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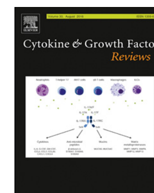
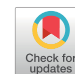


Augmenter of liver regeneration: Essential for growth and beyond



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ABSTRACT

Liver regeneration is a well-orchestrated process that is triggered by tissue loss due to trauma or surgical resection and by hepatocellular death induced by toxins or viral infections. Due to the central role of the liver for body homeostasis, intensive research was conducted to identify factors that might contribute to hepatic growth and regeneration. Using a model of partial hepatectomy several factors including cytokines and growth factors that regulate this process were discovered. Among them, a protein was identified to specifically support liver regeneration and therefore was named ALR (Augmenter of Liver Regeneration). ALR protein is encoded by GFER (growth factor erv1-like) gene and can be regulated by various stimuli. ALR is expressed in different tissues in three isoforms which are associated with multiple functions: The long forms of ALR were found in the inner-mitochondrial space (IMS) and the cytosol. Mitochondrial ALR (23 kDa) was shown to cooperate with Mia40 to insure adequate protein folding during import into IMS. On the other hand short form ALR, located mainly in the cytosol, was attributed with anti-apoptotic and anti-oxidative properties as well as its inflammation and metabolism modulating effects. Although a considerable amount of work has been devoted to summarizing the knowledge on ALR, an investigation of ALR expression in different organs (location, subcellular localization) as well as delineation between the isoforms and function of ALR is still missing. This review provides a comprehensive evaluation of ALR structure and expression of different ALR isoforms. Furthermore, we highlight the functional role of endogenously expressed and exogenously applied ALR, as well as an analysis of the clinical importance of ALR, with emphasis on liver disease and *in vivo* models, as well as the consequences of mutations in the GFER gene.

1. Introduction

In ancient times the liver was regarded as the seat of the soul, life and intelligence. Therefore, Greek mythology focused on the 'indestructible' liver with its extraordinary healing ability in the legend of Prometheus. After stealing fire from Zeus and giving it to mankind, Zeus punished Prometheus by chaining him to a rock where Zeus's eagle would feast on Prometheus's liver each day, and his liver would regrow at night until the eagle's return [1]. Despite the ancient myth, the term "liver regeneration" was only introduced into modern

Abbreviations: ACLF, acute on chronic liver failure; ADH1, alcohol dehydrogenase 1; ALDH1, aldehyde dehydrogenase 1; AKI, acute kidney injury; ALD, alcoholic liver diseases; ALR, augmenter of liver regeneration; AP1, activator Protein 1; APP, acute phase proteins; APR, aucte phase response; ARE, anti-oxidant response element; BARE, bile acid response element; BDL, bile duct ligation; C/EBPβ, CCAAT/enhancer binding protein β; CCC, cholangiocellular carcinoma; CHOP, C/EBP homologous protein; CNS, COP9 signalosome; CPT1α, carnitine palmitoyltransferase 1alpha; Drp1, dynamine-related protein 1; EGF-R, epidermal growth factor-Receptor; Egr-1, early growth response protein-1; ELOVL1, elongation of very long chain fatty acids protein 6; EMT, epithelial mesenchymal transition; ERK, extracellular signal regulated protein kinase; ESC, embryonic stem cells; FABP1, fatty acid binding protein 1; FAD, flavin adenin dinucleotide; FFA, free fatty acid; FOXA2, forkhead Box A2; FXR, farnesoid X Receptor; GFER, growth factor erv1-like; HDAC1, histone deacetylase 1; HF, hepatic failure; HNF4α, hepatocyte nuclear factor 4 alpha; HPC, hepatic progenitor cells; H/R, hypoxia / reoxygenation; HSC, hematopoietic stem cells; IDD, intrinsically disordered domain; IMS, intermembrane space; IP3R, inositol 14,5-triphosphate receptor; I/R, ischemia / reperfusion; IRI, ischemia / reperfusion injury; JAB1, C-Jun-activating domain binding protein 1; JNK, C-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MDR, multi drug resistance; Mia40, mitochondrial import and assembly 40; MIF, macrophage migration inhibitory factor; NAFLD, non-alcoholic fatty liver diseases; NASH, non-alcoholic steatohepatitis; NFκB, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; NK, natural killer cells; Nrf2, nuclear factor erythroid 2-related factor 2; ODC, ornithine decarboxylase; OMM, outer mitochondrial membrane; PI3K/Akt, phosphoinositide 3-kinase/AKT; RE, response element; ROS, reactive oxygen species; SHP, small Heterodimer Partner; STAT3, signal transducer and activator of transcription 3; tBHQ, tertiary butylhydroquinone; TNFα, tumor necrosis factor alpha; TRX, thioredoxin; ZO-1, zona occludens-1

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Table 1
Summary of studies investigating protein expression of ALR isoforms in different organs.

Organ	Species	Cell type	Remarks	Subcellular localization	Isoform (kDa)	Ref.
Brain	rat	neurons, glial cells	diencephalon, cerebellum, brainstem, cerebellum	nucleus, external envelope of mitochondria	23, 21	[56,57](1)
		glioma cell line T98G	–	cytosol	23, 21	[58]
	human	neuroblastoma cell line SH-5YSY	–	cytosol	15	[59](2)
Muscle	rat	skeletal muscle tissue	–	–	23, 21, 15	[56]
	human	muscle fibers	mitochondrial myopathy patients	mitochondrial innermembrane, cristae, cytosol	n.d.(3)	[55,60]
Kidney	rat	tissue	–	–	23, 21, 15	[56]
		renal tubular epithelial cells	induced ALR expression in renal cortex, outer strip of outer medulla upon acute kidney injury	cytosol and apical plasma membrane	15	[61,62]
		tissue	induced ALR expression after obstructive nephropathy		15	[82]
Testis	mouse	tissue	–	soluble, insoluble fractions	40, 38, 23 23 n.r.(4)	[23](5)
		spermatogonia, elongated sperm cells	seminiferous tubules	mitochondrial inner membrane	23, 21	[56]
	rat	tissue	–	–	23, 21	[56]
Blood	mouse	blood plasma	after hemorrhagic shock	–	ELISA(6)	[91]
	rat	blood from liver	negative in venous, arterial blood	–	23, 21	[56]
		serum	after portacaval shunt, endotoxemia, sepsis	–	ELISA(6)	[91]
		serum	after partial hepatectomy	–	21, ELISA(6)	[66,69]
	human	serum of patients	normal or with various liver diseases	–	ELISA(6)	[70,87,138]
		myloma cell line (U226)	–		23, 21	[124]
		leukemic T cells	–	–	23	[148]
Liver	mouse	tissue	–	–	40, 23 n.r.(4)	[23]
		tissue	–	–	23/22, 21/20(7)	[46,64]
		tissue	normal and after EtOH feeding		40, 38, 36 n.r.(4); 22, 20	[63]
		hepatocytes	normal, ALD, NASH liver tissue	–	40, 38, 36 n.r.(4)	[67]
	rat	tissue	–	–	40, 28, 23 n.r.(4)	[23]
		tissue	–	–	23/22, 21/20(7)	[56,64,65]
		tissue	–	cytosol	23, 21	[66]
		hepatocytes, sparely in non-paranchymal cells	–	cytosol	40, 38, 36 n.r.(4)	[69]
	human	tissue	liver: fetal, adult and tumor	cytosol nucleus	23, 15	[73]
		tissue	human liver samples (normal, cholestasis and HCC)	–	23, 21, 15	[64,72,88]
		tissue	human liver samples (normal, ALD, NASH, HCV)	–	40, 38, 36 n.r.(4)	[67]
		hepatocytes, cholangiocytes	–	cytosolic, perinuclear immunostaining	23	[71]
		hepatocytes, cholangiocytes	human liver sections (normal, ALD, HBV positive)	–	n.d.(3)	[46]
		hepatocytes, cholangiocytes	rarely in cholangiocytes and endothelial cells	cytosol, negative or barely in nucleus	n.d.(3)	[70,74]
		isolated primary human hepatocytes	–	cytosol	23, 21, 15	[64]
				mitochondria	23, 21	
		HepG2	–	cytosol, perinucleus		[34]
				mitochondria, cytosol	− 23, 21	[43,46,64,72]
		Hep3B, Huh7	–	–	23, 21	[64]
		cell line SMMC-7721	–	mitochondria / cytosol	23 / 15	[106](8)
Cervix	human	Hela cells	–	mitochondria	23	[103]

(1) Klissenbauer et al. have investigated the expression of ALR protein in different rat tissues, 23 kDa ALR was detected in heart. Moreover, 23 and 21 kDa ALR were detected in fat tissue, spleen and lungs.
(2) Polimeno et al. (2009) have pointed out the possibility of ALR up-take upon treatment with recombinant ALR (15 kDa).
(3) n.d., not determined.
(4) n.r. corresponds to non-reduced SDS-PAGE conditions that demonstrate analysis of ALR dimers, whereas all other studies analyzed ALR expression under reduced conditions.
(5) In addition Giorda et al. (1996) investigated the expression of ALR in mouse spleen and demonstrated the expression of 23 kDa ALR in soluble and insoluble fractions.
(6) ALR detection using ELISA includes all isoforms of ALR.
(7) Mouse and rat long forms of ALR have a calculated molecular weight of 22 kDa and 20 kDa. Due to restricted resolution of SDS-PAGE analysis murine long form ALR are also tagged as 23 and 21 kDa.
(8) Cao et al. (2009) investigated expression of ALR in SMMC-7721 cell line and detected 15 and 23 kDa ALR, the latter was localized in the mitochondria. Nevertheless, a recent report has suggested not considering the studies including BEL7402, SMMC7721 or SKHEP1 cell lines due to the highly possible contamination with Hela cells which was reported in different laboratories in Asia and Europe [149].

