Prostate cancer metastatic to bone has higher expression of the calcium-sensing receptor (CaSR) than primary prostate cancer.

Jie Feng, Georgia State University
Xiaojun Xu, Georgia State University
Bo Li, Emory University
Edward Brown, Brigham and Women’s Hospital
Alton Farris III, Emory University
Shi-Young Sun, Emory University
Jenny J. Yang, Georgia State University

Journal Title: Receptors & Clinical Investigation
Volume: Volume 1, Number 6
Publisher: Smart Science and Technology | 2014-09-27
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.14800/rci.270
Permanent URL: https://pid.emory.edu/ark:/25593/s5rbs

Final published version: http://dx.doi.org/10.14800/rci.270

Copyright information:
© 2014 Jie Feng, Xiaojun Xu, Bo Li, Edward Brown, Alton B Farris, Shi-yong Sun, Jenny J Yang
This is an Open Access work distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

Accessed January 5, 2020 1:07 PM EST
The calcium-sensing receptor (CaSR) is the principal regulator of the secretion of parathyroid hormone and plays key roles in extracellular calcium (Ca^{2+}) homeostasis. It is also thought to participate in the development of cancer, especially bony metastases of breast and prostate cancer. However, the expression of CaSR has not been systematically analyzed in prostate cancer from patients with or without bony metastases. By comparing human prostate cancer tissue sections in microarrays, we found that the CaSR was expressed in both normal prostate and primary prostate cancer as assessed by immunohistochemistry (IHC). We used two methods to analyze the expression level of CaSR. One was the pathological score read by a pathologist, the other was the positivity % obtained from the Aperio positive pixel count algorithm. Both of the methods gave consistent results. Metastatic prostate cancer tissue obtained from bone had higher CaSR expression than primary prostate cancer (P <0.05). The expression of CaSR in primary prostate cancers of patients with metastases to tissues other than bone was not different from that in primary prostate cancer of patients with or without bony metastases (P >0.05). The expression of CaSR in cancer tissue was not associated with the stage or status of differentiation of the cancer. These results suggest that CaSR may have a role in promoting bony metastasis of prostate cancer, hence raising the possibility of reducing the risk of such metastases with CaSR-based therapeutics.

**Keywords:** calcium-sensing receptor; prostate; cancer; bone; metastasis

**To cite this article:** Jie Feng, et al. Prostate cancer metastatic to bone has higher expression of the calcium-sensing receptor (CaSR) than primary prostate cancer. Receptor Clin Invest 2014; 1: e270. doi: 10.14800/rci.270.

**Copyright:** © 2014 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

**Introduction**

The calcium (Ca^{2+})-sensing receptor (CaSR) plays a central role in calcium homeostasis by sensing small changes in the level of extracellular calcium (Ca^{2+}) and regulating parathyroid hormone (PTH) secretion and renal...
calcium excretion so as to normalize Ca\(^{2+}\). Naturally occurring mutations cause familial hypercalciuric hyperparathyroidism (FHH) \(^{[1]}\), neonatal severe hyperparathyroidism (NSHPT) \(^{[2]}\) and autosomal dominant hypocalciuria with hypercalciuria (ADHH) \(^{[3]}\). The CaSR was first cloned from bovine parathyroid glands \(^{[4]}\) and belongs to class C of the G protein-coupled receptors (GPCR). CaSR also has been suggested to modulate adipocyte function \(^{[5]}\), carcinogenesis \(^{[6]}\), insulin secretion \(^{[7]}\), mineralization of the bony matrix \(^{[8]}\) and pathological calcification \(^{[9]}\), etc. Recently, much more attention has been paid to possible roles of CaSR in various types of cancer, including colon cancer \(^{[10, 11]}\), breast cancer \(^{[12, 13]}\), prostate cancer \(^{[14, 15]}\), ovarian cancer \(^{[16]}\), Leydig cell cancer \(^{[17]}\), gastric cancer \(^{[18]}\), insulinoma \(^{[19]}\), and glioblastoma \(^{[20]}\).

