Phosphatidylinositol 3-kinases (PI3Ks) play key roles in tumorigenesis. *PIK3CA*, which encodes PI3K complex catalytic subunit p110α, is one of the most frequently mutated oncogenes in human cancers.1 So, targeting p110α holds great promise for cancer therapy. Alpelisib, a small molecule inhibitor specifically targeting *PIK3CA*/p110α, has been approved by FDA to treat HR-positive and HER2-negative breast cancer patients harboring *PIK3CA* gene mutations.2 Most *PIK3CA*/p110α mutations occur at two hot spot regions: an acidic cluster (E542, E545, and Q546) in the helical domain and a histidine residue (H1047) in the kinase domain.3 Although all these hot-spot mutations activate the PI3-kinase activity, p110α helical domain mutations and kinase domain mutations promote tumorigenesis through different molecular mechanisms.4 Moreover, *PIK3CA* helical domain mutant tumors were less responsive to alpelisib treatment compared with *PIK3CA* H1047R mutant tumors in early clinical trials,5 while the mechanism has not been clearly clarified. Therefore, it is important to investigate the oncogenic mechanism and develop a more effective therapeutic strategy for tumors with *PIK3CA* helical domain mutations (Fig. 1).

The regulatory subunits of PI3K complex p85s normally stabilize p110 subunits and inhibit their enzymatic activity. Our previous studies have demonstrated that the p110α proteins with helical domain mutations can directly
interact with IRS1 to activate downstream AKT signaling. This process is independent of p85s. Whether p85s play important role in tumors with PIK3CA helical domain mutations remains unknown. Recently, we reported that p85\(b\) translocated into the nucleus and epigenetically regulated genes expression via stabilizing EZH1/2 proteins in PIK3CA helical mutant tumors. A combination of EZH inhibitors and the p110\(a\) inhibitor alpelisib induced the repression of tumors with PIK3CA helical domain mutations. This study indicates that simultaneously targeting EZHs and p110\(a\) could be a potential effective therapeutic strategy for PIK3CA helical domain mutant cancers.

We identified a nuclear localization signal (NLS) at amino acids 474 to 484 of p85\(b\) which mediated its nuclear translocation in cancer cells with a PIK3CA helical domain mutation. p85\(b\) could disassociate from p110\(\alpha\) helical-domain-mutated PI3K complex. p110-free p85\(b\) exposes NLS which is normally embedded in the interface of p85\(b\)/p110\(\alpha\) complex to trigger the nuclear translocation. Furthermore, our unpublished data indicates that the disassociation of p85\(b\)/p110\(\alpha\) rely on p85\(b\) tyrosine phosphorylation. These data suggest that p110-free p85\(b\) could actively translocate into the nucleus under certain physiological conditions.

Our study reveals the oncogenic function of nuclear-localized p85\(b\) in cancer development. Several studies showed p85\(b\) promoted the tumorigenesis through regulating PI3K activity and downstream AKT pathway. However, it is unknown whether p110-free p85s play important role in the tumorigenesis. In our study, we provided compelling evidence to show nuclear but not cytoplasmic p85\(b\) functions as an oncogene in tumors with PIK3CA helical domain mutations, which is independent of the canonical AKT signaling
pathway. In the patients with PIK3CA helical domain mutations, high PIK3R2/p85β levels were significantly correlated with poor overall survival. In cancer cells with PIK3CA helical domain mutations, depleting p85β or blocking p85β nuclear translocation could reduce cell proliferation, colony formation, and xenograft tumor growth. On the contrary, p85β didn’t show oncogenic function in cancer cells with PIK3CA kinase domain mutations or wild-type PIK3CA, which barely had nuclear-localized p85β. Together, we speculate p85β might perform oncogenic role through different signaling pathways in different cancers.

Our study demonstrates that the nuclear p85β stabilizes EZH1/2 and enhances H3K27 trimethylation in cancer cells with PIK3CA helical domain mutations. Nuclear p85β recruits deubiquitinase USP7 to histone methyltransferase EZH1/2 protein to prevent them from ubiquitin-mediated protein degradation, therefore enhancing the trimethylation of histone H3K27. As repressive histone mark, H3K27me3 was enriched at specific genome regions, especially the promoter region of downstream target genes such as tumor suppressor DLG2, thereby promoting the growth of PIK3CA helical domain mutant tumors. Thus, nuclear-localized p85β could directly involve in chromatin remodeling processes to serve as a transcriptional modulator in tumorigenesis.

Our study has important therapeutic implications. PIK3CA helical domain mutations promote tumorigenesis through both the canonical PI3K-AKT pathway and nuclear p85β-USP7-EZH1/2 pathways. It could partially explain that the patients with PIK3CA helical domain mutations are less responsive to alpelisib treatment. Therefore, simultaneously targeting EZHs and p110α could be a potentially effective therapeutic strategy for the patients with PIK3CA helical domain mutations. We observed that combination of alpelisib and EZH inhibitors inhibited the growth synergistically of CRC cell-derived xenograft (CDX) and patient-derived xenograft (PDX) tumors with a PIK3CA helical domain mutation, but only had additive effect on CRCs with either wild-type PIK3CA or PIK3CA H1047R mutation. As both EZH2 inhibitor tazemetostat and alpelisib are FDA-approved drugs for cancer, the evaluation of combinational effect of alpelisib and tazemetostat on the patients with PIK3CA helical domain mutations could be achieved in clinical trials soon. Currently, we have only tested the drug combination in CRC models. It is worth investigating whether this drug combination could be effective in other types of cancer patients harboring PIK3CA helical domain mutations.

In summary, our studies provide both conceptual advances to the field and therapeutic implications. Firstly, it sheds new light on the nuclear translocation and function of p85β. Secondly, nuclear-localized p85β is identified as an epigenetic regulator. Thirdly, a combination of an EZH inhibitor and a p110α inhibitor would be an effective approach to treat PIK3CA helical domain mutant cancers.

**Author contributions**

B.H. drafted the main text and figure. Y.H. revised the manuscript.

**Conflict of interests**

The authors declare no conflict of interest.

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