The Biofunctions of LIM Protein Ajuba in Human Cancer Cells

Chen Ning

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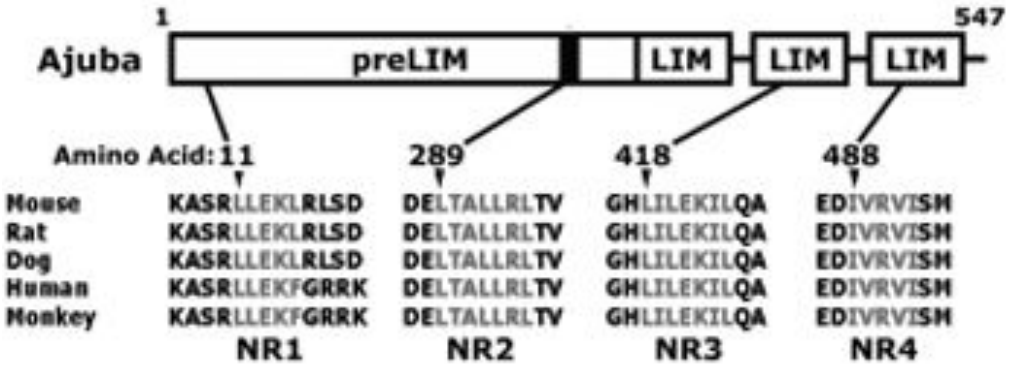
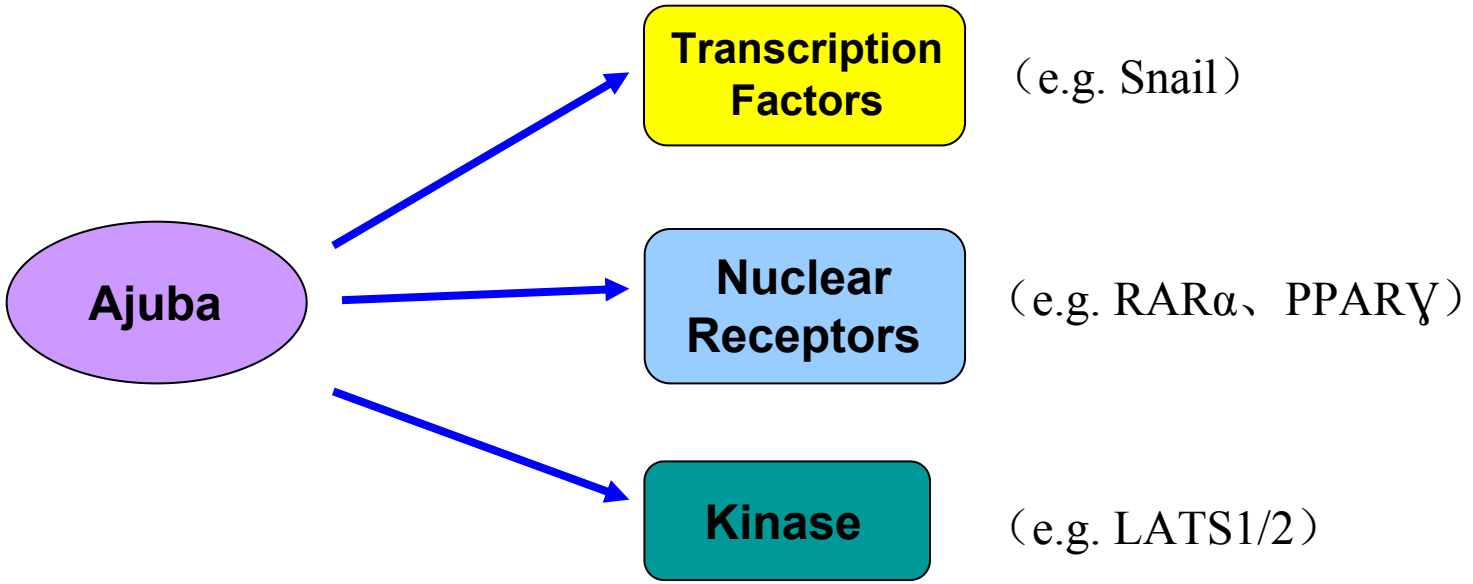
上海交通大学医学院
Shanghai Jiao Tong University School of Medicine

LIM Protein Family Member — Ajuba



- AJUBA is a multiple LIM domain-containing protein and belongs to the AJUBA/zyxin family of LIM proteins.
- Ajuba is characterized by three tandem C-terminal LIM domains and unique N-terminal regions designated the PreLIM regions.

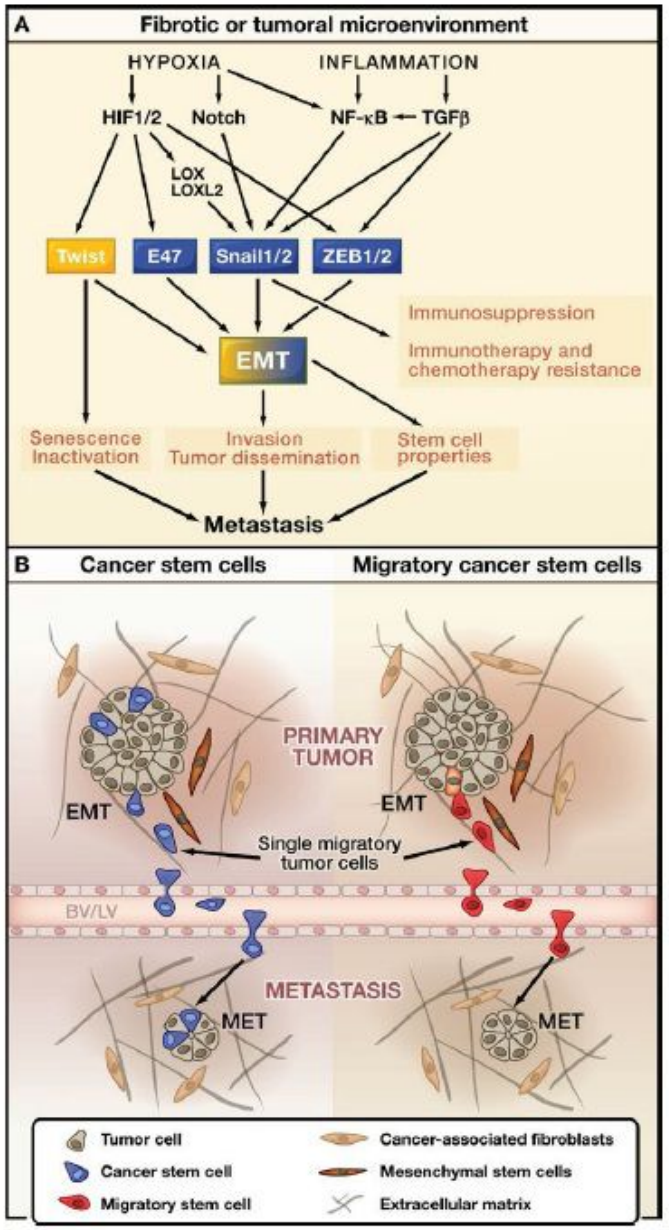
- The AJUBA protein is predominantly cytoplasmic, yet is recruited to E-cadherin-adhesive complexes during epithelium formation and can shuttle between the nucleus and cytoplasm.
- The AJUBA protein may function as a scaffold protein to assemble multiple cytoplasmic protein complexes involved in the processes of cell adhesion, migration, mitosis, and cell differentiation.



