Research Article

Gas Protein Expression Is an Independent Predictor of Recurrence in Prostate Cancer

Lijuan Wang, Guihua Jin, Chenchen He, Xijing Guo, Xia Zhou, Meng Li, Xia Ying, Le Wang, Huili Wu, and Qing Zhu

Department of Medical Oncology, The First Affiliated Hospital, Xi’an Jiao Tong University of Medical College, Xi’an 710061, China

Correspondence should be addressed to Qing Zhu; newzhuqing1972@yahoo.com

Received 30 December 2013; Accepted 27 February 2014; Published 31 March 2014

Academic Editor: Jianying Zhang

Copyright © 2014 Lijuan Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. T393C polymorphism in the gene GNAS1, which encodes the G-protein alpha s subunit (Gas) of heterotrimeric G protein, is significantly associated with the clinical outcome of patients suffering from several cancers. However, studies on the role and protein expression of Gas subunit in prostate cancer were still unavailable.

Methods. The immunohistochemical staining was used to assess Gas expression through tissue microarray procedure of 56 metastatic PCas, 291 localized PCas, and 67 benign hyperplasia (BPH). Gas expression was semiquantitatively scored and evaluated the correlation with pathologic parameters and biochemical recurrence of prostate-specific antigen (PSA).

Results. Gas expression was localized in nuclear and cytoplasm in prostate cancer cells and downregulated in metastatic PCa compared to localized PCa and BPH (P < 0.001). Gas was inversely associated with PSA level and Gleason scores; patients with low expression of Gas had adverse clinicopathological features. In multivariable Cox regression analysis, high Gas expression and Gleason scores were independent predictors of both PSA progression-free and overall survival.

Conclusions. Gas down-expression is associated with adverse clinicopathological features and clinical PSA biochemical recurrence of prostate cancer. Gas is an independent predictor to help determine the risk of PSA progression and death.

1. Introduction

Prostate cancer (PCa), the most frequently diagnosed malignancy, has become the second leading cause of cancer-related deaths among men in Western countries [1, 2]. Endocrine therapies which aimed at inhibiting the androgen receptor (AR) function was the mainstay of treatment for advanced prostate cancer based on that the androgen signaling will promote the proliferation of prostate cancer cell. Unfortunately, most of treated patients progressed toward castration-resistant prostate cancer (CRPC) from castration-dependent prostate cancer. And CRPC characterized by aggressive growth and ability to colonize distal organs, which made CRPC still incurable and the median survival time for patients with CRPC was only 12 months [3]. The status of AR was highly predictive of prostate cancer patients that will benefit from endocrine therapy but was not correlated with a better clinical outcome [4, 5]. Prostate-specific antigen (PSA) is a protein produced by the prostate gland cells. The PSA test measures the level of PSA in a man’s blood. Although many published studies have assessed the performance of candidate biomarkers in predicting time to relapse of prostate cancer following radical prostatectomy [6, 7], no molecular markers suitable for routine clinical practice that can identify those prostate cancer patients with a high risk of early clinical progression or prostate cancer-specific mortality have been found.

G-proteins are composed of α, β, and γ subunits and α subunit are classified into 4 families: Gas, Gai/o, Gasq/11, and Gai12/13. Each of them has multiple members with different expression specificity [8, 9]. Although Gas is the most extensively characterized and clinically relevant, literature is not unanimous on the role of Gas in different types of cancers. In lung cancer, Choi et al. found that Gas could augment cisplatin-induced apoptosis of lung cancer cells through upregulating Bak expression by increasing transcription and by decreasing the rate of protein degradation [10] and the efficacy of radiotherapy of lung cancer may be improved by
modulating Gas signaling pathway [11]. But in cervical cancer or intrahepatic cholangiocarcinoma (ICC), the situation was just opposite. Cho et al. found Gas inhibited cisplatin-induced apoptosis by increasing transcription of X-linked inhibitor of apoptosis protein (XIAP) and by decreasing degradation of XIAP protein in HeLa cervical cancer cells [12]. In ICC, Schmitz et al. also found a significant association of both unfavorable disease-specific overall survival and recurrence-free survival with the homozygous TT genotype of the GNAS1 gene which encoded Gas protein [13, 14]. However, they also reported that T393C polymorphism in the gene GNAS1 was significantly associated with favorable clinical outcome of patients suffering from bladder cancer, chronic lymphocytic leukemia [15], and renal cell carcinoma [16].

