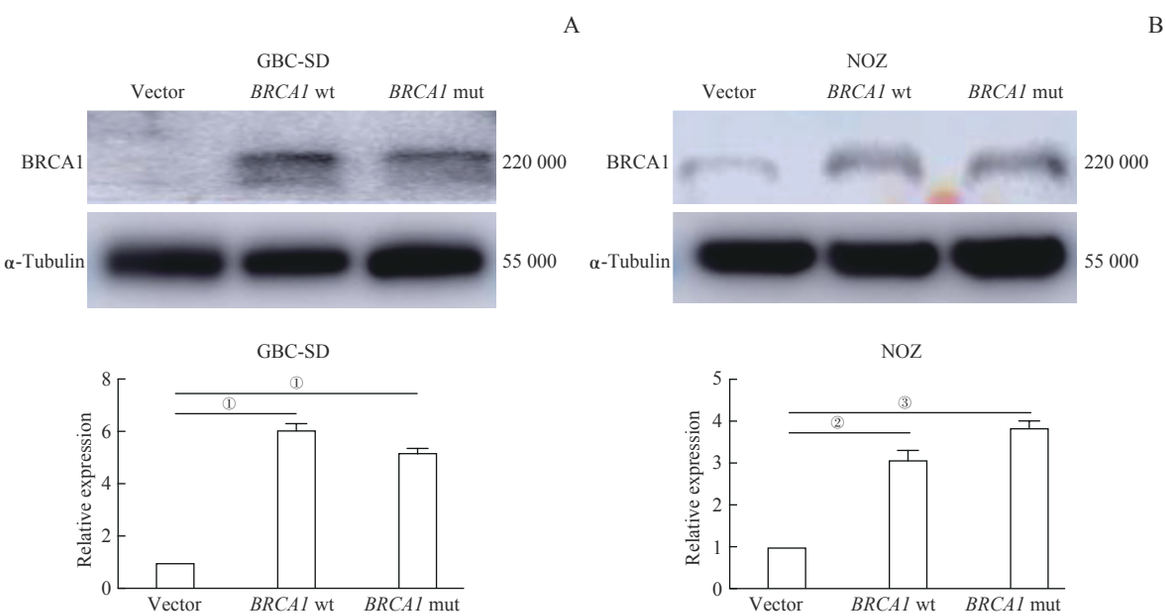
1.3 统计学分析

采用SPSS 22.0软件进行数据统计分析。定量资料用$\bar{x}\pm s$表示。组间比较采用独立样本t检验。$P<0.05$表示差异有统计学意义。

2 结果

2.1 过表达慢病毒感染后的胆囊癌细胞系稳定表达野生型或突变型BRCA1蛋白

用Western blotting对GBC-SD和NOZ细胞系Vector组、*BRCA1* wt组及*BRCA1* mut组目的蛋白BRCA1的表达情况进行验证。如图1A及B所示，对照过表达慢病毒载体感染的GBC-SD和NOZ细胞（Vector组）几乎无内源性BRCA1表达，*BRCA1*野生型慢病毒及*BRCA1* R1325K突变慢病毒感染后的细胞（*BRCA1* wt组、*BRCA1* mut组）则有相应野生型或突变型BRCA1蛋白的稳定表达，且表达量较高。



Note: A. Western blotting was used to detect BRCA1 protein expression in GBC-SD cell line. B. Western blotting was used to detect BRCA1 protein expression in NOZ cell line. ①P=0.000, ②P=0.003, ③P=0.001.