Ajuba Regulates N-cadherin Expression and Affect CRC Cell Migration via Interacting with Twist

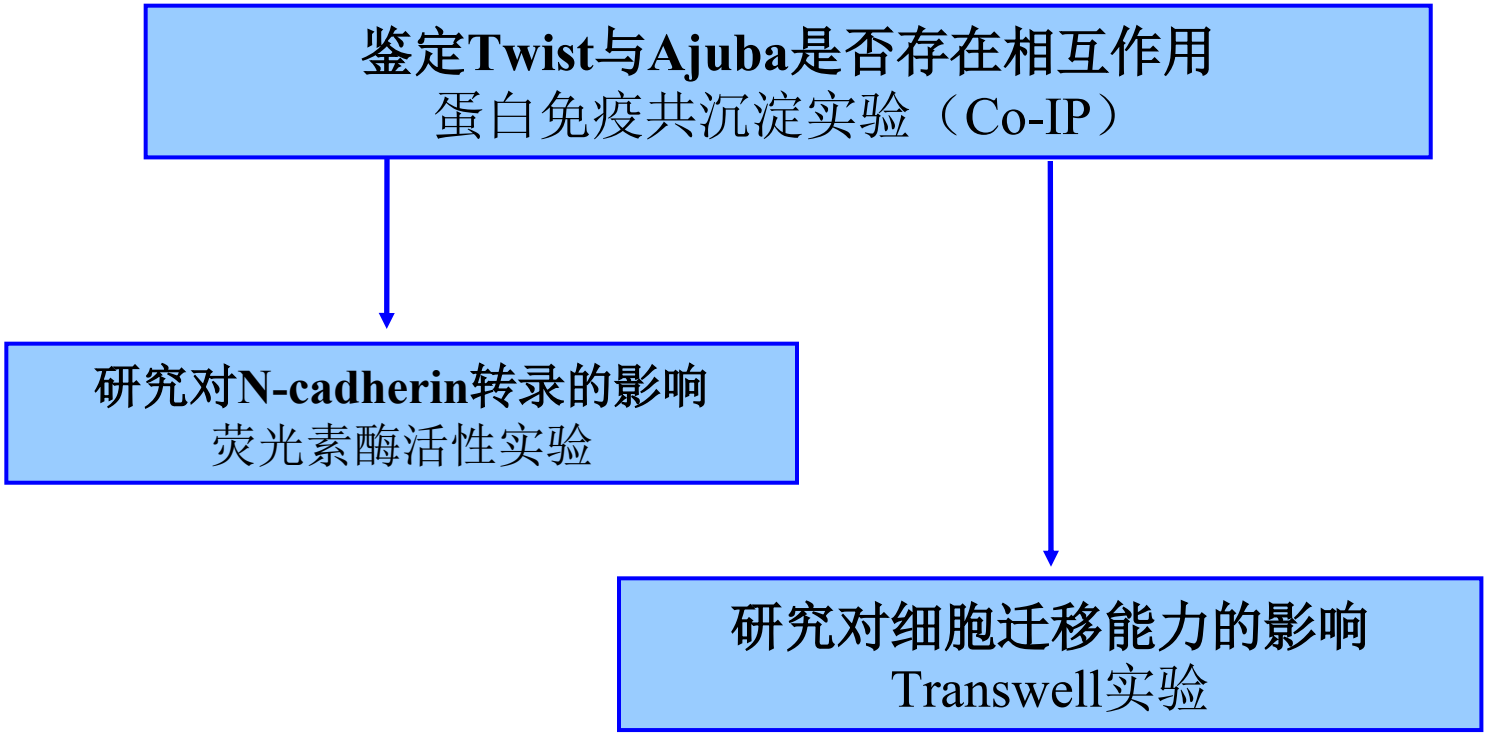
Twist

- The transcription factor Twist, a master regulator of embryonic morphogenesis, plays an essential role in metastasis.
- Ectopic expression of Twist results in loss of E-cadherin-mediated cell-cell adhesion, activation of mesenchymal markers, and induction of cell motility, suggesting that Twist contributes to metastasis by promoting an epithelial-mesenchymal transition (EMT).

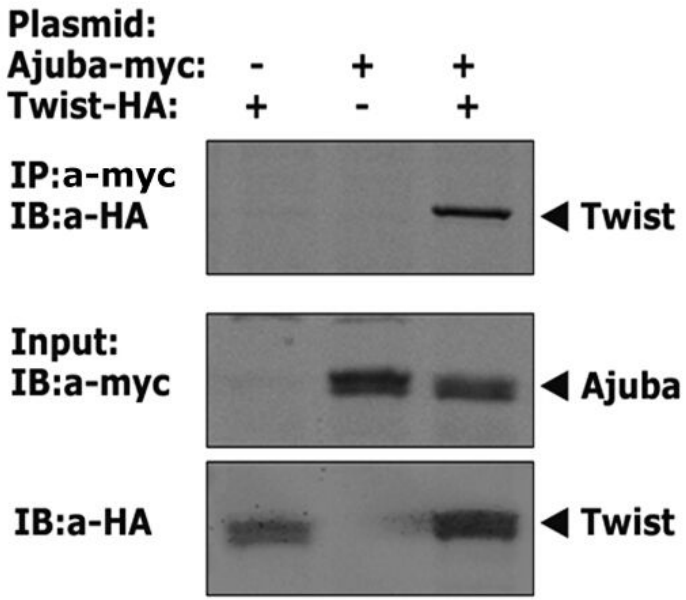


Thiery JP, Acloque H, Huang RY, et al.Epithelial-mesenchymal transitions in development and disease [J].Cell, 2009, 139(5):871-890.

