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Influence of adipocytokines in periprostatic adipose tissue on prostate cancer aggressiveness

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ABSTRACT

Objective: To evaluate the correlation between the level of adipocytokines expression in periprostatic adipose tissue and the prostate cancer aggressiveness.
Patients and method: The periprostatic adipose tissues were collected from 30 patients who underwent radical retropubic prostatectomy. The subcutaneous adipose, periprostatic adipose tissues and prostate cancer tissue from the same patient were collected from 10 patients for match research. The expression level of IL-6, Leptin and Adiponectin was detected by immunohistochemistry and by Real-time quantitative PCR in periprostatic adipose tissues.
Result: There were differences in the positive rates of IL-6, Leptin and Adiponectin expression in the periprostate adipose between prostate cancer and control ($P < 0.001$, $P = 0.032$, 0.003). Nothing but the "IL-6 expression intensity" was seen in difference with the aggressiveness of prostate cancer ($P = 0.001$), and was relevant with the prostate cancer aggressiveness ($r_s = 0.668$, $P < 0.001$); The mRNA expression of IL-6 in periprostatic adipose tissues of prostate cancer was higher than that of control ($P = 0.049$), and the mRNA expression of Adiponectin was lower ($P < 0.0001$); IL-6 mRNA expression in periprostate adipose tissue and prostate cancer tissue were higher than that in subcutaneous adipose ($P < 0.001$, $P = 0.001$); IL-6 mRNA expression in periprostate adipose was correlated with that in prostate cancer tissue ($r = 0.663$, $p = 0.036$); Adiponectin mRNA expression in prostate cancer tissue was lower than that in periprostate adipose ($P = 0.006$), and Adiponectin mRNA expression in periprostate adipose was correlated with that in prostate cancer tissue ($r = 0.707$, $p = 0.022$).
Conclusion: IL-6, Leptin and Adiponectin were expressed in the periprostatic adipose tissues, which constitute the microenvironment of prostate cancer aggressiveness. There might be intimate relationship between periprostate adipose and prostate cancer tissue.

1. Introduction

Obesity has effect on prostate cancer detection, treatment decisions and efficacy, and even results in increased risk of biochemical recurrence after radical prostatectomy [1,2]. In particular, increasing evidences reveal adjacent adipose tissue plays an important role in progression of cancer, including prostate cancer [3,4]. The periprostatic adipose is thought of as metabolic organ and can produces large number hormones and cytokines, including tumor necrosis factor-α, interleukin-6, leptin and adiponectin, which play a role in the biology of prostate cancer [5,6]. Correlation of periprostatic adipose with prostate cancer aggressiveness is mostly based on epidemiological studies, which is lack of experimental studies. Some studies showed a close association between the serum level of adipocytokines and the progression of prostate cancer [7,8], whereas others found adverse results [9,10]. Our study aims to evaluate correlation of adipocytokines expression in periprostatic adipose tissue with prostate cancer aggressiveness, so as to preliminarily discover the mechanism that the periprostatic adipose tissue affects the prostate cancer aggressiveness.

2. Material and method

2.1. Tissue collection

Studies were performed with full approval of the Xin Hua hospital affiliated to Shanghai Jiao Tong University school of Medicine. After patient consent was obtained, the periprostatic adipose tissues were collected from 30 patients who underwent radical

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retropubic prostatectomy (RRP). According to Carrlo PR [11], the patients were stratified into three groups. The control group was collected from 10 patients who underwent radical cystectomy; Meanwhile, the subcutaneous adipose, periprostatic adipose tissues and prostate cancer tissue from the same patient were collected from 10 patients who underwent radical retropubic prostatectomy (RRP) for match research. The periprostatic adipose tissues from anterior-lateral PP, the subcutaneous adipose from surgical incision and prostate cancer tissue from surgical removal of prostate were immediately processed for examination.

2.2. Immunohistochemistry

Paraffin embedded tissue blocks were cut to 5 μm sections. Each section was deparaffinized and rehydrated using xylene and ethanol; 3% H_2O_2 was used to inactivate the endogenous peroxidase for 30 min, Followed by 3 rinses in phosphate buffered saline (PBS) for 3 min each. Antigen retrieval was applied to 0.25% trypsin at room temperature for 20 min. Samples were again washed with PBS 3 times for 3 min each. The slide was blocked with goat serum, and placed in a humid chamber for 30 min at room temperature. Then the slide was incubated with primary antibody overnight at 4 °C. On the following day, the slide was washed 3 times for 3 min each with PBS. Then the secondary antibody was incubated for 60 min at room temperature, followed by PBS washing, 3 times for 5 min each. Diaminabenzidine (DAB) substrate was used for detection and hematoxylin was used for counterstaining. The samples were then dehydrated and mounted for visualization.

Immunohistochemical evaluation: The tissue of staining intensity (no staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3), and staining extent (% of positive cells; <5% = 0, 5–25% = 1, 26–50% = 2, 51–75% = 3, >75% = 4). The scale = staining intensity × staining extent; Scale ⩽ 1: −; 2 ⩽ scale ⩽ 5: +; 6 ⩽ scale ⩽ 9: ++; 9 ⩽ scale ⩽ 12+++. The levels of tissue staining were accessed independently by 3 pathologists who were blinded to the clinical and pathologic characteristics.

