HOXC8通过激活GDF15转录表达促进乳腺癌细胞的生长及迁移

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摘要 同源盒(homeobox, HOX)基因家族由包含同源盒结构域(homeodomain)的转录因子组成, 其表达失调与多种癌症的发生发展有关。HOXC8作为HOX家族成员之一, 它的表达可促进乳腺癌细胞的增殖与转移等。为进一步探索HOXC8在乳腺癌中的作用机制, 通过转录组测序、Western blot和实时荧光定量PCR等实验发现, 在乳腺癌细胞中HOXC8可上调生长分化因子15(GDF15)的表达。通过染色质免疫沉淀及荧光素酶报告等实验, 证明了HOXC8可结合到*GDF15*基因的启动子上, 作为转录因子来激活GDF15的表达。在乳腺癌细胞中, GDF15的表达能显著增强细胞的活力, 促进锚定非依赖性生长、迁移以及细胞板状伪足/丝状伪足的形成。进一步证明, 在乳腺癌细胞中HOXC8是通过诱导GDF15表达促进细胞生长和迁移的。此外, 通过已知的肿瘤数据库分析, 该研究发现在高侵袭性乳腺癌标本中*HOXC8*及*GDF15*基因均显示较高的扩增频率, 且二者表达水平的上调与乳腺癌患者的预后不良呈显著的相关性。根据上述实验数据, 该研究认为HOXC8-GDF15轴在乳腺癌的进展中起着重要作用, 有望成为乳腺癌治疗的新靶点。

关键词 乳腺癌; HOXC8; GDF15; 转录调控; 迁移

HOXC8 Promotes Growth and Migration of Breast Cancer Cells by Activating GDF15 Transcription

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Abstract HOX (homeobox) gene family consists of homeodomain-containing transcription factors and their dysregulation is related to development of various cancers. Previously, it had been reported that HOXC8 which was a member of HOX gene family, promoted migration and metastasis of breast cancer cells. HOXC8 expression was found by RNA-seq, Western blot and RT-PCR analyses to result in up-regulation of GDF15 (growth and differentiation factor 15) in breast cancer cells. HOXC8 was demonstrated by chromatin immunoprecipitation and luciferase assays to bind to the promoter of *GDF15*. As transcription factor, it was to activate GDF15 expression. Moreover, GDF15 expression promoted cell viability, anchorage-independent growth, migration and the formation of filopodium/lamellipodium of breast cancer cells. It was further demonstrated that HOXC8 promoted cell

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growth and migration by inducing GDF15 expression in breast cancer cells. Additionally, *HOXC8* or *GDF15* gene was amplified in invasive breast cancer and up-regulation of HOXC8 or GDF15 was statistically associated with poor prognosis of breast cancer patients. Altogether, this study showed that HOXC8-GDF15 axis played an important role in breast cancer progression and might present a promising target for breast cancer treatment.

Keywords breast cancer; HOXC8; GDF15; transcriptional regulation; migration

在全球范围内, 乳腺癌一直是对妇女健康造成严重威胁的主要癌症之一。虽然针对乳腺癌的诊断和治疗已取得了长足进展, 但肿瘤转移仍然是导致乳腺癌治疗失败的主要因素[1-2]。肿瘤转移使肿瘤细胞获得了较高的增殖、迁移和侵袭等能力, 然而肿瘤转移的分子机制仍有待于进一步的研究阐明[3-4]。

HOXC8是同源盒(homeobox, HOX)家族的成员之一, 在哺乳动物细胞中HOX家族包含39个成员[5-6]。研究发现, HOXC8参与了多种癌症的发生发展过程[7-11]。在这些肿瘤中, HOXC8的异常表达与肿瘤细胞转移有显著的关联。有研究证实, 在乳腺癌中HOXC8的表达水平上调, 并促进了乳腺癌细胞的转移[8,12]。作为HOX家族的成员之一, HOXC8通常作为转录因子通过调控相关基因的表达, 进而参与肿瘤的发生发展过程[8,13]。

生长分化因子15(growth and differentiation factor 15, GDF15)属于转化生长因子-β(transforming growth factor-β, TGF-β)超家族中的一员[14], 参与许多生理活动的调控, 并被证明与多种癌症的发生发展具有密切联系[15-17]。在乳腺癌中研究证实, GDF15能够促进肿瘤细胞的上皮–间质转化(epithelia-mesenchimal transition, EMT)过程, 但人们对其在肿瘤细胞的增殖及迁移中的作用尚未有深入的探究[18-19]。在近期的一项研究中, 研究人员发现, GDF15的表达能够抑制乳腺癌细胞的转移, 然而该研究仅主要在正常的乳腺上皮细胞MCF-10A中进行[20]。 因此, GDF15在乳腺癌中的功能尚未明确, 有待进一步的研究。

为进一步探索HOXC8在乳腺癌中的作用机制, 我们对HOXC8的表达是否可促进乳腺癌细胞的增殖与转移等进行了相关的实验研究。

1 材料与方法

1.1 细胞与试剂

人乳腺癌细胞株MDA-MB-231与MCF7均购自中国科学院上海细胞库, 并定期对其进行支原体检测。HOXC8抗体(15448-1-AP)和GDF15抗体(27455-1-AP)购自武汉三鹰生物技术有限公司; β-actin抗体(sc-1616)购自Santa Cruz Biotechnology公司; TRIzol RNA提取试剂盒、Lipofectamine 2000 & 3000以及ECL SuperSignal试剂盒等均购自Thermo Scientific公司; 其他化学试剂购自Sigma公司。

1.2 方法

1.2.1 *质粒构建* GDF15与HOXC8的shRNA敲低序列由Invitrogen Block-iT RNAi Designer设计, 通过聚合酶链反应(polymerase chain reaction, PCR)扩增并将其克隆至载体pLV-RNAi vector(BioSettia, 美国)上, 然后再将*GDF15*和*HOXC8*基因克隆到慢病毒表达载体pCDH-CMV-MCS-EF1-Puro(System Biosciences, 美国)上, 将*GDF15*启动子克隆到pGL4.23荧光素酶报告载体中(Promega, 美国)上。引物序列见表1。

1.2.2 *Western blot、实时荧光定量PCR与荧光素酶报告基因检测* 细胞总蛋白提取物用适当浓度的SDS-PAGE电泳分离后, 用相应的抗体进行Western blot实验。使用TRIzol提取总RNA后进行逆转录得到cDNA, 并将其作为模板在ABI7900系统中进行实时荧光定量PCR(Real time PCR, RT-PCR)检测。将已构建*GDF15*启动子的报告载体用Lipofectamin 3000转染至细胞内, 24 h后裂解细胞, 使用双荧光素酶报告系统(dual luciferase system, Promega)进行荧光检测。相关引物见表1。