研究步骤

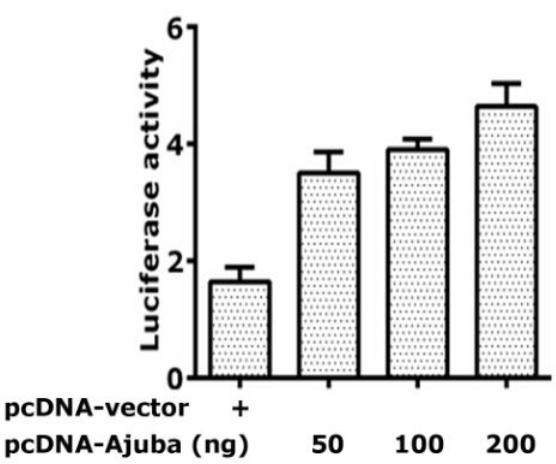

鉴定Twist与Ajuba是否存在相互作用
蛋白免疫共沉淀实验（Co-IP）
研究对N-cadherin转录的影响
荧光素酶活性实验
研究对细胞迁移能力的影响
Transwell实验

Ajuba interacts with TF Twist

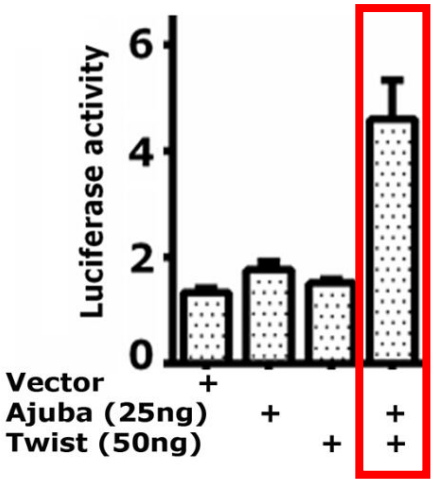


Ajuba与Twist通过外源性免疫共沉淀实验证明存在相互作用

Ajuba Regulates N-cadherin Expression synergizing with Twist

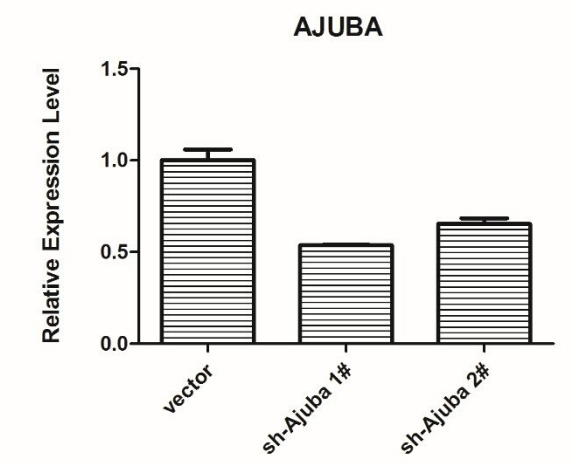


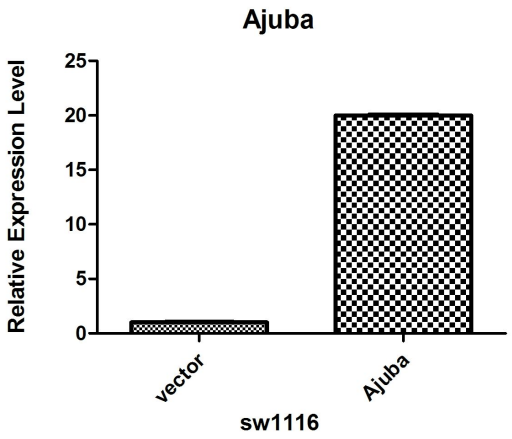
外源性Ajuba蛋白浓度梯度实验

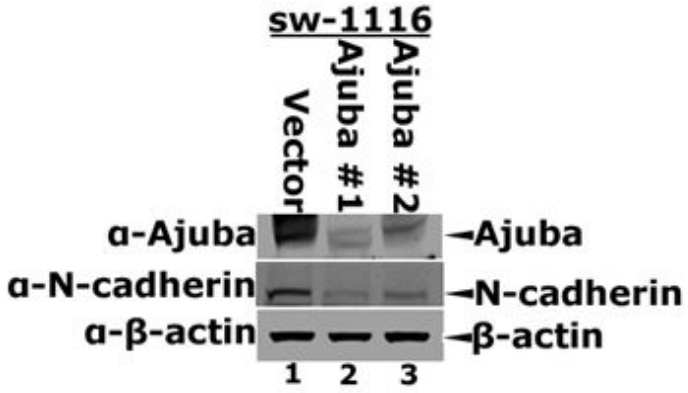


Ajuba与Twsit共转染报告基因实验

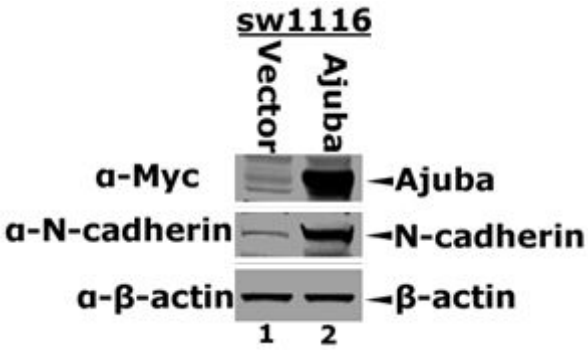
Ajuba Enhances Colorectal Cancer Cell Migration





