การประเมินสารบ่งชี้ สำหรับการกระจายตัวของโรคมะเร็งต่อมลูกหมากไปที่กระดูก

THE EVALUATION OF DIAGNOSTIC MARKERS FOR BONE METASTASIS OF PROSTATE CANCER

หัวหน้าโครงการ ผศ. ดร. ปรัชญา คงทวีเลิศ[1]

ผู้ร่วมวิจัย อาจารย์ วรรณรัตน์ ยิ่งสังข์[1]

ศ. นพ. สุพจน์ วุฒิการณ์[2]

[1]ภาควิชาชีวเคมี คณะแพทยศาสตร์

[2]ภาควิชาศัลยศาสตร์ คณะแพทยศาสตร์

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คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

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ปรัชญา คงทวีเลิศ[1], วรรณารัตน์ ยิ่งสังข์[1], พีระพรรณ โปธาเจริญ[1],
และ สุพจน์ วุฒิการณ์[2]
[1]ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
[2] ภาควิชาศัลยศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

บทคัดย่อ

ได้ทำการศึกษาประเมินการนำเอาสารบ่งชี้ทางชีวเคมี เพื่อการวินิจฉัยโรคมะเร็งต่อมลูกหมากที่มีการกระจายตัวไปยังกระดูก จากการศึกษาพบว่า hyaluronan (HA) ซึ่งเป็นสารชีวโมเลกุลชนิดหนึ่งที่พบได้ทั่วร่างกาย โดยเฉพาะอย่างยิ่งเนื้อเยื่อเกี่ยวพัน เมื่อเปรียบเทียบปริมาณในซีรั่มแล้ว มีปริมาณสูงมากขึ้นอย่างมีนัยสำคัญในซีรั่มของคนไข้โรคมะเร็งของต่อมลูกหมาก ทั้งที่มีการกระจายตัวไปยังกระดูก และที่ไม่มีการกระจายตัวไปยังกระดูก ส่วนสารบ่งชี้อีกอย่างหนึ่งคือ chondroitin sulfate ซึ่งใช้เป็นสารบ่งชี้ถึงการเปลี่ยนแปลงเมตาบอลิสม์ของกระดูกอ่อน พบว่าเมื่อตรวจวัดด้วยแอนติบอดีย์ชนิด 3B3 (3B3 epitope) จะมีปริมาณสูงมากอย่างเป็นนัยสำคัญเช่นกันทั้งในสภาวะที่มีการกระจายตัว และไม่มีการกระจายตัวไปยังกระดูก นอกจากนั้น จากการศึกษานี้ พบว่าเมื่อตรวจวัดสารบ่งชี้ชนิด chondroitin sulfate โดยการใช้แอนติบอดีย์ชนิด WF6 (WF6 epitope) ไม่มีระดับสูงขึ้นทั้งในกลุ่มที่มี และไม่มีการกระจายตัวของมะเร็งต่อมลูกหมาก ไปยังกระดูก อีกทั้ง ไม่มีความแตกต่างไปจากซีรั่มของคนปรกติอีกด้วย จากการศึกษาครั้งนี้พอสรุปได้ว่า HA และ 3B3 epitope สามารถใช้เป็นสารบ่งชี้สำหรับโรคมะเร็งต่อมลูกหมากได้ แต่ไม่สามารถใช้แยกความแตกต่างของมะเร็งที่มี และไม่มีการกระจายตัวได้ โดยที่ WF6 epitope ไม่สามารถนำมาใช้เป็นสารบ่งชี้โรคมะเร็งต่อมลูกหมากได้

The evaluation of diagnostic markers for bone metastasis of prostate cancer

Prachya Kongtawelert[1] ,Wannarat Yingsung[1], Peraphan Pothacharoen[1],

and Supot Wudhikarn[2]

[1]Department of Biochemistry, Faculty of Medicine, Chiang Mai University

[2] Department of Surgery, Faculty of Medicine, Chiang Mai University

ABSTRACT

The connective tissue biochemical markers have been used to evaluate their potential diagnostic tool for bone metastasis of prostate cancer. The hyaluronan (HA) which is a biomolecule distributed throughout the body especially connective tissues shows significantly higher in prostate cancer with and without metastasis to the bone than in normal ones. The marker of cartilage metabolism which is chondroitin sulfate epitopes (3B3's epitope) that have been proposed to be a marker for bone metastasis of prostate cancer also increased in both groups of cancer serum samples which were significantly higher than in normal. Furthermore, it was found that there was no increasing in the level of another chondroitin sulfate epitope which detected by monoclonal antibody WF6 (WF6's epitope) in both group of prostate cancer. It was suggested that both HA and 3B3's epitope could be used as a tumor marker for prostate cancer but could not be used to differentiate between metastatic and non-metastatic to the bone of prostate cancer. And the WF6 which demonstrated as a specific marker for cartilage degradation was not increased in cancer serum samples

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INTRODUCTION

In recent years, the incidence of prostate cancer in the word was exploded. It is the third common cause form cancer in men of all ages and is the most common cause of death from cancer in men over 75 years old. Recent studies suggest that an inherited predisposition may be responsible for 5-10 % of prostate cancer; dietary fat may be liked to prostate cancer.