1.2.3 *染色质免疫沉淀(chromatin immunoprecipitation, ChIP)* 在直径为15 cm培养皿中将细胞培养至70%汇合后, 用1%甲醛溶液固定并收集细胞。使用超声破碎仪将染色质破碎至大约500 bp大小, 加入2 μg HOXC8抗体或IgG作为阴性对照, 4 °C孵育过夜后加入40 μL蛋白质G-agarose, 10 000 r/min离心1 min, 沉淀DNA, 用PCR或RT-PCR方法分析实验结果。

1.2.4 *MTT实验检测细胞活力* 在24孔细胞培养皿中, 每孔加入大约5×10^3个细胞进行培养, 培养到相应的时间点, 添加适量MTT溶液, 37 °C孵育数小

表1 用于克隆、shRNA、Real-time PCR等引物序列

Table 1 Primer sequences used in cloning, shRNA and Real-time PCR studies

引物名称 Primer name	序列 Sequence
GDF15 2.0 Kb promoter F	5′-TCC AGG AGG AGG AGT TTG GGG CCAT-3′
GDF15 1.5 Kb promoter F	5′-GGA TTT TGG AGT GGG CTG AAG T-3′
GDF15 1.0 Kb F	5′-CTA AGT TTC TGT CCA GAA TTC T-3′
GDF15 0.5 Kb promoter F	5′-CCA CCT CTC CAG TGA GAG TCT C-3′
GDF15 promoter R	5′-AAG TAG CGC TTG GTG GTG GGA TTA CAT-3′
ChIP F1	5′-CTG TGA GGA TGG CTT CAA GGT-3′
ChIP R1	5′-GCA AAA AGC CCC TCT TCC AG-3′
ChIP F2	5′-GGC ATG TAA TCC CAC CAC CA-3′
ChIP R2	5′-AGG CAC GTG GAT CAT CTG AC-3′
ChIP F3	5′-CCA GAC AAA AGG ATG GGG TT-3′
ChIP R3	5′-TCC AGG AAG TCT TCT CCA ACA TTA-3′
HOXC8 shRNA1	5′-AAA AGC AAT ATC CCG ACT GTA AAT CTT GGA TCC AAG ATT TAC AGT CGG GAT ATT GC-3′
HOXC8 shRNA2	5′-AAA AGC CTC ATG TTT CCA TGG ATG ATT GGA TCC AAT CAT CCA TGG AAA CAT GAG GC-3′
GDF15 shRNA1	5′-AAA AGA CCA ACT GCT GGC AGA ATC TTT GGA TCC AAA GAT TCT GCC AGC AGT TGG TC-3′
GDF15 shRNA2	5′-AAA AGC AAG AAC TCA GGA CGG TGA ATT GGA TCC AAT TCA CCG TCC TGA GTT CTT GC-3′
GDF15 PCR F	5′-GAG CTG GGA AGA TTC GAA CA-3′
GDF15 PCR R	5′-AGA GAT ACG CAG GTG CAG GT-3′
HOXC8 PCR F	5′-GGC AAA CTT ACA GCC GGT AT-3′
HOXC8 PCR R	5′-TTC AAT CCG ACG TTT TCG T-3′
β-actin PCR F	5′-TGG ATC AGC AAG CAG GAG TAT G-3′
β-actin PCR R	5′-GCA TTT GCG GTG GAC GAT-3′
GDF15 cloning F	5′-ATC TAG AAC AGC CAT GCC CGG GCA AGA ACT CAG GAC G-3′
GDF15 cloning R	5′-AAG AAT TCT CAT ATG CAG TGG TTT GGC TAA CAA G-3′

时, 并在560 nm波长处检测吸光度值。

1.2.5 软琼脂克隆形成实验检测细胞锚定非依赖性生长能力 在6孔板中, 首先将0.6%琼脂糖均匀铺于孔内制备底层胶, 之后用包含2×10^{4}个细胞的0.3%琼脂糖构成上层胶。在细胞培养箱中培养3~4周后, 加入氯化碘二甲酸酯(INT)溶液对细胞团块进行染色, 并在显微镜下进行计数与拍照。

1.2.6 细胞迁移实验 首先将Transwell(Costar公司)在10 μg/mL的胶原蛋白溶液中进行包板处理, 然后在每个孔中加入大约5×10^{5}个细胞。37 ℃孵育4~6 h后, 移除上层的残余细胞, 用结晶紫溶液对下层细胞进行染色, 并在显微镜下计算迁移的细胞数量。

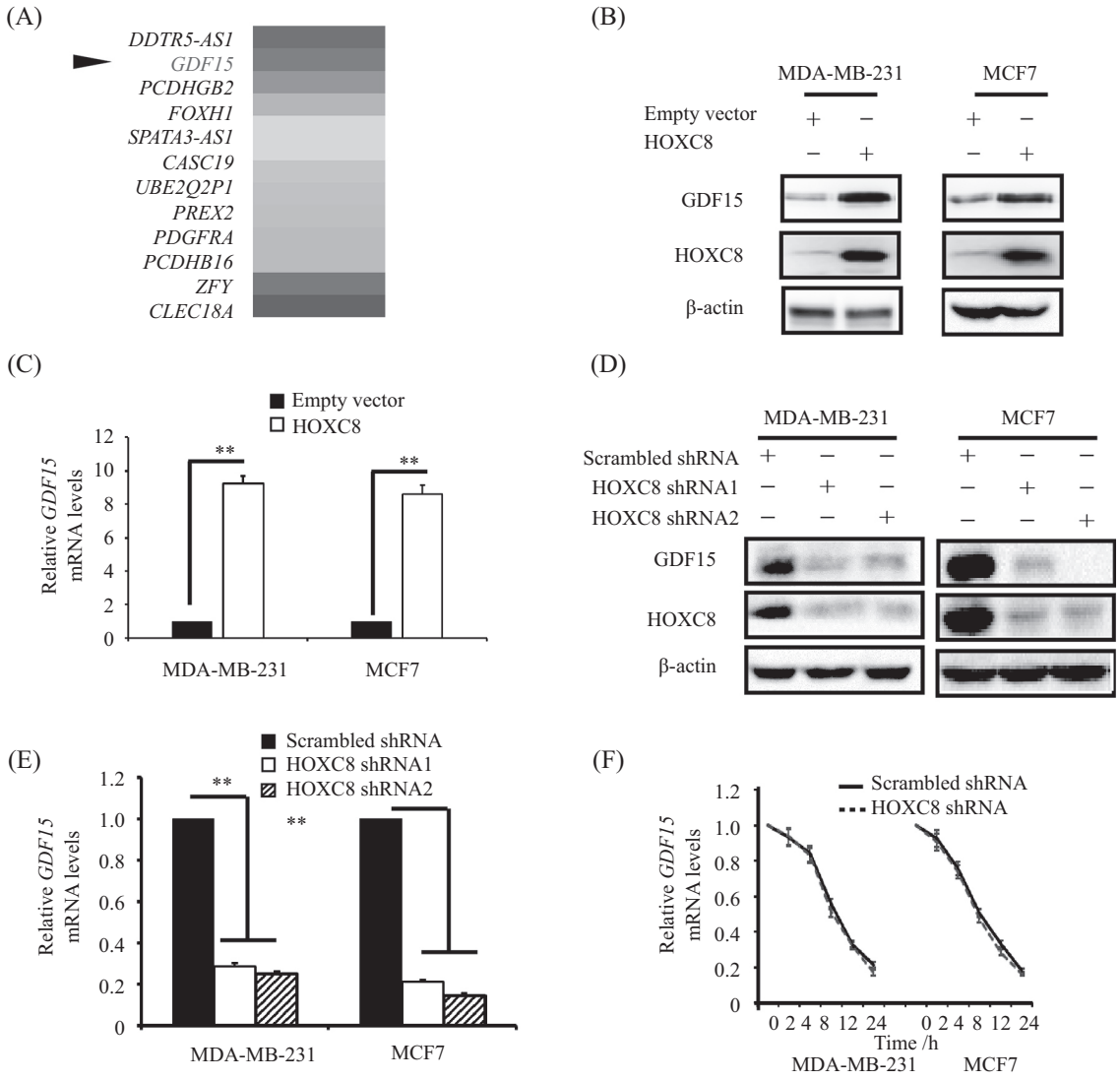
1.2.7 免疫荧光染色 将2×10^{4}个细胞接种于放置有灭菌盖玻片的6孔板中进行培养, 大约18 h后, 用4%多聚甲醛溶液进行细胞固定。0.2% Triton X-100溶液处理细胞10 min, 3% BSA溶液室温进行封闭, 然后滴加罗丹明标记的鬼笔环肽溶液, 室温孵育30 min, 最后在共聚焦显微镜下观察细胞骨架。

