Journal of Genetics and Gene Therapy

Extended Abstract

The Effects of p110 Synthetic Inhibitors in Mouse Neutrophils and Macrophages

Samira Khaliq

University of Balochistan, Pakistan.
Email: samkh811@yahoo.com

Abstract

Phosphatidylinositol 3-Kinases (PI3Ks) are a family of lipid kinases that synthesize PtdIns(3,4,5)P3, and in doing so, relay signals from external cues, regulating multiple signaling pathways such as cell proliferation,growth, survival, motility, and metabolism. Hyperactivation of PI3K signaling cascades is one among the foremost common events in human cancers. Tremendous efforts are dedicated to the event of effective PI3K inhibitors for cancer therapy. Initial PI3K-directed drugs in clinical trials, consisting largely of non-isoform-selective pan-PI3K inhibitors, haven't yielded exciting results. However, recent preclinical studies have demonstrated that different PI3K isoforms play divergent roles in cellular signaling and cancer, suggesting that inhibitors targeting individual isoforms may be able to achieve greater therapeutic efficacy. Isoform-selective inhibitors are now emerging in the clinic, and have had promising success. ClassI PI3K catalytic isoforms share a conserved domain structure. They utilize the same lipid substrates and generate the same lipid products. Despite their similarities, accumulating evidence indicates these isoforms have distinct roles in mediating PI3K signaling in physiological and oncogenic contexts.PI3K has been reported to be either pro- or anti-inflammatory in several model systems. We hypothesized that this might flow from to different activities of the p110 and isoforms of PI3K.The role of class I PI3kinase isoforms is well studied in processes like chemotaxis, cell differentiation,apoptosis, and ROS production in myeloid cells. However, little is known about their involvement in phagocytosis and the exact mechanism underlying the regulation of this process.This study was aimed at investigating the involvement of p110 isoforms in Fc-mediated phagocytosis of IgG tagged SRBC in mouse neutrophils, macrophages and RAW cell line.p110γ-/- neutrophils, p110γ-/- macrophages, p110γ KD in Raw cells or Inhibition of p110γ by AS-603 (p110γ specific inhibitor) all showed significant reduction of uptake of IgG tagged SRBCs as compared to untreated cells. RFP-tagged p110γ localized at the base of protrusions forming the phagosome, within 25 seconds of the initial contact between the particle and the cell surface but re-localized away from the phagosome within the next 30 seconds in wild type RAW cells. Since p110γ is known to have a RBD,when these cells were incubated with Ras activation inhibitor specific to act downstream of RTK signalling, it was observed that in 57% of the cells p110γ did not localize to the phagosome whereas in 20% of the cells it did localize initially but did not relocate and move away. On the other hand, when primary neutrophils, macrophages and RAW cells, were treated with Ras activation inhibitor, they showed a comparable decrease in phagocytic uptake to the cells which were treated with LY294002 or AS-603 alone. Phagocytic uptake did not show any significant reduction when a combination of LY294002 and Ras activation Inhibitor or AS604-850 and Ras activation inhibitor was used.This indicates that p110γ has a role in regulating Fc mediated phagocytosis and that this role of p110γ is Ras dependent and directly or indirectly modulating cytoskeleton activity at these sites via Rac1 in myeloid cells.For prophylactic treatment, mice were administered CAL-101 (idelalisib/GS-1101/Zydelig17) (0.05 mg/mouse) or IC87114 (0.5 mg/mouse) (both from Selleck Chemicals LLC, TX, USA), intraperitoneally or orally18 twice a day, 24 h prior to infection. Intraperitoneal or oral injection of CAL-101 and/or IC87114 was continued every 12 h for 2 weeks. For therapeutic treatment, mice were infected with L. donovani promastigotes and after 1 week or 2 weeks CAL-101 was administered intraperitoneally every 12 h

for an additional period of 2 weeks. For combination therapy, mice were infected with L. donovani promastigotes and after 2 weeks were treated intraperitoneally with CAL-101 (0.05 mg/mouse), amphotericin B (Fungizone, Bristol-Myers Squibb, Montreal, Canada; 0.1 mg/kg), combined CAL-101 and amphotericin B or PBS once daily for 5 consecutive days and sacrificed at 1 week or 3 weeks after last treatment. Additionally, 1 week after the last combined CAL-101 and amphotericin B treatment, some mice were treated with anti-IFN-γ MAb (1 mg/mouse intraperitoneally) as previously described.19,20 Mice were sacrificed 2 weeks post-anti-IFN-γ mAb administration. In all experiments, control mice received DMSO in PBS (vehicle control). ANA-1 cells were grown in complete RPMI medium23 and infected with 7 day stationary-phase L. donovani or L. major promastigotes at a cell-to-parasite ratio of 1:5 for 5 h. Free parasites were washed away and infected macrophages were further cultured for 24 and 72 h at 37 °C in the presence of IC87114 (0, 1 and 10 µM) or CAL-101 (0, 0.1 and 1 µM) or amphotericin B (10 nM).25–27 At the end of the cultures, the level of infection was determined by counting haematoxylin/eosin-stained cytospin preparations under a Zeiss Primo Star (Zeiss, Melville, NY, USA) light microscope with a ×100 (oil) objective. Neutrophilic inflammation is central to chronic inflammatory diseases such as rheumatoid arthritis and chronic obstructive pulmonary disease (COPD), which impose an increasing social and economic burden on our aging population. Treatment of COPD by next-generation combination therapy with inhaled corticosteroids and newer bronchodilators are viewed as maintenance pharmacotherapies but they do not specifically target cellular inflammation. The anti-inflammatory phosphodiesterase-4 inhibitor, roflumilast, targets systemic inflammation associated with COPD and reduces moderate to Chronic obstructive pulmonary disease (or COPD) is a serious condition that causes the lungs to become inflamed for long periods of time, leading to permanent damage of the airways. Immune cells referred to as neutrophils promote inflammation after an injury, or during an infection, to assist the healing process. However, if they're active for too long, they'll also cause tissue damage and drive inflammatory diseases including COPD. To limit damage to the body, neutrophils usually have a very short lifespan and die by a regulated process known as apoptosis. Finding ways to stimulate apoptosis in neutrophils could also be key to developing better treatments for inflammatory diseases. Cells contain many enzymes known as kinases that control apoptosis and other cell processes. Drugs that inhibit specific kinases are effective treatments for a few sorts of cancer and other conditions, and new kinase-inhibiting drugs are currently being developed.However, it remains unclear which kinases regulate apoptosis in neutrophils or which kinase-inhibiting drugs may have the potential to treat COPD and other inflammatory diseases.