There is no early warning sign of prostate cancer. Many prostate cancer symptoms are nonspecific and may be similar to those caused by benign conditions such as infection of prostate enlargement. Patients diagnosed with prostate cancer when it is still localized to the gland have a 5-year survival rate of 100%. Advanced prostate cancer is not curable.

Metastatic prostate cancer occurs by both homogenous and lymphatic routes, especially along the pelvic and abdominal great vessels (Merphy *et al.*, 1982). In advanced stages, it can be metastasize to various of the body, most commonly the lymph node and bone. The bones that are the most commonly involved are vertebrae, abnormally dense bone.

Diagnostic of bone metastasis is the accomplished by the bone scan. The problem with bone scans is that they are considered sensitive in the initial diagnostic, but the specificity is relatively low. They are also not suitable for monitoring the short-term response to treatment. Other markers are acid and alkaline phosphatase in serum, which increase when cancer spread to bone.

Proteoglycan (PGs) are abundantly and ubiquitously distributed macromolecules that contain a core protein to which side-chain glycosaminoglycans (GAGs) are attached covalently. PGs are involved in both normal and neoplastic cell growth by participating in cell-cell an cell matrix adhesion, cell migration and

proliferation (Wight *et al.*, 1992) and in regulation growth factor activities (Ruoslahti *el al.*, 1991 and Iozzo *et al.*, 1984).

GAGs consist of unbranched polymers of repeating disaccharide units containing a hexosamine and uronic acid. The uronic acid-containing GAGs are chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), dermatan sulfate (DS), heparan sulfate (HS), hyaluronic acid (HA) and heparin, keratan sulfate (KS), the most only exception, contains galactose without uronic acid. Alterations in the GAG content have been studies in various tumors and prosthetic GAG level have also been shown to very tumor (De Klerk *et al.*, 1990 and Kofoed *et al.*, 1990).

Due to immunological knowledge, immunoassay that recognized biochemical molecule form bone which are nonspecific PGs fragments and fragments containing either hyaluronan or CS have been developed. According to the specific of each antibody, the assay may reflect those PGs synthesis.

Thus, in this study we evaluated and developed markers (CS and HA) in human serum from bone metastatic compared with non-bone metastatic prostate cancer to figure out which marker is suitable for diagnostic bone metastasis.

MATERIAL AND METHODS

Clinical Samples. Healthy subjects: serum samples were collected from 20 apparently healthy individuals, age 50-70 years. All subjects were examined to be free from articular, bone, liver, endocrine, or other chronic disorders by the physician. None was currently taking any medication known to modified arthritic disease or influence joint metabolism. Joint disease subjects: serum samples from individual who had been previously diagnosed with non-bone metastatic (negative for bone scanning) and bone metastasis (positive for bone scanning) according to criteria.

Treatment of human serum samples. Prior to 3B3 competitive immunoassay, the samples were digested with chondroitinase ABC (equal volume of 0.1 U/ml in chondroitinase ABC buffer : 0.1M sodium acetate, Tris-HCl, pH 7.3), incubated at 37°C for 16 h (overnight) followed by heating at 100°C for 10 min. The digested samples were centrifuged in microfuge for 10 min and the supernatants were collected and kept frozen until analysis.

Enzyme-linked immunosorbent assays.

The 3B3 competitive ELISA was modified form synovial fluid assay as described by Hazell, *et al.*, porcine aggrecan core protein (chondroitinase ABC-digested porcine laryngeal cartilage aggrecan) was used to coat plate (50 ng/ml) and was the standard antigen (range 4 - 2000 ng/ml).

The WF6 competitive ELISA was described by Tiengburanathum, 1996 with some modification by using shark proteoglycan (A1-fraction) (10 µg/ml) coat the plates and shark proteoglycan (A1D1-fraction) as the standard antigen (range 19-10,000 ng/ml).

The hyaluronan using B-HABP competitive ELISA based assay was described previously, (Surangkul, 1998), HA from umbilical cord (Sigma - Aldrich) was used to coat plates (10 μg/ml) and Hyaluronan (Healon®) was used as the standard antigen (range 19-10,000 ng/ml).

