# Altered expression of Akt signaling pathway parameters in prostate needle biopsies derived from benign, adjacent and cancerous tissue

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Received September 21, 2009; Accepted December 18, 2009

### DOI: 10.3892/or\_00000758

Abstract. PTEN, p-Akt and p27kip1 are known to be altered in prostate cancer. The aim of the present study was to determine the addition of molecular markers to a classical histopathological approach to enhance the sensitivity in detection of malignant or premalignant lesions within prostatic biopsies. Forty-two fine needle biopsies from malignant, tumor adjacent and benign areas were obtained from 14 patients scheduled for a prostatic biopsy. Biomarker expression was determined by immunohistochemistry and correlated to different localizations. We observed a reduction of Akt signaling proteins in cancer tissue compared to benign controls with significantly lower expression of p27kip1 (P=0.0024), PTEN (P=0.0045) and p-Akt (P=0.028). A pathologist histopathologically classified the tumor adjacent tissue obtained from areas distinctly apart from the primary tumor as benign in all cases. In these regions we observed an intermediate expression of Akt signaling proteins without significant difference in relation to the findings in the malignant samples. The expression of Akt signaling proteins is reduced in prostate cancer compared to normal prostate tissue. The intermediate expression of these proteins in tumor adjacent tissue warrants further investigations into the role of Akt signaling in the carcinogenesis and early detection of prostate cancer. There seems to be a marked difference between the molecular and histopathological characterization of prostate tissue. Molecular markers might further augment the histopathological diagnosis suggesting the need for earlier repeated prostate biopsy in case of microscopic malignancy.

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Key words: prostate cancer, needle biopsies, PTEN, p-Akt, p27kip1

Patterns of diagnosis and treatment of prostate cancer have

Introduction

changed dramatically in the past years. Whereas the histopathological heterogeneity of prostate cancer is well-known (1-3), the molecular mechanisms of prostate carcinogenesis remain poorly understood. Disturbances of proliferation and apoptosis are fundamental events in early carcinogenesis and might be useful in characterizing tissue that is histologically normal but at high risk for neoplastic growth. Genetically altered but phenotypically normal looking cells in the vicinity of a cancer focus are referred to as field effects. Identification of these effects could play an essential role in research and clinical practice. In Western countries millions of prostate biopsies are performed annually with the great majority being negative for cancer. Benign prostatic tissue is sampled very commonly due to the lack of imaging tools that fail to detect the cancerous tissue. Therefore, biomarkers identifying high risk non-cancer tissue in prostate needle biopsies could be very useful for classifying patients with negative biopsies according to their need for close follow-up or early re-biopsy.

This study examined the expression of Akt pathway proteins PTEN, p-Akt and p27<sup>kip1</sup> in benign, adjacent benign and prostate cancer tissue in biopsy cores.

PTEN, p-Akt, p27kip1 are important check points of cell cycle control, tumor growth and differentiation in prostate cancer (4). The PTEN tumor suppressor gene is one of the most frequently deleted genes in various human cancers, including prostate cancer (5,6). The main function of PTEN relies on its phosphatase activity and subsequent antagonism of the PI3K/Akt pathway (7,8). Loss of PTEN function results in accumulation of PIP3 and activation of its downstream effectors, including Akt (9-11). As a serine/threonine protein kinase, Akt functions by phosphorylating key intermediate signaling molecules, leading to an increase in cell metabolism, cell growth and cell survival (12). Furthermore, Akt activation seems to be important for the progression of prostate cancer to an androgen-independent state (13). The p27kip1 protein regulates cell cycle progression from the G1-phase to S-phase by its inhibitory interaction with the cyclin E/cdk2 complex. Loss of p27kip1 expression has been shown to be a negative

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prognostic marker in various carcinomas as well as in prostatic carcinoma (14). Low levels of p27<sup>kip1</sup> may be as much a result of CDKN1B alterations as of (15) PTEN loss, because its function is mediated by the Akt signaling pathway (16).

