











Advances in nanotechnology-based drug delivery in targeting PI3K signaling in respiratory diseases

Yinghan Chan¹, Ronan MacLoughlin^{2,3,4}, Flavia C Zacconi⁵, Murtaza M Tambuwala⁶, Ritesh M Pabari³, Sachin Kumar Singh⁷, Terezinha de Jesus Andreoli Pinto⁸, Gaurav Gupta⁹, Dinesh Kumar Chellappan^{*,10} & Kamal Dua^{*,11,12}

¹School of Pharmacy, International Medical University (IMU), Bukit Jalil, 57000 Kuala Lumpur, Malaysia

²Aerogen, IDA Business Park, Dangan, Galway H91 HE94, Ireland

³School of Pharmacy & Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin D02 YN77, Ireland

⁴School of Pharmacy & Pharmaceutical Sciences, Trinity College, Dublin D02 PN40, Ireland

⁵Departamento de Química Orgánica, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Av Vicuña Mackenna 4860, Macul, Santiago 7820436, Chile

⁶School of Pharmacy & Pharmaceutical Sciences, Ulster University, Coleraine, Londonderry, Northern Ireland, UK

⁷School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi GT Road, Phagwara, 144411, Punjab, India

⁸Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo 05508-000, Brazil

⁹School of Pharmacy, Suresh Gyan Vihar University, Jagatpura, Jaipur 302017, India

¹⁰Department of Life Sciences, School of Pharmacy, International Medical University (IMU), Bukit Jalil 57000 Kuala Lumpur, Malaysia

¹¹Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, NSW 2007, Australia

¹²Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute (HMRI), University of Newcastle, New Lambton Heights, Newcastle, NSW 2305, Australia

*Author for correspondence: Tel.: +6129 514 7387; Kamal.Dua@uts.edu.au

**Author for correspondence: Tel.: +60 126361308; Dinesh.Kumar@imu.edu.my

“Nanomaterials offer great prospects to be developed as advanced and versatile drug delivery platforms targeting PI3K signaling in the treatment of CRDs owing to their unique physicochemical properties, at the same time, they have been shown to improve the pharmacokinetics of existing therapeutics”

First draft submitted: 3 March 2021; Accepted for publication: 24 March 2021; Published online: 17 May 2021

Keywords: chronic respiratory disease • drug delivery • nanomaterials • phosphoinositide 3-kinase

Inflammation is the natural defense mechanism within the human body to combat and eliminate hazardous stimuli, such as irritants, pathogens and damaged cells. Although inflammation can be beneficial in the resolution of injury, chronic inflammation is a concern, as chronically inflamed tissues often amplify the inflammatory response by recruiting immune cells from the bloodstream, leading to excessive self-targeted aggressiveness that results in disease. With respect to the respiratory system, excessive airway inflammation is the hallmark of various CRDs, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis and acute respiratory distress syndrome [1,2]. CRDs are among the main contributors to major public health burdens as they account for a significant proportion of global morbidity and mortality, thereby warranting the development of new targeted therapeutic approaches to tackle the increasing impact brought by these diseases. PI3Ks are one of the main families of kinases that play a central role in mediating signaling cascades that lead to the initiation and maintenance of inflammatory responses. As aberrant PI3K signaling can result in the development of CRDs, the selective targeting of individual PI3K isoforms is a promising therapeutic approach for the regulation of immune responses [3]. Nanomaterials offer great prospects to be developed as advanced and versatile drug delivery platforms targeting PI3K signaling in the treatment of CRDs owing to their unique physicochemical properties, at the same time, they have been shown to improve the pharmacokinetics of existing therapeutics [4,5]. This commentary describes the

roles of PI3Ks in the pathogenesis of CRDs and the applications of advanced drug delivery systems in targeting PI3K signaling for the management of CRDs.

PI3Ks & their signaling pathways

PI3Ks are intracellular signaling proteins involved in a myriad of cellular events and they have been widely considered as potential targets in the treatment of various human diseases. The PI3K family can be divided into three subtypes depending on their structure, namely classes I, II and III. Class I PI3Ks can be further classified into class IA which comprises of isoforms PI3K α , PI3K β and PI3K δ , as well as class IB which comprises of the PI3K γ isoform. While the roles of classes II and III PI3Ks in inflammatory responses are currently not fully understood, many studies have reported that class I PI3Ks play central roles in multiple steps of the inflammatory cascades. Structurally, class I PI3Ks exist as heterodimeric complexes where p110, a catalytic subunit, is associated with a particular regulatory subunit, such as p85, p55, p50, p101, and p84 [6,7]. Upon activation by cell surface receptors such as insulin, growth factors and G-protein-coupled receptors (GPCRs), class I PI3Ks initiate signaling pathways by phosphorylating phosphoinositol lipids at the D-3 position of the inositol ring, which then converts phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂) into phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P₃) that acts as a second messenger in the recruitment of cytosolic signaling proteins possessing pleckstrin homology domains to specific locations within the plasma membrane or endomembrane. Representative effectors of class I PI3Ks include protein serine/threonine kinases (protein kinase B/Akt), protein kinase C, PDK1 and MAPK. In summary, PI3K is regarded as a hub in which signals are first relayed prior to specialization into secondary signaling [6–8].

