

Identification of gene targets of anticancer activity of the leaf extract of Ashwagandha by gene silencing approaches

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Ashwagandha (*Withania somnifera*) is a proud herb in Indian traditional system of home medicine. It forms a common ingredient of health supplements, tonics and Indian home remedies designed to promote health and quality of life. Though sustained through experience and history, there are only a limited laboratory studies and experimental evidence to its effects. We first prepared extract from leaves of Ashwagandha and investigated its anticancer activity in in vitro cancer cell culture and in vivo nude mice tumor assays. Because of its strong tumor inhibitory activity the extract was named as (i-Extract). Chemical analysis of i-Extract revealed the presence of Withanolides including Withanone, Withaferin-A and Withanolide-A in the extract. We found that Withanone-enriched extracts possessed strongest activity and it caused selective killing of cancer cells. In order to investigate the mechanism of selective tumor cell killing by i-Extract, we adopted gene-silencing approaches including gene silencing by siRNA and by ribozymes. These screenings for gene targets revealed that the tumor suppressor pathway and oxidative stress pathway are critically involved in selective tumor cell killing by i-Extract. We demonstrate that i-Extract causes selective activation of wild type p53 in cancer cells as endorsed by increased expression of p21WAF1. Furthermore, the cells with mutant p53 were seen to acquire wild type p53 activity and underwent apoptosis. Consistent with these findings, i-Extract caused effective tumor suppression of mutant p53 tumors in nude mice assays. Gene silencing by randomized ribozyme library and the bioinformatics analysis revealed the involvement of oxidative pathways suggesting that i-Extract causes oxidative stress to cancer cells. We analyzed oxidative stress response of normal and cancer cells in response to UV treatment with and without i-Extract and i-Factor and found that whereas cancer are sensitized to UV induced killing, the normal cells are protected by treatment of i-Extract. We present the first mechanisms of action of Ashwagandha leaf extract demonstrating their selective cancer cell killing and normal cell protecting activities.

Reference:

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