The situation was even more complicated in prostate cancer. There were studies reported no association was found between GNAS T393T genotype and prostate cancer [17, 18]. But Liu et al. identified that membrane caveolae-associated Gas was involved in androgen receptor (AR) transactivation by modulating the activities of different PI3K isoforms [19]. More importantly, it had been reported that the expression of Gas decreased 30% to 40% after neoplastic transformation [20]. And the levels of Gas and Gαi subunits correlated inversely with serum prostate specific antigen in patients with prostate cancer [20], which indicated an important regulatory role of Gαs and Gαi for cell proliferation and neoplastic transformation in human prostate cancer and they may have prognostic value. Therefore, more in-depth investigations are necessary to address this controversy and identify the role of Gas in prostate cancer.

Thus, we assessed the potential of Gas as a prognostic marker by determining the level of Gas protein expression in a series of 347 postradical prostatectomy prostate cancer tissue microarrays (TMA) which include 56 metastatic PCAs and 291 localized PCAs and 67 benign prostatic hyperplasia (BPH) samples were collected as control. This research project was approved by the Ethical Committee of the Xi’an Jiao Tong University, and all the patients had been given their fully written informed consent.

Data were collected on patients with disease baseline and clinicopathologic characteristics as well as 2 treatment outcomes: time to progression and prostate cancer-specific mortality (PCSM). Prostate cancers were graded based on the Gleason system by 2 independent pathologists at the First Affiliated Hospital, College of Medicine, and Xi’an Jiao Tong University in a blind and consecutive manner to ensure adequate diagnosis and grade. The TNM staging system was used to describe the extent of Prostate cancer in patients (based on the AJCC Cancer Staging Manual, Seventh Edition, 2010, Springer, New York, Inc.). TNM stages IIA and IIB were considered TNM stage II.

2.2. Immunohistochemistry (IHC). Paraffin-embedded section of normal and tumor tissue was stained for Gas expression. Immunohistochemistry for Gas was performed as previous reported with slight modification. Briefly, slides were deparaffinized in xylene and rehydrated in a graded alcohol series before endogenous peroxidase activity was blocked with 3% H2O2 in methanol. After nonspecific protein binding was blocked, the primary antibody diluted into recommended concentration for Gas, which was purchased from Abcam (ab58810), was applied overnight in a humidity chamber at 4°C. Biotinylated secondary antibody was applied for 30 min at room temperature after washing with PBS for 3 times. Visualization was performed using DAB chromogen for 2 to 3 minutes. Negative control was conducted by replacing the primary antibody with preimmune rabbit serum.

2.3. Evaluation of Staining. To evaluate Gas expression, we used the immunoreactive score (IRS) as previously implemented by Tischler et al. [21], based on the intensity of immune staining and the quantity of stained cells. The intensity of staining was arbitrarily graded as absent (0), weak (1+), moderate (2+), and strong (3+). The percentage of stained cells was used to quantify the reaction as negative (0% of positive cells), 1+ (<10% positive cells); 2+ (10–50% of positive cells); 3+ (51–80% of positive cells); 4+ (>80% of positive cells). The final value of the analysis of each tissue sample was then expressed as an absolute value through the obtained score by multiplying the two individual scores (i.e., intensity of staining score times the percentage of stained cells score) then generates a final score ranging from − (no expression) to + (weak expression), ++ (moderate expression), or +++ (strong expression). And we identified − and + as negative for Gas expression and ++ and +++ as positive Gas expression. Examples of scoring according to staining intensity and the percentage of stained cells are shown in Figure 1.

2.4. Statistical Analysis. SPSS version 13.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. P values < 0.05 were considered significant. Mann–Whitney test was used to calculate the correlation between numerical variables. 2 tests
Figure 1: Immunohistochemically stained localized and metastatic PCa tissues from patients. (a) localized PCa tissues without Gαs expression (−); (b) localized PCa tissues with weak Gαs expression (+); (c) localized PCa tissues with moderate Gαs expression (++) (d) localized PCa tissues with strong Gαs expression (+++); (e) metastatic PCa tissues without Gαs expression (−); (f) metastatic PCa tissues with weak Gαs expression (+); (g) metastatic PCa tissues with moderate Gαs expression (++); (h) metastatic PCa tissues with strong Gαs expression (+++). Representative images were taken under a microscope (×20).

were used to evaluate differences in frequency of categorical-variable groups. Spearman’s rank correlation was used to analyze the correlation between continuous variables. PSA progression-free and overall survival curves were constructed by the Kaplan-Meier method and compared using the log-rank test. To evaluate the role of prognostic variables, a series of Cox proportional hazards models were fitted to PSA progression-free and overall survival data. Since PSA was a continuous estimate, with the median PSA level for the entire cohort of patients (n = 347) as 34.9 ng/mL, we divided the cohort into those with PSA levels ≤ 35 ng/mL and > 35 ng/mL. The following parameters were included: PSA levels (≤ 35 ng/mL, > 35 ng/mL); extraprostatic extension (Yes, No); involvement of surgical margins (No, Yes); involvement of seminal vesicles (No, Yes); involvement of pelvic nodes (N0, N+); Gleason scores (2–6, 7, 8–10).

