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BCSC-1与IQGAP1对乳腺癌细胞迁移的影响及其在浸润性导管癌中的临床意义

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摘要 该研究旨在探讨BCSC-1与IQGAP1对乳腺癌细胞迁移的影响及其在乳腺浸润性导管癌中的临床意义。免疫沉淀实验检测乳腺癌细胞MCF-7中BCSC-1与IQGAP1是否存在相互作用; 细胞免疫荧光实验检测MCF-7细胞中BCSC-1与IQGAP1的共定位情况; Western blot检测BCSC-1、IQGAP1分别过表达对对方表达水平的影响; 细胞划痕实验、细胞免疫荧光实验结合RNA干扰检测BCSC-1与IQGAP1在迁移的MCF-7、MDA-MB-231细胞中的定位情况及二者在细胞膜前缘定位的相互依赖性; Western blot检测各种乳腺癌细胞中BCSC-1与IQGAP1的蛋白表达情况; 免疫组织化学染色法检测BCSC-1与IQGAP1在乳腺浸润性导管癌与癌旁组织中的表达情况及与乳腺浸润性导管癌临床病理特征关系的相关性。在MCF-7细胞中BCSC-1与IQGAP1存在相互作用及共定位; BCSC-1、IQGAP1的分别过表达并不影响彼此的表达水平; 无论MCF-7和MDA-MB-231是处于随机迁移状态, 还是处于定向迁移状态, BCSC-1都定位在乳腺癌细胞胞质以及膜前缘, 与IQGAP1存在着共定位分布, 且二者对将彼此募集到膜前缘具有相互依赖性; 在多种乳腺癌细胞中, BCSC-1呈低表达而IQGAP1呈高表达; 与癌旁组织相比, 在浸润性导管癌组织中, BCSC-1的表达下调(P<0.05), IQGAP1的表达上调(P<0.05); 在浸润性导管癌组织中, BCSC-1的表达水平与乳腺癌的组织学级别、淋巴结转移有关(P<0.05), 与ER、PR和HER2的状态无关(P>0.05), 而IQGAP1的高表达水平与淋巴结转移有关(P<0.05), 与HER2的状态有关(P<0.05); BCSC-1和IQGAP1的表达呈负相关(R=–0.416, P<0.05)。BCSC-1和IQGAP1存在相互作用, 协同调控乳腺癌细胞迁移, 二者参与了乳腺浸润性导管癌的发展, 并与其转移性相关。

关键词 BCSC-1; IQGAP1; 细胞迁移; 浸润性导管癌

The Effect of BCSC-1 and IQGAP1 on the Migration of Breast Cancer Cells and Their Clinical Significance in Invasive Ductal Carcinoma

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Abstract This study aims to investigate the effect of BCSC-1 and IQGAP1 on the migration of breast cancer cells and their clinical significance in invasive ductal carcinoma of the breast. The interaction between BCSC-1 and IQGAP1 in breast cancer cell MCF-7 was detected by immunoprecipitation assay. Cell immunofluorescence assay was used to detect the co-localization of BCSC-1 and IQGAP1 in MCF-7 cells. Western blot was used to detect the respective effect of BCSC-1 overexpression and IQGAP1 overexpression on the expression level of IQGAP1 and BCSC-1. Cell scratch assay, cell immunofluorescence assay combined with RNA interference were used to detect the localization of BCSC-1 and IQGAP1 in migrating MCF-7 and MDA-MB-231 cells, as well as their interdependence of localization to the leading edge of membrane. Western blot was used to detect the protein expression of BCSC-1 and IQGAP1 in various breast cancer cells. Immunohistochemical staining was used to detect the expression of BCSC-1 and IQGAP1 in invasive ductal carcinoma of the breast and adjacent tissues, as well as their correlation with the clinical and pathological characteristics of invasive ductal carcinoma of the breast. BCSC-1 interacted with IQGAP1 and co-localized with IQGAP1 in MCF-7 cells; BCSC-1 and IQGAP1 expression did not influence each other. No matter whether MCF-7 cells and MDA-MB-231 cells were in a random migration state or a directional migration state, BCSC-1 was located in the cytoplasm and membrane front of breast cancer cells, co-located with IQGAP1, and their localization to the leading edge of membrane were interdependent; BCSC-1 was downregulated and IQGAP1 was upregulated in a variety of breast cancer cells; BCSC-1 was downregulated ($P<0.05$), while IQGAP1 was upregulated in invasive ductal carcinoma tissues compared with adjacent tissues ($P<0.05$). In invasive ductal carcinoma, the low expression of BCSC-1 was related to the histological grade and lymph node metastasis of breast cancer ($P<0.05$), but not to the status of ER, PR and HER2 ($P>0.05$), while the high expression of IQGAP1 was related to lymph node metastasis ($P<0.05$) and HER2 status ($P<0.05$). The expression of BCSC-1 and IQGAP1 showed a negative correlation ($R=-0.416$, $P<0.05$). BCSC-1 and IQGAP1 interact to synergistically regulate the migration of breast cancer cells. They are involved in the development of invasive ductal carcinoma of the breast and are related to its metastasis.

Keywords BCSC-1; IQGAP1; cell migration; invasive ductal carcinoma

乳腺癌是世界范围内女性最常见的恶性肿瘤, 也是女性最常见的与癌症相关的死亡原因, 其发病率呈逐年上升趋势[1-2]。因为缺乏早期特定症状或生物标志物, 大多数乳腺癌患者往往在晚期被诊断, 因此, 早期诊断和靶向治疗对乳腺癌尤为重要。根据世界卫生组织乳腺肿瘤分类, 乳腺癌最常见的组织学类型是浸润性导管癌(invasive ductal carcinoma, IDC)[3]。乳腺癌的发生涉及多种因素、基因和信号通路, 但其发病机制尚未被完全阐明。

乳腺癌候选抑癌基因-1(breast cancer suppressor candidate-1, *BCSC-1*), 位于人类染色体11q23-q24, 编码分子量为86 kDa的蛋白质, 是从早期乳腺癌患者细胞中发现的一个抑癌基因[4]。BCSC-1在多种肿瘤(包括鼻咽癌、宫颈癌和黑色素瘤)细胞或组织中表现为表达缺失或低表达[4-8]; 在之前的研究中, 我们发现BCSC-1在乳腺癌和食管鳞状细胞癌中低表达[9-11]。然而, 这些研究还不足以阐释BCSC-1在肿瘤发生和恶性发展中的作用和分子机制。

IQ结构域GTP酶激活蛋白1(IQ-domain GTPase-activating protein 1, IQGAP1), 在组织细胞中广泛表达, 分子量190 kDa。它通过与100多个结合蛋白的相互作用, 参与了多种生物学功能, 包括细胞骨架动力学、细胞间黏附、细胞运动/侵袭, 细胞增殖等[12]。研究发现, IQGAP1在多种肿瘤(包括乳腺癌)中过表达。作为很多信号通路的支架蛋白, IQGAP1促进了肿瘤的发生发展[13-15]。