In breast and prostate cancer, CaSR has been suggested to participate in bony metastasis. It has been implicated in a vicious cycle of bony metastases through its modulation of parathyroid hormone related peptide (PTHrP) secretion by cancer cells \(^{[21]}\). Mihai et al. found that most breast cancer patients with a high expression of CaSR in malignant tissue obtained from the breast had bony metastases. They suggested that CaSR can serve as a biomarker to predict the potential risk of bony metastasis in breast cancer patients \(^{[22]}\). Liao et al. found that PC-3 prostate cancer cells (originally obtained from a bony metastasis) have higher levels of CaSR mRNA than LNCaP cells (obtained from a lymph node). Increasing the extracellular calcium concentration stimulates growth of PC-3 cells but not of LNCaP cells \(^{[23]}\). Knockdown of CaSR expression reduces growth of PC-3 cells both in vitro and in vivo in a murine model of prostate cancer metastasis \(^{[23]}\). However, a direct comparison of CaSR expression level in the bony metastases with that in the primary cancers in prostate is still lacking. Therefore, the relative levels of CaSR expression in primary prostate cancers and in metastases to bone and other sites as well as the associated implications for the metastatic process are not clear.

In this study, we performed immunohistochemistry (IHC) to detect CaSR expression in various benign and malignant prostatic tissues on human prostate cancer tissue microarrays. Our results identified a higher expression level of CaSR in bony metastases of prostate cancer than that in specimens of primary prostate cancer.

### Materials and Methods

#### Tissue microarrays

Tissue arrays containing both prostate cancer tissue and normal prostate tissue (PR807 and PR955) were purchased from the Biomax Company (Rockville, MD www.biomax.us). The tissue samples, including (1) normal prostate tissue, (2) primary prostate cancer tissue in patients with or without bony metastases, (3) prostate cancer tissue from bony metastases, and (4) prostate cancer tissue from abdominal wall metastases in two tissue microarrays, which were combined to enlarge the sample size. Altogether, there were 24 samples of normal prostate tissue, 108 samples of primary prostate cancer tissue (i.e., obtained from the prostate gland), and 4 samples of prostate cancer tissue obtained from bony metastases (Table 1). Among the 108 prostate cancer patients represented by the two arrays in which cancer tissue was obtained from the prostate, 12 of them had coexistent bony metastases while 96 of them did not. Tissue specimens were not available from the bony metastases in these 12 patients.

#### Immunohistochemistry (IHC)

The slides were first deparaffinized by heating at 60°C for 2 hours. Then they were boiled in 10 mM citrate sodium solution (pH 6.0) for 10 min for antigen retrieval, followed by incubation in 3% H\(_2\)O\(_2\) for 5-10 min to block endogenous peroxidase. After blocking in normal goat serum for 20 min, the slides were incubated with rabbit polyclonal anti-CaSR antibody raised against a synthetic...
peptide whose sequence is within the first third of the receptor’s N-terminal extracellular domain (Sigma, St. Louis, MO) at 4°C overnight. The specificity of the antibody was documented by negative control IHC (Supplemental Figure 1) (i.e., by omitting the first antibody) and the western blot (Supplemental Figure 2) as described in Results. Biotinylated anti-rabbit antibody was used as secondary antibody. Staining was visualized using 3,3′-Diaminobenzidine (DAB) tetrahydrochloride, and slides were counterstained with hematoxylin.

**Analysis of IHC staining**

The tissue microarrays were read by a pathologist (B.L.) blinded to the identity of the tissue sections. The staining intensity in any given tissue section was given a grade of 1, 2 or 3. A higher grade indicates a higher intensity of staining. The area ratio stands for the percentage of the area stained for CaSR over the total epithelial cellular area. The final pathological score was obtained by multiplying the intensity grade by the area ratio. The tissue microarray was also scanned using a Hamamatsu/Olympus Nanozoomer 2.0HT whole slide image scanner (Hamamatsu Photonics K.K., Hamamatsu, SZK). The whole slide image was viewed in the Aperio ImageScope program (Vista, CA) and analyzed with the Aperio positive pixel count algorithm similar to methods previously described [24]. The default hue (brown) for the positive pixel count algorithm was used. The positivity (number of positive pixels over total number of pixels) of

**Fig 2. Pathological score and positivity% for CaSR expression in each group, as quantitated in prostate tissue microarrays.**

