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Cisplatin-induced apoptosis involves a Fas-ROCK-ezrin-dependent actin remodelling in human colon cancer cells

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ABSTRACT

In human colon cancer cells, cisplatin-induced apoptosis involves the Fas death receptor pathway independent of Fas ligand. The present study explores the role of ezrin and actin cytoskeleton in relation with Fas receptor in this cell death pathway. In response to cisplatin treatment, a rapid and transient actin reorganisation is observed at the cell membrane by fluorescence microscopy after Phalloidin-FITC staining. This event is dependent on the membrane fluidification studied by electron paramagnetic resonance and necessary for apoptosis induction. Moreover, early after the onset of cisplatin treatment, ezrin co-localised with Fas at the cell membrane was visualised by membrane microscopy and was redistributed with Fas, FADD and procaspase-8 into membrane lipid rafts as shown on Western blots. In fact, cisplatin exposure results in an early small GTPase RhoA activation demonstrated by RhoA–GTP pull down, Rho kinase (ROCK)-dependent ezrin phosphorylation and actin microfilaments remodelling. Pretreatment with latrunculin A, an inhibitor of actin polymerisation, or specific extinction of ezrin or ROCK by RNA interference prevents both cisplatin-induced actin reorganisation and apoptosis. Interestingly, specific extinction of Fas receptor by RNA interference abrogates cisplatin-induced ROCK-dependent ezrin phosphorylation, actin reorganisation and apoptosis suggesting that Fas is a key regulator of cisplatin-induced actin remodelling and is indispensable for apoptosis. Thus, these findings show for the first time that phosphorylation of ezrin by ROCK via Fas receptor is involved in the early steps of cisplatin-induced apoptosis.

1. Introduction

Cisplatin or cis-diamminedichloroplatinum II (CDDP) is one of the most known potent anticancer agents, displaying clinical activity against a variety of solid tumours. Platinum-DNA adducts, which are formed following the uptake of the drug into the cell nucleus, activate several signal transduction pathways and culminate in the activation of apoptosis.[1] However, the contributions of other DNA-independent targets, which have been so far underestimated, could also play an important role in cisplatin cytotoxicity.[2] Elucidating more thoroughly the cytotoxic mechanisms of cisplatin could be a benefit to refine anticancer therapeutic approaches based on this compound.

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Cisplatin-induced cell death implicates both Fas receptor-dependent pathway and mitochondria-dependent pathway. We have previously shown that exposure of HT29 or HCT116 human colon cancer cells to cisplatin induces Fas receptor clustering and activation in a ligand-independent manner.[3] The type I transmembrane protein Fas (CD95/Apo-1) triggers apoptosis in a variety of cell types. Upon engagement, the Fas-associated death domain protein (FADD) and procaspase-8 are rapidly recruited to the intracellular death domain of Fas receptor, forming the Death-Inducing Signalling Complex (DISC), which then leads to activation of a caspase cascade and irreversible apoptosis.[4] Recent studies have shown that apoptosis induced by Fas agonists or chemotherapeutic agents involves the aggregation of the Fas receptor into membrane lipid rafts enriched in cholesterol and sphingolipids.[5–9] Moreover, actin cytoskeleton is involved in Fas-mediated apoptosis, regulating Fas clustering and internalisation[10–12] with a recent demonstration of a key role of RhoA–ROCK-dependent ezrin–radixin–moesin phosphorylation in this cell death pathway.[13]

Actin cytoskeleton is central in the regulation of membrane-associated signalling and membrane trafficking. The ezrin–radixin–moesin (ERM) family proteins constitute the link between plasma membrane proteins and actin cytoskeleton. Among them ezrin has been shown to be involved in Fas-mediated apoptosis.[12]

