PIKfyve inhibition and involvement in cancer immunotherapy

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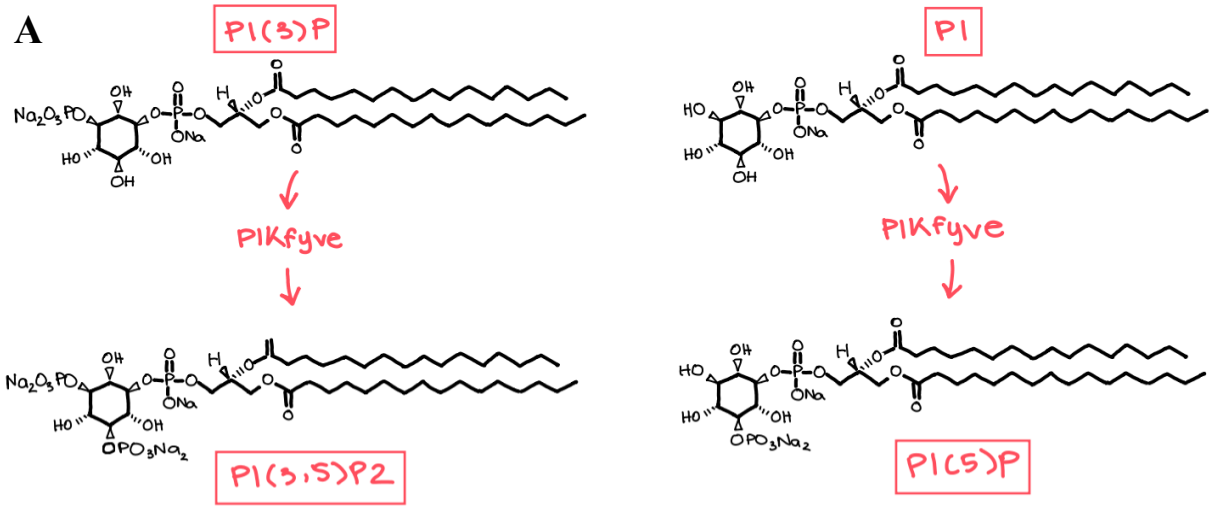
Summary

PIKfyve inhibition has led to a wide variety of implications of cancer treatment. Studying PIKfyve has led to a prominent discovery to improve cancer patients' reactions to immunotherapy treatment—a non-invasive fashion of alleviating cancer conditions. Targeting the PIKfyve gene has been found to enhance major histocompatibility complex class I (MHC-I) and CD8+ T cell activity, improving immunotherapy efficacy. Furthermore, PIKfyve negatively regulates dendritic cells (DCs) function through suppression of the NF-kB pathway. PIKfyve deletion in DCs, or treatment with Pikfyve inhibitor apilimod enhances DC-dependent T cell immunity reducing tumor growth. This paper summarizes PIKfyve function in the body and its implications in treating disease.

1. Introduction to PIKfyve

1.1 Protein Structure

PIKfyve, or also known as PhosphoInositide Kinase containing a FYVE finger (FAB1/YOTB/Vac1/EEA1) domain, is part of a family of phosphatidylinositol-3,5 biphosphate, PI(3,5)P2, synthesizing enzymes. Specifically, PIKfyve phosphorylates position 5 of PIP (phosphatidylinositol) or PI(3)P (phosphatidylinositol 3-phosphate) to form PI5P or PI(3,5)P2 (Fig. 1a). PIKfyve may also appear as a protein kinase, displaying autophosphorylation. Human PIKfyve is 2090 amino acids long with many inner domains—a FYVE PI3P-binding zinc-finger domain (aa 154-219), a DEP domain (aa 365-440) and a C-terminal PIP kinase region (aa 1172-2085) (Fig. 1b) [1].



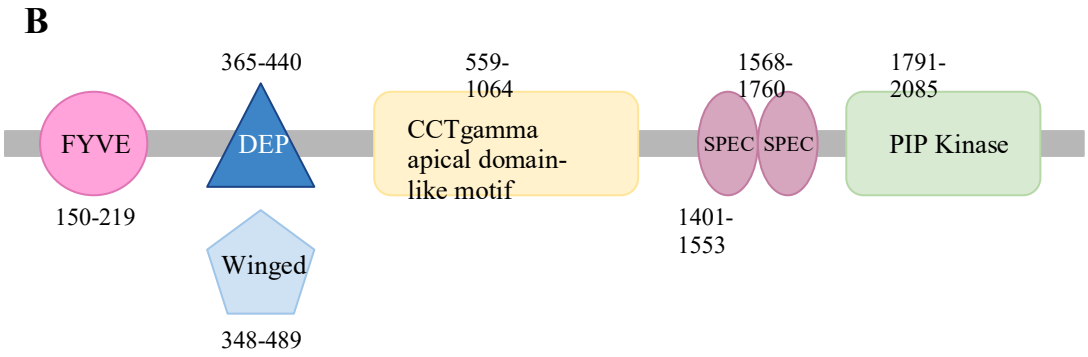


Fig. 1 Function and protein structure of PIKfyve: A simplified representation of the PIKfyve function for protein synthesis and the gene's domain structure. Amino acid numbers of the protein sequence are provided.

