ALOX5-AP in the Tumor Microenvironment

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Abstract:

The arachidonate 5-lipoxygenase–activating protein (ALOX5-AP) plays an essential role in leukotriene biosynthesis and is also known as a significant regulator in the tumour microenvironment (TME). Recent research has highlighted the ALOX5-AP as a key modulator of tumour progression through its regulation of leukotriene biosynthesis and inflammatory signaling pathways. In the TME, ALOX5-AP plays a double role in various cancers; ALOX5-AP positively contributes to prognosis and immunotherapies for osteosarcoma (OS) through utilizing CD8 T cells. On the other hand, it negatively impacts the TME in other cancers, such as acute myeloid leukemia (AML), ovarian cancer, and breast cancer. Its immunosuppressive effects are a result of inflammation by initiating the function of tumor-associated macrophages (TAMs) and M2 macrophages. This review synthesizes current findings on the molecular level, connecting the role of ALOX5-AP in tumour immune evasion, angiogenesis, and other processes. Furthermore, the therapeutic implications of targeting ALOX5-AP in various cancers, such as OS and AML, are explored. Overall, ALOX5-AP proves to be a promising biomarker target considering the current academic conversations regarding cancer immunology.

Keywords: arachidonate 5-lipoxygenase–activating protein (ALOX5-AP), tumour microenvironment (TME), osteosarcoma (OS), acute myeloid leukemia (AML

1. Introduction

The tumour microenvironment comprises all things in the system surrounding a tumour, including cancer cells types, immune cell types, endothelial cells, the extracellular matrix (ECM), and other tissue cell types (de Visser & Joyce, 2023). This highly structured ecosystem is actively involved in a tumour's growth, development, metastasis, and responses to treatments; cancer is not solely focused on the tumour itself, but this microenvironment it is in (Weber & Kuo, 2012). In the TME, cancer cells are surrounded by non-malignant cell types, and recruit and reprogram such cells to create a tumour-supportive environment, thus causing the tumor growth and metastasis. Interactions between cells include many other factors, such as integrins, cadherins, chemokines, and cytokines (Bejarano et al., 2021; de Visser & Joyce, 2023).

The structure and composition of a TME varies drastically depending on where it is located in the body, and even between patients with the same type of cancer. Specifically, immune cells– which can be both tumor-promoting or tumor suppressing–and stromal cell types– which are non-cancerous cells–are unique from organ to organ, given the different macrophage populations in each one (Anderson & Simon, 2020). During metastasis and other forms of invasion, the TME must further alter its composition to allow cancerous cells to break off and move to other parts of the body. Even blood vessels in the TME can serve as a route for metastasis, while supplying oxygen and nutrients for the tumour to grow (Anderson & Simon, 2020). In different cancers, the TME undergoes immune regulation by various genes to promote or suppress tumour proliferation, invasion, and survival (Ye et al., 2021).

The arachidonate 5-lipoxygenase activating protein (ALOX5-AP), or FLAP is a protein that is responsible for producing leukotrienes in the TME (Ye et al., 2021). Leukotrienes (LTs) are a type of fatty acids that are inflammatory and lipid mediators produced by immune cells. (Jo-Watanabe et al., 2019). Arachidonic acid (AA), a polyunsaturated fatty acid created and excreted by membrane phospholipids, is oxidized by the arachidonate 5-lipoxygenase (5-LO) enzyme in this pathway, resulting in the production of leukotrienes in the nuclear membrane of proinflammatory and immunomodulating lipid mediators or cells (Gur et al., 2018). In the 5-LOX pathway, for example, ALOX5-AP is responsible for binding 5-LO to AA, making LTs such as LTB4, LTC4, and LTD4 (Gur et al., 2018). A simplified visualization of this pathway is illustrated below in Figure 1. The ALOX5-AP pathway helps with inflammatory response and is also expressed in multiple types of cancer cell lines, thus showing a fundamental role in mediating cancer development (Ye et al., 2021). Specifically, this gene is shown to be associated with primary neuroblastoma patients, esophageal squamous cell carcinoma patients, and even cancers such as OS in the bones (Bai et al., 2018; Chen et al., 2023; Song et al., 2024). Another association is with colorectal carcinoma, the second-most common diagnosed cancer in Canada, because of its microsatellite instability; this is a type of error made in the DNA due to short repeated sequences, causing the spread of cancerous cells

(Kennedy & Harris, 2023). As a crucial part of leukotriene synthesis, its molecular structure and abilities thus present possible opportunities for future interventions, as well as the development of new medicines by utilizing its anti-inflammatory properties (Haeggstrom, 2018). Nevertheless, it is important to note that it has links to both pro-tumorigenic and anti-tumorigenic effects depending on different contexts and cancers (Kahnt et al., 2024; Song et al., 2024). This review will discuss the roles of ALOX—5-AP in the TME in prognosis and immunotherapy, immune cell infiltration and immunosuppression, and next steps in targeting the gene in combined therapies.

