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Omics approaches in cystic fibrosis research: a focus on oxylipin profiling in airway secretions

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Cystic fibrosis (CF) is associated with abnormal lipid metabolism, intense respiratory tract (RT) infection, and inflammation, eventually resulting in lung tissue destruction and respiratory failure. The CF RT inflammatory milieu, as reflected by airway secretions, includes a complex array of inflammatory mediators, bacterial products, and host secretions. It is dominated by neutrophils and their proteolytic and oxidative products and includes a wide spectrum of bioactive lipids produced by both host and presumably microbial metabolic pathways. The fairly recent advent of "omics" technologies has greatly increased capabilities of further interrogating this easily obtainable RT compartment that represents the apical culture media of the underlying RT epithelial cells. This paper discusses issues related to the study of CF omics with a focus on the profiling of CF RT oxylipins. Challenges in their identification/quantitation in RT fluids, their pathways of origin, and their potential utility for understanding CF RT inflammatory and oxidative processes are highlighted. Finally, the utility of oxylipin metabolic profiling in directing optimal therapeutic approaches and determining the efficacy of various interventions is discussed.

Keywords: cystic fibrosis; inflammation; omics; oxylipins; respiratory tract secretions

Introduction

Cystic fibrosis (CF) is a genetic disease ascribed to mutations in the *CFTR* gene, which codes for the widely expressed CFTR chloride channel. Clinically, the disease manifestations include pancreatic insufficiency, intestinal malabsorption, and respiratory tract (RT) defects in fluid and electrolyte balance, mucociliary clearance, host defenses against microbes, and heightened inflammatory/immune responses. The resulting nutritional deficiencies include abnormalities in lipid absorption, including lipophilic antioxidant micronutrients, whereas the RT abnormalities are dominated by progressively more severe bacterial infection and the overly aggressive inflammatory response leading to lung destruction and premature death.[1]

Inflammation and oxidative stress in the CF RT

The interrelated RT pathobiology of CF involves chronic airway infection by microorganisms overcoming CF RT host defenses, and a dysregulated and heightened RT inflammatory response characterized by a progressively more intense sustained neutrophil recruitment and activation.[2] Abundant evidence supports the ongoing pro-oxidative environment of CF RT secretions,[3–5] which is largely attributed to activation of the NADPH oxidase (Nox-2) and further exacerbated by the catalytic activity of myeloperoxidase (MPO) of the recruited airway neutrophils, including contributions of pro-oxidant species released from microorganisms, such as *Pseudomonas aeruginosa*, and from endogenous RT

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epithelial cells (i.e., Duox enzymes). The presence of elevated levels of the nonenzymatic lipid peroxidation products of arachidonic acid (AA) (i.e., isoprostanes) in CF plasma, buccal mucosa cells, breath condensate, and bronchoalveolar lavage fluids attests to the pro-oxidant status of the CF RT. Although active RT oxidative processes represent a well-recognized hallmark in CF RT secretions affecting both host and resident microbe biology,[4,6] efficacious anti-inflammatory and antioxidant therapies in CF have not met with major success. For example, inhaled GSH trials have failed to improve any standard biomarkers of RT oxidative stress.[7,8] *N*-acetylcysteine (NAC) administration trials in CF patients have also failed to improve RT biomarkers of oxidative stress or yield convincing evidence of efficacy.[9] It can be concluded that, despite two decades of efforts, there is no strong case for supplemental antioxidant administrations beyond those to sustain normal plasma levels of the lipophilic antioxidants (e.g., vitamin E).

Omic approaches in CF

Newer methods for more qualitative and quantitative characterization of the pathobiology driven by *CFTR* mutations giving rise to CF phenotypes are integrating with the myriad of molecular biology that has emerged in the two decades since discovery of the *CF* gene. These new-era technologies include those focused on systems-level approaches to global analysis of cell, tissue, and organ CF tissues.[10,11] As illustrated in Figure 1, these technologies focus on mutant CFTR effects on cellular message levels (genomics/transcriptomics), cellular protein and protein networks (proteomics), lipids and lipid metabolic pathways (lipidomics), and overall integrative omics (metabolomics). All of the new omics approaches are in the quest of further understanding CF pathophysiology that could reveal strategies for improved therapeutic approaches and clinical patient outcomes.

