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The anti-malarial chloroquine suppresses proliferation and overcomes cisplatin resistance of endometrial cancer cells via autophagy inhibition

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HIGHLIGHTS

- Chloroquine attenuated proliferation and induced apoptosis in endometrial cancer cells in part via autophagy inhibition.
- Autophagy inhibition by either chloroquine or knockdown of ATG5/7 improved cisplatin-sensitivity in Ishikawa endometrial cancer cells.
- Cisplatin-resistant Ishikawa cells, with an increased basal level of autophagy, were more sensitive to chloroquine than parental cells.

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ABSTRACT

Objective. The anti-malarial drug chloroquine (CQ) is also known as an autophagy inhibitor. Autophagy can promote tumor growth by fueling the necessary energy metabolism and inducing resistance to chemotherapy and/or irradiation in various human cancers. However, the role of autophagy in endometrial cancer has not yet been established. We investigated the anti-tumor effects and autophagy inhibition caused by CQ in endometrial cancer cells.

Methods. Cell proliferation and cell cycle were assessed in response to CQ in six endometrial cancer cell lines by using an MTT assay and/or flow cytometry. To assess the level of autophagy, western blotting and an immunofluorescence assay were used to measure LC3 expression. The effects of knockdown of either *ATG5* or *ATG7*, both of which are indispensable for induction of autophagy, were assessed via an MTT assay. Sensitivity to CQ was compared between parental and cisplatin-resistant (CP-r) Ishikawa endometrial cancer cells.

Results. CQ suppressed proliferation in all six endometrial cancer cell lines in a dose-dependent manner, whereas it increased the population of apoptotic cells. Inhibition of autophagy via knockdown of either *ATG5* or *ATG7* decreased the sensitivity to CQ. Additionally, sensitivity to cisplatin was improved by knocking down *ATG5* or *ATG7*. Finally, CP-r Ishikawa cells, with a high basal level of autophagy, were more sensitive to CQ than parental Ishikawa cells.

Conclusions. Our data suggest that autophagy is involved in endometrial tumor growth and cisplatin resistance. Furthermore, our data support a therapeutic role for CQ in endometrial cancer cells with upregulation of autophagy.

1. Introduction

Endometrial cancer is one of the most common gynecologic malignancies [1]. Systemic therapy for endometrial cancer consists mainly of platinum-based chemotherapy. However, when the cancer recurs after such chemotherapy, there are only a few options for further treatment. Therefore, novel therapeutic drugs are needed, especially for tumors with platinum-resistance.

Autophagy, which literally means “self-eating,” is a major cellular catabolic process that involves lysosomal degradation of organelles and cytoplasmic materials [2]. Autophagy is divided into three categories: macroautophagy, microautophagy, and chaperone-mediated autophagy. In this paper, we refer to macroautophagy as autophagy, since it is the main form of autophagy. The autophagy process consists of four stages: initiation, elongation, fusion to lysosomes, and degradation [3]. The

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initiation stage occurs near the endoplasmic reticulum and involves the formation of an isolation membrane. In the second stage, the isolation membrane elongates and closes to form an autophagosome that engulfs cytoplasmic materials. During the third stage, autophagosomes fuse to lysosomes. Finally, the components of the cytoplasmic material are degraded by lysosomal enzymes [2]. Proteins encoded by autophagy-related genes (ATGs) such as *ATG5* and *ATG7* regulate these sequential stages. Indeed, cells without either ATG5 or ATG7 fail to induce autophagy [2]. Autophagic activity is commonly assessed by determining the expression level of microtubule-associated protein 1 light chain 3 (LC3), another autophagy-related protein. Among the three human LC3 isoforms (LC3A, LC3B, and LC3C), LC3B is mainly used to assay autophagy [4]. The cytosolic form of LC3 (LC3-I; 16 kDa) is conjugated to phosphatidylethanolamine to form an LC3-phosphatidylethanolamine conjugate (LC3-II; 14 kDa), which is subsequently recruited to the autophagosomal membrane where it is degraded. Therefore, LC3-II is a marker of autophagosome formation. The p62 protein, also called sequestosome 1 (SQSTM1), forms protein aggregates that are degraded by autophagy [5]. Upon autophagy induction, the level of p62 decreases, whereas the protein accumulates when autophagy is inhibited. Therefore, the p62 protein is commonly used as a marker for autophagic flux.

The role of autophagy in cancer seems to be rather complex and is a topic of debate. Autophagy is reported to suppress carcinogenesis by eliminating oncogenic molecules and damaged organelles [6]. On the other hand, once invasive cancers are established, autophagy promotes the growth of the tumors via intracellular recycling of degraded metabolites, which fuels the cancer cell metabolism [6]. In addition, autophagy is enhanced by chemotherapy and radiation therapy, and functions as an adaptive response that mediates resistance to these treatments [7]. In particular, autophagy was demonstrated to correlate with cisplatin-resistance in ovarian cancer cells [8]. As cisplatin is a key chemotherapeutic agent for the treatment of endometrial cancer, autophagy inhibition might be a promising strategy to treat endometrial cancers with high levels of autophagy.

