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• 泌尿系统肿瘤专题 •

雄激素阻断治疗后前列腺癌组织中肿瘤干细胞比例变化

陈　伟，王国民

Androgen Deprivation Therapy Can Change Proportion of Prostate Cancer Stem Cells

CHEN Wei, WANG Guomin

Department of Urology, Zhongshan Hospital, Fudan University, Shanghai 200032*, China*

Corresponding Author: WANG Guomin, E-mail:wangguomin@zs-hospital.sh.cn

Abstract:Objective　To study the effect of androgen deprivation therapy on prostate cancer stem cells in prostate cancer tissues and to investigate the mechanisms of the formation of androgen independent prostate cancer. **Methods**　Eighteen patients were divided into 2 groups, the ADT (androgen deprivation therapy) group and non-ADT group. The proportions of cancer stem cells in preoperative and postoperative prostate cancer tissues were compared with immunofluorescence. **Results**　In the non-ADT group, the differences of cancer stem cell proportion between preoperative and postoperative specimens were not prominent[(3.56 ±1.33)% *vs.* (3.78±1.39)% ,n = 9, t =-0.686, P = 0.512]. But in the ADT group, compared with preoperative specimens, the proportion of prostate cancer stem cells was significantly increased after undergoing androgen deprivation therapy for 3 months [(3.44±1.81)% *vs.* (9.22±1.71)% ,n = 9, t = -6.353, P = 0.000]. In the tissue adjacent to carcinoma, stem cell markers were also increased after ADT. **Conclusion**　Androgen probably is involved in the differentiation of prostate stem cells or prostate cancer stem cells. The formation of androgen independent prostate cancer may be related to prostate cancer stem cells.

Key words: Cancer stem cells; Androgen independent prostate cancer; Androgen deprivation therapy

摘　要：目的　研究雄激素阻断治疗（androgen deprivation therapy，ADT）对前列腺癌组织中肿瘤干细胞比例的影响，探讨激素非依赖性前列腺癌形成的机制。**方法**　实验分为ADT治疗组和无ADT治疗组，应用免疫荧光技术比较两组前列腺癌患者术前术后标本中肿瘤干细胞的比例变化。**结果**　无ADT组术前穿刺标本和前列腺根治切除术后标本肿瘤干细胞比例差异无统计学意义[（3.56±1.33）% *vs.*（3.78±1.39）%, n=9, t=-0.686, P=0.512]，在ADT组，接受雄激素阻断治疗3月后，与穿刺标本比较，根治切除标本中前列腺癌肿瘤干细胞比例明显升高[（3.44±1.81）% *vs.*（9.22±1.71）%，n=9, t=-6.353, P=0.000]。在癌旁组织，也可见ADT后干细胞标志的增加。**结论**　雄激素可能参与前列腺和前列腺癌组织中干细胞的分化；雄激素非依赖性前列腺癌的形成可能与前列腺癌肿瘤干细胞有关。

关键词：肿瘤干细胞；雄激素非依赖性前列腺癌；雄激素阻断治疗

中图分类号：R737.25　　**文献标识码**：A

0　引言

前列腺癌是老年男性常见的肿瘤，在过去的几十年里，雄激素被看作是前列腺癌的重要促进因子，是前列腺癌的病因之一。因此，雄激素阻断治疗（androgen deprivation therapy, ADT）成为前列腺癌的常规治疗[1-2]。然而，大多数前列腺癌在ADT后变为雄激素非依赖性前列腺癌（androgen independent prostate cancer, AIPC）[3]，肿瘤侵袭性更强，更容易转移[4-5]。前列腺癌初治应用ADT有效，逐渐转为AIPC的机制目前仍不清楚，不表达雄激素受体的前列腺肿瘤干细胞(cancer stem cells, CSCs)选择性存活也许是AIPC的成因。因为其独特的生物学特性，肿瘤干细胞抵抗放疗[6]、化疗和激素治疗[7-11]。