medicine in the early 19th century [1] and is now described as a well-orchestrated and complex process, triggered by hepatocellular death. This could be induced by toxins, viral infections or by tissue loss due to trauma or surgical resection [2–6]. However, the liver's regenerative capacity becomes insufficient when exposed to chronic damage. In addition, numerous numbers of world's population are affected by chronic liver diseases with an apparent lack of satisfying therapeutic options. Therefore, scientists became interested in identifying the factors that regulate liver regeneration which might maintain or restore the vitally important hepatic function [7]. Several growth factors like

HGF (hepatocyte growth factor) or EGF (epidermal growth factor) as well as cytokines like TNFα (tumor necrosis factor α) or IL6 (interleukin 6) were identified to be involved in the process of liver regeneration. Interestingly, a novel factor, ALR (Augmenter of Liver Regeneration) has emerged as a regulator of liver regeneration with additional vital roles in liver functions, but also found to be important in other organs. Although a considerable amount of work has been devoted to summarizing the knowledge on ALR (see previous reviews [8–14]), an investigation of ALR expression in different organs as well as delineation between the ALR isoforms and the function of endogenous and exogenous ALR are still missing. Therefore, we aspired to revise the knowledge about ALR and to gather evidence of the expression of different ALR isoforms. We also reviewed the functional role of the endogenously over-expressed or exogenously applied ALR and its clinical importance in the hopes of minimizing the gaps in the knowledge about ALR and provoking additional research to further explore the capacities of this extraordinary protein.

The first scientific evidence of the liver's ability to regenerate was confirmed by performing partial hepatectomy in rats and showing the restoration of the liver mass [15]. In 1975 LaBrecque and Pesch [16] prepared an extract from weanling rat livers and demonstrated that injecting this substance into partially hepatectomized rats stimulates liver regeneration and therefore named it Hepatic Stimulatory Substance (HSS). Several attempts were applied to further identify and characterize the molecule responsible for the stimulation of liver regeneration [17–19]. After progressive purification of HSS, a fraction with augmenting effect following a 40% hepatectomy in rats was obtained containing three protein bands (14, 30 and 35 kDa) [20]. The 30 kDa band was chosen for further sequencing and was published in 1994 by Hagiya et al., who first coined the term "Augmenter of liver Regeneration" (ALR) [21]. The sequenced rat protein consisted of 125 amino acids with a molecular weight of 15 kDa under reducing conditions and 30 kDa under non-reducing conditions [21]. The published rat sequence was then corrected in 1995 [22] and proposed the presence of three ATGs and therefore opened the possibility of multiple isoforms of ALR. Afterwards, the sequence of human and mouse ALR were published in 1996 [23]. Moreover, Hepatopoietin (HPO) was cloned and sequenced from human fetal liver tissue in 1997 and was proved to be identical with ALR, therefore HPO is also referred to as ALR [24].

ALR protein is encoded by GFER (growth factor erv1-like) gene (NCBI Gene ID: **2671**) that consists of 3 exons and 2 introns [22,25] and can be regulated by different stimuli. In this review we focus on publications, investigating ALR, HPO or GFER. This is of note and critical, since there are some reports using the term "ALR" as a synonym for HSS, but using, in their studies, a purified protein fraction (HSS) instead of a single sequenced protein (e.g [26–28].). Furthermore, a few reports indicated that HSS has a molecular size of 15 kDa (similar to ALR) but then claimed that ALR is different from HSS [29], without presenting a sequence for HSS. Due to the ambiguity about HSS protein/fraction, articles on HSS were only taken into consideration, if a clear indication of gene name and molecular size were given.

2. ALR isoforms and structure

The first gene sequence of ALR was reported in 1994 [21] and was later corrected in 1995, where an additional G was added at position 266. This generated two additional in-frame ATG initiation sites and raised the possibility of different ALR isoforms [22]. Indeed, the expression of different ALR isoforms in mouse, rat and human organs has been thoroughly investigated and the existence of three isoforms of ALR have been shown (Table 1). Furthermore, the sequence of full length ALR shows 74% homology to mouse ALR and 73% homology to rat ALR (Fig. 1A). The longest transcript of human ALR encodes a 205 amino acid protein corresponding to a molecular weight of 23 kDa [30]. Moreover, the smallest transcript of ALR encodes a 125 amino acid protein corresponding to a molecular weight of 15 kDa (short form ALR, sfALR) [25]. The sfALR is 80 amino acids shorter than 23 kDa long form ALR (lfALR) at the N terminus [30] (Fig. 1B). Those 80 amino acids are also referred to as "N80". N80 includes (i) N-terminal domain which is described as an IDD (intrinsically disordered domain) and acts as a crucial targeting signal from the cytosol into the mitochondria [31]. (ii) C-terminal of N80 contains the CRAC motif (71–74 aa) (Fig. 1B). CRAC motif acts as a recognition site in the disulfide relay system of mitochondrial inter-membrane space (IMS). CRAC mediates the "recycling" of Mia40, an oxido-reductase, in charge of oxidative protein folding in the IMS [31–33]. Moreover, it was previously shown that mutating the cysteine residues (C71 or C74) in ALR's CRAC motif leads to preventing the reaction with Mia40 [33]. Nevertheless, Mia40 can still exchange electrons with ALR through the CEEC motif (142-145 aa) (corresponds to CXXC motif in Erv1, yeast homologue of ALR) (Fig. 1B) but this exchange is less efficient due to the "buried" structure of the CEEC motif. This argues for a specific hydrophobicity near the CRAC motif of long form ALR that drives the reaction between Mia40 and the N terminus of ALR [33]. In addition, Lu et al. have demonstrated that interaction between ALR and JAB1 occurs *via* amino acids 81-143 (corresponds to 1 to 63 aa in sfALR) [34] (Fig. 1B). Furthermore, the CEEC motif (Fig. 1B) neighbors the FAD (flavin adenin dinucleotide) binding domain and is affected by the reaction between Mia40 and FAD. Moreover, the "CEEC-FAD" region of ALR is referred to as "redox active center" that donates electrons to Mia40 [33].

Additionally, disulfide bonds formation in ALR (Fig. 1B) is of importance for ALR functionality. C95 and C204 are responsible of forming head-to tail dimers of all isoforms of ALR and therefore stabilizing the structure [35]. The dimerization also facilitates the binding to the FAD molecule by noncovalent bonds [36,37]. Other cysteins, C171 and C188 form "intramolecular" disulfide bonds that flank the FAD molecule. It is worth to mention that C154 and C165 (non-conserved cysteines) in human ALR (Fig. 1A and B) may account for oligomers formation often seen during the preparation of recombinant human ALR and mutating those cysteines prevented the formation of oligomers in recombinant human ALR [33].

3. Regulation of ALR

The human ALR gene (GFER) (growth factor *erv1*-like) has been mapped on chromosome 16 next to the polycystic kidney disease gene (PKD1) [38]. Analysis of ALR gene (Fig. 2) has shown that it consists of 3 exons and 2 introns [22,25]. ALR gene promoter has been shown to be TATA-less and to have some characteristics of an oncogene and a growth factor [39]. It further showed an initiator-like element with three tandem repeats (-66, -1 bp) [39]. It was shown that regulatory elements of ALR promoter might exist in the region between -447 and -49 bp. interestingly, it was found that a putative AP1/AP4 binding site (-375, -369 bp) is responsible for negative transcriptional activity of ALR by AP1 (Activator Protein 1) in HepG2 cells and AP4 (Activator Protein 4) in COS-7 cells [40]. Additionally, it was reported that ALR expression could be attenuated by EGF (epidermal growth Factor) *via* C/EBPβ (CCAAT/enhancer binding proteins) binding to its binding site (-292, -279 bp) within ALR promoter [41]. Analyzing a selected promoter region (-252, -49 bp) from the transcriptional start site has shown that HNF4α (hepatocyte nuclear factor 4 alpha) binds to a binding site (-209, -204 bp) and reduces ALR expression [42]. In addition, SP1 (specific protein 1) positively regulates ALR expression by binding to its binding site at (-152, -145 bp) [42]. Interestingly, a recent study including the first intron of GFER gene (-733 to +527 bp) [43] investigated the potential regulatory elements in the introns that may affect the expression of different genes [44,45]. It was demonstrated that HNF4α binds at (+421, +432 bp) to ALR promoter and induces ALR expression [43] despite the presence of an upstream HNF4α binding site with repressing activity. However, the inducing effect of HNF4α is diminished upon SHP (Small Heterodimer Partner) activation *e.g.* by bile acids [43]. Exposure of hepatocytes to oxidative stress

