Urol Res (1990) 18:87–92

Urological Research


*Invited review*

# Placental-like alkaline phosphatase in seminoma

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Accepted: April 30, 1989

**Summary.** Tumor marker identification in testicular cancer has contributed to early detection and monitoring of non-seminomatous disease. A placental alkaline phosphatase-like (PLAP-like) enzyme derived from seminomas has recently been focused upon as a possible marker for this disease. The biochemistry of the PLAP-like enzyme is reviewed, as well as its occurrence in tissue and sera from healthy persons and patients with testicular cancer.

**Key words:** Placental alkaline phosphatase – Seminoma – Testicular tumors

## Human alkaline phosphatases

The abundance in nature of alkaline phosphatases indicates an involvement in fundamental biochemical processes. Phosphatases hydrolyse phosphoric esters that occur as intermediary metabolites in all cells. Specific phosphatases are those that hydrolyse esters with the phosphoric acid bound to defined organic components. The nonspecific phosphatases are classified as alkaline or acid, depending on their pH optimum. Alkaline phosphatases hydrolyse a wide range of phosphatase esters at pH 9–10. The enzymes are integrated glycoproteins in membranes of many cells, including gut, neutrophil leukocytes, osteoblasts, liver and blood vessel endothelium. The enzymes are active in a dimeric state and dependent on divalent cations. They may be secreted from the cell membrane and detected in the circulation of healthy individuals. Human alkaline phosphatase (ALP) EC 3.1.3.1. comprises three genetically different isozymes: tissue unspecific or liver alkaline phosphatase (AP or LAP), intestinal alkaline phosphatase (IAP) and placental alkaline phosphatase (Plap) [8, 14, 56]. The proteins of these three isozymes are encoded by multilocus enzyme genes [52], which are considered to be descendents of a common ancestral gene [16]. Mutation or further duplication might then explain the occurrence of multiple enzymes in the system [36, 52, 55]. Placental alkaline phosphatase (PLAP, EC 3.1.3.1.) is a membrane-bound enzyme of 120 kD, normally synthesized by placental syncytiotrophoblasts and released into maternal circulation after the 12th week of pregnancy [13]. PLAP is extremely heat stable, resisting heat treatment up to 65 °C. Furthermore, PLAP is distinguished from AP and IAP by electrophoretic mobility, immunocatalytic reactions and inhibition by certain amino acids. Inhibition by several tripeptides is a characteristic of the placental enzyme, while the PLAP-like enzyme in normal testis or in seminomas is inhibited instead by L-leucine [26, 37]. PLAP shows a unique degree of allelic polymorphism, being the most polymorphic enzyme in man. Three common alleles and several rare alleles have been described at the placental gene locus [13, 36]. Recently, cDNAs encoding all three isozymes have been cloned and sequenced [17, 18, 39, 65], revealing 52% homology between AP and PLAP molecules [65] and 86% homology between IAP and PLAP [18] at the amino acid level. The PLAP protein precursor consists of 535 amino acids which, after cleavage of the signal peptide, gives a mature protein of 513 amino acids. The enzyme-active amino acid sequence Asp-Ser-Gly of PLAP is identical to that of AP. The C-terminal end of the molecule contains hydrophobic amino acids and may represent the membrane anchoring domain. A seminoma-derived PLAP related gene was recently cloned and found to have a 98% homology with PLAP [40]. A peptide representative of a non-homologous region induced an immunoreactivity that distinguishes the PLAP-like seminoma enzyme from PLAP.

Tumor associated PLAP was first demonstrated in serum of a patient with oat cell carcinoma of the lung [12] and was named Regan isoenzyme after the patient. The Regan isoenzyme was found to be identical to PLAP in terms of amino acid inhibition, heat stability and electrophoretic migration. Another type of tumor associated PLAP was reported in 1970 [43] and called the Nagao isoenzyme. This enzyme could be distinguished from the Regan isoenzyme as well as from PLAP with respect to amino acid inhibition. It was therefore regarded as a PLAP-like enzyme. This detection of reexpressed placen-

