MAPK signaling in cisplatin-induced death: predominant role of ERK1 over ERK2 in human hepatocellular carcinoma cells

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Hepatocellular carcinoma treatment by arterial infusion of cis-diaminedichloroplatinum-II (cisplatin) exhibits certain therapeutic efficacy. However, optimizations are required and the mechanisms underlying cisplatin proapoptotic effect remain unclear. The mitogen-activated protein kinase (MAPK) pathway plays a key role in cell response to cisplatin and the functional specificity of the isoform MAPK/ERK kinase 1 and 2 (MEK1/2) and ERK1/2 could influence this response. The individual contribution of each kinase on cisplatin-induced death was thus analyzed after a transient or stable specific inhibition by RNA interference in the human hepatocellular carcinoma cells Huh-7 or in knockout mice. We demonstrated here that ERK1 played a predominant role over ERK2 in cisplatin-induced death, whereas MEK1 and MEK2 acted in a redundant manner. Indeed, at clinically relevant concentrations of cisplatin, ERK1 silencing alone was sufficient to protect cells from cisplatin-induced death both in vitro, in Huh-7 cells and ERK1−/− hepatocytes, and in vivo, in ERK1-deficient mice. Moreover, we showed that ERK1 activity correlated with the induction level of the proapoptotic BH3-only protein Noxa, a critical mediator of cisplatin toxicity. On the contrary, ERK2 inhibition upregulated ERK1 activity, favored Noxa induction and sensitized hepatocarcinoma cells to cisplatin. Our results point to a crucial role of ERK1 in cisplatin-induced proapoptotic signal and lead us to propose that ERK2-specific targeting could improve the efficacy of cisplatin therapy by increasing ERK1 prodeath functions.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and still presents an increasing incidence and a poor long-term survival (1). Although conventional cytotoxic chemotherapy has not provided clinical benefits for advanced HCC, several studies using cis-diaminedichloroplatinum-II (cisplatin) by hepatic arterial infusion or in transcatheter hepatic arterial chemoembolization have shown encouraging results. Withal, the response rate remains inadequate (2). A better understanding of cisplatin mechanism of action is thus required to decrease or circumvent intrinsic and acquired resistances that limit the clinical efficacy of the drug (3). Even if non-genomic effects have been shown, the main putaminor activity of cisplatin is believed to involve DNA adduct formation. In fact, DNA damages could trigger a cell cycle arrest and the initiation of apoptosis in targeted cells (4,5). Nevertheless, the molecular mechanisms ensuring the signal transduction between cisplatin-mediated genotoxic stress and cell death remain unclear.

Numerous signaling pathways are activated upon cisplatin treatment. Among those, the mitogen-associated protein kinase (MAPK) pathway could have an important impact on the fate of the injured cell (6). Based on structural differences, three major subfamilies are found in the MAPK superfamily: the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases and the p38 kinases. All these kinases have been shown to be activated by cisplatin in various cell types (7). However, ERK mobilization seems to be a critical step in cisplatin response (8). Indeed, the Raf/MAPK/ERK kinase (MEK)/ERK pathway has been generally recognized as an important survival regulator. Nonetheless, an increasing body of evidence suggests that this pathway could also have a proapoptotic function in response to certain stimuli and depend-ent upon the duration of activation and the cell type (9). In that sense, MEK/ERK inhibition has been shown to confer resistance to cisplatin in different cell lines (8,10–13) and a prodeath function of ERK was revealed in vivo in a mouse model of cisplatin-induced nephrotoxicity (14). Nevertheless, the proapoptotic role of ERK appears to be dependent on the cellular context since chemical inhibition of the MEK/ERK pathway also sensitized other cell lines to cisplatin (15,16).