Many of the omics approaches have been targeted toward increased understanding of how mutated CFTR impacts on basic cellular functions beyond its primary role in chloride ion transport.[12–19] Other omics studies have focused on CF tissues,[18,20–22] CF blood compartments,[23–25] bronchoalveolar lavage fluid,[26,27] and exhaled breath condensate.[28,29] These latter two fluids have the advantage that they are collected directly from the RT, the tissue of greatest impact by CF. However, these two methods have the disadvantages that they are approximately 100 times and 10,000 times diluted with water, respectively, and neither collects secretions exclusively from the airways, the lung compartment most directly affected in CF. Several of the omic interrogations have incriminated the involvement of inflammatory-immune system genes in determining the severity of a given mutant *CFTR* genotype's phenotype.[30–32] Other omic studies have presented evidence suggesting a mechanistic link between mutations in CFTR and acquisition of the proinflammatory phenotype in the CF RT.[33] Mutant CFTR effects on CF neutrophil function may even represent manifestations of one such activity modulating neutrophil function,[34] although this may be related to the compromised chloride ion transport function associated with mutant CFTR.[35] A recent paper has presented evidence for an even more widespread mutant CFTR modulation of function in cells of the inflammatory/immune system that is generally recognized.[36]

The overall field of lipidomics has progressed somewhat slower than that of genomics and

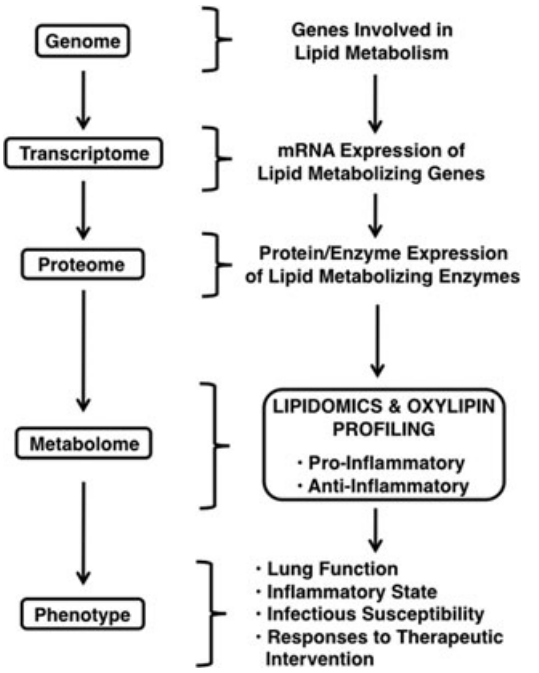

Figure 1. Generalized and lipid-specific scheme to illustrate omics pathways from gene to phenotype with regard to understanding CF airway biology and pathobiology.

proteomics. Contributing factors could include the diversity of biomolecular lipid classes, including fatty acids and their derivatives (amides, esters, and oxygenated species), phosopholipids, di- and triglycerides, sphingolipids, sterols, and numerous nonenzymatic oxylipids (e.g., isoprostanes). Lipid isolation from complex tissue matrices, extraction efficiencies, availability of standards, bioinformatic tools, and integration with imaging and other omics databases are only just being refined. Nonetheless, technologies are advancing rapidly.[37,38] Some of these studies have addressed oxidized phosopholipids,[39] including those in CF patients.[40,41] Many papers have focused on lipidomic profiling of inflammatory lipid mediators (lipoxins) including the well-known eicosanoids synthesized from AA (20:4), as well as the major omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA, 20:5) and docosohexaenoic acid (DHA, 22:6).[42–45] Two excellent recent reviews have detailed protocols and addressed lipid mediator profiling in various CF tissue matrices.[46,47]

