HEMIDESMUS INDICUS INDUCES IMMUNOGENIC CELL DEATH IN HUMAN COLORECTAL CANCER CELLS

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Background: The immunogenicity of malignant cells has recently been recognized as a critical determinant of efficacy in cancer therapy. Defined cytotoxic or genotoxic agents promote the generation of anticancer immune responses, potentially leading to tumor eradication, by inducing, in malignant cells, immunogenic cell death (ICD). ICD-inducing agents elicit a form of endoplasmic reticulum (ER) and oxidative stress that activate a danger pathway which involves the mobilization of the ICD mediators, the so-called damage-associated molecular patterns (DAMPs). DAMPs are endogenous molecules, such as calreticulin (CLR), heat shock proteins (HSP), and ATP, that acquire immunostimulant properties when exposed on the outer cellular membrane or released in the extracellular matrix in a defined spatial-temporal manner. When mobilized, DAMPs act as both danger signals and adjuvant molecules for the innate immune system. The ability of different synthetic chemotherapeutic drugs to induce ICD has been characterized in detail. In contrast, there is a paucity of data regarding natural products of potential clinical relevance. A variety of natural anticancer compounds have been successfully characterized. However, their immunogenic potential has not been analyzed in comparable detail. The aim of this study was to evaluate Hemidesmus indicus (H.i.) decoction's ability to induce ICD in a colon carcinoma cell line (DLD1).

Methods: The research was performed on a MMR-1deficient human colon adenocarcinoma cell line (DLD1). H.i. decoction was prepared according to the procedures described in the Ayurvedic Pharmacopeia of India. The plant (voucher #MAPL/20/178) was collected from Ram Bagh in Rajastan, India, authenticated by Dr. MR Uniyal, Maharishi Ayurveda Product Ltd (Noida, India). H.i. cytotoxic potential was assessed with or without the antioxidant N-acetyl-cysteine or the ER stress inhibitors tauroursodeoxycholic acid or AMGPERK44. Cell viability was assessed through the 4-methylumbelliferyl heptanoate assay, using the microplate reader Victor X3(Perkin Elmer). Specific protein-antibody binding was evaluated by flow cytometry [FACScalibur (Becton Dickinson); Guava EasyCyte 6-2L (Merck); CytoFLEX (Beckman Coulter)]. Fluorochrome-labeled monoclonal antibodies were obtained from Becton Dickinson and Abcam. For the analysis of extracellular ATP levels, the kit ATPLite 1step (Perkin Elmer) was used. In order to assess dendritic cells (DC) maturation, co-culture of DLD1and human immature DC (4:1) were treated with H.i. decoction and then the expression of specific cluster of differentiations on DCs were analyzed by flow cytometry.

Results: H.i. induces a dose-dependent decrease in the number of viable cells, which lies on its ability to increase ROS levels and promote ER stress, two crucial events required for the exploitation of ICD immunogenicity. Accordingly, H.i. positively modulates the expression and mobilization of characteristic DAMPs, including CLR, HSP70, and ATP. Prompted by these observations, we addressed H.i.'s ability to promote the activation of immature DC. Our data consistently indicate that H.i.-treated tumor cells are able to promote the transition from an immature to a mature state of in vitro generated antigen-presenting cells.

Discussion and conclusions: This was the first report where the ability of a botanical drug to trigger all in vitro hallmarks of ICD was demonstrated. Indeed, we clearly proved that H.i. decoction is able to induce an immunogenic type of cell death, as indicated by its ability to induce ER and oxidative stress, to activate a characteristic pattern of markers, including CLR, HSP70, and ATP, and to promote iDCs maturation. On the whole, the antitumor profile of H.i. that emerges from this study provides a clear rationale for the design of *in vivo* experiments in order to address the "endogenous vaccine" power of H.i.-treated tumor cells.