

Natural product drug discovery using zebrafish screening: Anti-obese constituents hunting by combination with culture cells and mice experiments.

Yasuhito Shimada^{1,2,3}, Liqing Zang^{2,4}, Hiroko Nakayama^{2,4}, Kanae Hata⁴, Izumi Matsuoka⁴, Youngil Kim⁵, Djong-Chi Chu⁵, Lekh Raj Juneja⁵, Norihiro Nishimura^{2,4}

¹Dept. Int Pharm., Grad. Sch. Med., Mie Univ., ²Mie Univ. Zebrafish Drug Screen. Cen., ³Mie Univ. Adv. Sci. Res. Cen., Bioinfo., ⁴Grad. Sch. Reg. Innov., Mie Univ., ⁵Rohto Co., Ltd.

Natural products have been a great source for drug discovery against several types of cancer, cardiovascular diseases, and obesity and its related diseases throughout human history. However, some of the target diseases, such as obesity, are hard to perform screening study to identify the functional natural products and their therapeutic constituents. For decades, zebrafish has become a suitable model animal for phenotype-based drug screening also in lipid and glucose metabolism, while the throughput is still limited, called "small-scale screening". Here, we performed the anti-adipogenic screening using mouse 3T3-L1 preadipocytes and zebrafish obesogenic test, and compared these results of 42 natural products. Preadipocyte and zebrafish obesogenic testing were performed in a 96-well (single fish per well) and 6-well plate format (5 fish per well), respectively. As a result, 14 and 6 compounds reduced lipid accumulation in mouse adipocytes and zebrafish, respectively. Of these, 4 compounds, nobiletin, green tea extract, moringa leaf powder, and red pepper extracts suppressed lipid accumulation both in 3T3-L1 adipocytes and zebrafish. In addition, we modified these protocols with robotic manipulation to improve throughput as medium scale screening. We finally confirmed these hit compounds showed anti-obesity effects in diet-induced obese mice. In summary, we developed combination protocol for discovery anti-obese compounds using culture cells, zebrafish and mice. We further performed drug screening to find therapeutic constituents to improve visceral obesity.