Research Institute of Clinical and Experimental Lymphology – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

Novosibirsk State University, Novosibirsk, Russia

The lithium effects on morphology and apoptosis in hepatocellular carcinoma cells

Gogaeva I.S., Taskaeva Iu.S., Obanina N.A., Makarova V.V., Bgatova N.P.

Background

Hepatocellular carcinoma (HCC) is a highly malignant type of liver cancer characterized by molecular changes that affect on apoptosis processes: mutations in the P53 tumor suppressor gene (TP53), increased expression of transforming growth factor β (TGF-β), the anti-apoptotic phenotype and the resistance to extrinsic apoptosis. The dysregulation of cell death and proliferation mechanisms, as well as the imbalance of pro- and anti-apoptotic signals in HCC require the targeted chemotherapy to enhancing HCC apoptosis. We have previously shown that lithium salts decrease viability and stimulate apoptosis in hepatocellular carcinoma-29 cells in vitro. The aim of this study was to investigate the lithium effects on cell morphology and HCC-29 apoptosis in vivo.

BGRS/SB-2020: 12th International Multiconference “Bioinformatics of Genome Regulation and Structure/Systems Biology”
06-10 July 2020, Novosibirsk, Russia

Materials and Methods

Male CBA mice of 6–8 weeks of age, with weights of 18–20 g were used in experiment. For tumor induction, $1 \cdot 10^6$ HCC-29 cells were transplanted into the abdominal cavity, after 10 days, ascitic fluid was removed and $2 \cdot 10^6$ HCC-29 cells suspended in 100 µL of PBS were injected into the right thigh muscle. Mice were randomly divided into two experimental groups (five mice in each). Experimental groups were as follows: the mice with intact tumor (Control) and mice, received 20 mM lithium carbonate (LC).

Transmission electron microscopy (TEM). The tumor tissue was processed at the JEM 1400 electron microscope (JEOL, Japan).

Immunofluorescent staining (IF-F). Frozen sections of tumor tissue were analyzed by immunofluorescence staining using anti-Bad, anti-Bcl-2 and anti-caspase-3 primary antibodies. Fourteen fields per group were captured (total area was 0.08 mm^2 for each group).

Statistical analysis. Data are presented as mean (M) ± standard deviation (SD). Mann–Whitney nonparametric tests were used to assess differences by statistical package Statistica 6.0 (StatSoft, USA). Statistically significant differences were considered at $P < 0.05$.

Results

An ultrastructural study of lithium-treated tumor tissue revealed typical morphological manifestations of apoptosis (nucleus fragmentation, chromatin condensation, a decrease in cell volume and cytopodia, plasma membrane blebbing) – fig. 1, 2.

Immunofluorescent staining showed an increase in Bad-positive and Caspase-3-positive cells and a decrease in Bcl-2-positive cells after lithium administration – fig. 3, 4.

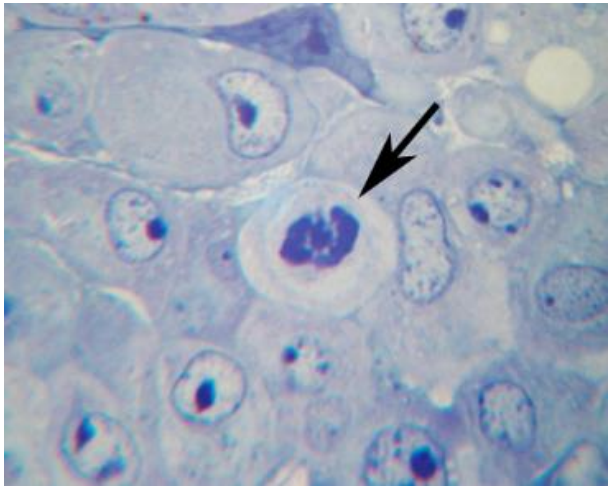

Figure 1. Toluidine Blue, × 400.

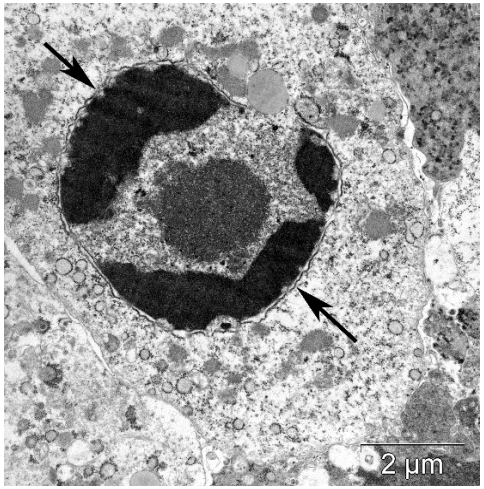

Figure 2. TEM.

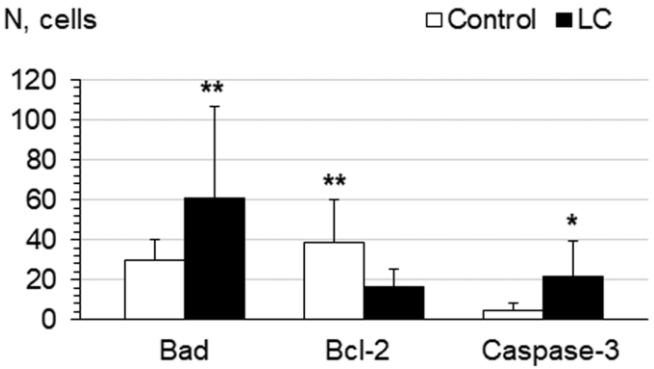

Figure 3. Analysis of Bad, Bcl-2 and Caspase-3 expression in HCC cells by IF-F.