Significance of PI3Ks in CRDs

Asthma is an example of a CRD characterized by airway hyper-responsiveness and inflammation, with an increased expression of cytokines, chemokines, growth factors and adhesion molecules, as well as enzymes. Typically, exposure to allergens induces Th2 differentiation of T cells which leads to release of cytokines that promote allergic inflammation. It has been demonstrated that class I PI3Ks are involved in the activation, differentiation and proliferation of T cells, with PI3K δ and PI3K γ being the predominant isoforms. Upon engagement of T cell receptor by antigens, PI3K δ will be activated by tyrosine kinase signaling cascades; at the same time, PI3K γ will be recruited by the activation of G proteins via GPCRs and chemokine receptors [7,9]. PI3Ks are also involved in mediating airway smooth muscles and epithelial cell responses. The contraction of airway smooth muscles and accumulation of contractile proteins are typically modulated by the activation of PI3K via the regulation of Rho kinase. Further, the secretion of IL-6 by airway smooth muscles is regulated by PI3K, in which IL-6 is a cytokine central to airway remodeling and development of airway hyper-responsiveness in the pathogenesis of asthma [9]. In addition, PI3K δ promotes the expression of IL-17 via the regulation of NF- κ B signaling cascade, as proven in the study by Park *et al.*, which reported that airway inflammation and hyper-responsiveness were attenuated by the suppression of PI3K δ [10]. Increased eosinophils leading to induction of pro-inflammatory cytokines, Th2 cytokines, chemokines and reactive oxygen species (ROS) is another key feature of asthma, whereby it has been found that inhibition of PI3K γ suppresses eosinophil chemotaxis. This has been proven in multiple studies which demonstrated that PI3K inhibition led to reduced eosinophilia and airway hyper-responsiveness in models of allergen challenge. Studies also revealed that both PI3K δ and PI3K γ are responsible for the activation of mast cells, in which they function in cascade with PI3K δ acting as the initial response to IgE, followed by PI3K γ to maximize degranulation [7,9,11]. In summary, PI3K is a crucial signaling molecule that is involved in almost all aspects of asthma pathogenesis, thus, inhibition of PI3K may be therapeutically advantageous as it can prevent mast cell degranulation, inhibit the recruitment of immune cells, suppress mucus hyperproduction, as well as to facilitate bronchodilation.

A similar strategy can also be employed to manage other inflammatory CRDs, such as COPD. In contrast to asthma, chronic inflammation in COPD primarily involves Th1-induced neutrophils and macrophages. It has been established that PI3K signaling is strongly induced in COPD and it is correlated with increased susceptibility to lung infection. Studies have also reported that PI3K activation is necessary for the migration of neutrophils and infiltration of monocytes into the lung. Moreover, the release of ROS in neutrophils is found to be dependent on the activation of both PI3K γ and PI3K δ in a biphasic manner [8,12]. Cystic fibrosis is another example of a CRD which is a result of a mutation in the gene responsible for encoding cystic fibrosis transmembrane conductance regulator. In cystic fibrosis, the lungs of patients may rapidly develop inflammation characterized by an influx of

polymorphonuclear cells, which is correlated with an imbalance between anti- and pro-inflammatory mediators. As the ability of PI3K δ and PI3K γ in modulating the influx of pro-inflammatory mediators has been widely documented in the inflammatory models of asthma and COPD, specific targeting of these PI3K isoforms may be exploited as an alternative management approach in the management of cystic fibrosis [8,13].

Applications of advanced drug delivery systems

Due to the involvement of PI3Ks in immune responses, isoforms of PI3Ks and their signaling pathways have been explored as promising targets in the design and development of therapeutics for the management of CRDs. Although various PI3K-targeted compounds have been developed, some of them face limitations including poor bioavailability and pharmacokinetic variability, incidences of adverse reactions, as well as poor targeting ability.