本实验室前期利用过表达BCSC-1的乳腺癌细胞MCF-7, 以BCSC-1抗体进行免疫沉淀串联质谱分析(immunoprecipitation-mass spectrometry, IP-MS), 发现IQGAP1是BCSC-1的新的潜在互作蛋白。目前尚无关于BCSC-1和IQGAP1相互间作用的报道。本研究拟采用免疫沉淀技术和细胞免疫荧光染色, 确证BCSC-1和IQGAP1在乳腺癌细胞中是否存在相互作用, 利用RNA干扰手段研究BCSC-1和IQGAP1对

乳腺癌细胞迁移的影响，还采用免疫组织化学方法检测BCSC-1和IQGAP1在乳腺浸润性导管癌中的表达情况，并分析二者在IDC中与各临床病理特征之间的关系，以期为研究乳腺癌发展发展的分子机制奠定基础，并为乳腺癌的早期诊断和靶向治疗提供潜在的生物标志物和分子靶点。

1 材料与方法

1.1 材料

组织微阵列和临床组织标本。组织微阵列包含40例乳腺癌病例，其中包括37例浸润性导管癌、2例浸润性小叶癌和1例髓样癌，以及40例正常乳腺组织病例，购自西安艾莉娜生物科技有限公司(编号：BR804b)。在患者接受任何治疗之前，我们从山东第二医科大学附属医院病理中心收集了41例浸润性导管患者的癌组织及其配对的癌旁组织标本。所有患者均签署了知情同意书。本研究获得了山东第二医科大学医学伦理委员会的批准(伦理审批号：2021YX050)。

乳腺癌细胞MCF-10A、MCF-7、MDA-MB-231、T47D、ZR75-1、HS578T、HCC1143、MDA-MB-453和MDA-MB-468购自中国科学院上海细胞库；293T细胞为本实验室保存；pLV-Neo慢病毒载体购自北京英茂盛业生物科技有限公司；pLV-Neo-Flag-BCSC-1、pcDNA3.1-BCSC-1、pcDNA3.1-IQGAP1及其相应对照质粒为本实验室构建；DMEM培养基、胰蛋白酶、胎牛血清购自北京索莱宝科技有限公司；兔抗人BCSC-1抗体由南京金斯瑞生物科技有限公司制备；兔抗人IQGAP1抗体购自CST公司；兔抗人GAPDH抗体、β-actin抗体、HRP标记的羊抗兔IgG均购自Sigma公司；ECL化学发光检测试剂盒购自Amersham Biosciences公司；蛋白A/G琼脂糖珠(protein A/G agarose beads)购自Santa Cruz公司；G418、Lipofectamine 2000、BCA蛋白浓度测定试剂盒、RIPA细胞裂解液和蛋白质相对分子质量标志物、微丝红色荧光探针、TRITC标记羊抗兔IgG(H+L)、FITC标记羊抗兔IgG(H+L)、Alexa Fluor 350标记羊抗兔IgG(H+L)、DAPI染色液均购自上海碧云天生物技术股份有限公司；SP免疫组织化学试剂盒购自北京中杉金桥生物技术有限公司。siRNA靶向人*BCSC-1*(NM_014622.4)的序列为：5′-GAG TTT ACC TAT AGG CTG TTA-3′；siRNA靶向人*IQGAP1*(NM_003870.4)的序列为：5′-AGG ACC TGC TGC AGC TAC A-3′。所有的siRNA均由上海吉玛制药技术有限公司合成。

1.2 方法

1.2.1 慢病毒颗粒的制备及细胞转染 将pLV-Neo-Flag-BCSC-1，其相应的空载体，慢病毒辅助包装载体psPAX2、pMD2.G质粒，分别进行高纯度质粒抽提，按照Lipofectamine 2000说明书转染293T细胞，分别收集病毒液上清，过滤，在6孔板内感染乳腺癌细胞MCF-7。24 h后换液，感染72 h后，弃掉培养基，每孔加入含G418(4 mg/mL)的培养基筛选阳性克隆细胞，挑取、扩大培养并进行Western blot鉴定，获得过表达BCSC-1及空载对照慢病毒的MCF-7细胞。将pcDNA3.1-BCSC-1及其相应对照质粒用Lipofectamine 2000转染至MDA-MB-231，按之前描述的方法[10]构建稳定过表达BCSC-1及空载对照质粒的MDA-MB-231。pcDNA3.1-IQGAP1及其相应对照质粒，靶向BCSC-1和IQGAP1的siRNA，分别进行瞬时转染，按照Lipofectamine 2000说明书进行。

1.2.2 Western blot检测 收集各组培养细胞的沉淀，加入RIPA细胞裂解液提取细胞总蛋白。用BCA法进行蛋白定量。经SDS-PAGE电泳分离后，将蛋白转移至硝酸纤维素膜上。5%脱脂奶粉室温封闭1 h，加入BCSC-1一抗(1:1 000)、IQGAP1一抗(1:1 000)、β-actin一抗(1:5 000) 或GAPDH一抗(1:2 000)，室温孵育2 h或4 °C过夜。加相应的二抗IgG/HRP(1:2 000)室温孵育2 h。PBST洗膜后用ECL法显影、定影。

1.2.3 免疫沉淀实验 MCF-7细胞总蛋白裂解液中加入2 μL相应抗体，4 °C孵育6 h。再加入30~40 μL蛋白A/G琼脂糖珠，4 °C孵育过夜。细胞裂解液洗免疫沉淀物3~5次，然后进行Western blot检测。

1.2.4 细胞免疫荧光实验 将乳腺癌细胞接种(1×10^3/皿)在玻底小皿上培养12 h后，在4%多聚甲醛中室温固定15 min。将细胞与抗BCSC-1抗体(1:200)、抗IQGAP1(1:200)在4 °C孵育过夜。然后用TRITC标记羊抗兔IgG(1:100)、FITC标记羊抗兔IgG(1:100)或Alexa Fluor 350标记羊抗兔IgG(1:100)室温孵育1 h。PBST清洗3次后，用肌动蛋白追踪器红色–罗丹明(1:50)室温孵育40 min用于F-肌动蛋白染色。通过DAPI(1:50)室温孵育15 min对细胞核进行染色。加入抗荧光衰减封片剂封片，用激光共聚

焦显微镜观察拍照。

1.2.5 细胞划痕实验 将乳腺癌细胞接种在玻底小皿上培养, 当细胞密度达到80%~90%时, 用200 μL枪头垂直于玻底小皿以均匀的力度对细胞进行划痕, 无菌PBS冲洗细胞3次, 去除划痕细胞, 然后向玻底小皿中加入2 mL培养基, 培养8 h后, 按照1.2.4进行细胞免疫荧光实验, 观察划痕处细胞定向迁移情况。

