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ISOLATION AND FUNCTIONAL CHARACTERIZATION OF BROCCOLI SPROUTS NANOVESCICLES AS POTENTIAL MIRNA CARRIER IN HUMAN INTESTINAL CACO2 CELLS

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Broccoli are widely consumed in the world and are considered natural functional foods for their high content of a number of secondary metabolites with a recognised beneficial role for human health, namely glucosinolates which are almost exclusively present in vegetables of the Brassicaceae family. Young seedlings (sprouts), in particular, represent enriched sources of vitamins, minerals, and health promoting bioactive substances with a higher nutritional value than adult plants. In order to systematically evaluate the biological and health related potential of compositional changes induced by elicitation (application of particular environmental conditions) we established an experimental pipeline for the controlled and reproducible production of broccoli sprouts aqueous extracts with different content of bioactive molecules and assessment of their protective effect on an in vitro model of inflammation based on human intestinal Caco-2 cells and on other cell and animal model systems (Ferruzza et al., 2016, Pharmaceuticals, 9(3); Masci et al., 2015, Oxidative Medicine and Cellular Longevity, 781938; Rubattu et al., 2015, Journal of Hypertension, 33(7), 1465-79; Rubattu et al., 2017 Cell death & disease, 8, e2891). Beside phytochemicals, plant derived nanovescicles and miRNAs have been reported to have health related effects in animals. In fact, dietary derived plant miRNAs were found in human sera and shown to exert a biological effect in human cell lines. Moreover, the plant derived nanovescicles have raised a great interest as potential natural carriers of different types of bioactive molecules and drugs able to regulate the expression of human target genes involved in several pathologies. Nonetheless, the mechanism of uptake and the trans-kingdom activity of plant miRNA and nanovescicles is still higly debated. Therefore, to further extend our analysis, we aimed to purify and characterize nanovescicles and miRNAs present in the broccoli sprouts extracts to investigate if they could play a role in the protective effect observed in our experimental model. A protocol for vescicles isolation, staining and loading with selected miRNAs has been set-up along with a qPCR protocol for absolute quantification of miRNAs present in vescicles prepared from broccoli sprouts. In addition, we have established an experimental system to test vescicles and miRNAs uptake in Caco2 cells. Analysis of the biological activity of a plant miRNA, alone or carried by broccoli sprouts vescicles, on a human gene target in Caco2 intestinal cells is currently underway.