1.2.8 生物信息学分析与统计学分析 使用cBioPortal (http://www.cbioportal.org)网站提供的乳腺癌数据库对*HOXC8*或*GDF15*基因表达进行分析, 该网站提供开放访问的临床癌症数据。Kaplan-Meier分析是以*HOXC8*和*GDF15*基因表达的中位阈值作为基因表达高/低来进行计算的。统计学分析是从至少3个独立实验中收集的数据平均值±标准差($\bar{x}\pm s$)进行分析, *t*测试(双尾)用于比较两组数据的差异, 当$P<0.05$时, 认为差异具有统计学意义。

2 实验结果

2.1 在乳腺癌细胞中HOXC8调控GDF15的表达

作为HOX家族的一员, HOXC8通常作为转录因子来调节其靶基因的表达。为了进一步阐明HOXC8在乳腺癌细胞中的作用, 我们在MDA-MB-231细胞中转染了HOXC8表达质粒, 并进行了转录组测序分析(图1A)。在MDA-MB-231细胞中, 我们发现HOXC8的表达诱导了*GDF15*基因的表达。为了检



A: 通过RNA-seq检测HOXC8过表达细胞中*GDF15*表达水平; B: Western blot检测HOXC8过表达细胞中GDF15的蛋白水平; C: RT-PCR检测HOXC8过表达细胞中*GDF15* mRNA表达水平; D: Western blot检测HOXC8沉默细胞中GDF15的蛋白水平; E: RT-PCR检测HOXC8沉默细胞中*GDF15* mRNA表达水平; F: 2 μg/mL放线菌素D(actinomycin D)处理HOXC8沉默细胞后, RT-PCR检测*GDF15* mRNA表达水平。**P<0.01。

A: the expression level of *GDF15* was detected in HOXC8 overexpressed cells by RNA-seq; B: the protein level of GDF15 was detected by Western-blot in HOXC8 overexpressed cells; C: the mRNA levels of *GDF15* was determined in HOXC8 overexpressed cells by RT-PCR; D: the protein level of GDF15 was measured in HOXC8 silenced cells by Western blot; E: the mRNA levels of *GDF15* was determined in HOXC8 silenced cells by RT-PCR; F: the mRNA levels of *GDF15* was determined by RT-PCR, after the HOXC8 silenced breast cancer cells were treated with 2 μg/mL actinomycin D. **P<0.01.

图1 HOXC8调控乳腺癌细胞中GDF15的表达

Fig.1 HOXC8 regulates the expression of GDF15 in breast cancer cells

测转录组测序的准确性, 我们进行了Western blot和RT-PCR实验, 结果表明在MDA-MB-231和MCF7细胞中, HOXC8的表达增强了GDF15蛋白与mRNA的水平(图1B和图1C), 而HOXC8 shRNA敲低则导致MDA-MB-231或MCF7细胞中GDF15蛋白质或mRNA水平的下降(图1D和图1E)。此外, 用放线菌素D处理细胞不同时间后, 我们发现HOXC8的shRNA敲低不会影响*GDF15* mRNA的稳定性(图1F), 表明HOXC8有可能是通过调节*GDF15*基因的转录来影响其表达水平的。

2.2 HOXC8调控*GDF15*的转录

为了进一步研究HOXC8对GDF15表达的调控, 我们将*GDF15*全长启动子, 即片段1(−2078—+103) nt[21]通过PCR扩增并克隆到荧光素酶报告载体pGL4.23中。我们发现HOXC8的表达显著增强了*GDF15*启动子的转录活性(图2A), 而HOXC8敲低