1.2.6 免疫组织化学染色 采用SP免疫组织化学试剂盒检测BCSC-1和IQGAP1在乳腺癌组织中的表达情况, 操作严格按说明书进行。将组织微阵列置于100 °C的EDTA缓冲液中20 min进行抗原修复,然后在4 °C下分别与兔抗人的BCSC-1抗体(1:500)和兔抗人IQGAP1抗体(1:500)孵育过夜。使用PV-9000聚合物检测系统和二氨基联苯胺(DAB)进行免疫染色。将厚约5 μm的乳腺癌组织石蜡包埋切片在二甲苯中脱蜡, 并在分级乙醇(乙醇浓度为: 100%、95%、85%、75%)中复水, 其余操作同组织微阵列的步骤。以PBS代替一抗作为阴性对照。结果判断标准: 每张切片在高倍镜下随机选取视野计数500个细胞, 以出现棕黄色颗粒作为阳性细胞。根据染色强度和阳性细胞率相结合的标准对蛋白质表达情况进行评分。染色强度分级如下: 0, 阴性; 1, 弱; 2, 适度; 3, 强。阳性细胞率分级: 0, 0%~5%; 1, 6%~25%; 2, 26%~50%; 3, 51%~75%; 4, 76%~100%。通过将上述两个分数相乘, 得出每个样本的最终免疫反应性分数。0~4分定义为阴性表达(–); 阳性表达(+)得分为5~12。

1.2.7 统计学分析 所有数据均以均数±标准差($\bar{x}\pm s$)表示, 使用SPSS 26软件进行数据分析。采用卡方检验分析BCSC-1、IQGAP1表达与临床病理特征的关系。使用Spearman相关分析评估BCSC-1和IQGAP1之间的相关性。以$P<0.05$表示差异有统计学意义。

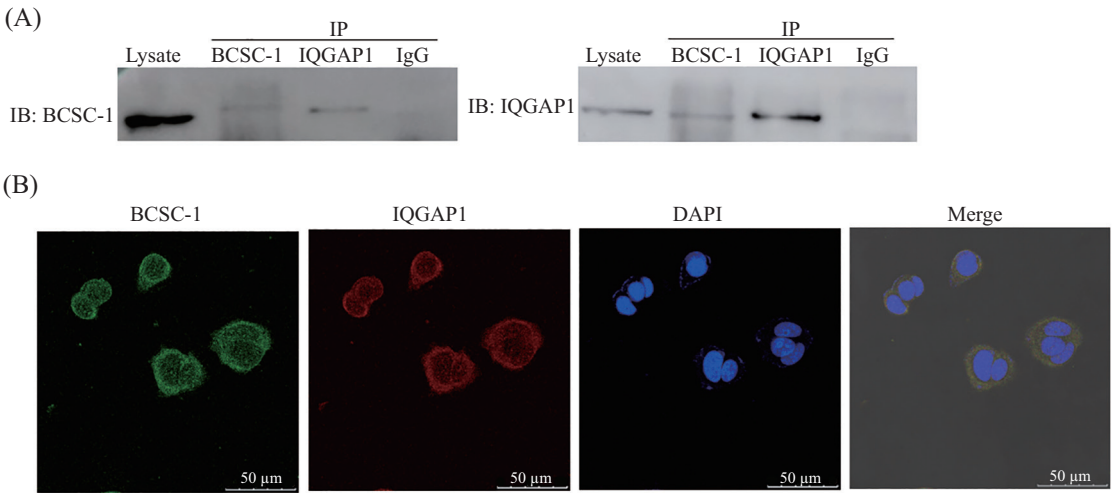
2 结果

2.1 BCSC-1与IQGAP1存在相互作用

提取MCF-7细胞总蛋白进行免疫沉淀和Western blot, 结果显示, BCSC-1免疫沉淀物中检测到IQGAP1蛋白的存在, 同时在IQGAP1免疫沉淀物中检测到BCSC-1蛋白的存在(图1A)。进行细胞免疫荧光染色发现, 在MCF-7细胞中, BCSC-1与IQGAP1存在共定位, 二者都分布在细胞膜、细胞质和细胞核中(图1B)。

2.2 BCSC-1和IQGAP1表达水平的改变不影响对方的表达

与空载对照细胞相比, BCSC-1在MCF-7细胞和MDA-MB-231细胞中稳定过表达后, IQGAP1蛋白的表达水平都没有明显变化(图2A和图2B)。将IQGAP1的过表达质粒及其空载对照质粒瞬时转染MCF-7细胞, BCSC-1蛋白的表达水平没有明显变化(图2C)。

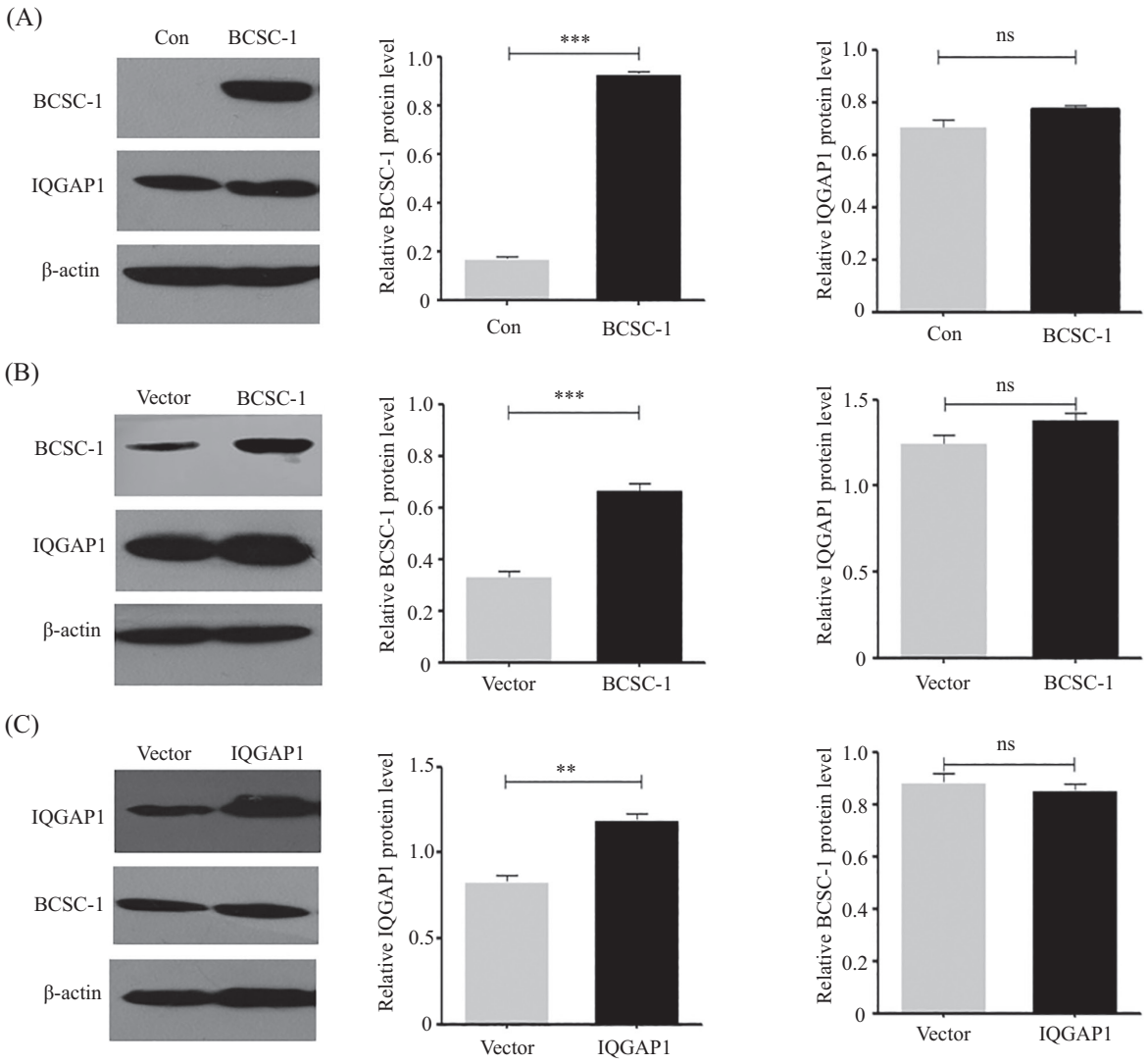


A: 免疫共沉淀实验检测BCSC-1与IQGAP1在乳腺癌细胞MCF-7中相互作用的情况; B: 细胞免疫荧光检测BCSC-1与IQGAP1在MCF-7细胞中共定位的情况。

