Mitochondrial quality control as potential therapy

Mitochondria are dynamic organelles that are present in almost every cell type of the human body, where they carry out many essential functions. They are responsible for producing 90% of the body's energy via oxidative phosphorylation to generate adenosine triphosphate (ATP). In addition, they produce biosynthetic intermediates, function in oxygen sensing and play a role in mediating calcium homeostasis, cell growth and programmed cell death (apoptosis). When mitochondria become 'old' or damaged and are no longer able to efficiently produce energy they are eliminated from the cells by a process known as mitophagy, where autophagosomes engulf and remove them from the internal cell environment, and they are then degraded by lysosomes.

Originally mitophagy was considered a phenomenon that was only observed *in vitro*, but recent conclusive evidence clearly demonstrates that it is a natural and continuous homeostatic process that occurs *in vivo*[1]. It is a part of the normal cellular turnover of mitochondria and is essential to keeping cells healthy. If this process is impaired or fails it leads to the accumulation of dysfunctional mitochondria, which induces oxidative stress within a cell, leading to cellular degeneration. The role of mitochondrial dysfunction and the impairment of mitophagy in disease and pathology is starting to be elucidated, highlighting the importance of finding therapeutic strategies that help promote mitochondrial clearance and quality control.

Mitochondrial quality control and human disease

It is emerging that mitophagy has a particularly critical role in Parkinson's disease (PD), which affects around 10 million people worldwide. The exact mechanisms underlying PD are unclear, however mitochondrial dysfunction is increasingly appreciated as a key determinant. Given the post-mitotic (non-dividing) nature of adult neurons, removal of dysfunctional mitochondria is essential to preventing the gradual accumulation of oxidative stress, Ca2+ dysregulation, and subsequent impaired neuronal function and survival. Failure of mitochondrial quality control may lead to degeneration or death of the highly active *substantia nigra* neurons in the brain, a pathological mechanism that results in PD.

In mitophagy, the enzymes E3 ubiquitin ligases such as parkin (also known as PARK2) and the protein kinase PTEN-induced putative kinase 1 (PINK1) collaborate to ubiquitylate, or 'tag' mitochondrial membrane proteins of damaged mitochondria with ubiquitin, a 7.5 kDa regulatory protein. This tagging promotes the clearance of these poorly functioning mitochondria from normal healthy cells. Definitive genetic studies have provided a clear link between inhibitory mutations in parkin and PINK1 to PD, thus directly implicating low ubiquitylation and poor mitochondrial quality control in disease susceptibility. Identification of strategies that increase ubiquitylation and mitophagy in PD may lead to novel therapeutics. As such, Ubiquitin Specific Peptidase 30 (USP30), a 517-amino acid protein that is found in the mitochondrial outer membrane, has emerged as a promising new target for the mitochondria-related pathological degeneration experienced in PD[2].

Increasing USP30 expression removes ubiquitin molecules attached by parkin onto damaged mitochondria and blocks parkin's ability to promote mitophagy, while reducing USP30 activity enhances mitochondrial degradation in neurons. Therefore, inhibition of USP30 could be a potential strategy for the treatment of PD by promoting regular mitochondrial clearance and quality control. This also means that PD patients with parkin mutations linked to reduced mitophagy could be therapeutically compensated by inhibiting USP30.

Mission Therapeutics has developed specific and potent small molecule inhibitors of USP30. This was no small feat as until now the development of potent deubiquitinase (DUB) inhibitors suitable for clinical development proved challenging due to specificity and selectivity issues. Over 100 human DUBs have been identified, and this large family of intracellular proteins, expressed in many different human cell types, have many different links to disease pathologies. By combining new insights from structural research, improved understanding of DUB enzymology and biology and technological developments with novel chemistry, we have confirmed that DUBs are druggable, novel therapeutic targets[3]. As a result, our first-generation USP30 (and other DUB) inhibitors are now progressing towards clinical development.

Mission Therapeutic scientists are currently evaluating USP30 inhibitors for their potential to treat PD. Using a range of *in vitro* cell line models, including induced pluripotent stem cells (iPSC) derived from patients with sporadic and familial PD, and the mitochondrial outer membrane protein TOM20 as a tracking and visualisation marker, data generated from these studies illustrate that USP30 inhibitors increase mitochondrial substrate ubiquitylation and mitophagy. USP30 inhibitors have also been shown to provide protection against neuron loss in an *in vivo* model of PD. Currently these compounds are under preclinical evaluation for progression to clinical development.

There is a significant unmet medical need for the treatment of mitochondrial diseases, as current therapies are limited to treating certain symptoms only. Rare mitochondrial diseases are progressive and currently there is no cure. As a result of the pivotal roles that mitochondria play in the generation and regulation of energy metabolism, mitochondrial diseases can affect almost any part of the body, including the cells of the brain, nerves, muscles, kidneys, heart, liver, eyes, ears or pancreas. This can result in debilitating physical, developmental, and cognitive disabilities with symptoms including poor growth; loss of muscle coordination; muscle weakness and pain; seizures; vision and/or hearing loss; gastrointestinal issues; learning disabilities; and organ failure.

Rare mitochondrial diseases are chronic, genetic disorders that are caused by various inherited or acquired mutations, such as point mutations or deletions, in either the mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) that code for mitochondrial proteins. Around 200 mutations in mtDNA and 2,000 mutations in nDNA are linked to such diseases. Mitochondrial diseases that result from defects in

mtDNA are characterised by heteroplasmy and therefore contain a mixture of mitochondria with normal as well as mutated mtDNA. The mtDNA mutations reduce the activity of the ATP generation machinery harboured within the mitochondrial membranes.