Oxylipin profiling of CF RT airway secretions

The term *oxylipin* (sputum) covers a broad spectrum of compounds, many of which have high biological activities and which are formed from unsaturated fatty acids in a cascade of reactions, most of which include at least one step of mono- or dioxygen-catalyzed oxygenation. There are several reasons for focusing on RT oxylipins in CF, a disease known to be associated with abnormalities of lipid absorption, both in extracellular and cellular lipid constituencies.[48–56] AA levels appear higher, whereas longer chained 20:5 and 22:6 fatty acids appear reduced, probably largely (but not only) as a function of their reduced absorption and dysregulated metabolism.[57,58] Recently, genes in the AA–prostagladin–endoperoxide synthetase pathway have been reported as possible modulators of disease severity in CF patients.[59] A wide spectrum of oxidative lipid products, most notably those of 20:4, 20:5, and 22:6, are increasingly being recognized to be important regulators of the intensity and duration of acute and chronic inflammatory pathways,[43,45,59–64] including those initiated in the lung by P450 pathways upon microbial exposure.[65] Of relevance, there are several reports of abnormalities of polyunsaturated lipid oxidation products, such as the lipoxins in inflammatory RT CF secretions, that have been reported.[66,67]

As of 2011, we are not aware of any published studies applying broad-spectrum oxylipin profiling methods to investigate the intensely infected and inflamed CF RT. The resident pool of oxylipins can be expected to be embedded in a complex matrix of CF sputum[68,69] that itself has both pro-oxidant and antioxidant properties.[3] Although it is relatively easy to collect freshly expectorated RT secretions from CF patients, specimen processing including measures to prevent further artifactual oxidation, lipid extraction methods, and strategies for evaluating extraction efficiency and data expression present practical, yet surmountable challenges. In several cases, key validation compounds (quantitative internal standards) of interest are not readily available from individual or corporate investigators. This has particularly presented obstacles in the determination of several of the more recently described oxidized polyunsaturated fatty acids with novel

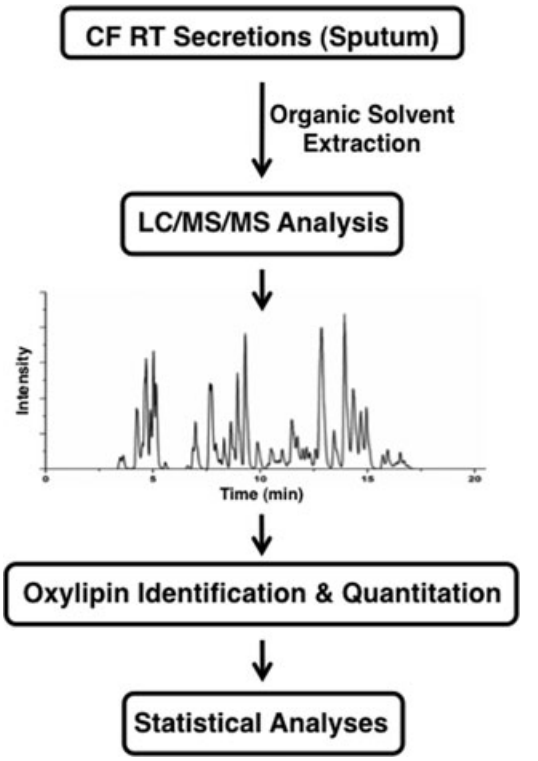

Figure 2. Generalized scheme illustrating an LC/MS/MS metabolomic approach to comprehensively evaluate bioactive oxylipin profiles in CF RT secretions.

anti-inflammatory properties. The CF mucus oxylipin profile can be expected to represent the omics of not only most inflammatory cells and RT epithelial cells but also reflect the contributions of a wide spectrum of airway microbiota[70] including *P. aeruginosa*, in the context of their residence in the CF airway.[71,72]

