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Research report

Cathepsin L knockdown enhances curcumin-mediated inhibition of growth, migration, and invasion of glioma cells

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ABSTRACT

Curcumin can be used to prevent and treat cancer. However, its exact underlying molecular mechanisms remain poorly understood. Cathepsin L, a lysosomal cysteine protease, is overexpressed in several cancer types. This study aimed to determine the role of cathepsin L in curcumin-mediated inhibition of growth, migration, and invasion of glioma cells. Results revealed that the activity of cathepsin L was enhanced in curcumin-treated glioma cells. Cathepsin L knockdown induced by RNA interference significantly promoted curcumin-induced cytotoxicity, apoptosis, and cell cycle arrest. The knockdown also inhibited the migration and invasion of glioma cells. Our results suggested that the inhibition of cathepsin L can enhance the sensitivity of glioma cells to curcumin. Therefore, cathepsin L may be a new target to enhance the efficacy of curcumin against cancers.

1. Introduction

Glioma is a common and aggressive malignant human brain tumor with poor prognosis. This tumor seriously harms human health and accounts for an incidence of 3 cases per 100,000 individuals (Westermark, 2012). Malignant gliomas are treated through surgical resection, radiation, and chemotherapy. Despite various treatments, most patients with gliomas die within 1 year after diagnosis (Mangiola et al., 2010; Van Meir et al., 2010). Gliomas are also characterized by critical prognostic factors, namely, rapid growth rate and highly diffuse infiltration; however, these factors contribute to the failure of current therapies (Gagliano et al., 2010; Claes et al., 2007). Furthermore, many genes associated with an increased risk of glioma are often mutated or correlated with specifically acquired mutations (Melin and Jenkins, 2013). Therefore, the pathogenesis of gliomas should be elucidated to develop new therapeutic targets and to improve therapeutic effects.

Cathepsin L (CTSL), a lysosomal cysteine protease, is a potential therapeutic target in cancer treatment (Lankelma et al., 2010; Navab et al., 2008). CTSL is overexpressed in several types of human carcinomas, including breast, lung, gastric, colon, melanoma, and glioma (Qin et al., 2016; Chen et al., 2011; Miyamoto et al., 2011; Chauhan et al., 1991; Stabuc et al., 2006; Strojnik et al., 2005). The level of CTSL expression is also associated with the degree of malignancy (Skrzydlewska et al., 2005). CTSL upregulation promotes angiogenesis (Rebbaa et al., 2009), transformation (Goulet et al., 2007), and differentiation (Duncan et al., 2008). Furthermore, CTSL is associated with the growth, survival (Primon et al., 2013), cycle (Goulet and Nepveu, 2004), migration, and invasion (Strojnik et al., 1999; Kirschke et al., 2000) of tumor cells. CTSL inhibition with radiotherapy significantly impedes the growth of glioma stem cells, promotes apoptosis, improves the radiosensitivity (Wang et al., 2016) and downregulation of CTSL, and suppresses cancer invasion and migration by inhibiting EMT (Zhang, 2015). Therefore, CTSL knockdown is necessary to regulate cell growth, migration, and invasion. The vulnerability of glioma cells to drugs can be enhanced by targeting the increased CTSL levels in glioma, by administering CTSL inhibitors, or by genetically manipulating CTSL expression. Thus, CTSL may be a potential therapeutic target for glioma treatment.

Curcumin(Cur) is a natural compound present in turmeric (*Curcuma longa* Linn), a rhizomatous plant commonly used as a spice (Prasad et al., 2014). Curcumin possesses potent anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic activities. It also exhibits a broad spectrum of suppressive

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activities in various tumors, including glioblastoma, lung cancer, ovarian cancer, and prostate cancer (Jordan et al., 2016; Ravindran et al., 2009; Lin et al., 2007). This substance is involved in different anti-tumor mechanisms, such as apoptosis, cell cycle arrest, and migration and invasion inhibition (Wang et al., 2015; Zhang et al., 2015; Ma et al., 2014; Chen et al., 2015). So far the most commonly recognized mode of curcumin action on cancer cells is the induction of apoptosis as was revealed by Ewa Sikora group (Wolanin et al., 2006; Grazyna et al., 2016). Several proteins are also implicated in curcumin-related mechanisms. For instance, Bcl-2 protein decreases in response to curcumin. Therefore, this protein may play a key role in curcumin-induced apoptosis of Caki cells (Karunagaran et al., 2005; Woo et al., 2003). Nevertheless, the precise molecular mechanisms of curcumin-mediated inhibition of tumor growth, migration, and invasion should be elucidated.

Our laboratory studies have revealed that curcumin activates autophagy and triggers the differentiation cascade in GICs isolated from human GBMs (Zhuang et al., 2012). Autophagy and apoptosis also contribute to curcumin-induced death of K562 cells (Jia et al., 2009). The upstream signal regulation of autophagy and apoptosis has been proposed as a new strategy to enhance the anti-tumor activities of curcumin. The activation of autophagy and apoptosis is accompanied by an increase in the activity of lysosomal cathepsins (Boland and Campbell, 2004; Kaasik et al., 2005); this phenomenon suggests that lysosomal hydrolases are involved in cell death pathways. As a lysosomal cathepsin, CTSL mediates autophagy and apoptosis (Li et al., 2016). Our study aimed to determine whether CTSL participates in the regulation of apoptosis and cycle arrest. This study also aimed to investigate whether CTSL is involved in curcumin-induced inhibition of migration and invasion and to reveal the underlying mechanisms. We found that the inhibition of CTSL may represent a novel therapeutic target to reinforce the efficacy of cancer chemotherapy.