3. Results

3.1. Histopathologic and Clinical Information. The median Gleason score of all patients was 7 (range: 2–10). 145 patients (41.8%) presented Gleason score of 2–6, 127 (36.6%) patients presented Gleason score of 7, and the remaining 75 cases (21.6%) presented Gleason score between 8 and 10. 49 patients (11.5%) presented TNM stage I; 125 (36.0%) patients presented stage II; 117 (33.7%) presented stage III, and 56 (16.1%) patients presented TNM stage IV. PSA progression was observed in 229 (66.0%) patients at a median interval of 123.5 month (range 7–167). Other clinico pathological features are summarized in Table 2. Moreover, prostate cancer patients who had higher Gleason scores (P < 0.001 and P < 0.001, resp.), higher TNM stages (P < 0.001 and P < 0.001, resp.), higher preoperative PSA level (P < 0.001 and P < 0.001, resp.), positive surgical margin (P = 0.009 and P < 0.001, resp.), angiolymphatic invasion (P = 0.004 and P = 0.032, resp.), extraprostatic extension (P = 0.031 and P < 0.001, resp.), and seminal vesicle invasion (P = 0.046 and P = 0.007, resp.) present shorter overall survival and PSA progression-free survival (Tables 5 and 6). PSA progression and overall survival time correlated with TNM stage, Gleason score, extraprostatic extension, positive surgical margins, and seminal vesicle invasion demonstrate the representability of study group.

3.2. Expression of Gαs in Human Prostate Cancer. To determine the prevalence and clinical significance of Gαs in prostate cancer tissues, we determined the expression of Gαs protein by immunohistochemistry in a retrospective cohort of 347 tumor tissue samples from prostate cancer patients and 67 samples from patients who were diagnosed with benign prostatic hyperplasia (BPH) after tumor resection. Among the 347 patients, 114 patients had not expression of Gαs (−); 73 patients were weak expression (+); 86 patients were moderate expression (++), and 74 patients were strong expression (+++) (as shown in Figure 1). Thus, as we described in the methods section, there were 160 (46.1%) samples positive for Gαs expression and 187 (53.9%) samples negative for Gαs.
expression in our PCA cohort. We also found that positive ratio of Gαs expression was downregulated in metastatic PCA compared to localized PCA and BPH (P = 0.012 and P < 0.001, respectively, as shown in Table 1). In patients with BPH, there were 42 (62.7%) samples positive for Gαs expression and 25 (37.3%) samples negative for Gαs expression. In the patients with localized PCA, there were 151 (51.2%) samples positive for Gαs expression and 140 (48.1%) samples negative for Gαs expression. Whereas in the patients with metastatic PCA, there were 9 (16.1%) samples positive for Gαs expression and 47 (83.9%) samples negative for Gαs expression. Gαs antibody mainly showed positive nuclear and cytoplasmic staining in prostate cancer cells. IHC staining for Gαs was sharp and reliable, no background or nonspecific staining was observed.

3.3. Correlation of Gαs with Histopathologic and Clinical Information. Then, we further evaluated the relationship between Gαs expression and clinical features of prostate cancer patients by Pearson chi-square test or Fisher’s exact test. We found that the positive ratio of Gαs expression was decreased as the level of Gleason score or preoperative PSA increased. These result showed that there was inverse correlations between Gαs expression and preoperative PSA and Gleason score and TNM stage at diagnosis (P = 0.030, P < 0.001, and P < 0.001, respectively, Table 2). But we found no specific correlation between Gαs expression and the rest of pathological parameters (age; angiolymphatic invasion; extraprostatic extension; positive margin; seminal vesicle invasion; positive lymph node) that we evaluate in the present analysis. In the localized PCA specimens, the expression of Gαs was correlated with preoperative PSA level, Gleason score, and TNM stage (P = 0.028, P = 0.016, and P = 0.011, respectively, Table 3). However, in metastatic PCA specimens, the expression of Gαs was only associated with preoperative PSA level and Gleason score much more significantly (P < 0.001, and P < 0.001, resp., Table 4).

