DOCTORAL THESIS

Exploration of the anticancer mechanisms of novel chemotherapeutic adjuvants involving autophagy and immune system reprogramming in the treatment of pancreatic cancer

Zhang, Zhu

Date of Award:
2020

Abstract

Pancreatic cancer is known to be one of the most life-threatening cancers characterized by aggressive local invasion and distant metastasis. The high basal level of autophagy in pancreatic cancer may be responsible for the low chemotherapeutic drug response rate and poor disease prognosis. However, the clinical application of autophagy inhibitors was unsatisfactory due to their toxicity and minimal single-agent anticancer efficacy. Hence, oncologists begin to consider the tumor microenvironment when exploring new drug targets. In the present study, the anti-tumorigenic mechanisms of two major phytochemicals derived from Chinese medicinal herbs had been investigated against pancreatic cancer development.

Calycosin is a bioactive isoflavonoid of the medicinal plant *Astragalus membranaceus*. Our results have shown that calycosin inhibited the growth of various pancreatic cancer cells both *in vitro* and *in vivo* by inducing cell cycle arrest and apoptosis. Alternatively, calycosin also facilitated MIA PaCa-2 pancreatic cancer cell migration *in vitro* and increased the expression of epithelial-mesenchymal transition (EMT) biomarkers *in vivo*. Further mechanistic study suggests that induction of the Raf/MEK/ERK pathway and facilitated polarization of M2 tumor-associated macrophage in the tumor microenvironment both contribute to the pro-metastatic potential of calycosin in pancreatic cancer. These events appear to be associated with calycosin-evoked activation of TGF-β signaling, which may explain the paradoxical drug actions due to the dual roles of TGF-β as both tumor suppressor and tumor promoter in pancreatic cancer development under different conditions.

Isoliquiritigenin (ISL) is a chalcone obtained from the medicinal plant *Glycyrrhiza glabra*, which can be a precursor for chemical conversion to form calycosin. Results

have shown that ISL decreased the growth and EMT of pancreatic cancer cells *in vitro*, probably due to modulation of autophagy. ISL-induced inhibition of autophagy subsequently promoted reactive oxygen species (ROS) production, leading to induction of apoptosis in pancreatic cancer cells. Such phenomenon also contributed to the synergistic growth-inhibitory effect in combined treatment with the orthodox chemotherapeutic drug 5-fluorouracil. In addition, ISL-induced tumor growth inhibition *in vivo* was further demonstrated in a tumor xenograft mice model of pancreatic cancer. ISL promoted apoptosis and inhibited autophagy in the tumor tissues. Study on immune cells indicates that ISL could reduce the number of myeloid-derived suppressor cells (MDSCs) both in tumor tissue and in peripheral blood, while $CD4^+$ and $CD8^+$ T cells were increased correspondingly. *In vitro* test has revealed that ISL inhibited the polarization of M2 macrophage along with its inhibition of autophagy in M2 macrophage. These immunomodulating effects of ISL had reversed the pro-invasive role of M2 macrophage in pancreatic cancer.

In conclusion, calycosin acts as a "double-edged sword" on the growth and metastasis of pancreatic cancer, which may be related to the dual roles of TGF-β and its influence on the tumor microenvironment. Alternatively, ISL consistently inhibited the growth and metastatic drive of pancreatic cancer through regulation of autophagy and reprogramming of the immune system. The differential modes of action of these compounds have provided new insights in the development of effective pancreatic cancer treatment adjuvants.

Keywords: pancreatic cancer, calycosin, Isoliquiritigenin, TGF-β, autophagy, anticancer immunity

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