The MEK/ERK signaling pathway consists of a highly conserved three-tier cascade, which typically transmits signals from cell membrane to the nucleus. Indeed, upon activation by the MAPK kinase MEK1 and/or MEK2, the MAPK ERK1 and ERK2 are translocated into the nucleus and phosphorylate a variety of substrates. Due to a high sequence homology, the MEK1 and MEK2 kinases, as well as ERK1 and ERK2, have been regarded as redundant isoforms (17). However, it is now clear that, in some cases, these proteins can also carry out specific functions (18). For instance, a predominant role of MEK1 and ERK2 over, respectively, MEK2 and ERK1 was highlighted by gene inactivation experiment in mice. MEK1 or ERK2 knockout is lethal at the embryonic stage of mice development, in contrast to MEK2−/− or ERK1−/− mice, which are viable, fertile and appear normal (19–22). Using RNA interference experiments, a specific function of MEK1 and ERK2 was also shown in the proliferation of various cancer cell lines, including HCC cells (23–26). However, the hypothesis of a complete functional redundancy among these protein kinases remains open. It has been proposed that the two isoforms ERK1 and ERK2 could participate in cell proliferation, but at different rates, linked to their expression level (27,28). Hence, the relative level of protein expression between the two isoforms could account for the apparent specificity of the kinase in some cell lines (29).

Although the notion of functional kinase specificity for the regulation of cell proliferation is still under debate, few studies have attempted to test the specific roles of MEK1/2 or ERK1/2 and their involvement in cell survival regulation. However, the elucidation of specific roles of these kinases is of particular interest because of the attraction shown toward MEK/ERK as a therapeutic target, and the potential improvement of efficiency of anticancer drugs, like cisplatin, in cells silenced for MEK1/MEK2 or ERK1/ERK2. In this study, we show that the MEK/ERK pathway is necessary to the cisplatin-induced death of transformed hepatocytes and demonstrate that ERK1 is a critical mediator of cisplatin cytotoxicity. Indeed, ERK1 silencing protected cells from clinically relevant doses of cisplatin, whereas ERK2 targeting sensitized cells by increasing ERK1 activity.

Materials and methods

Animals and treatments

Wild-type MF1 and ERK1−/− mice were obtained, respectively, from Harlan (UK) and Gilles Pagès (Nice, France). Animals were given food and water ad

Abbreviations: Cisplatin, cis-diaminedichloroplatinum-II; DEVD-AMC, Asp-Glu-Val-Asp-7-amino-4-methylcoumarin; EdU, 5-ethynyl-2′-deoxyuridine; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; shRNA, small hairpin RNA; siRNA, small interfering RNA.

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Viability assays were performed as described previously (31). Twenty-four hours after injections, blood was collected and mice were killed to collect livers. Serum aspartate aminotransferase and alanine aminotransferase were measured according to the IFCC primary reference procedures and using the Olympus AU2700 Autoanalyzer® (Olympus, Tokyo, Japan).

Cell culture and reagents
The human HCC Huh-7/D12 (HPACC, no. 01042712) cells were cultured as described (31). Hepatocytes were isolated from mice liver by in situ perfusion and cultured as described (32). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and propidium iodide (PI) were from Sigma–Aldrich (St-Quentin-Fallavier, France). Caspase-3/7 were selected following incubation with puromycin (4.5 µg/ml). siRNA transfection experiments were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. For transient transfection experiments, cells were treated to untreated cells. 

Results
To examine Huh-7 cell response to cisplatin, cells were first treated with the chemotherapeutic drug for different time points and the cell viability was analyzed. As shown in Figure 1A, cisplatin treatment led to a dose- and time-dependent increase in Huh-7 cell death, which appeared to be principally apoptosis as observed with caspase-3/7 activity measurements (Figure 1B and 1C). Cisplatin-induced death has been shown to be dependent on the MAPK pathways in different types of tumor cell lines (7). Therefore, we then examined if cisplatin could activate this pathway in our model. Contrary to other models demonstrating that c-Jun N-terminal kinases or p38 activities are required for DNA damage-induced apoptosis (33–35), no activation of the p38 and c-Jun N-terminal kinase pathways could be detected after cisplatin treatment in the human hepatocarcinoma cells Huh-7 (data not shown).

