Short Communication

Evaluation of Placental Alkaline Phosphatase Expression as A Potential Target of Solid Tumors Immunotherapy by Using Gene and Protein Expression Repositories

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Abstract

Placental alkaline phosphatase (PLAP) is a membrane enzyme mainly expressed in the placenta. PLAP is shown to be expressed in ovarian cancer (OV), however, there is little known about its expression in other cancers. Using gene and protein expression deposited data, we surveyed PLAP expression across malignant and normal human tissues to explore the potential of PLAP as an immunotherapy target. We detected more than two-fold increased PLAP expression in multiple solid tumors including ovarian cancer, testicular germ cell tumors (TGCT), and uterine corpus endometrial carcinoma (UCEC) compared with matched normal tissues. We also showed association of PLAP expression with high mortality pancreatic adenocarcinoma (PAAD). Altogether, our results suggest that PLAP can be a promising target for immunotherapy of multiple cancers, especially OV, TGCT, and UCEC.

Keywords: Alkaline Phosphatase Placental, Immunotherapy, Neoplasms

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Placental alkaline phosphatase (PLAP) also known as alkaline phosphatase, placental type (ALPP) is a membrane-bound glycosylated dimeric enzyme, which was first detected in the serum during pregnancy and shown to be originated from the placenta (1). In human, PLAP is encoded by *ALPP* gene located on chromosome 2 (2). There are three other distinct but related alkaline phosphatase isoenzymes: Alkaline phosphatase, placental-like 2 (PLAPL2), Alkaline phosphatase, intestinal (ALPI), and Alkaline phosphatase, tissue-nonspecific (ALPL). *PLAPL2* and *ALPI* genes are located together with *ALPP* gene on chromosome 2, whereas *ALPL* gene is located on chromosome 1. This redundancy in alkaline phosphatase isoenzymes is associated with different expression patterns throughout healthy tissues (3), while PLAP is believed to be primarily expressed in the placenta. Among human malignancies, PLAP is reportedly expressed in testicular seminoma (4), ovarian cancer (OV) (5) and endometrial cancer (6, 7).

A number of characteristics make PLAP an attractive candidate of antigen-targeting immunotherapy: i. Being Pa membrane-bound protein, PLAP is an accessible cell surface target for specific binding molecules such as antibodies, ii. The seemingly limited expression of PLAP in healthy tissues and increased expression in malignant tumors suggests that it might serve as a tumor specific antigen with low off-tumor expression. iii. Alkaline phosphatase activity is reported to induce tumor progression in different cancers such as prostate cancer (8), head and neck squamous cell carcinoma (9), and OV (10). Thus, targeting PLAP may also enhance tumor control by reducing tumor-derived alkaline phosphatase activity.

To provide a comprehensive view of PLAP expression across different human cancers, we analyzed RNA-Seq data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) using Gene Expression Profiling Interactive Analysis (GEPIA) gene expression analysis application (11).

Results showed that *ALPP* mRNA expression was statistically significantly higher ($P<0.05$) in 12 different cancers (Fig.1A), namely: OV, testicular germ cell tumors (TGCT), uterine corpus endometrial carcinoma (UCEC), pancreatic adenocarcinoma (PAAD), bladder urothelial carcinoma (BLCA), stomach adenocarcinoma (STAD), esophageal carcinoma (ESCA), uterine carcinosarcoma (UCS), rectum adenocarcinoma (READ), head and neck squamous cell carcinoma (HNSC), clone adenocarcinoma (COAD), and acute myeloid leukemia (LAML). However, this increase in *PLAP* expression had different magnitudes for different cancers, with OV, TGCT, and UCEC showing more than two-fold increase compared with paired normal tissues. We also performed similar analysis on two well-known immunotherapy targets, Mesothelin (codded by MSLN gene) (12) and HER-2 (codded by *ERBB2* gene) (13) as a reference for comparison (Fig.S1, See Supplementary Online Information at www.celljournal.org).

