Hot spot oncogenic mutations of Phospatidylinositol 3-Kinase α: Establishment of a new membrane-based activity assay and identification of novel specific allosteric inhibitors.

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ABSTRACT

In cancer treatment, chemotherapeutic drugs aim at inhibiting proliferating cancer cells. The main drawback of these treatments is lack of specificity, which causes serious side effects on healthy cells. PIK3CA encodes the catalytic subunit of class I PI3Kinase (p110α), which phoshorylates plasma membrane phosphatidylinositol-4,5-bisphosphate at position 3 of the inositol ring, to produce phosphatidyl-inositol-3,4,5-trisphosphate (PIP_3). This gene is one of the most frequently mutated in human cancer. Although mutations occur throughout PIK3CA, two hotspot mutations (H1047R in the kinase domain and E545K in the helical) are the most common ones, representing 80% of the mutations found in cancer. The mechanism responsible for the catalytic benefit of these mutations is not yet fully understood. Here we report the development of an assay that assesses quantitatively the enzymatic activity of PI3K. The conditions of the assay are optimized to rely on vesicle membranes reconstituted with PIP2 and lipids isolated from cancer cells, thereby recapitulating the catalytic properties of the membrane bound enzyme. Using this method, we show that the H1047R mutant of PI3Kinase owes its catalytic benefit, over the wt enzyme, to its interaction with the lipid membrane. Additionally, we provide proof of principle that the assay can be used for screening “de novo” designed molecules, or libraries of compounds, and we identify a lead compound that acts allosterically and inhibits specifically the H1047R mutant. These data demonstrate the strength of this assay in identifying new specific drugs against the oncogenic mutants of PI3Kinase, with minimal side effects for healthy cells.