图1 *BRCA1*野生型及*BRCA1* R1325K突变过表达慢病毒感染后胆囊癌细胞系GBC-SD和NOZ中BRCA1蛋白的表达情况

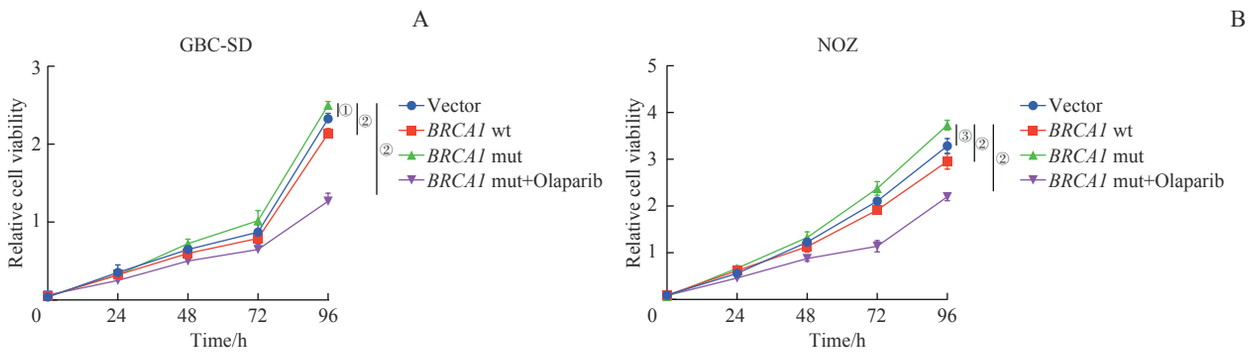
Fig 1 Expression of BRCA1 protein in gallbladder cancer cell lines GBC-SD and NOZ after *BRCA1* wild-type and *BRCA1* R1325K mutation overexpression lentivirus infection

2.2 *BRCA1* R1325K突变对胆囊癌细胞增殖能力的影响

*BRCA1*野生型和突变型胆囊癌稳转细胞系在Olaparib处理0、24、48、72、96 h后进行CCK8检测。如图2A、B所示，在GBC-SD和NOZ细胞中，*BRCA1* wt组的细胞增殖能力与Vector组相比差异没有统计学意义（均P>0.05），而*BRCA1* mut组的细胞增殖能力较Vector组明显增强（均P<0.05）；但在加入20 μmol/L Olaparib（适用于*BRCA1*/2突变的靶向药）处理96 h之后，*BRCA1* mut组GBC-SD和NOZ细胞的增殖能力均受到了显著抑制（均P<0.05）。