则明显降低了*GDF15*启动子的转录活性(图2B)。这些实验数据提示, 在乳腺癌细胞中HOXC8参与了*GDF15*基因转录的调控。

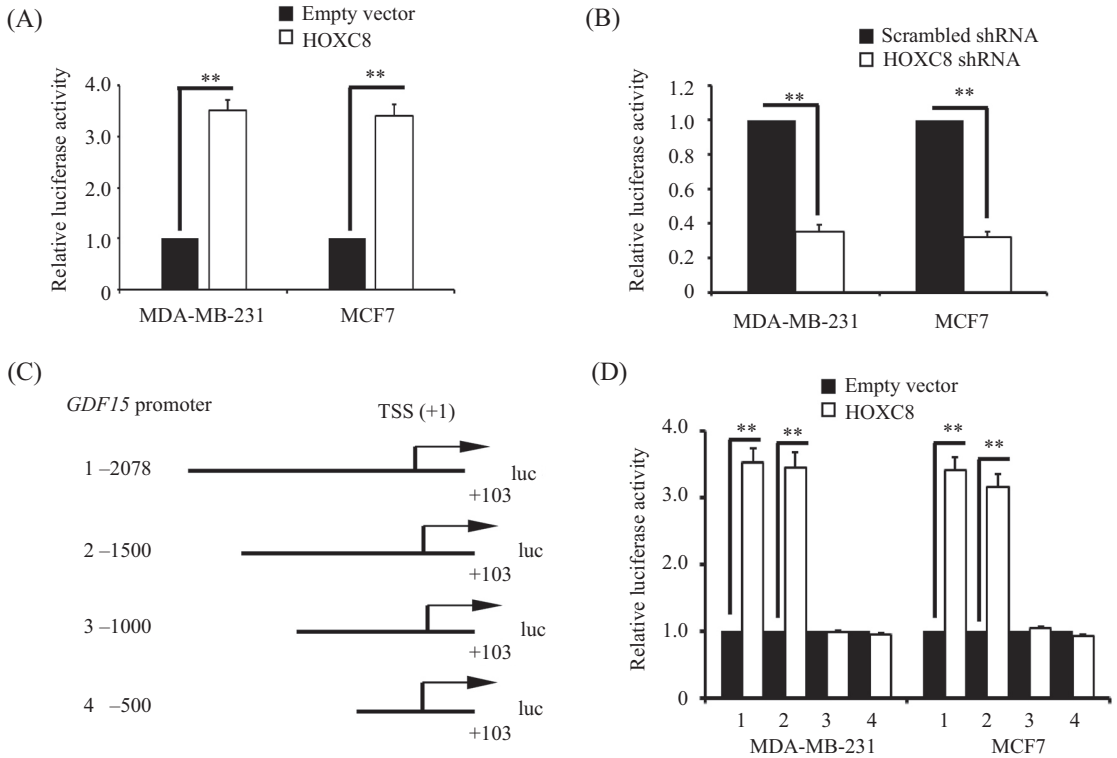
为鉴定HOXC8调控*GDF15*启动子的区域, 我们构建了三种不同长度的*GDF15*启动子的荧光素酶报告基因载体片段2(−1500—+103) nt、片段3(−1000—+103) nt、片段4(−500—+103) nt(图2C)。与*GDF15*全长启动子相比, 我们发现HOXC8表达增强*GDF15*启动子片段2的转录活性, 但对*GDF15*启动子片段3或4的转录活性没有影响(图2D)。该实验结果表明, *GDF15*启动子片段2(−1500—+103) nt是HOXC8调控*GDF15*转录的重要部分。

2.3 HOXC8在乳腺癌细胞中与*GDF15*启动子相结合

为鉴定HOXC8在*GDF15*启动子上的结合位点, 我们通过JASPAR网站(http://jaspar.genereg.net)进行分析, 发现在*GDF15*启动子上可能存在4个HOXC8结合位点(图3A)。根据分析结果, 我们针对这些位点设计了相应的PCR引物(图3A), 然后使用HOXC8抗体进行ChIP实验。ChIP实验结果显示, HOXC8结合到*GDF15*启动子上位点−1038 nt或−1016 nt处(图3B和图3C)。RT-PCR表明, 与IgG对照相比, HOXC8在该位点的结合水平上升了10倍以上。上述结果表明, 在乳腺癌细胞中HOXC8可结合到*GDF15*启动子−1038 nt或−1016 nt的位点上。

2.4 GDF15表达促进乳腺癌细胞的生长与迁移

接着, 我们在乳腺癌细胞中进行了GDF15的敲低或外源性表达来检测其功能。MTT实验显示, GDF15的表达显著增强了细胞活力(图4A), 而沉默GDF15的表达则抑制了细胞活力(图4B)。在琼脂克

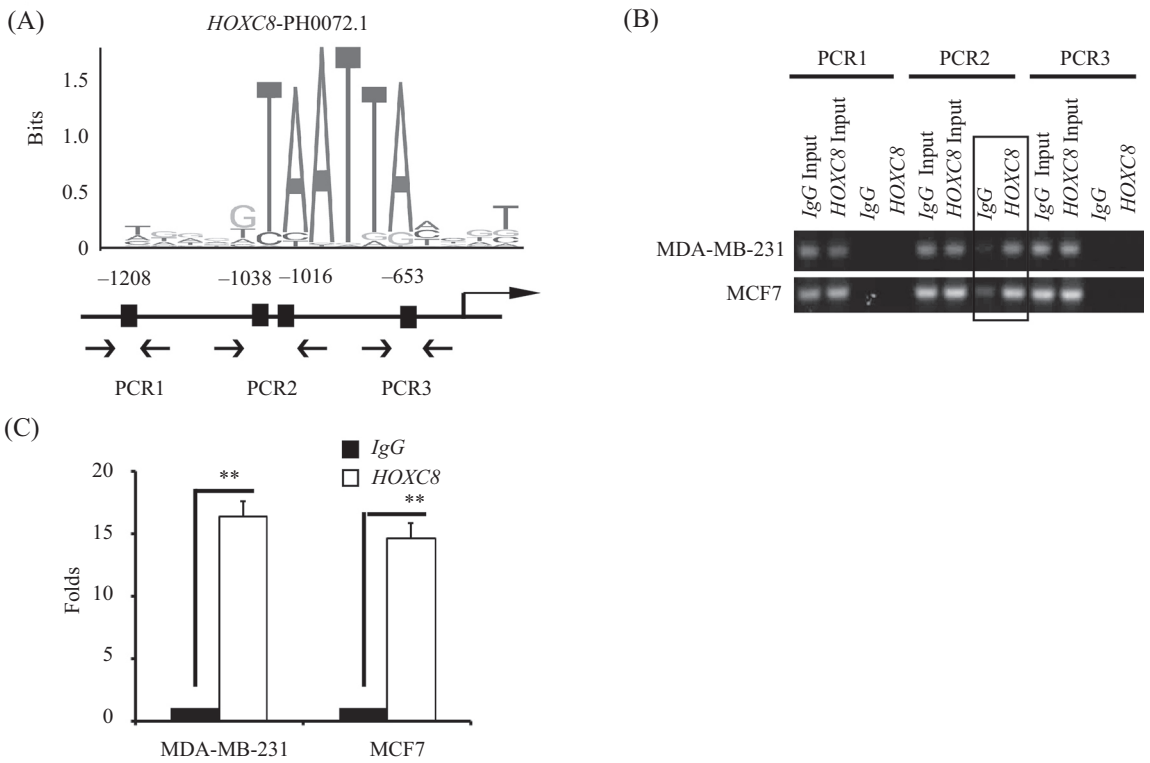


A: 共转染含有*GDF15*启动子(−2078—+103) nt的荧光素酶报告载体和HOXC8表达载体24 h后, 检测荧光素酶活性; B: 共转染含有*GDF15*启动子(−2078—+103) nt的荧光素酶报告载体和HOXC8 shRNA载体24 h后, 检测荧光素酶活性; C: 用PCR法扩增不同长度的*GDF15*启动子, 并克隆到荧光素酶报告载体pGL4.23中, TSS: 转录启动站点; D: 检测共转染不同长度*GDF15*启动子的荧光素酶报告载体和HOXC8表达载体的乳腺癌细胞的荧光素酶活性。**P<0.01。

A: the luciferase activity was measured after 24 h cotransfected with *GDF15* promotor reporting vector (−2078—+103) nt and HOXC8 expressed vector; B: the luciferase activity was measured after 24 h cotransfected with *GDF15* promotor reporting vector (−2078—+103) nt and HOXC8 silenced vector; C: different truncates of *GDF15* promotor were amplified by PCR and cloned into pGL4.23 vector, TSS: transcription start site; D: cotransfected with reporting vectors contain different truncates of *GDF15* promotors and HOXC8 expressed vector, luciferase activity was measured. **P<0.01.