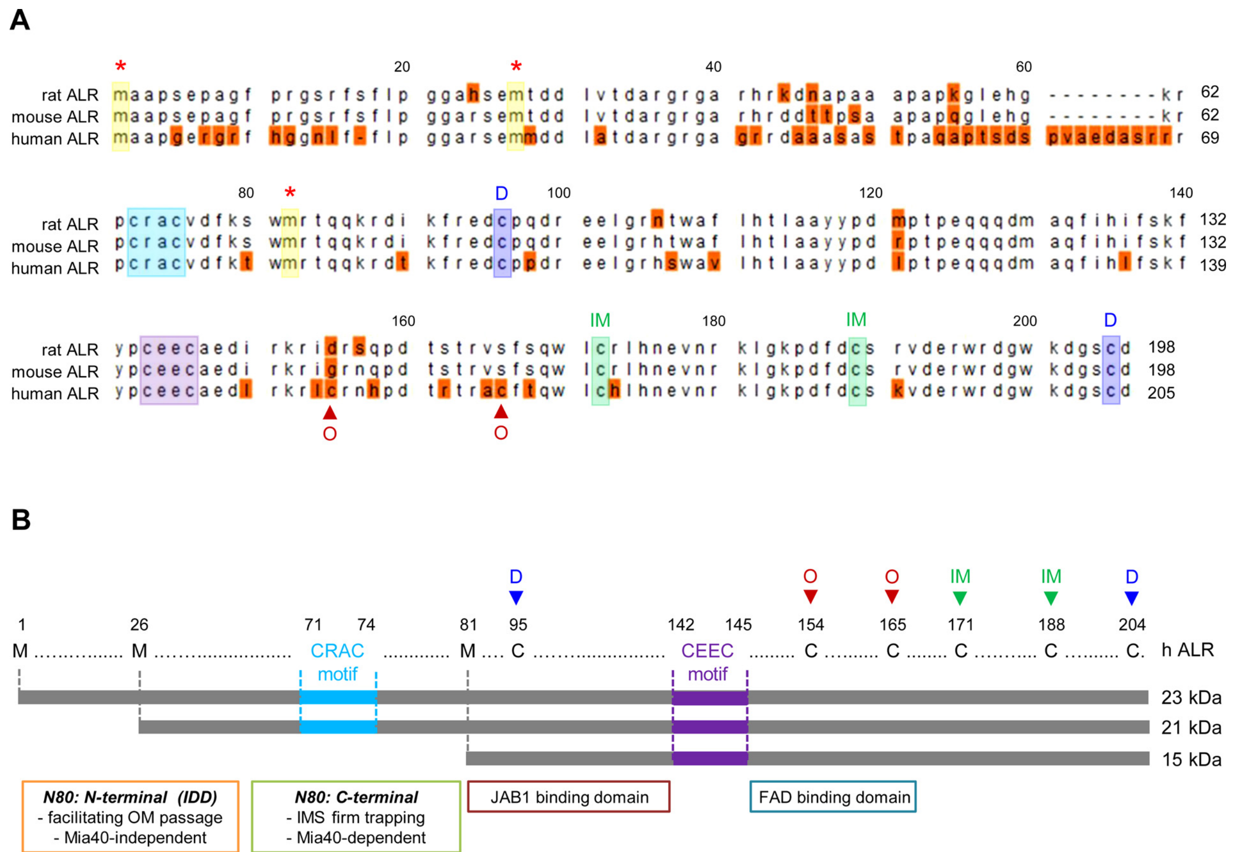

Fig. 1. (A) An illustration of ALR protein sequence homology in human, mouse and rat is shown. Sequences were obtained from Pubmed search tool; rat ALR NP_037354.2, mouse ALR NP_075527.2 and human ALR NP_005253.3. Dashes indicate absence of the amino acid (aa), orange background represents the non-conserved aa in ALR sequence, red stars above "m" indicate the initial methionines for different ALR isoforms, and red arrows below indicate the non-conserved cysteines responsible for oligomerization upon recombinant protein preparation. Conserved CRAC as well as CEEC motifs (CXXC motif) are highlighted as well as cysteines responsible of dimerization and intramolecular disulfide bonds. Rat and mouse full length ALR (198 aa) correspond to a molecular weight of approx. 22kDa, and human full length ALR (205 aa) to approx. 23 kDa. (B) Sequence and functional domains of human ALR protein isoforms. Long forms (e.g. 23 kDa) of ALR possess additional 80 amino acids (N80) at the N terminus of the short form ALR (15 kDa). N80 harbors the IDD at (N80-N terminal) and CRAC motif at (N80-C terminal), which are responsible for mitochondrial localization and Mia40 recognition. The CEEC motif (corresponds to CXXC in yeast homologue Erv1) and the neighboring FAD binding domain are both essential to "recycle" Mia40 by catalyzing the electron transfer from ALR to Mia40. JAB1 binding domain is indicated in the 3 isoforms responsible for interaction with JAB1 and activation of AP1. Disulfide bonds formed by cysteines are indicated by arrows; D: functional dimerization; O: Oligomerization (non-conserved cysteines) and IM: intramolecular bonds; IDD, intrinsically disordered domain.

induces Nrf2 (nuclear factor erythroid 2-related factor 2), which in turn, by binding to an anti-oxidant response element (ARE) located between (-27, -19 bp), induces ALR expression [46]. Interestingly, involvement of Nrf2 in ALR regulation was further confirmed by studies showing ALR induction upon treatment with known Nrf2 inducers such as tBHQ (tertiary butylhydroquinone), HBV (hepatitis B virus) infection [46], phenethyl isothiocyanate and sulforaphane [47,48]. In addition, FOXA2 (Forkhead Box A2, also known as HNF3β) binds to a binding

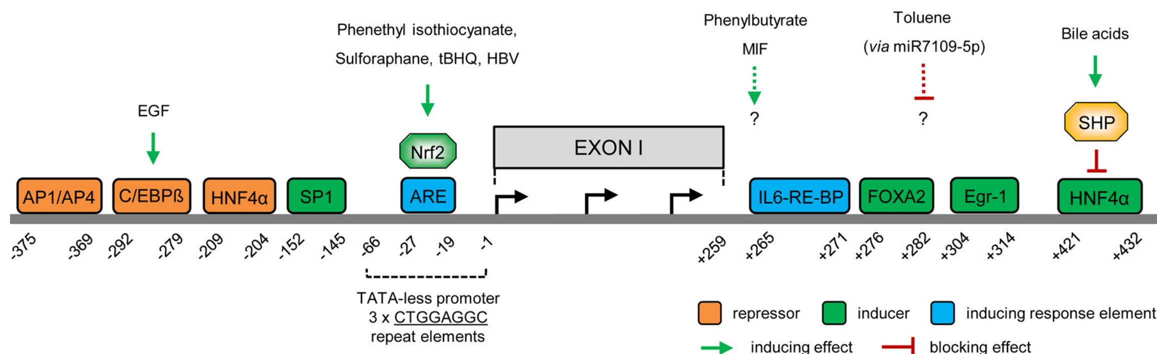

Fig. 2. Schematic illustration of ALR promoter region (ALR NCBI reference sequence: NG_016288.1), transcription factors and response elements involved in ALR regulation. SP1, FOXA2, Egr-1 and HNF4α (+421, +432) are inducers of ALR expression. However, the induction by HNF4α (+421, +432) is reversed by SHP which is activated by bile acids. Furthermore, AP1/AP4, HNF4α (-209, -204) and C/EBPβ (induced by EGF) are repressing factors of ALR expression. IL6-RE-BP increases the activating effect of FOXA2 whereas ARE (-27, -19) induces ALR expression by binding of Nrf2 to this response element. The latter could be activated by HBV infection, tBHQ, sulforaphane and phenethyl isothiocyanate. Moreover, HDAC1-inhibitor phenylbutyrate and MIF activate the expression of ALR whereas Toluene exposure represses ALR expression *via* miR7109-5p. IL6-RE-BP, IL6 response element binding protein.

site (+276, +282 bp) within ALR promoter inducing ALR expression and this binding is enhanced by IL6 [49]. Moreover, ALR is also regulated by Egr-1 (early growth response protein-1) that binds to its binding site (+304, +314 bp) and induces ALR expression [43].

Furthermore, phenylbutyrate, a HDAC1 (histone deacetylase 1) inhibitor and demethylation reagent, was shown to induce the expression of ALR in the brain of a Huntington's disease mouse model [50]. This proposes the possibility that ALR could be regulated by histone acetylation and/or methylation [50] and therefore by epigenetic mechanisms [51,52]. Additionally, MIF (macrophage migration inhibitory factor), a pluripotent cytokine involved in cell cycle, cell proliferation and liver regeneration, has been demonstrated to increase the promoter activity of ALR [53]. Interestingly, a recent report has suggested that ALR may be regulated by miRNAs and showed that Toluene exposure up-regulated miR7109-5p, which may regulate ALR [54].