A: the interaction between BCSC-1 and IQGAP1 was detected by co-IP in breast cancer cell MCF-7; B: the co-localization of BCSC-1 and IQGAP1 was detected by cell immunofluorescence in MCF-7 cells.

图1 BCSC-1与IQGAP1存在相互作用

Fig.1 BCSC-1 interacts with IQGAP1



A: Western blot检测过表达BCSC-1的MCF-7细胞和空载对照细胞中的IQGAP1蛋白水平; B: Western blot检测过表达BCSC-1的MDA-MB-231细胞和空载对照细胞中的IQGAP1蛋白水平; C: Western blot检测瞬时过表达IQGAP1的MCF-7细胞和空载对照细胞中的BCSC-1蛋白水平。**P<0.01; ***P<0.001; ns: no significance.

A: Western blot was used to detect IQGAP1 protein level in MCF-7 cells overexpressing BCSC-1 and empty control cells; B: Western blot was used to detect IQGAP1 protein level in MDA-MB-231 cells overexpressing BCSC-1 and empty control cells; C: BCSC-1 protein level was detected by Western blot when IQGAP1 overexpressing plasmid and control plasmid were transiently transfected into MCF-7 cells. **P<0.01; ***P<0.001; ns: no significance.

图2 BCSC-1和IQGAP1的分别过表达对乳腺癌细胞中IQGAP1、BCSC-1表达水平的影响

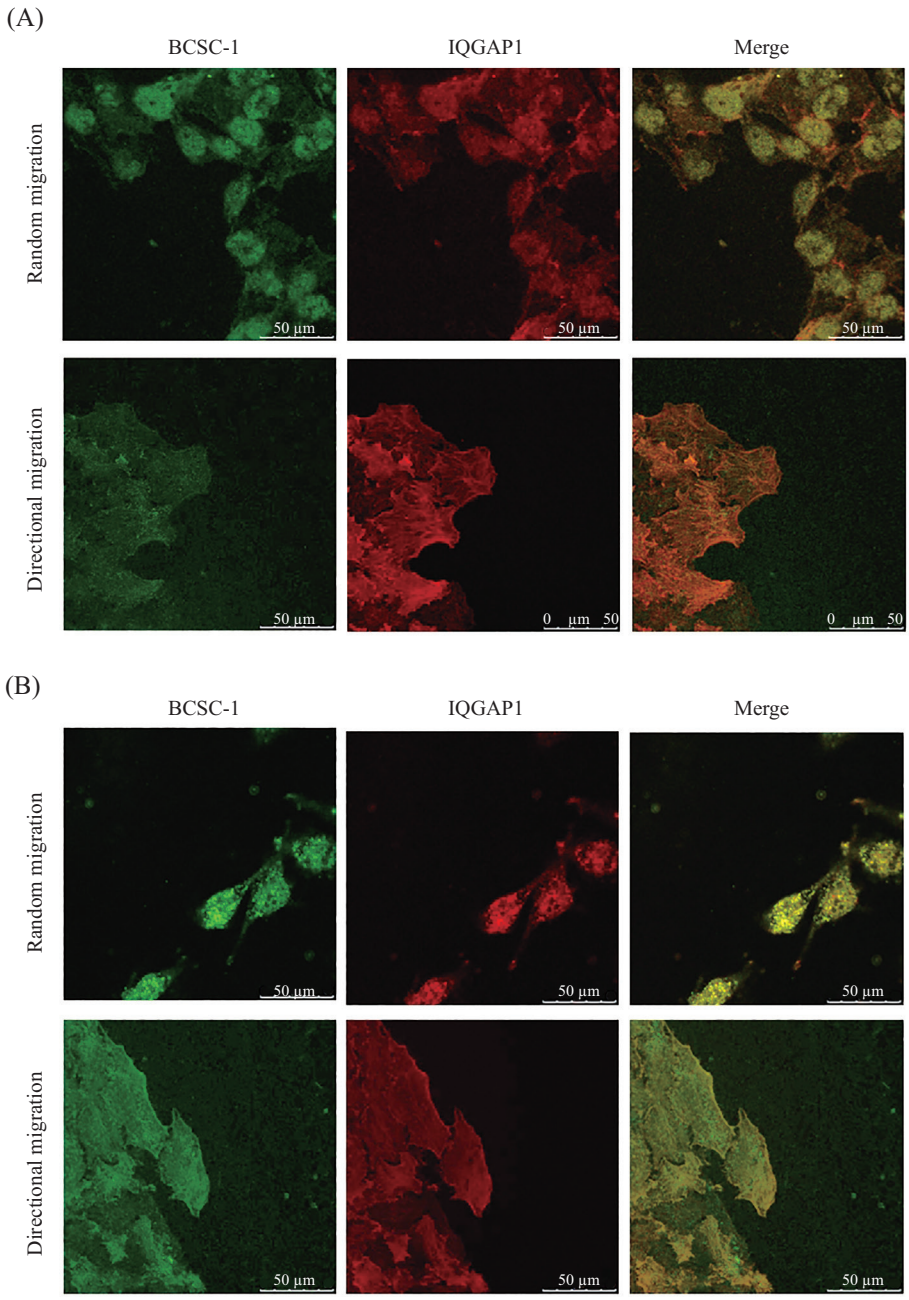
Fig.2 Effect of BCSC-1 and IQGAP1 respective overexpression on the expression of IQGAP1, BCSC-1 in breast cancer cells

2.3 BCSC-1与IQGAP1的复合物参与乳腺癌细胞迁移

无论乳腺癌细胞MCF-7和MDA-MB-231是处于随机迁移状态, 还是处于定向迁移状态, BCSC-1都定位在乳腺癌细胞胞质以及膜前缘, 且与IQGAP1存在着共定位分布(图3)。

2.4 BCSC-1与IQGAP1对乳腺癌细胞迁移的影响具有相互依赖性

采用RNA干扰技术在MCF-7细胞中分别敲低BCSC-1、IQGAP1的表达后, 对方的表达都没有明显改变(图4A), 然后进行细胞免疫荧光染色观察。结果显示, 在对照细胞中, BCSC-1、IQGAP1、F-actin都明显定位于细胞膜上; BCSC-1敲低后, 其在细胞膜上的分布亦减少, IQGAP1不再定位在细胞膜上, 主要分布在胞质中, F-actin也不能集中在细胞膜上, 膜分布减少。同时细胞形态发生改变: 细胞变大, 形态不规则, 细胞膜上皱褶明显; 同样地, IQGAP1敲低后, 其在细胞膜上的分布亦减少, BCSC-1也不再定位在细胞膜



A、B: 细胞免疫荧光检测BCSC-1和IQGAP1在乳腺癌细胞MCF-7(A)和MDA-MB-231(B)中在随机迁移状态(上图)和定向迁移状态(下图)的共定位。

A,B: cell immunofluorescence was used to detect the co-localization of BCSC-1 and IQGAP1 in breast cancer cells MCF-7 (A) and MDA-MB-231 (B) with random migration state (upper image) and directional migration state (below image).