图2 HOXC8在乳腺癌细胞中调控*GDF15*的转录

Fig.2 HOXC8 regulates *GDF15* transcription in breast cancer cells



A: 由JASPAR提供的HOXC8在*GDF15*启动子上可能的结合位点; B: 用HOXC8抗体进行ChIP实验, 沉淀的DNA使用(A)中所示的引物进行PCR扩增, 方框显示为阳性的结果; C: 用HOXC8抗体进行ChIP, 然后通过实时荧光定量PCR进行检测。**P<0.01。

A: the bind site of HOXC8 in *GDF15* promotor was provided by JASPAR; B: the antibody against HOXC8 was applied in ChIP assay and the precipitated DNA was amplified by PCR with primer which had shown in (A) and the positive results were indicated by rectangular box; C: the result from ChIP assay was examined by RT-PCR. **P<0.01.

图3 在乳腺癌细胞中HOXC8结合到*GDF15*启动子上

Fig.3 HOXC8 binds to *GDF15* promotor in breast cancer cells

隆形成实验中, GDF15的表达也显著地增强了细胞锚定非依赖性生长能力(图4C), GDF15的敲低则明显抑制了细胞锚定非依赖性生长能力(图4D)。

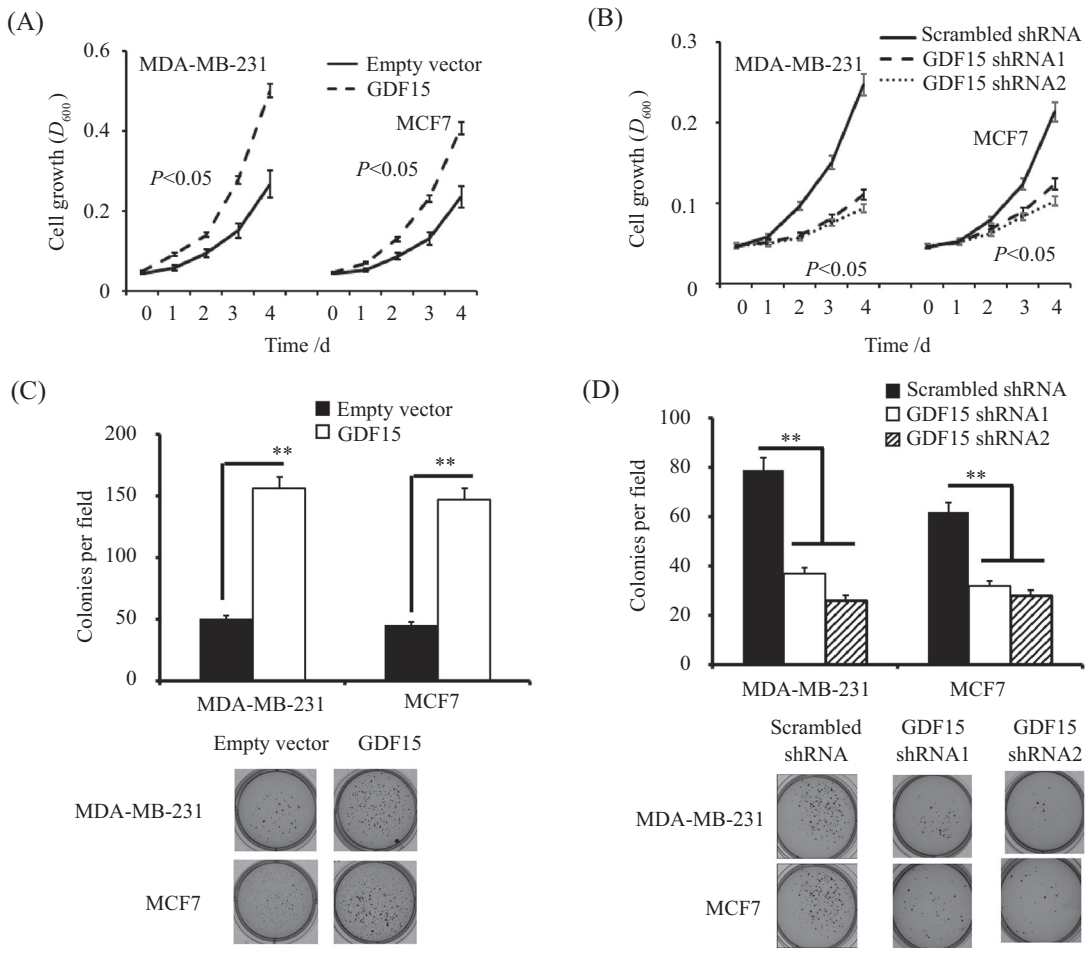
在Transwell迁移实验中, GDF15的表达增强了细胞迁移能力(图5A), 而GDF15的表达沉默则抑制了细胞迁移能力(图5B)。用鬼笔环肽对细胞骨架进行免疫荧光染色实验, 结果显示外源性表达GDF15诱导了MCF7细胞板状伪足或丝状伪足的形成(图5C), 提示GDF15可能通过诱导细胞板状伪足或丝状伪足的形成来增强细胞的迁移运动能力。总的来说, 这些实验结果表明, GDF15表达促进了乳腺癌细胞的生长和迁移。

2.5 HOXC8通过激活GDF15的表达来促进乳腺癌细胞的生长与迁移

在我们先前的研究中, 我们发现HOXC8的表达能促进乳腺癌细胞的生长和迁移等[12], 所以我们在HOXC8 shRNA敲低的细胞中进行GDF15的外源性过表达来进行拯救实验。MTT、琼脂糖克隆形成和Transwell迁移等实验结果表明, HOXC8敲低显著抑制了细胞活性(图6A)、锚定非依赖性生长(图6B)或迁移等(图6C), 而这些抑制作用几乎都可被GDF15的外源性表达所逆转(图6A~图6C)。这些数据表明, HOXC8对乳腺癌细胞生长和迁移的作用是通过激活GDF15的表达来实现的。

