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ORIGINAL ARTICLE

# Altered expression of farnesyl pyrophosphate synthase in prostate cancer: evidence for a role of the mevalonate pathway in disease progression?

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**Abstract**
*Background* Preclinical studies demonstrated effects of drugs inhibiting the mevalonate pathway including nitrogen-containing bisphosphonates (N-BPs) and statins on tumor growth and progression. The exact role of this pathway in prostate cancer (PC) has not been identified yet. Herein, we evaluate the expression of farnesyl pyrophosphate synthase (FPPS), the key enzyme of the mevalonate pathway, in PC.
*Patients and methods* Prostate cancer (PC) and benign prostate tissue of 114 men who underwent radical prostatectomy were constructed to a tissue microarray. Immunohistochemical staining of FPPS was quantified by the Remmele/Stegner immunoreactivity-score. Patients' clinical follow-up was assessed. IRS was correlated to pathological and clinical data. The impact of FPPS expression on clinical course was assessed univariate and multivariate.
*Results* Mean IRS in PC and benign tissue was 5.7 (95% CI 5.0–6.5) and 2.6 (2.1–3.0, $p < 0.0001$). Mean IRS in PC tissue of patients with organ-confined and locally advanced disease ($pT \geq 3$) was 5.09 (4.22–5.96) and 6.87 (5.57–8.17, $p = 0.035$). IRS of PC tissue significantly correlated with Gleason score ($p = 0.03$). Patients with PC tissue IRS >3 showed shorter recurrence-free survival compared to the remaining ($p = 0.01$). Increased FPPS expression is an independent risk factor for early biochemical recurrence ($p = 0.032$).
*Conclusions* This is the first study on FPPS in PC specimens. The association of FPPS with established histopathological risk parameters and biochemical recurrence implicates a contribution of the mevalonate pathway to PC progression. Further functional analysis is required to explore the role of this pathway in PC and to investigate whether FPPS expression affects the response of PC cells to N-BPs.

**Keywords** Prostate cancer · Bisphosphonates · Farnesyl pyrophosphate synthase · Zoledronic acid · Mevalonate pathway

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## Introduction

Several studies indicate that inhibition of the mevalonate pathway shows direct effects on growth and progression of prostate cancer (PC) [1]. This pathway is essential for production of cholesterol and isoprenoids being essential for cell membrane integrity [2]. Moreover, it is required for prenylation of proteins involved in cell cycle regulation and angiogenesis [3]. The most relevant drugs inhibiting this pathway are nitrogen-containing bisphosphonates (N-BPs) and statins. Both have been discussed to exert

anti-tumor activity beyond their effects on bone and cholesterol metabolism [4, 5]. The molecular target of N-BPs is the enzyme farnesyl pyrophosphate synthase (FPPS) being part of the mevalonate pathway. FPPS conjoins geranyl-pyrophosphate (GPP) with isopentenyl pyrophosphate (IPP) to farnesyl pyrophosphate (FPP). Zoledronic acid—a N-BP—is standard for the treatment of metastatic bone disease in PC [6]. It prevents skeletal-related events including fracture, spinal cord compression and pain [6]. N-BPs improve bone mineral density in men receiving androgen-deprivation therapy [6]. Beside its effects on bone metabolism and osteoclast activity, various studies indicated effects of N-BPs on tumor growth, cancer progression and metastatic potential of PC [7]. The underlying mechanism has not been identified yet. It is assumed that major parts of their antitumor effects are mediated by an inhibition of prenylation of small GTPases such as Ras and Rho.

Statins are used to attenuate cholesterol synthesis by inhibition of hydroxy-$\beta$-methylglutaryl-CoA ($\beta$-HMG-CoA) reductase. The role of statins for chemoprevention of PC and inhibition of disease progression is controversially discussed [1]. Nevertheless, evidence exists that intake of statins directly affects tumor biology and prognosis of PC patients [8, 9]. Both cholesterol-dependent and independent mechanisms are responsible for these effects [1].

Despite a considerable amount of evidence for the pathophysiological role of the mevalonate pathway, the exact mechanisms remain unclear. Only little is known about the role of enzymes and intermediates of the mevalonate pathway for PC progression. Herein, we aimed to investigate the expression of FPPS in PC tissue and its correlation with histopathological parameters and individual disease course.