4. Location of ALR

Although initially discovered in the liver, expression of ALR mRNA was detected in several tissues and was found to be highly expressed in the liver, testis, brain and kidneys. Moreover, low levels of ALR mRNA were detected in the heart, muscles, spleen and lungs [22,23]. Interestingly, it was shown that mRNA expression of ALR in muscle tissues differs between males and females between 18 and 35 years of age [55]. A variety of studies were published analyzing the expression of ALR protein expression, including its isoforms and their respective cellular localization. Table 1 summarizes reports with detailed information about ALR isoform (molecular weight), organ and organelle affiliation that point out the expression of different ALR isoforms in total of three in brain, muscle, kidney and liver. In the rat brain, ALR was found to expressed in its long forms (23 and 21 kDa) [56,57] in neurons and glial cells located in the nucleus and mitochondria [57]. Furthermore, this was partly confirmed in a study demonstrating cytosolic expression of these isoforms in a human glioma cell line [58]. In addition, cytosolic expression of 15 kDa ALR (short form ALR, sfALR) was detected in human neuroblastoma cell lines [59]. All ALR isoforms (23, 21 and 15 kDa) have been identified in rat muscle tissue [56] and other studies performing electron microscopy demonstrated ALR localization in the mitochondrial inter-membrane space or associated to mitochondrial cristae in human muscle fibers [55,60]. Moreover, all three ALR isoforms were detected in rat kidneys [56] and the short form ALR (15 kDa) expression was reported in the renal cortex as well as medulla of rat kidneys after injury [61,62]. In addition, testis of mouse [23,56] and rat [56] also showed positive expression of long form isoforms (23 and 21 kDa). However, since ALR was first detected in the livers of weanling rats, the hepatic expression of ALR isoforms has been thoroughly investigated. The first report demonstrating ALR protein expression by Giorda et al. [23] showed western blots with ALR bands of 40, and 23 kDa under non-reduced conditions in rat and mouse liver tissue, and a 28 kDa band was also detected in rat liver. In the meantime, the expression of ALR under reducing conditions showed 2 bands (~23 and ~21 kDa) in mouse [46,63,64] and rat [56,64–66]. Using non-reducing conditions revealed expression of 3 bands (40, 38 and 36 kDa) of ALR in rats [67] and in normal as well as disease-mouse models (NASH and ALD) [63,67]. Interestingly, in a study that used liver-specific knock-out of ALR (by an albumin-Cre/LoxP system) it was shown that ALR protein expression, after being repressed at birth, reappeared in the livers of the KO-mice after 1 year. The same study indicated the presence of oval cells (hepatic progenitor cells, HPC) in the KO-mice [67] which might explain the recurred expression of ALR by activation of HPCs and consequently giving rise to cholangiocytes or hepatocytes [68]. Gandhi and his colleagues did not describe ALR expression in cholangiocytes [63,67,69], but based on the findings that ALR is expressed in epithelial cells of the human liver, both in hepatocytes and cholangiocytes [46,70,71], a potential expression of ALR in HPC might be proposed. So far, expression of ALR in non-parenchymal liver cells could not be detected [46,71] except noted in one study [69] with low expression of ALR in stellate, Kupffer and hepatic endothelial cells, which was not detectable after 2 days of cell culture. It is worth to mention that in human liver tissue, hepatocytes and hepatoma cell lines under reducing conditions ALR is expressed up to all three isoforms (23, 21, 15 kDa) and under non-reducing conditions in several bands (40, 38, 36 kDa) [67]. Furthermore, it was demonstrated that hepatoma cell lines (HepG2, Huh7 and Hep3B) express the 23 and 21 kDa ALR [43,46,64,72] in the mitochondria as well as cytosol [64]. Moreover, primary human hepatocytes (PHH) were analyzed and showed expression of 3 isoforms of ALR in the cytosol, whereas 23 and 21 kDa were additionally found in the mitochondria [64]. Another study by Li et al. using human fetal, adult normal and tumorous liver tissue reported 23 kDa ALR in the cytosol and its 15 kDa isoform in the nucleus [73]. In addition, expression of ALR was investigated in normal [64,71,72], HCC (hepatocellular carcinoma) [71–74], CCC (cholangiocellular carcinoma) [71], and HBV positive [46] liver tissue and demonstrated reduced ALR expression in normal liver compared to diseased tissues. The expression of three isoforms of ALR (23, 21, 15 kDa) is attributed most likely to the presence of 3 initial in-frame ATG initiation site in ALR gene [22]. Therefore it is of interest to find out why more or less isoforms of ALR are expressed in different tissues and under conditions of disease. It was hypothesized that in rats the 22 kDa isoform is post-translationally modified by dimerization to form the observed 40, 38 and 36 kDa bands under non-reducing conditions [69]. Recently published data by the same group showed two isoforms of ALR (22 and 20 kDa) under reducing conditions [63]. Considering the estimated molecular sizes on SDS-PAGE gels and the detection of 22 and 20 kDa bands of ALR under reducing conditions, the three bands (40, 38, 36 kDa), detected under non-reducing conditions, may be due to the formation of homo- and hetero-dimers of the 22 and 20 kDa ALR.

Finally, contradictory evidence about the expression of ALR isoforms may be due to technical limitations like resolution of gels used in SDS-PAGE or the specificity and sensitivity of anti ALR antibodies for immune detection. For example, in a former publication our group demonstrated the expression of a 23 kDa ALR in normal, cirrhosis, HCC and CCC samples under reduced conditions, but also showed multiple unresolved bands under non-reducing conditions [71]. Nevertheless, more recent studies of our group using various new developed anti-ALR antibodies have shown that ALR is expressed in human liver in 3 different isoforms: 23, 21 and 15 kDa [64,72]. Therefore, the variable findings in detecting ALR isoforms might in part be attributed to reduction of protein lysates, the quality of gel resolution and the quality of antibodies.

5. Clinical importance of ALR

5.1. Mutations in GFER gene

Over the last years, the clinical importance of ALR has been underlined by studies that identified several mutations in ALR gene GFER (Supplementary Fig. S1) leading to severe mitochondrial disorders. The first described mutation in GFER (R194H) was reported to cause an infantile mitochondrial disorder [75]. Di Fonzo et al. reported three children who suffered from, among others, progressive myopathy, partial combined respiratory chain deficiency and development delays. Later on, the mutation (R194 H) was characterized structurally and enzymatically in both lfALR (23 kDa) and sfALR (15 kDa) and was demonstrated to adversely affect the stability of both isoforms of ALR (characterized by weaker FAD binding and lower thermal stability) with minimal effects on its enzyme activity (characterized by minor changes to interaction with Mia40 and cytochrome C) [76]. However, in a more recent study it was emphasized that the mutated site (R194H in human ALR that corresponds to R182H in yeast Erv1) causes lower thermal stability and the quick release of the FAD cofactor but also pointed out the loss of enzyme activity (interaction with Mia40) [77].

Furthermore, North et al. [78] described a patient at the age of 18 months with neonatal onset of lactic acidosis, poor feeding, irritability, hepatomegaly, bilateral cataracts and adrenal insufficiency. This patient was diagnosed with a mitochondrial disorder, but the genetic etiology was unknown. At the age of 19 years old, the patient was presented with significant muscle weakness in the face, neck, trunk and proximal portions of all extremities [79]. Using "Next generation sequencing" (NGS) the R194H mutation as well as another mutation in GFER gene (Q125X; C.373C > T) (Fig. S1) were identified in a study and were suggested to cause frame shift variations or truncation of ALR [80]. Interestingly, in a recent report, the authors suggested using rapid targeted genomics to analyze genetic defects in GFER gene in critically ill infants, since they identified R194H mutation in a 5 day old infant suffering from lactic acidosis and dysmorphic features [81]. This might open the possibility that investigating ALR mutation may contribute to adjustment of treatment plans and improvement in pre-symptomatic and prenatal testing.

5.2. ALR and liver diseases

The mRNA and protein expression of ALR have been investigated in specific organs linked to various diseases and using different injury models. For example, performing an ischemia/reperfusion injury (IRI) mouse and a hypoxia/re-oxygenation (H/R) model induced expression of sfALR (15 kDa) in the kidneys [61,62,82,83]. Acute kidney injury (AKI) also induced the expression of ALR [61,62]. Moreover, the expression of ALR was reported to be induced in muscle biopsies from patients with mitochondrial myopathies [60] and was shown to be inversely correlated with the tumor grading in samples from patients with colorectal carcinoma and colon cancer cell lines [84].

ALR expression was thoroughly analyzed in samples from patients with various liver diseases (see summary in Table 2) and was found to be strongly increased in diseases associated with normal or abnormal regeneration like cirrhosis or HCC. Several reports have shown that the expression of ALR is increased in liver tissue from patients with HCC [70–72,85], CCC [71] and cirrhosis [71,86,87]. Interestingly, ALR protein expression in HCC correlates inversely with tumor grading and its angio-invasion [72,85] and this was found presumably to be attributed to expression of the cytosolic 15 kDa ALR isoform (sfALR) [72]. Furthermore, ALR mRNA [64] and protein [67] expression were shown to be reduced in the liver of patients with non-alcoholic steatohepatitis (NASH). In hepatic samples from patients with alcoholic liver diseases (ALD) ALR expression was either reduced [67] or not altered [46] compared to normal liver tissue. Moreover, ALR mRNA expression has been investigated in liver tissues from patients with cholestatic liver diseases and was found to be significantly reduced [88].

Infection with hepatitis B virus leads to induction of several genes among them target genes of Nrf2 such as ALR [46]. Hence increased ALR mRNA and protein expression was found in HBV positive liver tissues [46]. Furthermore, ALR serum levels were found to be induced in patients with HBV-related acute, chronic and severe hepatitis or cirrhosis [87]. Serum levels of ALR might be considered a candidate for prognosis of HBV related-liver failure since it was found to be induced in patients with HBV-related ACLF (acute-on chronic liver failure) compared to normal patients and to be also induced in the surviving ACLF patients compared to dead patients [89]. In addition, patients with improved hepatic failure (HF) show increased serum levels of ALR protein compared to patients with deteriorating HF [70]. On the other hand, HCV infection impairs induction of Nrf2 [90] and therefore as expected, ALR expression is not altered in HCV positive liver tissue [46,67]. Interestingly, hepatic ALR expression is low or even unchanged after insulting the liver by fat, alcohol or HCV infection,

Table 2
Summary of studies investigating hepatic expression of ALR in various human liver diseases.