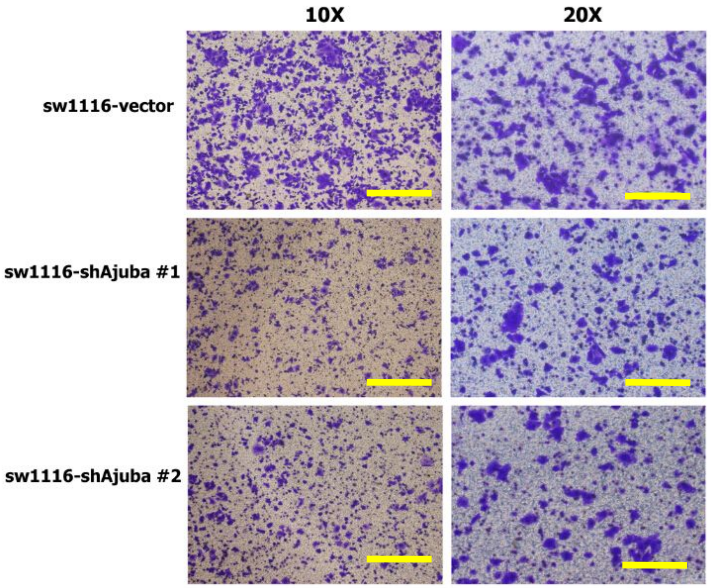


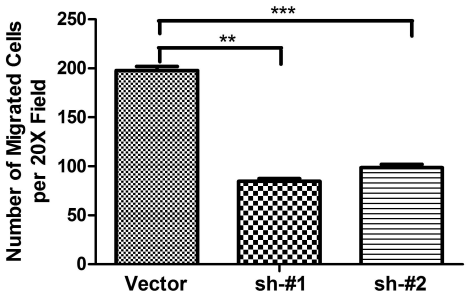
构建并鉴定SW1116-shAjuba稳转细胞



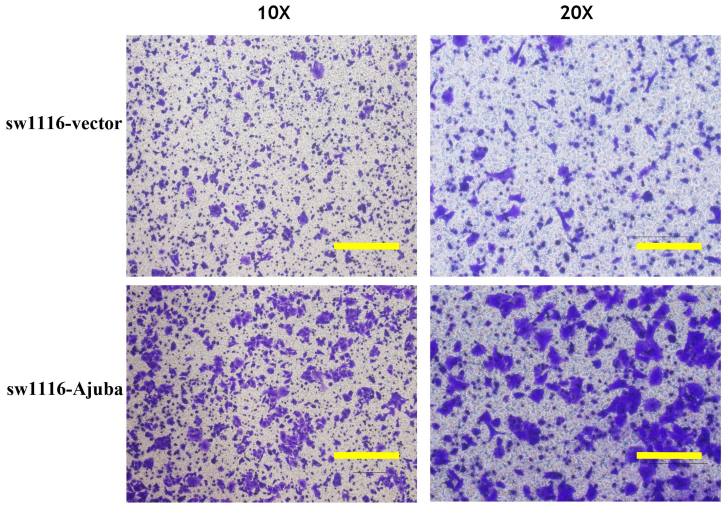
构建并鉴定SW1116-Ajuba稳转细胞

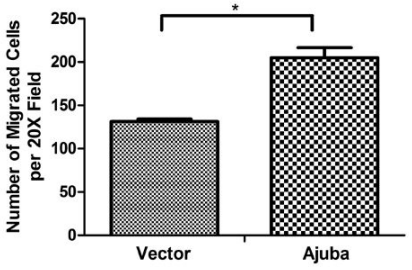
Ajuba Enhances Colorectal Cancer Cell Migration





Transwell侵袭实验检测SW1116-shAjuba稳转细胞迁移能力

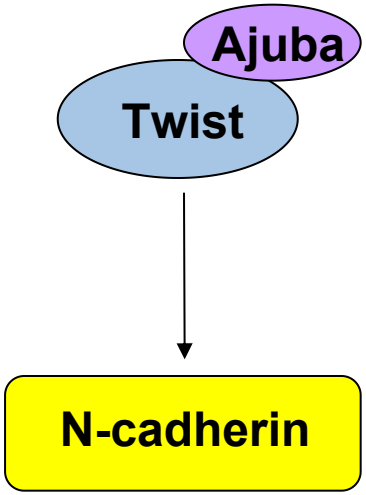




Transwell侵袭实验检测SW1116-Ajuba稳转细胞迁移能力

Conclusion

- LIM protein Ajuba can interact with TF Twist in CRC cells.
- We found Ajuba is a new coactivator of Twist, which affects mesenchymal biomarker N-cadherin expression and CRC cell migration.

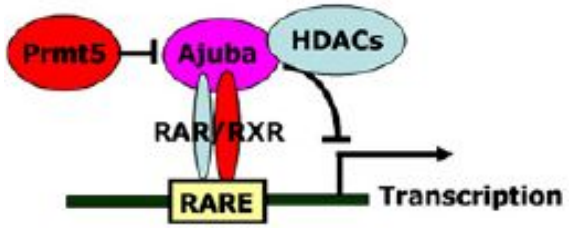


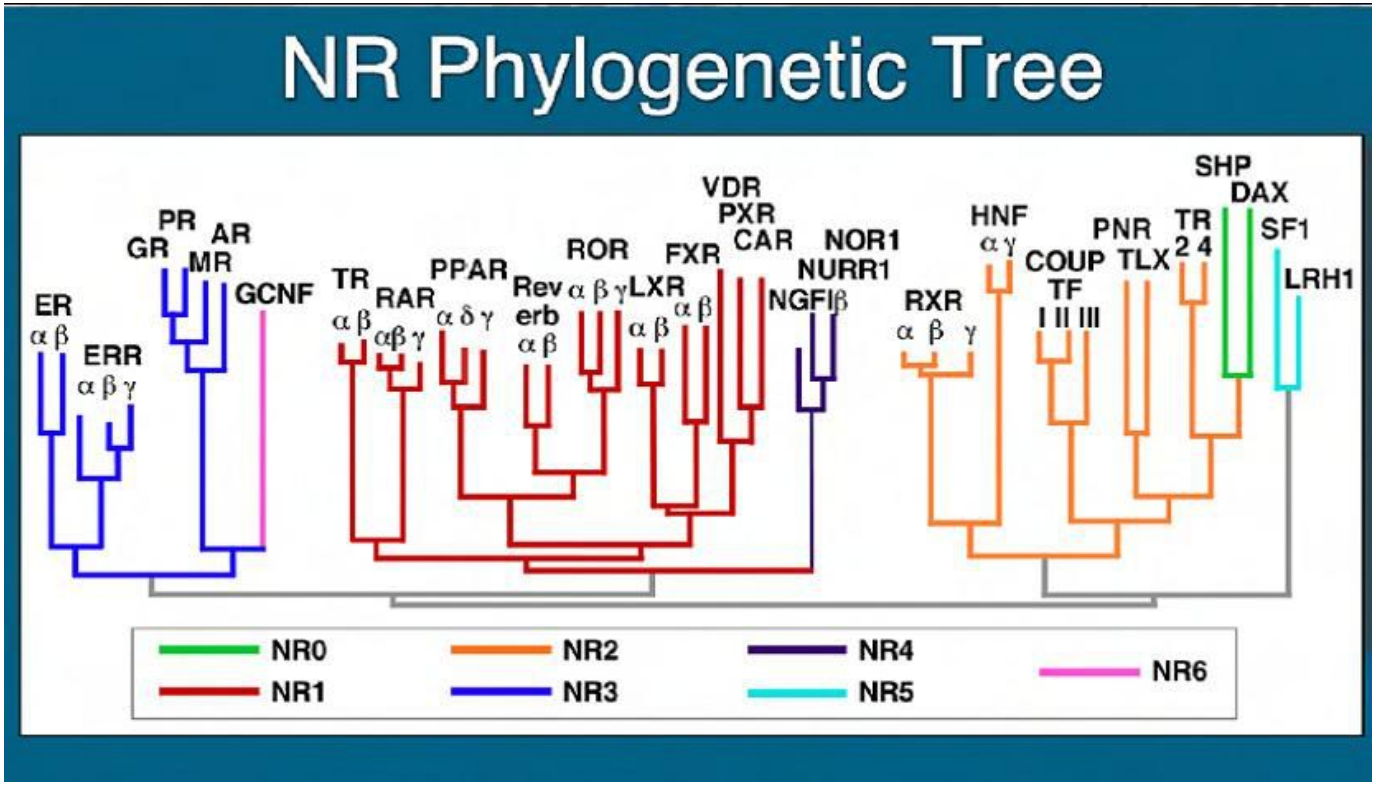
Ajuba regulates CDK6 mediated G1/S transition via interacting with ERα in BrCA

LIM protein Ajuba functions as a nuclear receptor corepressor and negatively regulates retinoic acid signaling