图3 BCSC-1与IQGAP1的复合物参与乳腺癌细胞迁移

Fig.3 The complex of BCSC-1 and IQGAP1 participates in breast cancer cell migration

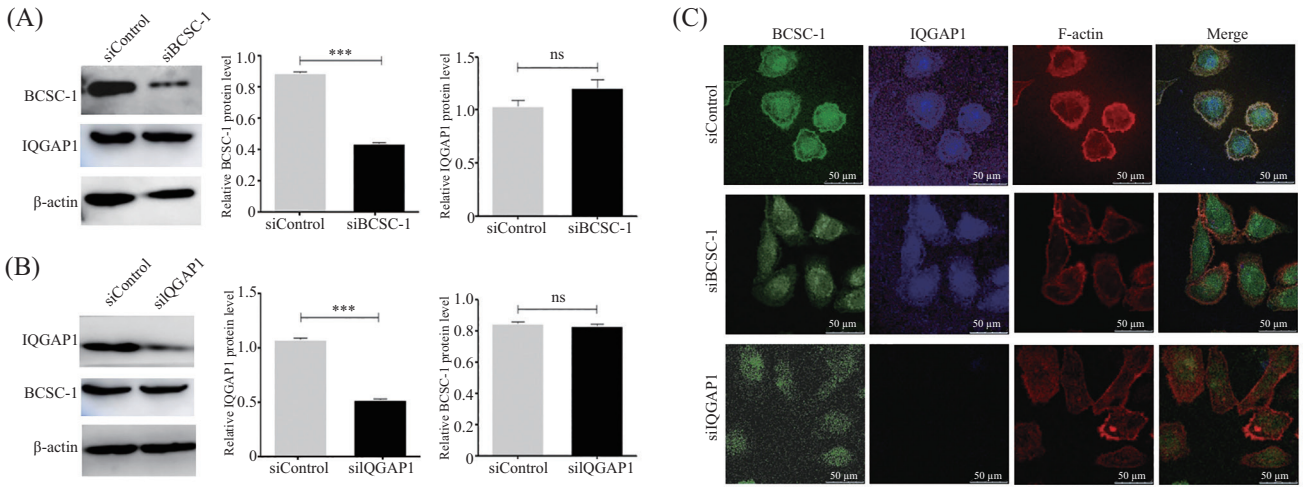
上, 主要分布在胞质中, 细胞膜上的F-actin亦减少, 细胞呈现与BCSC-1敲低后类似的形态改变(图4B)。

2.5 BCSC-1与IQGAP1在各种乳腺癌细胞中的表达情况

与MCF-10A相比, BCSC-1在乳腺癌细胞T47D和MDA-MB-468中明显高表达, 在MCF-7、MDA-MB-231、ZR75-1、HS578T、HCC1143和MDA-MB-453中明显低表达或不表达; 而IQGAP1在各种乳腺癌细胞中均高表达, 尤其在T47D和HCC1143中呈显著高表达(图5)。

2.6 BCSC-1与IQGAP1在浸润性导管癌组织及癌旁组织中的表达情况

与癌旁组织相比, 浸润性导管癌组织中BCSC-1的表达水平显著降低($P<0.05$), 而IQGAP1的表达水



A: Western blot检测BCSC-1经RNA干扰后在MCF-7细胞中BCSC-1、IQGAP1的表达情况; B: Western blot检测IQGAP1经RNA干扰后在MCF-7细胞中IQGAP1、BCSC-1的表达情况; C: 细胞免疫荧光检测RNA干扰后BCSC-1和IQGAP1在MCF-7细胞中的定位情况。***P<0.001; ns: no significance.

A: Western blot was used to detect the expression of BCSC-1 and IQGAP1 after RNA interference targeted BCSC-1 in MCF-7 cells; B: Western blot was used to detect the expression of IQGAP1 and BCSC-1 after RNA interference targeted IQGAP1 in MCF-7 cells. C: cell immunofluorescence was used to detect the co-localization of BCSC-1 and IQGAP1 after RNA interference in MCF-7 cells. ***P<0.001; ns: no significance.

图4 BCSC-1与IQGAP1对乳腺癌细胞迁移的影响具有相互依赖性

Fig.4 The effects of BCSC-1 and IQGAP1 on migration of breast cancer cells are interdependent

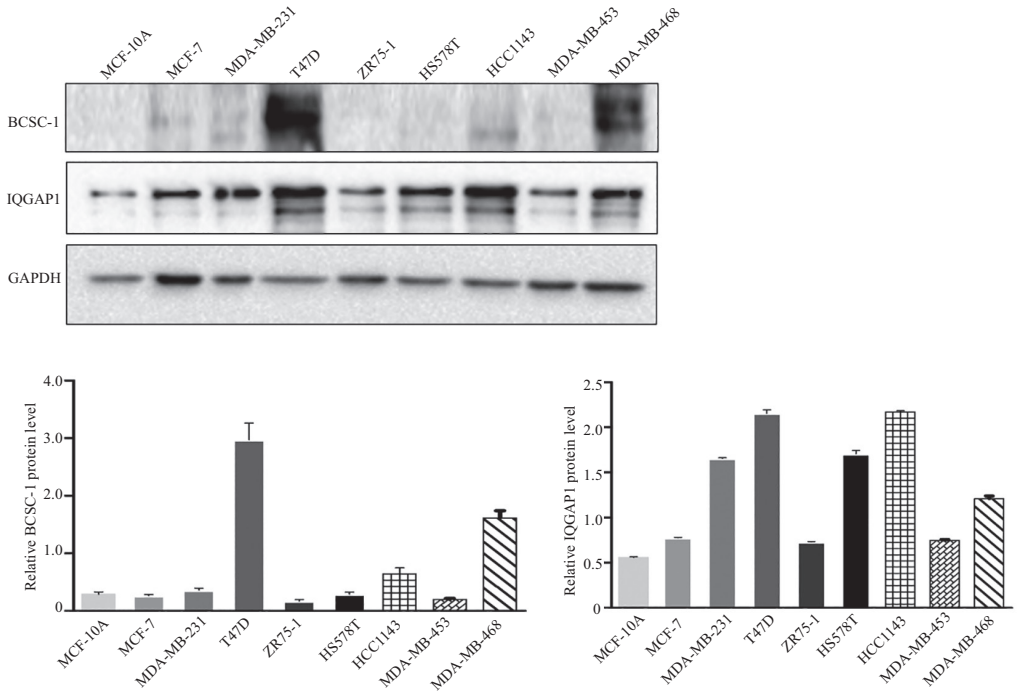


图5 BCSC-1与IQGAP1在各种乳腺癌细胞中的表达情况

Fig.5 Expression of BCSC-1 and IQGAP1 in various breast cancer cells

平显著升高(P<0.05)(图6和表1)。

2.7 BCSC-1与IQGAP1在浸润性导管癌组织中的表达与临床病理特征的关系

BCSC-1的低表达与乳腺癌的组织学级别、淋巴结转移有关(P<0.05), 与ER、PR和HER2的状态无关(P>0.05)。而IQGAP1的高表达与淋巴结转移有关(P<0.05), 且与HER2的状态有关(P<0.05)(表2)。