2.3 *BRCA1* R1325K突变对胆囊癌细胞克隆形成能力的影响

如图3A~D所示，克隆形成实验结果提示：与Vector组相比，过表达野生型*BRCA1*基因对胆囊癌

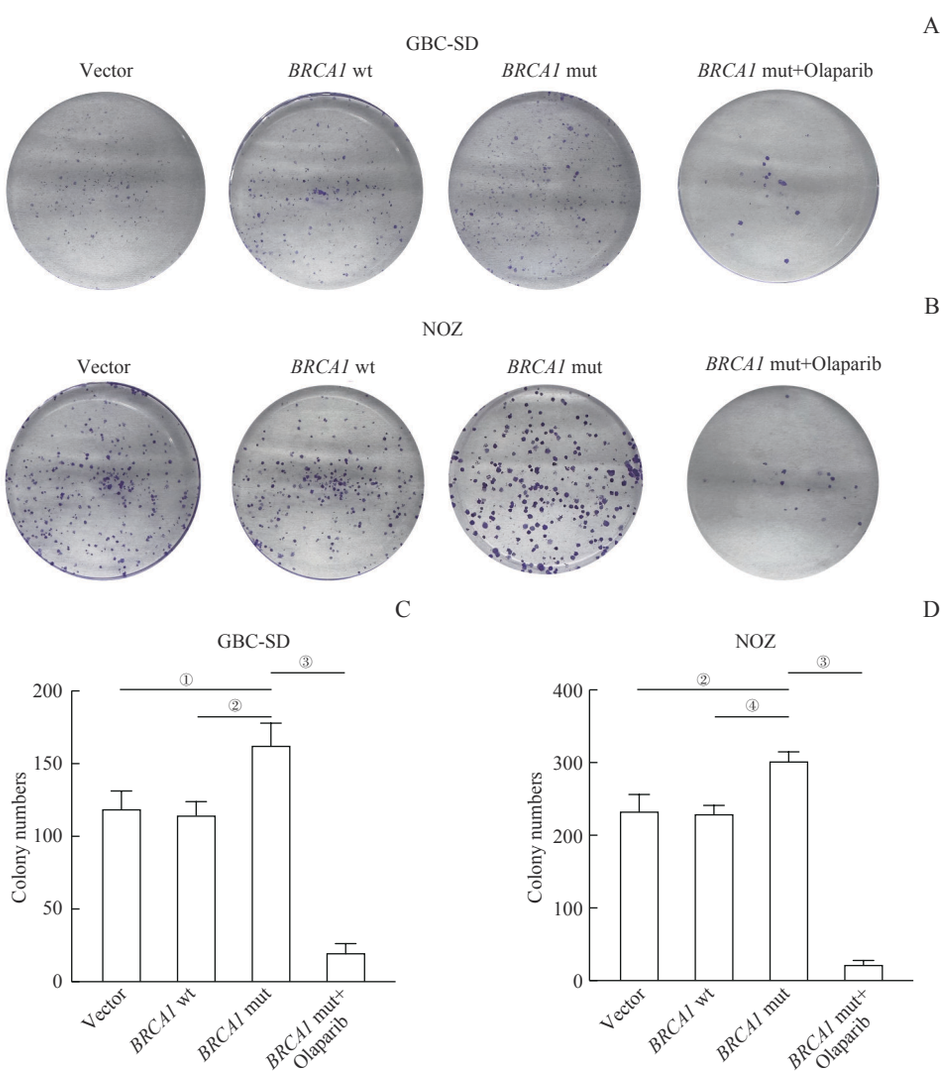


Note：A. Proliferation curves of GBC-SD cell line. B. Proliferation curves of NOZ cell line. ①*P*=0.002, ②*P*=0.000, ③*P*=0.001.

图2　*BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ增殖能力的影响

Fig 2　Effects of *BRCA1* R1325K mutation on proliferation of gallbladder cancer cell lines GBC-SD and NOZ

