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Original article

Targeting urothelial carcinoma cells by combining cisplatin with a specific inhibitor of the autophagy-inducing class III PtdIns3K complex

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Abstract

Background: Cisplatin-based regimens are routinely employed for the treatment of urothelial carcinoma. However, therapeutic success is hampered by the primary presence of or the development of cisplatin resistance. This chemoresistance is executed by multiple cellular pathways. In recent years, the cellular process of autophagy has been identified as a prosurvival pathway of cancer cells. On the one hand, autophagy enables cancer cells to survive conditions of low oxygen or nutrient supply, frequently found in tumors. On the other hand, autophagy supports chemoresistance of cancer cells. Here, we aimed at investigating the involvement of autophagy for cisplatin resistance in different urothelial carcinoma cell lines.

Materials & Methods: We analyzed the expression levels of different autophagy-related proteins in cisplatin-sensitive and cisplatin-resistant urothelial carcinoma cell lines. Furthermore, we performed cell viability assays and caspase activity assays with cells treated with cisplatin, non-specific or specific autophagy inhibitors (chloroquine, 3-methyladenine, SAR405) or combinations thereof.

Results: We found that autophagy-related proteins are up-regulated in different cisplatin-resistant urothelial carcinoma cells compared to the sensitive parental cell lines. Furthermore, inhibition of autophagy, in general, or of the autophagy-inducing class III PtdIns3K complex, in particular, sensitized both sensitive and resistant urothelial carcinoma cells to cisplatin-induced cytotoxic effects.

Conclusion: We propose that targeting the autophagic machinery might represent a suitable approach to complement or even increase cisplatin efficacy in order to overcome cisplatin resistance in urothelial carcinoma.

Keywords: Autophagy; Cisplatin; Urothelial carcinoma; VPS34; Chemoresistance

1. Introduction

Bladder cancer (BC) is the fifth most common cancer in the developed world, with approximately 400,000 new cases diagnosed per year and 150,000 deaths worldwide [1]. In industrial countries, about 90% of BCs are urothelial carcinomas (UC) which may be further classified into muscle-invasive and non–muscle-invasive cancers. These UC subtypes are distinct in clinical behavior and molecular alterations [2]. Comprising up to one-third of UC, muscle-invasive tumors often progress to metastatic disease and patients face a poor prognosis with only 50% to 60% survival after 5 years [3]. Although platinum-based chemotherapy is the standard first-line treatment for advanced UC, its impact on cancer-specific survival is limited [4]. Despite frequent initial treatment responses, overall survival does not exceed 12 to 16 months in metastatic patients [5]. Its anticancer efficacy mainly originates from the formation of bivalent DNA intrastrand crosslinks blocking transcription and replication [6,7]. Subsequently generated DNA double-strand breaks stimulate DNA damage response and initiate the intrinsic mitochondrial apoptosis pathway [8–10]. However, cisplatin treatment frequently leads to the development of chemoresistance, and the molecular mechanisms of resistance are multifaceted [8]. Several factors have been

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suggested to determine the response to cisplatin treatment, including factors regulating mechanisms of apoptosis, DNA repair and transport, as well as phenotype plasticity [8,11,12]. However, the mechanisms underlying cisplatin resistance of UC cells have not been clearly identified yet, and current preclinical research aims at increasing efficacy of cisplatin treatment or re-sensitizing cisplatin-resistant cells for cytotoxic effects.

In recent years, autophagy has emerged as an attractive target for cancer therapy [13–15]. During autophagy, intracellular cargo becomes engulfed by double-membraned vesicles termed autophagosomes. Autophagosomes fuse with lysosomes, and within the resulting autolysosomes, the engulfed cargo becomes degraded [16]. Autophagy occurs at basal levels in most cell types, but can also be actively induced upon stress conditions like nutrient deprivation or treatment with anticancer drugs. The induction of autophagy is centrally regulated by 2 kinase complexes: (1) the ULK1 protein kinase complex consisting of the Ser/Thr protein kinase unc51-like kinase 1 (ULK1) and the interacting proteins autophagy-related (ATG) protein 13 (ATG13), ATG101, and RB1-inducible coiled-coil 1 (RB1CC1; alternatively termed FAK family kinase-interacting protein of 200 kDa, FIP200) and (2) the class III phosphatidylinositol 3-kinase (PtdIns3K) lipid kinase complex consisting of the catalytic subunit vacuolar protein sorting 34 (VPS34; alternatively termed phosphatidylinositol 3-kinase catalytic subunit type 3, PIK3C3) and the interacting proteins VPS15/PIK3R4, Beclin 1, ATG14, and nuclear receptor-binding factor 2 (NRBF2) [16,17]. The activation of these 2 complexes initiates autophagosome biogenesis, most likely at specific subdomains of the endoplasmic reticulum (ER) [18]. Several additional ATG proteins are involved in the formation of autophagosomes; among them the ubiquitin-like protein microtubule-associated proteins 1A/1B light chain 3 (MAP1LC3 or briefly LC3), which can be conjugated to phosphatidylethanolamine and thus be recruited to the autophagosomal membrane [19]. Anticancer therapies frequently induce autophagy as a prosurvival response that contributes to chemoresistance [14,20]. Consequently, drugs that inhibit autophagy are tested in clinical trials in combination with different anticancer drugs to increase their cytotoxic potential. Several of these trials make use of chloroquine/hydroxychloroquine, which raise the lysosomal pH and thus block fusion of autophagosomes and lysosomes [14]. So far, more specific inhibitors targeting the kinase activities of ULK1 or VPS34 have only been assessed in preclinical studies. These inhibitors include the ULK1 inhibitor MRT68921 or the VPS34 inhibitor SAR405 [21,22].

