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Original contribution

Cellular interactions of the phosphorylated form of AKT in prostate cancer☆

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Summary Phospho-Akt (P-Akt1) promotes proliferation and increased survival in vitro and plays an important role in prostate cancer (PCa) progression as well as the prediction of the probability of recurrence. In this study, the goal was to demonstrate the involvement and impact of P-Akt1 on cellular interactions, biomechanisms, and pathways in PCa. Tissue microarrays from 640 PCa patients were immunostained with various antibodies. Ki-67 was used to measure proliferation index, and terminal deoxynucleotidyl transferase–mediated dUTP biotin nick end labeling was used for apoptotic index. Increased expression of P-Akt1 was associated with an increased proliferation but inversely correlated with apoptotic index. Higher levels of P-Akt1 are associated with both higher levels of cytoplasmic p27 and higher levels of nuclear p27, suggesting an involvement in both cytoplasmic entrapment and phosphorylation of p27. P-Akt1 expression significantly correlated with nuclear and cytoplasmic staining of FHKR and GSK. The strongest correlations were found with the P- forms of both, suggesting enzyme kinetics in the latter. Here, phosphorylation is the principal method of FHKR and GSK inactivation. P-Akt1 correlated with nuclear transcription factor kappa B, suggesting a role in the inhibition through phosphorylation of nuclear transcription factor kappa B. The results of the current study are unique because of the scope of the markers and the size of the population used. In vitro– and in vivo–derived information of P-Akt1 and its downstream effectors demonstrates significant involvement in PCa. Our data suggest that PCa uses multiple mechanisms to regulate this pathway and substantiate the concept of redundancy in cancer pathway regulation. Consequently, new hypothesis-driven studies can be derived from this information.

1. Introduction

There is increasing evidence showing that phospho-Akt (P-Akt1) could play a key role in prostate cancer (PCa) progression. Akt, also known as *protein kinase B*, consists of a family of highly conserved serine/threonine kinases including Akt1, Akt2, and Akt3. P-Akt1 has been involved in many human cancers. Amplification of P-Akt1 has been

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identified, but not modified or mutated genes. P-Akt1 seems to be involved in the progression of tumors rather than in their initiation. Transgenic mice with targeted overexpression of P-Akt1 in the breast tissues showed developmental abnormalities but no tumors [1]. Other studies suggest that interaction with other pathways is needed for metastatic progression.

Three major domains make up Akt: an N-terminal pleckstrin homology domain, a kinase catalytic domain, and a hydrophobic C-terminal. The second carries a phosphorylation site that is necessary for Akt activation (T308 for Akt-1). A second phosphorylation site is present in the C-terminal (s-473 for Akt-1) and is necessary for maximal activation of the molecule [2].

The regulatory role of Akt in the biology of PCa cells is very complex, and it is usually activated by phosphorylation to P-Akt. In this setting, P-Akt1 is truly the orchestrator of numerous prosurvival and antiapoptotic pathways. It affects survival genes positively, whereas it downregulates the antiapoptotic pathways. Among the most important are the Forkhead family, glycogen synthethase kinase (GSK), nuclear transcription factor kappa B (NFκB), and the cyclin kinases p21 and p27.

Upstream activation of Akt is regulated by phosphoinositide 3-kinase (PI3K), which in turn is activated by tyrosine kinase and G-coupled receptors. Following receptor activation, phosphoinositide 3-kinase phosphorylates phophatidylinosistil-4,5-biphosphate (PIP_2) to PIP_3. This step is negatively regulated by phosphatases such as PTEN [3] and SHIP. The main substrate for PTEN is PIP_3, which is dephosphorylated and therefore inactivated. However, the loss of PTEN is thought to be followed by a strong activation of Akt [4]. On the other hand, PIP_3 recruits Akt to the cytoplasmic membrane, permits conformational alteration, and subsequently promotes phosphorylation by phosphoinositide-dependent kinase PDK-1, as it interacts with Akt and PDK-2 becomes then an S473 kinase. Furthermore, integrin-linked kinase can also be involved in facilitating phosphorylation of S473 [5]. PTEN and cell location have both been shown to be related to the functional regulation of the integrin-linked kinase and Akt. Nonetheless, nuclear translocation of Akt in cells treated with growth factors is the result of sustained activation of Akt and seems to be needed for the modification of other nuclear factors such as FKHR [6].

Increased P-Akt1 activity has been associated with PCa progression and hormone independence in prostate cell lines [7]. More interestingly, P-Akt1 expression may have clinical implications in PCa because increased P-Akt1 expression has been associated with higher Gleason grades 8-10 [8-10].

2. Materials and methods

2.1. Cohort enrollment and follow-up

More than 6400 patients underwent radical prostatectomies at one of the Baylor College of Medicine–affiliated institutions and willingly provided tissues. Of these, 1291 were operated on by a single surgeon between 1983 and 1998, without any previous form of adjuvant therapy such as radiation or hormonal therapy. Entry criteria for this retrospective cohort study to create a radical prostatectomy tissue array included the following: (1) no preoperative treatment, (2) operated on by a single surgeon between 1983 and 1998, (3) radical prostatectomy specimen in the tissue bank, (4) PCa present in the surgical specimen and sufficiently large to be cored for microarrays. A total of 640 patients fulfilled the above-mentioned criteria and were cored to produce a large outcomes array.

2.2. Radical prostatectomy specimens

Radical prostatectomy specimens from these patients were processed using whole-mount slides according to procedures previously described [11]. After surgery, the prostate specimens were sliced into 5 mm–thick tissue whole mounts. The tissue slices were then fixed in 10% neutral buffered formalin and embedded in paraffin according to a routine procedure. A single pathologist performed the pathologic analysis including pathologic stage, surgical margins, capsular penetration, seminal vesicle invasion, biopsy and prostatectomy primary and secondary Gleason grades, lymph node status, tumor volume, and geographic location. The clinical and pathologic data of patients who fulfilled the entry criteria were available for analysis in the Baylor Prostate Specialized Programs of Research Excellence (SPORE) data bank. The clinical follow-up data include prostate-specific antigen (PSA) *recurrence* (defined as PSA >0.4 ng or 2 consecutive rises), clinical metastasis, and death.

2.3. Tissue microarray

Slides from all 640 radical prostatectomy specimens were reviewed and mapped. The tissue microarrays were built using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). The *index tumor*, defined as the largest focus and/or highest Gleason score tumor, was identified and circled on the slide. Areas of normal peripheral zone away from the tumor were also circled as well as areas of Benign Prostatic Hyperplasia (BPH). Triplicate 0.6-mm cores were obtained from the circled areas of tumor, normal peripheral zone, and Benign Prostatic Hyperplasia (BPH) and transferred on to a recipient paraffin block. Sausage internal controls, which included up to 10 different types of tissues within each 0.6-mm control core, were also placed with the standard controls. A database was built for every block produced, including the coordinates of each core and the area and case of origin. The final tissue array set consisted of 15 blocks with 9 cores for every one of the 640 patients for a total of approximately 6000 cores.

A second microarray set was built using all the metastatic tissues (met array) from the Baylor prostate Specialized