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· 临床研究 ·

CD11b 表达与人工髋关节置换术后无菌性松动的相关性分析 *

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摘要 目的: 分析髋关节周围滑膜组织 CD11b 表达水平与人工髋关节置换术后无菌性松动的相关性。**方法:** 以 2006 年 5 月至 2016 年 5 月于西京医院接受人工髋关节置换的患者为研究对象,对其髋关节周围滑膜组织进行 CD11b 免疫组化染色,并随访术后 5 年和 10 年无菌性松动的发生情况,通过单因素分析及 logistic 回归分析讨论 CD11b 表达与无菌性松动之间的相关性。**结果:** 共 300 例患者纳入研究,全部获得随访,CD11b 表达阳性患者 163 例,阳性率为 54.33%;术后 5 年松动患者 29 例,术后 5 年无菌性松动发生率为 9.67%;术后 10 年无菌性松动患者 49 例,术后 10 年无菌性松动发生率为 16.33%;单因素分析结果表明 CD11b 表达阳性患者 5 年及 10 年松动率均高于 CD11b 表达阴性患者($P<0.05$);logistic 回归分析结果表明 CD11b 过表达是髋关节置换术后无菌性松动发生的危险因素。**结论:** 髋关节周围滑膜组织 CD11b 过表达是人工髋关节置换术后无菌性松动发生的危险因素,该分子或可作为无菌性松动的辅助诊断指标及潜在治疗靶点。

关键词: CD11b; 无菌性松动; 相关性分析

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Analysis of the Correlation between CD11b Expression and Aseptic Loosening*

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ABSTRACT Objective: To analyze the correlation between CD11b expression and aseptic loosening. **Methods:** CD11b of synovial tissue around the hip joint from patients received total hip replacement during 2006 May to 2016 May were immunohistochemical stained, and a follow-up was performed to detect the occurrence of aseptic loosening. Single factor analysis and logistic regression analysis was used to investigate the correlation between CD11b expression and aseptic loosening occurrence. **Results:** 300 patients were followed up; the number of CD11b positive patients was 163, CD11b positive ratio was 54.33%; 29 patients (9.67%) suffered from aseptic loosening 5 years after operation and 49 patients (16.33%) suffered from aseptic loosening 10 years after operation; the aseptic loosening ratio of CD11b-positive patients was higher than that of CD11b-negative patients ($P<0.05$); logistic regression analysis suggested that CD11b over-expression was the risk factor of aseptic loosening. **Conclusions:** CD11b over-expression was the risk factor of aseptic loosening and CD11b might be an auxiliary diagnosis indicator and potential therapeutic target of aseptic loosening after total hip replacement.

Key words: CD11b; Aseptic loosening; Correlation analysis

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前言

人工关节置换术是本世纪发展最成熟的术式之一,可显著改善患者关节功能,提高生活质量,多项报道已证实人工关节置换术后假体 15 至 20 年生存率高达 90%以上^[1,2],但无菌性松动已成为置换术后最重要的长程并发症之一,并已成为造成

翻修的主要原因之一^[3]。关于无菌性松动发生机制及预防的研究主要基于实验研究、临床研究、病例报告等方面^[4,5],除了假体材料、假体设计、固定方式、手术方式、应力遮挡等因素可影响无菌性松动的发生外,个体因素亦参与该疾病的发生和发展^[6],目前对于早期无菌性松动发生机制尚未明确,仍缺少针对该并发症的有效预防和治疗手段。CD11b 是破骨细胞表面的标志分

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子,而破骨细胞参与了无菌性松动的病理过程^[7,8],本研究通过髋关节周围滑膜组织免疫组化染色和统计学分析,对 CD11b 的表达水平与无菌性松动的相关性进行初步分析。

1 材料与方法

1.1 病例

2006 年 5 月至 2016 年 5 月在西京医院接受人工髋关节置换的患者,纳入标准:(1)原发疾病诊断明确,如骨性关节炎,股骨颈骨折等;(2)符合髋关节置换术的手术指征;(3)患者签署知情同意书。排除标准:(1)髋关节感染及假体周围骨折患者;(2)不能获得完整随访患者^[9]。共计 300 例患者,随访 10 年。

1.2 试剂

抗 CD11b 单克隆抗体(Abcam,英国),辣根过氧化物酶(Horseradish Peroxidase,HRP)标记的二抗(Santa Cruz,美国),免疫组化试剂盒(碧云天,中国),无水乙醇,二甲苯,盐酸。