2.8 BCSC-1与IQGAP1在浸润性导管癌组织中的相关性分析

在浸润性导管癌组织中, BCSC-1呈低表达, 而

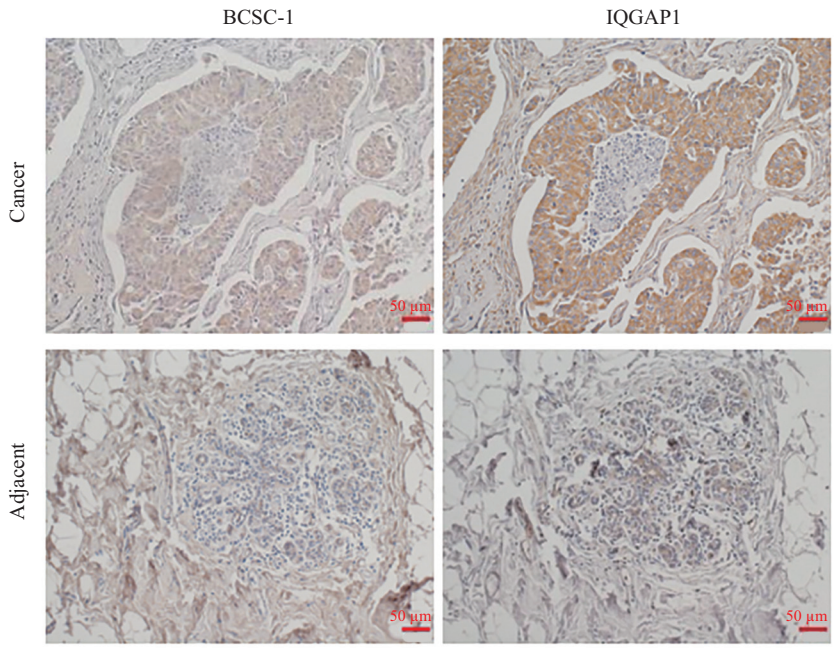


图6 BCSC-1与IQGAP1在浸润性导管癌组织及癌旁组织中的表达情况

Fig.6 Expression of BCSC-1 and IQGAP1 in invasive ductal carcinoma and adjacent tissues

表1 BCSC-1和IQGAP1在浸润性导管癌和癌旁组织中表达比较

Table 1 Expression of BCSC-1 and IQGAP1 in invasive ductal carcinoma tissues compared with adjacent tissues

组别 Groups	*n*	BCSC-1 低表达率/% Low expression rate /%	BCSC-1 高表达率/% High expression rate /%	BCSC-1 *P* 值 *P* value	IQGAP1 低表达率/% Low expression rate /%	IQGAP1 高表达率/% High expression rate /%	IQGAP1 *P* 值 *P* value
Invasive ductal carcinoma tissues	78	55 (70.5%)	23 (29.5%)	<0.01	31 (39.7%)	47 (60.3%)	<0.01
Adjacent tissues	37	11 (29.7%)	26 (70.3%)		28 (75.7%)	9 (24.3%)	

IQGAP1呈高表达, 二者表达水平之间存在显著的负相关关系(R=–0.416, P<0.05)(图7和表3)。

3 讨论

文献已报道, BCSC-1的过表达能够降低人鼻咽癌细胞的致瘤性和转移性, 导致细胞中E-cadherin、α-catenin和p53的表达水平增加, 并诱导细胞周期阻滞[6]。BCSC-1的过表达也能够抑制人肺癌细胞的恶性增殖, 这种抑制作用与细胞周期阻滞和黏附分子CD44表达水平增加有关[16]。而我们以前的研究发现, BCSC-1在乳腺癌组织中低表达, 通过抑制NF-κB通路的活性降低MMP-7、MMP-9、OPN的表达水平, 进而抑制乳腺癌细胞的迁移和侵袭[10]; 乳腺癌组织中低表达的BCSC-1与高表达的MMP-14呈负相关[11]。以上研究证实了*BCSC-1*具有抑癌基因的特性, 但关于其在肿瘤包括乳腺癌中的作用及详细的机制尚需要被阐明。

前期研究中, 我们通过IP-MS筛选出BCSC-1的潜在相互作用蛋白IQGAP1。本研究通过免疫共沉淀(Co-IP)和免疫荧光共定位实验证实, BCSC-1与IQGAP1在乳腺癌细胞中形成稳定的蛋白复合物, 但二者互作并不影响彼此的表达水平。作为多功能支架蛋白, IQGAP1通过整合多种信号通路参与细胞迁移调控——这一过程在肿瘤转移中具有关键作用[13]。具体而言, IQGAP1可通过两种机制促进细胞迁移: 在迁移细胞前缘与Cdc42/Rac1相互作用, 或直接结合F-actin, 进而调控肌动蛋白细胞骨架的动态重组, 该过程还受到Ca^{2+}/钙调蛋白的精细调控[17-19]。值得注意的是, IQGAP1介导的细胞迁移调控涉及PI3K、MAPK和Wnt等多条信号通路[20-22]。基于上

表2 BCSC-1和IQGAP1在浸润性导管癌患者中的表达水平与临床病理特征的比较

Table 2 Comparison of expression levels of BCSC-1 and IQGAP1 with clinicopathological features in patients with invasive ductal carcinoma

临床特征 Clinical features	*n*	BCSC-1 低表达率/% Low expression rate /%	BCSC-1 高表达率/% High expression rate /%	BCSC-1 *P* 值 *P* value	IQGAP1 低表达率/% Low expression rate /%	IQGAP1 高表达率/% High expression rate /%	IQGAP1 *P* 值 *P* value
Age, years				0.818			0.096
＜50	29	20 (69.0%)	9 (31.0%)		15 (51.7%)	14 (48.3%)	
≥50	49	35 (71.4%)	14 (28.6%)		16 (32.7%)	33 (67.3%)	
Tumor size				0.264			0.464
≤2 cm	54	36 (66.7%)	18 (33.3%)		20 (37.0%)	34 (63.0%)	
>2 cm	24	19 (79.2%)	5 (20.8%)		11 (45.8%)	13 (54.2%)	
Histological grade				0.013			0.158
I-II	56	44 (78.6%)	12 (21.4%)		25 (44.6%)	31 (55.4%)	
III	22	11 (50.0%)	11 (50.0%)		6 (27.3%)	16 (72.7%)	
Clinical stage				0.235			0.432
I	42	32 (76.2%)	10 (23.8%)		15 (35.7%)	27 (64.3%)	
II-III	36	23 (63.9%)	13 (36.1%)		16 (44.4%)	20 (55.6%)	
Lymph node metastasis				0.033			0.003
Yes	57	44 (77.2%)	13 (22.8%)		17 (29.8%)	40 (70.2%)	
No	21	11 (52.4%)	10 (47.6%)		14 (66.7%)	7 (33.3%)	
ER				0.412			0.518
+	56	38 (67.9%)	18 (32.1%)		21 (37.5%)	35 (62.5%)	
–	22	17 (77.3%)	5 (22.7%)		10 (45.5%)	12 (54.5%)	
PR				0.826			0.894
+	46	32 (69.6%)	14 (30.4%)		18 (39.1%)	28 (60.9%)	
–	32	23 (71.9%)	9 (28.1%)		13 (40.6%)	19 (59.4%)	
HER2				0.616			0.010
+	51	35 (68.6%)	16 (31.4%)		15 (29.4%)	36 (70.6%)	
–	27	20 (74.1%)	7 (25.9%)		16 (59.3%)	11 (40.7%)	