Liver disease	Expression (1)	Study subject	Ref.
Hepatocellular carcinoma (HCC)	up	HCC *vs.* surrounding non-tumorous tissue (n = 4) R, HCC *vs.* surrounding cirrhotic tissue (n = 5) R,I,W	[71]
		HCC (n = 12) vs. non-tumorous (n = 5) (w/o HBV nor alcohol) I(2)	[46]
		HCC (n = 22) *vs.* para-cancerous liver tissue (n = 22) R,I	[74]
		HCC (n = 20) *vs.* normal (n = 10) E, *vs.* hepatic failure (HF) R,I	[70]
	up	HCC high vs. low ALR expression (n = 53) I; HCC (w/o angioinvasion n = 2 vs. with angioinvasion n = 2) *vs.* normal W	[72]
		HCC high (n = 25) *vs.* low (n = 19) ALR expression R, HCC w/o angioinvasion (n = 30) *vs.* HCC with angioinvasion (n = 14) R	[85]
Cholangiocyte carcinoma (CCC)	up	CCC *vs.* adjacent non-tumorous tissue (n = 5) R, CCC *vs.* normal (n = 3) I,W	[71]
Hepatitis- HBV infection	up	chronic (n = 24) *vs.* normal (n = 10) E	[70]
		acute (n = 5), chronic (n = 30), severe (n = 15) HBV *vs.* normal (n = 10) E	[87]
		acute (n = 10), fulminant (n = 7) *vs.* normal (n = 58) E	[138]
		tumorous (n = 7) *vs.* non-tumorous (n = 8) I, positive (n = 8) *vs.* negative (n = 5) I; HBV (n = 10) *vs.* HCV (n = 10) R	[46]
-HCV infection	up	chronic (n = 14) *vs.* normal (n = 58) E, chronic (n = 9) *vs.* normal (n = 2) R	[138]
	not altered	HCV infection (n = 7) *vs.* normal (n = 5) W	[67]
Cirrhosis	up	cirrhosis (n = 25) *vs.* normal (n = 18), with n = 14 alcohol-induced, n = 7 HCV-induced and n = 4 autoimmune diseases induced cirrhosis R	[71]
		cirrhosis (n = 30) *vs.* normal (n = 10) E	[87]
		cirrhosis (n = 5) *vs.* normal (n = 58) E, cirrhosis (n = 5) *vs.* normal (n = 2) R	[138]
Hepatic failure (HF)	down	deteriorating HF (n = 12) *vs.* improved (surviving) HF (n = 6) E	[70]
		acute liver failure (n = 2) *vs.* normal (n = 2) W	[86]
Acute on chronic liver failure (ACLF)	up	HBV related ACLF (n = 20) *vs.* normal (n = 10) E	[89]
	down	dead ACLF patients (n = 20) *vs.* survival ACLF patients (n = 10) E	
Non-alcoholic fatty liver diseases (NAFLD)	down	NASH (n = 29) and Steatosis (n = 27) *vs.* normal (n = 17) R	[64]
		NASH (n = 5) *vs.* normal (n = 5) W	[67]
Alcoholic liver diseases (ALD), alcoholic steatohepatisis (ASH)	not altered	ALD (n = 4) *vs.* normal (n = 5) (non-tumorous tissue) I(2)	[46]
	down	ASH (n = 5) *vs.* normal (n = 5) W	[67]
Cholestatic liver diseases	down	cholestasis (n = 45) *vs.* normal (n = 13) R; Cholestasis (n = 5) *vs.* normal W	[88]

Detection of all three ALR isoforms: I, immunohistochemistry; E, ELISA of serum samples; R, qRT-PCR. Detection of particular ALR isoforms: W, western blot.
(1) Represent the expression of ALR under pathophysiological conditions compared to normal (less diseased) patients.
(2) Dayoub et al. (2013) investigated ALR protein expression in liver sections from patients with HCC and non-tumorous tissue (with or without HBV or alcohol) by IHC and quantitatively evaluated the intensity of anti-ALR staining (from 0 to 3).

respectively. But as a consequence of these insults progression to cirrhosis or even tumorigenesis occurs with induction of ALR expression as part of the regenerative response that occurs regardless of the underlying etiology [71]. Although the previously mentioned studies have indicated a dysregulation of ALR expression, only a few of them have pointed out if one or all isoforms are affected. Therefore and due to the different functions of ALR isoforms, it is of great importance to investigate which isoforms of ALR are regulated in a certain diseases and the cellular compartment (cytosol or mitochondria) in which the differential regulation takes place.

5.3. ALR and in vivo models

Multiple studies have reported the use of mouse and rat models that mimic diseases conditions in order to explore the effect of ALR in those diseases. It was reported that ALR serum levels are elevated in rats after PCS (portacaval shunt) surgery, endotoxemia or sepsis, and in a mouse model of hemorrhagic shock [91]. Furthermore, ALR serum levels are increased in rats after tissue loss by partial hepatectomy (PH) [66,69] and return to normal levels after 12 h [69] or 36 h [66]. On the contrary, hepatic level of ALR after PH remains unchanged (after 40% PH) or decreases (after 70% PH) [69]. ALR liver-specific knock-out mice have been shown to endure accelerated steatosis and to develop liver tumors after 1 year [67]. The same mouse model was used to investigate the alcohol-induced injury and was shown to accelerate injury [63]. Interestingly, using hepatocytes transfected with ALR (23 kDa) expression plasmid have shown that over-expressed ALR enhances the effect of anti-tumor agents [92] and reduces IRI injury in steatotic liver [93]. Further, the subcutaneous-injected hepatoma cells expressing sfALR (15 kDa [72],) showed reduced tumor growth in mice due to their reduced metastatic ability. Injecting ALR (23 kDa) expression plasmid into mice with CCl_4-induced injury suppresses apoptosis and attenuates the acute injury [86] which is underlined with a study that reported increased ALR serum levels after CCl_4-injury in rats [89]. Interestingly, ALR-knock-in and knock-out models in Zebrafish have been established and demonstrated the functional role of ALR in vertebrate hepatogenesis [94]. It is worth to mention that the physiological effects of ALR in spermatogenesis were investigated by an ALR transgenic mouse model driven by the human TSPY (testis-specific protein, Y-encoded) promoter that allows the transgene to be specifically activated in the testes [95]. This study highlighted that temporal expression of ALR is required for normal testicular development and spermatogenesis. The previous studies on animal models emphasize the importance of ALR in different liver diseases and encourage the consideration of ALR as a potential future therapeutic option. However, extensive work should still be applied to verify the different effects of recombinant or transfected ALR (short or long form) for the further establishment of recombinant protein or gene therapies. Therefore, the next paragraph is devoted to the delineation between the transfection of ALR isoforms and application of recombinant ALR.

6. Function of ALR

Different isoforms of ALR have been associated with different subcellular localization and therefore specific functions. Several studies have investigated the role of the 23 kDa ALR (long form, lfALR) and the 15 kDa ALR (short form, sfALR) related to different diseases and pointed out the beneficial effects of the individual ALR isoforms. In the next paragraph we summarize the studies that investigated the over-expression of lfALR or sfALR as well as the studies that explored the impact of ALR silencing (effects all isoforms) on cellular functions under normal and aberrant physiological conditions.

6.1. The functional role of mitochondrial lfALR (23 kDa)

As implied before, the 23 kDa of ALR is located in the mitochondria (see the location of ALR) and the mitochondrial targeting of ALR is attributed to the IDD domain in the initial 80 amino acids at the N terminal of lfALR (see the structure of ALR). In mammalian cells, lfALR is a part of the disulfide relay system that recycles Mia40 (mitochondrial import and assembly 40) in the inter-mitochondrial space (IMS) while transporting proteins into the IMS. Mia40 interacts with its substrates by its CPC motif [96,97] and oxidizes those substrates, which leads to substrate folding and trapping in the IMS. Furthermore, reduced Mia40 is re-oxidized -“recycled”- by the CRAC and CXXC motif in ALR, which itself obtains electrons from cytochrome C [32,33,98]. For more detailed information about the role of ALR in mitochondrial biogenesis we would like to refer to recent review articles highlighting this subject in more detail [11–14,99].

Besides its role in mitochondrial protein import, there has been contradictory evidence about the role of ALR in the maturation and export of cytosolic Fe-S cluster proteins. Fe-S proteins were reported to be involved in enzymatic catalysis, DNA synthesis and repair, iron homeostasis and heme synthesis [100]. Lange et al. [101] have suggested that ALR, may be involved in the maturation of cytosolic Fe-S proteins. The authors claimed that ALR interacts with Atm1 (an ABC transporter in the inner membrane of the mitochondria) and facilitates the export of Fe-S proteins to the cytosol [101]. However, later it was suggested that, in yeast cells, ALR plays neither a direct nor an indirect role in the maturation of Fe-S cluster [102]. Nevertheless, a recent study in mammalian cells has pointed out the role of ALR in exporting MitoNEEt to the outer mitochondrial membrane (OMM). MitoNEEt is a Fe-S protein that is synthesized in the mitochondrial matrix. Upon synthesis, MitoNEEt translocates through the inner membrane (IM) of the mitochondria by ABCB7 and then through the IMS by ALR to the OMM where it contributes to cell proliferation [103]. However, whether ALR is involved in the maturation and export of other Fe-S protein still requires further investigations.

