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Autophagy modulation by antitumor protein lactaptin

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Motivation and Aim: Lactaptin, the proteolitic fragment of human milk kappa-casein, induces apoptosis of various cultured cancer cells. Earlier we have demonstrated lactaptin treatment led to processing LC3-I to LC3-II form in treated cells that was defined as autophagy. Combination of lactaptin with autophagy inhibitor chloroquine (CQ) strongly increased their cytotoxicity against various cancer cells. We supposed that lactaptin besides apoptosis induced also pro-survival autophagy in treated cancer cells. However, lactaptin-dependent autophagosome formation and autophagic flux have not been demonstrated yet.
Methods and Algorithms: Lactaptin analog (RL2) produced in *E. coli* was used for experiments. Autophagosome formation has been detected by transmission electron microscopy. Changes in autophagy-related protein were detected by Western Blot analysis. Lysosome dynamic was monitored by fluorescent microscopy. Autophagy inhibitors and inducers were used to increase or decrease lactaptin-dependent autophagy.
Results: Effective suppression of RL2-induced autophagy by inhibitor CQ was confirmed by electron microscopy: in the presence of RL2 and CQ the number of autophagosomes two-times increased without the implementation of catabolic processes (autophagic flux). It was found that spermidine and 3-methyladenine (3MA) did not affect the cytotoxicity RL2 *in vitro*, but CQ, Ku55933 and rapamycin increased cytotoxic effect of RL2 *in vitro*. Thus, RL2 stimulates pro-survival autophagy of cancer cells, and its inhibition potentiates the cytotoxic effect of RL2 *in vitro*. It was shown that RL2 treatment decreased p62 while in combination with inhibitors of autophagy (3MA, CQ, Ku55933) we detected up-regulation of p62.
Tumor growth inhibition and survival outcomes after RL2 and CQ treatment were estimated using mice, bearing cyclophosphamide-resistant lymphosarcoma RLS. We demonstrated that intravenous injections of RL2 (12 mg/kg) in combination with CQ (50 mg/kg) enhanced the antitumor effect of monotherapy. Tumor growth inhibition was 24.9 % for RL2, 78.4 % for CQ and 86.5 % for RL2 in combination with CQ. It was shown that survival rate in the group receiving RL2 and CQ was 100 % compared to 22.2 % in the control.
Conclusion: Autophagy inhibitors potentiate the cytotoxic effect of RL2 *in vitro*. Combination of lactaptin analog with CQ enhanced antitumor effect in mice.
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