Biochemical Importance of Placental Alkaline Phosphatase Isoenzyme as a Predictor Marker of Lung Malignancy

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Abstract:

Lung cancer is the main cause of cancer-related death worldwide and conventional diagnostic strategies must be improved. Developments of a simple method or techniques which would enable researchers to identify and validate the early screening biomarker of lung cancer patients.
The aims of this article were to review the background documents on the state of the art of the scientific literature in studies that used Placental alkaline phosphatase in the diagnostic of lung cancer also to suggest areas where further research is needed, either to deal with gaps in the knowledge related to employ the heat stability of Placental alkaline phosphatase or assessment the quantitation methods of the isoenzyme.

Introduction

Cancer is a wide term, It can be labeled as an illness that outcome once cellular changes cause the uncontrolled growth and division. Most of the body's cells have particular functions and fixed lifetimes. However, cell death is part of a natural phenomenon called apoptosis. A cell takes directions to die so that the body can substitute it with a newer one that functions better. Cancerous cells lack the mechanisms that train them to stop dividing and to die (1).

Lung cancer is a malignant lung tumor considered by uncontrolled cell growth in lung tissues (2). It could be classified according to histological type (3). This classification is important for determining both the management and predicting outcomes of the disease. For therapeutic purposes, two broad classes are distinguished: non-small cell lung cancer and small-cell lung carcinoma (4).

Non-small-cell lung carcinoma(NSCLC)

The three main subtypes of Non-small cell lung carcinoma are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (5). Rare subtypes include pulmonary enteric adenocarcinoma (6).Nearly 40% of lung cancers are adenocarcinoma, which usually comes from peripheral lung tissue (3)**.** Although most cases of adenocarcinoma are associated with smoking, adenocarcinoma is also the most-common form of lung cancer among people who have smoked fewer than 100 cigarettes in their lifetimes ("never-smokers") (7) and ex-smokers with a modest smoking history **(5)** . A subtype of adenocarcinoma, the bronchioloalveolar carcinoma, is more common in female never-smokers, and may have a better long-term survival (8).

Squamous-cell carcinoma

It causes about 30% of lung cancers. They typically occur close to large airways. A hollow cavity and associated cell death are commonly found at the centre of the tumor **(7).** Nearly 9% of lung cancers are large-cell carcinoma. These are so named because the cancer cells are large, with excess cytoplasm, large nuclei, and conspicuous nucleoli **(3).**

Small-cell lung carcinoma (SCLC)

In SCLC, the cells contain dense neurosecretory granules (vesicles containing neuroendocrine hormones), most cases arise in the larger airways (primary and secondary bronchi) (9)**.**

60-70% have extensive disease (which cannot be targeted within a single radiation therapy field) at presentation time **(5).** For others types, four main histological subtypes were recognised, although some cancers may contain a combination of different subtypes**,** such as adenosquamous carcinoma Rare subtypes include carcinoid tumors, bronchial gland carcinomas, and sarcomatoid carcinomas **(3).**

Lung cancer diagnostic techniques

- **Imaging Tests**; Imaging tests create pictures of the inside of the body by using X-rays, magnetic fields, sound waves, or radioactive particles. (10)
- **chest X-ray** : is a type of high-energy radiation that goes through the body and onto film to produce a picture. A chest X-ray produces pictures of the organs in the chest, including the lungs, airways, heart, and blood vessels (11) (12)**.**
- **Computed tomography(CT or CAT scan);** uses a computer linked to an X-ray machine to make detailed pictures of the inside of the body. Three-dimensional (3D) views of the organs and tissues can be created. A CT scan can provide specific information about the size, shape, and position of masses or nodules in the lung (13) (14)**.**
- **Magnetic resonance imaging (MRI)**: is used in lung cancer to find out whether the cancer has spread to the brain or spinal cord**.** MRI scans provide detailed pictures of areas inside the body by using radio waves and strong magnets. The energy from the radio waves is absorbed and then released in a pattern that a computer translates into images. A contrast dye is usually injected intravenously prior to the MRI to make clearer images (15) (16)**.**
- **Positron emission tomography (PET) scan:** It done by using radioactive sugar which is given intravenously to the patient. Because cancer cells grow rapidly, they absorb more of the radioactive sugar than healthy cells. one hour after, patient would place on a table in the PET scanner for approximately 30 minutes while a special camera creates a picture of the areas in the body that absorbed the radioactive sugar (17).
- **Biopsies :** Tissue biopsies are tests in which small amounts of tissue are removed for examination to find out if a person has lung cancer. Currently, tissue biopsies are the only way to confirm a diagnosis of lung cancer (18) (19)**.**