In recent years, the development of advanced drug delivery systems using nanomaterials has gained significant attention due to their potential for targeted delivery and their sustained release profile when compared with unconjugated compounds. Nanotechnological advancements have allowed surface functionalization of site-selective ligands, which can further improve targeting toward specific PI3K isoform while enhancing the cellular uptake, bioavailability, biodistribution and biocirculation time of loaded compounds. Nanomaterials may also possess intrinsic biological activities that offer synergistic benefits along with those of the loaded compounds. Overall, utilization of advanced drug delivery systems is highly beneficial in the development of novel therapeutics targeting PI3K signaling in CRDs, as it allows customization where the engineering of such nanovehicles can be tailored to meet specific applications [5,14,15].

There have been multiple studies performed in recent years to evaluate the feasibility of advanced drug delivery systems in targeting PI3Ks for managing CRDs. A study by de Oliveira *et al.* formulated resveratrol (RSV)-loaded lipid-core nanocapsules (RSV-LNCs) composed by biodegradable polymers to evaluate their efficacy in attenuating acute lung injury. It was reported that RSV-LNCs remarkably improved lung function by preventing the accumulation of leucocytes and neutrophils in the lung tissue via blockade of the PI3K/Akt signaling pathway. Conversely, unloaded RSV was found to be ineffective in preventing the production of pro-inflammatory cytokines in lung tissues [16]. Similarly, Gholizadeh *et al.* loaded dactolisib, a potent class I PI3K inhibitor into polymeric nanoparticles and evaluated their anti-inflammatory potential. It was demonstrated that the nanoparticles could effectively deliver dactolisib to TNF- α activated cells, suggesting that they can be developed as novel therapeutics for inflammatory CRDs by targeted inhibition of PI3Ks [17]. Further, Jang *et al.* investigated the effects of silver nanoparticles on mucus hyperproduction, which is a key feature in allergic airway inflammation. Results indicated that the administration of silver nanoparticles significantly reduced the expressions of PI3Ks in lung tissues, which led to the suppression of mucus hyperproduction via the downregulation of PI3K/HIF-1 α /VEGF signaling pathway [18]. On the other hand, Poerio *et al.* developed liposomes loaded with phosphatidylinositol-5-phosphate (PI-5-P) and evaluated their potential in limiting tissue-damaging inflammatory response in cystic fibrosis associated infections [19]. PI-5-P is a second messenger that promotes bacterial phagocytosis by modulating the PI3K/Akt signaling pathway. The results showed that the liposomes remarkably downregulated NF- κ B and the production of IL-1 β , IL-6 and TNF- α . The infiltration of pulmonary neutrophils was also remarkably reduced, while enhancing bacterial uptake, phagosome acidification and intracellular bacterial killing in cystic fibrosis macrophages. Taken together, these findings suggest that liposomal nanovehicles enable a targeted approach in enhancing phagocytosis and ameliorate chronic inflammatory response in cystic fibrosis patients [19]. Furthermore, a recent study by Pooladanda *et al.* engineered iRGD peptide-conjugated liposomes loaded with nimbolide (iRGD-NIMLip), an anti-inflammatory phytomedicine, in treating acute respiratory distress syndrome, which shares many of the clinical manifestations as those associated with the recent outbreak of the COVID-19 [20]. It was reported that iRGD-NIMLip attenuated lipopolysaccharide-induced expressions of inflammatory genes via the PI3K/Akt/mTOR signaling pathway. Oxidative stress and cytokine storm were also remarkably suppressed by iRGD-NIMLip in contrast to free nimbolide. Thus, these findings suggest that advanced drug delivery system can be employed as novel therapeutics targeting chronic lung inflammation and other pathological consequences associated with COVID-19 and other CRDs [20].

Conclusion

Although PI3Ks regulate various key processes in the inflammatory responses of human body against damage and infections, dysregulated PI3K signaling in the lung tissues may lead to augmented self-perpetuating damage, resulting in the development of CRDs. Advanced nano-based drug delivery systems have the potential to be