本课题拟研究雄激素阻断治疗对前列腺癌组织中肿瘤干细胞的影响，以CD44和整合素α2β1作为前列腺肿瘤干细胞标志，探讨激素非依赖性前列腺癌的形成机制。

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作者单位：200032上海，复旦大学中山医院泌尿外科

通信作者：王国民，E-mail：wangguomin@zs-hospital.sh.cn

作者简介：陈伟(1978-)，男，博士，主治医师，主要从事泌尿系肿瘤的基础和临床研究

1 资料与方法

1.1 患者资料

18例前列腺癌病理石蜡切片，包括术前前列腺穿刺活检病理及根治性前列腺切除术后的肿瘤病理。其中，9例在根治性前列腺切除前接受3个月的ADT。此外，还检测2例去势术后5年和7年后重复穿刺的病理切片（未手术），共20例患者的标本。

1.2 免疫组织化学

（1）石蜡切片脱蜡至水。（2）1.5%H_2O_2 室温孵育10 min，消除内源性过氧化物酶活性。PBS清洗5 min×2次。（3）10%山羊血清孵育1 h。（4）加入PE标记的抗-CD44单克隆抗体 (10 μl/ml, Novus Biologicals, LLC. Littleton) 和FITC标记的抗-整合素α2β1单克隆抗体 (10 μl/ml, Life Span Bioscience, Seattle) 37°C 孵育1 h。PBS洗涤5 min×3次。（5）防淬灭封片剂封片。（6）荧光显微镜观察CD44和整合素α2β1的表达。蓝色为DAPI细胞核染色，标记细胞核；红色为CD44阳性细胞；绿色为整合素α2β1阳性细胞；橙色为CD44和整合素α2β1双阳性细胞; CD44和整合素α2β1阳性部位均定位于细胞膜。

1.3 统计学方法

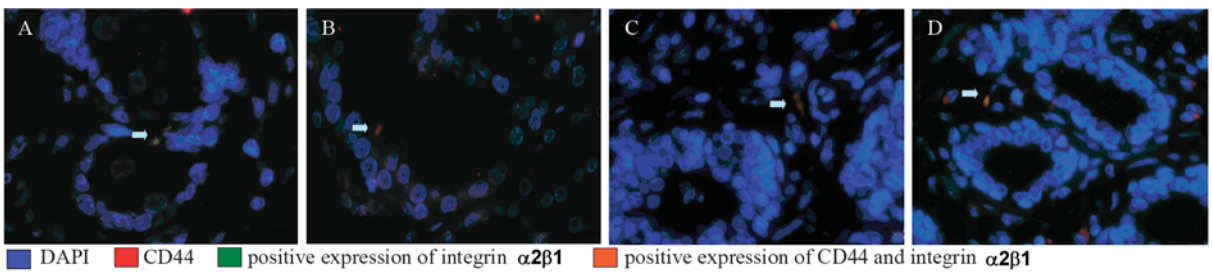
观察5个高倍视野（40倍），计数细胞不少于1 000个，比较术前术后标本中CD44和整合素α2β1双阳性细胞比例的平均值，作 t 检验，P<0.05为差异有统计学意义。

2 结果

18例前列腺癌组织均可见CD44和整合素α2β1表达。在非ADT治疗组，前列腺癌组织（穿刺活检标本）含有(3.56±1.33)% (n=9)的CD44和整合素α2β1双阳性细胞，根治性前列腺切除后，该组前列腺癌组织（根治切除标本）中CD44和整合素α2β1双阳性细胞增加至 (3.78±1.39)% (n=9, t=-0.686, P=0.512)，治疗前后双阳性细胞比例差异无统计学意义。在ADT治疗组，ADT开始前的前列腺癌组织（穿刺活检标本）含有(3.44±1.81)% (n=9)的CD44和整合素α2β1双阳性细胞，患者接受3月的ADT后，在根治性前列腺切除获得的前列腺癌组织中CD44和整合素α2β1双阳性细胞增加至 (9.22±1.71)% (n=9, t=-6.353, P=0.000)。结果提示ADT治疗后，CD44和整合素α2β1双阳性细胞显著增加。除此之外，接受5年和7年ADT治疗后，穿刺标本中CD44和整合素α2β1双阳性细胞增至18.7%，比初诊时穿刺标本平均比例增加4.3倍，见图1A、1B。在癌旁组织中，ADT同样增加了CD44和整合素α2β1双阳性细胞比例，见图1C、1D。提示雄激素阻断也使正常的前列腺组织的前列腺干细胞比例增加。术前穿刺肿瘤标本与术后切除肿瘤标本中CD44和整合素α2β1双阳性细胞比例变化，见图2。

