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MKP1 repression is required for the chemosensitizing effects of NF-κB and PI3K inhibitors to cisplatin in non-small cell lung cancer

María Cortes-Sempere [a,b,1], Sharmila Chattopadhyay [g,1], Ana Rovira [e,f], Vanessa Rodriguez-Fanjul [a,b], Cristobal Belda-Iniesta [c], Marian Tapia [e,f], Paloma Cejas [c], Rosario Machado-Pinilla [a,b], Cristina Manguan-García [a,b], Isabel Sánchez-Pérez [a,b], Manuel Nistal [d], Carmen Moratilla [a], Javier de Castro-Carpeño [c], Manuel Gonzalez-Barón [c], Joan Albanell [e,f], Rosario Perona [a,b,*]

[a] Translational Oncology Unit C.S.I.C./U.A.M: Instituto de Investigaciones Biomédicas C.S.I.C./U.A.M., C/Arturo Duperier, 4, Madrid 28029, Spain
[b] CIBER de Enfermedades Raras (CIBERER) Valencia, C/Álvaro de Bazán, 10 Valencia, Spain
[c] Medical Oncology Department, Hospital La Paz, Madrid, Spain
[d] Pathology Department Hospital La Paz, Madrid, Spain
[e] Medical Oncology Department, Hospital del Mar-IMAS, Barcelona, Spain
[f] Cancer Research Program, IMIM-Hospital del Mar, PRBB, Barcelona, Spain
[g] Department of Pathology, University of California, San Diego, CA, United States

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ABSTRACT

Treatment of non-small cell lung cancer (NSCLC) with cisplatin has a level of antitumor activity still modest. We have shown previously that MKP1/DUSP1 inhibits cisplatin-induced apoptosis in NSCLC cells and is overexpressed in tumors from most patients with stage I–II NSCLC. Here, using different NSCLC cell lines we found that MKP1 and NF-κB are differentially expressed. We studied whether targeting MKP1, NF-κB or both affects cisplatin-induced cell death. MKP1 is expressed in H460 and H727 cells. H727 and H1299 cells showed constitutive phosphorylation of Akt and increased NF-κB activity than did H460 cells. H460-MKP1-siRNA-expressing cells (but not H727-MKP1-siRNA or H1299-MKP1-siRNA cells) exhibit a marked increase in cisplatin response compared with parental cells. Treatment with the PI3K inhibitor LY294002 or the NF-κB inhibitor BAY11-7082 enhanced cisplatin antitumor activity in parental H1299 cells but only weakly affected responses of H727 and H460 cells. MKP1-siRNA expression enhanced the chemosensitization effect of LY294002 and BAY11-7082 on H727 and H460 cells. Additionally, NSCLC cell lines with higher NF-κB-constitutive activation were the most sensitive to PS-341 (Bortezomib), a non-specific NF-κB inhibitor. This finding suggests the proteasome as a suitable strategy in treating NSCLC tumors with high constitutive NF-κB activity. Altogether, these results showed that either an activated PI3K/Akt/NF-κB pathway and/or high MKP1 was linked to reduced sensitivity to cisplatin in NSCLC cells. Inhibition of NF-κB or PI3K potently enhanced cisplatin cytotoxicity in cells with endogenous or genetically induced low MKP1 levels. These findings support the potential improvement in cisplatin responses by co-targeting NF-κB or Akt and MKP1.

1. Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Systemic treatment of advanced/metastatic NSCLC and, more recently, of high-risk surgically

Abbreviations: CDDP, cisplatin; TNF-α, tumor necrosis factor alpha.

* Corresponding author. Address: Translational Oncology Unit C.S.I.C./U.A.M: Instituto de Investigaciones Biomédicas C.S.I.C./U.A.M., C/Arturo Duperier, 4, Madrid 28029, Spain. Tel.: +34 91 5854463; fax: +34 91 5854401.
E-mail address: RPerona@iib.uam.es (R. Perona).

[1] These authors contributed equally to this manuscript.

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treated patients, involves mainly the use of cisplatin-based chemotherapy. Cisplatin is a DNA-interactive agent that induces lesions in the DNA by forming monoadducts and intra- and interstrand cross-links [1]. Cell death is then mediated by inducing a sustained activation of *N*-terminal c-Jun kinase (JNK) and p38 kinase [2–4]. Unfortunately, the clinical efficacy of cisplatin in NSCLC patients is still far from optimal. This may be related, at least in part, to the presence of various survival pathways in malignant cells that limit or counteract platinum antitumor effects. Upon cisplatin exposure, tumor cells activate survival pathways, including those mediated by dual specific phosphatase MKP1 (DUSP1) [2–4], PI3K [5] and the transcription factor nuclear-factor kappaB (NF-κB) [6]. Gaining further knowledge on the role of these pathways in chemoresistance should lead to novel strategies to enhance cisplatin activity.