3.4. Correlation of Gαs Expression with PSA-Free and Overall Survival. In our retrospective cohort with 347 patients, we got the detailed follow-up information of 15 years. At the time of our analysis, 121 patients died and 256 patients progressed. Median time to PSA progression for the whole cohort was 123.5 months (range 7–167 months), while the median time to death was 123.5 months (range 3–179 months). We found that patients with negative expression of Gαs had a higher ratio of PSA progression than those with positive Gαs expression (Table 2). More importantly, negative Gαs expression was associated with PSA progression-free survival and overall

### Table 1: Comparison of Gαs expression among different pathological categories.

| Variable               | Number | Gαs positive | Gαs negative | χ²  | P   |
|------------------------|--------|--------------|--------------|-----|-----|
| All patients           | 347    | 160          | 187          |     |     |
| Metastatic PCa         | 56     | 17           | 39           |     |     |
| Localized PCa          | 291    | 143          | 148          | 6.67| 0.012*|
| BPH                    | 67     | 42           | 25           | 12.77| <0.001**|

*Significant differences of Gαs expression in metastatic PCa compared to localized PCa.

**Significant differences of Gαs expression in metastatic PCa compared to BPH.

### Table 2: Characterization of the cohort of 347 prostate cancer samples.

| Variable               | Number | Gαs positive | Gαs negative | χ²  | P   |
|------------------------|--------|--------------|--------------|-----|-----|
| All patients           | 347    | 160          | 187          |     |     |
| Age at diagnosis (years) |       |              |              |     |     |
| ≤73                    | 211    | 96           | 115          | 0.081| 0.826|
| >74                    | 136    | 64           | 72           |     |     |
| Clinical stage at diagnosis |       |              |              |     |     |
| I                      | 49     | 31           | 18           |     |     |
| II                     | 125    | 61           | 64           | 9.358| <0.001*|
| III                    | 117    | 51           | 66           |     |     |
| IV                     | 56     | 17           | 39           |     |     |
| Gleason score at diagnosis |     |              |              |     |     |
| 2–6                    | 145    | 83           | 62           | 15.692| <0.001*|
| 7                      | 127    | 54           | 73           |     |     |
| 8–10                   | 75     | 23           | 52           |     |     |
| Preoperative PSA (ng/mL) |       |              |              |     |     |
| <35                    | 184    | 99           | 85           | 9.334| 0.003*|
| ≥35                    | 163    | 61           | 102          | 1.096| 0.350|
| Angiolympathic invasion |        |              |              |     |     |
| Presence               | 114    | 48           | 66           | 1.096| 0.350|
| Absence                | 233    | 112          | 121          |     |     |
| Extraprostatic extension |       |              |              |     |     |
| Presence               | 105    | 53           | 52           | 0.993| 0.351|
| Absence                | 242    | 135          | 107          |     |     |
| Positive surgical margin |       |              |              |     |     |
| Presence               | 128    | 60           | 68           | 0.048| 0.911|
| Absence                | 219    | 100          | 119          |     |     |
| Seminal vesicle invasion |       |              |              |     |     |
| Presence               | 172    | 78           | 94           | 0.079| 0.830|
| Absence                | 175    | 82           | 93           |     |     |
| Positive lymph node    |        |              |              |     |     |
| Presence               | 34     | 14           | 20           | 0.369| 0.590|
| Absence                | 313    | 146          | 167          |     |     |
| PSA progression         |        |              |              |     |     |
| Presence               | 213    | 89           | 124          | 4.153| 0.047*|
| Absence                | 134    | 71           | 63           |     |     |

*Significant differences of Gαs expression among different clinical factors groups in prostate cancer samples.
We further conducted a multivariate Cox regression analysis to assess whether Gαs was a prognostic predictor of survival independent of age, Gleason scores, TNM stages, preoperative PSA, positive margin, angiolymphatic invasion, extraprostatic extension, seminal vesicle invasion, and positive lymph node. Multivariate analysis showed that negative Gαs expression was a strong independent predictor of outcome providing survival information (both PSA progression-free or overall survival) above other independent prognostic features (TNM stage, Gleason score), with a hazard ratio of 4.328 and 3.904 and a 95% confidence interval of 1.876–8.432, \( P < 0.001 \) (negative Gαs group versus positive Gαs group) and 1.278–5.873, \( P < 0.001 \) (negative Gαs group versus positive Gαs group). In our cohorts, PSA, positive margin, angiolymphatic invasion, extraprostatic extension, and seminal vesicle invasion were not independently associated with outcome at the multivariable level.

### 4. Discussion

It has been reported that the expression of Gαs correlated inversely with serum prostate specific antigen in patients with prostate cancer and the expression of Gαs decreased 30% to 40% after neoplastic transformation [20]. But there was no study concerning the role of Gαs protein in the prognosis of prostate cancer patients. In the present study, we characterized the expression pattern of Gαs protein in a large number of tissues derived from prostate cancer patients, consisting of localized and metastatic PCa, and assessed the utility of Gαs as a prognostic marker in these patients. In agreement with previous reports, we confirmed that Gαs expression was localized in nuclear and cytoplasm in neoplastic cells. Moreover, we found that expression of Gαs was downregulated in metastatic PCa compared to localized PCa and BPH. And Gαs was inversely associated with PSA level and Gleason scores both in localized and metastatic PCa. At the univariate level, Gαs, Gleason scores, TNM stages, preoperative PSA level, positive margin, angiolymphatic invasion, extraprostatic extension, and seminal vesicle invasion were all significantly associated with PSA progression-free and overall survival. But in multivariable Cox regression analysis, only high Gαs expression and Gleason scores were independent predictors of both PSA progression-free and overall survival. These findings support the potential clinical utility of incorporating Gαs into clinical nomograms to help determine the risk of PSA progression and death.