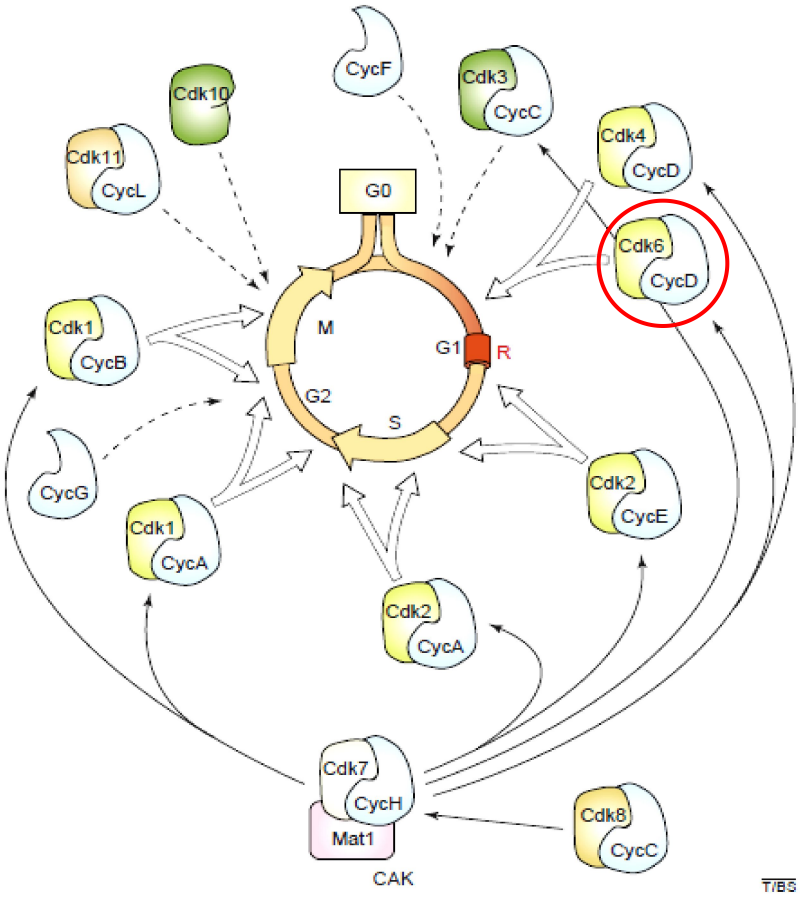
Zhaoyuan Hou[a], Hongzhuang Peng[a], David E. White[a], Dmitri G. Negorev[a], Gerd G. Maul[a], Yunfeng Feng[b], Gregory D. Longmore[b], Samuel Waxman[c], Arthur Zelent[d], and Frank J. Rauscher III[a,1]

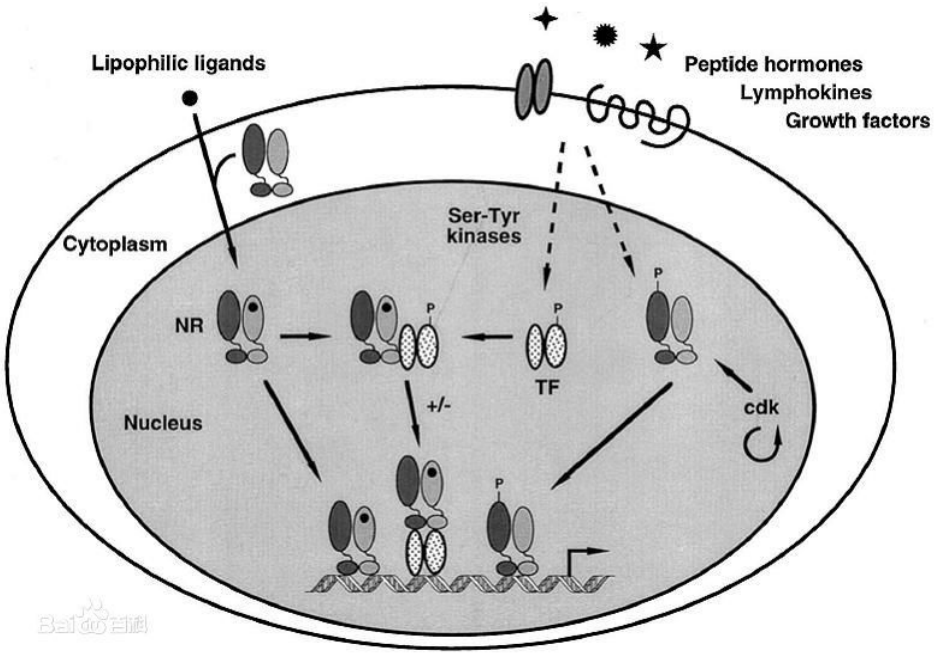
[a]The Wistar Institute, Philadelphia, PA 19104; [b]Department of Medicine, Washington University, St. Louis, MO 63110; [c]Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029; and [d]Section of Hemato-Oncology, Institute of Cancer Research, London, United Kingdom