Chloroquine (CQ) is an autophagy inhibitor. The drug was discovered in 1934 and has been used as an anti-malarial agent since then [9]. In Japan, CQ was withdrawn from the market in 1974 because instances of retinopathy due to the use of CQ had been reported [10]. A safety screening protocol for CQ-induced retinopathy has since been established [11], and CQ is currently considered as a useful drug for various applications. Because of its anti-inflammatory effects, CQ and its derivative hydroxychloroquine have been used to treat rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome [12–14]. As CQ exhibits anti-tumor effects in vitro and in vivo by suppressing autophagy, many clinical trials have been performed, often in combination with anticancer drugs [15,16]. CQ was shown to block the fusion of autophagosomes to lysosomes and the subsequent degradation step, thereby inducing autophagosome accumulation [17]. To date, only a few studies have been conducted on the role of autophagy in endometrial cancer.

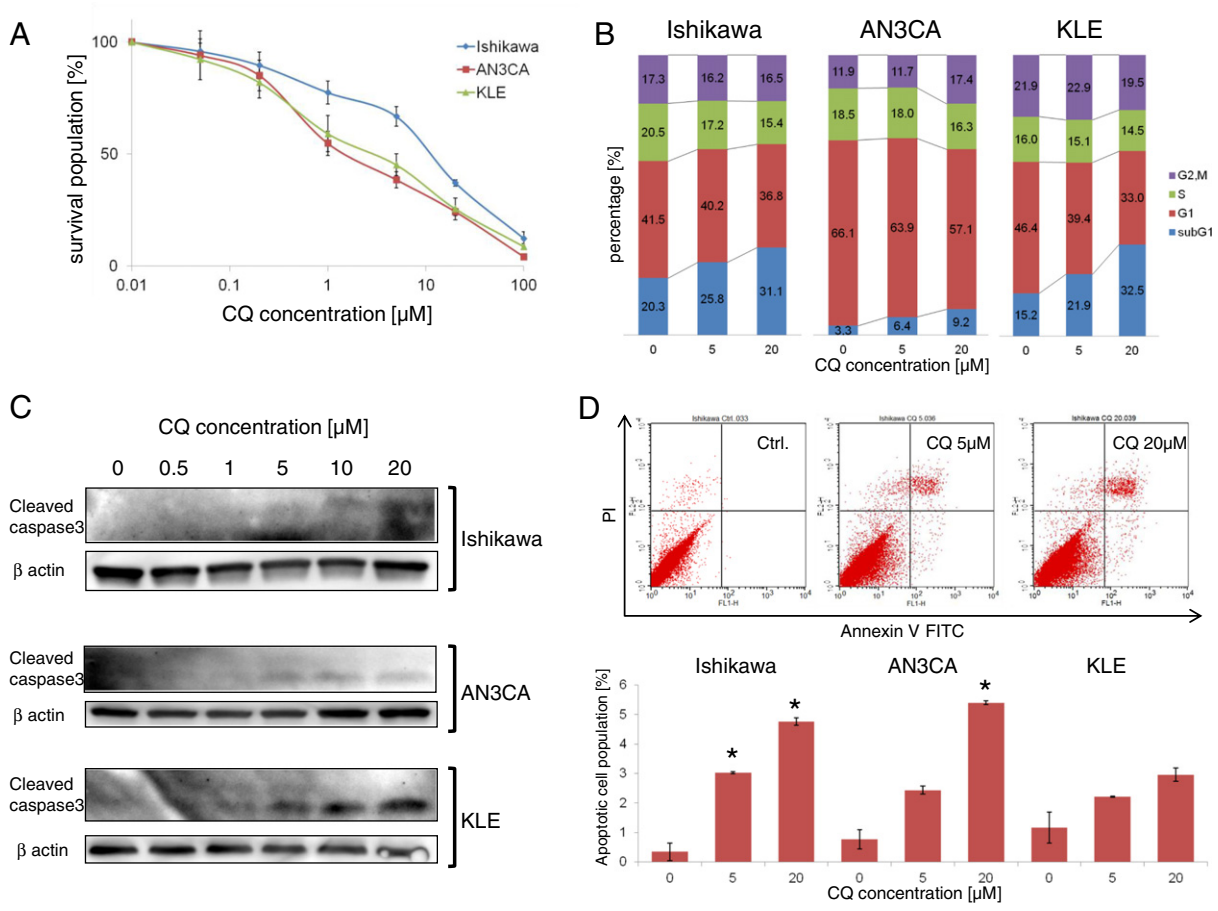

Fig. 1. CQ suppresses the proliferation of endometrial cancer cells in a dose-dependent manner by induction of apoptosis. (A) Effect of CQ on endometrial cancer cells. Ishikawa, AN3CA, and KLE cells were cultured in the presence of various concentrations of CQ for 72 h, and the cell viability was analyzed by using an MTT assay. The results are shown as the mean ± SE of four samples. (B) Flow cytometric analysis of the cell cycle was performed for the three endometrial cancer cell lines using the concentrations of CQ indicated. The results are shown as the mean of three independent experiments. (C) Induction of cleaved caspase-3 by CQ was evaluated by western blotting. Cells were exposed to CQ for 48 h at the indicated concentrations. (D) The three endometrial cancer cell lines were treated with CQ (0 μM, 5 μM, or 20 μM) for 24 h. The cells were harvested and stained with annexin V-FITC and PI, and the apoptotic cell population was determined as the double-positive fraction of the total population using FACS. The results are shown as the mean ± SE of three independent experiments. The upper panel shows FACS data of one of three experiments for Ishikawa cells. $^{*}P < 0.05$.