Inflammatory Twins from PI3K Gang Brought to Justice?

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PI3 kinase inhibitors are hot property. In this issue of *Chemistry & Biology*, Williams et al. add a dual PI3Kδ/γ inhibitor to the collection and show that its anti-inflammatory profile in vitro is quite different from pan-PI3K inhibitors, but bears an uncanny resemblance to that of the glucocorticoid drugs.

In 2007, Rommel et al. suggested that PI3Kδ and PI3Kγ were partners in crime in inflammation—working in concert and terrorizing our immune systems like cellular versions of Monty Python's London gangsters, the cruel-but-fair Piranha Brothers (http://www.ethelthefrog.com/?tag=youtube). Williams et al. (2010) describe the compound SW14, their version of the determined Superintendent Harry "Snapper" Organs, single-handedly bringing down these kinase twins, compiling a broad dossier of evidence against them, and foreshadowing an exciting approach to treating inflammatory and immunological diseases.

From the first identification of PI3 kinase as a fundamental component of cell signaling, PI3K inhibitors have been postulated as drugs of the future for treating a range of disease states. In particular, the first generation inhibitors wortmannin and LY294002 were shown to block the oxidative burst after neutrophils were treated with fMLP, which identified PI3K as a potential target for inflammatory and immunomodulatory diseases (Arcaro and Wymann, 1993; Vlahos et al., 1995). In the intervening years, progress across the field has included the elucidation of PI3K isoform structures and localization, and the development of genetic and pharmacological systems that define the pathways regulated by PI3K isoforms (Hirsch et al., 2008). By 2003, both PI3Kγ and PI3Kδ (or Doug and Dinsdale) had been identified as validated drug targets in immune and inflammatory disease (Rommel et al., 2007).

Further studies have shown that the PI3Kγ and PI3Kδ isoforms have overlapping but nonredundant roles in the inflammatory response, and the leap to dual selectivity inhibitors that do not touch the more widespread PI3Kα and PI3Kβ isoforms was an attractive next step—attractive but not necessarily simple. Previously developed PI3K inhibitors had either modest selectivity or tend to cluster along PI3Kα/γ and PI3Kβ/δ lines (Knight et al., 2006). However, in 2006, Shokat's and Williams' groups were able to co-crystallize a delta selective compound, PIK39, into the PI3Kγ gamma catalytic domain, revealing an extraordinary alteration in the binding site to accommodate a bulky quinazoline structure (Figure 1) (Knight et al., 2006). The detective's hunch was that the same shift, centered on a methionine residue, occurs and is favored in PI3Kδ, explaining the remarkable selectivity of IC87114 and analogs (Sadhu et al., 2003). Further, they asserted that dual selectivity PI3Kδ/γ inhibitors would be achievable by rational design. In this article, the synthetic achievement and the demonstrable selectivity are described (Williams et al., 2010). For what must have been a thrilling confirmation of the design principle, the X-ray structure of this family in the PI3Kδ isoform matched their prediction precisely. In compounds such as SW14, they had the tool to evaluate the effects of combined PI3Kδ/γ inhibition in comparison to pan-PI3K and PI3Kδ-only inhibitors (Berndt et al., 2010). In the first set of in vitro experiments, they confirmed the PI3Kδ activity associated with macrophage colony stimulating factor (M-CSF/CSF-1) action on THP-1 monocytes and the PI3Kγ activity associated with chemokine, monocyte chemoattractant protein-1 (MCP-1) confirming the selectivity profile of these compounds in a cellular context.

In many such stories, the next stop is at in vivo animal models, but the authors next took an unconventional step, subjecting the compounds to a battery of in vitro assays using the proprietry BioMAP® system from BioSeek (Biologically Multiplexed Activity Profiling), importantly using primary human cell preparations. In this approach, combinations of primary cell types are simultaneously activated to replicate intricate cell and pathway interactions normally found in disease physiology (Kunkel et al., 2004). In this profiling approach, human umbilical vein endothelial cells (HUVECs), alone or in combination with peripheral blood mononuclear cells (PBMCs), are stimulated with various inflammatory signaling molecules (IL-1β, TNFα, IFN-γ, IL-4, histamine, lipopolysaccharide, and Superantigen), with 40 different readouts (mainly ELISA-derived levels of receptors, cytokines, cell adhesion molecules, and second messengers) collected and compared to other compounds of other classes. Thankfully, in this case the results cluster into digestible pieces; the studies show that the most effective and T-cell selective inhibitors of inflammatory signals inhibit both PI3Kδ and PI3Kγ, but not PI3Kα and PI3Kβ.