Despite these challenges, our laboratory groups have embarked upon developing methods (both sample preparation and analytical) to ascertain the profile of oxylipins in RT secretions from subjects capable of producing spontaneously expectorated sputum, as is the case for most adult CF patients. We have found that liquid–liquid solvent extraction of freshly obtained whole expectorated sputum is the most highly efficient method for recovering oxylipins. We preserve/stabilize the specimens with butylated hydroxyltoluene, triphenylphosphine, and a broad spectrum COX inhibitor (indomethacin). The specimens are immediately frozen on dry ice and we take all practical steps to minimize exposure to air (it would be preferable to overlay an inert gas) during the subsequent work-up procedures. We subsequently use a state-of-the-art LC/MS/MS analytical method previously optimized,[73] and which is capable of screening nearly 100 diverse oxylipins within a single LC run of 20 minutes (Fig. 2). Thus far, we have been capable of identifying greater than 30 oxylipins in adult CF sputums. As illustrated in Figure 2, employing this analytical procedure, and coupled with multiple statistical methods, we have established that this oxylipin metabolomic method provides a potentially unique view into the complex inflammatory-immune processes occurring in the CF airway. A general scheme that illustrates our method is depicted in Figure 2. Of the detected oxylipins, approximately 75% are derived from AA, 20% from linoleic acid (LA), and a much smaller quantity from EPA and DHA. The predominant source of oxylipin metabolites in CF sputum is the 12-lipoxygenase pathway (12-LOX; ∼50%), followed by 5-lipoxygenase (5-LOX; ∼35%), cyclooxygenase (∼5%), 15-LOX (∼3%), and the remainder coming from P-450 dependent pathways. Not surprisingly, the predominant oxylipin metabolites present in the CF RT are typically regarded as proinflammatory. Much lower levels of anti-inflammatory oxylipins were detected including Resolvin E1. Interestingly, Lipoxin A4 was not detected in any of the CF sputum specimens we examined,

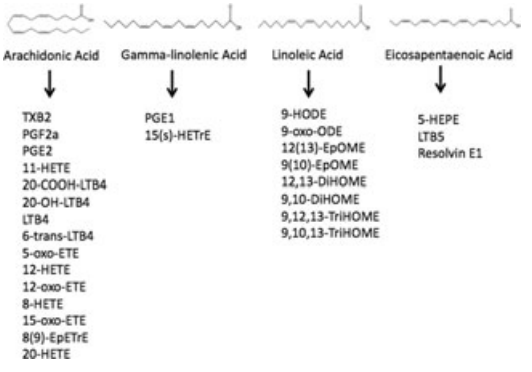

Figure 3. **Summary of oxylipins detected in adult CF sputum as a function of the parent unsaturated fatty acid.**

suggesting a deficiency of this anti-inflammatory lipid mediator. Figure 3 summarizes the oxylipins we have detected in CF sputum to date using our liquid chromatography/massspectrometry/mass spectrometry (LC/MS/MS) method, inclusive of their parent fatty acid. Preliminary analyses using the oxylipin metabolome in aggregate, as opposed to individual metabolite measurements, provides a more robust association with overall lung function (assessed by forced expiratory volume in 1 second [FEV1], % of predicted). The complete data and discussion of these findings are the topic of a recently accepted manuscript.[74]

Potential microbial contribution to oxylipins in CF sputum

Although the prevailing wisdom would indicate that oxylipins present in the CF airway likely primarily arise from host cells and tissues/lipid substrates, the presence of large numbers of bacteria (particularly *P. aeruginosa*) begs the question as to the possible involvement of microorganisms in the synthesis and metabolism of the analyzed RT oxylipins. *P. aeruginosa* contain primarily short chain saturated fatty acids,[75] thus they are not a source of the parent fatty acids of the oxylipins we have detected in our studies. However, recent studies have revealed that *P. aeruginosa* express a secreted cytotoxin (ExoU) with phospholipase activity capable of liberating free unsaturated fatty acids (LA and AA) from host cells.[76] Moreover, *P. aeruginosa* express a number of fatty acid metabolizing enzymes, including dioxygenases, hydroperoxide isomerases, and arachidonate 15-lipoxygenase,[77,78] that may directly contribute to the oxygenation of fatty acids in the CF airway.