Background





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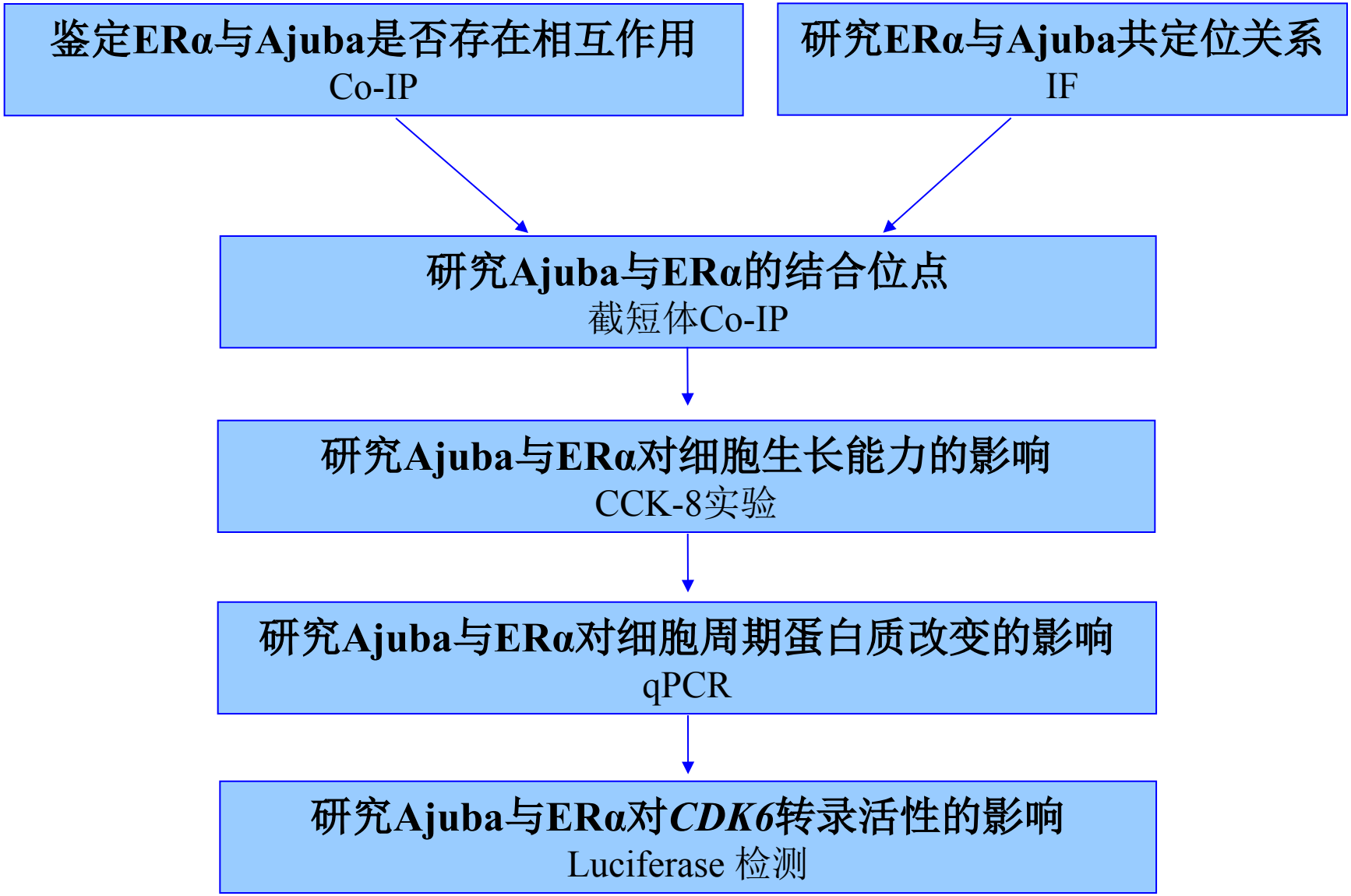
研究步骤


鉴定ERα与Ajuba是否存在相互作用
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研究ERα与Ajuba共定位关系
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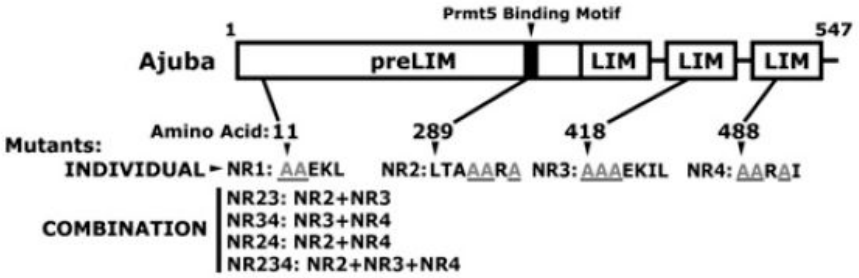
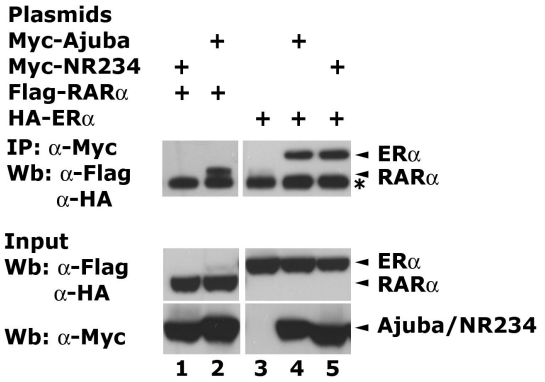
qPCR
研究Ajuba与ERα对*CDK6*转录活性的影响
Luciferase 检测