GBC-SD和NOZ细胞的克隆形成能力无明显影响；但*BRCA1* mut组相较于Vector组和*BRCA1* wt组，胆囊癌细胞的克隆形成能力均明显提高（*P*<0.05）；在加入20 μmol/L Olaparib后，*BRCA1* mut组胆囊癌细胞的克隆形成能力显著下降（*P*<0.05）。



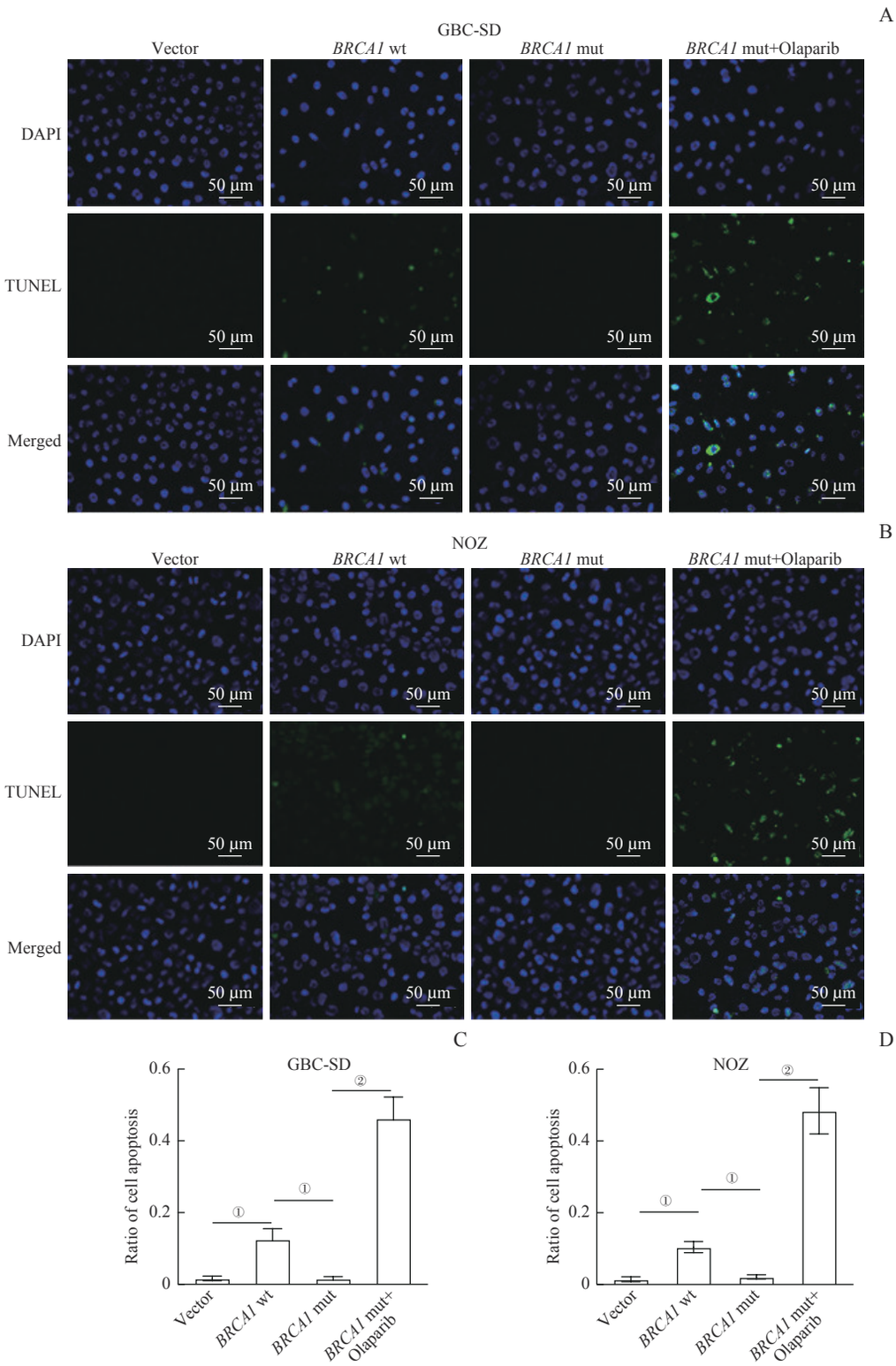
Note：A. The representative pictures of colony assay in GBC-SD cell line. B. The representative pictures of colony assay in NOZ cell line. C. The statistical graph of colony numbers for GBC-SD cell line. D. The statistical graph of colony numbers for NOZ cell line. ①*P*=0.015, ②*P*=0.008, ③*P*=0.000, ④*P*=0.001.