We then compared *ALPP* gene expression across different stages of cancers. Interestingly, in cancers with more than two-fold increase in *ALPP* expression (OV, TGCT, and UCEC), slightly higher levels of expression were observed in stage I compared with later stages. In contrast, in cancers with less than two-fold but still statistically significant increase in *ALPP* expression (PAAD, BLCA, STAD, ESCA, UCS, READ, HNSC, COAD, and LAML), stage I showed lower expression than the later stages (Fig.1B). This observation suggests that in the latter group of cancers *ALPP* expression correlates with cancer progression. To further examine correlation of *ALPP* with the aforementioned cancers prognosis, we compared the overall survival rate of patients with a higher and lower expression of *ALPP* using GEPIA survival analysis. Interestingly, *ALPP* expression showed significant correlation with mortality in PAAD (Fig.1C). To further investigate whether *ALPP* expresses among cancer surface markers, we also used the QSurface tool (14) which can analyze the expression profile of cell surface markers in 14 cancer subtypes. Expression profiles of surface markers for OV, TGCT, and UCEC are not provided in the QSurface, however, we could find statistically significant increase in the *ALPP* expression, with more than 2-fold change, in BLCA, HNSC, and STAD (Fig.2A, B).

To survey the PLAP protein expression in human cancers, we queried PLAP in the Pathology Atlas of Human Protein Atlas (HPA) (15). Results showed that PLAP protein expression was detected with at least one antibody in eight different types of cancer types, namely TGCT, UCEC, OV, liver hepatocellular carcinoma (LIHC), STAD, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), PAAD, and BLCA (Fig.3). Among them, the most robust detections were observed in TGCT, UCEC, and OV with all four antibodies. These results confirm that *PLAP* expression is detectable at the protein level in human cancers.