## Patients and methods

### Patients

Samples of 114 men (median age, 65 years; median PSA, 9.04 ng/ml; median Gleason score, 6; 12 × T2a, 6 × T2b, 59 × T2c, 17 × T3a, 20 × T3b, 20 × R1, 5 × pN1, 115 × M0) who underwent radical prostatectomy were constructed to a tissue microarray (TMA). Patients were staged and graded according to the AJCC TNM classification system. The study was approved by the IRB (no. 290/2010BO2).

### Tissue microarray (TMA)

To obtain representative cores for TMA construction, the obtained specimens were HE-stained and suitable areas were selected. TMA preparation was conducted as described previously [10]. Two cores of each PC and corresponding benign prostatic tissue were integrated.

### Immunohistochemistry: confirmation of antibody specificity

Before definitive analysis, the Abgent FPPS Rabbit Ig Center (No. AP2418b, Abgent, San Diego, CA, USA) antibody was evaluated for its ability to specifically bind FPPS. The antibody was combined with a specific blocking peptide (Abgent) to assess the staining specificity in prostate tissue. Staining was performed with and without blocking peptide.

### Staining protocol

Staining was performed manually using a polyclonal FPPS antibody (center). Sections at 5 µm were deparaffinized through xylene and rehydrated by serial dilutions of ethanol. Antigen retrieval was performed by incubation in a 10 mM citrate buffer. To quench endogenous peroxidase, the sections were incubated in 3% $H_2O_2$. The antibody was used in a dilution of 1:200 and incubated overnight at +4°C. As secondary antibody, we used rabbit IgG (Vectastain kit PK 6200, Vector, Burlingame, CA, USA). For visualization, a DAB Substrate kit (Zytomed, Berlin, Germany) was used. Counterstaining was accomplished by hematoxylin, and slides were mounted. Liver cirrhosis tissue served as positive control, and as negative control, the primary antibody was omitted.

### Analysis

TMA slides were evaluated in a blinded manner by two independent investigators, and divergent results were re-evaluated. Expression of FPPS was quantified according to Remmele and Stegner [11]. This score covers both the percentage of positive cells and the intensity of staining. IRS was calculated as 'percentage of positive cells' (0% = 0; <10% = 1; 10–50% = 2; 51–80% = 3; >80% = 4) × 'staining intensity' (0 to −3) = IRS. As in some specimens, expression analysis was difficult, and additional staining intensities were defined: 0.5 = very low but visible intensity, 0.3 = extreme low but visible intensity. An IRS of 0 was interpreted as no expression. An IRS of $0 > \text{IRS} \leq 3$ was interpreted as low expression, an IRS of $3 < \text{IRS} < 8$ as moderate expression and an IRS $\geq 8$ as strong expression.

### Follow-up

The postoperative clinical course was determined by questionnaires. Following radical prostatectomy patients

had follow-up according to the EAU guidelines. Two consecutive values of PSA >0.2 ng/ml define the international consensus of recurrence [12]. Patients not reaching a nadir of <0.1 ng/ml were excluded from biochemical recurrence analysis. The development of metastases was assessed by imaging.

### Statistical analysis

Results were correlated to pathological and clinical data by Wilcoxon–Kruskal–Wallis and linear regression analyses (JMP 7.0, SAS Inc., Cary, NC, USA). $p$ values <0.05 were considered significant. Kaplan–Meier curves were used to estimate biochemical recurrence-free survival. Kaplan–Meier analysis was performed for patients with no or low FPPS expression (IRS $\leq$ 3) in tumor areas versus patients with moderate and high FPPS expression (IRS > 3). This threshold was selected a priori. To identify risk factors for biochemical recurrence, uni- and multivariate Cox proportional hazard analyses were performed.

## Results

### Expression of FPPS

The antibody showed clear specificity to FPPS in prostate tissue. No staining was observed in the presence of the peptide. PC tissue showed intense cytoplasmatic staining for FPPS (Fig. 1a).

Tissue cores from 16 patients were not evaluable and patients were excluded. Mean IRS of FPPS in PC was 5.7 (95% CI 5.0–6.5) compared to 2.6 (95% CI 2.1–3.0) in benign tissue ($p < 0.0001$). Representative specimens of tumor areas showing strong FPPS staining compared to low expression in normal tissue areas are shown in Fig. 1b.

Mean IRS in Gleason score <7, =7 and >7 was 4.85 (95% CI 3.85–5.84), 6.32 (95% CI 5.13–7.51) and 7.83 (95% CI 3.82–11.83) ($p = 0.03$). Linear regression analysis confirmed a correlation between Gleason score and IRS in cancer tissue ($p = 0.007$). Mean IRS in locally advanced disease (>pT2) was 6.87 (95% CI = 5.57–8.17) versus 5.09 (95% CI 4.22–5.96) in patients with organ-confined disease ($p = 0.035$). Expression of FPPS in areas of benign tissue did not differ significantly in both groups ($p = 0.83$).