employed as both therapeutics and vectors in the management of CRDs via PI3K-targeted approaches, as they possess multiple key advantages attributed to their unique physicochemical characteristics as compared with those of conventional therapeutics. Additionally, modern nanotechnological approaches allow for surface and structural functionalization of drug delivery systems, thereby enhancing their targeting capability and therapeutic efficacies. Unfortunately, to date, the studies conducted in this area of research remain relatively scarce. Hence, more in-depth studies should be performed to elucidate the exact targeting mechanisms involved, which allows for future clinical translation of safe and effective novel therapeutics in the management of CRDs.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1. Aghasafari P, George U, Pidaparti R. A review of inflammatory mechanism in airway diseases. *Inflamm. Res.* 68(1), 59–74 (2019).
2. Lee KY, Ito K, Maneechotesuwan K. Inflammation to pulmonary diseases. *Mediators Inflamm.* 2016, 7401245 (2016).
3. Jeong JS, Kim JS, Kim SR, Lee YC. Defining bronchial asthma with phosphoinositide 3-kinase delta activation: towards endotype-driven management. *Int. J. Mol. Sci.* 20(14), 3525 (2019).
4. Chan Y, Ng SW, Chellappan DK *et al.* Celastrol-loaded liquid crystalline nanoparticles as an anti-inflammatory intervention for the treatment of asthma. *Int. J. Polym. Mater. Polym. Biomater.* doi:10.1080/00914037.2020.1765350 (2020) (Epub ahead of print).
5. Chan Y, Ng SW, Mehta M, Gupta G, Chellappan DK, Dua K. Sugar-based nanoparticles for respiratory diseases: a new paradigm in the nanoworld. *Future Med. Chem.* 12(21), 1887–1890 (2020).
6. Stokes CA, Condcliffe AM. Phosphoinositide 3-kinase δ (PI3K δ) in respiratory disease. *Biochem. Soc. Trans.* 46(2), 361–369 (2018).
7. Yoo EJ, Ojiaku CA, Sunder K, Panettieri RA. Phosphoinositide 3-kinase in asthma: novel roles and therapeutic approaches. *Am. J. Respir. Cell Mol. Biol.* 56(6), 700–707 (2017).
8. Marwick JA, Chung KF, Adcock IM. Phosphatidylinositol 3-kinase isoforms as targets in respiratory disease. *Ther. Adv. Respir. Dis.* 4(1), 19–34 (2010).
9. Medina-Tato DA, Ward SG, Watson ML. Phosphoinositide 3-kinase signaling in lung disease: leucocytes and beyond. *Immunology* 121(4), 448–461 (2007).
10. Park SJ, Lee KS, Kim SR *et al.* Phosphoinositide 3-kinase δ inhibitor suppresses interleukin-17 expression in a murine asthma model. *Eur. Respir. J.* 36(6), 1448–1459 (2010).
11. Ghigo A, Damilano F, Braccini L, Hirsch E. PI3K inhibition in inflammation: toward tailored therapies for specific diseases. *BioEssays* 32(3), 185–196 (2010).
12. Pirozzi F, Ren K, Murabito A, Ghigo A. PI3K signaling in chronic obstructive pulmonary disease: mechanisms, targets, and therapy. *Curr. Med. Chem.* 26(16), 2791–2800 (2018).
13. Adam D, Roux-Delrieu J, Luczka E *et al.* Cystic fibrosis airway epithelium remodelling: involvement of inflammation. *J. Pathol.* 235(3), 408–419 (2015).
14. Chan Y, Ng SW, Mehta M *et al.* Advanced drug delivery systems can assist in managing influenza virus infection: a hypothesis. *Med. Hypotheses* 144, 110298 (2020).
15. Sharma VR, Sharma AK, Punj V, Priya P. Recent nanotechnological interventions targeting PI3K/Akt/mTOR pathway: a focus on breast cancer. *Semin. Cancer Biol.* 59, 133–146 (2019).
16. de Oliveira MTP, Coutinho D de S, de Souza ÉT *et al.* Orally delivered resveratrol-loaded lipid-core nanocapsules ameliorate LPS-induced acute lung injury via the ERK and PI3K/Akt pathways. *Int. J. Nanomedicine* 14, 5215–5228 (2019).
17. Gholizadeh S, Kamps JAAM, Hennink WE, Kok RJ. PLGA-PEG nanoparticles for targeted delivery of the mTOR/PI3kinase inhibitor dactolisib to inflamed endothelium. *Int. J. Pharm.* 548(2), 747–758 (2018).
18. Jang S, Park JW, Cha HR *et al.* Silver nanoparticles modify VEGF signaling pathway and mucus hypersecretion in allergic airway inflammation. *Int. J. Nanomedicine* 7, 1329–1343 (2012).
19. Poerio N, de Santis F, Rossi A *et al.* Liposomes loaded with phosphatidylinositol 5-phosphate improve the antimicrobial response to *pseudomonas aeruginosa* in impaired macrophages from cystic fibrosis patients and limit airway inflammatory response. *Front. Immunol.* 11, 532225 (2020).
20. Pooladanda V, Thatikonda S, Sunnapu O *et al.* iRGD conjugated nimbolide liposomes protect against endotoxin induced acute respiratory distress syndrome. *Nanomedicine* 33, 102351 (2021).