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ORIGINAL ARTICLE

Keratin 18 provides resistance to Fas-mediated liver failure in mice

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ABSTRACT

Background Keratins are intermediate filament proteins of epithelial cells with pivotal functions for cell integrity. They comprise keratins 18 [K18] and 8 [K8] in hepatocytes. Keratins are of major importance for an intact cellular microarchitecture and have protective functions in human liver diseases. In mice, K8 has been demonstrated to protect against Fas-antibody-induced liver failure by direct interaction with apoptotic regulators, while the role of K18 remains unresolved.

Materials and methods We analysed effects of K18 deficiency on Fas-induced liver failure in mice. We determined survival and analysed induction of apoptosis after injection of the agonistic Fas antibody Jo2 into $K18^{-/-}$ and wild-type control mice by TUNEL assay and fluorometrically analysed caspase-3, -8 and -9 activities 1, 2 and 3 h after Jo2 injection.

Results In $K18^{-/-}$ mice, survival of Fas-antibody treated mice was significantly shorter than that of wild-type controls (P = 0·02). However, shortened survival of $K18^{-/-}$ mice was caused by increased hepatic damage but was not correlated to enhanced induction of apoptotic pathways, as neither numbers of TUNEL positive apoptotic cells nor activities of caspases-3, -8 and -9 differed between $K18^{-/-}$ and $K18^{+/+}$ mice at any point of time.

Conclusion $K18^{-/-}$ mice are significantly more susceptible to Fas-antibody-induced liver failure. The cytoprotective effect of K18 is not explained by a differential activation of caspases-3, -8 and -9, suggesting that K18 does not directly interfere with apoptotic regulators. Importantly, however, K18 exerts significant protective functions by other mechanisms.

Keywords Apoptosis, Fas, fulminant hepatic failure, keratin 18, liver.

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Introduction

Keratins are highly abundant and make up about 0·3% of total mouse liver proteins [1]. Epithelial cells express at least one type I (K9-K20) and one type II (K1-8) keratin. Keratins of either type combine as non-covalent heterodimers to form intermediate filament proteins. Intermediate filaments in hepatocytes are comprised of K8 and K18 heterodimers.

K8/K18 heterodimers are essential for maintaining structural integrity of hepatocytes. For instance, K8/K18 intermediate filament proteins help to maintain integrity of the hepatocellular surface membrane in response to mechanical stress [2–4]. Beyond their mechanical functions, keratins control a variety of other cellular functions such as adhesion to other cells and cell motility [5]. This is exemplified in keratin 8-deficient hepatocytes, which spread more slowly in cell culture. Furthermore, attachment of K8-null hepatocytes to each other was more efficient owing to increased ß1-integrin expression on the cell surface. Finally, K8-null hepatocytes revealed reduced general protein synthesis but accelerated G1/S phase transition of the cell cycle [5]. These alterations of cellular functions induced by knockout of keratins are not completely understood, but they demonstrate the complex role of keratins within the cellular system beyond their mechanical functions.

Alterations of keratins not only influence physiological cellular functions but also have an effect on severity of liver diseases in different mouse models. Transgenic mice which express the Arg89/Cis mutation of K18 reveal fragile hepatocytes, mild chronic hepatitis and increased susceptibility to apoptosis [3]. Of note, $K8^{-/-}$ mice are markedly more sensitive to

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Fas-antibody- or Concanavalin A-induced liver failure [6], suggesting an overall cytoprotective function of keratins for hepatocytes. Also, transgenic mice overexpressing the Arg89/Cys K18 mutation are more susceptible to Fas-mediated liver injury, while tumour necrosis factor-induced apoptosis is not affected [7]. $K18^{-/-}$ mice are viable and express an apparently normal phenotype, apart from accumulation of K8-containing Mallory bodies and formation of enlarged hepatocytes in old mice [8,9]. Similar to $K8^{-/-}$ mice, $K18^{-/-}$ mice exhibit multinuclear giant cells and anisokaryosis in their livers, probably indicating aberrant cell cycle regulation [8].

Keratins also serve as markers for hepatocellular stress. This was demonstrated in human liver injury in which K8/18 mRNA and protein levels increase up to threefold [10,11]. Furthermore, keratin hyperphosphorylation [12,13] as well as apoptosis-related K18 fragments indicated progression of human liver disease [14–16]. Keratins are probably involved directly in human liver disease, as a strong correlation was found between K8/18 variants and liver cirrhosis [17–19]. Variants of keratins 8 and 18 can alter keratin solubility and phosphorylation state rendering individuals more susceptible to end-stage liver disease, which may be a pivotal co-factor for liver damage in addition to alcohol or viral infection [13]. This hypothesis is supported by the observation of a 3·3-fold increase in keratins 8 and 18 variants in patients with end stage liver disease compared with that in blood bank controls [19]. Furthermore, a keratin 8 mutation [18] and keratin 18 mutations [17] have been found to be preferentially associated with cryptogenic liver disease in human. In addition, exonic K8 variants were significantly correlated with the development of liver fibrosis in a German cohort of 329 patients with chronic hepatitis C infection [20]. Taken together, these data clearly implicate a cytoprotective function of keratins in the human liver and have identified altered expression of keratins as a cofactor accelerating hepatocellular injury by other agents.

