***Connecting inflammatory bowel and neurodegenerative diseases: microRNAs as a shared therapeutic intervention***

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We read with interest the recent article by Zhang *et al* that reported a higher risk of developing dementia in patients with inflammatory bowel disease (IBD), with the largest increase in Alzheimer's disease (AD)[1]. These findings align with a growing body of evidence which links gut inflammation or leaky gut with neurodegeneration. Lee *et al* discussed the known shared pathophysiological links between IBD and Parkinson's disease (PD), underscoring the importance of genetic overlap, microbiota-gut-brain axis, autoimmunity, mitochondrial function and autophagy[2]. We would like to highlight another less-explored biological connection: microRNAs (miRNAs).

MicroRNAs are small non-coding RNAs, which regulate gene expression at the post-transcriptional level by silencing targeting mRNA(s). Intriguingly, miRNAs have been implicated in the pathogenesis of both IBD and neurodegenerative diseases (NDDs). miRNAs have emerged as important regulators of gut and blood-brain barrier (BBB) integrity[3-4]. Complementing these findings, we recently found significantly upregulated miR-23a-3p and miR-150-5p in the blood of patients who had undergone successful intestinal microbiota transplantation (IMT) for recurrent *C. difficile* infection (rCDI)[5]. Furthermore, we demonstrated the cytoprotective effects of combining these two IMT-regulated miRNAs in intestinal epithelial cell (IEC) lines exposed to *C. difficile* toxin B (TcdB)[5]. We validated these circulating miRNA signatures in rCDI-IMT and toxin-treated murine mucosal tissues and *ex vivo* human colonoids; we found that miR-23a-3p and miR-150-5p targets inflammatory genes *IL-12B* and *IL-18*, respectively[5]. Of note, serum miR-150-5p-p is downregulated in PD patients compared with healthy controls, where adenylate kinase 3 (AK3) was verified as a target of miR-150-5p in BV2 microglial cells[6]. Moreover, miR-150-5p is also protective of the BBB[4]. Similarly, miR-23a-3p is decreased in plasma-derived extracellular vesicles in AD[7].

We generated and evaluated the cytoprotective properties of polymer-based nanoparticles (NPs) loaded with miR-23a-3p and miR-150-5p in IEC and brain-derived cell lines. The NP formulations protected the intestinal epithelial barrier (IEB) from colitogenic toxins (TcdA+B) and lipopolysaccharide (LPS) (Fig 1A), with our preliminary data suggesting that this formulation has broad (non-toxin-specific) effects. The miRNA-NPs increased intracellular levels of miR-23a-3p and miR-150-5p in all IECs tested without cytotoxicity effects (representative data, Fig 1B). The NPs also effectively increased intracellular miR-23a-3p and miRNA-150-5p levels in three brain-derived cell lines (SH-SY5Y, LUHMES, A-172 glioma/glial) (Fig 2A) and demonstrated protective effects against LPS (Fig 2B).

Obefazimod (ABX464) is a recently discovered small quinolone which promotes specifically the upregulation of miR-124 (also implicated in PD pathogenesis[8]) in immune cells[9] and, in landmark clinical trials, has demonstrated safety and profound anti-inflammatory activity in induction and maintenance of UC[10]. We questioned if Obefazimod may affect miR-124 levels in neuronal cells and could similarly protect from LPS. Our data indicate that Obefazimod reduced miR-124 levels in brain-derived cells (Fig 2C), and that Obefazimod elicits cell type-specific protective effects in SH-SY5Y but not LUHMES or A-172 (Fig 2D). These findings do not exclude the possibility that Obefazimod elicits protective effects secondary to its gut anti-inflammatory action. Full methodological details, extended figure legends, and information on nanoparticle characterisation (Fig S1) can be found in the supplementary materials.

Overall, our preliminary data suggest that both miR-23a-3p and miR-150-5p in combination may elicit protective effects on the gut and brain. We surmise that future miRNA-based therapeutics could increase the resilience of the IEB and thus delay or even prevent the onset of NDDs such as PD. We believe that attention should now focus on uncovering other commonly dysregulated miRNAs such as those involved in mitochondrial dysfunction which may facilitate the development of dual therapy approaches. It will also be important to identify common pathways involving miRNAs with functional overlap across inflammatory disease states. To realise the full therapeutic potential of miRNA drug candidates, several major challenges associated with the miRNA delivery process will need to be overcome. Nonetheless, miRNA therapeutics will likely play a prominent role in treating disorders of the microbiota-gut-brain axis.

