

Prostatic acid phosphatase in breast cyst fluid

Hakan ERBAS *PhD*, Oya ERTEN *MD* and Mehmet Emin IRFANOGLU* *MD*

*Departments of Biochemistry and *Surgery, Faculty of Medicine, University of Trakya, TURKEY*

Abstract

Prostatic Acid Phosphatase (PAP) is mostly found in the epithelial cells and secretions of the prostate gland. It has also been found to be present in several tissues and biological fluid. Gross cystic breast disease is the commonest benign breast condition and several studies have shown that women with palpable breast cysts may have a higher risk of developing breast cancer. There are two types of breast cyst and women with apocrine breast cyst may have a higher risk of developing breast cancer than women with breast cysts lined by flattened epithelium. The growth inhibitory effect of transforming growth factor beta (TGF- β) on epithelial cells suggests a potential protective role in breast cancer. TGF- β is secreted as a high molecular weight complex in a biologically inactive or latent form and activation of TGF- β is necessary for the exertion of its effects on target cells. Prostate specific antigen (PSA) has been found in breast cyst fluid (BCF) and it may have a protective effect on the development of several carcinomas by activating TGF- β . As a similar molecule to PSA, PAP may also involve in this mechanism. We investigated the presence of PAP in two groups of BCF using an ELISA kit. PAP was found to be present in BCF but there was not a statistically significant difference between the two cyst groups. The presence of PAP in BCF may suggest its possible role in the development of breast cancer from cystic breast diseases. A possible role of PAP on TGF- β activation needs further investigation.

Key words: Prostatic acid phosphatase, breast cyst fluid, breast cancer, transforming growth factor beta.

INTRODUCTION

Human Prostatic acid phosphatase (PAP) is a major phosphatase and a differentiation marker in normal, well-differentiated prostate epithelial cells.¹ PAP, a 100-kDa glycoprotein containing two subunits of approximately 50 kDa each, was first described in 1936² and has served as a tumour marker of prostate cancer. PAP levels in serum, caused by PAP-secreting tumour cells, are elevated in patients at all stages of prostatic carcinoma³ and it was suggested to be a more accurate indicator of micrometastatic disease compared to the Gleason score and prostate specific antigen (PSA) level.⁴ In addition, PAP is a promising target molecule in specific immunotherapy for metastatic androgen-independent prostate cancer.⁵

The tissue distribution of PAP has been believed to be restricted to the prostate and prostate carcinoma.^{6,7} However, there were several reports showing that PAP is expressed in tissues other than the prostate and prostate cancer.⁸⁻¹² It has been reported to be

immunohistologically positive in neuroendocrine tumours in the pancreas,¹³ small intestine and pancreas endocrine tumours,¹⁴ hindgut-origin tumours,⁹ pancreatic islet cell carcinoma¹⁵ and adenocarcinomas of the urinary bladder.¹⁶ It has also been suggested to be a tumour marker of intravascular large B-cell lymphomas¹⁷ and found to be expressed in oesophageal and lung squamous carcinoma cells.¹⁸ Recently, PAP was revealed to be expressed in different types of adenocarcinomas, including colon, gastric and breast cancer.¹⁹

Gross cystic breast disease is the commonest benign breast condition affecting 1 in 20 women in the western world. There are two groups of breast cysts: lined either by metaplastic epithelium (intracystic Na/K less than 3, type I) or flattened epithelium (intracystic Na/K greater than 3, type II).²⁰ Patients with type I cysts are more likely to develop further cysts than women with type II cysts and patients who develop large numbers of cysts almost always have type I cysts.²¹ Several large studies have shown the risk of breast cancer in women with gross cystic

disease to be 1.7 to 7.5 times higher and women with apocrine breast cysts may have a higher risk of developing breast cancer than women who have breast cysts lined by flattened epithelium.²²⁻²⁴ Histological risk factors for breast cancer have been also reported to be more common with type I cysts.²²

Transforming growth factor-beta (TGF- β) family comprises a superfamily of ligands that includes the TGF β s, activins, Mullerian inhibiting substance and bone morphogenic proteins that are potent regulators of cell growth and development.²⁵ TGF- β has been isolated from a variety of tissues and has a broad spectrum of effects on many cell types. TGF- β stimulates the growth of cells of mesenchymal origin²⁶ and inhibits the growth of epithelial, endothelial, fibroblast, neuronal, lymphoid and hematopoietic cells as well as normal cells.²⁵

Although, five different members of the TGF- β subfamily have been identified to date, three of these isoforms, TGF- β_1 , TGF- β_2 and TGF- β_3 , are present in mammals, including humans. Their amino acid sequences ranges from 64-82%. The TGF- β dimers consist of two chains of the same type yielding the homodimers.^{25,27} After synthesis, two chains of pro-TGF- β associate to form a disulfide-bonded dimer. Therefore, the mature TGF- β needs to be released from the latent complex in an event called activation.²⁸ TGF- β can be activated *in vitro* by multiple mechanisms, including proteolysis, enzymatic deglycosylation and acid treatment.²⁸ However, the *in vivo* mechanism of this activation is still mainly unknown.

The previous finding of PSA in breast cancer²⁹ and breast cyst fluid (BCF)³⁰ and PAP in different types of malignancies, particularly breast cancer¹⁹ suggests that PAP may also be present in breast cyst fluid. Therefore, the aim of this study was to investigate the presence of PAP in the two groups of breast cysts.

MATERIALS AND METHODS

Patient samples

Breast cyst fluid was obtained by fine needle aspiration from 32 women (19 apocrine and 13 flattened cysts) attending the Breast Clinic in the Department of General Surgery. Samples were centrifuged for 20 minutes at 1000g and the supernatant stored at -70°C until assayed.