1.2 Role in Lysosomes, Endosomes, and Transcription

PIKfyve can alter the inner ion homeostasis and membrane fission and formation of tubules, along with influencing the pH within the endomembrane system in mammalian cells and yeast.
As a result of inhibiting PIKfyve, ion channels driven by P1(3,5)P2 are inactivated, leading to the swelling of the lysosome by an influx of water.

In endosomes, PIKfyve is responsible for generating Stage I melanosomes, the retrograde traffic of endosomal proteins to the trans-Golgi network (TGN), and endocytic recycling to the plasma membrane.

PIKfyve can also play a part in yeast cell transcription, as PI(3,5)P2 modulates the assembly of the Tup1/Cyc8/Cti6 transcription complex via direct interaction of Tup1 and Cti6. For mammalian cells, PIKfyve contributes to IL-12 expression by transcriptional upregulation of the repressor ATF3. Additionally, PIKfyve regulates transcription factor EB (TFEB), which plays a key role in autophagy and lysosomal biogenesis [2].

PIKFYVE, including lipid phosphatase Fig4 (Sac) and a scaffold protein Vac14, combine to form a regulatory complex that localizes to endosomes and lysosomes, regulating endosome-to-TGN retrograde transport and lysosome fission. Targeting PIKfyve, which plays a crucial role in early embryonic development, is embryonic lethal [3].

1.3 Role in Cancer, Viral Infections, Neurodegenerative Diseases

As a class III lipid kinase that was discovered over two decades ago, PIKfyve is a potential target for diverse biological treatments and diseases.

Historically, PIKfyve's relevance was first revealed in a study on apilimod, a drug developed to inhibit production of interleukins IL-12 and IL-23 via Toll-like receptor (TLR), disrupting lysosomal function. Apilimod was ineffective for treating autoimmune diseases such as rheumatoid arthritis within the last decade, unfortunately due to the lack of knowledge concerning apilimod's specific molecular targets. By implicating PIKfyve, interleukin blockage was mediated by improving TLR-induced signaling, thereby proving PIKfyve's potential [4].

Additionally, Gayle et al 2019 found Pikfyve inhibitor apilimod to induce B-NHL cytotoxicity through impairing endolysosomal membrane traffic, disrupting lysosomal homeostasis and function. Through discovering a kinase domain mutation presenting resistance and knockdown approaches, apilimod was shown to have high specificity selective cytotoxic activity in B-NHL compared with normal cells and was driven by PIKfyve inhibition. Supported by a genome-wide CRISPR screen, apilimod's disruption of lysosomal homeostasis shows to be a new approach to treat B-NHL [5]. Apilimod can also trigger non-apoptotic death through excessive vacuolation in cancer cells. However, there are limitations as apilimod exhibits low plasma levels in patients treated with maximum oral dosage and is inactivated in cultured cells. To overcome these obstacles, we need to further develop drug combinations of PIKfyve inhibitors with effective pharmacokinetics and understand the mechanisms behind the PIKfyve inhibitors' vacuolation in sensitive cancer cells [6].

Campos et al 2020 reported PIKfyve kinase inhibitors involvement in anti-myeloma activity in vivo and ex vivo. They found that PIKfyve inhibitors disrupted lysosomal function and autophagic flux. Overall, PIKfyve proved to hold clinical potential in anti-myeloma strategies [7].

Cheng et al 2024 researched how targeting PIKfyve-driven lipid homeostasis caused pancreatic ductal adenocarcinoma (PDAC) to upregulate *de novo* lipid synthesis through upregulating the ACACA and FASN genes. The results suggest co-targeting PIKfyve and FASN or ACACA through KRAS-MAPK-directed therapies as a therapeutic strategy for PDAC which induces synthetic lethality for PDAS by disrupting lipid metabolism through PIKfyve inhibition [8].

PIKfyve presents diverse uses, as it is involved in the entry of viruses into host cells. Various affected viral diseases include the Ebola virus and coronaviruses such as SARS-CoV-2. Kang et al 2020 reported the pharmacological inhibition of PIKfyve by apilimod suppressed the release of the viral Ebola and SARS-CoV-2 genome into the cytoplasm by blocking fusion sites on endosomes, preventing entry into various mammalian cell lines [9].

For PIKfyve's role in neurodegenerative diseases, PIKfyve has been shown to act as a regulator of distributing tau aggregates, decreasing the lysosomal delivery and progression of tauopathies. This was done with the PIKfyve inhibitor YM201636, also shown to improve motor neuron survival developed from pluripotent stem cells [10].