3 讨论

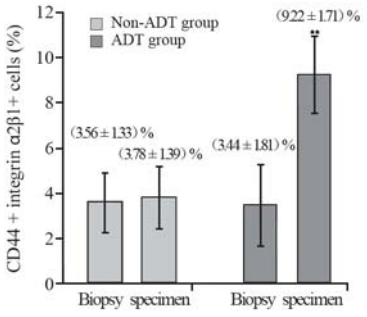
前列腺癌由激素依赖性转为激素非依赖性的机制尚不清楚。激素非依赖性前列腺癌（AIPC）的形成可能与长期的雄激素阻断使最初占少数的前列腺肿瘤干细胞或祖细胞比例逐渐增加，占据大多数比例有关。Collins等[12-13]认为，前列腺癌肿瘤干细胞既不表达雄激素受体（AR），也不对雄激素受体发生反应。只有分化好的腺腔细胞表达AR并对雄激素发生反应。雄激素阻断后，对雄激素有反应性的分化好的腺腔细胞被杀灭，而对其无反应的前列腺肿瘤干细胞可在低雄激素环境中存活下来。由此，AIPC可能是相对未分化的前列腺肿瘤干细胞或祖细胞占多数的细胞群。研究证明，CD44与整合素α2β1联合，可作为前列腺干细胞和前列腺肿瘤干细胞的标志物[12-13]。本课题



A:cancer tissues from biopsy; B:cancer tissues from prostatectomy after ADT; C:peritumoral tissues from biopsy;D:peritumoral tissues from prostatectomy after ADT

图1 雄激素阻断治疗后前列腺癌组织及癌旁组织干细胞比例的变化 (免疫组织化学法 ×20)

Figure1 The change of the proportion of stem cells in prostate cancer tissues and peritumoral tissues after ADT (IHC ×20)



In non-ADT group, the differences of cancer stem cell proportion between preoperative and postoperative specimens were not prominent(*P*=0.512);In ADT group, compared with preoperative specimens, the proportion of prostate cancer stem cells was significantly increased after undergoing androgen deprivation therapy for 3 months(*P*=0.000),**:*P*<0.01

图2　ADT治疗后前列腺癌组织中CD44和整合素α2β1双阳性细胞比例的变化

Figure2　The change of the proportion of CD44+ integrin α2β1+ cells in prostate cancer tissue after ADT

检测了雄激素阻断治疗（ADT）前后前列腺组织和前列腺癌组织中的干细胞标志物CD44和整合素α2β1。结果显示，ADT治疗后，前列腺干细胞标志物CD44 和 整合素 α2β1阳性细胞较治疗前比例增高。这提示，雄激素可能参与前列腺肿瘤干细胞或正常前列腺中前列腺干细胞的分化。Liu[14] 等报道， CD44 和 整合素 α2β1在 AIPC 细胞系 PC3 和 DU145高表达 。在我们之前的研究中 [15], Du145 细胞系中肿瘤干细胞比例较原发瘤高。 以上研究结果均提示AIPC可能与前列腺肿瘤干细胞和雄激素水平变化有关。因此，研究更佳的治疗方案若能调整雄激素水平，控制肿瘤干细胞，则对延缓AIPC的形成，改善患者的预后具有重要意义。

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