图3　*BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ克隆形成能力的影响

Fig 3　Effects of *BRCA1* R1325K mutation on colony formation ability of gallbladder cancer cell lines GBC-SD and NOZ

2.4 *BRCA1* R1325K突变对胆囊癌细胞凋亡的影响

根据文献[21]报道，*BRCA1*的高表达在某些肿瘤中可促进凋亡，而其突变则能产生抑制凋亡的作用，故我们用TUNEL实验对胆囊癌细胞GBC-SD和NOZ的凋亡情况进行了检测。如图4A~D所示，Vector组胆囊癌细胞未见明显凋亡，在过表达野生型*BRCA1*



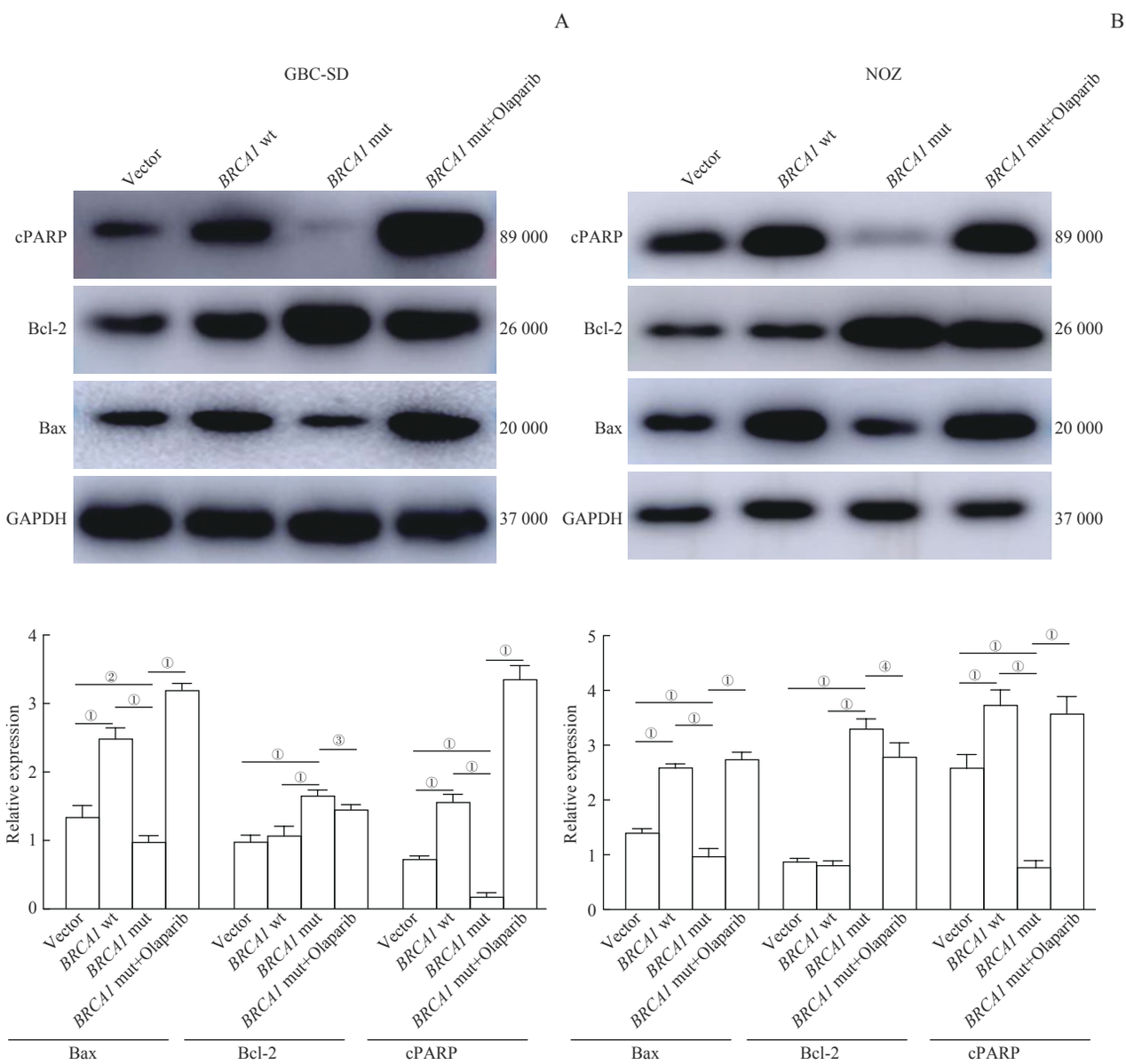
Note: A. The representative pictures of TUNEL assay in GBC-SD cell line. B. The representative pictures of TUNEL assay in NOZ cell line. C. The statistical graph of apoptosis ratio for GBC-SD cell line. D. The statistical graph of apoptosis ratio for NOZ cell line. ①P=0.001, ②P=0.000.

图4 *BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ凋亡的影响

Fig 4 Effects of *BRCA1* R1325K mutation on apoptosis of gallbladder cancer cell lines GBC-SD and NOZ

后，可观察到细胞出现部分凋亡，而过表达*BRCA1* R1325K突变则未见细胞凋亡，表明过表达野生型*BRCA1*可以促使胆囊癌细胞系发生凋亡，而突变型*BRCA1*无此效应。但在加入20 μmol/L Olaparib处理后，*BRCA1* mut组重新出现凋亡现象。

进一步，我们用Western blotting对于*BRCA1* R1325K突变前后的凋亡相关蛋白的表达情况进行了检测。如图5A和B所示，相较于*BRCA1*野生型，过表达突变型*BRCA1*后，cleaved PARP及促凋亡蛋白Bax的表达量降低，凋亡抑制蛋白Bcl-2的表达量升高，提示*BRCA1* R1325K突变后的胆囊癌细胞有一定的抗凋亡能力。而加入20 μmol/L Olaparib处理后，Bcl-2的表达量则降低，并且cleaved PARP及Bax的表达量升高。综上所述，初步认为，野生型*BRCA1*的过表达会促使胆囊癌细胞发生凋亡，*BRCA1* R1325K突变则能够产生抗凋亡的作用。



Note：A. Effects of *BRCA1* R1325K mutation on expression of apoptosis-related proteins in GBC-SD cell line. B. Effects of *BRCA1* R1325K mutation on expression of apoptosis-related proteins in NOZ cell line. cPARP—cleaved PARP. ①*P*=0.000, ②*P*=0.004, ③*P*=0.002, ④*P*=0.009.