A: Comparison of pathological scores of normal prostate tissue, primary prostate cancer tissue, and prostate cancer tissue from bone. B: Comparison of pathological scores of primary prostate cancer tissue from patients with bony metastasis and primary prostate cancer tissue from patients without bony metastasis. C: Comparison of positivity% of normal prostate tissue, primary prostate cancer tissue, and prostate cancer tissue from bone. D: Comparison of positivity% of primary prostate cancer tissue from patients with bony metastasis and primary prostate cancer tissue from patients without bony metastasis.
Western Blot

Cell lysates of cells were extracted and loaded onto an SDS-PAGE gel. After electrophoresis, equal amounts of protein from each sample were transferred to a nitrocellulose membrane. Then the membrane was blocked, incubated with Sigma anti-CaSR antibody and then secondary antibody sequentially. The protein bands were then visualized using an enhanced chemiluminescence reaction system (Bio Rad, Hercules, CA).

Statistical analysis

Statistical analyses of differences between groups were carried out by R software (R Development Core Team) using Student’s t test, the Wilcoxon test, or the Kolmogorov-Smirnov test according to whether the samples were normally distributed or not. If both groups were normally distributed with the same variance, Student’s t test was used. If neither of the two groups had a normal distribution, the Wilcoxon test was used. If one group was normally distributed but the other was not, the Kolmogorov-Smirnov test was used. A value of P < 0.05 was taken as indicating a statistically significant difference. All of the tests are two-sided. Pearson’s correlation was used to analyze the correlation among groups. P < 0.05 was taken to indicate a statistically significant correlation.

Results

Patient information

Figure 1 and Supplemental Figure 3 shows normal prostate tissue with a regular glandular structure. CaSR is expressed both in the cell membrane and in the cytoplasm of all of the epithelial cells. Primary prostate cancer tissue also expresses CaSR in all of the cancer cells with the same cellular localization, regardless of bony metastases or not. Prostate cancer tissue obtained from bone does not typically have a glandular structure, and the cancer cells cluster together, expressing much more CaSR than in prostate cancer sections obtained from the prostate or from non-bony metastases, as shown by the deep brown color in the bony metastases. Two cases of prostate cancer tissues obtained from the abdominal wall also did not have a glandular structure but show less staining than the cancer tissues obtained from bone.

CaSR expression levels in patient tissues were analyzed using two complementary methods. As shown in Table 1, the median pathological score of normal prostate tissue was 180 with a 25th percentile of 95 and 75th percentile of 214, and the median pathological score of all prostate cancer tissue samples obtained from prostate was 190 with a 25th percentile of 115 and 75th percentile of 240. All of the 4 prostate cancer tissues obtained from bone had the same pathological score of 300 (i.e., all had intensity grades of 3 with 100% of the cells staining positive for CaSR). Two cases of prostate cancer tissues obtained from the abdominal wall had a score of 120.

The Aperio positive pixel count algorithm was applied to measure the areas and intensities in IHC results. The positivity%, which describes the number of positive pixels over the total number of pixels of each sample, was used, in addition to the use of the pathological score, to analyze the expression level of CaSR. The semi-quantitative values obtained by this method are summarized in Table 2 for detailed comparison. The median positivity% of normal prostate tissue was 0.07 with a 25th percentile of 0.03 and 75th percentile of 0.14, and the median positivity% of all prostate cancer tissue samples obtained from prostate was 0.07 with a 25th percentile of 0.03 and 75th percentile of 0.16. All of the 4 prostate cancer tissues obtained from bone had a positivity% of around 0.39. Therefore the use of these two different methods yielded quite consistent results, showing a significant correlation by linear regression between

