Epigenetic silencing of triple negative breast cancer hallmarks by pharmacological concentrations Withaferin A

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Withaferin A (WA) isolated from *Withania somnifera* (Indian ginseng or Ashwagandha in Ayurvedic medicine) has recently become an attractive phytochemical under investigation in various preclinical studies for treatment of different cancer types. Pharmacological levels of WA trigger clinically relevant anticancer effects specific to triple negative breast cancer cells.

We determined genome-wide DNA methylation changes in weakly-metastatic and aggressive, metastatic BC cell lines, following 72h treatment to a sub-cytotoxic concentration of WA. In contrast to the DNA demethylating agent 5-aza-2'-deoxycytidine (DAC), WA treatment of MDA-MB-231 cells rather tackles an epigenetic cancer network through gene-specific DNA hypermethylation of tumor promoting genes including ADAM metalloproteinase domain 8 (ADAM8), urokinase-type plasminogen activator (PLAU), tumor necrosis factor (ligand) superfamily, member 12 (TNFSF12), and genes related to detoxification (glutathione S-transferase mu 1, GSTM1), or mitochondrial metabolism (malic enzyme 3, ME3). Gene expression and pathway enrichment analysis further reveals epigenetic suppression of multiple cancer hallmarks associated with cell cycle regulation, cell death, cancer cell metabolism, cell motility and metastasis. Remarkably, DNA hypermethylation of corresponding CpG sites in PLAU, ADAM8, TNFSF12, GSTM1 and ME3 genes correlates with receptor tyrosine-protein kinase erbB-2 amplification (HER2)/estrogen receptor (ESR)/progesterone receptor (PR) status in primary BC tumors. Moreover, upon comparing differentially methylated WA responsive target genes with DNA methylation changes in different clinical subtypes of breast cancer patients in the cancer genome atlas (TCGA), we found that WA silences HER2/PR/ESR-dependent gene expression programs to suppress aggressive TNBC characteristics in favor of luminal BC hallmarks, with an improved therapeutic sensitivity. In this respect, WA may represent a novel and attractive phyto-pharmaceutical for TNBC treatment.