图5　*BRCA1* R1325K突变对胆囊癌细胞系GBC-SD和NOZ凋亡相关蛋白表达情况的影响

Fig 5　Effects of *BRCA1* R1325K mutation on expression of apoptosis-related proteins in gallbladder cancer cell lines GBC-SD and NOZ

3 讨论

作为胆道系统最常见的恶性肿瘤，胆囊癌起病隐匿，手术依然是其主要的治疗手段。但胆囊癌早期临床症状不典型，多数患者就诊时即已失去手术切除的机会，局部进展、转移性或复发患者只能进行化学治疗（化疗）及其他综合治疗，目前大多难有令人满意的疗效和预后。靶向药物和免疫治疗通过各种机制起到抑制和杀伤肿瘤的作用，其中分子靶向治疗具有特异性强、不良反应小的特点，在多种实体瘤的治疗中发挥了巨大作用。但目前胆囊癌缺乏特异性分子标志物，对于潜在的靶向药物敏感度较低，因此特异性肿瘤标志物的寻找将为胆囊癌的靶向治疗提供新的方向，具有十分重要的意义。

*BRCA*家族基因是一种十分重要的抑癌基因，其高表达在某些肿瘤中有抑制增殖与促进凋亡的作用[21-24]。其他研究[9-11]表明，*BRCA*家族基因突变与乳腺癌、卵巢癌、胰腺癌、前列腺癌、宫颈癌等肿瘤的发病密切相关。Olaparib是美国食品和药物管理局（FDA）批准的靶向*BRCA1*和*BRCA2*（*BRCA1/2*）突变的药物，被称为聚ADP-核糖聚合酶抑制剂（PARPi）。在临床试验中，PARPi改善了包括乳腺癌、卵巢癌、胰腺癌和前列腺癌在内的多种*BRCA1/2*突变肿瘤患者的预后[25-29]。我们在前期的临床工作中发现了1例*BRCA1* R1325K突变的胆囊癌病例，该患者肿瘤进展较其他无突变的胆囊癌患者迅速，但对于靶向药Olaparib反应良好，在口服Olaparib之后，肿瘤缩小降期并成功实现了R_0切除，达到了转化治疗目的。基于此，我们对于*BRCA1* R1325K在胆囊癌发生和发展的作用产生了兴趣，进而使用胆囊癌细胞系GBC-SD和NOZ构建了相应的稳转细胞系，再通过CCK8、Western blotting、TUNEL等实验验证了*BRCA1* R1325K突变对胆囊癌发生和发展的促进作用；同时探究了抑制剂Olaparib对*BRCA1* R1325K突变胆囊癌的抑制作用，这些也为相似临床病例的治疗提供了一定的借鉴。

目前，*BRCA1*突变在胆囊癌中的研究仍属罕见，且多为临床病例，已有报道的位点如*BRCA1* Q858*[30]、*BRCA1* p. S451Lfs*20[31]等，但R1325K位点突变尚属首例。本文从细胞层面，对*BRCA1* R1325K突变促进胆囊癌发生和发展的作用进行了验证，并发现其能够升高凋亡抑制蛋白Bcl-2的表达、降低促凋亡蛋白Bax的表达，为进一步探究*BRCA1* R1325K突变促进增殖及抑制凋亡的具体机制奠定了基础。根据既往文献报道，BRCA1参与转录调节过程，能够与p53蛋白的C端区域结合，形成复合物并能作为共激活分子调控其下游基因的表达[32]，其对PI3K/Akt通路也有调节作用[33-34]。我们已发现*BRCA1* R1325K突变能够影响Bcl-2/Bax的表达，而p53、PI3K/Akt也能够直接或间接调控Bcl-2/Bax的表达[35-39]。由此我们推测，*BRCA1* R1325K可能是通过上调p53表达或激活PI3K/Akt信号通路等机制调节Bcl-2、Bax及其下游通路分子如Cyt-C、Caspase-3等相关蛋白的表达，从而抑制胆囊癌细胞的凋亡，这与为我们后续的机制探索提供了一定的思路。

总之，本研究发现*BRCA1* R1325K能够促进胆囊癌增殖并抑制其凋亡，这一效应能够被Olaparib所抑制，这为胆囊癌发生和发展机制的探究提供了新思路，也为胆囊癌的临床治疗提供了新的靶点。而在后续的工作中，我们将进一步深入探究*BRCA1* R1325K突变促进胆囊癌细胞增殖并抑制其凋亡的具体机制，并结合体内实验和临床样本验证，同时联合多中心收集病例，开展相应的临床研究，或许有望找到胆囊癌诊断和治疗的新靶点，并为胆囊癌的个体化治疗提供新的思路。

利益冲突声明/Conflict of Interests

所有作者声明均不存在利益冲突

All authors disclose no relevant conflict of interests

作者贡献/Authors′Contributions

杨婧潇、李茂岚参与了实验设计以及论文的写作和修改。杨婧潇、贾子尧、朱逸荻、张飞负责实验操作。杨婧潇、李怀峰、吴文广、吴向嵩负责数据分析。所有作者均阅读并同意了最终稿件提交。

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参·考·文·献

[1] SCHMIDT M A, MARCANO-BONILLA L, ROBERTS L R. Gallbladder cancer: epidemiology and genetic risk associations[J]. Chin Clin Oncol, 2019, 8(4): 31.

