Peer Review File

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Reviewer A

The review paper discusses the potential of alkaline phosphatases in diagnosis, prognosis, and treatment of cancer. The manuscript needs some revisions before being accepted for publication.

Comment 1: Please clearly explain the novelty of your work, as previous studies have already reviewed the use of ALP as a discriminating marker in various cancer.
Reply 1: First of all, we reviewed ALP as a marker in different tumors, we also outlined that ALP expression may be up-regulated or down-regulated in different tumors, the role of ALPs in cancer is complex and context-dependent. Secondly, we also proposed that ALP should be detected together with other molecules to improve the diagnostic accuracy
Changes in the text: We added this idea to the "Abstract" part. It is also reflected in Figure 1 and Table 1.(see Page 02, line 45-46).

Comment 2: In the abstract, authors stated that "ALPs might be a potential tumor biomarker, further research is needed to better understand the mechanisms underlying ALP dysregulation in cancer and to identify potential therapeutic targets." However, authors did not underly mechanism in the review. It would be better to strongly link the associated regulation pathways.
Reply 2: The expression of ALPs in cancer is complex and context-dependent. Its abnormal expression is associated with multiple molecular pathways. Activation of the Wnt/β-catenin pathway is associated with increased ALP expression in multiple cancer types, including colon, liver, and bone cancers.β-catenin translocates to the nucleus and promotes the transcription of ALP gene, leading to increased ALP production. Activation of BMP pathway leads to increased ALP expression in cancer cells, particularly in bone metastases of solid tumors, where tumor cells acquire osteoblastic features. In the tumor microenvironment, changes in Extracellular matrix (ECM) composition and stiffness can modulate ALP expression in cancer cells. Activation of integrin-mediated signaling pathways, such as focal adhesion kinase (FAK) or Rho GTPases, can upregulate ALP expression and promote cancer cell invasion and metastasis. MiR-122 targets ALP in hepatocellular carcinoma (liver cancer) and is associated with reduced ALP activity.
More and more studies are exploring how ALP affects the occurrence and development of tumors, Therefore, we can detect the abnormal expression of upstream regulatory molecules in specific tumors, and then design targets in the upstream molecular pathways to control ALP expression.

Changes in the text: We added this idea to the "Regulation pathways of ALP in cancer" part. (see Page 09-10, line 322-336).

Comment 3:The authors might add additional illustrations as a form of figure to make the reader easier to understand rather than only the dominant text as a present form. The review did not provide the quantitative data to support their statements.
Reply 3: Thanks for your suggestion. We have added additional figure.
Changes in the text: (see Page 10, line 338-340,or Fig.2).

Reviewer B

The paper titled "Do alkaline phosphatases have great potential in the diagnosis, prognosis, and treatment of tumors?" is interesting. This article reviews application progress of ALPs in cancer, and we hypothesize that ALPs might be a potential tumor biomarker, further research is needed to better understand the mechanisms underlying ALP dysregulation in cancer and to identify potential therapeutic targets. However, there are several minor issues that if addressed would significantly improve the manuscript.
1) Comment 1: What is the relation between ALP gene overexpression and gene amplification by polyploidy of chromosomes 1 and 2 in these lesions? It is recommended to add relevant content.
Reply 1: The ALP gene overexpression and gene amplification by polyploidy of chromosomes 1 and 2 may be related in certain lesions, although it is important to note that the specific relationship can vary depending on the context and specific biological system. In certain lesions or diseases, such as certain types of cancer, genetic alterations can occur, leading to the overexpression of specific genes and the amplification of certain chromosomal regions. In this context, it is possible that ALP gene overexpression and gene amplification by polyploidy of chromosomes 1 and 2 are associated in these lesions. The increased number of chromosomes 1 and 2 due to polyploidy could contribute to the amplified copy number of the ALP gene, leading to its overexpression. Therefore, we need to further distinguish between these two conditions in clinical research, such as DNA copy number analysis, DNA sequencing and other methods.
Changes in the text: We added this content to the "Conclusions and Perspectives" part. (see Page 11, line 366-375).