**Table 1.** Incidence of elevation of serum PLAP levels in patients with testicular tumor

| Author | Year [reference] | Antibody | Method | Seminoma | | | | NSGCT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | active | (%) | NED | (%) | active | (%) | NED | (%) |
| Wahren B et al. | 1979 [63] | poly | RIA | 10/19 | 53 | 7/26 | 27 | 12/20 | 60 | | |
| Lange PH et al. | 1982 [30] | poly | ELISA | 16/28 | 57 | 0/33 | 0 | 4/22 | 18 | | |
| Javadpour N et al. | 1983 [23] | poly | ELISA | 12/32 | 40 | 6/49 | 12 | | | | |
| Jeppson A et al. | 1983 [24] | poly | RIA | 9/21 | 43 | 9/68 | 13 | | | | |
| Dass S et al. | 1984 [ 7] | poly | RIA | 31/51 | 61 | | | 14/57 | 25 | | |
| | | | | 22/33[a] | 67 | | | | | | |
| Nustad K et al. | 1984 [46] | poly | RIA | 149/413 | 36 | | | 55/376 | 15 | | |
| Cooper EH et al. | 1985 [ 6] | mab | ELISA | 25/41 | 61 | 2/11 | 18 | 1/10 | 10 | 2/18 | 11 |
| Epenetos AA et al. | 1985 [10] | mab | ELISA | 11/11 | 100 | 10/70[b] | 14 | | | 5/42 | 12 |
| | | | | 6/6[a] | 100 | | | | | | |
| Horwich A et al. | 1985 [20] | mab | ELISA | 15/16 | 94 | 16/46[b] | 35 | | | | |
| Pledger DR et al. | 1985 [49] | poly | ELISA | 4/7 | 57 | | | | | | |
| Tucker DF et al. | 1985 [59] | mab | ELISA | 14/16 | 88 | | | 7/21 | 33 | | |
| | | | | 7/13[a] | 54 | | | | | | |
| Wahren B et al. | 1986 [64] | mab | ELISA | 46/73 | 63 | 3/68[b] | 4 | 2/15 | 13 | | |
| | | | | 5/11[a] | 45 | | | | | | |
| Yamamoto H et al. | 1988 [68] | mab | ELISA | 18/21 | 86 | | | 3/20 | 15 | | |
| | | | | 3/6[a] | 50 | | | | | | |

[a] mixed tumors containing seminoma component
[b] including patients with smoking habits

tal and fetal genes during malignancy founded the concept of oncodevelopmental biology. Elevations of serum PLAP or PLAP-like enzyme levels have been reported in a variety of malignancies, including those from the prostate: 18% [53], stomach: 36% [42], pancreas: 27–30% [15, 42], colon: 10–54% [15, 42, 51], breast: 5–23% [42, 57, 61, 62], lung: 9–40% [15, 42, 50, 51, 61], uterus: 5–68% [15, 27, 33, 57, 61], ovary: 35–50% [7, 15, 33, 42, 45, 50, 57] and testis (see Table 1). The frequency of elevations in these malignancies differs considerably between investigations and relatively high frequencies have been found repeatedly only in ovarian and testicular cancers.

Seminomas make up approximately 40% of all testicular germ cell tumors. Although the survival rate for patients with seminoma is high, the course is fatal in around 10%. Management of the disease would be facilitated if a biochemical marker were available for monitoring the disease. It is well known that alphafetoprotein (AFP) and human chorionic gonadotropin (HCG) are invaluable in the monitoring of non-seminomatous germ cell tumors (NSGCT). Elevation of the two markers has been found in patients with seminoma, but the incidence was only 10–20% [22, 68]. Moreover, it is believed that elevation of AFP indicates the presence of non-seminomatous components [30]. Thus, no available marker had been established for seminoma when Wahren et al. presented the potential of PLAP in 1979 [63]. Thereafter, several investigations were published on PLAP in seminoma tissue as well as in serum from patients with seminoma. In this article we review those reports and summarize the enzyme's biological and immunohistochemical characteristics.

## AP and PLAP-like molecules in normal testicular and seminoma tissues

The existence of trace amounts of PLAP in normal testis was reported by Chang et al. [5]. The thermostability and enzyme inhibition properties suggested that this was the D-variant of PLAP. The finding in normal testis was confirmed immunologically but the characteristics differed from the D-variant of PLAP as regards reactivity with MAbs [35, 37]. On the basis of the reactivity with a monoclonal antibody (MAb) and enzyme inhibitors, Millan et al. [37] proposed an expression of a new locus of ALP in normal testis.