#### Materials and methods

*Patients*. The study included 14 patients subjected to prostate needle biopsies who had been diagnosed with prostate cancer between 2002 and 2006 at Tübingen University Hospital. The age range was 53-74 (median 64.5) years. The Ethics Committee of the institution approved the study. The proportion of tumor cells within the needle biopsy ranged from <25% to >75%.

Procedures. Representative tissue biopsies from patients with prostate cancer were selected, for this purpose one tumor biopsy cylinder, one adjacent, but benign cylinder and one benign distant biopsy cylinder was chosen for the study. Thus, glass slides contained 42 samples including 14 benign, 14 'adjacent' benign and 14 primary tumor tissue samples. Expression of PTEN, p-Akt and p27kip1 was determined by immunohistochemistry. The biopsy containing slides were deparaffinized, rehydrated and immersed in 3% hydrogen peroxide solution to block endogenous peroxidase activity. Antigen retrieval was accomplished by microwave heating specimens in a 0.01 citrate buffer for 15 min. Biomarker expression was immunohistochemically detected by commercially available antibodies (PTEN and p27kip1 monoclonal mouse, p-Akt polyclonal Cell Signaling Technology, Inc., Beverly, MA, USA). The optimal dilutions were: PTEN and p27<sup>kip1</sup>, 1:200; and p-Akt, 1:150. After 12 h of incubation (PTEN, 2 h) the sections were washed in TBS and incubated with a secondary biotinylated antibody (Vectastatin Elite ABC Kit, Vecto Laboratories, Inc., Burlingame, CA, USA) for 60 min. The DAB system (Vector) was used for visualization according to the manufacturer's instructions. Sections were briefly rinsed in tap water, counterstained with Mayer's hematoxylin solution and then mounted. For negative control, the primary antibody was replaced by non-immune serum. All biopsy staining were assessed by two independent investigators (A.S.M. and J.H.) in a blinded manner so that neither of them knew the origin of the biopsies. The staining reaction was classified according to a semi-quantitative IHC reference scale ranging from 0 to 3 as previously described (17-19).

Statistical analysis. Score values nested into the three respective groups benign, adjacent and tumor showed unequal variance. Score values were therefore dichotomized into high expression with a score >8 and low expression with a score value <9. A global Pearson Chi-square test was used to analyze the overall difference in the frequency of the dichotomized score values between groups. A significant global test with P<0.05 was followed by a post-hoc analysis using the Pearson Chi-square test comparing each group with the other two groups. Post-hoc P-values were corrected for multiple comparisons using the Bonferroni-Holm correction.



Figure 1. (A) Score values for the three different sample types belonging to each patient. Score values belonging to one patient are connected and presented in the same colour. (B) Mosaic plots for the frequency of high [(light grey) score >8] or low [(dark grey) score <9] expression within the three groups. Benign, 0; adjacent, 1 and tumor, 2.

#### Results

Fig. 1A shows the score values for the three different sample types belonging to each patient. Score values belonging to one patient are connected and presented in the same color. The score values nested into groups do not show equal variance. For all three different markers benign tissue samples do not reach score values <9. Fig. 1B shows mosaic plots for the frequency of high (score >8) or low (score <9) expression within the three groups. For p27<sup>kip1</sup> (n=40, x<sup>2</sup>=12.0, P=0.0025), PTEN (n=41, x<sup>2</sup>=11.7, P=0.0029) and p-Akt (n=41, x<sup>2</sup>=7.4, P=0.025) significant trends pointing at decreased frequencies of higher expression values of these tumor suppressors were found in the tumor tissue samples. Post-hoc analysis only showed significant differences for the benign tissue compared to the tumor tissue for p27kip1 (n=28, x2=11.3, P=0.0024), PTEN (n=29,  $x^{2}=10.1$ , P=0.0045) and p-Akt (n=29,  $x^{2}=6.8$ , P=0.028), but no differences for the adjacent group to the benign group for any of the markers.