In all assays, The bound antibody was detected by O-PD substrate in citrate buffer, pH 5.0. The reaction was stopped with 4M sulfuric acid and the absorbance was determined using a microplate reader at 492/690nm. Standards and samples were analysed in triplicate, the concentration of each epitope was calculated from the standard curve which performed by using DeltSoft II software on Macintosh computer

Statistical analysis. The serum 3B3, WF6 epitopes and HA for the healthy and patient groups were expressed as means and SDs. Comparison between prostate cancer and healthy subjects were evaluated with unpair t-test, for this analysis, $P < 0.05$ (two-tail test) was considered significant. Statistic calculations were performed by StatView 512+ software on Macintosh computer.

RESULTS AND DISCUSSION

The most commonly metastasis of prostate cancer are lymphnode and bone. Bone scan and serum phosphatases level remain the gold standard of matastatic prostate cancer, but they allow neither early detection of metastasis nor efficient monitoring because of their poor sensitivity and relative large precision error. Clearly, few identified patients at high risk of matastatic prostate cancer, there is a need for better techniques for investigating and monitoring of bone metastasis. Alternatively, specific and sensitive biochemical markers reflecting abnormalities of cartilage metabolism may be useful. According to the hypothesis of loss of normal balance between synthesis and degradation of macromolecules that provide cartilage and bone with their biomechanical and functional properties. Concomitant changes occurring in structure and metabolism of cartilage and bone tissue (Carvero *et al.*, 2000). Thus, for a comprehensive assessment of abnormalities in bone metabolism associated with cancer metastasis, molecule of each of these different tissues (cartilage and bone) may represent potentially valuable biochemical markers.

The developed assays using these two mAb (3B3 and WF6) were used to investigate chondroitin sulfate epitopes in prostate cancer and normal human serum samples. The expression 3B3 epitope was significantly higher in both bone metastasis (149.63 $\pm$ 106.43 ng/ml) and no bone metastasis (123.75 $\pm$ 118.6 ng/ml) groups when compared with normal serum (14 $\pm$ 14 ng/ml) (both $p = 0.001$). But there is no significantly difference between both groups of prostate cancer ($p > 0.05$) (Table 1, and Figure 1-2). The WF6 epitope expression showed that it was not significantly difference between both group of pathological samples (metastatic and non-metastastic prostate cancer) when compare with normal serum ($p > 0.05$) as shown in Table 1 and Figure 3.

In conclusion, this study suggest that the cartilage degradation markers, HA and chondroitin sulfate, which detected by HA-binding proteins and two monoclonal antibodies (3B3 and WF6) which recognized a specific pattern of sulfated chondroitin sulfate of proteoglycans could not be used as a marker for metastatic stage to the bone of prostate cancer. A better markers could be used to investigate further to this application such as bone sialoglycoproteins, N-telopeptide, and type I bone collagen (Brawer *et al.*, 1998).

Table 1 The amount of hyaluronan, native chondroitin sulfate (WF6 epitope) and unsaturated chondroitin sulfate (3B3 epitope) in normal, bone metastatic , and no bone metastatic prostate cancer human serum samples.

	HA (ng/ml)	WF6 (μg/ml)	3B3 (ng/ml)
Normal (n=20)	130.09 ± 82.53	1.18 ± 0.81	14.41 ± 14.15
Bone metastasis (n=10)	445 ± 302.43[a]	0.61 ± 1.25[b]	149.63 ± 106.43[c]
No bone metastasis (n=13)	368.02 ± 405.50[d]	0.73 ± 0.71[e]	123.75 ± 118.67[f]

Significant value (against normal):