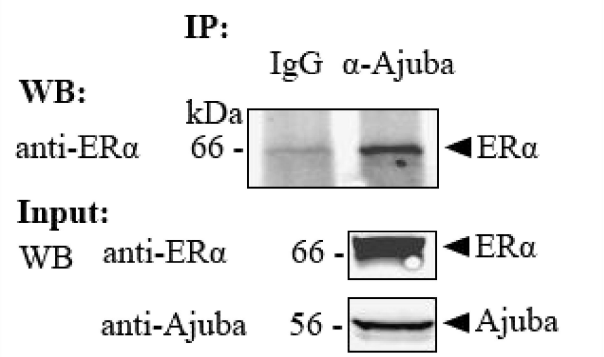
Ajuba interacts with ERα



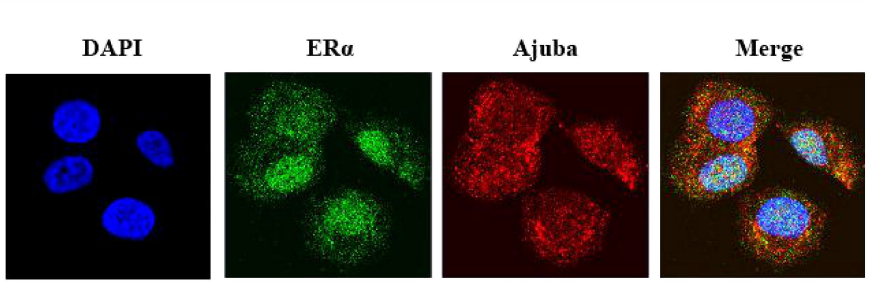
Ajuba蛋白突变体示意图



Ajuba与核受体ERα存在相互作用

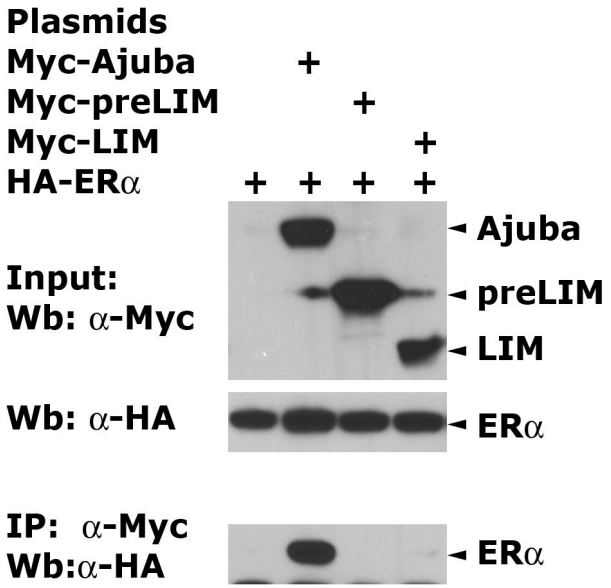


T47D细胞内源性ERα与Ajuba Co-IP实验

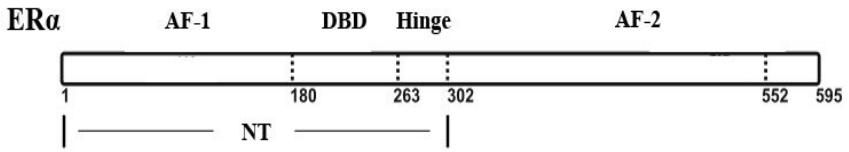


IF鉴定ERα与Ajuba细胞内定位

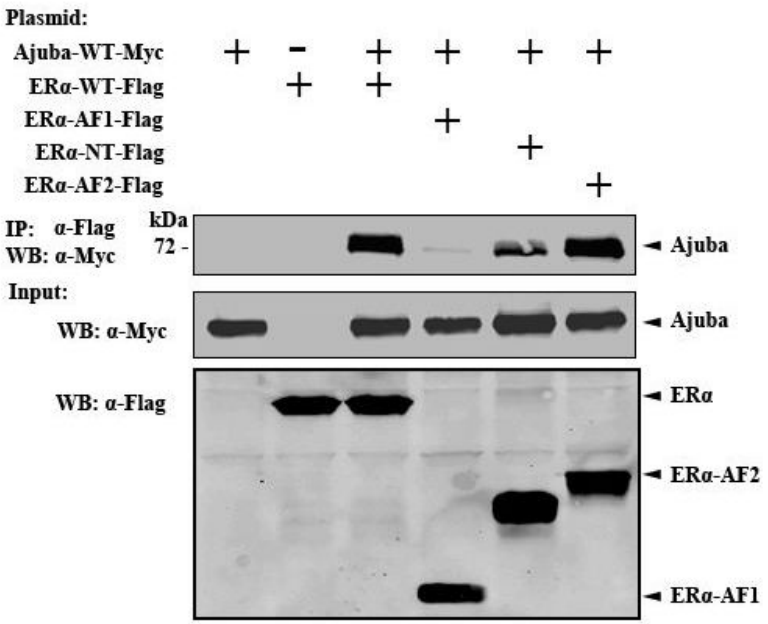
Binding motifs of Ajuba-ERα interaction



Ajuba与ERα全长结合

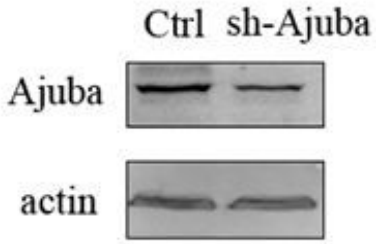


ERα蛋白结构示意图

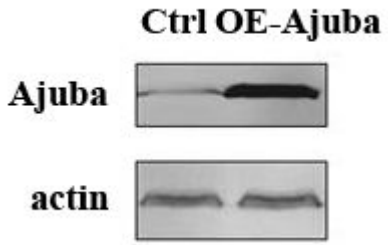


ERα C端与Ajuba相互结合

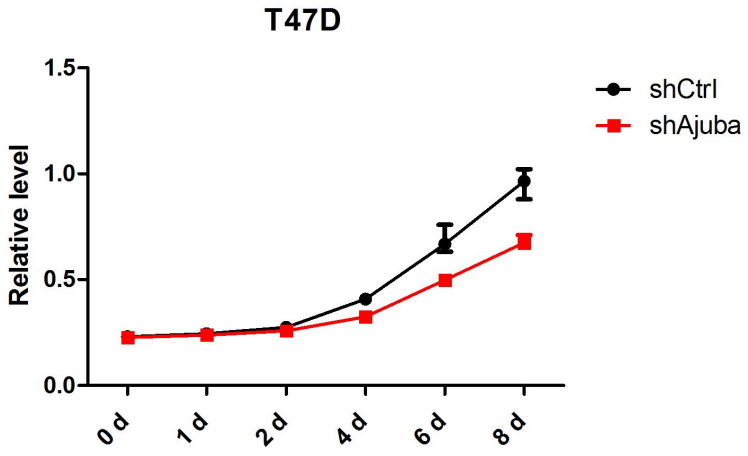
Ajuba enhances cell growth



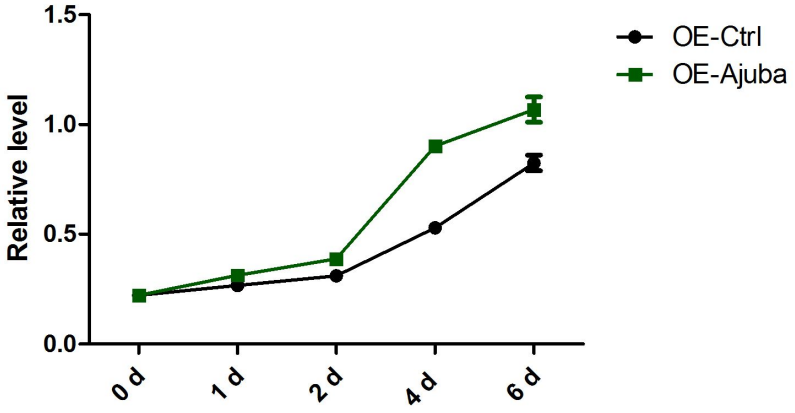
构建T47D-shAjuba稳转细胞



构建T47D-OEAjuba稳转细胞

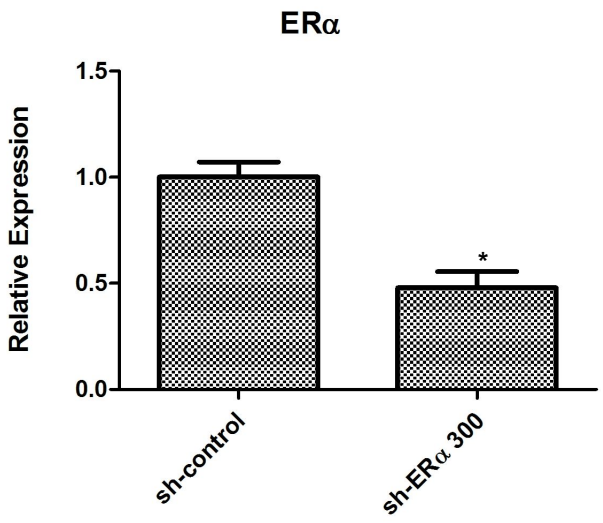


CCK8检测T47D-shAjuba细胞生长变化

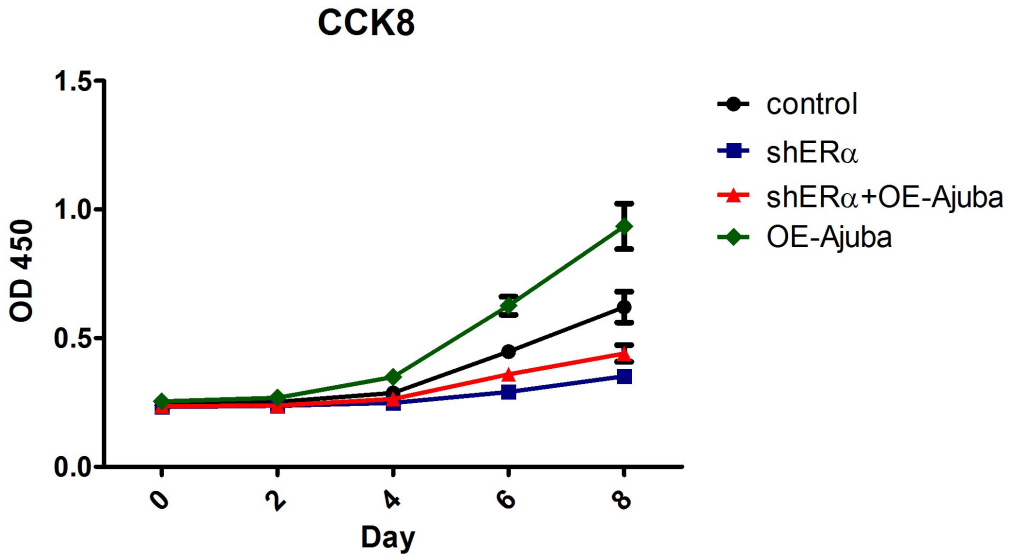


CCK8检测T47D-OEAjuba细胞生长变化