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The MEK/ERK pathway is necessary to cisplatin toxicity. (A) Huh-7 cells were treated with increasing concentrations of cisplatin (1–30 µg/ml) for up to 72 h and cell viability was assessed by MTT assays. (B and C) Caspase-3/7 activities, expressed as Vmax, were quantified by DEVD-AMC fluorometric assay after 24 h treatment with different doses of cisplatin (B) or after a treatment with cisplatin (5 µg/ml) for 24 or 48 h (C). (D) Huh-7 cells were treated with cisplatin (5 µg/ml) and arrested at the indicated times to analyze ERK1/2 phosphorylation by western blot. (E) ERK1/2 activation status was analyzed in the liver of wild-type mice 24 h after injection of cisplatin (45 mg/kg; i.p.) or of a saline solution. For each condition, two representative samples were shown. (F and G) Cells were pretreated for 1 h with dimethyl sulfoxide or with the MEK inhibitor U0126 (50 µM) and then exposed to different concentrations of cisplatin. ERK1/2 phosphorylations were monitored by western blot (F) and MTT assays were performed after 48 h of cotreatment with U0126 (50 µM) and cisplatin (G). (H) Cell viabilities were also assessed after 24–72 h treatment with cisplatin (5 µg/ml) ± U0126 (50 µM) and (I) cell cycle was analyzed by PI staining of DNA after 48 h treatment by cisplatin (5 µg/ml) ± U0126 (50 µM). Representative figures of cell cycle analysis are depicted and quantifications of cells in S phase are shown. Data are representative of at least three independent experiments (mean ± SD; *P < 0.05; **P < 0.005).
available at Carcinogenesis Online). Rottlerin has also been shown to block the cells in G1 phase, as does the inhibition of MEK activity (36). Same cell viabilities were obtained with cisplatin treatment when cells were pretreated with or without rottlerin (Supplementary Figure 2B, available at Carcinogenesis Online). Thus, rottlerin treatment did not affect cisplatin-induced death, reinforcing the positive role of the MEK/ERK pathway in cisplatin toxicity.

**ERK1 is predominantly involved in cisplatin toxicity**

Several studies in the literature, including results of our group, have reported that MEK1 and MEK2, or ERK1 and ERK2, may have specific functions, notably in cell proliferation (37,38). To identify functional specificities of these kinases in cisplatin-induced toxicity, we first generated stable knockdown clones for MEK1 and MEK2 and tested if a differential involvement of these proteins could be seen. As assessed by MTT assays, the specific inhibition of MEK1 or MEK2 had no impact on the death induced by cisplatin. Indeed, stable or transient silencing of MEK1 or MEK2 expression did not alter cisplatin toxicity (Supplementary Figure 3A–D, available at Carcinogenesis Online). Thus, MEK1 and MEK2 may compensate for each other’s activities in cisplatin-induced cell death. To validate this hypothesis, we analyzed the ERK1/2 activation by cisplatin. After 24 h treatment, an increase in ERK phosphorylation could be seen even if MEK1 or MEK2 were silenced (Supplementary Figure 3E, available at Carcinogenesis Online). Hence, one isoform seems to be sufficient to fully activate ERK1/2 during cisplatin injury.

To investigate if the death signal induced by cisplatin was specifically or preferentially mediated by ERK1 or ERK2, we next generated...
stable knockdown clones for each kinase by shRNA (Figure 2A) and tested their sensitivity toward cisplatin (Figure 2B and 2C). Surprisingly, shERK1 and shERK2 cells displayed different response profiles to cisplatin treatment. Indeed, the selective suppression of either ERK1 or ERK2 activity significantly protected the cells from the toxic effects of high cisplatin concentrations (20–30 µg/ml). However, at the concentration of 5 µg/ml of cisplatin, the peak serum concentration reached during chemotherapy (39), ERK2 inhibition sensitized Huh-7 cells, whereas ERK1 knockdown seemed to have no impact on low-dose cisplatin toxicity.

**ERK1 and ERK2 are both involved in cisplatin action**

To validate the protection observed at high cisplatin concentrations, we examined the apoptosis response of the cloned cells. The caspase-3/7 activities were lower in the shERK1 and shERK2 cloned cells than in control cells, when treated with a high dose of cisplatin. (Figure 2D). In addition, a 2-fold decrease in sub-G1 accumulation was observed (Figure 2E). To confirm that the protection was not due to side-effects of the cloning process, Huh-7 cells were transiently transfected with shRNA targeting ERK1 or ERK2, or both plasmids (Figure 2F). Similar

![Figure 3](https://academic.oup.com/carcin/article-abstract/34/1/38/2463845)
to the results obtained with stable cell clones, the silencing of ERK1 or ERK2 protected the cells from high doses of cisplatin (Figure 2G). Hence, both ERK1 and ERK2 seem to be involved in cisplatin-induced toxicity.