In this study, we made use of the urothelial carcinoma cell line (UCC) RT-112 and its respective cisplatin-resistant subline RT-112$^{CisPt\text{-}R}$ [12]. We observed that the expression levels of several autophagy-related proteins are increased in RT-112$^{CisPt\text{-}R}$ cells compared to the parental line. Furthermore, it appears that basal autophagy is increased in the resistant cells, but they still remain responsive to autophagy-inducing stimuli. The inhibition of autophagy either by chloroquine or the VPS34-Beclin 1 complex-targeting inhibitors 3-MA or SAR405 complemented or even increased the cytotoxic effects of cisplatin in both parental and RT-112$^{CisPt\text{-}R}$ cells. Furthermore, we obtained similar results with other UCCs representing the heterogeneity of this disease. Accordingly, we hypothesize that the inhibition of the autophagy-inducing VPS34-Beclin 1 complex represents a promising approach to increase the efficacy of cisplatin or to overcome cisplatin resistance in UC.

2. Material and methods

2.1. Antibodies and reagents

Antibodies against β-actin (clone AC-74, Sigma-Aldrich, #A5316), ATG13 (Sigma-Aldrich, #SAB4200100), ATG14 (MBL, #PD026), Beclin 1 (Santa Cruz, #sc-11427 or Sigma-Aldrich, #B6186), Caspase-3 (R&D Systems, #AF-605-NA), GAPDH (Abcam, #ab8245), LC3B (Cell Signaling Technology, #2775), PARP (Enzo, #BML-SA250), RB1CC1 (Bethyl Laboratories, #A301–574A), α-Tubulin (Sigma-Aldrich, #T5168), ULK1 (clone D8H5, Cell Signaling Technology, #8054), and VPS34 (Thermo Fisher Scientific, #PA1–46456) were used. IRDye 800- or IRDye 680-conjugated secondary antibodies were purchased from LI-COR Biosciences (926–68070, 926–68071 and 926–32211). Other reagents used were 3-MA (Sigma-Aldrich, #M9281), Bafilomycin A_1 (Sigma-Aldrich, #B1793), Chloroquine (Sigma-Aldrich, #C6628), Cisplatin (Accord Healthcare GmbH, PZN: 00370955), DMSO (Sigma-Aldrich, #D4540), Q-VD-OPh (MP Biomedicals, #03OPH109), and SAR405 (Selleck Chemicals, #7682).

2.2. Cell lines and cell culture

All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FCS and 4.5 g/l D-glucose in a humidified atmosphere at 37°C and 5% CO_2. The Cisplatin-resistant sublines were generated over several months by increasing dosages of cisplatin added with every passage up to concentrations of 12, 1, 2, 7, 3.5, or 1.5 μg/ml to RT-112, J82, 253J, T24, 5637, and SW-1710 cells, respectively. Accordingly, the respective concentration of cisplatin was added to the media of the cisplatin-resistant sublines with every passage. For amino acid starvation, RT-112 cells were washed once with PBS and incubated for 2 hours in EBSS (Gibco, #24010–043).

2.3. Microscopy

RT-112 and RT-112$^{CisPt\text{-}R}$ cells were cultured in regular medium or medium containing 12 μg/ml cisplatin, respectively. Phase contrast images were captured using an Axio Observer A1 microscope (Carl Zeiss) with a magnification of 200× (Objective: ZEISS, LD A-Plan 20×/0.30 Ph1).