## Discussion

As early stage prostate cancer is curable a timely diagnosis is essential. Furthermore, accurate detection of prostate cancer is critical for the appropriate management of patients. In most cases, a prostate biopsy is the initial means of making a diagnosis.

A major current problem in prostate cancer is to predict the behaviour of early and potentially localised disease (15). Some cases may remain dormant for many years without progressing, while others will progress rapidly. Therefore it is important to identify the patients who need to be treated and separate them from those who can be managed by active surveillance, for example in elderly or comorbid patients, thus sparing the latter of the adverse consequences of unnecessary treatment (20,21). If tests are to be developed that will allow the prediction of clinical behaviour of patients diagnosed with prostate cancer, for example, following prostate-specific antigen (PSA) screening, it must be possible to perform the test on biological specimens obtained from the patients at the time of diagnosis, which would usually only include trans-rectal ultrasound (TRUS)-guided needle biopsy specimens, blood or urine.

Over the past several years we have appreciated that the utility of the needle cores goes well beyond the presence or absence of cancer. These needle cores also contribute to risk assessment in patients found to have cancer. Accurate grading is important to clinical decision-making. However, a general problem is that the main growth or expression pattern of the tumor tissue may not be present in the needle biopsy, especially in small tumors. This is mostly due to a lack of representative tumor areas in the biopsy, that can be caused by a very heterogeneous and clustered growth of prostate cancer throughout the gland (22).

To gain insight into the molecular mechanisms of carcinogenesis and tumor markers to predict a definitive prognosis and to find cellular functions suitable for therapeutic interference is the focus of many current investigations. Thus, more data are available on Akt expression in prostate cancer. The mechanism by which Akt signaling facilitates tumorigenesis and progression is multifactorial. However, inhibition of apoptosis and facilitation of cell cycle entry appear to be of great importance, as suggested by mounting evidence in prostate cancer and other malignancies. Loss of p27kip1 protein expression in radical prostatectomy specimens has been shown to be an adverse prognostic factor in patients with clinically localized prostate cancer (14,23-27), and in those undergoing salvage prostatectomy after radiation therapy failure (28). In a previous study, we were able to demonstrate altered expression of p27kip1 in histologically benign tissue areas in whole mounted prostate cancer specimens (18). We concluded that, in adjacent benign tissue of whole prostate specimens, p-Akt and p27kip1 act similarly to that in cancerous tissue. These areas are part of the tumor mass, extending the histologically maligned region. In a study from 1999 our group postulated a significant correlation of decreased p27kip1 expression with prostate cancer progression (29). Several studies confirmed these findings (14,30). Because of this, loss of p27kip1 has been suggested as a component of molecular staging (27,31).

Whang *et al* (32) and Latini *et al* (33) postulated that PTEN alterations appear to be a late event, possibly influencing metastatic potential and progression to androgen independence rather than tumorigenesis. Moreover, PTEN mutations are more commonly found in metastatic compared to localized prostate cancers (32,34-40). A role for PTEN mutations in resistance to chemotherapy is also proposed (41). Gene therapy approaches using transfected wild-type PTEN are being developed (42). PTEN clearly plays an important role in the progression of prostate cancer.

Despite the small number of patients in our study, this is the first study on these proteins and a possible alteration in benign tissue in prostate needle biopsies. Further investigation in Akt pathway will show its implication in the prognosis and therapy of prostate cancer and the possible diagnostic use in biopsy specimen. Further plans call for a multivariate model in a larger prospective study set comprised of various biomarkers and clinical variables to predict outcomes following negative biopsies.

## Acknowledgements

Funding: Grant sponsor: Federal Ministry of Education and Research; Grant number: Fö 01KS9602; Grant sponsor: Fortune program; Grant number: F 1481010; Grant sponsor: Biomedical Science Exchange Program (BMEP).

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