**Figures**

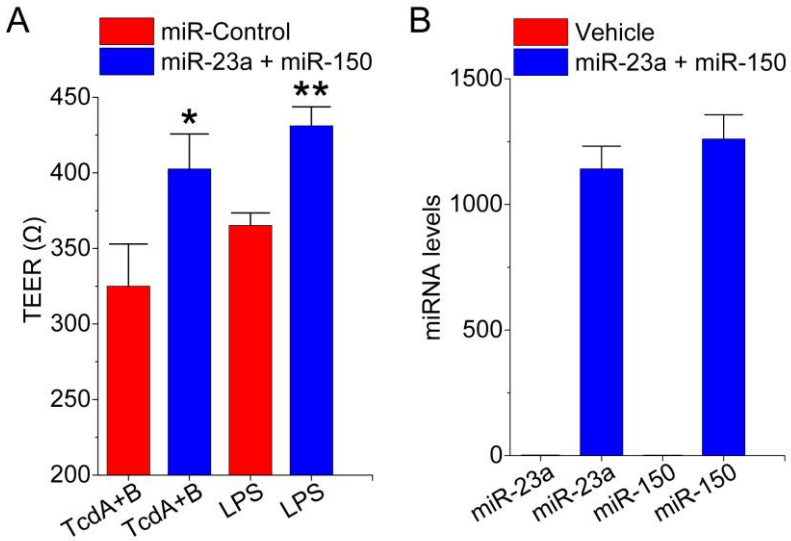


Figure 1. Effects of miR-23a and miR-150-loaded NPs on (A) transepithelial electrical (TEER) resistance of CaCO2/TC-7 cells treated with LPS or TcdA+B and (B) miRNA levels in NCM356 cells.

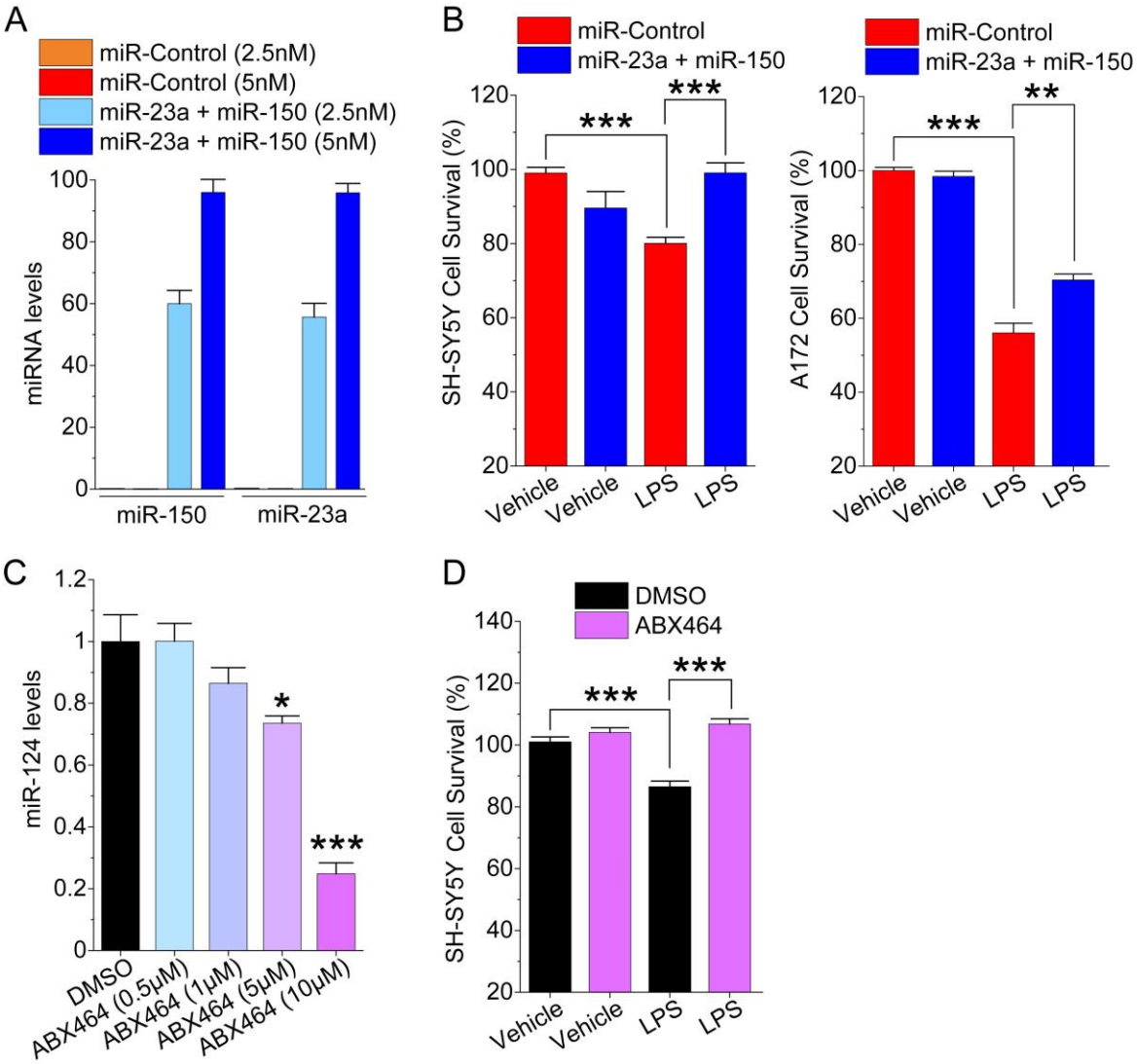


Figure 2. Effects of miR-23a and miR-150-loaded NPs on (A) miRNA levels in SH-SY5Y, and (B) cell survival of SH-SY5Y and A172 cells. Effects of Obefazimod (ABX464) on (C) miR-124 levels in differentiating LUHMES and (D) cell survival of SH-SY5Y cells.

See Supplementary for extended figure legends and methodology.

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