MKP1 is an immediate early gene regulated at the transcriptional level by mitogenic, inflammatory, and DNA-damaging stimuli [3,7,8]. Dephosphorylation and inactivation of JNK by MKP1 protects against cisplatin-induced apoptosis [2]. With regards to NSCLC, we have reported that MKP1 plays an important function in NSCLC tumor growth and in response to cisplatin treatment [9]. NSCLC cells expressing a small interfering RNA (siRNA) of MKP1 were more sensitive to cisplatin and grew more slowly when injected into nude mice. Chemical compounds that inhibit, non-specifically, MKP1 expression also induced higher sensitivity to cisplatin. Furthermore, these effects of MKP1 were very specific because inhibition or overexpression of an MKP1-related phosphatase, MKP2, did not alter tumor growth or response to cisplatin in NSCLC cells [9]. These results suggested MKP1 as a novel target for tumor regression and, particularly, for chemosensitizing NSCLC to cisplatin. Since high expression of MKP1 has been reported in NSCLC [7] MKP1 may have implications to improve lung cancer treatment strategies. In addition to MKP1, both PI3K/Akt and NF-κB survival pathways can also limit cisplatin antitumor effects in lung cancer [5]. Further characterization of these pathways is needed, taking in consideration that inhibitors of PI3K and NF-κB are in extensive preclinical and early clinical development.

Class I PI3K comprises a family of heterodimeric complexes, each composed of a p110 catalytic subunit and an adaptor subunit that exists predominantly as p85 [10,11]. PI3K phosphorylates PI(4)P and PI(4,5)P to produce PI(3,4)P2 and PI(3,4,5)P3, which recruit proteins such as Akt/protein kinase B [10] to the cytoplasmic membrane. In turn, Akt inactivates pro-apoptotic proteins such as BAD and caspase-9, thus promoting cell survival [12–14]. With regards to NSCLC, increased gene copy number of PI3K catalytic subunit α and phosphorylated Akt expression has been observed in clinical specimens. In addition, inhibition of PI3K/Akt by pharmacological or genetic approaches reduced proliferation in some NSCLC cancer cell lines [15–18].

NF-κB comprises a family of inducible transcription factors that, among many other roles, protect cells from apoptosis induced by several chemotherapeutic agents [6,19]. In unstimulated cells, NF-κB forms cytoplasmic dimmers associated with a family of inhibitory molecules known as IκBs [20,21]. There are two known NF-κB pathways [22]. In the canonical pathway, activation of NF-κB involves the phosphorylation of IκBs through the IκB kinase signalosome complex [23–25]. This is followed by rapid ubiquitin-dependent degradation by the 26S proteasome. This allows NF-κB dimmers, mainly p65/p50, to translocate to the nucleus where they stimulate expression of target genes. In the non-canonical pathway, the p100-RelB complex is activated by an IκKα homodimer [26]. Notably, cisplatin activates NF-κB through MEKK1, and this activation is modulated by c-Jun, the main substrate of the JNK pathway [27]. This effect, again, can limit cisplatin-induced cell death. Many current antitumor therapies seek to block NF-κB activity as a means of inhibiting tumor growth or sensitizing the tumor cells to chemotherapy [28]. Bortezomib (PS-341, Velcade™) is a potent and selective inhibitor of the chymotryptic activity of the 20S proteasome with a cytotoxic activity in several malignant cell lines. It has clinically proven efficacy in patients with multiple myeloma and malignant lymphoma [29]. The earliest mechanism of action attributed to Bortezomib was the inhibition of NF-κB signaling by stabilizing IκB. Besides, proteasome inhibitors have a number of additional mechanisms of action beyond NF-κB inhibition, due to the effect on proteolysis such as stabilization of pro-apoptotic proteins as p53, Bax, while reducing levels of some antiapoptotic proteins such as Bcl2.

Here, we have examined the relative role of MKP1, PI3K/Akt and NF-κB survival pathways in preventing cisplatin-induced cell death. Our data revealed that MKP1 played a key role in determining the ability of PI3K and NF-κB inhibitors to sensitize NSCLC cells to cisplatin cytotoxicity. In cells with undetected MKP1 expression, inhibition of either PI3K or NF-κB was sufficient to enhance cisplatin effects while in cell lines with detectable expression of MKP1 this was not observed. We then addressed whether MKP1 expression was a marker or a cause of these differential chemosensitizing effects. To this end, NSCLC cell lines were interfered for MKP1 expression with siRNA. Downmodulation of MKP1 expression resulted in a dramatic enhancement of the chemosensitizing effects of both PI3K and NF-κB inhibitors in NSCLC. The data strongly support that MKP1 and PI3K, or MKP1 and NF-κB, signaling pathways contribute independently to cisplatin resistance in NSCLC cells. A concerted inhibition of these pathways emerges as a novel strategy to improve cisplatin antitumor activity.

2. Materials and methods

2.1. Cell culture, antibodies, and reagents

H460, H727, H1299, H23 and A549 cell lines were purchased from the American Type Culture Collection, maintained in RPMI supplemented with 10% fetal-bovine serum. Antibodies used were as follows; unless indicated otherwise, these reagents were from Santa Cruz Biotechnologies, Santa Cruz, CA: anti-MKP1 (M18,), anti-pJNK (V7391, Promega, Madison, WI), anti-JNK1 (C-17), anti-p38 (C20), anti-relB (C19), anti-pP38 (9211S, Cell Signaling