Fas-mediated liver failure is a well-established animal model to study liver injury in response to apoptotic stimuli by injection of the agonistic Fas-antibody Jo2 [21]. Fas-mediated liver injury is dependent on caspase-3 activation [22]. Caspases are cytoplasmic proteases which are activated post-translationally by proteolytic cleavage and which are centrally involved in the sequence of events leading to apoptotic cell death. Induction of apoptosis first involves activation of the so-called 'activator' caspases up-stream in the signal cascade. Next, activated activator caspases cleave and activate the so-called 'effector' caspases down-stream in the apoptotic signal pathway. Activator caspases are caspase-8 and caspase-9, while caspase-3 is the central effector caspase. Caspase-8 (also called FLICE) can be directly activated by the death domain of the Fas receptor complex. Alternatively, apoptotic signals can be transmitted via the mitochondrial pathway involving caspase-9. Caspase-3 is the central effector caspase cleaving proteolytically several substrates which subsequently induce the specific changes of apoptotic cell death and ultimately lead to the execution of the programmed cellular damage. Proteolysis by caspase-3 includes a broad spectrum of cell components, including keratin 18.

While keratin 8 has a protective role in Fas-mediated liver injury [6], the function of keratin 18 has not been examined until now. To clarify the role of K18 for protection of liver cells, we analysed the effect of keratin 18 knockout on Fas-mediated liver failure. We studied activation of caspases-3, -8 and -9 as well as numbers of apoptotic cells in $K18^{-/-}$ vs. $K18^{+/+}$ mice after induction of Fas-mediated liver failure to find out if K18 also acts directly on induction of hepatocyte apoptosis.

Experimental procedures

Fas-antibody-induced liver failure

Experiments were performed in $K18^{-/-}$ mice and wild-type controls ($K18^{+/+}$) on a mixed genetic background of the mouse strains Ola, FVB and N. $K18^{-/-}$ and $K18^{+/+}$ mice were housed under standard conditions. All procedures were performed according to approved protocols, following the recommendations for the proper use of laboratory animals and in agreement with the German legal requirements for animal protection (AZ 50·263·2-BN24,20104). Liver failure was induced by intraperitoneal application of 500 μg kg^{-1} Fas-antibody Jo2 (IgG isotype, containing < 0·01 lipopolysaccharide/μg antibody – BD Pharmingen, Franklin Lakes, NJ, USA).

Survival

In the first set of experiments, survival was determined after Jo2 application in seven $K18^{+/+}$ vs. ten $K18^{-/-}$ mice without further intervention. Differences in survival between both groups were analysed by the Kaplan–Meier method using the SPSS PC+ software package (SPSS Inc., Chicago, IL, USA).

Apoptotic changes 1, 2 and 3 h after Jo2 application

To analyse induction of apoptosis at various time points after Jo2 application, 34 mice were injected intraperitoneally with the Jo2 antibody: Six $K18^{-/-}$ and six $K18^{+/+}$ were killed after 1 h, six $K18^{-/-}$ and six $K18^{+/+}$ after 2 h and eight $K18^{-/-}$ and seven $K18^{+/+}$ mice after 3 h by cervical dislocation. Livers were immediately shock-frozen in liquid nitrogen and stored at −80 °C for further analysis.

TUNEL assay

The TUNEL test (TdT-mediated dUTP nick-end labelling) was performed using the *'In situ* cell death detection Kit,

POD' (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Briefly, liver tissue was fixed with 4% paraformaldehyde (Sigma Chemicals, Munich, FRG) for 1 h at 4 °C. Endogenous peroxidase activity was blocked by incubating with 0·03% H_2O_2 (Peroxidase Blocking Reagent; Dako, Carpinteria, CA, USA) and cells were permeabilized by 0·1% Triton X-100 in 0·1% sodium citrate. TUNEL reaction mixture was applied at 37 °C for 1 h and visualized by horse radish peroxidase-conjugated sheep anti-fluoresceine antibody ('converter POD'; Roche Diagnostics) and 3-amino-9-ethylcarbazole. Sections were then counterstained with hemalaun for 5 s. Serial negative control sections were treated in the same way without terminal deoxynucleotidyl transferase. TUNEL staining was quantified by counting TUNEL positive liver cells in relation to TUNEL negative liver cells in at least 10 high power fields at 400-fold magnification.