The prognostic significance of Gαs as a biomarker for prostate cancer is likely to its biological functions. Gαs is a member of GTP-binding protein superfamily and could independently regulate a variety of effectors including adenylate cyclases, phospholipase Cβ, and ion channels [22, 23]. However, Gαs and T393C polymorphism in the gene GNAS1 which is encoded by Gαs plays distinct roles in different cancers. Gαs could augment cisplatin-induced apoptosis of lung cancer cells [10], but it inhibited cisplatin-induced apoptosis in cervical cancer or intrahepatic cholangiocarcinoma (ICC) [12]. T393C polymorphism in the gene GNAS1 was significantly associated with favorable clinical outcome of
patients suffering from bladder cancer, chronic lymphocytic leukemia [15], and renal cell carcinoma [16], and significantly associated with unfavorable clinical outcome of patients suffering from ICC and breast cancer [13, 14]. In prostate cancer, previous study reported that low expression level of Gαs was found in T2 stage PCa compared to high levels in normal controls [20]. More importantly, the expression of Gαs was found downregulated in hormone refractory C4-2B and PC3 cell lines compared to hormone sensitive LNCaP and RWPE-1 cell lines. All these studies indicating the
functionality and expression of Gs are selectively modified in human prostate adenocarcinoma and downregulated Gs levels may play an important regulatory role for cell proliferation and neoplastic transformation in prostate cancer. Thus, we did our efforts to investigate and validate whether Gs functioned as an efficient prognostic biomarker to predict the outcome of PCa patients. Consistent with previous studies, we found that the expression of Gs was much lower in metastatic PCa than it is in localized PCa. Furthermore, the patients who had the low expression of Gs tend to have shorter progression-free survival and overall survival time, nonetheless, metastatic or localized PCa. More importantly, multivariable Cox regression analysis proved that Gs was an independent predictor of prognosis in prostate cancer.

Though the answer to the discrepancy of Gs function in different cancers was still unclear, and the role of Gs in prostate cancer was also in dispute, our results were relatively easy to understand for Gs had a close relationship with EGFR. EGFR belongs to ErbB oncogene family which also includes ErbB-2, 3, and 4 and is comprehensively expressed in epithelial cells including prostate cancer cells. EGFR are known to regulate cell proliferation, differentiation, angiogenesis, and survival [24]. In prostate cancer, EGFR is elevated along with disease progression. It has been reported that EGFR was highly expressed in DU145 and PC3 cell lines which were hormone-independent human prostate cancer cell lines and responsive to EGF stimulation [25–27]. It was also found that prostate cancer bone metastases express significantly higher level of EGFR [28]. More importantly, EGFR expression increased as prostate cancer progressed from an androgen-dependent to an androgen-independent stage [29]. di Lorenzo et al., found 41%, 76%, and 100% EGFR expression in radical prostatectomy hormone-sensitive and hormone-refractory metastatic patients in a cohort consisting of 76 patients with androgen-dependent and androgen-independent prostate cancer, respectively [30]. And there was a significant association between EGFR expression and higher Gleason score [31]. Many studies confirmed that overexpression of EGFR contributes significantly to the progression of prostate cancer [31–34].

Zheng et al. reported that overexpression of the stimulatory Gs promotes ligand-dependent degradation of epidermal growth factor (EGF) receptors and Texas Red EGF, and knock-down of Gs expression by RNA interference (RNAi) delays receptor degradation [35]. Recently, they demonstrated that EGF-induced, proliferative signaling occurs from