Table 1
Primer sequences for Actβ, Adiponectin, Leptin and IL-6.

Gene	Primer sequences (5′–3′)	Size (bp)
Actβ	Forward: CTCCCTGGAGAAGAGCTACGAGC Reverse: CCAGGAAGGAAGGCTGGAAGAG	101
Adiponectin	Forward: AACATGCCCATTCGCTTTACC Reverse: TAGGCAAAGTAGTACAGCCCA	107
Leptin	Forward: AAAGTCCAAGATGACACCAAAACCC Reverse: TTGGAGGAGACTGACTGCGTGTG	86
IL-6	Forward: AACAAATTCGGTACATCCTCGACG Reverse: TTTTCTGCCAGTGCCTCTTTGC	101

2.3. Real-time quantitative PCR (Q-PCR)

The total RNA was extracted using TRIZol reagent (Invitrogen life Technologies). The RNA (2 μg) was used to synthesize cDNA. For Q-PCR amplification, a 20 μl reaction volume, containing 10.0 μl 2X SYBR Premix Ex Tap (Roche, 04913914001, Germany),

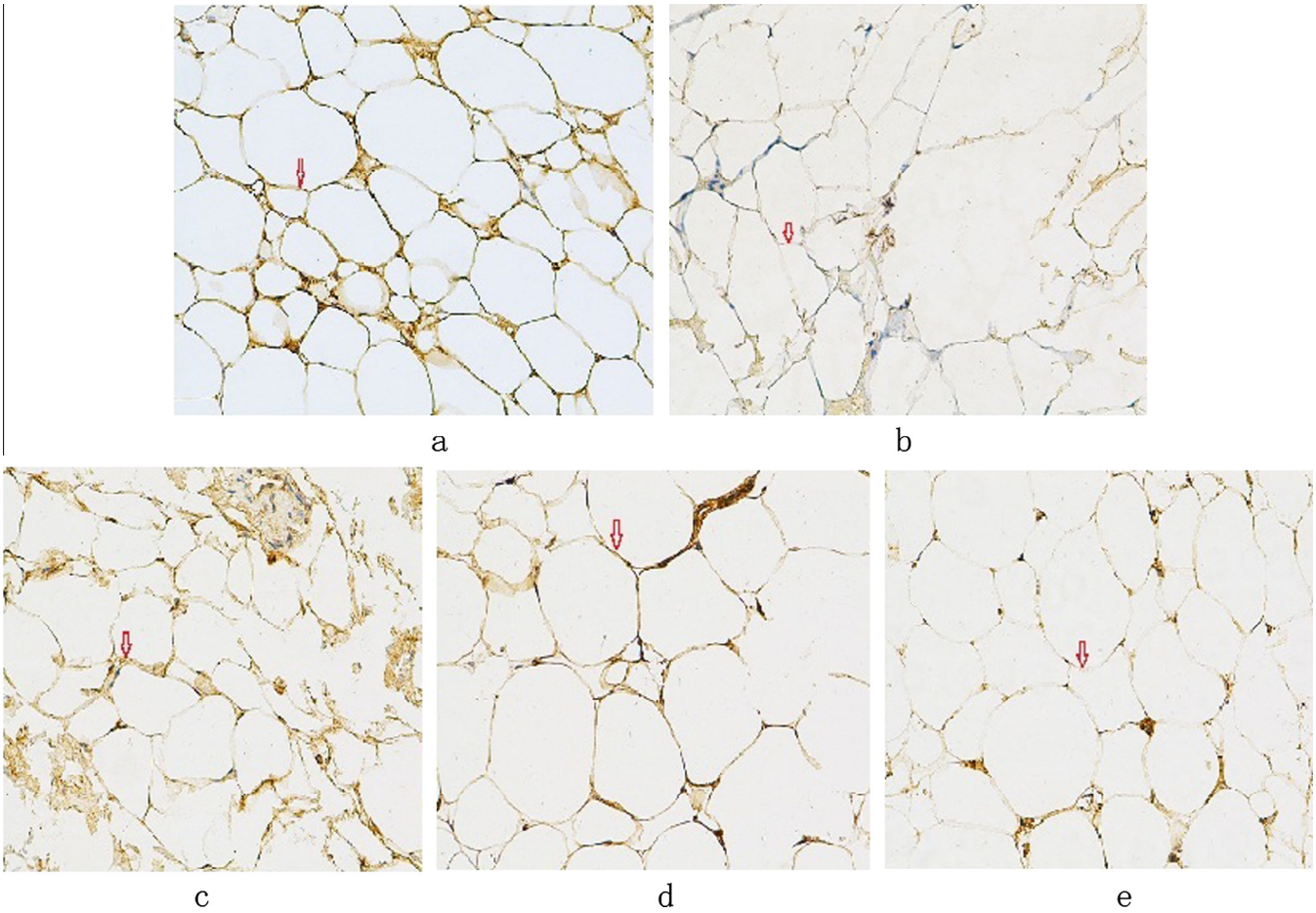

Fig. 1. IL-6 expression detected with immunohistochemistry in periprostate adipose tissue of prostate cancer and control. ((a) is prostate cancer; (b) is control (200X); (c) is high-risk group; (d) is intermediate-risk group; (e) is low-risk group (200X)).