2.6 高表达的HOXC8和GDF15与乳腺癌患者的预后不良显著相关

为了进一步研究HOXC8和GDF15在乳腺癌中的作用, 我们使用cBioPortal网站(www.cbioportal.org)对HOXC8及GDF15进行了分析。通过分析数据库中总共9 131个乳腺癌临床样本的数据, 我们发现*HOXC8*或*GDF15*基因显现出较高的基因扩增频率, 特别是在高侵袭性的乳腺癌中(图7A~图7C)。重要的是, *HOXC8*及*GDF15*基因扩增的乳腺癌患者的整体存活率显著下降(P=0.024 2)(图7D)。这些临床样本的数据显示, *HOXC8*和*GDF15*基因在高侵袭性乳腺癌中表达上调, 而HOXC8与GDF15表达的上调则



A: MTT实验检测GDF15外源性表达对乳腺癌细胞活力的影响; B: MTT实验检测GDF15 shRNA沉默对乳腺癌细胞活力的影响; C: 软琼脂实验检测GDF15外源性表达对乳腺癌细胞锚定非依赖性生长的影响; D: 软琼脂实验检测GDF15表达沉默对乳腺癌细胞锚定非依赖性生长的影响。n=3, **P<0.01。

A: the effect of GDF15 over-expressed breast cancer cells on the viability was measured by MTT assay; B: the effect of GDF15 silenced breast cancer cells on the viability was measured by MTT assay; C: the effect of GDF15 ectopic expressed breast cancer cells on the anchorage-independent growth was measured by soft agar colony formation assay; D: the effect of GDF15 silenced breast cancer cells on the anchorage-independent growth was measured by soft agar colony formation assay. n=3, **P <0.01.

图4 GDF15促进乳腺癌细胞的增殖

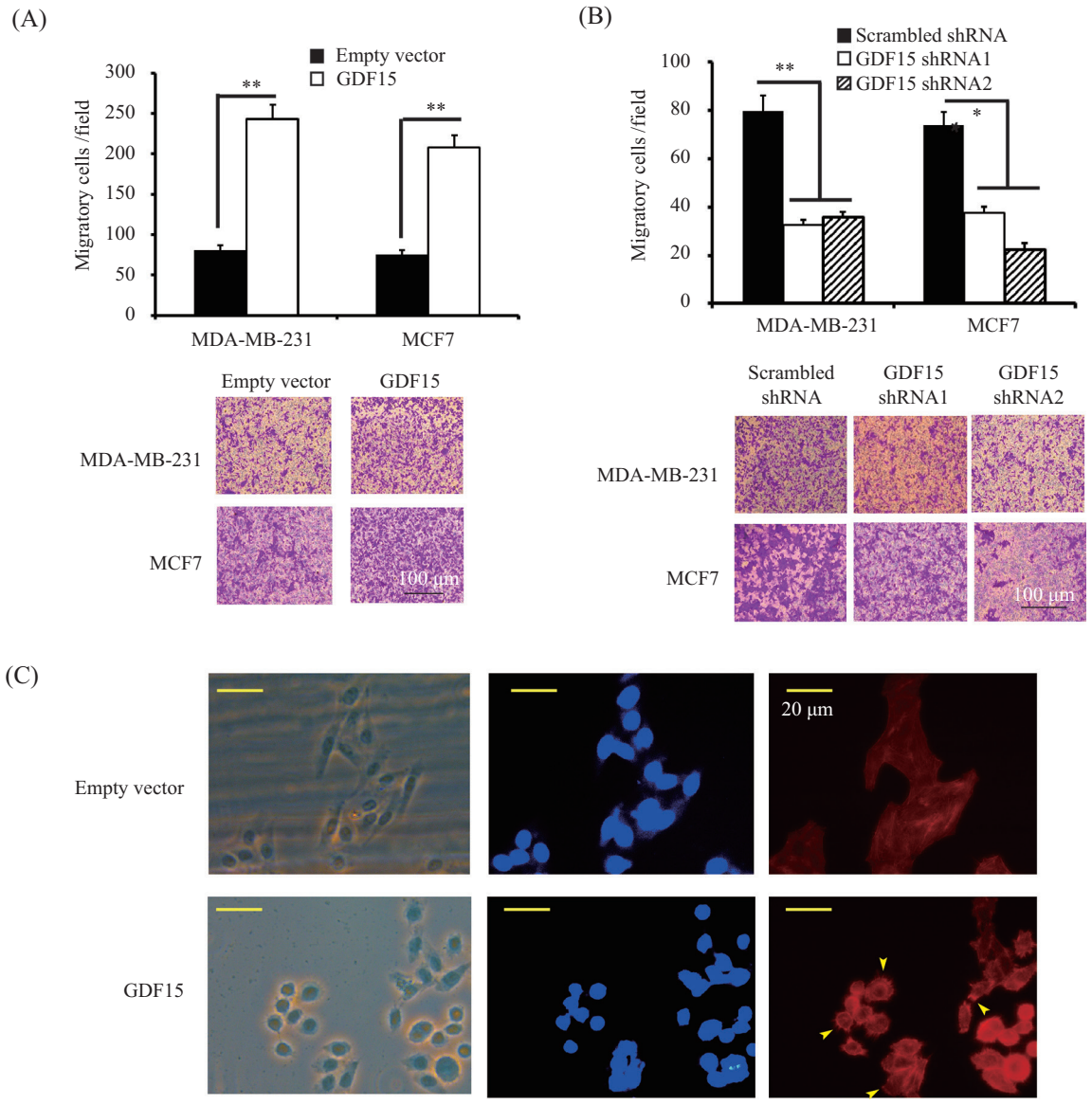
Fig.4 GDF15 promotes breast cancer cells proliferation

与乳腺癌患者预后不良显著相关。

3 讨论

在哺乳动物细胞中, HOX基因家族包含39个成员。根据其在不同染色体上的分布及排列顺序, 将HOX基因的家族成员分为A、B、C及D四组, 每组中包含9~11个基因, 并以1~13对其分别进行命名[7,22]。所有的HOX分子均为转录因子, 包含一个保守的由60或61个氨基酸组成的同源域, 可特异性地识别DNA序列来调节下游基因的转录活性[7,23]。早期的研究发现, *HOX*基因在胚胎生成中发挥着关键作用, 后期的研究则证明*HOX*基因同样在肿瘤的发生发展中发挥着重要的作用[7,22]。不难理解, 因为胚胎生成和肿瘤的发生发展均具有细胞的增殖分化等相似的生物学过程等。HOXC8作为HOX家族的一员, 在多种类型的癌症包括乳腺癌、宫颈癌和肺癌等中, 其表达能显著促进肿瘤细胞的增殖与转移[9,12,24]。由于*HOX*基因均编码转录因子, 因此HOXC8主要是通过调控其下游靶基因的转录表达来发挥其作用的。为了进一步探索HOXC8在乳腺癌中的作用机制, 我们通过转录组测序、Western blot及ChIP等实验来筛选鉴定其下游靶基因的表达, 结果表明*GDF15*是*HOXC8*的下游靶基因之一。