Caspase-3, -8 and -9 activity assays

Caspase activities were measured by cleavage of specific fluorogenic substrates as previously published elsewhere [23,24]. Substrates were Ac-DEVD-afc (Ac-Asp-Glu-Val-asp-afc, Bachem, Heidelberg, Germany) for caspase-3, Ac-LETD-afc (Ac-Leu-Glu-Thr-Asp-afc, Alexis, Grünberg, Germany) for caspase-8 and Ac-LEHD-afc (Ac-Leu-Glu-His-Asp-afc, Bachem) for caspase-9.

Mouse liver was homogenized in 25 mM N-[2-hydroxyethyl]piperazine-*N*'-[2-ethanesulfonic acid] (HEPES) (pH 7·5) buffer containing 0·1% Triton X-100, 5 mM MgCl2, 2 mM dithiothreitol (DTT) and a protease-inhibitor cocktail (Complete^{TM}; Roche Diagnostics), and centrifuged at 40 000 *g*. Ten microlitres of the supernatant was added to 1·500 µL of 100 mM HEPES buffer (pH 7·4) containing 2 mM DTT. After addition of the fluorogenic substrate (12·5 µM Ac-DEVD-afc, Ac-LETD-afc or Ac-LEHD-afc), fluorescence was measured in 5 min intervals (400 nm/505 nm; Shimadzu, RF-5301PC fluorometer). The increase in fluorescence was linear between 5 and 35 min after adding the fluorogenic substrate. Caspase-3, caspase-8 and caspase-9 activities were calculated from the slope as fluorescence units per mg protein per min reaction time and converted to pmol of substrate cleaved per mg protein per min based on a standard curve for amino-4-trifluoromethyl coumarine (afc). Protein concentration in the supernatant was determined by Bio-Rad *DC* Protein Assay (Bio-Rad, Munich, Germany). Enzyme activity is expressed as mean ± standard deviation. In parallel control experiments, specificity of the fluorometric signal was confirmed by adding specific caspase inhibitors of caspase activities to the reaction mixture [caspases-3 inhibitor: z-DEVD-fmk (z-Asp-Glu-Val-DL-Asp-fmk, Bachem), caspase-8 inhibitor: Ac-IETD-CHO (Ac-Ile-Glu-Thr-Asp-CHO, Alexis), caspase-9 inhibitor: Ac-LEHD-CHO (Ac-Leu-Glu-His-Asp-aldehyd, Bachem)].

Western blotting for active caspases-3

Western blots for active caspase-3 in murine livers were performed as previously published elsewhere [24]. Extracts from mouse livers were prepared by lysis in RIPA (RadioImmuno Precipitation Assay) buffer (pH 7·55) containing PBS (Phosphate-buffered saline), NP-40 1%, 0·5% Na-deoxycholat, 0·1% SDS (Sodium dodecyl sulphate) and protease-inhibitor cocktail (Complete^{TM} Roche) followed by centrifugation at 40 000 *g*. Ten micrograms total protein of each sample was loaded on a 10% SDS–PAGE (Sodium Dodecyl Sulphate–PolyAcrylamid Gel Electrophorese) gel. After electrophoresis, protein was transferred to a PVDF (Polyvinylidenfluoride) membrane (Bio-Rad). Blots were blocked overnight with 5% non-fat dry milk in TBST (Tris-buffered saline Tween 20) (50 mM Tris, pH 7·5, 150 mM NaCl, 0·05% Tween 20) at 4 °C and probed with rabbit polyclonal antibodies against the p17 subunit of caspase-3 (not reacting with procaspase-3) (R&D, AF 835, Minneapolis, MN, USA) in 5% non-fat dry milk/TBST. Immunoblots were then processed with secondary horseradish peroxidase conjugated secondary antibody.

Bands of caspase-3 were detected using the ECL (Enhanced Chemiluminescens) + Western Blotting Detection system (Amersham Biosciences, Buckinghamshire, UK) and a high performance chemiluminescence film (Hyperfilm^{TM} ECL; Amersham Biosciences).

Immunostaining procedures and hemalaun/eosin stainings

Sections from frozen liver tissue were stained by an avidin–biotin technique. Shortly, endogenous biotin was blocked using Dako Biotin Blocking System (Dako, Hamburg, Germany). The sections were incubated with caspase-3 antibody (R&D, Cat. AF 835) in 'antibody diluent with background reducing components' (Dako) at room temperature for 90 min. After washing in PBS, biotin-coupled secondary antibody (Dianova, Hamburg, Germany) was applied for 30 min. Bound antibody was detected with 3-amino-9-ethylcarbazole (Dako). All sections were then counterstained with hemalaun.

Furthermore, randomly selected sections of livers, which were obtained 3 h after Jo2 application, were stained with hemalaun/eosin to analyse the degree of liver damage.

Statistical analysis

All statistical calculations were performed using the SPSS PC+ software package. Data are given as mean ± standard deviation. Differences between the groups were calculated by the non-parametric Mann–Whitney *U*-test.