A: p= 0.004; b: p=0.001; c: p= 0.0123; d: p=0.003: e: p=0.001; f: p=0.006

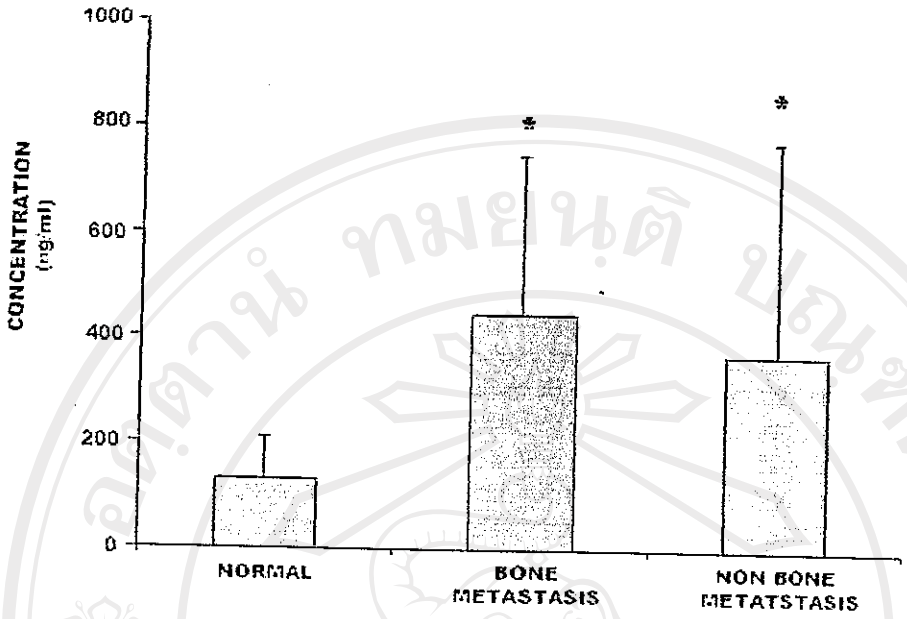

Figure 1. Concentration of hyaluronan (HA) in normal human serum compared with bone metastatic and non bone metastatic prostate cancer.

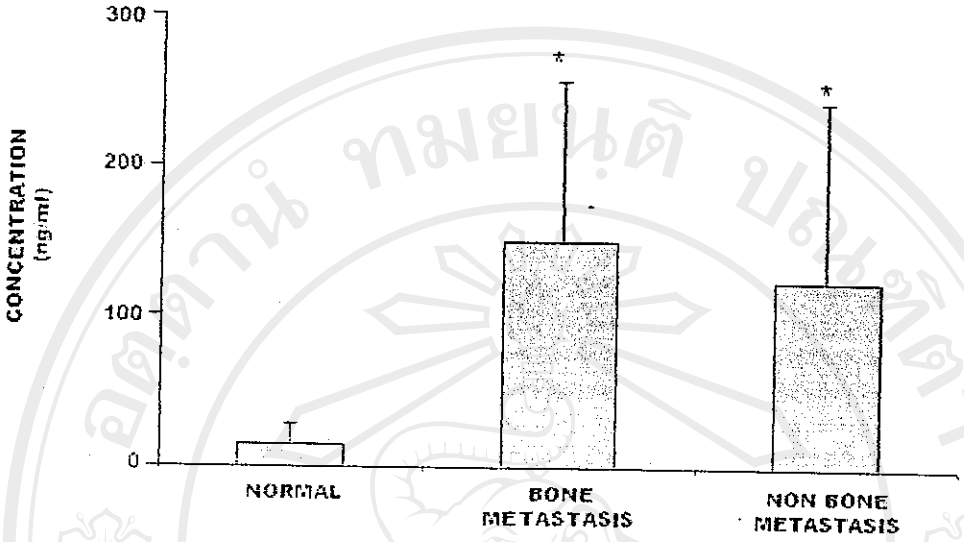

Figure 2. Concentration of chondroitin 6-sulfate epitope (3B3) in normal human serum compared with bone and non bone metastatic prostate cancer.

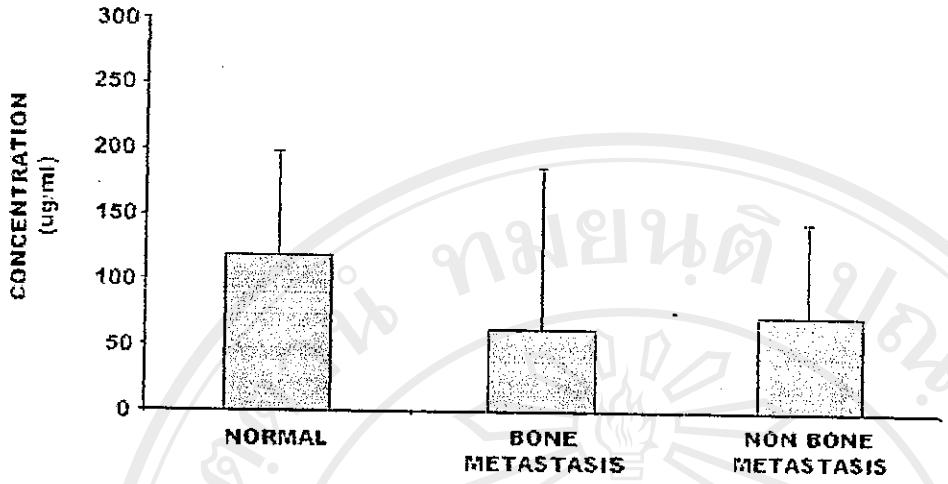

Figure 3. Concentration of native chondroitin 6-sulfate epitope (WF6) in normal human serum compared with bone metastatic and non bone metastatic prostate cancer.

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