Ajuba enhances cell growth via coacting with ERα

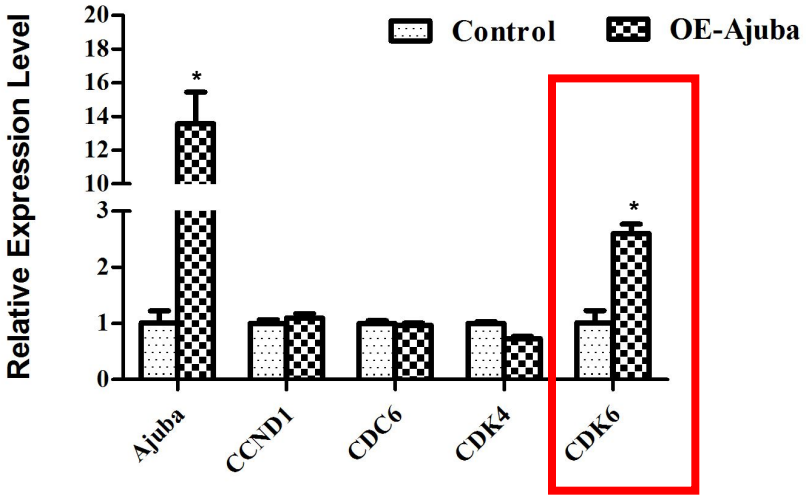


构建T47D-shERα稳转细胞

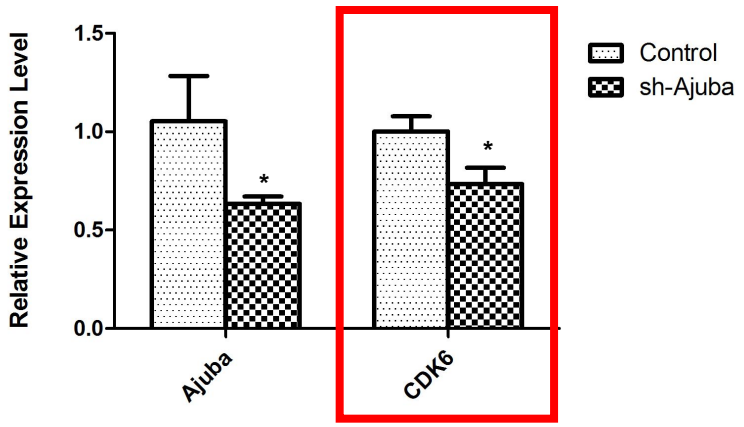


CCK8检测T47D稳转细胞生长差异

Ajuba regulates *CDK6* gene expression by interacting with ERα

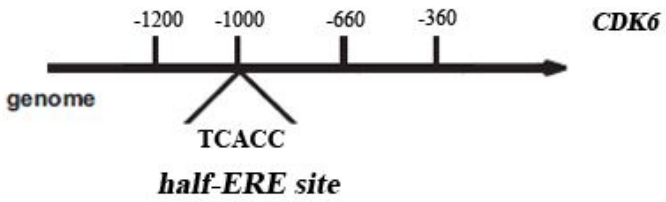


T47D-OEAjuba细胞中生长周期相关基因表达水平

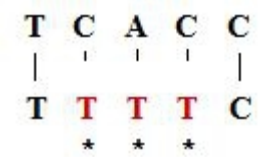


T47D-shAjuba细胞*CDK6*基因表达水平

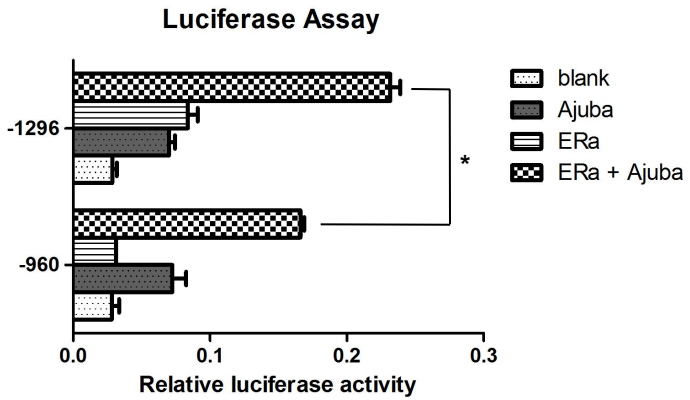
Ajuba regulates *CDK6* gene expression by interacting with ERα



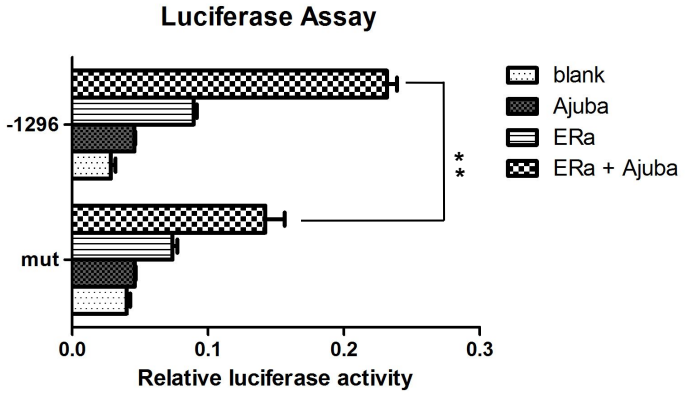
*CDK6*启动子区示意图



half-ERE位点突变示意图



Ajuba、ERα对*CDK6*启动子的作用



*CDK6*启动序列突变后转录活性下降

Conclusion

- LIM protein Ajuba can interact with Nuclear Receptor ERα.
- Ajuba regulates CDK6 expression and affects BrCA cell growth by funcitioning as a coactiator of ERα.
- We found CDK6 is a new downstream target of ERα.