Genetic engineering of probiotics: a new pharmacological tool for inflammatory and obesity-linked disorders

Martina Vincenzi[1,*], Luisa Seguella[1], Chiara Corpetti[1], Jie Lu[2], Silvia Basili Franzin[1], Irene Palenca[1], Aurora Zilli[1], Daniele Caprioli[1], Walter Sanseverino[3], Giovanni Sarnelli[4], Giuseppe Esposito[1]

1 Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy;
2 Department of Anatomy and Cell biology, China Medical University, N0.77 Puhe Road, Shenbei New District, Shenyang City, Liaoning Province, PR China;
3 Sequentia Biotech SL, Carrer del Dr. Trueta, 179, 08005 Barcelona, Spain;
4 Department of Clinical Medicine and Surgery, University of Naples "Federico II", Via Pansini 5, 80131, Naples, Italy.

**martina.vincenzi@uniroma1.it*

Mood and metabolic disorders are tightly interrelated, suggesting a shared pathological process. Compelling evidence displays that a high-fat diet (HFD) elicits intestinal dysbiosis and persistent low-grade inflammation in the small intestine that remodel the epithelial barrier, leading to disruption of the neuroepithelial circuits that control energy homeostasis by the gut-brain axis. Therefore, therapies targeting the restoration of intestinal microbial niche and barrier function are promising candidates to counter peripheral metabolic challenges that affect behaviors controlled by the brain. The response of the intestine to the consumption of high-fat meals is mediated by N-oleoylethanolamine (OEA), an endogenous N-acylethanolamine that belongs to a group of autacoid local inflammation antagonism amides (ALIAmides). Recently, OEA was found to shape the intestinal microbiota profile towards a "lean-like phenotype", ameliorating pathological profiles of metabolic diseases. Further, OEA displays beneficial effects in several cognitive paradigms and preserves the epithelial barrier integrity, acting as an intestinal "gate-keeper". Here, we developed an "intestinal OEA factory" for the in-situ and controlled release of OEA by using a probiotic-based delivery system. We engineered the Lactobacillus paracasei F19 (LP) to express the human N-acylphosphatidylethanolamine-preferring phospholipase D (NAPE-PLD) gene and to produce OEA in response to dietary ultra-low oleate supply. The ability of LP to integrate himself with resident microbiota and its anaerobic nature provide a system for the continuous release of OEA in the intestine with an inherent biosafety feature. We treated 12-week HFD male mice with oleate-probiotic formulations and assessed their impact on metabolic and behavioral dysfunctions, and microbiota-gut-brain signaling after 8 weeks of the treatment. NAPE-expressing LP (pNAPE-LP) led to significant weight loss and metabolic dysfunction betterment in HFD-treated mice. Further, a parallel improvement of depressive- and anxiety-like phenotypes was associated with duodenal barrier function retrieval, the restoration of the *Firmicutes/Bacteroidetes* ratio, and an increase in beneficial bacteria, such as *Lactobacillus, Prevotella,* and *Parabacteroides.* The HFD-driven changes both in the enteric and central nervous system were prevented by pNAPE/oleate treatment. Collectively, our data suggest that these effects were mediated by the oleate-dependent release of OEA by pNAPE-LP since no significant effects were observed in HFD mice treated by the native probiotic alone (pLP). This oleate-regulated delivery system of OEA is a safe and efficient probiotic-based strategy for the treatment of metabolic syndrome and related behavioral disorders.