Results

Survival after Jo2 application

To assess the effect of K18 expression on survival in Fas-induced liver failure, we determined survival after a single intraperitoneal injection of 500 μg kg^{-1} Jo2 in seven $K18^{+/+}$ vs. ten $K18^{-/-}$ mice. In $K18^{-/-}$ mice, we observed increased distress and more severe clinical illness (reduced mobility, social contact and neglect of fur care) after Jo2 application than that in the wild-type control animals. Of note, survival of $K18^{-/-}$ mice was significantly shorter than that of $K18^{+/+}$ mice (P = 0·022). Two out of seven $K18^{+/+}$ mice died of fulminant hepatic failure 244 and 520 min after Jo2 application, while five mice survived. In contrast, nine out of ten $K18^{-/-}$ mice died 273, 322, 330, 358, 461, 520, 520 and 520 min after Jo2 application (Fig. 1). On macroscopic inspection, livers of mice which died after Fas application were heavily swollen and enlarged due to haemorrhage into the liver parenchyma (Fig. 2a,b). On microscopy, we found destroyed liver architecture and severe haemorrhage in the sinusoids already 3 h after injection of Jo2 in $K18^{-/-}$ mice, while Jo2 injected wild-type mice revealed essentially normal liver histology at the same time (Fig. 2c,d). To study pathways of apoptosis induction in more detail, we repeated intraperitoneal injection of Jo2 in 20 $K18^{-/-}$ and 19 wild-type mice. To obtain livers for analysis of apoptosis induction, we killed 6–8 mice from either group at 60, 120 and 180 min respectively.

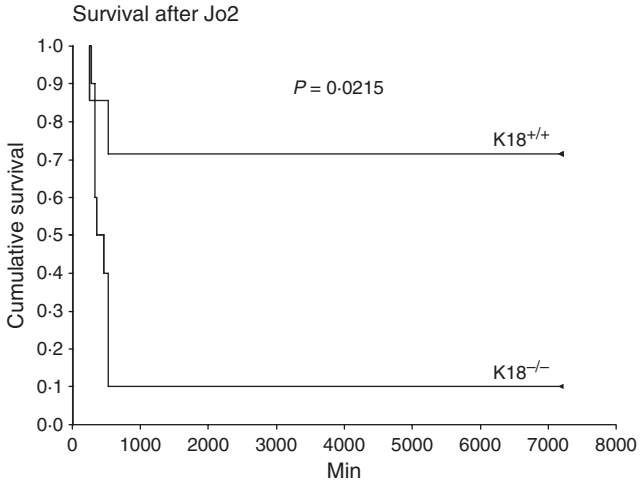

Figure 1 Survival after Jo2 application. Seven $K18^{+/+}$ vs. ten $K18^{-/-}$ mice were treated with Fas-antibody and survival was observed. More $K18^{-/-}$ mice (9/10) than $K18^{+/+}$ mice (2/7) died and survival time was significantly shorter in $K18^{-/-}$ mice than that in $K18^{+/+}$ mice (P = 0·022).

TUNEL test

To quantify the degree of Jo2-induced apoptosis in murine livers *in situ*, we performed TUNEL tests 1, 2 and 3 h after application of the antibody. The highest numbers of TUNEL-positive cells were observed both in $K18^{-/-}$ and $K18^{+/+}$ mice at 3 h after Jo2 application. However, numbers of TUNEL-positive apoptotic cells/high power field in the livers did not differ between $K18^{+/+}$ and $K18^{-/-}$ mice at each of the studied time points (Fig. 3).

Caspase-3 activation

Activation of the effector caspase-3 was studied with three different methods. In immunohistochemistry, activated caspase-3 was found in the cytoplasm of hepatocytes after induction of Fas-induced liver failure (Fig. 4). Hepatocytes with activated caspase-3 were predominantly found in the periportal region, while centrolobular hepatocytes did not express activated caspase-3. To further quantify caspase-3 activity, we performed fluorometric measurements of caspase-3 activity in liver specimens obtained 1, 2 and 3 h after induction of liver failure (Fig. 5a). Caspase-3 activities increased from 1 to 3 h in both groups of animals. However, we did not find any significant differences in caspase-3 activity between $K18^{-/-}$ and $K18^{+/+}$ mice at any particular time. These data could be confirmed by analysis of Western blots, which also did not reveal any difference of the active caspase-3 cleavage products (Fig. 6).

Caspase-8 and -9 activation

Parallel to caspase-3 activities we also studied activation of activator caspases-8 and -9 by fluorometry. Here, we found activation of both caspases 1, 2 and 3 h after application of Jo2 antibody. However, caspase-8 and -9 activities were not higher in $K18^{-/-}$ than that in wild-type mice at any time after Jo2 injection (Fig. 5b,c).

Discussion

Our data demonstrate that keratin 18 exerts protection against Fas-induced liver injury as death from liver failure occurred quicker and much more frequently in $K18^{-/-}$ mice than in wild-type mice.