2) Comment 2: What are the methods for analyzing alkaline phosphatase activity in clinical samples? What is the biggest problem faced? How to overcome it? It is recommended to add relevant content.
Reply 2: There are several methods available for analyzing ALP activity in clinical samples. Such as enzyme assays, which measure the enzymatic activity of ALP by

monitoring the conversion of substrate to product. Different substrates can be used, such as p-nitrophenyl phosphate (p-NPP) or 5-bromo-4-chloro-3-indolyl phosphate (BCIP). If we want to improve the sensitivity and specificity of detecting and quantifying ALP activity, enzyme-linked immunosorbent assay (ELISA) or chemiluminescent immunoassay (CLIA) can be used. While ALP isoenzymes can be separated and analyzed using methods like electrophoresis or high-performance liquid chromatography (HPLC). This helps to identify different forms of ALP and distinguish them from other isoenzymes.

Although there are so many methods to analyze ALP activity. The biggest problem often faced in ALP analysis is the interference from other enzymes or compounds present in clinical samples. Some compounds can lead to false-positive or false-negative results, affecting the accuracy of ALP activity measurements. To overcome this problem, various measures can be taken: (1) Selective Inhibitors: Addition of specific inhibitors can help suppress interfering enzymes while measuring ALP activity. For example, levamisole is commonly used to inhibit interference from intestinal alkaline phosphatase. (2) Sample Preparation: Proper sample preparation techniques, such as sample dilution or filtration, can help remove interfering substances and improve the accuracy of ALP activity measurements. (3) Validation and Quality Control: Regular validation and quality control should be performed to monitor the accuracy and precision of the analytical methods used for ALP analysis. This ensures reliable and consistent results.

Changes in the text: We added this content to the "**Challenges of alkaline phosphatases in tumor diagnosis**" part. (see Page 09, line 301-320).

3) Comment 3: What is the prognostic value of ALP and LDH together with host-related factors in patients with unresectable advanced GC? It is recommended to add relevant content.

Reply 2: High levels of ALP and LDH are associated with advanced tumor stage, increased metastasis, and worse overall survival in patients with unresectable advanced GC.Higher levels of ALP and LDH are indicative of greater tumor volume and aggressiveness. They are associated with larger tumor size, lymph node involvement, and the presence of distant metastases, all of which contribute to a poor prognosis.

Higher baseline levels of ALP and LDH have been associated with poorer response to chemotherapy and targeted therapies in GC. Monitoring ALP and LDH during treatment can help assess treatment efficacy and identify patients who may benefit from alternative therapies. It's important to note that the prognostic value of ALP and LDH, along with host-related factors, may vary depending on other tumor characteristics, patient demographics, and the specific treatment regimen. Assessing these factors in combination with ALP and LDH can provide a more comprehensive prognostic evaluation for patients with unresectable advanced GC.

Changes in the text: We added this content to the **"Alkaline phosphatases in gastric cancer"** part. (see Page 08, line 265-280).

4) Comment 4:What is the application of alkaline phosphatase isoenzymes in the diagnosis of tumor metastasis? It is recommended to add relevant content.
Reply 2: Intestinal and placental ALP isoenzymes are not usually associated with tumor metastasis diagnosis, but their measurement can help exclude non-tumor-related causes of elevated ALP levels. Bone,and Liver-specific ALP isoenzymes are involved in bone metabolism. By assessing the levels and ratios of ALP isoenzymes, along with total ALP, healthcare professionals can obtain valuable information regarding the potential presence and source of tumor metastasis. The combination of ALP isoenzyme analysis with other clinical and imaging investigations can aid in the diagnosis, staging, and monitoring of tumor metastasis, particularly in cases where bone or liver involvement is suspected.
Changes in the text: We added this content to the **"Alkaline phosphatases isozymes**" part. (see Page 04, line 102-103,105-109).

5) Comment 5:The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "ALP bouncing and LDH normalization in bone metastatic castration-resistant prostate cancer patients under therapy with Enzalutamide: an exploratory analysis, Transl Androl Urol, PMID: 34804841". It is recommended to quote the article.
Reply 1: Thanks for your suggestion. We supplemented this in the **"Alkaline phosphatases in gastric cancer"** part.
Changes in the text: See Page 08, line 265-280, and reference[32].

6) Comment 6:It is recommended to add the polymorphism at the enzyme level.
Reply 1: Thanks for your suggestion. We supplemented this in the "Conclusions and Perspectives" part.
Changes in the text: See Page 11, line 376-384.