THE INHIBITION OF PhIP-INDUCED CELL CYCLE CHANGES BY Diallyl Sulfide IN MCF10A CELLS: A POSSIBLE MECHANISM OF BREAST CANCER PREVENTION

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Abstract: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a dietary carcinogen produced during the cooking of meat at high temperatures. PhIP is bioactivated by cytochrome p450 1A2, cytochrome B5 reductase and Sulfur estrogen transferases resulting in reactive species including nitrenium ions and Reactive oxygen species which causes DNA adducts and strand breaks. These genetic perturbations lead to mutations that give rise to breast, colon and prostate cancer. Failure to repair these mutations in oncogenes and tumor suppressor genes may result in breast cancer induction. Garlic organosulfur compounds, such as diallyl sulfide (DAS), have been shown to possess antiproliferative effects that may contribute to their chemopreventive properties. Therefore, the purpose of this study was to determine the effect of DAS on PhIP-induced alterations in cell cycle distribution and gene expression of cell cycle regulatory genes. MCF-10 A human breast epithelial cells were plated and treated for 24, 48 and 72 hours with PhIP (100μM), DAS (100μM) and N-OH PhIP (5μM). Combination groups were pretreated with 100μM of DAS 6 hours prior to dosing with PhIP (100μM) or N-OH PhIP (5μM). Untreated and DMSO treated cells served as negative and vehicle controls, respectively. Cell cycle distribution was determined by flow cytometric analysis. The G2/G1 ratio was used as a means to identify those cells undergoing abnormal cell cycle arrest. While DAS did not significantly affect the G2/G1 ratio of PhIP treated cells, it did show a time dependent increase in the G2/G1 ratio significantly greater than that of N-OH PhIP alone. The induction of abnormal cell cycle arrest is presumably in response to the DNA damage observed in earlier studies. Furthermore, quantitative real time RT-PCR was used to determine the gene expression of key cell cycle regulatory genes. The alterations in gene expression levels observed in this study supported the results observed in the flow cytometric portion of this study. Reductions in cyclins and cyclin dependent kinases in the PhIP combination group at 48 hours supported the reductions in S phase. Additionally, the modified expression of genes associated with cell cycle arrest at 72 hours in PhIP combination cells and 48 hours in N-OH PhIP combination cells, support the alterations in cell cycle progression observed. These results could provide a possible mechanism for the reductions in cellular proliferation observed in MCF-10A cells treated with DAS prior to PhIP and N-OH PhIP treatment. Therefore, the ability of DAS to induce cell cycle arrest via modulation of key cell cycle regulatory genes upon genotoxic damage provides a potential mechanism for its chemopreventive properties.

Keywords: PhIP, Diallyl Sulfide, Cell Cycle and Gene Expression

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