Measurement of Na and K

Intracystic Na and K concentrations were

measured by an auto analyser (Beckman Coulter).

Determination of prostatic acid phosphatase activities

Prostatic acid phosphatase activities were determined using an ELISA kit which provides an enzyme immunoassay for the specific recognition of acid phosphatase 2 (DRG International, Inc., USA). It is a solid phase enzyme-linked immunosorbent assay.

Statistics

The results were expressed as median \pm SD. The Mann-Whitney U test was used for statistical analysis of the two groups. Spearman Correlation Coefficient analysis was performed to test the possible relations between prostatic acid phosphatase and Na/K ratios and $p < 0.05$ was considered statistically significant.

RESULTS

Although PAP concentrations were slightly higher in the low electrolyte ratio group compared with the high electrolyte ratio group (Table 1, Figure 1), this difference was not statistically significant ($p > 0.05$).

There was also no statistically significant correlation between breast cyst fluid PAP levels and Na/K ratios ($p > 0.05$).

DISCUSSION

Relatively little is known about the pathophysiology and pathogenesis of gross cystic disease of breast. In an attempt to elucidate the endocrinology of cystic breast disease, many investigators have measured different constituents in breast cyst fluid. Higher intracystic concentrations of certain mitogenic polypeptides, such as transforming growth factor α ,³¹ epidermal growth factor and gastrin releasing peptide³² and sex hormones, in particular estradiol,³³ were present in the low electrolyte ratio group than in the high electrolyte ratio group. This may provide an explanation for the higher risk of breast cancer which has been observed in the low electrolyte ratio group in view of the roles which these substances may have in mammary carcinogenesis.³⁴ In human breast, a preponderance of growth stimulation over growth inhibition may lead to cellular over-production and, possibly, malignant transformation. However, this complex mechanism is still mainly unknown.

TABLE 1. Intracystic concentrations of prostatic acid phosphatase in the two cyst sub-groups.

	Na/K < 3 (n = 19)	Na/K > 3 (n = 13)	p
	median±SD	median ±SD	
PAP (ng/ml)	0.23±0.15	0.05±0.10	0.448 (NS)

To date there has not been very much attention paid to the presence of substances in breast cyst fluid which exert growth inhibitory effects on mammary epithelial cells. One of those substances could be TGF- β_2 which was found to be present in significantly higher levels in the high electrolyte ratio group which is associated with a lower risk of breast carcinoma.³¹ Transforming growth factor beta (TGF- β) is involved in the regulation of cell growth and function, particularly during development and repair. Its growth inhibitory effect on epithelial cells suggests a potential protective role in breast cancer.³¹ TGF- β is secreted as a high molecular weight complex in a biologically inactive or latent form and activation of TGF- β is necessary for the exertion of its effects on target cells.

The activation of TGF- β in the breast could be a key point of this complex mechanism. TGF- β activation was first described by Lawrence *et al.* in cultured cells which contained TGF- β activity after treatment with acid.³⁵ It has also been shown that alkalinisation, exposure to urea or guanidine hydrochloride and heating at 100 °C for 3 minutes would similarly afford

this activation *in vitro*.³⁶ Enzymatically, some proteases such as plasmin, cathepsin D, calphain and Kato III cells, glycosidases, integrin $\alpha_v\beta_6$ and thrombospondin have also shown to have some ability on the TGF- β activation *in vivo*.³⁷⁻⁴²

In addition to these proteases, in a previous study, we have found that PSA, a serine protease, is also present in breast cyst fluid.³⁰ PSA has been also shown to be present in about 30% of breast cancers²⁹ and has been suggested to be a favourable prognostic indicator for women with breast cancer.⁴³ We could not find any statistical difference in PSA concentrations between the two cyst groups, like PAP levels in this study. Recently, we have shown that plasmin was also present in BCF and its concentrations are significantly higher in type II (flattened) cysts (paper submitted for publication)

It is clear that *in vivo* TGF- β activation is a complex mechanism which may involve several substances. Proteases may play an important role in the activation of TGF- β . In breast cyst fluid, the finding of several different proteases like cathepsin D, thrombospondin, PSA, plasmin and PAP in this study may support this idea although, it has not been shown to activate TGF- β yet. However, it is an enzyme, like PSA and in similar mechanism it may connect to TGF- β activation. Proteases, including PAP may be involved in different stages of this activation process.

In conclusion, there was no statistically significant difference in PAP levels between the two cyst groups. PAP has been shown to be present in different cancer types, in particular breast cancer. The presence of PAP in BCF may also suggest its possible role in the development of breast cancer from cystic breast diseases. One of the possible mechanisms of PAP action may be through the activation of TGF- β in the similar manner of PSA. Cathepsin D, PSA, thrombospondin, plasmin and PAP have been found to be present in BCF and together, they may help to cleave TGF- β from its latent proteins. This complex mechanism including the exact role of PAP in TGF- β activation needs to be elucidated.

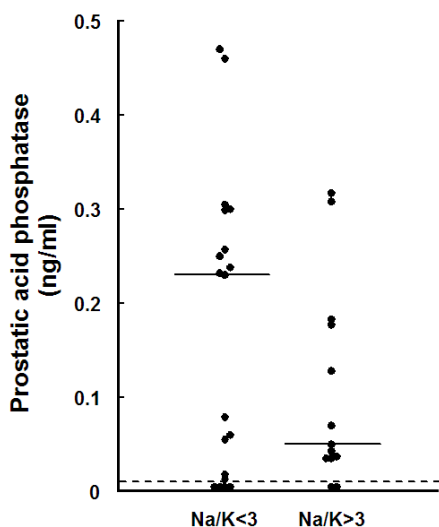


FIG. 1. PAP levels in two groups of breast cyst. Continuous line represents the median, dashed line represents the detection limit.

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