MASTER'S THESIS

Differentiation inducing effect of isoflavonoids on neuroblastoma cells

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Abstract

Neuroblastoma, an embryonal tumor of the nervous system, is one of the major solid tumors that usually occur in children. It is a group of primitive and clinically aggressive neoplasm. In my present study, murine neuroblastoma Neuro-2A was used as a cell model to study the anti-tumor activities of two selected isoflavonoids, namely biochanin A and genistein. These two isoflavonoids have been previously reported to mediate anti-tumor activities in mammalian cancer cells.

Biochanin A and genistein not only can inhibit the growth of Neuro-2A, but also induce morphological differentiation of this tumor cell. Morphological differentiation was observed as early as 1 day after isoflavonoids treatment. Neuro-2A is a heterogenous population of cells. Two subclones, namely BU-1 and BU-2, were isolated for the subsequent studies. The cytotoxic and growth inhibitory effects of both biochanin A and genistein on Neuro-2A, BU-1 and BU-2 cells were similar. The degree of differentiation in isoflavonoids treated BU-1 cell was higher than Neuro-2A and BU-2 cells. Morphological features of biochanin A- and genistein-treated BU-1 cells are different. Biochanin A treated BU-1 cells are generally elongated in shape, whereas genistein treated BU-1 cells produce extensive neurite network. Both biochanin A and genistein induce morphological differentiation in a dose- and time dependent manner. Results from limiting dilution analysis reveal that isoflavonoids treated BU-1 cells undergo terminal differentiation at 6 days after treatment.

Axonal (NF-H and tau) and dendritic (MAP-2) markers were employed to characterize the nature of induced neuritic outgrowth in differentiated BU-1 cells. Laser scan confocal microscopy was used to study the distribution of these neuronal markers. A mixed phenotype of axon- and dendrite-like processes was observed 3 days after biochanin A and genistein treatment. In terminally differentiated BU-1 cells (after 6 days of incubation), axonal property was observed in the neurites.

Neuropoietic cytokoines are important in controlling neuronal growth and differentiation in the nervous system. In this study, the roles of some selected neuropoietic cytokines (IL-6, CNTF and LIF) upon isoflavonoids induced differentiation of neuroblastoma BU-1 cells was examined. The mRNA expression of neuropoietic cytokine genes was examined using Reverse-transcription Polymerase chain reaction (RT-PCR). The level of expression was semi-quantified by cycle titration and dot blot hybridization. The expression of CNTF was not significantly altered in isoflavonoids-treated BU-1 cells. However, the expression profile of IL-6 and LIF was different in biochanin A- and genistein-treated BU-1 cells. Our results suggest that IL-6 and LIF possibly interact with other neuropoietic cytokines in controlling neuronal differentiation of BU-1 cells.

In summary, biochanin A and genistein may act as anti-cancer compounds upon neuroblastoma cells. The induced neuronal differentiation and also the nature of isoflavonoids driven neuritogenesis may be under the control of neuropoietic cytokines. The role of cytokines on isoflavonoids induced neuronal maturation remains to be investigated. The biological effects of isoflavonoids on the development of neurons should also be studied. Hopefully, the underlying mechanism of cellular differentiation of neuroblastoma can be elucidated in future.

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