The observation of reduced resistance of $K18^{-/-}$ mice to Fas-induced liver injury in our study corresponds to recent data of Gilbert and co-workers, who described that hepatocytes from K8-null mice were three- to fourfold more sensitive to Fas-mediated apoptosis than those from wild-type controls [6]. Nevertheless, the mechanisms of protective effect of keratins differ between K8 and K18. Gilbert and co-workers provided evidence for a prominent targeting of the Fas receptor from the Golgi area to the surface in $K8^{-/-}$ hepatocytes to explain the K8

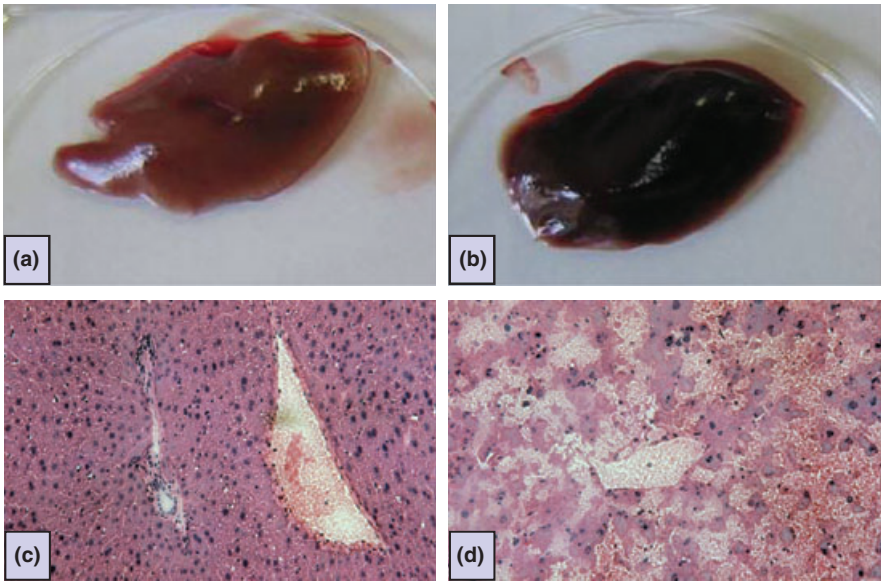

Figure 2 (a, b) Livers after Jo2 application: Liver of a wild-type mouse (a) compared with liver of a K18$^{-/-}$ mice explanted directly after Fas-induced death (b). Compared with the apparently normal control liver, the K18$^{-/-}$ liver is strongly swollen and dark-red due to the seepage of blood into the liver parenchyma. (c, d) Hemalaun/eosin staining 3 h after Jo2 application: Compared with wild-type mice with almost unaffected liver parenchyma (c), K18$^{-/-}$ mice revealed grossly destroyed liver architecture with haemorrhage into the sinusoidal spaces (d).

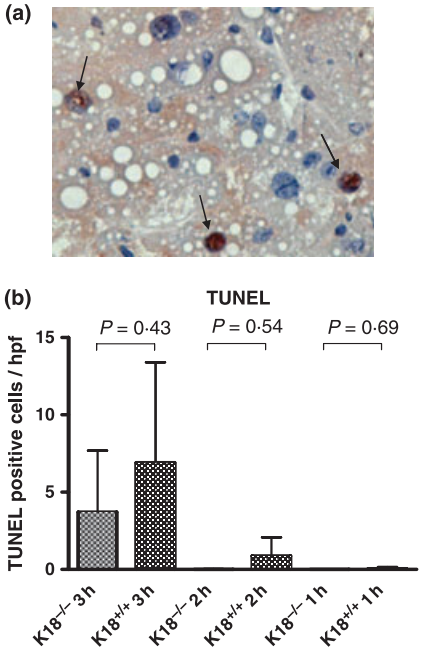

Figure 3 Apoptotic cells after Jo2 application. (a) TUNEL test: Representative example of a TUNEL test in K18$^{-/-}$ mice 3 h after Jo2 application. Arrows indicate positive TUNEL staining in hepatocytes undergoing apoptosis. (b) Number of TUNEL-positive apoptotic cells after Fas-antibody injection in six K18$^{-/-}$ and six K18$^{+/+}$ mice after 1 h, in six K18$^{-/-}$ and six K18$^{+/+}$ mice after 2 h and in eight K18$^{-/-}$ and seven K18$^{+/+}$ mice after 3 h. Significant differences in the numbers of TUNEL-positive cells could not be found between K18$^{-/-}$ and K18$^{+/+}$ mice at any point in time.