Generally, PIKfyve also plays a critical role in transcription, cellular homeostasis, and membrane trafficking. PIKfyve activity triggers the activity of Atg 18, known for its involvement in autophagy and autophagolysosome formation—a vacuole that is a fusion of autophagosomes and lysosomes, responsible for breaking down proteins and junk in cells [3].

PIKfyve is a key factor in initiating autophagic flux and lysosomal biogenesis. For PIKfyve's involvement in cancer and immune responses, PIKfyve is responsible for degrading MHC-I by autophagic flux, which plays a role in cell-mediated immunity and T cell recognition. MHC-I binds to peptide fragments of pathogens to signal the proper T cells for recognition, which is vital for initiating anti-tumor immunity and immunotherapy. [2]

2. Immunotherapy

Immunotherapy is a prominent solution to improving cancer patients' conditions in a non-invasive fashion [11]. However, as patients of certain cancer subsets fail to respond to treatment, the rising need for a solution is critical. Currently, the lack of understanding concerning how cancer cells adapt to the tumor microenvironment by genetically manipulating MHC-I is prohibiting the development of an adaptable treatment to override cancer cells' ability to resist immunotherapies. In particular, understanding first how cancer cells can downregulate MHC-I and hinder T cells to escape antitumor immunity is a must [12].

To combat the resistance to clinical immunotherapies in certain cancer patients and to reveal what genes within our cells downregulate MHC-I naturally, scientists have come across PIKfyve, a gene that plays a role in autophagy. Thus, they have looked at PIKfyve as a viable strategy to enhance immunotherapy responsiveness and efficacy for unresponsive cancer patients.

Bao et al. 2023 reported that genetically and pharmacologically manipulating the PIKfyve gene led to enhanced cancer cell killing via CD8+ T cells and upregulating MHC-I [2].They reported that by pharmacologically inhibiting PIKyve and genetic knockout in two cancer cell lines (KPC1361 pancreatic cancer cell line and B16-F10 melanoma cell line), MHC-I surface expression increased, allowing for increased CD8+ T cell recognition of cancer cells. This improves immunotherapy by slowing tumor progression and enhanced antitumor responses in a T cell- and MHC-I-dependent environment.

3. Dendritic Cells (DCs)

Choi et al. 2024 utilized a commercial screening library investigating 25 Phase I/Phase II/FDA-approved protein kinases inhibitors (ALK, AURKA, BTK, CSF1R, EGFR, FGFR1, FLT1, FLT3, IGF1, IGFR1, IKBKB, JAK1, JAK2, KDR, KIT, MET, MTOR, NTRK1, PDGFRA, PIKFYVE,

PTK2, RAF1, RET, SRC, and SYK) to find the relevance of these gene targets in ICB-induced tumor immunity [13].

The scientists utilized published single cell RNA-seq (scRNA-seq) datasets to find PIKfyve expressed in immune cells across multiple cancer types [14]. Through a study of patients with melanoma treated with ICB, PIKfyve expression was lower in DCs of the responding patients while high PIKfyve expression in DCs in ICB non-responding patients [15]. This suggested that higher PIKfyve expression was associated with worse overall survival. PIKfyve may play a significant role in cancer immunity and ICB-associated outcomes by being a potential therapeutically targetable gene.

Researchers found that PIKfyve gene targeting and pharmacological inhibition led to enhanced DCs function through regulation of the alternate/non-canonical NF-κB pathway. Through treatment with apilimod—a PIKfyve inhibitor—and loss of PIKfyve in DCs, this resulted in decreased tumor growth, enhanced DC-dependent T cell immunity, potentiated ICB efficacy, and reduced tumor progression in vivo. Overall, researchers found that PIKfyve is a negative control for DCs and PIKfyve inhibition is a potential strategy for cancer immunotherapy and vaccine treatment.

By conducting RNA-seq studies, the researchers found that a gene signature of enhanced DC maturation was enriched in Pikfyve KO cDCs. Additionally, they also found enrichment of the "TNF_SIGNALING_VIA_NFκB" gene set in Pikfyve KO versus WT cDCs. NF-κB is known to play a critical role in driving the maturation and acute activation of DCs as an important transcription factor. Through posteriori analysis, the activation of downstream NF-kB genes was validated, leading the researchers to discover that this signature was positively enriched in Pikfyve KO versus WT cDCs [13].

4. Conclusion

Overall, PIKfyve inhibition has diverse impacts. Inhibition can improve CD8+ T cell function by increasing MHC-I expression through genetic targeting of PIKfyve and pharmacological PIKfyve inhibition while also playing a potential role in neurodegenerative diseases via DCs. Furthermore, this gene targeting can activate dendritic cells, leading to enhanced CD8+ T cell antitumor immunity through the NF-kB pathway.

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