作为TGF-β超家族的成员之一, GDF15参与了



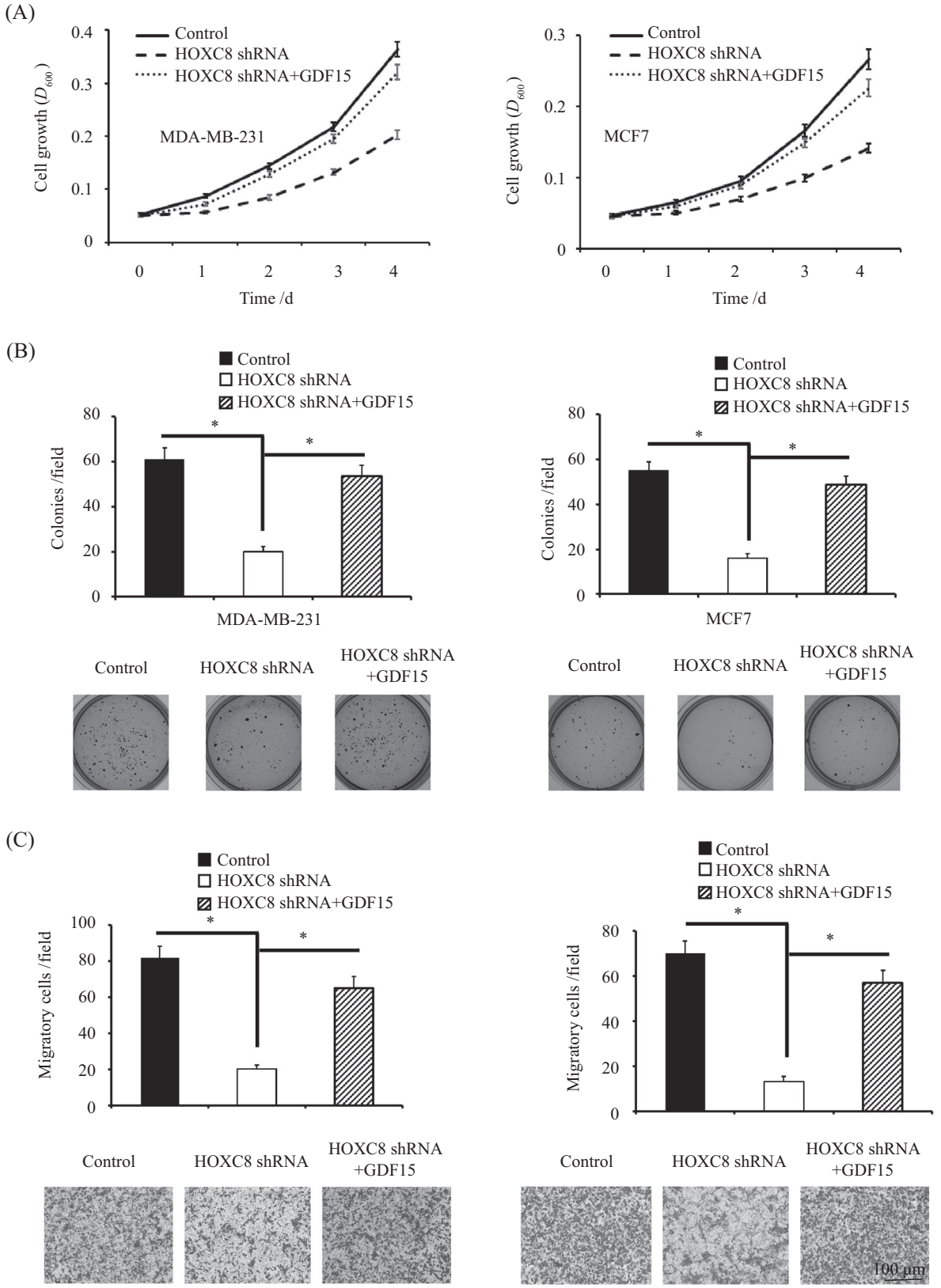
A: Transwell实验检测GDF15外源性表达对乳腺癌细胞迁移能力的影响; B: Transwell实验检测GDF15表达沉默对乳腺癌细胞迁移能力的影响; C: 荧光显微镜观察空载体或GDF15表达载体转染的MCF7细胞的形态。箭头所指为板状伪足或丝状伪足。

A: the effect of GDF15 over-expressed breast cancer cells on migration was measured by Transwell assay; B: the effect of GDF15 silenced breast cancer cells on migration was measured by Transwell assay; C: the micromorphology of MCF7 cells transfected by GDF15 over-expressed and empty vetor was monitored by Fluorescence microscope. Arrows indicated the lamellipodia and filopodium.

图5 GDF15促进乳腺癌细胞的迁移

Fig.5 GDF15 promotes breast cancer cells migration

多种肿瘤如乳腺癌、胰腺癌、胃癌、前列腺癌和结直肠癌等的发生发展过程。然而, GDF15在肿瘤中的具体作用仍不十分清楚。例如, 在前列腺癌中, GDF15的表达促进了癌症的生长和转移, 且GDF15在患者血清中的表达水平也显著升高[25-26]; 然而, 另一项研究表明, 转移性前列腺癌中GDF15表达水平下调, 低表达GDF15的患者显示预后较差[27]。在乳腺癌中, PKB/Akt通路的激活诱导了GDF15的上调, GDF15的高表达促进乳腺癌细胞获得类似干细胞的特征[28-29], 然而, 另有报道显示, 抑制GDF15表达可能促进乳腺癌的转移[20]。因此, 针对GDF15在乳腺癌发展过程中的作用, 有必要通过进一步的研究探究其作用及机制。在本研究中, 我们证明了GDF15表达显著增强了乳腺癌细胞的生长和迁移能力, 而GDF15的沉默则显著抑制了细胞的生长和迁移。同时, 临床样本数据库的分析也证明了GDF15的高表达与患者总生存率的降低显著相关。这些数据表明, 在乳腺癌中GDF15表达促进了肿瘤的发展。