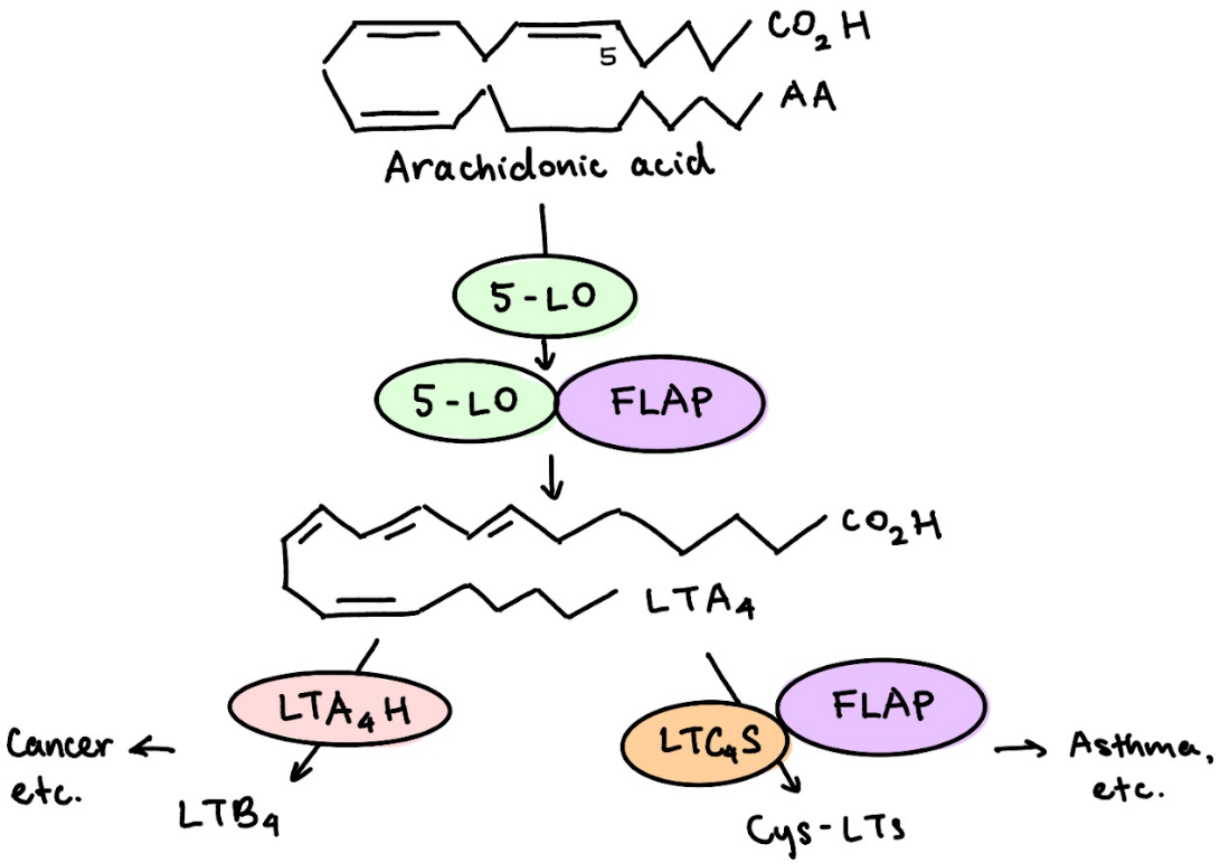

Figure 1: 5-LOX pathway of ALOX5-AP from AA, binding process, to production of different LTs.

2. Double Role in Cancer Prognosis

2.1 ALOX5-AP & positive regulation in osteosarcoma immunotherapy

In OS, ALOX5-AP has been linked to CD8 T cell infiltration (Y. Chen et al., 2023). Chen et al., have found that a higher expression of ALOX5-AP was correlated with lower risk in the cancer (Y. Chen et al., 2023). As shown in Figure 2, ALOX5-AP was also correlated with increased immune cell infiltration (Han et al., 2023). Thus, ALOX5-AP acts as a biomarker to predict cytotoxic CD8 lymphocyte infiltration (Ge et al., 2022). Similarly, ALOX5-AP can help with improved prognosis and

immunotherapies by increasing the ALOX5-AP abundance in OS (Ye et al., 2021). The gene has also shown to suppress tumour progression by inhibiting the Wnt/β-catenin signaling pathway in OS (Han et al., 2023). Being confirmed as a tumor suppressor in OS, its overall lower expression shows poor prognosis (Han et al., 2023; Xie et al., 2020).

