Molecular mechanisms of Ying Bu Bo extracts to promote apoptosis inhibit proliferation and metastasis through protein phosphatase 2A activation of HA22T hepatocarcinoma cells by *in vitro* and *in vivo*

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Abstract

The use of herbs as alternative cancer therapies has attracted a great deal of attention owing to their lower toxicity. Whether Ying Bu Bo (YBB) induces liver cancer cell apoptosis, inhibit proliferation and metastasis remains unclear. In this study, we investigated the effect of YBB extracts (YBBEs) on HA22T human hepatocellular carcinoma cells *in vitro* and in an *in vivo* mouse xenograft model. HA22T cells were treated with different concentrations of YBBEs and analyzed with MTT assay, flow cytometry, Western blot analysis, TUNEL, JC-1 staining and siRNA transfection assays. Additionally, the HA22T-implanted xenograft nude mice model was applied to confirm the cellular effects. YBBEs showed a strong inhibition of HA22T cell viability in a dose dependent manner and significantly reduced the cell proliferative proteins as well as induced cell cycle arrest in G2/M phase. YBBEs-induced apoptosis, up-regulated death receptor apoptotic pathway markers as well as mitochondrial proteins, and suppressed the survival proteins in a dose-dependent manner. Pro-survival Bcl-2 family proteins were inhibited and the pro-apoptotic ones were increased. YBBEs also demonstrated a high level of suppression of HA22T cell proliferation by cell migration inhibition by Boden chamber migration and invasion assays. When HA22T cells were treated with YBBEs, the cell migration-promoting proteins, FGF-2, uPA, MMP2/9, β-cartenin, p-GSK-3β, TBX-3, and IL8 were downregated, however the migration-inhibiting proteins, PAI, GSK-3β, APC and b-TrCP/HOS were significantly upregated. It was also discovered that there was a decrease of the amount of β-catenin in the nucleus, meaning a significant nuclear export of that protein. In addition, PP2A siRNA or PP2A inhibitor totally blocked the YBBEs cell proliferation, metastasis inhibition and induced HA22T apoptosis. Finally, in the HA22T-implanted nude mice model, it was further confirmed that YBBEs inhibited tumor cell proliferation, metastasis and increased tumor cell apoptosis *in vivo*. All these results suggest that YBBEs is a potential candidate to inhibit HA22T hepatocellular carcinoma cell proliferation,metastasis and promote apoptosis via PP2A *in vitro* and *vivo*. In the near future, we would like to further investigate the YBBEs anticancer effect by preclinical studies and clinical trials.