Biomarkers of lung cancer

Due to its high incidence rate and poor prognosis, lung cancer, as the leading cause of cancer-related mortality worldwide (20)**.** It has become a serious and growing disease burden throughout the world. Therefore, scientific researchers aimed to develop a more reliable diagnostic modality to identify early-stage lung cancer is an urgent priority. Tumor markers measured in serum could be a tool for identifying patients with high risk of recurrent disease. The usefulness of different tumor markers in lung cancer diagnostics, prognostics and disease monitoring has been studied intensely, but often with conflicting results. Many biochemical markers were investigated their prognostic role in lung cancer such as Carcinoembryonic antigen (CEA), Cancer antigen (CA 125), Carbohydrate antigen (CA 19–9), Human epididymis protein 4 (HE4) and Neuron-specific enolase (NSE). (21)

Carcinoembryonic antigen (CEA)

Is a glycoprotein produced during embryonal and fetal development. In adults it is produced in low amounts by the gastrointestinal tract, the pancreas and liver. Elevated CEA in cancer is hypothesized to be caused by a loss of repression of CEA-encoding genes (22). In lung cancer, the use of CEA has been reported for differential diagnosis of malignant lung tumor, monitoring of therapy in advanced stages of disease and for detection of recurrent disease**(36).** Several

studies have suggested CEA as a prognostic marker in non-small cell lung cancer (NSCLC) but results are conflicting (23).

Cancer antigen 125 (CA-125)

Is a glycoprotein produced in fetal tissue, also in mesothelial cells in adults. It has been extensively studied as a tumor marker for screening and management of ovarian cancer (24) (25).It has reported that CA 125 as a marker for worse prognosis in lung cancer (26).

Carbohydrate antigen 19–9(CA 19-9):

Marker used in management of pancreatic tumors. It has also been studied in lung cancer. CA 19–9 in bronchoalveolar lavage fluid, but not in serum, has been identified as a potential diagnostic marker of lung cancer in a study by Ghosh et al. (27) Carcinoembryonic Antigen (CEA) and Cytokeratin Fragment 19 (CYFRA 21-1) are two predictive markers that have been extensively studied in lung cancer. Oncofetal glycoprotein CEA is overexpressed in approximately 35-60% of patients with NSCLC. A number of studies demonstrates that elevated preoperative serum CEA concentrations predict a poor prognosis in early-stage NSCLC (28) (29).

Human epididymis protein 4(HE4)

Is a protein expressed in tissues such as genital tract and respiratory epithelium. Overexpression of the protein has been detected in ovarian cancer but also in lung adenocarcinoma and other cancers. It has been suggested as a tumor marker useful in diagnosing ovarian cancer, especially in premenopausal women **(39).** In lung cancer it has been suggested as a potential diagnostic (30) and prognostic marker (31).