Furthermore, over-expression of lfALR (23 kDa) has been investigated in various disease models (summarized in Table 3). Expression of lfALR promotes liver growth during hepatogenesis [94], reduces fibrotic injury [104,105], protects against radiation-induced oxidative stress [106] and attenuates acute liver injury by acetaminophen or CCl_4 [86,107] as well as the IRI-induced injury [93]. The latter has been attributed to autophagy activation (by promoting p62 degradation and LC3II conversion) and apoptosis repression [86,107]. In addition, lfALR has been reported to reduce ER stress by reducing Ca^{+2} release into cytosol [108] and to promote the anti-tumor effects of doxorubicin by increasing its cellular retention [92]. Moreover, lfALR has been associated with anti-metastatic effects in HCC by reducing the ERK phosphorylation [85]. Furthermore, lfALR has been shown to increase the pluripotency of embryonic stem cells (ESC) by preserving the mitochondrial integrity and function in ESCs [109]. Interestingly, a study in hematopoietic stem cells (HSC) demonstrated that binding of ALR to JAB1 blocks JAB1 interaction with $p27^{kip1}$, which promotes cell cycle arrest and restricts the abnormal proliferation of HSC and the subsequent exhaustion [110]. This was confirmed by ALR knock-down that caused reduced $p27^{kip1}$ nuclear retention and therefore a hyper-proliferative response of the HSC [110]. On the other hand, it was shown that lfALR does not contribute to reducing the bile acid-induced apoptosis [111].

It is worth to mention that over-expression of lfALR in the testis of the mice (transgenic mouse) was reported to cause abnormal spermatogenesis and reduced fertility in those mice [95]. Furthermore, it was suggested that continuous over-expression of lfALR in mammalian cells leads to the accumulation of lfALR not only in the mitochondria but also in the cytosol [112], which is also in agreement to observations of cytosolic occurrence of long form ALR as reported in Table 1. This finding raises the question whether the above mentioned effects of over-expressed lfALR (*e.g.* altering gene expression and activating pathways) may be due to its cytosolic accumulation rather than its mitochondrial localization. On the other hand, if the reported effects

Table 3
Summary of the studies investigating the over-expression of lfALR (23 kDa).

Disease / Model	Function of lfALR (23 kDa)	Molecular mechanism	Target cell /Host	Ref.
Radiation (oxidative stress)	protects against radiation-induced oxidative stress, improves cell viability	reduces ROS, mitochondrial dysfunction, apoptosis; cytochrome C release and caspase 3/7 activity	cell lines (HepG2, SMMC-7721, BEL-7402, 293FT, L02)	[106]
Acute liver injury (oxidative stress): acetaminophen or CCl_4-induced	protects liver by activation of autophagy, reduction of apoptosis, promoting proliferation	increases p62 degradation, LC3II conversion, enhances ATG5, ATG7 and Beclin-1, reduces release of AIF, cytochrome c and caspase 3 activation	injection into mouse tail or transfection into AML12 cells	[86,107]
Liver fibrosis (e.g. induced by colchicine)	protects against and reduces fibrosis, enhances regeneration	reduces the expression of TIMP1, collagen I and III; increases the survival rate *via* deactivation of hepatic stellate cells	injection into rat caudal vein; transfection into SMMC7721, HSC-T6	[104,105]
IRI liver injury and steatosis	reduces inflammation and necrosis caused by IRI	enhances oxygen consumption and ATP production	transfection into mice or HepG2 cells	[93]
Lipotoxicity (palmitic acid treatment)	reduces ER stress by reducing Ca^{2+} release to the cytosol	reduces IP3R expression, induces IP3R and Bcl2 interaction, reduces mitochondrial Bax expression	transfection into HepG2 cells	[108]
Cholestasis (GCDCA)	no reduction of caspase 3/7 activity	–	transfection into Huh7-NTCP cells	[111]
HCC and anti-tumor agents (doxorubicin)	sensitizes hepatocytes to anti-tumor effects of doxorubicin	reduces expression of ABCB1 and ABCG2 (MDR proteins) partially by blocking Snail/Akt pathway	transfection into HepG2 cells (xenografts in mice)	[92]
HCC and metastasis	reduces HCC metastasis	–	transfection into MHCC97H and xenografts in mice	[85]
Hepatogenesis	promotes liver growth	–	injection into Zebrafish embryos	[94]
Hematopoietic stem cells (HSC)	restricts abnormal proliferation of HSC	interacts with JAB1 and inhibits the JAB1-$p27^{kip1}$ interaction	transfection into HSC	[110]
Embryonic stem cells (ESC)	promotes ESC pluripotency, preserves mitochondrial structural integrity and function	reduces expression of Drp1	transfection into mouse ESC (embryonic stem cells)	[109]

AIF, apoptosis inducing factor; NTCP, Na + -taurocholate co-transporting polypeptide; TIMP1, tissue inhibitor of metalloproteinases 1; GCDCA; glycodeoxycholic acid.

Table 4
Summary of the studies investigating over-expression of sfALR (15 kDa).

Disease / model	Function of sfALR (15 kDa)	Molecular mechanism	Host, traget cell	Ref.
–	interacts with thioredoxin (TRX)	interacts with thioredoxin by the CXXC motif	infection of BS-C-1 cells (Kidney)	[119]
–	interacts with BNIPL (apoptotic protein)	–	transfection into BEL-7402 cells	[120]
–	activates AP1	interacts with JAB1 (nucleus) and with MIF (cytosol) in a MAPK independent manner	transfection into COS-7 and HepG2 cells	[34,53,116,117]
–	activates AP1, NFκB and c-Jun phosphorylation	interacts with thioredoxin	transfection into COS-7 cells, Yeast Y190 cells	[36]
Acute hepatic injury, hepatic failure	increases cell proliferation and survival rates	–	intravenous injection into rats after CCl_4-induced liver injury	[150]
HCC and metastasis	reduces cell motility and EMT	reduces Snail expression, induces E-Cadherin and ZO-1 expression	transfection into HepG2 cells	[72]
NAFLD	reduces the severity of fatty acid injury	suppresses JNK phosphorylation and fatty acid synthesis genes, induces miR122	mice on methionine choline deficient (MCD) diet	[115]
Acute phase response (APR)	induces expression of fibrinogen β, haptoglobin	induces STAT3 phosphorylation	transfection into HepG2 cells	[113]
NAFLD	attenuates ER stress, lipoapoptosis, alters lipid metabolism genes, reduces TAG levels	reduces DR5, Bax and CHOP expression and JNK phosphorylation, increases ATP synthesis, alters expression of CPT1α, FABP1, ELOVL6	transfection into HepG2 and Huh7 cells	[64]
Cholestasis	reduces bile acid synthesis and -induced apoptosis	preserves STAT3 activation, reduces HNF4α and DR5 expression	transfection into HepG2 cells	[88]

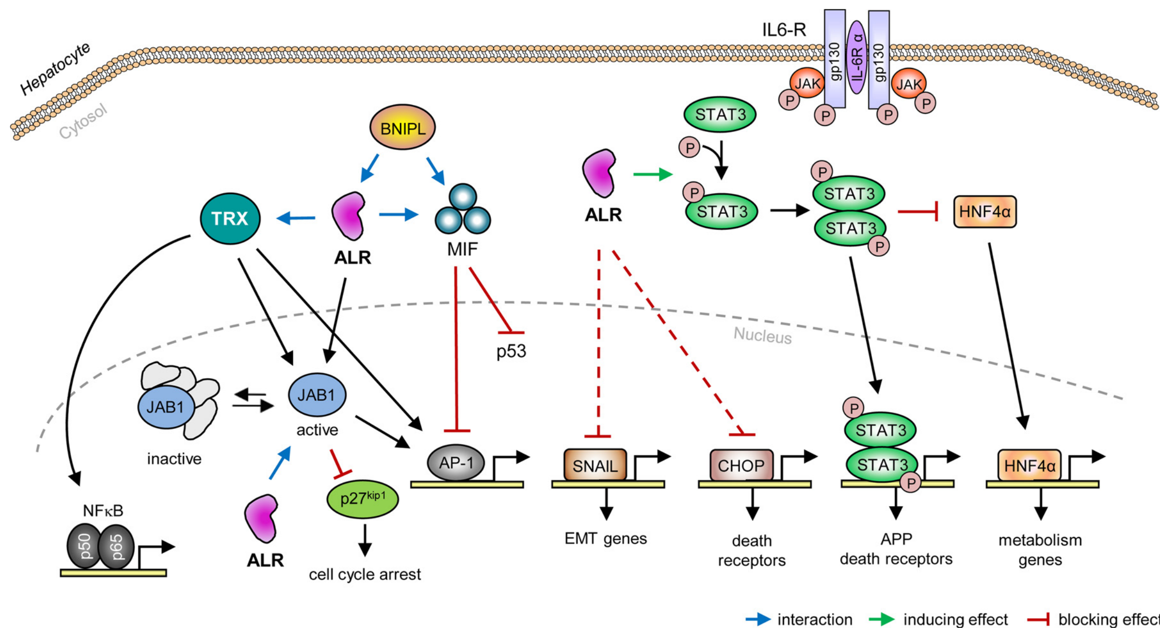

Fig. 3. An illustration of how over-expressed sfALR affects hepatocytes. ALR promotes IL6 signaling by increasing STAT3 phosphorylation and thereby regulating STAT3 target genes. Furthermore, STAT3 activation by sfALR blocks HNF4α and reduces its trans-activating effects. sfALR also indirectly reduces the expression of SNAIL and CHOP (by reducing ER stress) and thereby their target genes. ALR interacts with MIF (contains a CXXC motif) and blocks its inhibitory effects on p53 and AP1, which results in their activation and tumor suppression by p53. ALR also interacts with thioredoxin (contains a CXXC motif) and promotes AP1 and NFκB activation. Moreover, endogenous ALR interacts with JAB1 in the nucleus and thereby activates AP1. ALR, MIF and thioredoxin bind to JAB1 to prevent JAB1 interaction with $p27^{kip1}$ which increases the nuclear retention of $p27^{kip1}$ and therefore induces cell cycle arrest at G1 phase. In addition, the pro-apoptotic protein BNIPL interacts with ALR and MIF and regulates cell survival. BNIPL, Bcl-2/adenovirus E1B.

are indeed due to the mitochondrial pool of lfALR, an investigation of the molecules intermediating the effect between a mitochondrial protein and altered gene expression or pathways activation would possibly be of great importance.