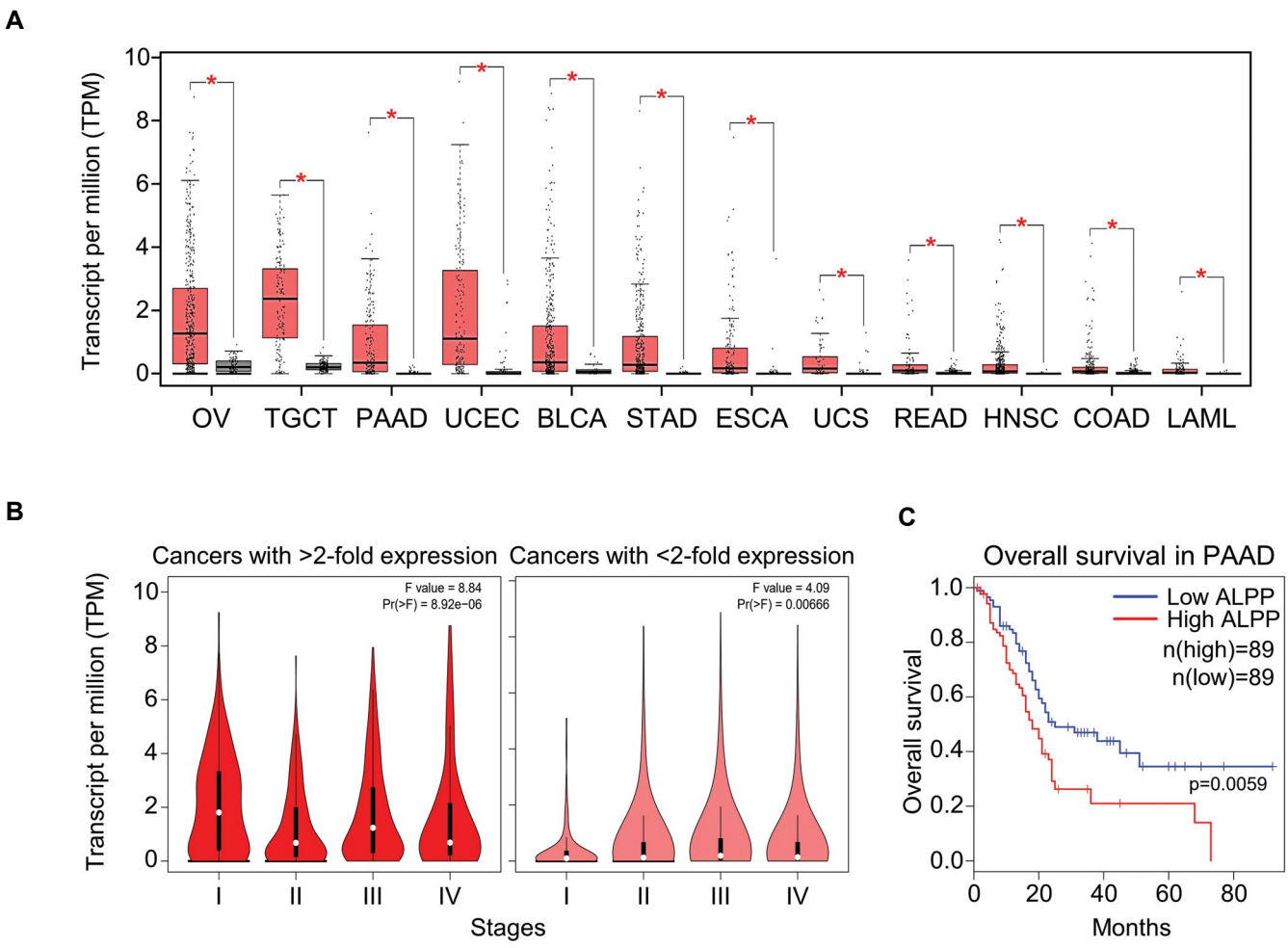

Fig.1: Differential expression of *PLAP* gene in different cancers based on data deposited in The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. **A.** The expression of *PLAP* gene was statistically significantly higher in cancerous tissues (red) than matched normal tissues (gray). **B.** Expression *PLAP* gene across different stages of cancers: (left) including OV, TGCT and UCEC, with >2-fold overexpression and (right) including PAAD, BLCA, STAD, ESCA, UCS, READ, HNSC, COAD and LAML with <2-fold overexpression. **C.** Survival rate analysis comparing PAAD tumors with high and low levels of *PLAP* expression shows that highest expression was associated with poor prognosis in patients with PAAD. *; P<0.05, BLCA; Bladder urothelial carcinoma, COAD; Clone adenocarcinoma, ESCA; Esophageal carcinoma, HNSC; Head and neck squamous cell carcinoma, LAML; Acute myeloid leukemia, OV; Ovarian cancer, PAAD; Pancreatic adenocarcinoma, READ; Rectum adenocarcinoma, STAD; Stomach adenocarcinoma, TGCT; Testicular germ cell tumors, UCEC; Uterine corpus endometrial carcinoma, and UCS; Uterine carcinosarcoma.