More recently, we have demonstrated that cisplatin induces a rapid inhibition of Na^+/H^+ exchanger-1 (NHE1), leading to intracellular acidification which promotes acid sphingomyelinase activation, ceramide generation, membrane fluidification and Fas aggregation into lipid rafts.[14] The present study further explores this cascade by evaluating the role of ezrin and actin cytoskeleton in cisplatin-induced apoptosis. We show for the first time that cisplatin induces actin cytoskeleton reorganisation, which depends on membrane fluidification. Concomitantly, cisplatin induces ezrin phosphorylation through ROCK, and co-localisation of Fas receptor with ezrin at the cell membrane of HT29 cells. All these events are dependent on Fas. Altogether, these data suggest for the first time that Fas receptor triggering is indispensable for actin microfilament rearrangement during cell death induced by cisplatin in human colon cancer cells.

2. Materials and methods

2.1. Chemicals

Cisplatin (CDDP) was from Merck, latrunculin A (LTN A) and water-soluble cholesterol (CHOL) were from Sigma–Aldrich. Rho inhibitor (Rho Inh) was from Cytoskeleton. Hoechst 33342 and fluorescein-tagged phalloidin (Phalloidin-FITC) were from Molecular Probes.

2.2. Cell culture and treatments

HT29, HCT116 and SW480 human colon carcinoma cell lines (American Tissue Culture Collection, Biovalley) were cultured in Eagle's minimum essential medium (Eurobio) complemented with 10% foetal calf serum (FCS) (v/v) (GibcoBRL) and 2 mM L-glutamine (GibcoBRL). For all experiments, the cells, growing in exponential phase, were treated with 25 μM CDDP for different times. When indicated, the cells were pre-treated for 2 h or overnight with various chemical compounds.

2.3. Cell death assays

2.3.1. Hoechst staining

Apoptotic index was measured as previously described.[14] At least three independent experiments were performed per inhibitor tested.

2.3.2. Caspase-3 activity

Caspase-3 activity was measured using the substrate DEVD-AMC (N-Acetyl-Asp-Glu-Val-Asp-AMC; Calbiochem) as previously described.[14] Three experiments were performed in triplicate for each experimental condition tested.

2.4. Determination of membrane fluidity by EPR spin-labelling method

The membrane fluidity of cells was determined by a spin-labelling method using electron paramagnetic resonance (EPR) as previously described.[14] A decrease in the membrane order parameter S reflects an increase in the membrane fluidity.

2.5. Analysis of F-actin labelling

After treatment, the cells were fixed in 4% paraformaldehyde (Sigma–Aldrich) for 20 min, washed in PBS 1X and then preincubated with PBS–BSA 2% (w/v)–saponin 0.2% (w/v) for 30 min before staining with fluorescein-tagged phalloidin (Phalloidin-FITC) (1:500, Sigma) for 30 min. The samples were viewed using a fluorescent DMRXA2 Leica microscope with a 40× NA 1.32 lens equipped with standard fluorescent filters. The images of F-actin were acquired with a CoolSNAP ES camera using MetaMorph software.

2.6. Immunofluorescence microscopy analysis

After treatment, the cells were fixed in 4% paraformaldehyde (Sigma–Aldrich) for 15 min, washed in PBS 1X and then preincubated with PBS–BSA 2% (w/v)–saponin 0.2% (w/v) for 30 min before incubation for 2 h with either polyclonal rabbit IgG anti-Fas (1:100, AbCam), mouse monoclonal IgG anti-ezrin (1:100, Biogenesis), or isotype-matched controls. The cells were then washed in PBS and stained for 45 min with TRITC-labelled goat anti-rabbit IgG (Molecular Probes) or FITC-labelled goat anti-mouse IgG (Jackson Immunoresearch Laboratories). The fluorescent images of ezrin and Fas stainings were analysed using a DMRXA Leica microscope, a COHU high performance CCD camera and the metavue software. A Z-series of images has been taken after image acquisition.

2.7. Isolation of membrane microdomains

The membrane microdomains of HT29 cells were isolated as previously described.[8] After ultracentrifugation, 1mL fractions were collected from the top of the gradient. Measurement of cholesterol content was performed with Infinity cholesterol