1.3 方法

1.3.1 无菌性松动的诊断 髋关节 X 片显示假体周围存在明显透亮线,假体位置发生偏移并具备相应临床症状和体征,实验室检查排除排除细菌感染及其他自身免疫性疾病的患者诊断为无菌性松动^[10]。

1.3.2 免疫组化染色 样本采集前患者已签署知情同意书,并通过西京医院伦理委员会批准。术中取髋关节周围滑膜组织,4%多聚甲醛固定 24 h 后进行石蜡包埋,切片后进行抗原修复,加入抗 CD11b 一抗,4℃孵育过夜,加入二抗,室温孵育 20 min 后,进行显色,复染,封片,于镜下观察。

1.3.3 判定标准 将棕褐色及棕黄色染色的细胞记为 CD11b 染色阳性细胞。高倍镜下随机取 5 个不同视野各计数 200 个细胞,阳性细胞数 < 2% 记为 "-", 阳性细胞数 3%~25% 记为 "+", 阳性细胞数 26%~50% 记为 "++", 阳性细胞数 > 50% 记为 "+++"。将 "-" 和 "+" 记为 CD11b 表达阴性,将 "++" 和 "+++" 记为 CD11b 表达阳性(CD11b 过表达)。

1.4 统计学分析

采用 SPSS.16.0 软件进行统计学分析,利用卡方检验和 logistic 回归分析评价 CD11b 表达水平与无菌性松动发生的相关性, $P < 0.05$ 认为具有统计学差异。

2 结果

2.1 患者 CD11b 表达情况

300 例标本中,CD11b 阳性数为 163,阳性率为 54.33%,染色结果如图 1 所示。

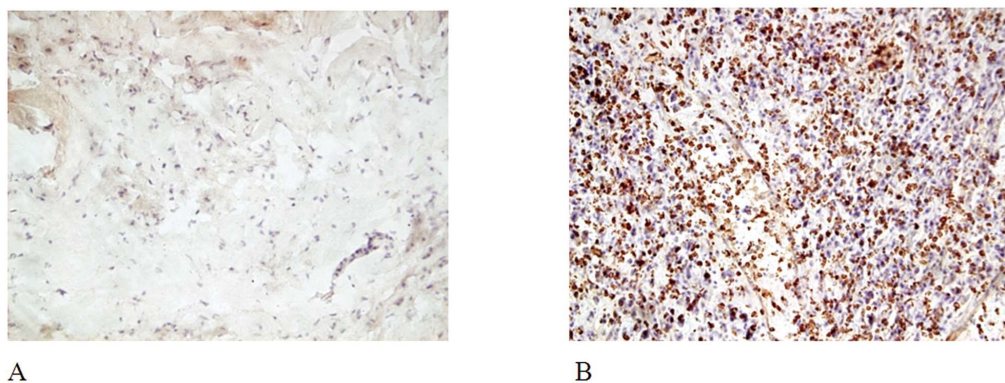


图 1 髋关节周围滑膜组织免疫组化染色(40)

Fig.1 Immunohistochemical staining of synovial tissue around the hip joint($\times 40$)

A:CD11b 表达阴性

B:CD11b 表达阳性

A: CD11b negative

B: CD11b positive

2.2 患者无菌性松动发生率

300 例患者中,术后 5 年无菌性松动患者 29 例,无菌性松动发生率为 9.67%;术后 10 年无菌性松动患者 49 例,无菌性松动发生率为 16.33%。

2.3 CD11b 的表达与无菌性松动发生的单因素分析

髋关节周围滑膜组织 CD11b 表达阳性患者无菌性松动发生率高于 CD11b 表达阴性患者($P < 0.05$)。结果如表 1 所示。

表 1 CD11b 表达与无菌性松动的相关性单因素分析

Table 1 Single factor analysis of the correlation between CD11b expression and aseptic loosening

Results	N	5 years	Chi-square Value	10 years	Chi-square Value
CD11b positive	163	23	8.07	40	17.59
CD11b negative	137	6 [#]		9 [#]	

Note: [#] $P < 0.05$ compared with group in the same period, 5 years: patients with aseptic loosening 5 years after operation, 10 years: patients with aseptic loosening 10 years after operation.

