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Up-regulation of p21WAF1/CIP1 expression stimulates G1-phase arrest of the cell cycle in cladosporol A-treated HT-29 cells

D. Zurlo[1], C. Leone[1], G. Assante[2], S. Salzano[3], G. Renzone[4], A. Scaloni[4], C. Foresta[1], V. Colantuoni[1] and A. Lupo[1]

[1]Universita` del Sannio, Dipartimento di Scienze Biologiche ed Ambientali, Benevento, Italy, 2 Universita` di Milano,
[2] Dipartimento di Patologia Vegetale, Milano, Italy,
[3] Istituto di Endocrinologia e Oncologia Sperimentale ‘‘Gaetano Salvatore’’ (IEOS)-CNR, Napoli, Italy,
[4] Laboratorio di Proteomica e Spettrometria di Massa, ISPAAM, Consiglio Nazionale delle Ricerche, Napoli, Italy

Chemoprevention is a clinical practice that is gaining favour in the treatment of degenerative diseases including cancer. The list of compounds able to interfere with the various steps of the tumorigenic process is growing and includes new molecules isolated from plants, fungi and microorganisms. Cladosporols, a series of compounds purified as secondary metabolites from Cladosporium tenuissimum, display antifungal activity. In this study we sought to investigate the antiproliferative properties of cladosporol A, the major isoform of this family of compounds. By assessing cell viability we show that cladosporol A inhibits the growth of several human colorectal cancer-derived cell lines in a time- and dose-dependent manner. Administered to HT-29 cells, cladosporol A causes a G1-phase arrest of the cell cycle, accompained by an early and robust induction of p21WAF1/CIP1. By RT-qPCR assays and transient transfections of a luciferase reporter gene, under the control of the p21WAF1/CIP1 promoter region, we demonstrate that the induction takes place at transcriptional level. Molecular dissection of this region, by deletion analysis, revealed the shortest fragment able to drive the full transcription. A series of Sp1 binding sites located at nucleotides were identified as crucial for the induction. Their site directed mutagenesis abolishes the induction. This is the first report of an antiproliferative effect induced by cladosporol A, mediated by transcription induction of p21WAF1/CIP1 in a p53-independent manner.