**Table 5: Univariate and Multivariate analysis of clinical factors in relation to overall survival.**

| Clinical Factor                  | Univariate HR (95% CI) | P       | Multivariate HR (95% CI) | P       |
|---------------------------------|------------------------|---------|--------------------------|---------|
| Negative Gs                     | 5.832 (3.232–10.763)   | <0.001* | 3.904 (1.278–5.873)      | <0.001* |
| Age at diagnosis                | 1.098 (0.921–1.284)    | 0.495   | 1.328 (0.493–4.187)      | 0.276   |
| Clinical stage at diagnosis     | 2.287 (1.639–3.121)    | <0.001* | 0.723 (0.298–1.114)      | 0.294   |
| Gleason score at diagnosis      | 3.809 (2.778–5.132)    | <0.001* | 2.153 (1.471–3.957)      | 0.004*  |
| Preoperative PSA                | 2.673 (2.007–3.297)    | <0.001* | 2.158 (0.622–3.192)      | 0.429   |
| Angiolympathic invasion         | 1.224 (1.098–1.989)    | 0.004*  | 1.472 (0.897–1.677)      | 0.172   |
| Extraprostatic extension        | 1.327 (1.211–2.019)    | 0.031*  | 0.819 (0.531–1.396)      | 0.491   |
| Positive margin                 | 2.127 (1.271–4.918)    | 0.009*  | 1.211 (0.682–2.198)      | 0.514   |
| Seminal vesicle invasion        | 1.778 (1.281–3.711)    | 0.046*  | 1.397 (0.723–2.187)      | 0.283   |
| Positive lymph node             | 1.698 (0.831–3.781)    | 0.064   | 0.931 (0.871–2.011)      | 0.592   |

**Table 6: Univariate and Multivariate analysis of clinical factors in relation to PSA progression-free survival.**

| Clinical Factor                  | Univariate HR (95%) | P       | Multivariate HR (95%) | P       |
|---------------------------------|---------------------|---------|-----------------------|---------|
| Negative Gs                     | 5.269 (1.187–7.589) | <0.001* | 4.328 (1.876–8.432)   | <0.001* |
| Age at diagnosis                | 1.132 (0.809–1.727) | 0.413   | 0.743 (0.239–3.158)   | 0.712   |
| Clinical stage at diagnosis     | 2.787 (1.131–4.238) | <0.001* | 2.135 (0.897–5.328)   | 0.117   |
| Gleason score at diagnosis      | 5.821 (3.496–10.825)| <0.001* | 3.219 (1.276–8.557)   | <0.001  |
| Preoperative PSA                | 1.784 (1.389–3.476) | <0.001* | 0.976 (0.597–2.911)   | 0.061   |
| Angiolympathic invasion         | 1.829 (1.142–2.109) | 0.032*  | 0.734 (0.549–1.291)   | 0.125   |
| Extraprostatic extension        | 2.352 (1.399–4.569) | <0.001* | 1.892 (0.897–3.219)   | 0.071   |
| Positive margin                 | 3.404 (1.778–6.091) | <0.001* | 2.199 (0.782–3.988)   | 0.084   |
| Seminal vesicle invasion        | 3.891 (1.584–5.822) | 0.007*  | 1.329 (0.806–1.986)   | 0.322   |
| Positive lymph node             | 2.012 (0.904–4.584) | 0.091   | 1.212 (0.814–1.507)   | 0.532   |

Cl: confidence interval; HR: hazard ratio; rec: recurrence.
* Significant relationships of clinical factors with overall survival.
EEA1 endosomes and was regulated by the Gαs through interaction with the signal transducing protein GIV (also known as Girdin). When Gαs or GIV was depleted, activated EGFR and its adaptors accumulate in EEA1 endosomes. Then EGFR signaling was prolonged and EGFR downregulation was delayed, which made cell proliferation greatly enhanced [36]. Basing on our finding that Gαs was downregulated in advanced PCa and the previous studies concerning the function of EGFR in PCa, we hypothesise that downexpression of Gαs inhibit the degradation of EGFR, then androgen receptors which are activated by EGFR were prolonged and cell proliferation increased, eventually causing tumor progression and hormone-resistant in prostate cancer. That maybe the underlying mechanism account for our results in which the expression of Gαs was downregulated in metastatic PCa and inversely associated with PSA level and Gleason scores. But experiments would be desirable to further clarify the relationship between Gαs and EGFR and identify their function in the progression of prostate cancer. However, such functional studies were beyond the scope of this study.

5. Conclusion

In summary, we discovered that Gαs is a promising biomarker of prostate cancer patients. To our knowledge, this is the first study to describe the predictive role of Gαs in prostate cancer. We found that the expression of Gαs was downregulated in metastatic prostate cancer compared to localized prostate cancer. And low expression Gαs was significantly associated with adverse clinopathological features. More important, Gαs was an independent prognostic predictor in prostate cancer. Although our results are promising, Gαs expression needs to be validated in relationship to outcome in the context carefully controlled clinical trials. If confirmed, application of Gαs immunohistochemical analysis should be technically straightforward and feasible. All in all, targeting Gαs could be a promising therapeutic strategy for enhancing the therapy effect of patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Lijuan Wang and Guihua Jin contributed equally to this work.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (nos. 81072117 and 81272200).