PLAP in seminoma tissues was first demonstrated by Wahren et al. in 1979 [63]. Several studies showing very high amounts of PLAP or PLAP-like enzyme in seminoma tissues have since been presented [19, 26, 67]. It has been demonstrated that PLAP-like enzyme in seminomas conforms to the testicular PLAP-like patterns of reactivity with MAbs [26, 38, 67]. We recently demonstrated similar characteristics of the carbohydrate moiety of PLAP-like enzyme in seminomas and in normal testes [28]. Measurement of each of the three major alkaline phosphatase isozymes [19] served to identify all phosphatase isozymes in the normal testis. Another major observation was that all the investigated tumors demonstrated highly increased levels of all three isozymes, not just PLAP. This indicates that the entire genome coding for alkaline phosphatases is activated in seminomas. The relative increase for AP is the same as for PLAP. This observation may have physiological implications and indicates that the catalytic properties of the enzymes might be of importance for tumor progression [8].

**Table 2.** Immunohistochemical studies of PLAP in germ cell tumors

| Author | Year [reference] | Antibody | Frequency of positive tumors | | | | | | Normal testis |
|---|---|---|---|---|---|---|---|---|---|
| | | | Seminoma | | NSGCT | | CIS | | |
| | | | (%) | | (%) | | (%) | | |
| Wahren B et al. | 1979 [63] | poly | 3/4 | 75 | 1/5 | 20 | | | – |
| Uchida T et al. | 1981 [60] | poly | 8/9 | 89 | 1/9 | 11 | | | – |
| Paiva J et al. | 1983 [47] | mabs | 6/7 | 86 | 7/7 | 100 | | | + |
| Epenetos AA et al. | 1984 [ 9] | mab | 7/7 | 100 | 7/7 | 100 | | | – |
| Jacobsen GK et al. | 1984 [22] | poly | 18/19 | 95 | 7/14 | 50 | 20/24 | 83 | – |
| Manivel JC et al. | 1987 [32] | poly | 55/56 | 98 | 58/79 | 73 | 52/53 | 98 | – |
| Hustin J et al. | 1987 [21] | poly | 14/15 | 93 | 10/18 | 56 | 17/18 | 94 | – |
| Bartkova J et al. | 1987 [ 2] | mab | 16/16 | 100 | 12/14 | 86 | | | – |
| Wick MR et al. | 1987 [66] | poly | 19/19 | 100 | 18/18 | 100 | 21/30 | 70 | NT |
| Brehmer E et al. | 1988 [ 4] | mabs | 12/12 | 100 | | | | | – |

NT = not tested

## Immunohistological studies

Reports on the immunohistological localization of PLAP-like enzyme in testicular tumors are listed in Table 2. The frequency of PLAP staining was very high in seminoma tissue (75–100%) regardless of the type of antibody used. The staining reaction product was mainly located to the cell membrane and occasionally in the cytoplasm, too [4, 22, 32, 60, 66]. The distribution and intensity of staining for PLAP varied between different tumors as well as between different areas of the same tumor [21, 22, 60]. Different patterns of reactivity with six MAbs among seminomas were demonstrated [47]. These observations imply heterogeneity both of the quantity of enzyme in each tumor cell and of the enzyme itself from an immunohistochemical point of view.

Positive reactivity was sometimes noted in NSGCT, although the frequency was usually lower (Table 2). Embryonal carcinoma was more likely to be positive for PLAP staining than teratoma [21, 22, 60]. This variation might be explained by different reagents and, perhaps more importantly, by varying histopathology of the tumors in the NSGCT group.

PLAP staining also occurs in carcinoma-in-situ (CIS) of the testis. The positivity in CIS were reported to be very frequent (70–98%). The germ cell elements in gonadoblastomas were also reported to be positive for PLAP [32]. Spermatocytic seminoma, which is believed to be a more differentiated type of seminoma, was shown to be completely negative for PLAP staining [21].