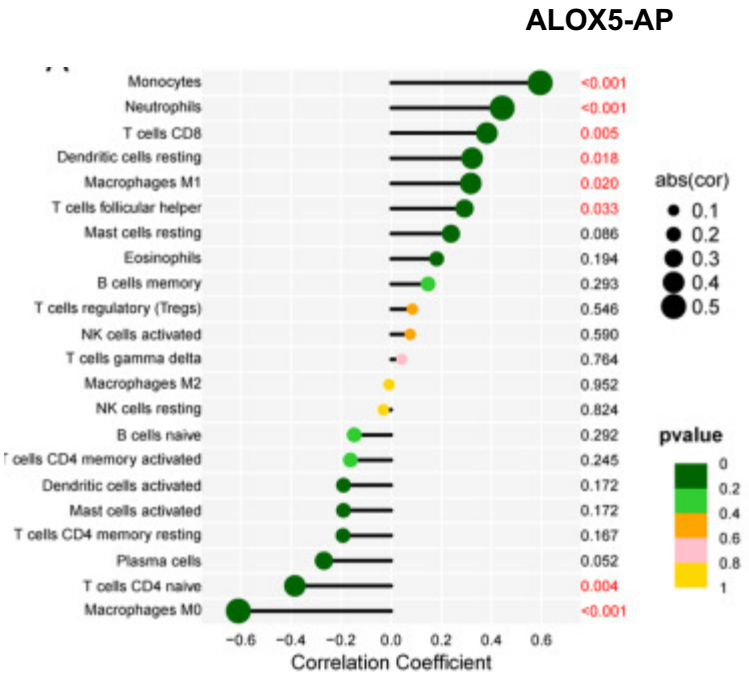

Fig. 2: Correlation between ALOX5-AP and infiltrating immune cells (Han et al., 2023).

The ability to act as a biomarker for such cancers is seen with OS as well, an aggressive bone cancer that is more common in early adolescence than adults (Xie et al., 2020). Research results show how ALOX5-AP acts as an excellent indicator for OS diagnosis, with a high degree of efficiency as well, applicable to both metastatic and non-metastatic cancer patients (Guan X., et al., 2020).

2.2 ALOX5-AP & negative impact in acute myeloid leukemia and other cancers

Moreover, acute myeloid leukemia (AML), one of the most common types of acute leukemia, AML is the most common among adults averaging a 5-year survival rate (X. Y. Chen et al., 2023). Here, ALOX5-AP was seen to be upregulated in AML being associated to DNA hypomethylation; a decrease in DNA methylation, which is a molecular change that affects DNA expression, thus contributing to different diseases and cancers (Song et al., 2024). In AML, gene methylation levels were lower than in normal conditions, predicting poorer prognosis in AML (X. Y. Chen et al., 2023). In other words, high expression of ALOX5-AP is correlated with poor prognosis in AML. Similarly, ALOX5-AP predicts poor prognosis in ovarian cancer, serving as another example of ALOX5-AP's role in negatively impacting a patient in the microenvironment (Ye et al., 2021). In the ovarian cancer TME, studies have confirmed ALOX5-AP's relation to M2 macrophage infiltration through

sequencing results (Ye et al., 2021). Overall, patients with higher M2 macrophage infiltration and ALOX5-AP abundance had the worse outcomes due to its ability to maintain M2 macrophage recruitment and polarization—the transformation of white blood cells to an anti-inflammatory phenotype (Xu et al., 2025). ALOX5-AP also increases the risk of breast cancer through multiple mechanisms in correlation with its biological activities, which will be discussed below.