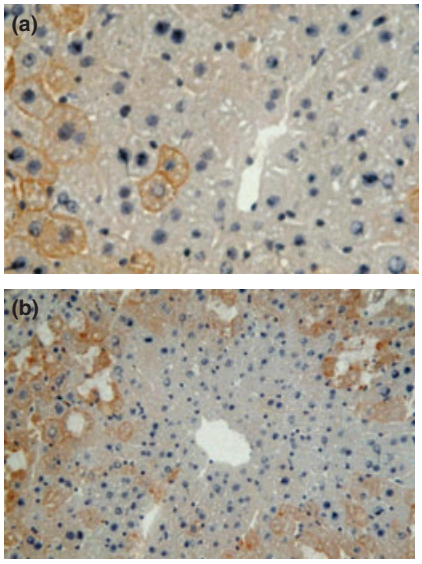

Figure 4 (a, b) Immunostaining of activated caspase-3. Activated caspase-3 was detected in the cytoplasm of hepatocytes. Caspase-3 staining hepatocytes were preferentially located in the periportal region of the lobules.

effect on survival. In contrast to K8$^{-/-}$ mice examined by Gilbert and co-workers, our K18$^{-/-}$ mice were apparently more susceptible to Fas-mediated liver damage via a pathway that did not involve differential activation of caspase-3, as in our study no difference in caspase-activity could be detected between K18$^{-/-}$ and K18$^{+/+}$ mice using three independent methods to assess caspase-3 activity. We also demonstrated

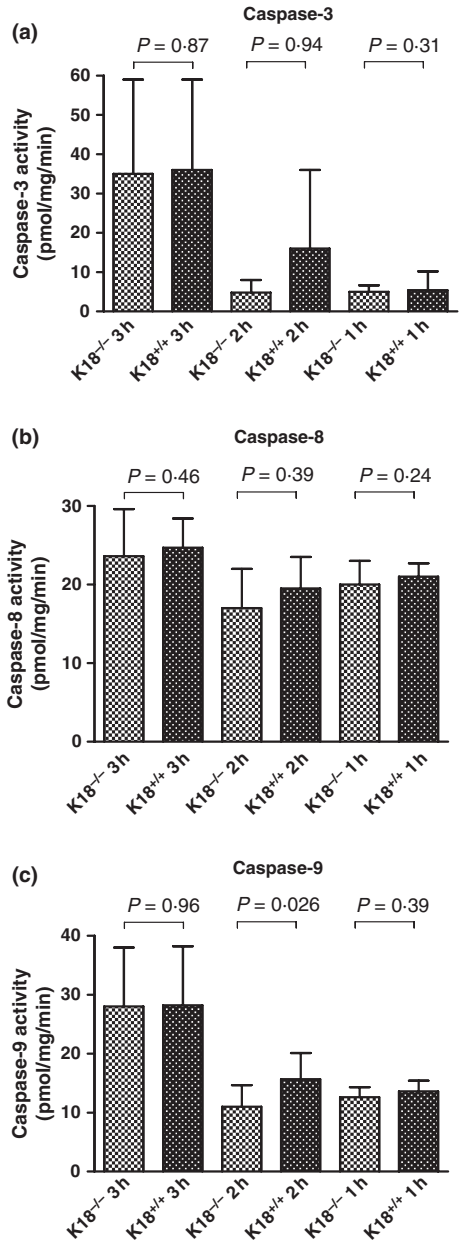

Figure 5 Fluorometric assay of intrahepatic caspases-3, -8 and -9 activity 1, 2 and 3 h after Jo2 application. Caspase activation was measured in six $K18^{-/-}$ and six $K18^{++}$ mice after 1 h, in six $K18^{-/-}$ and six $K18^{++}$ mice after 2 h and in eight $K18^{-/-}$ and seven $K18^{++}$ mice after 3 h. Activity of the effector caspase-3 was highest 3 h after Jo2, similar to the activation of the activator caspase-9, while the activator caspase-8 was activated as early as 1 h after Jo2. No difference in caspases-3, -8 and -9 activity was found between $K18^{-/-}$ and wild-type mice, despite an elevation in caspase-9 activity confined to the measurements 2 h after Jo2 injection.

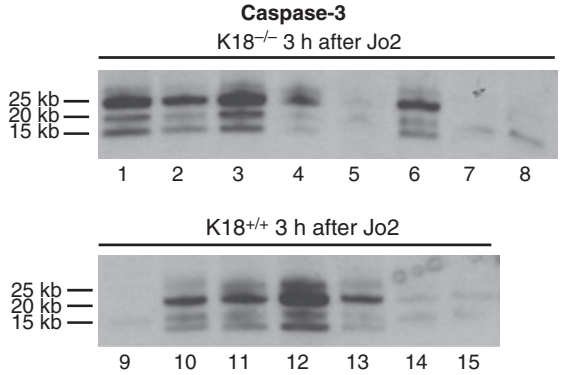

Figure 6 Western blot of caspase-3. Western blot analysis of caspase-3 in livers of eight $K18^{-/-}$ mice (no. 1–8) and seven $K18^{+/+}$ mice (no. 9–15) 3 h after Jo2. No significant difference between $K18^{+/+}$ and $K18^{-/-}$ mice was found concerning active caspase-3 cleavage products.