6.2. The functional role of cytosolic sfALR (15 kDa)

Short form ALR (sfALR, 15 kDa) is located in the cytosol [64] and presumably in the nucleus [73], due to the lack of the IDD (responsible for mitochondrial targeting) in the initial 80 amino acids found only in lfALR (see Fig. 1). However, the potential effects (summarized in Table 4) and molecular functions (Fig. 3) of sfALR have been investigated in different models. sfALR was shown to induce the response of hepatocytes to IL6 signaling by induced STAT3 (signal transducer and activator of transcription 3) phosphorylation, which increases the expression of *e.g.* acute phase proteins (APP) like fibrinogen β (FGB) and haptoglobin (HP) [113]. In addition, the activation of STAT3 might account for the anti-apoptotic effect of sfALR by reduced DR5 (death receptor 5) expression and caspase 3/7 activity during cholestasis [88] and free fatty acid (FFA)-induced toxicity [64]. Interestingly, the reduced DR5 expression by sfALR may also be due to reduced CHOP (C/EBP-homologous protein, an inducer of DR5 expression [114]) which is repressed upon attenuation of FFA-induced ER-stress by sfALR [64].

In addition, sfALR was attributed to have anti-metastatic effects by reducing the migratory and invasion activity of hepatoma cells and as well as maintaining an epithelial like state with less signs of aggressive proliferation (*in vitro* and *in vivo*). sfALR mediates this by reducing Snail expression which induces the expression of epithelial cell markers like CDH1 (E-Cadherin) and ZO-1 (Zona Occludens-1) [72]. Moreover, the hepatic metabolic capacity is affected by over-expression of sfALR *via* reducing the synthesis of bile acids due to reduced CYP7A1 (rate-limiting enzyme in bile acid synthesis) expression and reduced HNF4α expression [88]. Additionally, lipid metabolism genes (CPT1α, FABP1, ELOVL1) were reported to have altered expression in sfALR over-expressing cells under steatotic conditions [64], which could be due to repressed activation of JNK (C-Jun N-terminal kinase) [64,115] or the activation of miRNA expression *e.g.* miR122 [115].

The interactions of sfALR protein with other molecules have been also reported in multiple studies (Fig. 3). Cytosloic sfALR interacts with JAB1 (c-Jun-activating domain binding protein 1) [34,110,116,117] in the nucleus and might include the whole CSN (COP9 signalosome, consisting of COP9 and JAB1) [117]. This interaction results in AP1 activation [116,117] in a MAPK (Mitogen-activated protein kinase) - and JNK-independent manner (phosphorylation of c-Jun) [34]. Furthermore, it was shown that ALR activation of AP1 is dependent on the CXXC motif in ALR [116]. Moreover, cytosolic sfALR interacts with MIF (macrophage migration inhibitory factor) [53], which also results in AP1 activation due to MIF's ability to block AP1 [53], this interaction might as well block MIF effects on p53 and results in tumor suppression [118] which may contribute to the described anti-metastatic effects of ALR [72]. Furthermore, ALR interacts with thioredoxin (TRX) [119] by its CXXC motif (C142-C145) and contributes to the maturation of viral proteins [119]. This interaction results in oxidized TRX which therefore induces AP1, c-Jun and NFκB activity [36]. Additionally, ALR interacts with the pro-apoptotic protein BNIPL (Bcl-2/adenovirus E1B) and leads to growth inhibition in hepatoma cells (BEL7402) [120]. To summarize, although the studies on over-expressed ALR have given valuable knowledge on molecular links between ALR and many vital cellular process in several species, nevertheless, further investigations are still required to uncover the mechanisms of ALR's interference in cellular signaling and gene transcription.

6.3. ALR silencing

In order to further clarify the role of ALR for cellular functions, many studies have explored the effect of ALR silencing (all isoforms) and monitored the outcome of its absence on the development of various diseases. (Table 5 summarizes the main finding of the studies that inspected the effect of silencing ALR).

In the kidney, it was shown that ALR knock-down had no effect on

Table 5
Summary of studies investigating ALR silencing (all ALR isoforms).

Organ	Disease/ model	Effect of ALR silencing	Molecular mechanism	Target cell / Host	Ref.
Liver	–	reduced expression of the three isoforms of ALR	–	HepG2, Huh7, Huh7-sfALR cells	[64]
	hepatogenesis	suppressed liver growth	reduced hepatocyte proliferation without affecting apoptosis	zebrafish embryos	[94]
	hepatocytes survival	reduced cell viability and induced apoptosis	induced rounding, detachment of cells, increased LDH, cytochrome C release, Caspase 3 activity, reduced ATP	rat heptocytes	[122]
	partial hepatectomy	reduced hepatocyte proliferation and polyamine synthesis, increased pro-apoptotic proteins and caspase activity	–	rat	[65]
	radiation (oxidative stress)	inhibited cell viability, induced sensibility to radiation (HepG2), minimal effects on normal cell line	–	HepG2, L02 cells	[106]
	HCC growth	inhibited growth of HepG2 and xenografted HCC tumors in nude mice	–	HepG2 cells, xenografts in mice	[123]
	HCC and metastasis	promoted cell growth and migration *in vivo* and *in vitro*	induced EMT in hepatoma cells due to activated ERK pathway	HepG2 cells xenografts in mice	[85]
	doxorubicin treatment	reduced caspase 3 activity due to reduced cellular retention of doxorubicin	induced Snail and therefore ABCB1 and ABCG2 (export pump) expression	HepG2 cells, xenografts in mice	[92]
	steatosis, liver-specific ALR knock out (Albumin Cre-lox)	ALR-KO in mice results in accelerated hepatic steatosis after 2 weeks, development of liver tumors after 6 months of age	increased ratio of Bax /Bcl2, recruitment of NK and $CD8^+$ cells, induced IL1β, TNFα, IL6, increased ROS, mtDNA damage, reduced ATP levels and expression of ACACA, SREBP1c, CPT1α and PPARα	mice	[67]
	lipotoxicity, palmitic acid	induced ER stress by increasing Ca^{2+} release into the cytosol	induced expression of IP3R and Bax, reduced expression of Bcl2	HepG2 cells	[108]
	alcohol induced injury, liver-specific ALR knock out	accelerated alcohol induced liver injury in ALR-KO mice reduced lipid metabolism genes and alcohol metabolism genes	reduced expression of FASN, ACACA, SREBP1c, CPT1α, and activity of ADH1, ALDH1 and CYP2E1, augmentted oxidative stress, fibrosis, inflammation by EtOH feeding	mice	[63]
Stem cells	ESC	loss of mitochondrial function in ESC, reduced proliferation, enhanced apoptosis	triggered mitochondrial autophagy or mitophagy by increased Drp1	murine ESC (Embryonic stem cells)	[125]
	HSC	hyper-proliferative response and exhaustion in hematopoietic stem cells	enhanced binding of $p27^{kip1}$ to its inhibitor JAB1 leading to down-regulation	HSCs, bone marrow transplantation in mice	[110]
	–	induced apoptosis by cytochrome C release and impaired cardiac development in Zebrafish embryos	inhibition of ALR activity MitoBloCK-6 („ALR inhibitor“)	ESC, Hela cells, and zebrafish embryos	[126]
Kidney	H/R injury	no effect on viability, inhibited inflammatory response and cytokines production after H/R injury	blocked MAPK pathway and decreased nuclear translocation of NFκB	HK-2 cells	[83]
	I/R kidney injury	no effect on cell viability, increased autophagy and ROS production *in vitro*	activated AMPK/mTOR pathway	HK-2 cells	[121]
Glioma cells	H_2O_2 (oxidative stress)	reduced expression of clusterin (anti-oxidative protein) and Bcl2, induced Caspase 3 and 9 activity	–	T98 G cells (human)	[58]
Myeloma cells	–	promoted apoptosis	induced Bax expression, reduced Bcl2 expression and IL6 synthesis	U266 cells	[124]

Table 6
Summary of the studies investigating the role of recombinant ALR (15 kDa).