No significant correlation was observed between preoperative PSA or lymph node involvement and FPPS in PC tissue ($p = 0.13$ and 0.77).

### Correlation to clinical course

Follow-up data were available from 101 of 115 patients (87.8%). Median follow-up was 74 months (2–95): 28 (24.35%) showed biochemical recurrence, 11 (9.56%) developed metastasis, and 6 (5.22%) died from PC within the observational period. Five-year metastasis-free survival was 93.5%. Five-year cancer-specific survival was 95.7%.

In patients with moderate or strong FPPS expression (IRS > 3), 5-year biochemical recurrence-free survival was 62.9 versus 85.4% in patients with no/low FPPS expression (IRS $\leq$ 3) ($p = 0.01$, Fig. 1c).

As IRS > 3 in cancer areas was a risk factor for biochemical recurrence in univariate analysis, multivariate analysis controlling for 3 additional histopathological risk factors associated with early biochemical recurrence in univariate analysis was done (Table 1). FPPS expression was an independent risk factor for biochemical recurrence ($p = 0.032$).

## Discussion

There is evidence that drugs targeting the mevalonate pathway affect biology and aggressiveness of PC [1, 4]. This indicates that the pathway plays an essential role in the pathophysiology of PC. We investigated the expression of a key enzyme of this pathway, FPPS, in tissue samples of patients with localized PC and correlated its expression with established histopathological parameters and patients' outcome. Our results show an increased expression in PC and a significant correlation with factors indicating aggressive PC. Irrespective of the exact mechanism by which the mevalonate pathway is involved in PC biology, results from numerous studies justify the assumption that this pathway is a promising target for therapy of PC beyond the prevention of PC-related bone disease.

Nitrogen-containing bisphosphonates (N-BPs) are the treatment of choice for bone metastases in PC, breast cancer and multiple myeloma. Whereas in breast cancer phase III trials indicated an anticancer activity of N-BPs including improved outcomes of patients receiving zoledronic acid in addition to antihormonal therapy [13], evidence in PC predominantly comes from preclinical studies. Zoledronic acid in vitro inhibits survival and proliferation of cancer cell lines directly [7, 14]. Combining cytoreductive drugs with zoledronic acid led to an improved efficacy in vitro and in vivo [14, 15]. N-BPs influence tumor cell invasion and angiogenesis in PC [4, 16]. It is assumed that these effects are resulting from the prevention of prenylation of small GTPases. These GTPases, for example, Ras, are among the most frequently mutated oncoproteins in human tumors [17]. For activation of Ras and other G-proteins, prenylation and localization to the inner surface of the cell membrane is required. Subsequently, pathways critical for cell proliferation such as the PI3 kinase/Akt pathway and the Raf/Mak/Erk kinase