Neuron-specific enolase (NSE)

Is a glycolytic neurospecific isoenzyme found in tumors of neural and neuroectodermal origin such as small cell lung cancer (SCLC) and neuroblastoma. NSE is also found in erythrocytes, plasma cells and platelets and may be released to serum due to hemolysis in the procedure of venipuncture (32). In patients with NSCLC, NSE has been suggested as a prognostic marker and some studies have presented an association between increased NSE and shorter survival in *EGFR*-mutated NSCLC treated with tyrosine kinase inhibitors (TKI´s) (33) (34)

ALKALINE PHOSPHATASE:

Alkaline phosphatase (ALP) is a membrane-bound metalloenzymes which have an active site facing the extracellular space (35).They hydrolyze phosphate monoesters and are involved in several cellular events including protein phosphorylation, cell growth, and apoptosis. Based on their tissue distribution, Alkaline phosphatase is classified into: tissue specific alkaline phosphatase including placental ALP (PALP), germ cell ALP (GCALP), intestinal ALP(IALP) and tissue non-specific alkaline phosphatase (TNAP) including liver ALP(LALP) and bone ALP(BALP) (36) (37) (38).

Alkaline Phosphatase Isoenzymes and their Clinical Significant

In humans, four genes encode ALP enzyme isoforms: tissue non-specific ALP (TNALP), intestinal ALP (IALP). placental ALP ,the placental-like type and germ cell AP (39) .Summary of the alkaline phosphatase isoforms and their clinical significance were listed in the Table (1).
Some of the tumor-associated enzymes are attributed to the placental-like alkaline phosphatase, they are structurally related to the term placental alkaline phosphatase, For this reason the placental isoenzyme has attracted much interest (40). TNAP is mainly expressed in liver, bone and kidney but is also found in circulating leukocytes and colon and its expression within the intestine is increased during inflammation. (41). The function of TNAP is not entirely understood but its genetic absence has been linked to hypophosphatemia and therefore it is believed to play a role in bone matrix mineralization (42). Intestinal ALP is predominately expressed by the intestinal epithelium whereas the other three isoforms are not, intestinal ALP is expressed and secreted by intestinal epithelial cells and remains active within the mucosal membrane as well as the intestinal lumen. Table 1 showed the most well-known functions of alkaline phosphatase isoforms.

Table 1: List of alkaline phosphatase isoforms and their clinical significance (106).

Isoform	Location	Function
Tissue non-specific alkaline phosphatase (TNALP)	Liver Bone	Un known Genetic absence has been linked to hypophosphatemia
Intestinal alkaline phosphatase (IALP)	Intestinal Epithelial Cells	Detoxification of Bacterial Endotoxin. Dephosphorylation of Tri and Di phosphorylated nucleotide. Regulation of the Intestinal Microbiome. Regulation of Intestinal Lipid Absorption.

Placental alkaline phosphase (PALP)	Placenta	Tumor marker for Seminomas and Germ Cell Neoplasms Detoxification of Bacterial Endotoxin
Germ Cell (GCALP)	Germ Cell Neoplasms	Unknown

Detection and Quantitation techniques of ALP and their Isoenzymes

1. **Stability of denaturation to heat**

Liver, bone and intestinal ALPs are rapidly inactivated at temperature >65 °C. In contrast, placental ALP is remarkably thermostable. They may be heated at 65 °C for an hour or more without loss of activity (43).

2. Chemical inhibition

Various low molecular weight substances show differential inhibition of the different ALPs. The L/B/K ALPs are more sensitive to inhibition with L-homoarginine (Har) than placental, placental-like or intestinal ALPs. In contrast, placental, placental-like and intestinal ALPS are about 30 times more sensitive to inhibition with l-phenylalanine (Phe) than the L/B/K ALPs. L-Leucine (Leu) characteristically gives much stronger inhibition with placental-like ALP than with the other ALPs. Levamisole (Leva) is a particularly potent inhibitor of L/B/K ALP, but has little inhibitory effect on the other ALPs (44).

3. Immunological Techniques

The quantitative measurements of placental and intestinal ALP might be performed using polyclonal or monoclonal antisera. PLAP and intestinal alkaline phosphatase share some antigenic determinants and a cross-reactivity is observed with unabsorbed antisera against PLAP and intestinal ALP. However, monospecific antisera for each form of the enzyme can be prepared by absorption with purified Placental ALP or intestinal ALP (45).