述背景，我们探讨了BCSC-1-IQGAP1复合物在乳腺癌细胞迁移中的作用。在随机迁移和划痕诱导的定向迁移实验中，BCSC-1与IQGAP1均共定位于细胞膜前缘。通过RNA干扰实验发现，敲低任一蛋白均导致二者从细胞膜消失，膜上的F-actin也减少，表明二者在细胞迁移过程中存在功能依赖性。这一发现与已知的基因功能特征形成有趣对比：*BCSC-1*作为抑癌基因可抑制细胞迁移，其机制尚无研究报道；而*IQGAP1*作为癌基因则通过多种信号途径促进细胞迁移。这种定位依赖与功能拮抗的协调现象，提示二者可能通过不同信号通路发挥作用。我们提出以下机制假说：当形成膜定位复合物时，BCSC-1可能通过阻断IQGAP1与Cdc42/F-actin的相互作用抑制迁移；而BCSC-1缺失时，胞质定位的IQGAP1可能通过激活PI3K/MAPK等替代通路发挥促迁移作用。为验证这一假说，后续研究将结合亚细胞分级分离技术精确定位蛋白分布变化，并通过检测Cdc42激活状态、PI3K/MAPK通路活性等关键指标，系统阐明膜结合与胞质IQGAP1的功能异质性。

另外，本研究还发现在多种乳腺癌细胞中BCSC-1低表达或缺失表达，而IQGAP1呈高表达。该结果与我们在乳腺癌组织中的检测结果一致：在乳腺浸润性导管癌组织中BCSC-1呈低表达，而IQGAP1呈高表达。*BCSC-1*，又被称为*LOH11CR2A*，定位于人类染色体11q23-q24，该区域的杂合性缺失(loss of heterozygosity, LOH)，已在很多肿瘤包括乳腺癌、肺癌、卵巢癌中被报道[4-5]。尽管采用免疫组织化学手段和Western blot已发现IQGAP1高表

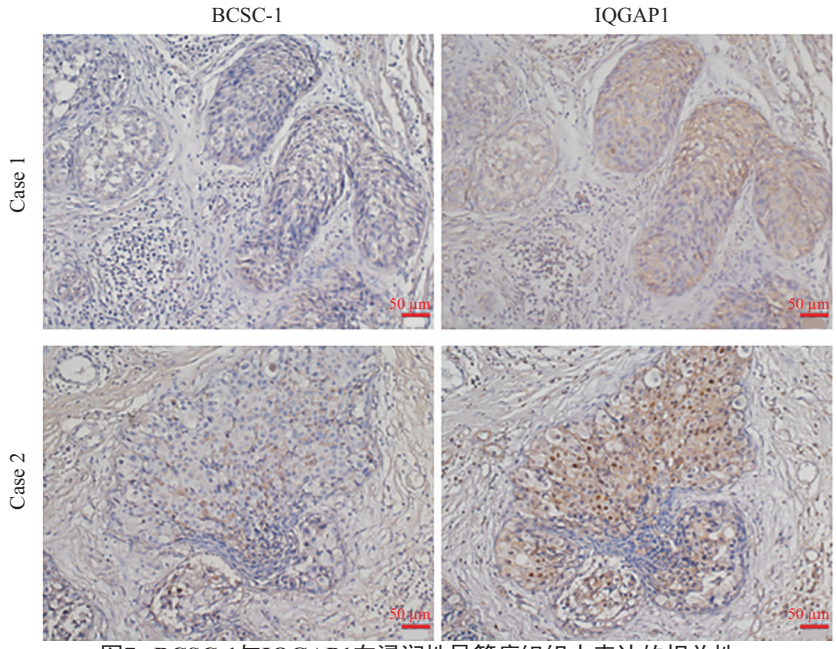


图7 BCSC-1与IQGAP1在浸润性导管癌组织中表达的相关性

Fig.7 The correlation between BCSC-1 and IQGAP1 expression in invasive ductal carcinoma

表3 BCSC-1和IQGAP1在浸润性导管癌患者中的表达呈负相关

Table 3 BCSC-1 expression negatively correlates with IQGAP1 in patients with invasive ductal carcinoma

BCSC-1	IQGAP1		*R*	*P*
	低表达 Low expression	高表达 High expression		
Low expression	15	40	–0.416	<0.01
High expression	16	7		

达于多种肿瘤, 但其高表达的原因目前报道还较少, 基因扩增是IQGAP1高表达于结直肠癌、胃癌的主要原因[22-23], 通过qRT-PCR检测到乳腺癌中*IQGAP1* mRNA水平升高[24]。因此乳腺癌以及其他肿瘤中IQGAP1高表达的原因还有待进一步研究。我们还发现在乳腺癌组织中BCSC-1和IQGAP1的表达呈负相关。这提示BCSC-1和IQGAP1在乳腺癌的发生发展中相互协调, 共同起重要作用。进一步的研究发现, BCSC-1的低表达与乳腺癌的组织学级别和淋巴结转移具有相关性, 这与以前我们报道的结果一致[10]。ER、PR和HER2状态在乳腺癌诊断和治疗中具有重要意义。本研究发现BCSC-1的表达与乳腺癌ER、PR和HER2的状态无关, 目前尚无此方面的研究报道。而IQGAP1在浸润性导管癌中的高表达, 与淋巴结转移有关, 与ER和PR的状态无关, 该结果与ZHAO等[14]的报道一致。此外, 本研究还发现IQGAP1与HER2的状态有关。尽管研究已发现, ER是IQGAP1的互作蛋白之一, IQGAP1与ER在乳腺癌细胞中存在相互作用, IQGAP1敲降能够减弱雌二醇诱导雌激素反应基因*pS2*、*PR*和*cyclin D1*转录的能力[26], 但我们目前的研究结果及其他学者的研究都没有支持IQGAP1与ER在乳腺癌组织学上的相关性。WHITE等[27]的研究显示, IQGAP1在HER2阳性的乳腺癌症组织中过表达, 能直接与HER2结合。敲除IQGAP1能够降低HER2的表达水平、磷酸化水平, 抑制其下游信号转导, 同时上调p27的表达从而抑制乳腺癌细胞增殖。重要的是, IQGAP1在曲妥珠单抗耐药的乳腺上皮细胞中过表达, 降低IQGAP1的表达水平既能增强曲妥珠抗体的抑制作用, 又能恢复曲妥珠疫苗耐药SKBR3细胞对曲妥珠的敏感性。因此, BCSC-1作为IQGAP1的互作蛋白, 在乳腺癌细胞中是否参与IQGAP1对HER2信号通路的调控, 以及这两个互作蛋白是否通过影响乳腺癌细胞迁移而参与调控浸润性导管癌的转移, 还需要做进一步的深入