In most investigations, normal testicular tissue showed no staining for PLAP, probably because the amount of antigen is very small compared to tumor tissue [4, 19]. One author reported that in the normal testis, cells along the basement membrane of the seminiferous tubules showed more PLAP activity than centrally located cells [47]. Definite staining was present in the primitive germ cells of the embryonic gonads [21]. Taking these observations together, the occurrence of PLAP appears to be related to a particular stage of germ cell differentiation. Testicular tumor pathology is often difficult and the totipotentiality of germ cells in developing into different phenotypes has fascinated pathologists [3, 41]. The presence of large amounts of PLAP-like enzyme in seminomatous tissues, which have determinants in common with the placental enzyme but are also clearly different, may provide a tool for studying the tissue of origin and evolution of seminomas.

## Heterogeneity of seminoma-derived PLAP

The PLAP-like enzyme in normal testis and seminomas has been shown to be heterogeneous in several respects. Several unique phenotypes of the PLAP-like enzyme have been distinguished. Using MAb probes, a unique phenotypic polymorphism of PLAP-like enzyme in normal testis was clearly differentiated from the well-described allelic variation of PLAP [38]. Antigenic heterogeneity, on the basis of reactivity with MAbs, was demonstrated not only in the enzyme extracted from seminoma tissues [26] but also in the circulating enzyme in seminoma sera [64]. This should be remembered when serum levels of the enzyme are evaluated with a MAb. We have shown that a combination of MAbs was able to increase the frequency of positive seminoma serum samples [64]. The variation of reactivity with different MAbs in the immunohistochemical staining of seminomas [47] is also an expression of the enzyme's antigenic variation between individuals. It is likely that post-translational modifications of the proteins add to the phenotypic diversity. Thus, increased glycosylation and perhaps blocking of antigenic sites could explain the shift in the PLAP-like phenotype observed in patients during follow-up [64].

Sera from seminoma patients assayed by catalytic and immunocatalytic assays (MICA) show divergent PLAP contents, the MICA resulting in two-fold higher values than the catalytic assay. Similar results were found in seminoma tissues. Pregnancy sera assayed in the same way give almost identical values with the MICA and the

catalytic assay. Since the catalytic assay includes a heat inactivation step and the MICA does not, it is concluded that the tumor associated PLAP-like form of the enzyme is partially heat sensitive compared with the pregnancy related PLAP [19]. The existence of a population of tumor derived PLAP-like enzyme that is partially heat sensitive may indicate a loss of information when catalytic techniques are used for detection.

Starch gel electrophoresis demonstrated a considerable heterogeneity of the seminoma enzyme within and between samples. Broad bands were seen, as well as electrophoretic patterns that diverged from the common PLAP phenotypes [19]. Isoelectric focusing revealed complex patterns of isoelectric point distributions in both catalytic and immunological activities. The heterogeneity seen after isoelectric focusing was shown to be due in part to a structural change of the carbohydrate moiety and to variation in hydrophobicity of the molecule [29]. The findings indicate both microheterogeneities within each examined tumor and polymorphism between the different tumors.

### Serum PLAP levels in patients with testicular tumors

In non-pregnant healthy persons, serum levels of PLAP are usually very low and significantly exceeded by the serum levels of tissue unspecific (liver, bone, kidney) and intestinal alkaline phosphatases [8]. The PLAP isozyme is detectable in normal human sera during pregnancy and in certain malignant conditions; identification is simple thanks to highly sensitive assays [19, 64]. The other two isozymes, the tissue unspecific (liver, bone, kidney) and the intestinal alkaline phosphatases, have not yet been studied thoroughly in sera of testis cancer patients but several reports have appeared on the PLAP-like enzyme (Table 1). The frequency of elevation was 36–100% in active seminoma, 10–60% in NSGCT. The elevation is rather moderate compared, for instance, with HCG. Assays using MAbs as reagents seem to give higher positive rates than the use of polyclonal sera, probably due to better sensitivity and specificity. The relationship between tumor burden (stage) and a positive serum value is not particularly firm [7, 24, 30, 68]. This favors the theory that seminomas of a particular differentiation produce (or secrete) the enzyme. In one report [46], however, the positive rate in seminoma was 30% in stage I versus 59% in stages II and III.