References

[1] C. la Vecchia, C. Bosetti, F. Lucchini et al., “Cancer mortality in Europe, 2000–2004, and an overview of trends since 1975,” Annals of Oncology, vol. 21, no. 6, pp. 1323–1360, 2009.
[2] A. Jemal, R. Siegel, J. Xu, and E. Ward, “Cancer statistics, 2010,” CA Cancer Journal for Clinicians, vol. 60, no. 5, pp. 277–300, 2010.
[3] H. I. Scher and C. L. Sawyers, “Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis,” Journal of Clinical Oncology, vol. 23, no. 32, pp. 8253–8261, 2005.
[4] R. Mori, Q. Wang, M. L. Quet et al., “Prognostic value of the androgen receptor and its coactivators in patients with DI prostate cancer,” Anticancer Research B, vol. 28, no. 1, pp. 425–430, 2008.
[5] H. Zhao, M. A. Coram, R. Nolley et al., “Transcript levels of androgen receptor variant AR-V1 or AR-V7 do not predict recurrence in patients with prostate cancer at indeterminate risk for progression,” Journal of Urology, vol. 188, pp. 2158–2164, 2012.
[6] K. K. Rasiah, M. Gardiner-Garden, E. J. D. Padilla et al., “HSD17B4 overexpression, an independent biomarker of poor patient outcome in prostate cancer,” Molecular and Cellular Endocrinology, vol. 301, no. 1–2, pp. 89–96, 2009.
[7] G. J. L. H. van Leenders, D. Dukers, D. Hessels et al., “Polycyogroup oncogenes EZH2, BMI1, and RING1 are overexpressed in prostate cancer with adverse pathologic and clinical features,” European Urology, vol. 52, no. 2, pp. 455–463, 2007.
[8] K. L. Pierce, R. T. Premont, and R. J. Lefkowitz, “Seven-transmembrane receptors,” Nature Reviews Molecular Cell Biology, vol. 3, no. 9, pp. 639–650, 2002.
[9] Y. Daaka, “G proteins in cancer: the prostate cancer paradigm,” Science’s STKE: Signal Transduction Knowledge Environment, vol. 2004, no. 216, p. re2, 2004.
[10] Y. J. Choi, J. M. Oh, S. Y. Kim, M. Seo, and Y. S. Juhnn, “Stimulatory heterotrimeric GTP-binding protein augments cisplatin-induced apoptosis by upregulating Bak expression in human lung cancer cells,” Cancer Science, vol. 100, no. 6, pp. 1069–1074, 2009.
[11] Y. J. Choi, S. Y. Kim, J. M. Oh, and Y. S. Juhnn, “Stimulatory heterotrimeric G protein augments gamma ray-induced apoptosis by up-regulation of Bak expression via CREB and AP-1 in H1299 human lung cancer cells,” Experimental and Molecular Medicine, vol. 41, no. 8, pp. 592–600, 2009.
[12] E. A. Cho, J. M. Oh, S. Y. Kim, Y. Kim, and Y. S. Juhnn, “Heterotrimeric stimulatory GTP-binding proteins inhibit cisplatin-induced apoptosis by increasing X-linked inhibitor of apoptosis protein expression in cervical cancer cells,” Cancer Science, vol. 102, no. 4, pp. 837–844, 2011.
[13] K. J. Schmitz, H. Langy, U. H. Frey et al., “GNAS T393C polymorphism is associated with clinical course in patients with intrahepatic cholangiocarcinoma,” Neoplasia, vol. 9, no. 2, pp. 159–165, 2007.
[14] U. H. Frey, H. Alakus, J. Wohlschlaeger et al., “GNAS T393C polymorphism and survival in patients with sporadic colorectal cancer,” Clinical Cancer Research, vol. 11, no. 14, pp. 5071–5077, 2005.
[15] U. H. Frey, H. Nückel, L. Sellmann et al., “The GNAS T393C polymorphism is associated with disease progression and survival in chronic lymphocytic leukemia,” Clinical Cancer Research, vol. 12, no. 19, pp. 5686–5692, 2006.
[16] U. H. Frey, G. Lümmen, T. Jäger et al., “The GNAS T393C polymorphism predicts survival in patients with clear cell renal cell carcinoma,” Clinical Cancer Research, vol. 12, no. 3, pp. 759–763, 2006.
[17] A. Eisenhardt, A. Scherag, K. H. Jöckel, H. Reis, H. Rübben, and W. Siffert, “Lack of association of the genotype in the GNAS foki polymorphism and prostate cancer,” *Urologia Internationalis*, vol. 87, no. 1, pp. 80–86, 2011.

[18] M. Watanabe, Y. Hirokawa, M. Tsuji et al., “Lack of involvement of the GNAS1T393C polymorphism in prostate cancer risk in a Japanese population,” *Anticancer Research A*, vol. 28, no. 6, pp. 3711–3716, 2008.