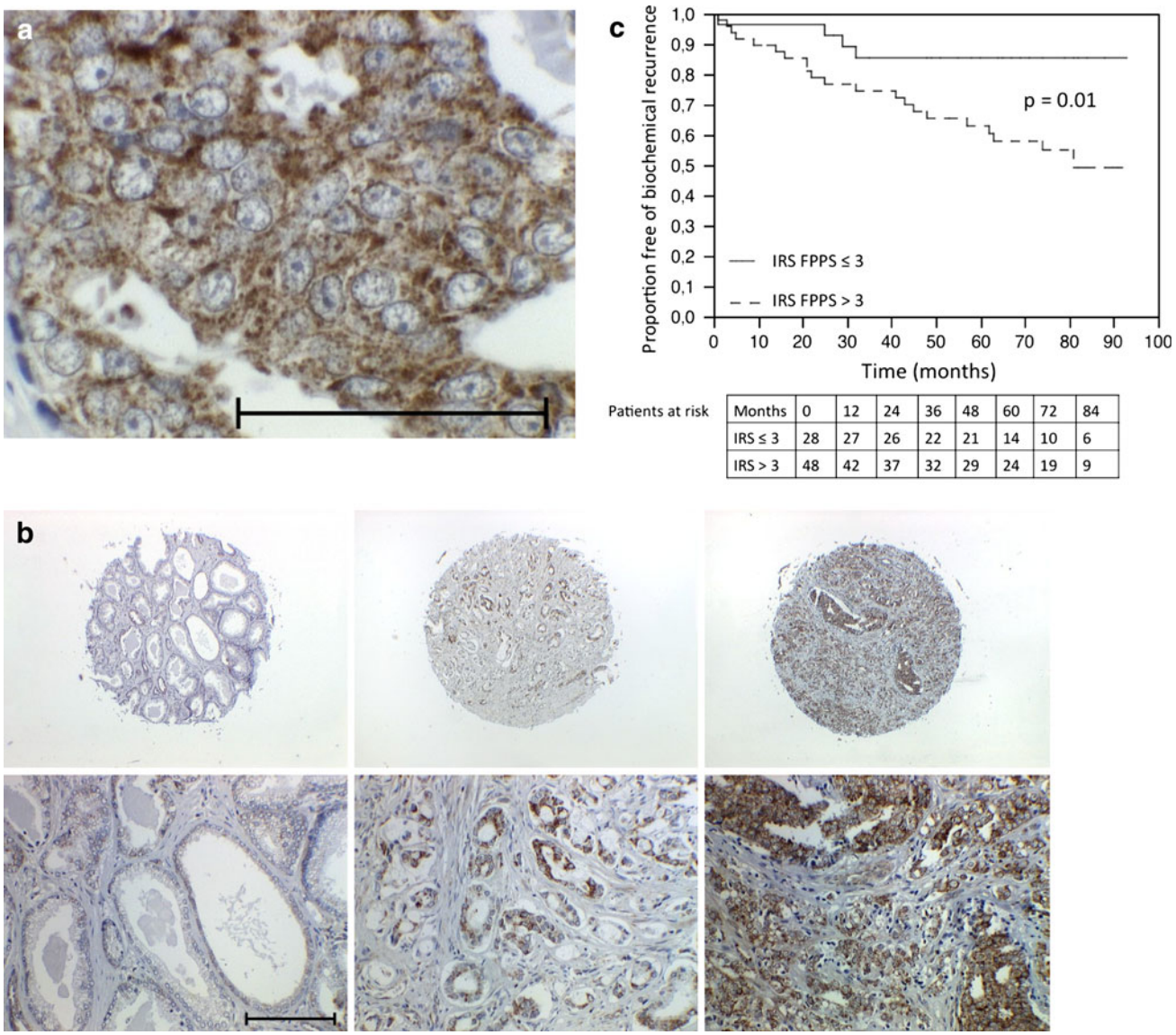


**Fig. 1** Immunohistochemical staining of PC and Kaplan–Meier analysis for biochemical recurrence-free survival. Cytoplasmatic staining (**a**) for FPPS, ×400, *bar* = 50 µm. **b** *Left* normal prostate, *middle*: low-grade PC (Gleason score 6) and *right* aggressive PC (Gleason score 8), IRS were 1, 6 and 12. Magnifications: ×25, *lower row* ×100, *bar* = 100 µm. **c** Kaplan–Meier *plot* for biochemical recurrence-free survival in patients with no/low versus moderate/high FPPS expression in PC tissue ($p = 0.01$)

pathway are activated [17]. N-BP-mediated anti-tumor effects can be prevented or partially reversed by mevalonate pathway intermediates [4].

To date, no evidence from phase III trial exists in PC demonstrating a benefit of N-BPs in the context of anti-tumor activity. Currently, four trials are investigating N-BPs regarding an effect on cancer outcome. The *Stampede*-trial compares androgen suppression alone with androgen suppression in combination with zoledronate, docetaxel, prednisone and celecoxib in patients with advanced or metastatic PC (NCT00268476). The ZEUS (Zometa European Study) trial randomizes patients without bone metastases and risk factors to standard therapy with or without zoledronic acid. Primary endpoint is the proportion of patients, who develop at least one bone metastasis after 48 months of therapy (NCT00294437). The RADAR study is evaluating whether zoledronic acid can prevent bone loss and bone metastases in patients under androgen-deprivation therapy (NCT00005073).

In vitro, N-BPs were used in concentrations far higher than those in the serum [18]. The results of other studies investigating N-BPs with clinically relevant doses show that inhibition of cancer growth can still be achieved [14]. Furthermore, efforts are made to extend plasma half-life and antitumor activity by developing liposome-encapsulated N-BPs [19].