4. Electrophoresis

In gel electrophoresis, isoenzyme fragments are drawn through a thick gel by an electric charge. Each isoenzyme has a distinct charge of its own because of its unique amino acid sequence. This enables gel electrophoresis to separate the fragments into bands for identification. The liver ALP moves rapidly toward the anode following bone ALP then intestinal ALP migrates slowly than

the bone ALP, whereas the placental ALP appears as a discrete band overlap the diffuse bone fraction (46).

Placental and placental like alkaline phosphatase isoenzymes and lung cancer:

Placental alkaline phosphatase (PALP) is polymorphic and heat stable enzyme. High levels of this enzyme is found in trophoblast of placenta. It is localized in apical and basal cells of syncytiotrophoblast plasma membrane (47). It is synthesized from placental syncytiotrophoblast from the twelfth week of pregnancy and is released into the maternal blood. This enzyme when infused into human subjects, has a biological half life of about seven days and large artificially induced changes in serum alkaline phosphatase concentration may persist for several weeks. In early pregnancy Placental ALP activity is low. Measurable levels of Placental ALP appear in maternal serum by the end of first trimester and increases progressively with gestational age (48). It has suggested to be involved in nutrient transport from mother to fetus and also in transport of maternal IgG to the fetus. Also, It has a role in active transport of phosphates, absorption of nutrients and uptake mechanism through the plasma membrane (49). Enzyme was involved in transfer of glucose and fatty acids across the cell membrane (50). The central core of PLAP, consisting of an extended β-sheet and flanking α-helices. The overall structure of Placental ALP is a dimer and each monomer contains 484 residues, four metal atoms, one phosphate ion, and 603 water molecules. The two monomers are related by a two-fold crystallographic axis (51).

PLAP is a dimeric enzyme, composed of subunits of approximately 65 kDa. The placental enzyme stems from the demonstration that PLAP and PLAP-like enzymes are produced by tumor cells in vitro and in vivo (52).

Placental-like placental alkaline phosphatase and placental alkaline phosphate are virtually identical in amino acid sequence (98% homology) and have a highly restricted tissue expression pattern, expressing in placental trophoblasts only. Both share high homology with the intestinal alkaline phosphatase (87% homology), and some homology with the tissue-nonspecific liver/bone/kidney phosphatase ALP (57% homology) (53)

Placental alkaline phosphatase is known to be highly heat stable, its activity being unchanged after 30min at 70°C in the presence of $Mg^{2+,}$ as found by (54).

It resembles in many properties the alkaline phosphatase called regan isoenzyme found by fishman et al. in nontrophoblastic tumors. PLAP has been reported in the sera of about 20% of patients with various cancers, although some have reported a prevalence as high as 95%**.** Substantial evidence of clinical utility has been lacking. (55)

Implications and contribution to the knowledge gap:

Most of detection methods of Alkaline phosphatase isoforms have many merits and limitations. Mainly, its consider as a high cost of equipment, low sample processing speed, physically large instruments and larger required sample volumes. Morever, most detection methods suffer from a

lack of sensitivity and specificity, especially in the discrimination between placental and intestinal alkaline phosphatase.

Researcher were needing to develop a detection method of placental alkaline phosphatase and make it available test in any simple lab, also to produce inexpensively and simply operate testing. Also, there is a needing for an experimental assessment to the performance of different techniques such as heating methods to measure the Placental alkaline phosphatase and compared with highly sensitive method (such as ELISA) to demonstrated and confirmed the accuracy and validity of the both methods. That might be encouraged a potential use of Placental alkaline phosphatase isoenzyme as a simple accessible and affordable biomarker for monitoring lung cancer patients. Moreover, using placental alkaline phosphatase to provide baseline information as a diagnostic marker without needing for advanced facilities.

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