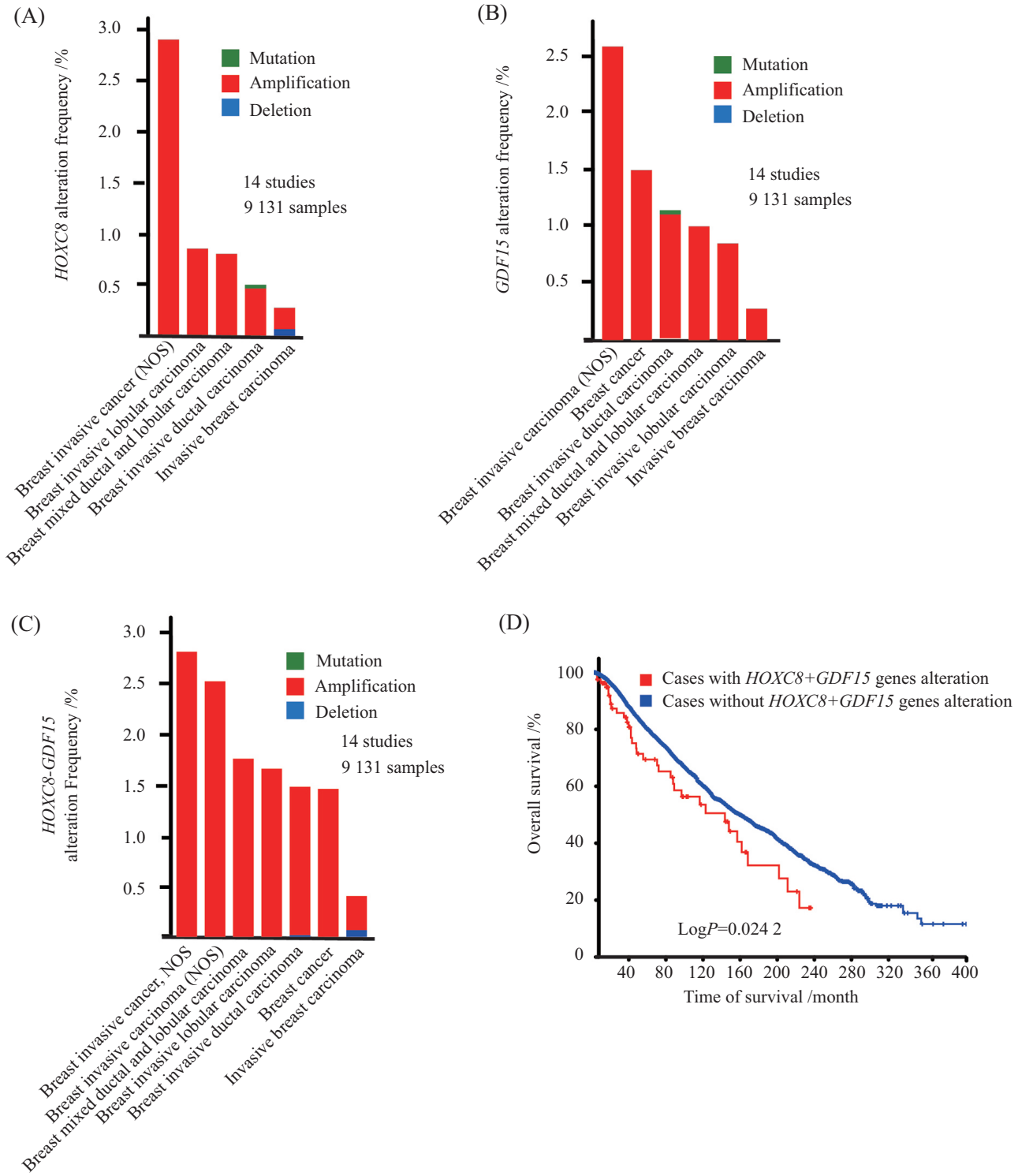


A: MTT实验检测细胞的活力; B: 软琼脂实验检测细胞的锚定非依赖性生长能力; C: Transwell实验检测细胞的迁移能力。n=3, *P<0.05.

A: MTT assay detected the viability of cells; B: soft agar colony formation assay determined the anchorage-independent growth of cells; C: Transwell assay determined the migration of cells. n=3, *P<0.05.

图6 HOXC8-GDF15轴在乳腺癌细胞的增殖与迁移中的作用

Fig.6 The function of HOXC8-GDF15 axis in proliferation and migration of breast cancer cells



用cBioPortal(www.cbioportal.org)网站的乳腺癌数据库对*HOXC8*及*GDF15*基因进行分析, 其中共包括14项研究, 合计9 131例乳腺癌标本。*HOXC8*基因(A)和*GDF15*基因(B)在多种乳腺癌中的扩增频率。*HOXC8*和*GDF15*基因(C)在多种乳腺癌中共同的扩增频率。*HOXC8*与*GDF15*基因表达的扩增导致乳腺癌患者的总生存率显著下降(D)。

Using the data base of breast cancer specimens from the cBioPortal website (www.cbioportal.org), we analysized *HOXC8* and *GDF15* genes expression in 14 studies including 9 131 samples of breast cancer. The amplified frequency of *HOXC8* (A) and *GDF15* (B) in different types of breast cancer. The co-amplified frequency of *HOXC8* and *GDF15* in different types of breast cancer (C). The significant decrease of overall survival rate of breast cancer patients which caused by the amplified expression of *HOXC8* and *GDF15* (D).

图7 在高侵袭性乳腺癌细胞中*HOXC8*与*GDF15*基因均显示高扩增性

Fig.7 Both *HOXC8* and *GDF15* genes show high amplification in invasive breast cancer cells

如上所述, GDF15在多种肿瘤细胞中表达失调, 然而*GDF15*转录调控机制在很大程度上仍不清楚。通过RNA-seq、Western blot等实验, 我们发现HOXC8参与了乳腺癌细胞中GDF15表达的调控。荧光素酶报告和ChIP等实验进一步显示, HOXC8作为转录激活因子直接结合到*GDF15*启动子上来调控其转录表达。然而, 通过对*GDF15*启动子序列的分析表明, *GDF15*启动子上还包含p53、Sp1和AP1/2等结合位点[30-31], 而TSA(Trichostatin A)抑制HADCs的活性也诱导了GDF15的表达[32], 说明了GDF15表达调控依赖于多种转录因子与表观遗传调控等之间的合作。因此, 需要来进一步研究来阐明HOXC8与其他转录因子或因素之间的合作, 这将有益于揭示GDF15在乳腺癌发生发展中的作用机制。

综上所述, 我们的研究表明, 在乳腺癌细胞中*GDF15*是HOXC8调控的靶基因, GDF15能显著地促进乳腺癌细胞的生长与迁移等, 而HOXC8则通过激活GDF15表达来促进肿瘤细胞的生长与迁移以及乳腺癌的发展。本研究同时表明, *HOXC8*和*GDF15*基因在高侵袭性乳腺癌中存在明显的表达上调, 而其上调与乳腺癌患者的预后不良显著相关。因此, HOXC8-GDF15轴有可能成为乳腺癌诊断和治疗的新靶点。

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