Isatin inhibits LSD1 regulated autophagy through SESN2-mTOR signaling pathway in neuroblastoma

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Abstract
Neuroblastoma (NB) is common in the pediatric tumors with low cure rate and high mortality, drug resistance makes chemotherapy more difficult, it threaten to children's health seriously. 1H-indole-2, 3-dione (isatin) is a second-hand of derivative of anticarcinogen indirubin which had been shown to be a monoamine oxidase inhibitor and have antitumor activity, in this study, we explained that SH-SY5Y cells was add to isatin at a dose-dependent level ranging from 50 to 200 µmol/L were capable of inhibiting tumor cell growth, included triggering apoptosis and inhibiting proliferation, invasion and metastasis, and assessed the potential anticancer capability of isatin on the induction of autophagy. The experimental result showed that cell SH-SY5Y disposed with isatin could induce autophagy effectively, at the same time, it also help suppress the growth of tumor cells. Furthermore, the article indicated that the induction of autophagy of isatin-mediated occurred in inhibition-lysine-specific demethylase 1(LSD1) and sestrin2 (SESN2) - dependent manner. Furthermore, we identified that SH-SY5Y cells after treating with isatin induced SESN2 expression via a mTOR-dependent signal pathway, which mechanism is that isatin inhibit LSD1-enzyme activation and combine the promoter regions of SESN2, so that given rise to a significant transcriptional induction of SESN2. In addition, western blot analysis indicated that SH-SY5Y cells with isatin-exposed can regulated activity of LC-3, Beclin1 and p62 which are correlated with autophagy. Collectively, all the results from this study illustrated that adding to isatin could occur to induce autophagy and restrain NB cell SH-SY5Y growth through mTOR-dependent transcriptional induction of SESN2, this supplied a new mechanism for understanding the anti-tumor ability of isatin on NB. Taken together, this paper demonstrate that isatin is a promising candidate for treating NB.

Full Text
Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures
Isatin inhibited SH-SY5Y cells proliferation. a CCK-8 assay showed that isatin inhibited the proliferation of SH-SY5Y cells. b Tablet cloning showed SH-SY5Y cells were treatment with 0 µmol/L, 50 µmol/L, 100 µmol/L, 200 µmol/L isatin for 48 h, cell cloning was significantly inhibited.

SH-SY5Y cells were treatment with isatin for 48 h, Cell morphology changed.
Flow cytometry to detect cell cycle and apoptosis. A SH-SY5Y cells were treatment with different isatin, flow cytometry to detect the change of cell cycle. B SH-SY5Y cells were treatment with different concentrations isatin, flow cytometry to detect the change of cell apoptosis. c was percentage of cell cycle after adding to different concentrations isatin in SH-SY5Y cells from a. d was percentage of cell apoptosis after adding to different concentrations isatin in SH-SY5Y cells from b.

DAPI staining to detect apoptosis. SH-SY5Y cells were treatment with 0 µmol/L, 50 µmol/L, 100 µmol/L, 200 µmol/L isatin, the morphology of the nucleus changed.
Isatin suppressed SH-SY5Y cells invasion and migration. a SH-SY5Y cells were treatment with 0 µmol/L, 50 µmol/L, 100 µmol/L, 200 µmol/L isatin, the invasion of cells were obviously inhibited. b SH-SY5Y cells were treatment with 0 µmol/L, 50 µmol/L, 100 µmol/L, 200 µmol/L isatin, the migration of cells was obviously inhibited.
q-PCR assay detect mRNA relative expression. In SH-SY5Y cells p53, LSD1, SESN2, Beclin1, P62 and LC3 changed (P<0.01) after adding to different concentrations isatin for 48 h.
Western Blot detect protein relative expression. In SH-SY5Y cells LSD1, SESN2, Beclin1, P62 and LC3 relative protein expression changed (P<0.01) after adding to different concentrations isatin for 48 h.