European Journal of Cancer 60 (2016) 59–68

Available online at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Original Research

The integrin-linked kinase-associated phosphatase (ILKAP) is a regulatory hub of ovarian cancer cell susceptibility to platinum drugs

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Received 27 August 2015; received in revised form 4 December 2015; accepted 25 February 2016
Available online 13 April 2016

KEYWORDS
ILKAP;
p90RSK;
Ovarian cancer;
Platinum drugs;
Hepatocyte growth factor

Abstract ***Background:*** Platinum drugs are the most powerful chemotherapeutic agents in the treatment of ovarian cancer. We demonstrated previously that unexpectedly ovarian cancer cells are sensitised to cisplatin (CDDP) by the hepatocyte growth factor (HGF), usually considered an anti-apoptotic factor.
Methods: We used quantitative polymerase chain reaction and Western blot analysis to evaluate gene and protein expression, immunofluorescence to evaluate protein localisation and functional assays to measure cell viability and apoptosis.
Results: In ovarian cancer cells, CDDP induced the phosphorylation, i.e. the activation, of the p90RSK. Surprisingly, a 48-h-long cell pre-treatment with HGF reverted this activation. HGF pre-treatment also resulted in the increased expression of the integrin-linked kinase (ILK)-associated phosphatase (ILKAP) that dephosphorylated the p90RSK. Conversely, CDDP down-modulated ILKAP expression. This impaired CDDP efficacy, as ILKAP silencing protected cells from CDDP-induced death. In line, the biochemical inhibition of the p90RSK or the combined silencing of the most expressed RSK isoforms, namely RSK1 and RSK2, increased the efficacy of CDDP. However, p90RSK inhibition was not sufficient to revert cell protection from death after ILKAP suppression, because of the simultaneous increased activity of the anti-apoptotic kinases ILK and ILK substrate AKT, which were both dephosphorylated, i.e. negatively regulated, by ILKAP. Only the combined inhibition of p90RSK and ILK reverted the effect of ILKAP suppression.

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http://dx.doi.org/10.1016/j.ejca.2016.02.022

Conclusions: As RSKs, ILK and AKT are vital kinases for ovarian cancer onset and progression, data suggest that ILKAP is a regulatory hub of ovarian cancer cell survival by controlling the activation of these kinases.

1. Introduction

The clinical use of platinum compounds was a milestone in the development of successful cancer chemotherapeutic agents [1]. Its action is based on the induction of DNA lesions, that trigger the recruitment and activation of the ataxia telangiectasia mutated and of the ataxia and RAD-related kinases, which in turn phosphorylate several key proteins that activate the DNA damage response, leading to cell cycle arrest and DNA repair or apoptosis [2]. However, platinum drugs, as other chemotherapeutic agents, can also activate signalling pathways responsible for cell protection and drug resistance. For example, cisplatin (CDDP) treatment of cells results in the activation of various branches of the mitogen-activated protein kinase system, including those mediated by extracellular signal-regulated kinases (ERK1/2), c-JUN N-terminal kinases and stress-activated protein kinases [3,4].

It is well established that cell sensitivity to chemotherapy depends on the balance between death and survival signals. We showed that apoptosis of human ovarian cancer cells treated with CDDP and paclitaxel is enhanced, unexpectedly, by the hepatocyte growth factor (HGF), known as a survival factor, which also activates cell proliferation, motility and invasiveness through its receptor encoded by the *MET* tyrosine kinase oncogene [5,6]. We show here that CDDP stimulated the activation of the p90RSK, while long-term treatment of cells with HGF induced its dephosphorylation. The RSKs are the immediate downstream effectors of ERK1/2 and mediate the growth-promoting, proliferative, and anti-apoptotic actions of factors that activate this pathway [7,8]. We show that HGF affected the expression of the integrin-linked kinase-associated phosphatase (ILKAP), that regulated the phosphorylation not only of ILK but also of the p90RSK.

2. Materials and methods

2.1. Cell lines and reagents

SK-OV-3, NIH:OVCAR-3, TOV21-G, OV-90, RMG1, OVCAR-4, OVCAR-5, IGROV-1 and OAW42 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) in 2011. OVCAR-8 cell line was from the NCI-60 collection and obtained from Charles River in 2011. All cell lines have been characterised by the provider and maintained as suggested. Recombinant human HGF was purchased from Raybiotech, Inc (Norcross, GA, USA). AZD6244, LY294002, BI-D1870 and ILK inhibitor Cpd22 were purchased from Calbiochem (Merk Millipore, Darmstadt, Germany) and used at the indicated doses. SCH77294 was from Selleckchem.

2.2. Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) was carried out as described previously [9]. Sequence of primers and PCR conditions can be obtained from the authors.

2.3. Western blot analysis

Western blot analysis was carried out as described previously [10]. The following antibodies were used: mouse monoclonal anti-vinculin from Sigma (St Louis, MO, USA), mouse monoclonal anti-P-ERK1/2 (Thr202/Tyr204), rabbit polyclonal anti-ERK1/2 antibodies, rabbit monoclonal anti-P-AKT (Ser473), rabbit monoclonal anti-AKT, rabbit monoclonal anti-ILK, rabbit polyclonal anti-RSK1, rabbit monoclonal anti-RSK2, rabbit monoclonal anti-P-RSK (Ser221), rabbit polyclonal anti-P-RSK (Ser380), rabbit monoclonal anti-P-BAD (Ser112), rabbit monoclonal anti-BAD, rabbit monoclonal anti-P-YB-1 (ser102), rabbit monoclonal anti-YB-1, rabbit monoclonal anti-P-GSK3β (Ser9) and rabbit monoclonal anti-pan RSK were all obtained from Cell Signalling Technology (Beverly, MA, USA). Rabbit anti-ILKAP antibody was purchased from Bethyl Laboratories, Inc (Montgomery, AL, USA). Goat polyclonal anti-Lamin B was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Bound antibodies were detected using the appropriate peroxidase-conjugated secondary antibody and revealed by enhanced chemiluminescence (Pierce, Rockford, IL, USA). Where indicated, band intensities were quantified using ImageJ software (Wayne Rasband, US National Institutes of Health, Bethesda, MD, USA).

2.4. Cell fractionation

Extraction and separation of cytoplasmic and nuclear protein fractions were obtained using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce) according to the manufacturer's protocol.