Statins are the second most commonly prescribed drug in the United States [20]. In addition to their cholesterol-

**Table 1** Histopathological risk factors for biochemical recurrence

| Parameter | HR (95% CI) | *p* value |
|---|---|---|
| Univariate analysis | | |
| Gleason score ≤7 versus >7 | 0.376 (0.16–0.81) | 0.01 |
| pR0 versus pR1 | 0.27 (0.10–0.67) | 0.006 |
| pT <3 versus pT ≥3 | 0.24 (0.11–0.51) | 0.0003 |
| FPPS IRS (tumor) ≤3 versus >3 | 0.29 (0.08–0.76) | 0.009 |
| Mutivariate analysis | | |
| Gleason score ≤7 versus >7 | 0.39 (0.13–1.51) | 0.16 |
| pR0 versus pR1 | 0.41 (0.16–1.17) | 0.09 |
| pT <3 versus pT ≥3 | 0.39 (0.16–0.95) | 0.038 |
| FPPS IRS (tumor) ≤3 versus >3 | 0.30 (0.07–0.91) | 0.032 |

Uni- and multivariate Cox proportional hazard analysis for identification of predictors of biochemical recurrence

lowering effects, studies have investigated their role in PC. A possible reduction in PC by statin intake is discussed controversially since studies have shown diverging results: Whereas one study showed a decreased risk of PC [21], other studies have found no link between statins and PC [22] or even an increased risk for stage I PC in patients with statin intake [23]. Others showed that statins do not decrease the overall risk for PC but lead to a reduction in PC progression [8]. A metaanalysis confirmed a significant reduction in advanced PC by statins [24]. Looking at results of our study, this effect might be due to an increased activity of the mevalonate pathway in advanced disease being prevented by drug-related inhibition. Statins significantly reduce the risk for biochemical recurrence after radical prostatectomy [9]. A shorter time to biochemical recurrence in patients with increased FPPS expression might imply involvement of the mevalonate pathway. Preclinical studies have shown that statins can reduce PC growth, induce apoptosis and inhibit angiogenesis [5]. Similar to N-BPs, an effect of statins on the activity of small GTPases and downstream pathways has been demonstrated [1].

Only few studies have focused on the role of FPPS in cancer. Notarnicola et al. [25] observed an increased activity of FPPS in colorectal cancer. Inhibition of FPPS by pamidronate led to a reduction in cell growth. Increased expression of FPPS attenuated paclitaxel-induced apoptotic cell death in human glioblastoma cells [26]. In prostate cells, expression of FPPS is regulated by steroid responsive elements playing an essential role in castration-resistant disease [27]. FPPS has been identified as an important mediator of $\gamma$–$\delta$–T-cell activity being essential for elimination of tumor cells by the immune system [28].

FPPS might be an indicator for increased activity of the mevalonate pathway and downstream molecular targets in PC. A possible antitumor effect of N-BPs and statins could be depending on FPPS. Whereas several markers have been discussed to be surrogates of N-BP efficacy [4], only limited effort has been made to identify markers associated with improved survival in the context of cancer treatment. A case study reported on a complete response to zoledronic acid in urothelial cancer in a patient having an oncogenic Ras mutation [29]. Zoledronic acid might be more effective in patients with increased Ras activity [30].

Zoledronic acid is also used in the adjuvant setting in several cancer types. FPPS could help to identify susceptible patients. PC studies mainly focus on the prevention of bone metastases. Secondary endpoints include progression-free survival helping to reveal a possible antitumor effect.

The main limitation is the relatively low number of patients especially of those with biochemical recurrence. Multivariate analysis could only control for 3 additional parameters which were all histopathological parameters associated with early recurrence in univariate analysis. Other relevant factors (e.g., PSA, race, age) were left out to prevent overfitting. Therefore, we cannot exclude that FPPS expression simply correlates with other risk factors for poor outcome rather than being an independent factor for early biochemical recurrence. As for example Gleason score was not an independent risk factor in our cohort, there is a need for external validation in larger cohorts with higher numbers of events. Multivariate analysis controlling for more factors can be performed to investigate the role of FPPS as an independent risk factor. Our study does not provide a sound explanation of the role of FPPS in PC. Further studies have to be performed to evaluate whether increased FPPS activity is associated with elevated activity of downstream pathways of the mevalonate pathway including PI3K/Akt and MAPK/ERK. Both are related to the aggressiveness of PC and are novel targets for treatment of advanced disease [17].

## Conclusions

This is the first study investigating the expression of FPPS in PC. Although this study does not provide functional analyses, the correlation of FPPS with established risk parameters indicates a potential contribution of the mevalonate pathway to the progression of PC. FPPS expression could be a prognostic indicator for early biochemical recurrence. Further functional analysis is required to explore the role of this pathway in PC and to investigate whether FPPS affects response of PC cells to N-BPs.

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**Conflict of interest** None.

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