that the initiator caspases-8 and -9 were not more activated in $K18^{-/-}$ mice than in wild-type controls. Finally, numbers of TUNEL-positive apoptotic hepatocytes did not differ between Fas antibody treated $K18^{-/-}$ and wild-type mice. Nevertheless, liver damage was more severe in the $K18^{-/-}$ mice. Taken together, these data congruently indicate that cytoprotection mediated by K18 is apparently not the result of a direct interaction with pro-apoptotic regulatory proteins. Thus, K18 must act via other mechanisms to reduce liver damage in response to Fas antibody. For instance, absence of an intact keratin cytoskeleton leads to an altered mechanical integrity of hepatocytes as demonstrated in K18 mutant mice [3]. Keratins have potent micromechanical properties for the cell [25]. They build a dynamic scaffold allowing rapid and localized restructuring involved in an intimate interaction with all aspects of cell behaviour, including migration, differentiation and proliferation [26]. Another possible mechanism to explain increased cytotoxicity might be accumulation of K8 positive aggregates in the hepatocytes due to the absence of K18 [9]. This accumulation could alter protein turnover, inducing a protein stress response and diminished resistance against Fas-induced liver failure.

Keratins are prominent targets of degradation in programmed cell death and keratin 18 is an important substrate of proteolytic cleavage by caspase-3 during apoptosis [27–31]. Interestingly, type-I (e.g. K18) but not type-II (e.g. K8) keratins are cleaved during apoptosis [32], indicating a major difference in the mechanisms by which K18 and K8 can interact with apoptotic stimuli such as Fas-antibody-induced liver failure. Two sites of proteolytic cleavage of K18 are known: The first cleavage site is the ^{393}DALD/S site, which is cleaved in the early phase of apoptosis. In addition to caspase-3, caspase-8 and caspase-7 are also involved in cleavage at this site. The second

cleavage site of K18 is the L12 linker region (by caspase-6) [29]. As caspase-3 is centrally involved in K18 cleavage, in the absence of K18, caspase-3 has greater capacity to cleave substrates other than K18, resulting in accelerated degradation of cellular constituents in K18$^{-/-}$ mice as compared with K18$^{+/+}$ wild-type controls. This mechanism may explain the reduced survival in K18$^{-/-}$ mice after induction of Fas-antibody liver failure in the presence of unaltered activities of caspases.

Taken together, keratin 18$^{-/-}$ mice are significantly more susceptible to Fas-antibody-induced liver failure. In contrast to K8, the cytoprotective effect of K18 cannot be explained by a differential activation of caspase-3 and brings indirect interactions of keratin 18 with apoptotic regulators and other functions of keratin 18 into focus of interest. Our data of a cytoprotective effect of K18 in Fas-induced liver failure in mice provide support for observations in man, where impaired integrity of keratins emerges as risk factor for liver damage and cirrhosis.