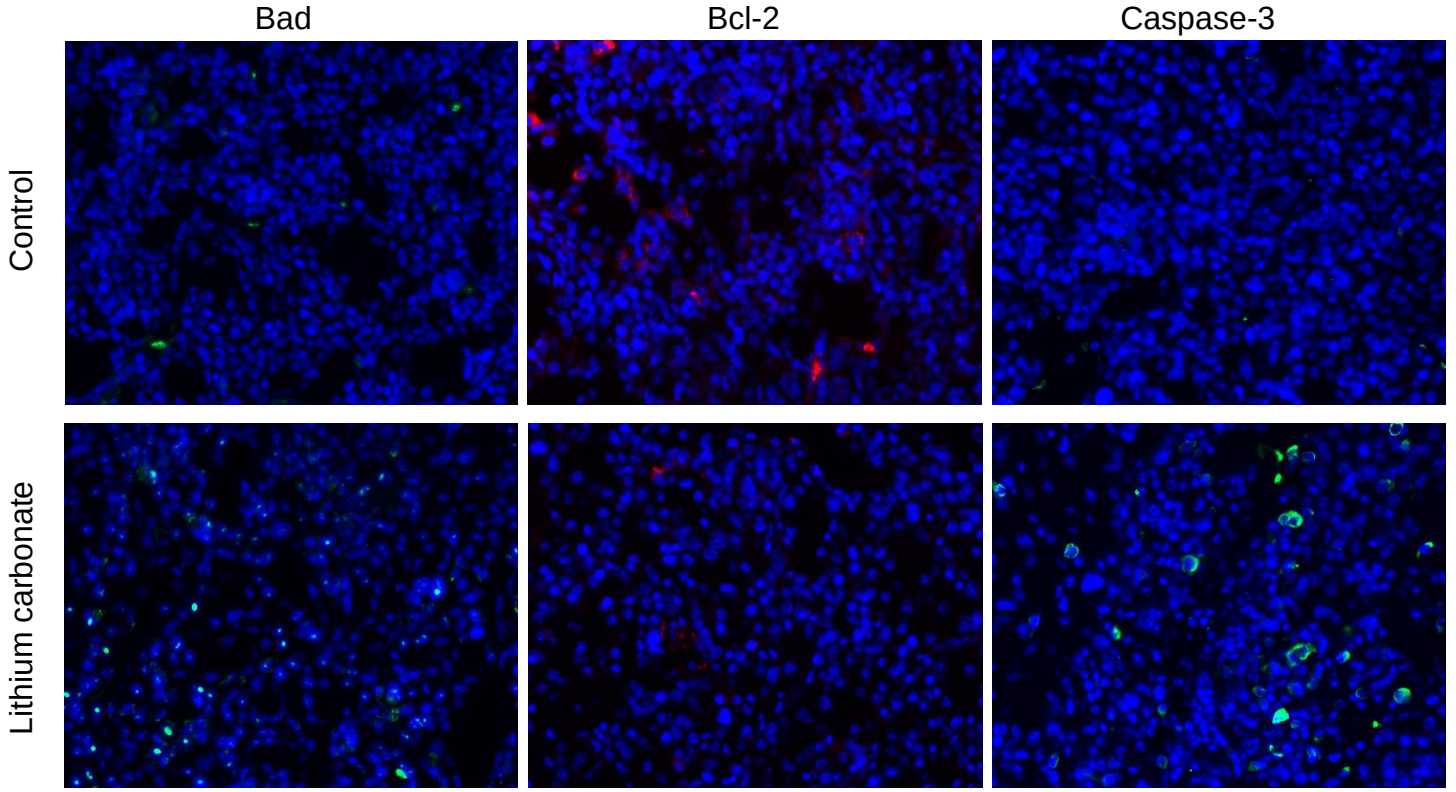

Figure 4. Immunofluorescence staining of HCC cells using anti-Bad, anti-Bcl-2 and anti-caspase-3 primary antibodies, × 400.

Conclusions

Apoptosis is a process of regulated cell death that develops as a result of intracellular changes (intrinsic pathway of apoptosis) or changes in the cell microenvironment (extrinsic pathway of apoptosis). The intrinsic pathway of apoptosis is initiated by disturbance of intracellular homeostasis, leading to permeabilization of the outer mitochondrial membrane, which occurs under the control of the regulating apoptosis Bcl-2 proteins family. The extrinsic apoptosis pathway is triggered mainly by death receptors, which results in the formation of a death-inducing signaling complex (DISC) and activation of caspase-8, -10, -3 and others. The results obtained in this study demonstrate the possible ability of lithium to influence on the mitochondrial membranes permeability and participate in the intrinsic and probably extrinsic apoptotic pathways. The molecular features of HCC that contribute to the apoptosis resistance determine the relevance of further research and the development of new strategies for enhancing apoptosis in HCC cells. Lithium administration can enhance pro-apoptotic chemotherapeutic drugs potential and overcome the resistance of tumor cells to apoptosis in HCC.