**ERK1 overactivation sensitizes Huh-7 cells to cisplatin**

To gain further insight in the potential sensitization of ERK2 silenced cells and test its relevance (Figure 2C–G), shRNA clone cells were treated with clinically relevant concentrations of cisplatin (1–5 µg/ml). As shown in Figure 3A and 3B, the two clones silenced for ERK2 were more affected by cisplatin toxicity than shERK1 and control cells. This was further confirmed by caspase-3/7 activity measurements (Figure 3C), quantification of sub-G1 accumulation (Figure 3D) and Annexin-V staining (data not shown). To verify that the sensitization was not restricted to cell clones, we performed siRNA-mediated silencing experiments in Huh-7 cells (Figure 3E). As shown in Figure 3F, cells transiently inhibited for ERK2 were also more sensitive to cisplatin. However, when cells were knocked down for both ERK1 and ERK2, this sensitization was lost and cell responds as controls cells (Figure 3F).

![Image](https://academic.oup.com/carcin/article-abstract/34/1/38/2463845)

**Fig. 4.** ERK1 is critical for cisplatin-induced death of normal and transformed hepatocytes. (A and B) Huh-7 cells were transiently transfected with shCtl or shERK1 and strongly selected by puromycin. (A) ERK1/2 phosphorylation status was analyzed by western blot in shRNA transiently transfected cells and ERK1 activity was evaluated by densitometric analysis. Results are expressed as ratio of control activity. (B) shRNA-transfected cells were treated for 48 h with increasing concentration of cisplatin. MTT assays were performed to evaluate cell viability. (C) Hepatocytes were isolated from wild-type or ERK1−/− liver mice and treated with increasing concentrations of cisplatin. After 24h, ERK1/2 phosphorylations were monitored by western blot. (D and E) Cell viabilities were assessed by MTT assays after 72 h of treatment (D) or after a treatment with cisplatin (5 µg/ml) for different time points (E). (F) Caspase-3/7 activities were quantified after 48 h of treatment with cisplatin, using the DEVD-AMC fluorometric assay. Results are expressed as relative units (RU) due to differences in basal caspase activities of hepatocytes preparations (varying from a Vmax of 1280–3850). The data are representative of at least three independent experiments (mean ± SD; *P < 0.05; ***P < 0.005).
activity was greatly increased in shERK2 cells under basal conditions, as well as after cisplatin treatment. However, total ERK activities were unchanged whatever the kinase silencing. To explain the lack of protection from low dose of cisplatin in shERK1 cells, we thus postulate that ERK1 was not fully inhibited. So, in order to completely inhibit ERK1 activity, transient transfections with shRNA were performed in Huh-7 cells, followed by a stronger selection with puromycin for 48h. Under this experimental condition, the quantification of protein expression revealed that less than 10% of ERK1 phosphorylation remained in the transfected cells (Figure 4A). Interestingly, a dose-dependent protection of Huh-7 cells from the low-dose effects of cisplatin could be seen when ERK1 was highly silenced (Figure 4B). To reinforce our hypothesis and eliminate residual ERK1 expression, we then compared cisplatin sensitivity of wild-type and ERK1−/− hepatocytes, a model which responds to cisplatin as Huh-7 cells (Figure 4C). MTT assays showed that hepatocytes deficient in ERK1 were more resistant to cisplatin toxicity than wild-type cells (Figure 4D). This protection was observed in a dose- and time-dependent manner (Figure 4E). Moreover, ERK1 deficiency led to an enhanced resistance to apoptosis (Figure 4F). Hence, ERK1 silencing could protect cells from low dose of cisplatin, whereas ERK1 overactivation, brought up by ERK2 targeting, could sensitize them.

**The loss of ERK1 attenuates cisplatin side effects**

To ascertain the proapoptotic role of ERK1, we then tested the ERK1 involvement in cisplatin side effects. For this purpose, wild-type or ERK1−/− mice were treated for 24h with a saline solution or a single dose of cisplatin (45 mg/kg; i.p.), in order to induce hepatotoxicity (30). As assessed by aspartate aminotransferase/alanine aminotransferase serum dosage, hepatic damages induced by cisplatin treatment were strikingly lower in ERK1−/− animals than in wild-type mice (Figure 5A and 5B). Caspase-3/7 activity measurements also showed that ERK1−/− hepatocytes were less subjected to apoptosis than wild-type cells (Figure 5C). Thus, all these results argue for a critical and predominant role of ERK1 over ERK2 in cisplatin mechanism of action.