[2] BAIU I, VISSER B. Gallbladder cancer[J]. JAMA, 2018, 320(12): 1294.

[3] MIRANDA-FILHO A, PIÑEROS M, FERRECCIO C, et al. Gallbladder and extrahepatic bile duct cancers in the Americas: incidence and mortality patterns and trends[J]. Int J Cancer, 2020, 147(4): 978-989.

[4] SHARMA A, SHARMA K L, GUPTA A, et al. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: recent update[J]. World J Gastroenterol, 2017, 23(22): 3978-3998.

[5] LAMARCA A, EDELINE J, MCNAMARA M G, et al. Current standards and future perspectives in adjuvant treatment for biliary tract cancers[J]. Cancer Treat Rev, 2020, 84: 101936.

[6] ROA J C, GARCÍA P, KAPOOR V K, et al. Gallbladder cancer[J]. Nat Rev Dis Primers, 2022, 8: 69.

[7] SUNG H, FERLAY J, SIEGEL R L, et al. Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA A Cancer J Clin, 2021, 71(3): 209-249.

[8] YOSHIDA K, MIKI Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage[J]. Cancer Sci, 2004, 95(11): 866-871.

[9] NA R, ZHENG S L, HAN M, et al. Germline mutations in *ATM* and *BRCA1/2* distinguish risk for lethal and indolent prostate cancer and are associated with early age at death[J]. Eur Urol, 2017, 71(5): 740-747.

[10] JOHANNSSON O, LOMAN N, MÖLLER T, et al. Incidence of malignant tumours in relatives of *BRCA1* and *BRCA2* germline mutation carriers[J]. Eur J Cancer, 1999, 35(8): 1248-1257.

[11] YEHIA L, KEEL E, ENG C. The clinical spectrum of *PTEN* mutations[J]. Annu Rev Med, 2020, 71: 103-116.

[12] WOOSTER R, WEBER B L. Breast and ovarian cancer[J]. N Engl J Med, 2003, 348(23): 2339-2347.

[13] NAROD S A. Which genes for hereditary breast cancer?[J]. N Engl J Med, 2021, 384(5): 471-473.

[14] DANENBERG E, BARDWELL H, ZANOTELLI V R T, et al. Breast tumor microenvironment structures are associated with genomic features and clinical outcome[J]. Nat Genet, 2022, 54(5): 660-669.

[15] KONSTANTINOPOULOS P A, NORQUIST B, LACCHETTI C, et al. Germline and somatic tumor testing in epithelial ovarian cancer: ASCO guideline[J]. J Clin Oncol, 2020, 38(11): 1222-1245.

[16] SUN S X, BRAZHNIK K, LEE M, et al. Single-cell analysis of somatic mutation burden in mammary epithelial cells of pathogenic *BRCA1/2* mutation carriers[J]. J Clin Invest, 2022, 132(5): e148113.

[17] FEILOTTER H E, MICHEL C, UY P, et al. BRCA1 haploinsufficiency leads to altered expression of genes involved in cellular proliferation and development[J]. PLoS One, 2014, 9(6): e100068.

[18] WERNER H. BRCA1: an endocrine and metabolic regulator[J]. Front Endocrinol (Lausanne), 2022, 13: 844575.

[19] MOSCHETTA M, GEORGE A, KAYE S B, et al. *BRCA* somatic mutations and epigenetic BRCA modifications in serous ovarian cancer[J]. Ann Oncol, 2016, 27(8): 1449-1455.

[20] FARMER H, MCCABE N, LORD C J, et al. Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy[J]. Nature, 2005, 434(7035): 917-921.

[21] HARKIN DP, BEAN JM, MIKLOS D, et al. Induction of *GADD45* and JNK/SAPK-dependent apoptosis following inducible expression of *BRCA1*[J]. Cell, 1999, 97(5): 575-586.

