Xanthohumol, a Novel Plant Extract, Alters Neuroendocrine Phenotype and Inhibits Growth of Carcinoid Cell Lines

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Abstract
Carcinoids tumors can lead to carcinoid syndrome, a set of debilitating symptoms due to the excess secretion of various bioactive hormones such as chromogranin A (CgA) and serotonin. Currently, the only potential curative treatment for carcinoid tumors is surgical resection; however, surgery is not an option for patients with metastasized carcinoid tumors. Thus, new therapeutic agents are required in order to improve the effectiveness of treatment for carcinoid syndrome. The purpose of this study was to determine the effectiveness of xanthohumol (XN), a prenylflavonoid anti-oxidant, on carcinoid tumor cells.

Human gastrointestinal carcinoid BON and bronchopulmonary carcinoid H727 cells were treated with XN (up to 20 µmol/L) or DMSO (drug carrier). Proteins were isolated after two days and were analyzed by Western blot for neuroendocrine markers. Cellular proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) growth assay. In order to determine the possible role of XN on proliferation, the Myr Akt plasmid was transfected in XN treated cells. Two days after treatment, proteins were isolated and the levels of phosphorylated Akt and NE markers were measured by Western blot.

Treatment with XN considerably reduced NE markers CgA and achaete scute complex-like 1 (ASCL1), along with phosphorylated Akt in both carcinoid cell lines. Furthermore, a significant dose dependent growth inhibition was observed with XN treatment. Importantly, cells transfected with Myr Akt plasmid did not alleviate the effect of XN.

Treatment with XN reduces phosphorylated Akt, but over-expression of Akt with the Myr Akt plasmid did not alter NE phenotype, indicating that XN may downregulate Akt at the transcriptional level. Our findings demonstrate for the first time the anti-proliferative effects of XN in carcinoid cell lines. The ability of XN to reduce NE markers and inhibit cell growth encourages the potential to use XN to treat and palliate patients with unresectable carcinoid cancer, and therefore warrants clinical investigation.

Background
Carcinoids are classified as neuroendocrine (NE) tumors that arise primarily in the gastrointestinal or respiratory tract. By nature these tumors are relatively slow growing; however, they have a tendency to metastasize. Carcinoids may lead to carcinoid syndrome, which is classified as a set of debilitating symptoms due to the excessive secretion of hormones such as serotonin and chromogranin A (CgA). Currently, the only potential curative treatment for carcinoid tumors is surgical resection. However, surgery is often times precluded as an effective treatment for patients with carcinoid syndrome due to the spreading of the tumor. Thus, new therapies and antitumor agents are required in order to improve the effectiveness of treatment for carcinoid disease. Xanthohumol (XN), a prenylflavonoid anti-oxidant, is present in hops. Hops are used for aroma, flavor, and bitterness in beer. XN has been shown to have antitumor properties with a broad spectrum of inhibitory mechanisms at the initiation, promotion, and progression stages of tumors. However, the effect of XN on carcinoid cell growth is not known. The purpose of this study was to determine the effectiveness of XN on carcinoid tumor cells.

Conclusions
• XN inhibits carcinoid cell proliferation by a dose-dependent manner.
• Treatment with XN results in suppression of the P33-Kinase pathway and concomitant reduction in NE markers.
• Over-expression of constitutively active Akt did not alter the effects of XN on NE phenotype, indicating the possibility that XN may downregulate Akt at the transcriptional level and XN may also regulate other pathways.
• Our findings encourage the potential to use XN as an effective agent for carcinoid disease and should be tested for its in vivo efficacy.

**Figure 1:** XN reduces neuroendocrine markers. BON and H727 cells were treated with varying concentrations of XN. Proteins were isolated and then analyzed by western blot to see the effects on the neuroendocrine (NE) markers; achaete scute complex-like 1 (ASCL1), CaKi2, and CgA. Treatment with XN reduced NE markers ASCL1 and CgA in both carcinoid cell lines. Importantly, continuous incubation for 4 days with XN resulted in a more pronounced effect on NE markers as compared to day 2.

**Figure 2:** XN inhibits growth dose dependently. Human gastrointestinal carcinoid BON and bronchopulmonary carcinoid H727 cells were treated with XN (up to 20 µmol/L) or DMSO (drug carrier). Cellular proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) growth assay over a six day period. A statistically significant dose dependent growth inhibition was observed with XN treatment. H727 showed considerable growth suppression with low concentrations of the XN compared to BON cells.

**Figure 3:** XN reduces phosphorylation of Akt. To determine the mechanism by which XN affects the P33-Kinase pathway, the Myr Akt plasmid was transfected in XN treated cells. Then the proteins were analyzed for phosphorylated Akt and NE markers by western blot. Similar to the cells transfected with the control empty vector, LNCK1, there was a reduction in levels of phosphorylated Akt and in NE markers with XN treatment in cells transfected with the Myr Akt plasmid. The results of the over expression of Akt did not alleviate the effect of XN indicating that the XN may be regulating Akt at a transcriptional level. These results suggest that the XN may regulate other signaling pathways which may play a role in cell proliferation.