Table 1. Patient information and CaSR expression level

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Pathological Score (%) (multiply intensity by area) Median (25th, 75th percentiles)</th>
<th>Positivity (%) Median (25th, 75th percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal prostate tissue</td>
<td>24</td>
<td>180 (95,214)</td>
<td>0.07 (0.03,0.14)</td>
</tr>
<tr>
<td>Primary prostate cancer tissue</td>
<td>108</td>
<td>190 (115,240)</td>
<td>0.07 (0.03,0.16)</td>
</tr>
<tr>
<td>Metastatic prostate cancer tissue from bone</td>
<td>4</td>
<td>300 (300,300)</td>
<td>0.39 (0.36,0.40)</td>
</tr>
</tbody>
</table>
Statistical analysis of the pathological scores showed that there was no difference in CaSR expression between the normal prostate tissues and the primary prostate cancer tissues (Fig. 2A) (P = 0.65). There were only 4 samples of prostate cancer tissue metastatic to bone, and consequently, the Kolmogorov-Smirnov test was used, since it can be employed with small sample sizes. The metastatic prostate cancer tissues obtained from bone have higher CaSR expression than the prostate cancer specimens obtained from prostate (P = 0.001) or the normal prostate tissue (P = 0.004). Among the 108 samples of prostate cancer obtained from prostate, 12 samples were from patients having bony metastasis (tissue from the bony metastases of these patients were not available), and these had the same CaSR expression in the primary prostate cancer specimens as that in the 96 samples from primary prostate cancer of patients having no bony metastases (P = 0.67). (Figure 2B)

The positivity% results are shown in Figure 2 C and D. The conclusions are the same. There was no difference in CaSR expression between the normal prostate tissues and the primary prostate cancer tissues (P = 0.97). The metastatic prostate cancer tissues obtained from bone have higher CaSR expression than the prostate cancer specimens obtained from prostate (P = 0.003) or the normal prostate tissue (P = 0.003). Samples from primary prostate cancer tissue of patients having bony metastasis had the same CaSR expression as the primary prostate cancer samples from patients having no bony metastases (P = 0.07).

Correlation of CaSR expression with cancer stage

The tissue array included 29 cases of stage 2 cancer, 44 cases of stage 3 cancer, and 33 cases of stage 4 cancer. The scatter plot in Figure 3A shows that there is no correlation between the pathological score for the CaSR and stage of the cancer (R^2 = 0.011, P = 0.54). The data on the stages of the patient’s cancers are shown in Supplemental Table 1. The positivity% results also showed that there is likewise no correlation (R^2 = 0.007, P = 0.41) as in Figure
Correlation of CaSR expression with Gleason score

Cancers with a higher Gleason score are more aggressive and have a worse prognosis. The scatter plot in Figure 3B shows that there is no correlation between the pathological score for CaSR expression and the Gleason score ($R^2 = 0.003$, $P = 0.08$). Information about the patients on whom a Gleason score was available is shown in Supplemental Table 2. There is also no correlation between Gleason score and positivity% ($R^2 = 0.009$, $P = 0.31$) as shown in Figure 3E.

Correlation between CaSR expression and prostate specific antigen (PSA) concentration

PSA, secreted by the epithelial cells of the prostate gland, is often elevated in the presence of prostate cancer or other prostate disorders. The normal PSA levels should be less than 4 ng/mL. The scatter plot in Figure 3C shows that there is no correlation between the pathological score for CaSR expression and the PSA concentration in blood ($R^2 = 0.013$, $P = 0.07$). Information about the patients in whom PSA values were available is shown in Supplemental Table 3. There is also no correlation between PSA and positivity% ($R^2 = 0.075$, $P = 0.05$) as shown in Figure 3F.

Discussion

In this study, higher expression level of CaSR were found to be associated with the metastatic prostate cancer tissues obtained from bone rather than primary prostate cancer tissues or normal prostate tissues, while no significant difference in CaSR expression between normal prostate tissue and primary prostate cancer tissue was observed. The metastatic prostate cancer tissues studied here that were obtained from bone have higher CaSR expression than primary prostate cancer tissues. For the second tissue microarray (designated PR955 by the supplier), most of the tissues were obtained from prostate. Some were obtained from metastatic sites: 6 from bone and 2 from the abdominal wall. The samples of prostate cancer obtained from bone in these arrays are very scarce and fragile. Sections from two such bony metastases were damaged. Therefore, only four were available for statistical comparison with the staining from the other tissues due to limitations of patient sample availability. However, these four prostate cancer tissues obtained from bone all showed the highest level of CaSR expression among the tissues studied here, as reflected by the largest possible pathological score of 300 for each specimen. 2 metastases from the abdominal wall both had scores of only 120. This may suggest that high expression of CaSR in the cancer tissue from bone is a consequence of their localization in the bony environment, e.g., expression of the CaSR may be upregulated by factors in the bony microenvironment (see below). It is also possible that bone selects for metastatic cells with high CaSR expression and resultant increased potential to metastasize to bone because bone is a “fertile field” [26] for them when they express a high level of CaSR.