3. ALOX5-AP & Immunosuppression

In the tumour microenvironment (TME), ALOX5-AP acts in many different ways. Studies have shown its link to immune cell infiltration within the TME, especially M2 macrophages, known to reduce and suppress immune responses and increase the growth of tumors as a result (Ye et al., 2021). M2 macrophages particularly create a tumour-promoting environment for cancer growth first by suppressing the immune system's ability to attack tumour cells. These TAMs also stimulate angiogenesis, increasing the formation of new blood and lymphatic vessels to supply tumours with oxygen and nutrients to help tumour growth (Xu et al., 2025). As a result, M2 macrophages help remodel the TME to create a supportive EMC in order to invade surrounding tissues and metastasize (Huang et al., 2024). Additionally, M2 macrophages can also induce hypoxic conditions in the TME, which also help with its progression. Furthermore, M2 macrophages are responsible for tumour initiation, relapse, and metastasis by promoting cancer stem cell (CSC) development; in particular, exosomes derived from M2 macrophages maintain CSCs (Zhang et al., 2023). ALOX5-AP's contribution to M2 macrophage polarization shows the potential to act as a biomarker for immunotherapies and targets against some cancers, like AML, ovarian, and breast cancer patients (Guan et al., 2020).

4. ALOX5-AP & Cancer Signaling

ALOX5-AP is involved in many signaling pathways that regulate cancer development and progression. One of these main pathways is the Wnt/β-catenin signaling pathway, known for its role in cell proliferation, differentiation, migration, apoptosis, and even tissue homeostasis (Song et al., 2024). While many cancers have been linked to this pathway's cascade dysregulation, ALOX5-AP is commonly linked to the Wnt/β-catenin pathway in OS. ALOX5-AP suppresses the Wnt/β-catenin pathway, which results in suppressing OS growth (Han et al., 2023).

However, ALOX5-AP is also linked to the PI3K/Akt/mTOR pathway, where it was found to be involved with the progression and migration of breast cancer development. Furthermore, ALOX5-AP may also influence the RhoA and focal adhesion signaling in breast cancer, affecting cancer migration (Zhou et al., 2020). By impacting the expression and activity of immune-related pathways, ALOX5-AP thus changes the TME and immune response for many different cancers, in different ways.

5. Targeting ALOX5-AP & Combined Therapies

Researchers are utilizing ALOX5-AP's role in promoting an immunosuppressive TME in different cancers to combine with its inhibitors in other therapies (Xu et al., 2025). Such processes

include focusing on immune checkpoint inhibitors (Gomes et al., 2018). As well, inhibiting ALOX5-AP itself can decrease immunosuppressive effects of TAMs like M2 macrophages by reducing its infiltration into the TME, and thus enhance the efficiency of current immunotherapies (Gur et al., 2018) . As summarized above, both ALOX5-AP as an immunosuppressor and anti-tumorigenic gene can act as a biomarker target for different cancers (Xie et al., 2020; Xu et al., 2025).

In other cancers, where ALOX5-AP serves as an anti-tumorigenic gene, combined therapies are being studied to increase its impact on patients. Although ALOX5-AP has tumour suppressor-like properties in cancers like OS, combining it with other genes in the AA pathway can enhance survival rates for these cancer patients (Gilbert et al., 2021). Specifically, 5-LOX does not significantly improve survival predictions alone; this is why 12-LOX, which exists in the same molecular pathway can improve overall predictive power (Orafaie et al., 2020). Nevertheless, combinations should be carefully studied before utilizing them in therapeutic settings. For example, different ALOX5-AP genotypes can respond differently to other genes and acids, like linoleic acid (Wang et al., 2008). When combined with high amounts, ALOX5-AP may be correlated with an increased risk of breast cancer. Consequently, further research regarding ALOX5-AP's role as a target *and* in combined therapies must be conducted in order to fully utilize its properties in developing new diagnostic and therapeutic strategies.

As a rising potential biomarker target in the ovarian cancer TME, so does its ability to perform as a immunotherapy target to re-educate macrophages towards the M1 antitumor macrophage instead (Lim et al., 2023). However, more research and studies must be conducted to fully enhance these indicator abilities of ALOX5-AP (Song et al., 2024). As such, cancers that are linked to M2 macrophage abundance can turn to immunotherapies that target the inhibition of ALOX5-AP, and thus the spread of cancerous cells; existing medications or recovery paths may see to be more effective and responsive as a prognostic biomarker with immunosuppression (Han et al., 2023).

6. Conclusion

In summary, ALOX5-AP contributes to M2 macrophage polarization in cancers like ovarian cancer and AML, resulting in improving tumour suppressor environments, and cancer promotion in growth. On the other hand, it also contributes to CD8 T cell infiltration, improving the function of tumour suppressor genes in osteosarcoma (Song et al., 2024). Depending on which cancer ALOX5-AP is correlated with, its abilities are involved with many different cancer signaling pathways, such as the Wnt/β-catenin pathway. Overall, it should be carefully considered as a therapeutic target in different cancers based on its expression in the respective TMEs.

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