Recent scientific data suggests that the removal of impaired mitochondria with mutant mtDNA can be achieved by activating the parkin-ubiquitin-mitophagy pathway[4]. Therefore, inhibiting USP30 might promote removal of impaired mitochondria which harbour significant mutant mtDNA, thus reducing associated oxidative stress and calcium dysregulation. Mission Therapeutics is testing the hypothesis that USP30 inhibition has the potential to provide a novel approach for reducing mitochondrial dysfunction in these types of mitochondrial diseases, with the goal of relieving or treating the associated symptoms.

Most recently, Mission Therapeutics has broadened its search for new potential therapeutic applications of improved mitochondrial quality control to more prevalent but equally debilitating conditions. A prominent modern public health concern is the development of organ fibrosis. This condition can occur in many different tissues in the body and is most often caused by inflammation or tissue damage. This results in collagen accumulation or fibrosis that can lead to scarring of vital organs such as the lung, liver, skin, eye, heart and kidney, causing irreparable damage and eventual organ failure. Fibrosis is estimated to be prevalent in 45% of all diseases and no clinically satisfactory therapeutic approach exists. It therefore represents a large unmet clinical need.

Idiopathic pulmonary fibrosis (IPF) is a specific type of lung fibrosis that is a rare chronic disease, which is ultimately fatal with the majority of people living only three to five years after diagnosis. Over time, tissue deep in the lungs becomes scarred, resulting in a progressive decline in lung function and shortness of breath. The current IPF drugs on the market, Pirfenidone and Nintedanib, are anti-fibrotic agents that slow down the reduction in lung volume characteristic of IPF patients. However, these compounds only slow the progression of the disease and do not act as a cure by halting or reversing the deterioration in lung function.

Recent scientific and clinical studies have demonstrated that insufficient or impaired mitophagy is involved in the pathogenesis of IPF[4]. Reduced mitophagy in IPF has been implicated in increasing apoptosis and cellular senescence in epithelial lung cells. It also enhances fibrogenic myofibroblast differentiation in lung fibroblasts that promotes the accumulation of fibrotic tissue. Activating mitophagy through USP30 inhibition therefore represents a promising therapeutic approach for treating IPF. At Mission Therapeutics we are currently testing USP30 inhibitors and comparing them to Pirfenidone in IPF disease models. Our key USP30 inhibitor prevents collagen deposition and the generation of an abnormal excess of cells, or hypercellularity, in an *in vivo* bleomycin-induced lung fibrosis model, with comparative efficacy to Pirfenidone. This efficacy has been shown in both preventative and therapeutic testing paradigms.

As well as IPF, kidney fibrosis represents an interesting and highly prevalent target condition. Kidney fibrosis may occur at any stage of kidney disease, from chronic kidney disease (CKD) through to end-stage renal disease (ESRD). Kidney fibrosis can develop as a result of cardiovascular disease such as hypertension or diabetes, both of which place immense strain on kidney function which promotes a fibrotic response. However, kidney fibrosis can also be idiopathic (i.e. without a known cause), and certain genetic mitochondrial diseases also present kidney fibrosis manifestations and associated symptoms. Pathologically, kidney injury and fibrosis are often associated with fragmented mitochondria and increased oxidative stress. Most recently, high levels of constitutive mitophagy have been reported in the main functional units of the kidney, known as the nephron. Mission Therapeutics has validated antibodies for USP30 which can show whether the protein is expressed in tissues of interest for disease; kidney nephrons (proximal tubules and the glomerulus) are strongly positive for USP30. Our key peripherally active USP30 inhibitor prevents fibrotic collagen deposition in a surgical model of obstructive kidney injury and fibrosis, with similar efficacy to gold standard experimental tools (TGF-beta receptor inhibitors).

Similar to our hypothesis for IPF, initial results indicate that activating mitophagy by inhibiting USP30 has the potential to be a promising therapeutic approach for treating kidney fibrosis.

Conclusion

USP30 is looking like a particularly promising therapeutic target. If all goes to plan these programmes will be the first to move towards the clinic. Our multi-pronged approach into human testing reflects the breadth of Mission's technology and chemistry platform and the range of diseases in which USP30 is implicated. Mission has compounds with structures likely to penetrate the central nervous system (CNS), which may be suitable for use against PD. Other compounds are unable to cross the blood-brain barrier and therefore will have their primary mechanism of action outside the CNS i.e. peripherally acting therapeutic drugs; these might also be useful for treating fibrosis. Mitochondrial disorders are characterised by both peripheral and CNS symptoms and therefore can be tackled by compounds with either peripheral and/or CNS distributions.

Our USP30 programme represents the first step on our journey to develop first-in-class compounds to fulfil unmet medical needs.

References:

1. McWilliams, TG. et al. Mito-QC illuminates mitophagy and mitochondrial architecture *in vivo*. 3. J Cell Biol. 2016 Aug 1; 214(3):333-45.

2. Bingol, B. et al. The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature*. 2014; 510, 370–375.

3. Harrigan J.A et al. Deubiquitylating enzymes (DUBs) and drug discovery: emerging opportunities. *Nat. Rev. Drug Discov*. 2017; 17, 57–78 http://dx.doi.org/10.1038/nrd.2017.152

4. Kurita et al. Pirfenidone inhibits myofibroblast differentiation and lung fibrosis development during insufficient mitophagy. *Respiratory Research*. 2017; 18:114 https://doi.org/10.1186/s12931-017-0600-3

This article was written by Paul Thompson, VP, Clinical Development, Mission Therapeutics, which is located in Cambridge, UK.