**ERK1 is responsible for cisplatin-induced Noxa upregulation**

To get into the molecular mechanism regulated by ERK1, we analyzed the effect of a global inhibition of ERK1/2 activities on the regulation of a panel of proapoptotic factors. Among proteins tested, only Noxa and Puma, two critical BH3-only proteins induced by DNA damages (40), were upregulated after cisplatin treatment. Interestingly, these increases were totally prevented by ERK1/2 inhibition (Figure 6A). To check whether Noxa and Puma upregulations were due to ERK1 activation, we then analyzed their expression in transiently knockdown Huh-7 cells after cisplatin treatment. As shown in Figure 6B, Noxa and Puma upregulation by cisplatin differentially responded to ERK1 silencing. Indeed, Noxa induction was lower in shERK1 cells, whereas Puma regulation was not altered. To confirm the importance of ERK1 for Noxa induction by cisplatin, we finally examined the effect of an ERK1 overactivation. For this purpose, we used the stable knockdown clones for ERK2 (Figure 6C). In these cisplatin sensitive cells, the treatment led to a drastic upregulation of Noxa, what strengthens our hypothesis.

**Discussion**

Investigation of cisplatin signal transduction is of particular interest in cancer therapy, particularly to circumvent resistances that limit the efficacy of the drug. In the present study, we have confirmed that the MEK/ERK pathway is necessary for cisplatin-induced death of human HCC cells. Indeed, the MEK/ERK signaling was the only MAPK pathway activated by cisplatin in Huh-7 cells or in HepG2 cells (13). Moreover, we have demonstrated by RNA interference assays that the kinases MEK1 and MEK2 transmitted the death signal of cisplatin to ERK1/2 in a redundant manner but that this signal transduction was preferentially carried out by the kinase ERK1, which regulated the expression of the apoptotic BH3-only protein Noxa.

Within the MEK/ERK pathway, the different isoforms MEK1 and MEK2 or ERK1 and ERK2 share a high sequence homology as well
as substantially equivalent spatiotemporal regulation. Consistently, we showed here that the kinases ERK1 and ERK2 were both activated and involved in cisplatin toxicity. However, whereas MEK1 and MEK2 showed redundant functions, we clearly demonstrated a predominant role of ERK1 over ERK2. First, ERK2 deficiency protects cells from high dose of cisplatin whereas ERK1 silencing protects from both high and low concentrations. Second, ERK1 inhibition protects cells at a higher level than ERK2 targeting and a dual knockdown of ERK1/2 did not increase this protection. Third, ERK2 silencing results in an elevated ERK1 activity and in an increased sensitivity to low doses of cisplatin. Moreover, when ERK1 overactivation was prevented, cisplatin sensitization was completely lost. By in vivo and ex vivo approaches, we also ascertained for the first time that Erk1−/− hepatocytes are more resistant to cisplatin-induced death than wild-type hepatocytes, establishing that ERK1 could have more general roles in apoptosis regulation. This finding is in agreement with our previous results showing that ERK1 was a negative regulator of normal hepatocyte survival (32, 41), as well as in other cell types, such as keratinocytes or early splenic erythroblasts (42, 43). Despite their high homology, ERK1 and ERK2 could thus be involved in the regulation of different processes in specialized cells. In that way, ERK2 may represent a potential target to improve the chemotherapeutic efficacy of cisplatin. Indeed, we have highlighted previously the importance of ERK2 in tumor cell proliferation (23,38). Here, we have shown that ERK2 targeting could increase the sensitivity of cell exposed to clinically relevant doses of cisplatin by increasing ERK1 activity.