Importance of bone environment

Bone is a favored metastatic site for some cancer cells [27]. These metastatic sites are characterized by high rates of bone turnover [28], with continuous breakdown of bone by osteoclasts, followed by replacement of the missing bone by osteoblasts. In some active lacunae where bone resorption is taking place, the extracellular calcium level can reach as high as 8-40 mmol/L [29]. The high calcium concentration within this microenvironment could induce the expression of the CaSR in cancer cells. High concentrations of calcium and calcimimetics (i.e., allosteric CaSR activators) have, in fact, been shown to upregulate expression of the CaSR in normal tissues, such as parathyroid gland [30, 31]. Elevated extracellular calcium concentrations stimulate parathyroid hormone related peptide (PTHrP) production by prostate cancer cell lines [32], which could increase bone resorption near bony metastases of prostate cancer [33], thereby producing a favorable environment for tumor growth and providing a growth advantage for metastatic cancer cells having high CaSR expression.

There is abundant literature addressing possible targets for the treatment of prostate cancer [34, 35]. The seed and soil theory is a popular one [36]. Cancer cells are regarded as the seeds and the bony environment as the soil. Some believe that the therapeutic target should be the seed. From the point of view of our study, treatments targeting both the seed (e.g., prostate cancer cells with high CaSR expression) and soil (i.e., high local levels of Ca$^{2+}$ in bone) could be a better therapeutic direction in the clinic. That is, a therapeutic approach combining inhibition of bone resorption using a bisphosphonate [37], for example, and suppression of CaSR activity with a calcilytic, e.g., a negative allosteric modulator of the receptor [38]. While such a combined approach has not been reported to our knowledge, decreasing the level of expression of the CaSR in PC-3 cells in a murine model of prostate cancer metastasis to bone, reduced the metastatic burden in bone [23].

Comparison with other studies

CaSR is considered to be an important factor in bony metastases of some types of cancer. Breast cancer tissues
from patients with bony metastases have higher expression of CaSR than that of breast cancer from patients without bony metastases. In our study, we didn’t see any differences between CaSR expression in the primary prostate cancers of patients with bony metastases and that in the primary prostate cancers that had not metastasized to bone. This might be due to the CaSR having different functions in different types of cancer [39, 40]. Huang et al. demonstrated that CaSR expression was significantly higher in more tumorigenic prostate cancer cell lines and in prostate cancer tissue specimens than in the normal prostate cells [41]. However, this study did not use IHC to detect the expression of CaSR protein in situ, but extracted the protein from the normal tissue and cancer tissue then performed western blot analysis. The number of tissue specimens examined was also small.

Adams, et al. reported that hematopoietic stem cells engraft in bone, at least in part, because of CaSR. Hematopoietic stem cells from CaSR-/- mice exhibited diminished adhesion to extracellular matrix proteins, even though they were normal in their capacities to differentiate, migrate and home to bone [42]. Therefore, if, as we have suggested, CaSR expression increases after prostate cancer cells arrive at bone, this increased CaSR could potentially enhance the capacity of cancer cells to localize in the bone by a similar mechanism(s).

Conclusions

Our tissue microarray study suggests that CaSR expression may increase after prostate cancer cells arrive at bone. This increase could result from the process of cancer cells adapting to the bone environment and, thereby, enhancing their capacity to colonize in bone. We cannot exclude, however, the possibility that small numbers of prostate cancer cells with high CaSR expression have greater metastatic potential for bone rather than the remaining prostate cancer cells localized in prostate. Stimulation of PTHrP secretion by the high level of CaSR expressed by this subpopulation of cells might enhance their capacity to establish metastases in bone. Given the limited number of prostate cancer tissues obtained from bony metastases studied here due to difficulties in obtaining such samples, it would be important to extend the study in the future to additional cases of bony metastases of prostate cancer.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgements

This work was supported in part by National Institutes of Health Grants GM081749 to JJY and a predoctoral fellowship to JF from the Brain and Behavior Program at Georgia State University. We thank Dr. Zhiren Liu for his valuable advice, Chen Zhang for help in preparation of this manuscript, Dr. Xue Wang for statistical analysis and other members of the Yang group for helpful suggestions.