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References

1 Zhong B, Zhou Q, Toivola DM, Tao GZ, Resurreccion EZ, Omary MB. Organ-specific stress induces mouse pancreatic keratin overexpression in association with NF-kappaB activation. *J Cell Sci* 2004;**117**:1709–19.
2 Loranger A, Duclos S, Grenier A, Price J, Wilson-Heiner M, Baribault H *et al.* Simple epithelium keratins are required for maintenance of hepatocyte integrity. *Am J Pathol* 1997;**151**:1673–83.
3 Ku NO, Michie S, Oshima RG, Omary MB. Chronic hepatitis, hepatocyte fragility, and increased soluble phosphoglycokeratins in transgenic mice expressing a keratin 18 conserved arginine mutant. *J Cell Biol* 1995;**131**:1303–14.
4 Toivola DM, Omary MB, Ku NO, Peltola O, Baribault H, Eriksson JE. Protein phosphatase inhibition in normal and keratin 8/18 assembly-incompetent mouse strains supports a functional role of keratin intermediate filaments in preserving hepatocyte integrity. *Hepatology* 1998;**28**:116–28.
5 Galarneau L, Loranger A, Gilbert S, Marceau N. Keratins modulate hepatic cell adhesion, size and G1/S transition. *Exp Cell Res* 2007;**313**:179–94.
6 Gilbert S, Loranger A, Daigle N, Marceau N. Simple epithelium keratins 8 and 18 provide resistance to Fas-mediated apoptosis. The protection occurs through a receptor-targeting modulation. *J Cell Biol* 2001;**154**:763–73.
7 Ku NO, Soetikno RM, Omary MB. Keratin mutation in transgenic mice predisposes to Fas but not TNF-induced apoptosis and massive liver injury. *Hepatology* 2003;**37**:1006–14.
8 Toivola DM, Nieminen MI, Hesse M, He T, Baribault H, Magin TM *et al.* Disturbances in hepatic cell-cycle regulation in mice with assembly-deficient keratins 8/18. *Hepatology* 2001;**34**:1174–83.
9 Magin TM, Schroder R, Leitgeb S, Wanninger F, Zatloukal K, Grund C *et al.* Lessons from keratin 18 knockout mice: formation of novel keratin filaments, secondary loss of keratin 7 and accumulation of liver-specific keratin 8-positive aggregates. *J Cell Biol* 1998;**140**:1441–51.
10 Omary MB, Ku NO. Intermediate filament proteins of the liver: emerging disease association and functions. *Hepatology* 1997;**25**:1043–8.
11 Marceau N, Loranger A, Gilbert S, Daigle N, Champetier S. Keratin-mediated resistance to stress and apoptosis in simple epithelial cells in relation to health and disease. *Biochem Cell Biol* 2001;**79**:543–55.
12 Toivola DM, Ku NO, Resurreccion EZ, Nelson DR, Wright TL, Omary MB. Keratin 8 and 18 hyperphosphorylation is a marker of progression of human liver disease. *Hepatology* 2004;**40**:459–66.
13 Strnad P, Stumptner C, Zatloukal K, Denk H. Intermediate filament cytoskeleton of the liver in health and disease. *Histochem Cell Biol* 2008;**129**:735–49.
14 Yagmur E, Trautwein C, Leers MP, Gressner AM, Tacke F. Elevated apoptosis-associated cytokeratin 18 fragments (CK18Asp386) in serum of patients with chronic liver diseases indicate hepatic and biliary inflammation. *Clin Biochem* 2007;**40**:651–5.
15 Luft T, Conzelmann M, Benner A, Rieger M, Hess M, Strohhaecker U *et al.* Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. *Blood* 2007;**110**:4535–42.
16 Hetz H, Hoetzenecker K, Hacker S, Faybik P, Pollreisz A, Moser B *et al.* Caspase-cleaved cytokeratin 18 and 20 S proteasome in liver degeneration. *J Clin Lab Anal* 2007;**21**:277–81.
17 Ku NO, Wright TL, Terrault NA, Gish R, Omary MB. Mutation of human keratin 18 in association with cryptogenic cirrhosis. *J Clin Invest* 1997;**99**:19–23.
18 Ku NO, Gish R, Wright TL, Omary MB. Keratin 8 mutations in patients with cryptogenic liver disease. *N Engl J Med* 2001;**344**:1580–7.
19 Ku NO, Lim JK, Krams SM, Esquivel CO, Keeffe EB, Wright TL *et al.* Keratins as susceptibility genes for end-stage liver disease. *Gastroenterology* 2005;**129**:885–93.
20 Strnad P, Lienau TC, Tao GZ, Lazzeroni LC, Stickel F, Schuppan D *et al.* Keratin variants associate with progression of fibrosis during chronic hepatitis C infection. *Hepatology* 2006;**43**:1354–63.
21 Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y *et al.* Lethal effect of the anti-Fas antibody in mice. *Nature* 1993;**364**:806–9.
22 Woo M, Hakem A, Elia AJ, Hakem R, Duncan GS, Patterson BJ *et al.* In vivo evidence that caspase-3 is required for Fas-mediated apoptosis of hepatocytes. *J Immunol* 1999;**163**:4909–16.
23 Fink K, Zhu J, Namura S, Shimizu-Sasamata M, Endres M, Ma J *et al.* Prolonged therapeutic window for ischemic brain damage caused

by delayed caspase activation. *J Cereb Blood Flow Metab* 1998;**18**:1071–6.
24 Leifeld L, Fink K, Debska G, Fielenbach M, Schmitz V, Sauerbruch T *et al*. Anti-apoptotic function of gelsolin in fas antibody-induced liver failure in vivo. *Am J Pathol* 2006;**168**:778–85.
25 Sivaramakrishnan S, DeGiulio JV, Lorand L, Goldman RD, Ridge KM. Micromechanical properties of keratin intermediate filament networks. *Proc Natl Acad Sci U S A* 2008;**105**:889–94.
26 Magin TM, Vijayaraj P, Leube RE. Structural and regulatory functions of keratins. *Exp Cell Res* 2007;**313**:2021–32.
27 Ku NO, Liao J, Omary MB. Apoptosis generates stable fragments of human type I keratins. *J Biol Chem* 1997;**272**:33197–203.
28 Caulin C, Ware CF, Magin TM, Oshima RG. Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. *J Cell Biol* 2000;**149**:17–22.
29 Schutte B, Henfling M, Kolgen W, Bouman M, Meex S, Leers MP *et al*. Keratin 8/18 breakdown and reorganization during apoptosis. *Exp Cell Res* 2004;**297**:11–26.
30 Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol* 1997;**138**:1379–94.
31 Leers MP, Kolgen W, Bjorklund V, Bergman T, Tribbick G, Persson B *et al*. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* 1999;**187**:567–72.
32 Ku NO, Strnad P, Zhong BH, Tao GZ, Omary MB. Keratins let liver live: mutations predispose to liver disease and crosslinking generates Mallory-Denk bodies. *Hepatology* 2007;**46**:1639–49.