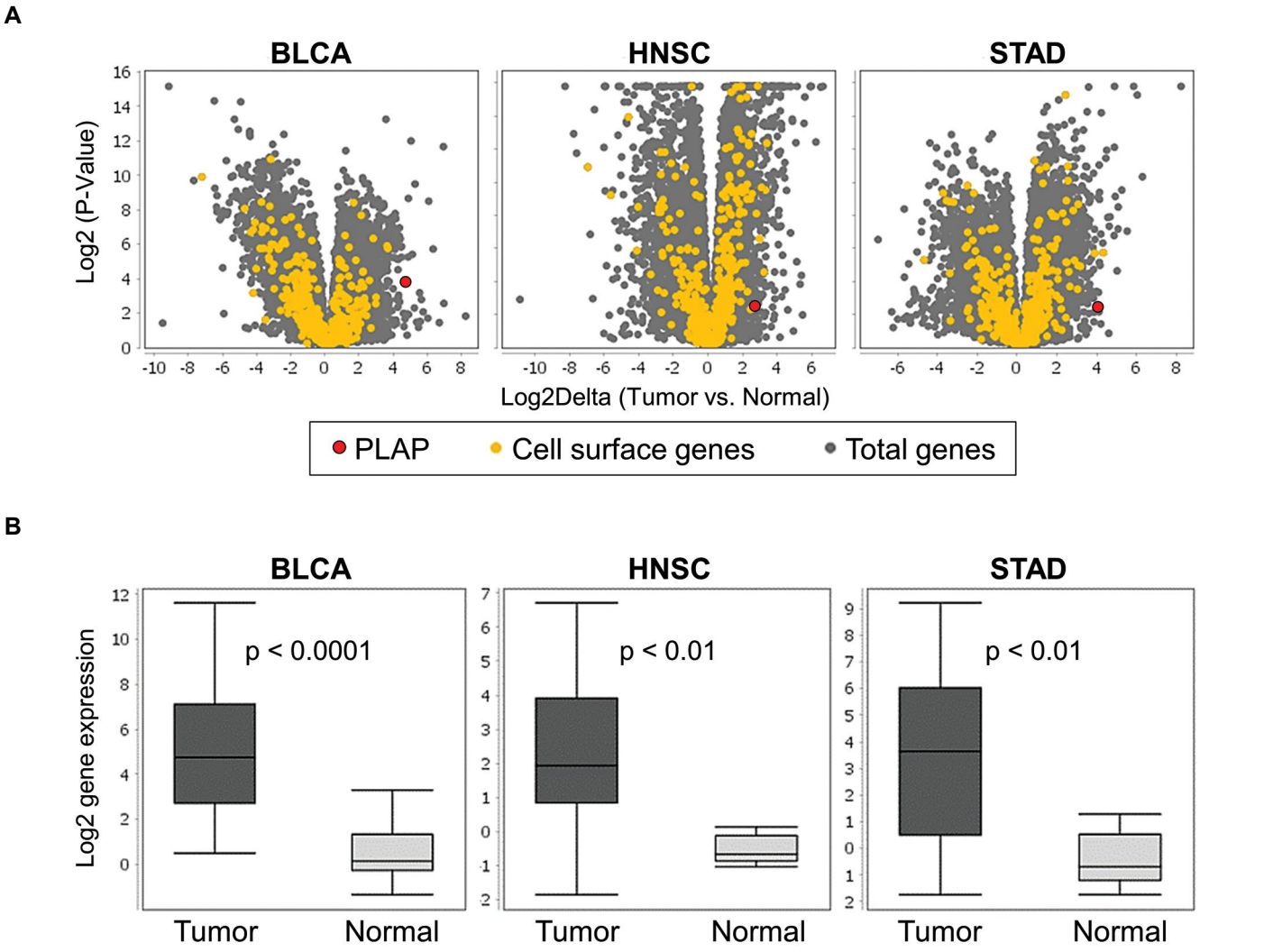

Fig.2: Evaluation of *PLAP* expression among surface protein coding genes in datasets of 14 different cancers provided by QSurface tool. **A.** Volcano plots: expression level of PLAP (red) among cell surface proteins (yellow) and total genes (gray) in the BLCA, HNSC, and STAD. **B.** Box plots: significant increase of PLAP expression in cancerous samples compared to matched normal tissues. BLCA; Bladder urothelial carcinoma, HNSC; Head and neck squamous cell carcinoma, and STAD; Stomach adenocarcinoma.

Another important aspect of targeting PLAP for immunotherapy is possible off-tumor expression of the protein. To address this safety concern, we surveyed PLAP protein expression in the Tissue Atlas of HPA (16) and its mRNA expression in HPA, GTEx, and FANTOM5 data. Not surprisingly, among normal tissues, the highest expression of PLAP protein and mRNA was observed in the placenta (Fig.4A). The low levels of PLAP protein and mRNA expression could be detected in the cervix and uterine tissues, although this expression is confined to glandular cells. Also, PLAP mRNA expression in lung had been reported in GTEx data, but HPA protein expression data did not show any sign of PLAP expression in this tissue (Fig.4B). These data further confirm limited expression of PLAP in somatic female organs which can be considered a favorable safety profile as a potential immunotherapy target.

Altogether, our survey on PLAP expression data in malignant and normal human tissues shows that this surface protein can be a suitable candidate target for immunotherapy. Available specific monoclonal antibodies against PLAP (17) can be used in different immunotherapy strategies such as conventional monoclonal antibody therapies, bispecific antibodies, and chimeric antigen receptor T cells. Our survey showed that *PLAP* expression in healthy somatic tissues is limited to a low-level expression in cervix and uterine. This potentially makes PLAP a safe target with low off-tumor toxicity, especially in male cancers such as TGCT. Our study confirmed the previous reports that have proposed PLAP as a tumor antigen in OV (3, 17). In one of such studies, PLAP expression was examined in 82 women with OV and it was suggested that PLAP expression can be considered as an early marker of OV (18). In line

with this report, our analyses also showed high PLAP expression in early stages of cancers with more than two-fold elevation in PLAP expression (Fig.1B), such as OV. Moreover, our findings suggest that PLAP can be a potent target for late stage PAAD and STAD. Especially, our results showed that PLAP expression was associated with poor prognosis in PAAD patients. To our knowledge, there are no publication pointing out the role of PLAP as a prognostic marker, although, another alkaline phosphatase, PLAPL2, is suggested to be a PAAD biomarker (19) and associate with poor survival in STAD (20). Additionally, the expression of the tissue non-specific alkaline phosphatase, ALPL, is shown to be associated with prostate cancer (8). Although further studies are required to confirm PLAP as a targetable cancer antigen, this study provides significant evidences suggesting that PLAP can serve as a safe and potent target for cancer immunotherapy.