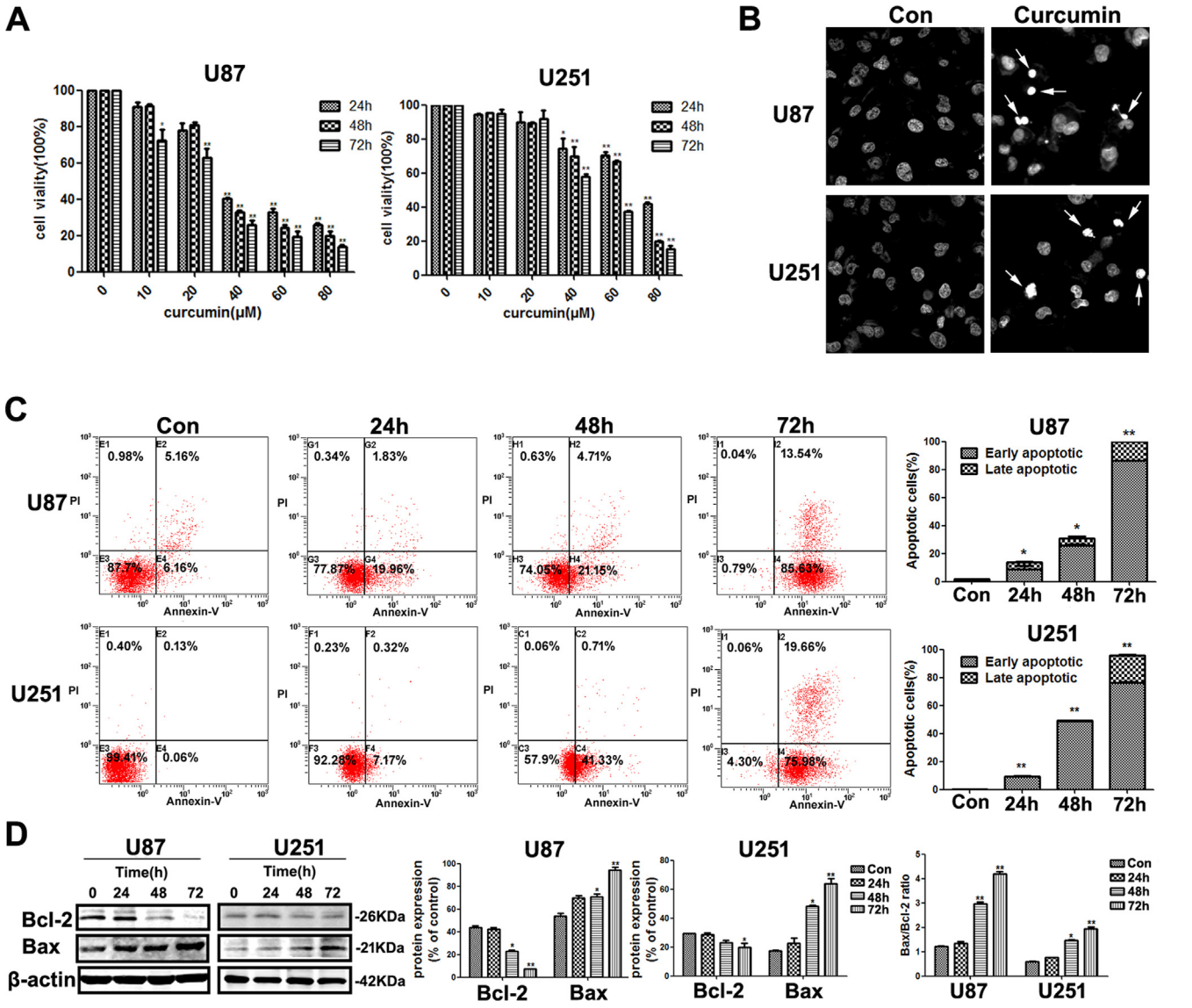

Fig. 1. Curcumin inhibits the proliferation of U87 and U251 cells and induces their apoptosis. (A) Dose- and time-dependent curcumin cytotoxicity determined through CCK-8 assay. Cells were treated with 0, 10, 20, 40, 60, and 80 μM curcumin for 24, 48, and 72 h. (B) Cells were treated with 20 μM curcumin for 48 h and stained with Hoechst 33258; nuclear condensation was observed through Hoechst staining (the arrowhead indicates an apoptotic nucleus, 40 × magnification). (C) Annexin V-FITC/PI staining and flow cytometric determination of the apoptosis of U87 and U251 cells treated with 20 μM curcumin for 24, 48, and 72 h demonstrated a remarkable increase in the percentage of apoptotic cells compared with the untreated control group. (D) U87 and U251 cells were treated with curcumin (20 μM) for 24, 48, and 72 h. Cell lysates were prepared, and protein level was determined through Western blot analysis. β-actin was used to normalize and verify protein loading. Results are expressed as mean ± SD. Statistical significance is indicated by $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.