Dual targeting of PI3Kδ/γ resulted effective inhibition of LPS-induced TNFα production and overall T cell activation. Particularly notable was the diminution of E-selectin production upon Superantigen treatment. PI3Kδ inhibition yielded similar but less efficacious results. Pan-PI3K inhibition (PIK90) was highly antiproliferative to both HUVECs and PMBCs, but somewhat surprisingly yielded a relatively diminished action on T cells compared to inhibition of PI3Kδ/γ. The authors speculate on the mechanism of the PI3Kδ/γ pathway, noting recently identified links between PI3Kγ and PDE4 signaling and the identified role of PDE4 inhibition in TNFα production, but the question

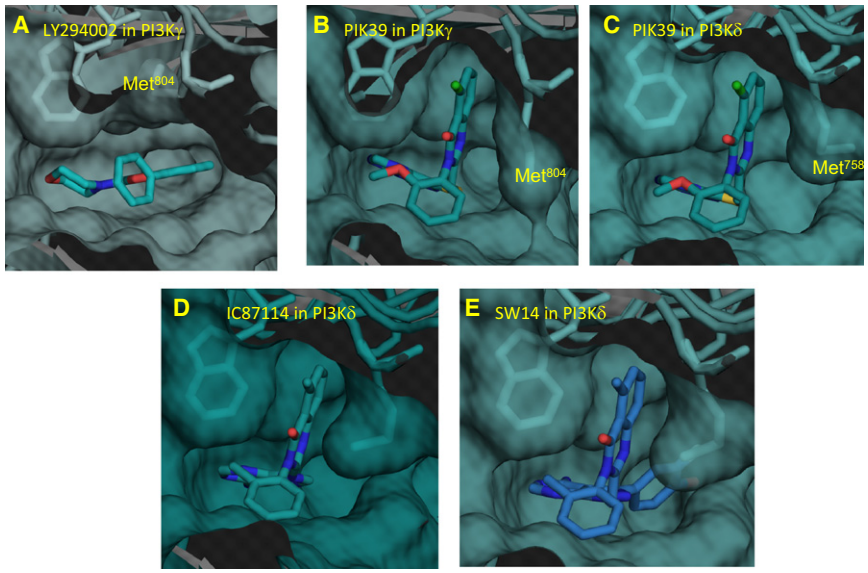

Figure 1. Crystal Structures of PI3K-Inhibitor Complexes
Poses of PI3K inhibitors in PI3Kγ and PI3Kδ showing (A) typical PI3K binding site conformer: LY294002 in PI3Kγ (PDB code: 1E7V), and (B-E) showing induced pocket adjacent to methionine: (B) PIK39 in PI3Kγ (2CHW); (C) PIK39 in PI3Kδ (2WXF); (D) IC87114 in PI3Kδ (2WXE); (E) SW14 in PI3Kδ (2WXH). Images were generated using Pymol.

remains open to further study. The apparent inhibition of this effect by additional PI3Kα/β inhibition shows the complexity of even this refined inflammatory cell system.

Last but not least, the authors asked if the anti-inflammatory signature is reminiscent of other anti-inflammatory drugs. Direct comparison of the profiles of SW14, SW18, and prednisolone showed significant similarities, especially with regard to their effect on TNFα production in the LPS-stimulated HUVEC/PBMC coculture. The BioMAP® profiles for each compound were analyzed using a Pearson correlation metric and visualized in two dimensions through a multi-dimensional scaling algorithm. Strikingly, compounds with significant activity against PI3Kδ/γ (SW14, SW18) were functionally linked to the glucocorticoid prednisolone. Remarkably, in this profiling the PI3Kδ/γ inhibitors are more similar to prednisolone than they are similar to pan-PI3K compounds, which themselves grouped with inhibitors mTOR, microtubule modulators, and estrogens, all of which have been linked to PI3K in other contexts. Interestingly, a similar link with prednisolone was previously observed in studies of PDE4 inhibitors and prednisolone (Kunkel et al., 2004). The demonstrated efficacy of prednisolone and the advanced clinical status of PDE4 inhibitors suggest that this might be a profile worth seeking in drugs irrespective of molecular mechanism.

The discovery of PI3Kδ/γ inhibitors that display up to 1,000-fold selectivity over PI3Kα/β is a worthy feat in itself, and such compounds can head toward the fraught trail of drug development, where pharmaceutical properties of inhibitors will also determine clinical progress. By providing a BioMAP® analysis, they have demonstrated the favorable anti-inflammatory properties against primary human cells. This is a strong commendation of the potential therapeutic effect, but not a replacement for whole human body. Time will tell if taking these gang leaders off the inflammatory paths will make them safe.

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