We succeeded in typing the serum PLAP-like enzyme from its reactivity with MAbs in 77% of patients with pure seminoma [64]. Type I enzyme in serum was found in over half (55%), which agrees with the finding that most seminoma tissues contain type I [26]. The second most common seminoma PLAP phenotype – and the most common in females – is type II; this was found in 15% of the patients.

The availability of sensitive markers, such as AFP and HCG for NSGCT, has led to the successful adoption of a surveillance policy following orchiectomy for clinical stage I disease [48]. In an analogous situation for seminoma patients we have shown that a measurement of PLAP after orchiectomy but before radiation was indeed relevant when assessing the precise stage of the disease [64, 68]. In patients with localized seminoma and elevated PLAP before orchiectomy, some still had a positive PLAP level after orchiectomy but before radiation therapy. This indicated that surgery had not been radical and pointed to a clinical staging error [68]. After radiation therapy, two out of four patients still had positive PLAP levels and these two patients also experienced a recurrence. The half-life of PLAP is 2–7 days; beyond that time, elevation of PLAP-like enzyme is a strong indication of residual disease or recurrence. In patients with testicular tumors, the addition of PLAP measurement to AFP, HCG and clinical examination improves selective screening and the accuracy of staging. In the management of advanced seminoma with chemotherapy, the pre-treatment level and the initial decrease of the marker may have prognostic significance. With PLAP monitoring, relapse might be detected before the tumor makes a clinical appearance [30, 44, 68].

Smoking is known to contribute to increased levels of some tumor markers such as the carcinoembryonic antigen (CEA) [1, 54]. This is apparently the case also for PLAP. It is believed that the smoke-affected lung tissue contributes these enzymes. The frequency of elevation of serum PLAP in smokers has been reported to be between 46 and 65% [34, 42, 58, 59]. We confirmed that 56% (23/41) of smoking but otherwise healthy persons had increased serum PLAP levels (unpublished data). Some values were even similar to those in patients with seminoma. The smoking habit of the patient evidently contributes to positive results in NED populations during follow-up [10, 20, 59]. The smoking habit of patients with testicular tumors should therefore be taken into account when evaluating serum PLAP levels.

### Immunolocalization

Radiolabelled antibodies with specificity against tumor antigens have attracted considerable interest as a means of detecting tumors and their metastases. In that PLAP and PLAP-like enzyme are normally bound in high amounts to the outer surface of the cytoplasmic membrane, they would seem to be suitable targets for radioimmunodetection. Both polyclonal and monoclonal antibodies against PLAP accumulated in experimental tumors which synthesized PLAP in mice, but not in tumors in which PLAP was non-measurable [25]. The amounts of labelled antibody bound to the tumor showed a positive, although not very high, correlation with the PLAP concentration. Smaller tumors had somewhat higher mean concentration ratios than larger tumors. This would be an advantage if the method were to be used for diagnostic purposes. Epenotos et al. [11] have in fact reported a clinical application of a radiolabelled MAb against PLAP for detection of tumors from the testis, ovary and cervix. They showed that an indium-111 labelled MAb can localize, with a high degree of accuracy, the presence of active disease in patients with PLAP-positive tumors. This was demonstrated in cases in which the tumors could not be detected with other imaging techniques, including ultrasonography and tomography.

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## Invited Comment

The great importance of markers in the management of non-seminomatous tumours of the testes is well established. The figures from the Irish Testis Tumour Registry indicate a significantly worse prognosis in patients who do not have proper marker studies before, during and after treatment.

Unfortunately, we have not so far had a reliable marker for seminoma. For this reason I found the new work reported here by Koshida and Wahren very exciting. They start with the best summary I have seen of the human alkaline phosphatases. The summary is very clear and very succinct.

The authors go on to give a strong pointer to the possibility that radiolabelled antibodies with specificity against tumour antigens may prove a very valuable means of diagnosing and pinpointing active seminomatous disease in patients with PLAP-positive tumours. This work should lead to a definite improvement in cure rates of seminoma. I am even tempted to hope that it will point the way to the development of many other valuable cancer markers of a similar type.

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