

Discovery & Development of Natural Product-Based Potential Immuno-Therapeutics for Melanoma

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Advanced melanoma has a very poor prognosis and incidence has risen rapidly over the past 30 years. Immunotherapies with immune checkpoint inhibitor antibodies approved recently by the FDA have demonstrated impressive therapeutic responses in about 30% of patients. However, the majority of patients do not respond, and the reasons for this are unclear. Strategies for cancer therapy aim to overcome key hallmarks of cancer including excessive proliferation, evasion of immune-mediated destruction, and avoidance of apoptosis by cancer cells. We recently discovered that natural products of the 17 β -hydroxywithanolide (17-BHW) class occurring in plants of the genus *Physalis* (family: Solanaceae), many of which are used in South American traditional medicines, were highly effective in inducing tumor cells to undergo apoptosis on exposure to death ligand proteins of the TNF (Tumor Necrosis Factor) family such as TRAIL (TNF-Related Apoptosis Inducing Ligand) or poly (I:C) [polyriboinosinic:polyribocytidylic acid, a synthetic polynucleotide (ds-RNA) viral mimetic which activates TLR (Toll-Like Receptor) signaling]. This led to the hypothesis that 17-BHWs combined with immunotherapeutic regimens could both increase cancer cell death and amplify anticancer immune responses. To test this hypothesis, we obtained 17-BHWs by an innovative aeroponic cultivation of several *Physalis* species and evaluated these and their derivatives in vitro for their ability to induce melanoma cells to undergo TRAIL and poly (I:C)-mediated apoptosis. Evaluation of over fifty of these 17-BHWs provided valuable information on their structure-activity relationships and led to the identification of the most promising 17-BHW as physachenolide C (PCC) encountered in *Physalis crassifolia* (yellow nightshade ground cherry), a plant collected in Arizona. Molecular studies suggested that the mechanism of action of PCC is due to a dramatic reduction in the levels of anti-apoptotic cFLIP [(FADD-like IL-1 β -converting enzyme)-inhibitory proteins], cFLIP_S and cFLIP_L, in melanoma cells. Intra-tumor administration of PCC and poly (I:C) in a xenograft M14 melanoma model provided therapeutic benefit resulting in complete tumor regression in about 90% of the mice compared to untreated mice. This regression was due to extensive melanoma cell apoptosis as assessed by Tunel staining of tumor tissue sections. Encouraged by these findings, forty-five semisynthetic analogues of PCC were prepared and tested. Some of these analogues were 3 to 6 fold more potent than PCC in promoting poly (I:C)-sensitized apoptosis in melanoma cells. As poly (I:C) is known to act as a powerful immune adjuvant, and TNF family death ligands are often produced by activated immune cells, our findings led to the conclusion that 17-BHWs when combined with various immunotherapeutic regimens may promote tumor regression and improve therapeutic outcome for patients with advanced metastatic melanoma.

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