References

8. Takaoka S, Yamaguchi T, Yano S, Yamauchi M, Sugimoto T. The Calcium-sensing Receptor (CaR) is involved in strontium ranelate-induced osteoblast differentiation and mineralization. J Bone Miner Res 2010; 25:627-631.
12. El Hiani Y, Lehen’kyi V, Ouaddid-Ahidouch H, Ahidouch A. Activation of the calcium-sensing receptor by high calcium induced breast cancer cell proliferation and TRPC1 cation


Supplements

**Suppl. Table 1. Pathological tumor (T) stage and positivity% for expression of CaSR in primary prostate cancer tissues of patients with different stages of prostate cancer in array PR955**

<table>
<thead>
<tr>
<th>T Stage</th>
<th>Cases (N)</th>
<th>Pathological Score (%) (intensity multiplied by area) Median (25th, 75th percentiles)</th>
<th>Positivity (%) Median (25th, 75th percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>29</td>
<td>180 (85,200)</td>
<td>0.06 (0.03,0.13)</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>190 (158,255)</td>
<td>0.06 (0.03,0.14)</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>180 (160,200)</td>
<td>0.08 (0.05,0.16)</td>
</tr>
</tbody>
</table>

The correlation P value for stage and pathological score is 0.54. The correlation P value for stage and positivity is 0.41.

**Suppl. Table 2. Pathological scores and positivity% for expression of CaSR in primary prostate cancer tissues of patients with different Gleason scores in array PR955**

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>Cases (N)</th>
<th>Pathological Score (%) (intensity multiplied by area) Median (25th, 75th percentiles)</th>
<th>Positivity (%) Median (25th, 75th percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>200</td>
<td>0.26</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>170</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>175 (89,221)</td>
<td>0.09 (0.04,0.23)</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>195 (160,255)</td>
<td>0.08 (0.04,0.14)</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>190 (93,214)</td>
<td>0.10 (0.02,0.17)</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>185 (150,200)</td>
<td>0.05 (0.03,0.10)</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>180 (145,200)</td>
<td>0.15 (0.04,0.24)</td>
</tr>
</tbody>
</table>

The correlation P value for Gleason score and pathological score is 0.08. The correlation P value for Gleason score and positivity is 0.31.

**Suppl. Table 3. Pathological scores and positivity% for expression of CaSR in primary prostate cancer tissues of patients with different PSA concentrations in array PR955**

<table>
<thead>
<tr>
<th>PSA(ng/mL)</th>
<th>Cases (N)</th>
<th>Pathological Score (%) (intensity multiplied by area) Median (25th, 75th percentiles)</th>
<th>Positivity (%) Median (25th, 75th percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>5</td>
<td>160 (160,200)</td>
<td>0.02 (0.02,0.03)</td>
</tr>
<tr>
<td>4-10</td>
<td>9</td>
<td>200 (160,200)</td>
<td>0.03 (0.02,0.04)</td>
</tr>
<tr>
<td>10-20</td>
<td>20</td>
<td>185 (120,200)</td>
<td>0.06 (0.04,0.14)</td>
</tr>
<tr>
<td>20-40</td>
<td>13</td>
<td>255 (190,285)</td>
<td>0.12 (0.08,0.16)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>10</td>
<td>198 (190,253)</td>
<td>0.10 (0.03,0.12)</td>
</tr>
</tbody>
</table>

The correlation P value for PSA and pathological score is 0.07. The correlation P value for PSA and positivity is 0.05.
**Suppl. Figure 1.** IHC staining of CaSR in prostate tissue with Sigma anti-CaSR antibody (right) and negative control (left). (×10)

**Suppl. Figure 2.** Western Blot of CaSR expression in prostate cancer cell line, PC-3 cells, using Sigma anti-CaSR antibody. GAPDH was detected as a loading control.

**Suppl. Figure 3.** IHC staining of CaSR in prostate tissue microarray PR955. Normal prostate tissue: H1-H11 and G9-G12. Primary prostate cancer: A1-A12, B1-B12, C1-C12, D1-D12, E1-E12, and F1-F12. Prostate cancer from bone: G3-G8 (G5 and G6 were damaged). Prostate cancer from abdominal wall: G1-G2. (×4)

**Suppl. Figure 4.** Correlation between pathological score and positivity%. ($R^2 = 0.35$, $P = 0.001$)