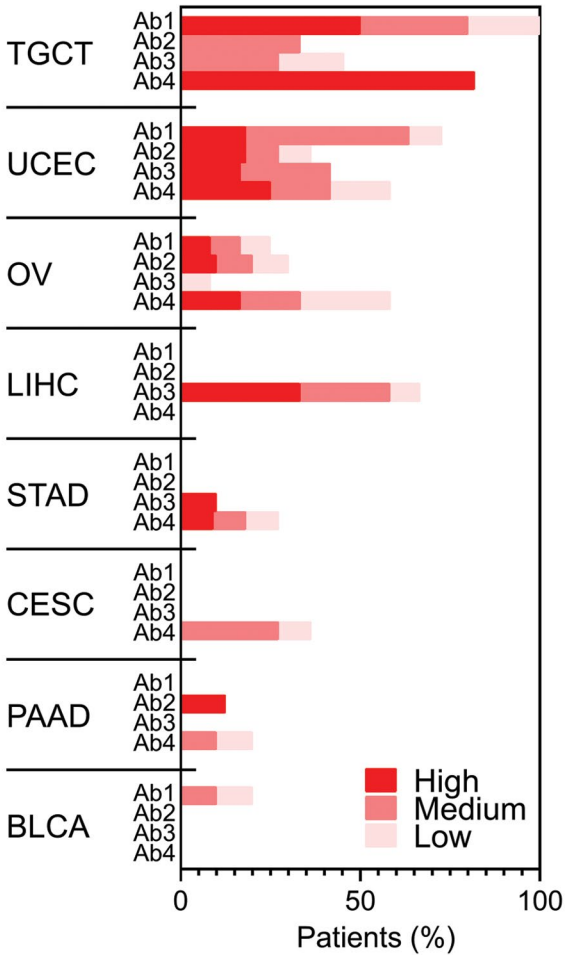

Fig.3: Prevalence of PLAP protein expression in cancer samples based on the Human Protein Atlas (HPA). The levels of PLAP expression are represented by different shades of red based on staining with four different antibodies: Ab1 (CAB026327), Ab2 (HPA051699), Ab3 (HPA038765), and Ab4 (HPA038764). BLCA; Bladder urothelial carcinoma, CESC; Cervical squamous cell carcinoma and endocervical adenocarcinoma, LIHC; Liver hepatocellular carcinoma, OV; Ovarian cancer, PAAD; Pancreatic adenocarcinoma, STAD; Stomach adenocarcinoma, TGCT; Testicular germ cell tumors, and UCEC; Uterine corpus endometrial carcinoma.

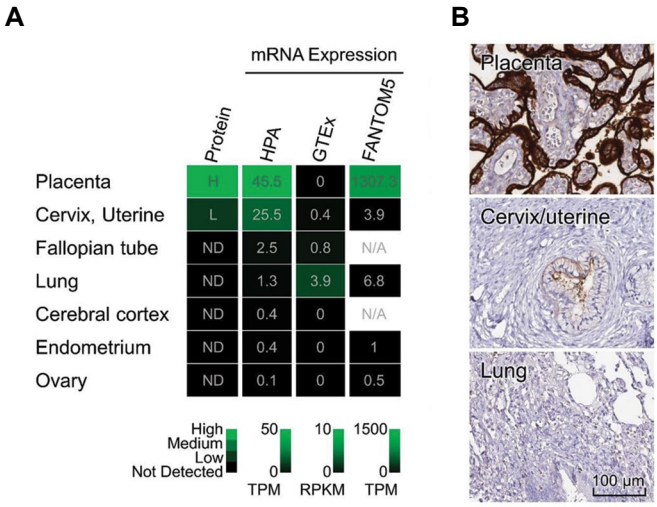

Fig.4: Expression of PLAP in healthy tissues. **A.** Expression of ALLP protein based on the Human Protein Atlas (HPA) scores and *ALLP* mRNA expression in HPA, GTEx and FANTOM5 databases is shown as a heatmap with the actual scores and values in each cell. Non-available data are indicated with N/A. **B.** Representative data from HPA showing immunohistology staining of PLAP in placenta, cervix/uterine, and lung with respectively high, low and undetectable expression levels. Low PLAP expression in cervix/uterine is limited to the glandular cells.

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Authors' Contributions

M.B.; Conception, data analyses, and drafting manuscript. S.P.; Data interpretation and drafting the manuscript. Both authors read and approved the final manuscript.

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