[19] J. Liu, H. Youn, J. Yang et al., “G-protein alpha-s and -12 subunits are involved in androgen-stimulated PI3K activation and androgen receptor transactivation in prostate cancer cells,” *Prostate*, vol. 71, no. 12, pp. 1276–1286, 2011.

[20] M. O. García-Fernández, R. M. Solano, M. Sánchez-Chapado, A. Ruiz-Villaespesa, J. C. Prieto, and M. J. Carmen, “Low expression of g alpha protein subunits in human prostate cancer,” *Journal of Urology*, vol. 166, no. 6, pp. 2512–2517, 2001.

[21] V. Tischler, F. R. Fritzschke, P. J. Wilde et al., “Periostin is upregulated in high grade and high stage prostate cancer,” *BMC Cancer*, vol. 10, article 273, 2010.

[22] H. R. Bourne, D. A. Sanders, and F. McCormick, “The GTPase superfamily: a conserved switch for diverse cell functions,” *Nature*, vol. 348, no. 6297, pp. 125–132, 1991.

[23] M. I. Simon, M. P. Strathmann, and N. Gautam, “Diversity of G proteins in signal transduction,” *Science*, vol. 252, no. 5007, pp. 802–808, 1991.

[24] Y. Yarden and M. X. Sliwkowski, “Untangling the ErbB signalling network,” *Nature Reviews Molecular Cell Biology*, vol. 2, no. 2, pp. 127–137, 2001.

[25] E. R. Sherwood and C. Lee, “Epidermal growth factor-related peptides and the epidermal growth factor receptor in normal and malignant prostate,” *World Journal of Urology*, vol. 13, no. 5, pp. 290–296, 1995.

[26] Y. S. Pu, M. W. Hsieh, C. W. Wang et al., “Epidermal growth factor receptor inhibitor (PD168393) potentiates cytotoxic effects of paclitaxel against androgen-independent prostate cancer cells,” *Biochemical Pharmacology*, vol. 71, no. 6, pp. 751–760, 2006.

[27] S. Kharait, R. Dhir, D. Lauffenburger, and A. Wells, “Protein kinase Cδ signaling downstream of the EGF receptor mediates migration and invasiveness of prostate cancer cells,” *Biochemical and Biophysical Research Communications*, vol. 343, no. 3, pp. 848–856, 2006.

[28] T. Zellweger, C. Ninck, M. Bloch et al., “Expression patterns of potential therapeutic targets in prostate cancer,” *International Journal of Cancer*, vol. 113, no. 4, pp. 619–628, 2005.

[29] E. Hernes, F. Fossà, A. Berner, B. Ottnes, and J. M. Nessler, “Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence,” *British Journal of Cancer*, vol. 90, no. 2, pp. 449–454, 2004.

[30] G. di Lorenzo, G. Tortora, F. P. D’Armiendo et al., “Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer,” *Clinical Cancer Research*, vol. 8, no. 11, pp. 3438–3444, 2002.

[31] D. C. Weber, J. C. Tille, C. Combescure et al., “The prognostic value of expression of HIFalpha, EGFR and VEGF-A, in localized prostate cancer for intermediate- and high-risk patients treated with radiation therapy with or without androgen deprivation therapy,” *Radiation Oncology*, vol. 7, p. 66, 2012.

[32] A. M. Traish and A. Morgentaler, “Epidermal growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth,” *British Journal of Cancer*, vol. 101, no. 12, pp. 1949–1956, 2009.

[33] E. R. Sherwood, J. L. van Dongen, C. G. Wood, S. Liao, J. M. Kozlowski, and C. Lee, “Epidermal growth factor receptor activation in androgen-independent but not androgen-stimulated growth of human prostatic carcinoma cells,” *British Journal of Cancer*, vol. 77, no. 6, pp. 855–861, 1998.

[34] S. Xu and Z. Weihua, “Loss of EGFR induced autophagy sensitizes hormone refractory prostate cancer cells to adriamycin,” *Prostate*, vol. 71, no. 11, pp. 1216–1224, 2011.

[35] B. Zheng, C. Lavoie, T. D. Tang et al., “Regulation of epidermal growth factor receptor degradation by heterotrimeric Gαs protein,” *Molecular Biology of the Cell*, vol. 15, no. 12, pp. 5538–5550, 2004.

[36] A. O. Beas, V. Taupin, C. Teodorof, L. T. Nguyen, and M. Garcia-Marcos, “Galphas promotes EEA1 endosome maturation and shuts down proliferative signaling through interaction with GIV (Girdin),” *Molecular Biology of the Cell*, vol. 23, pp. 4623–4634, 2012.