研究。

综上所述, BCSC-1和IQGAP1在乳腺癌细胞中存在相互作用, 二者相互依赖, 共同调控乳腺癌细胞的迁移。在乳腺浸润性导管癌中, 低表达的BCSC-1与高表达的IQGAP1呈负相关, 协同促进了乳腺癌的发生发展。本研究为乳腺癌机制的探索提供了新的证据, 为乳腺癌的诊治提供了新的思路。

参考文献 (References)

[1] BRAY F, LAVERSANNE M, SUNG H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. CA Cancer J Clin, 2024, 74(3): 229-63.

[2] PEDERSEN R N, ESEN B O, MELLEMKJÆR L, et al. The incidence of breast cancer recurrence 10-32 years after primary diagnosis [J]. J Natl Cancer Inst, 2022, 114(3): 391-9.

[3] CSERNI G. Histological type and typing of breast carcinomas and the WHO classification changes over time [J]. Pathologica, 2020, 112(1): 25-41.

[4] MARTIN E S, CESARI R, PENTIMALLI F, et al. The BCSC-1 locus at chromosome 11q23-q24 is a candidate tumor suppressor gene [J]. Proc Natl Acad Sci USA, 2003, 100(20): 11517-22.

[5] RASIO D, NEGRINI M, MANENTI G, et al. Loss of heterozygosity at chromosome 11q in lung adenocarcinoma: identification of three independent regions [J]. Cancer Res, 1995, 55(18): 3988-91.

[6] ZHOU Y Q, CHEN S L, JU J Y, et al. Tumor suppressor function of BCSC-1 in nasopharyngeal carcinoma [J]. Cancer Sci, 2009, 100(10): 1817-22.

[7] INDRA D M, MITRA S, SINGH R K, et al. Inactivation of CHEK1 and EI24 is associated with the development of invasive cervical carcinoma: clinical and prognostic implications [J]. Int J Cancer, 2011, 129(8): 1859-71.

[8] ANGHEL S I, CORREA-ROCHA R, BUDINSKA E, et al. Breast cancer suppressor candidate-1 (BCSC-1) is a melanoma tumor suppressor that down regulates MITF [J]. Pigment Cell Melanoma Res, 2012, 25(4): 482-7.

[9] ZHAO C L, YU W J, GAO Z Q, et al. Association of BCSC-1 with human esophageal squamous cell carcinoma [J]. Neoplasma, 2015, 62(5): 765-9.

[10] DI D, CHEN L, GUO Y, et al. BCSC-1 suppresses human breast cancer metastasis by inhibiting NF-κB signaling [J]. Int J Oncol, 2018, 52(5): 1674-84.

[11] DI D, CHEN L, GUO Y, et al. Association of BCSC-1 and MMP-14 with human breast cancer [J]. Oncol Lett, 2018, 15(4): 5020-6.

[12] ABEL A M, SCHULDT K M, RAJASEKARAN K, et al. IQGAP1: insights into the function of a molecular puppeteer [J]. Mol Immunol, 2015, 65(2): 336-49.

[13] WEI T, LAMBERT P F. Role of IQGAP1 in carcinogenesis [J]. Cancers, 2021,13(16): 3940.

[14] ZHAO H Y, HAN Y, WANG J, et al. IQ-domain GTPase-activating protein 1 promotes the malignant phenotype of invasive ductal breast carcinoma via canonical Wnt pathway [J]. Tumour Biol, 2017, 39(6): 1010428317705769.

[15] ZENG F, JIANG W, ZHAO W, et al. Ras GTPase-activating-like protein IQGAP1 (IQGAP1) promotes breast cancer proliferation and invasion and correlates with poor clinical outcomes [J]. Med Sci Monit, 2018, 24: 3315-23.

[16] 袁芳, 鞠吉雨, 唐媛媛, 等. BCSC-1基因异位表达对人小细胞肺癌细胞增殖的抑制效应[J]. 中国癌症杂志(YUAN F, JU J Y, TANG Y Y, et al. Inhibition of malignant proliferation of human small cell lung cancer cells by ectopic expression of BCSC-1 gene [J]. China Oncology), 2012, 22(5): 336-41.

[17] WATANABE T, WANG S, KAIBUCHI K. IQGAPs as key regulators of actin-cytoskeleton dynamics [J]. Cell Struct Funct, 2015, 40(2): 69-77.

[18] WATANABE T, WANG S, NORITAKE J, et al. Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration [J]. Dev Cell, 2004, 7(6): 871-83.

[19] BRIGGS M W, SACKS D B. IQGAP1 as signal integrator: Ca^{2+}, calmodulin, Cdc42 and the cytoskeleton [J]. FEBS Lett, 2003, 542(1/2/3): 7-11.

[20] MCNULTY D E, LI Z, WHITE C D, et al. MAPK scaffold IQGAP1 binds the EGF receptor and modulates its activation [J]. J Biol Chem, 2011, 286(17): 15010-21.

[21] GOTO T, SATO A, ADACHI S, et al. IQGAP1 protein regulates nuclear localization of beta-catenin via importin-beta5 protein in Wnt signaling [J]. J Biol Chem, 2013, 288(51): 36351-60.

[22] YERRAMILLI V S, ROSS A H, SCARLATA S, et al. IQGAP1 scaffolding links phosphoinositide kinases to cytoskeletal reorganization [J]. Biophys J, 2022, 121(5): 793-807.

[23] BERTUCCI F, SALAS S, EYSTERIES S, et al. Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters [J]. Oncogene, 2004, 23(7): 1377-91.

[24] SUGIMOTO N, IMOTO I, FUKUDA Y, et al. IQGAP1, a negative regulator of cell-cell adhesion, is upregulated by gene amplification at 15q26 in gastric cancer cell lines HSC39 and 40A [J]. J Hum Genet, 2001, 46(1): 21-5.

[25] ZENG F, JIANG W, ZHAO W, et al. Ras GTPase-activating-like protein IQGAP1 (IQGAP1) promotes breast cancer proliferation and invasion and correlates with poor clinical outcomes [J]. Med Sci Monit, 2018, 24: 3315-23.

[26] ERDEMIR H H, LI Z, SACKS D B. IQGAP1 binds to estrogen receptor-α and modulates its function [J]. J Biol Chem, 2014, 289(13): 9100-12.

[27] WHITE C D, LI Z, DILLON D A, et al. IQGAP1 protein binds human epidermal growth factor receptor 2 (HER2) and modulates trastuzumab resistance [J]. J Biol Chem, 2011, 286(34): 29734-47.