2.4 CD11b 的表达与 5 年及 10 年松动关系的 logistic 回归分析

经 LR 向前逐步法分析,CD11b 过表达是 5 年及 10 年无

菌性松动发生的危险因素,OR 值分别为 4.39 和 3.6。分析结果见表 2。

表 2 CD11b 表达与无菌性松动 5 年及 10 年发生率关系的 logistic 回归分析

Table 2 Logistic regression analysis of the correlation between CD11b over-expression and aseptic loosening after 5 and 10 years

Group	β Value	OR Value	95 % confidence interval	Wald	P Value
5 years	2.94	4.39	2.971~5.786	24.368	<0.05
10 years	3.17	3.62	2.684~5.315	18.341	<0.05

Note: OR, Odds ratio.

3 讨论

人工关节置换术作为治疗终末期骨关节疾病最有效的术式之一, 已为患者带来关节功能的重塑和生活质量的改善, 然而随着假体设计的不断改进, 关节周围骨折、关节感染等因素导致的移植失败已逐渐减少^[11,12], 而无菌性松动成为造成翻修的主要原因之一, 51 % 的人工全髋关节置换术后翻修及 41 % 的人工全膝关节置换术后翻修都是由无菌性松动导致的^[13,14], 这为患者带来生活质量的下降和沉重的医疗负担。假体的磨损颗粒释放到周围组织后, 被巨噬细胞所吞噬, 进而刺激肿瘤坏死因子 α 的 (tumor necrosis factor α , TNF- α) 的分泌, TNF- α 可刺激成骨细胞分泌粒细胞巨噬细胞刺激因子、白细胞介素 6、前列腺素 E2 等细胞因子, 这些细胞因子募集破骨细胞到达假体 - 骨界面, 并刺激破骨细胞的分化, 导致骨溶解的发生^[15]。破骨细胞是这一病理过程的核心效应细胞, 而巨噬细胞分化抗原-1 (Macrophage differentiation antigen-1, Mac-1, CD11b/CD18) 是主要表达在破骨前体细胞及破骨细胞表面的粘附分子。作为整合素家族的重要一员, 该分子不仅参与免疫应答和炎症反应, 也参与细胞间识别和信号转导^[16], CD11b 作为 Mac-1 的亚基之一, 在 Mac-1 介导的白细胞分化中发挥主要作用^[17]。

根据免疫组化染色结果及单因素分析结果, CD11b 表达阳性的患者其 5 年及 10 年无菌性松动的发生率均高于 CD11b 表达阴性的患者, 说明 CD11b 过表达与无菌性松动的发生密切相关。而 logistic 回归分析结果显示 CD11b 过表达是 5 年及 10 年无菌性松动发生的危险因素, 提示 CD11b 可能促进无菌性松动的发生。CD11b 作为破骨细胞表面的标志性分子, 已被证明在破骨细胞的分化中起促进作用, Hidetaka Hayashi 等^[18]发现抗 CD11b 抗体处理破骨前体细胞后, 破骨细胞的分化受到抑制。Alan şuğur 等^[19]的研究显示 CD11b 阳性的破骨前体细胞参与关节骨破坏的病理过程。以上研究结果证明了 CD11b 可通过增强破骨细胞的骨吸收, 加剧骨溶解, 导致无菌性松动的发生。

无菌性松动的发生与多种因素密切相关。金属蛋白酶诱导因子和 Caspase-3 的过表达已被证明与全髋关节置换术后无菌性松动的发生呈正相关^[20], 而假体周围界膜组织中 β - 防御素-3 在无菌性松动患者中表达也高于同组股骨颈骨折患者^[21]。此外, Jeffrey J. 等^[22]发现男性与年龄 <65 岁是人工全髋关节置换术后无菌性松动的危险因素, 肥胖 (身体质量指数, Body Mass Index, BMI $\geq 30 \text{ Kg/m}^2$)、吸烟及高体力劳动也与髋关节置换术后无菌性松动的发生密切相关^[13,23,24]。除了肥胖 (BMI $\geq 30 \text{ Kg/m}^2$) 外, 并未发现其他促进全膝关节置换术后无菌性松动发生的个体因素^[25]。尽管骨水泥固定仍为目前髋关节置换术的主流固定方式, 但骨水泥碎屑与假体无菌性松动的关系现已得到

证实^[26], 故非骨水泥型关节假体设计不断得到采用。

本研究通过随访接受人工髋关节置换术患者, 结合髋关节周围滑膜组织免疫组化染色单因素分析及 logistic 回归分析, 证明了 CD11b 过表达是临床无菌性松动发生的危险因素, 而 CD11b 分子可能成为诊断无菌性松动的辅助检查指标和治疗靶点。

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