A recent study has also identified an epoxide hydrolase produced by *P. aeruginosa*[79] that could potentially be used to convert epoxyeicosanoic acids (EETs), thus diminishing the anti-inflammatory functions of these oxylipins in the CF RT. The possibility that *P. aeruginosa*, and potentially other bacteria, may play a key role in the synthesis and metabolism of bioactive oxylipins in the CF airway is particularly exciting and remains a fertile area of investigation.

Translational implications

It is not difficult to envision how comprehensive oxylipin profiling could be incorporated into an increased understanding of the inflammatory versus anti-inflammatory bioactive lipid contributions to inflammatory-immune processes at RT apical cellular surfaces. Such mechanistic data could be incorporated into clinical trials focused on ameliorating the excessive RT inflammation in CF, and inform efficacies of systemic versus aerosolized treatments, when linked to appropriate patient outcome studies.[80–82]

RT oxylipin profiling in CF should provide dose-ranging insights with regard to nutritional and drug therapies targeted to modify bioactive lipid contributions to RT inflammatory pathobiology. These include the overly exuberant proinflammatory processes in CF that are possibly related to the high AA and low DHA/EPA concentrations observed in patients with CF, and the degree to which CF lipid and oxylipin abnormalities can be influenced by dietary[49,83–87] or pharmacological[88–90] interventions, including even antibiotics with anti-inflammatory activities that are used in treating CF patients,[91] statins,[92,93] and antiproteases.[94] It is important to note that some of these approaches are likely to modulate both host and microbe, and their interactions, in addition to solely affecting host inflammatory pathways. A theoretical list of emerging CF therapies and approaches that could influence CF RT lipidomics and reveal anti-inflammatory strategies are depicted in Table 1. Such approaches appear strengthened by recent data showing that select anti-inflammatory oxylipins (or their fatty acid precursors) appear to both facilitate antimicrobial activity and decrease inflammatory processes,[95] stimulating experimental activities designed for focal delivery of these compounds directly to the CF RT via inhalation routes.

Table 1. Therapeutic approaches that could influence CF RT oxylipin profiles

Modulators of cyclooxygenase (COX) and leukotriene pathways
Modulators of RT lipid metabolism
Statins
Phospholipase inhibitors
Cytochrome P450 modulators
PPAR gamma agonists
Soluble epoxide hydrolase inhibitors
Antibiotics with anti-inflammatory properties, such as azithromycin and tetracycline
Inhaled antiproteases
Sildenafil
Oral, systemic, or inhaled anti-inflammatory oxylipins

Clinical studies of the full inflammatory/anti-inflammatory profile of oxylipins in the CF RT are needed to more fully unveil significance of anti-inflammatory oxylipin-based therapies in the disease. Many of these oxylipins are already known to be therapeutic targets of and contributors of inflammatory-immune processes, but their efficacy within the RT airway fluids has not been extensively investigated. As therapeutic manipulation of both proinflammatory and anti-inflammatory oxylipin profiles are increasingly being proposed as effective RT disease modifiers, it can be speculated that more than one oxylipin target or pathway will be needed for addressing this aspect of anti-inflammatory therapy in CF.

Summary

The purpose of identifying CF omic signatures is to provide clinically useful clues for improved patient management and disease outcome. Further omic characterization of the infected, inflamed, and oxidizing RT fluids (separating the atmospheric environment from the underlying RT cells) should provide for new insights into CF disease pathobiology. Such omic approaches should in addition mechanistically inform new targets for anti-inflammatory and antioxidant therapies and complement clinical studies specifically designed to decrease overzealous RT inflammatory-immune pathways. Interestingly, eicosanoid lipidomics are starting to be examined in human breath condensates.[42] Finally,

detailed studies of the inflamed CF RT secretions could lead to further understanding and therapeutics of the broad swath of RT diseases characterized by activation of RT inflammatory-immune processes, including those initiated by toxic inhaled environmental agents.

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Conflicts of interest

The authors declare no conflicts of interest.

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