Two different studies have suggested that the apparent preponderant role of ERK2 in cell proliferation was related to the greater abundance of the kinase (27,28). More generally, it has been proposed that the kinases ERK1 and ERK2 are redundant in cell proliferation and that the specific features observed by RNA interference reflected their expression level. Since ERK1 is generally expressed at a lower level than ERK2 (29), its gene silencing will cause smaller effects on total ERK activity and thus will display less severe phenotypes than ERK2 inactivation. In our study, we have shown that ERK1 has a predominant role over ERK2 in cisplatin-induced death, although in our model, ERK1 activity in cisplatin-treated cells was lower than ERK2. Furthermore, the ERK1 silencing did not significantly alter the activation level of ERK2, whereas ERK2 silencing greatly increased ERK1 activation, suggesting that the observed protective effect is related to the loss of ERK1. Similarly and given compensation phenomena, ERK1/2 total activity was not altered in shERK1 or shERK2 cells and this was observed under basal conditions as well as following cisplatin treatment. Our data thus demonstrate that the effects of sensitization or protection are not related to changes in ERK total activity but are rather related to the ratio of activation between the two isoforms, especially in the level of activated ERK1. In a same way, we showed that the level of ERK1 activity correlated with the level of induction of the proapoptotic factor Noxa. ERK1 silencing reduced Noxa induction by cisplatin, whereas ERK2 inactivation stimulated it. Thus, ERK1 appeared as a crucial regulator of Huh-7 survival, but we could not totally rule out a role of ERK2. Indeed, the importance of the MAPK pathways for Noxa induction has previously been reported (10) as well as the

Fig. 6. ERK1 regulates cisplatin-induced Noxa expression. Expressions of different proapoptotic factors were analyzed after 24 h of treatment with cisplatin (5 μg/ml) in U0126-treated cells (50 μM) (A) and in Huh-7 cells silenced for ERK1 (B) or ERK2 (C). Noxa and Puma expression was evaluated by densitometric analysis and results expressed as fold induction. Data are representative of at least three independent experiments (mean ± SD; *P < 0.05).
inhibitory effect of U0126 on Puma induction by cellular stresses (44,45). However, in this study, we showed that ERK1 silencing did not succeed in reducing cisplatin-induced Puma induction, an event which could explain the differences of protection observed between ERK1-specific knockdown and ERK1/2 global inhibition. Puma regulation could thus be dependent on both ERK1 and ERK2 activity.

Far from its role in cisplatin toxicity, ERK1 could also carry out more general functions in the regulation of hepatocyte survival. Indeed, ERK1/2 activation by growth factors or stresses has been mainly defined as a source of antiapoptotic signals. The kinases could promote cell survival by stabilizing antiapoptotic factor like Mcl-1 or downregulating/inactivating proapoptotic members like Bim or Bad (14). ERK1, but not ERK2, could phosphorylate Bad on its ser75, what favors the sequestration of Bad by the 14-3-3 proteins and thus its inactivation (data not shown). ERK1 could thus be implied in the fine tuning of the cell death decisions. Accordingly, Bourbonnais et al. (47) have shown that ERK1 activation induced by collagen I signaling attenuated Fas-induced hepatocyte cell death. Depending on the stimulus, ERK1 activation may thus promote or prevent apoptosis in hepatocytes. This contradictory effect of ERK1 on cell survival could be explained by the hypothesis that a sustained activation of ERK transmits proapoptotic signals, whereas a transient activation could protect cells from death (48,49). Indeed, Bad phosphorylation on Ser75 by ERK1/2 has been shown to occur rapidly after EGF stimulation (50). Thus, ERK1 could act, in a first step, as a prosurvival kinase but its sustained activation could prime cell toward apoptotic engagement by stimulating the late expression of different proapoptotic factors like Noxa. Accordingly, Noxa induction was seen in Huh-7 cells 12h after cisplatin treatment.

In conclusion, we have shown that the MEK/ERK pathway is involved in the mechanism of action of cisplatin in tumoral and normal hepatocytes and that the proapoptotic signal is primarily mediated by the kinase ERK1. Our data highlighted the complexity of ERK1 and ERK2 regulations in hepatocyte fate responses and the specialization of the two kinases in the regulation of survival after cisplatin treatment in these differentiated cells. The development of therapeutic tools allowing specific targeting of ERK2 could thus be beneficial in order to inhibit cell proliferation, as demonstrated previously (23,38), without affecting ERK1 proapoptotic activities.

Supplementary material
Supplementary Figures 1–4 can be found at http://carcin.oxfordjournals.org/

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