[22] PAPADAKI C, TSAROUCHA E, KAKLAMANIS L, et al. Correlation of *BRCA1*, *TXR1* and *TSP1* mRNA expression with treatment outcome to docetaxel-based first-line chemotherapy in patients with advanced/metastatic non-small-cell lung cancer[J]. Br J Cancer, 2011, 104(2): 316-323.

[23] BUSACCA S, SHEAFF M, ARTHUR K, et al. BRCA1 is an essential mediator of vinorelbine-induced apoptosis in mesothelioma[J]. J Pathol, 2012, 227(2): 200-208.

[24] DEVICO MARCIANO N, KROENING G, DAYYANI F, et al. *BRCA*-mutated pancreatic cancer: from discovery to novel treatment paradigms[J]. Cancers, 2022, 14(10): 2453.

[25] DE BONO J, MATEO J, FIZAZI K, et al. Olaparib for metastatic castration-resistant prostate cancer[J]. N Engl J Med, 2020, 382(22): 2091-2102.

[26] GOLAN T, HAMMEL P, RENI M, et al. Maintenance olaparib for germline *BRCA*-mutated metastatic pancreatic cancer[J]. N Engl J Med, 2019, 381(4): 317-327.

[27] MOORE K, COLOMBO N, SCAMBIA G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer[J]. N Engl J Med, 2018, 379(26): 2495-2505.

[28] HUSSAIN M, MATEO J, FIZAZI K, et al. Survival with olaparib in metastatic castration-resistant prostate cancer[J]. N Engl J Med, 2020, 383(24): 2345-2357.

[29] ROBSON M E, TUNG N, CONTE P, et al. OlympiAD final overall survival and tolerability results: olaparib *versus* chemotherapy treatment of physician's choice in patients with a germline *BRCA* mutation and HER2-negative metastatic breast cancer[J]. Ann Oncol, 2019, 30(4): 558-566.

[30] XIE Y, JIANG Y, YANG X B, et al. Response of *BRCA1*-mutated gallbladder cancer to olaparib: a case report[J]. World J Gastroenterol, 2016, 22(46): 10254-10259.

[31] LI X F, GAO L M, QIU M, et al. Olaparib treatment in a patient with advanced gallbladder cancer harboring *BRCA1* mutation[J]. Onco Targets Ther, 2021, 14: 2815-2819.

[32] OUCHI T, MONTEIRO A N, AUGUST A, et al. BRCA1 regulates p53-dependent gene expression[J]. Proc Natl Acad Sci USA, 1998, 95(5): 2302-2306.

[33] ZHANG L, ZHOU Q, QIU Q Z, et al. CircPLEKHM3 acts as a tumor suppressor through regulation of the miR-9/BRCA1/DNAJB6/KLF4/AKT1 axis in ovarian cancer[J]. Mol Cancer, 2019, 18(1): 144.

[34] LIU X L, LIU H X, ZENG L, et al. BRCA1 overexpression attenuates breast cancer cell growth and migration by regulating the pyruvate kinase M2-mediated Warburg effect *via* the PI3K/AKT signaling pathway[J]. PeerJ, 2022, 10: e14052.

[35] MIYASHITA T, KRAJEWSKI S, KRAJEWSKA M, et al. Tumor suppressor p53 is a regulator of *bcl-2* and *bax* gene expression *in vitro* and *in vivo*[J]. Oncogene, 1994, 9(6): 1799-1805.

[36] MIYASHITA T, HARIGAI M, HANADA M, et al. Identification of a p53-dependent negative response element in the *Bcl-2* gene[J]. Cancer Res, 1994, 54(12): 3131-3135.

[37] RESHI L, WU H C, WU J L, et al. GSIV serine/threonine kinase can induce apoptotic cell death *via* p53 and pro-apoptotic gene Bax upregulation in fish cells[J]. Apoptosis, 2016, 21(4): 443-458.

[38] DASHZEVEG N, YOSHIDA K. Cell death decision by p53 *via* control of the mitochondrial membrane[J]. Cancer Lett, 2015, 367(2): 108-112.

[39] LAYANI-BAZAR A, SKORNICK I, BERREBI A, et al. Redox modulation of adjacent thiols in VLA-4 by AS101 converts myeloid leukemia cells from a drug-resistant to drug-sensitive state[J]. Cancer Res, 2014, 74(11): 3092-3103.

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