

# The Extra Virgin Olive Oil phenolic compounds (–)-oleacein and (–)-oleocanthal inhibit tumor cell autophagy

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Our group has recently shown that the antitumor Extra Virgin Olive Oil phenolic compounds (–)-oleocanthal and (–)-oleacein also behave as anti-angiogenic agents. Interestingly, it has been described that phenolic compounds found in the Mediterranean diet affect the autophagy pathway. Based on this background, we studied the modulatory effects of (–)-oleocanthal and (–)-oleacein on tumor cell autophagy. Methodologically, the tumor cell lines MDA-MB-231, MCF-7 and HT-1080 cell lines were used in in vitro cellular and molecular studies of the autophagy flux and key mediators of this process, and High Content Screening (HCS) System using Perkin Elmer Operetta for single-cell analysis was performed in these cells. Interestingly, (–)-oleocanthal and (–)-oleacein repressed the autophagy flux of MDA-MB-231 and MCF-7 submitted to autophagy-inducing conditions (severe starving) at doses in the low micromolar range. In addition, key autophagy mediators, like LC3 or WIPI2 proteins, were dramatically reduced in the same settings, as seen in immunohistochemical studies. Furthermore, preliminary results of HCS in tumor cells revealed depletory effects on autophagy by using specific dyes for this process at the single-cell level. Altogether, our results point to a drastic inhibitory effect of (–)-oleocanthal and (–)-oleacein on tumor cell autophagy at low doses. [Grants: PID2022-138181OB-I00, PID2019-105010RB-I00 and RTI2018-098560-BC22 (Spanish Government), UMA18-FEDERJA-220, and PY20\_00257 (Andalusian Government and FEDER). Funds from BIO 267 (Andalusian Government) M.B. is supported by “Juan de la Cierva – Incorporation Program” (IJC2018-037657-I), Spanish Ministry of Science and Innovation, Spain].