Organ	Effect of rALR	Remarks	Ref.
Liver	ALR binds to its receptor (ALR-R) on hepatoma cells, activates MAPK pathway, activates AP1	ALR binds to a surface protein (~75 kDa), phosphorylation of EGF-R, activation of MAPK pathway	[116,140,141]
	via EFG-R activates MAPK and PI3k/Akt signaling pathway	proliferation is partly induced by EGF-R signaling	[144]
	induces polyamine synthesis and proliferation	activates c-myc and ODC expression	[142]
	reduces CYP450 enzyme expression and activity	activates NFκB, reduces CAR expression	[143]
	reduces expression of acute phase proteins	reduces STAT3 phoshorylation	[113]
	reduces lipoapoptosis	reduces pro-apoptotic Bax and caspase 3/7 activity; no effect on ER-stress and DR5 expression	[64]
	reduces apoptosis (extrinsic induced, EtOH).	–	[145]
	anti-apoptotic effects, reduced cell damage after PH (rat)	reduces Bax, induces Bcl2 and clusterin expression	[66]
	normal liver tissue: reduction of IFN-γ expression and inhibition of lytic activity of liver resident Natural Killer cells; increase expression of mitochondrial transcription factor A (TFAM) and therefore capacity of oxidative phosphorylation	no direct effect of ALR on NK cells; proliferating hepatocytes protected from NK cell activity; enhanced mitochondrial gene expression and therefore activity supports regeneration	[137,151,152]
	promotes liver regeneration in rats after 40% partial hepatectomy (PH); activation of Kupffer cells (KC)	binds to cholera toxin-sensitive receptor on KC, activates NFκB, induces release of TNFα, IL6, iNOS	[139]
	hepatocytes transplantation (rat): promotes regeneration, attenuates acute injury, improves survival after	reduces pro-inflammatory cytokines (IL1β and TNFα), reduces apoptosis and $CD4^+$ recruitment	[133]
	induces survival and reduced organ rejection after liver transplantation in rats	reduces IL2, IFNγ and TNFα expression, promotes T cell apoptosis, induces IL10 expression	[134]
	reduces hepatic ischemia reperfusion injury (IRI) in rats	reduces serum transaminases, apoptosis and infiltration of inflammatory cells (neutrophiles)	[146]
	attenuates the injury after obstructive cholestasis (BDL) in rats	reduces serum transaminases, preserves mitochondrial DNA	[147]
	no ALR receptor and no induction of DNA synthesis in isolated rat hepatocytes (in vitro)	–	[69,122]
Kidney	attenuates renal tubular epithelial cell injury after liver transplantation in rats	reduces TNFα production, increases regenerating cells	[135]
	reduces gentamicin induced injury, improves renal dysfunction after acute kidney injury (AKI)	–	[62,83]
	reduces tubular injury and attenuated renal dysfunction after IRI in rats	activates PI3k/Akt pathway, increases Bcl2/Bax ratio, represses p53 activation, reduces apoptosis and cytokine production, inhibits NFκB	[61,129,130]
	attenuates the inflammatory response after hypoxia / reperfusion (H/R) injury	reduces expression of IL1β and IL6 by down-regulation of TLR4 and NFκB expression	[128]
	reduces renal fibrosis	represses TGFβ-receptor II, alleviates TGFβ1 signaling, reduces Smad2, NFκB phosphorylation and EMT in renal tubular cells	[131]
Glioma cells	reduces H_2O_2-induced oxidative stress in human glioma cells	reduces ROS production	[58]
Neuro-blastoma	induces cell viability after H_2O_2 injury and preserves mitochondrial integrity	reduces cytochrome C release	[59]
Pancreas	reverses diabetes after fetal pancreas transplantation (rat)	–	[136]
Lymphocytes	represses concanavalin A-induced apoptosis in activated human lymphocytes	reduces PARP cleavage, decreases Bax/Bcl2 ratio, reduces cytochrome C release	[132]

HK-2 cells viability [83,121] but aggravated the H/R injury by increased ROS production and apoptosis due to the activation on AMPK/mTOR pathway [121]. Moreover, ALR silencing inhibited cytokine production and the inflammatory response after H/R injury by blocking MAPK pathway [83]. Furthermore, it was suggested that ALR silencing decreases NFκB nuclear translocation in kidney cells [83]. On the contrary, it was reported that over-expression of sfALR (15 kDa isoform) does not affect NFκB's nuclear translocation in hepatocytes during cholestasis [88], which might suggest that cytosolic lfALR may be responsible for the activation of NFκB or that ALR isoforms possess organ-specific effects.

The anti-oxidative role of ALR was further evidenced in human derived glioma cells (T98 G) which showed that silencing of ALR reduces the expression of antioxidative protein clusterin and induces the activity of caspase 3 and 9 [58]. Additionally, ALR silencing sensitizes HepG2 cells to radiation induced oxidative stress [106]. ALR silencing suppresses liver growth [94], reduces cell viability and induces apoptosis in rat hepatocytes [122] as well as HepG2 cells [123]. In the liver, after partial hepatectomy ALR silencing resulted in reduced compensatory hepatocellular proliferation, increased pro-apoptotic proteins and caspase activity [65]. Interestingly, ALR anti-apoptotic effects were further confirmed by findings suggesting that ALR knock out causes induced apoptosis *via* increased expression of the pro-apoptotic Bax protein [108,124]. Furthermore, liver specific ALR knock-out (KO) mice developed steatosis accompanied by altered expression of lipid metabolism genes, enhanced ROS production, mitochondrial dysfunction, increased Bax expression and NK as well as $CD8^+$ cell recruitment to the liver [67]. Interestingly, it was shown that ALR-KO mice develop liver tumors within 6 months of age and therefore ALR silencing links non-alcoholic liver disease to hepato-carcinogenesis [67]. The same group showed later that ALR-KO mice suffer accelerated alcohol injury, which they attributed to changes in lipid metabolism genes, altered alcohol metabolism by ADH1 (alcohol dehydrogenase 1), ALDH1 (aldehyde dehydrogenase 1) and CYP2E1 (cytochrome P450 2E1) and augmented oxidative stress upon injury [63]. In addition, it was shown that diminishing ALR expression enhances free fatty acid-induced ER stress and lipoapoptosis [108] and reduces the anti-tumor effects of doxorubicin due to enhanced expression of export transporters and therefore reduced cellular retention of doxorubicin [92]. In contrast to Tang et al. [123], who showed reduced tumor growth of HepG2 in nude mice after ALR silencing, others reported promoted cell growth and EMT (epithelial mesenchymal transition) [85]. It is worth to mention that silencing ALR in HSC (hematopoietic stem cells) caused a hyper-proliferative response due to reduced JAB1 binding and therefore reduced nuclear retention of $p27^{kip1}$ [110]. In addition, ALR silencing in mouse embryonic stem cells (ESC) increases Drp1 (Dynamine-related protein 1) expression and activates mitochondrial autophagy, which results in enhanced apoptosis of ESC [125]. Interestingly, a chemical screen identified a molecule that is capable of inhibiting ALR activity, MitoBloCK-6 [126]. MitoBloCK-6 was shown to induce apoptosis by cytochrome C release in ESCs but not in differentiated cells which suggests a vital role of ALR in the ESC homeostasis [126].

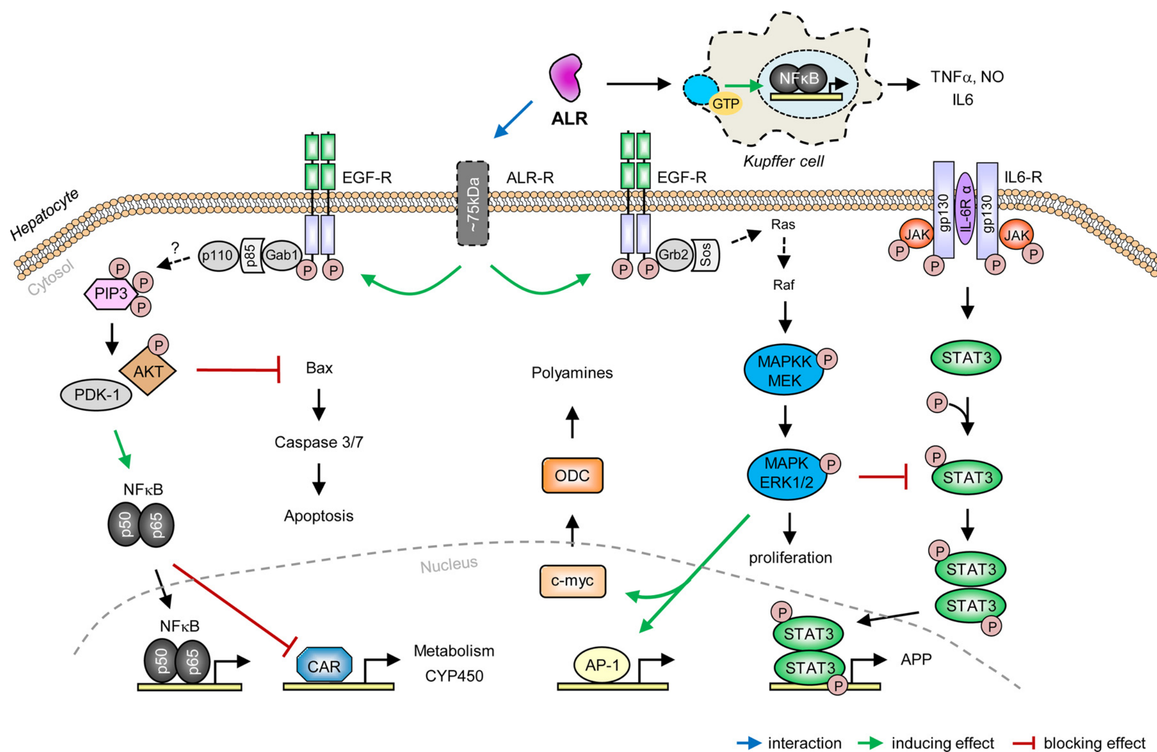

Fig. 4. An illustration of how exogenous ALR affects the liver. ALR binds to its cholera toxin-sensitive G-protein-coupled high affinity receptor on Kupffer cells to activate NFκB and increases the expression and release of NO, TNFα and IL6. Furthermore, ALR binds to its receptor (approx. 75 kDa) on hepatocytes and after phosphorylation of EGF-R on cytoplasmatic tyrosin sites, EGF-R signaling pathways PI3k/Akt and MAPK are activated. Activation of PI3k/Akt induces NFκB, attenuates Bax expression leading to reduced apoptosis and represses CAR expression and subsequently less target gene expression. Moreover, phosphorylated MAPK reduces STAT3 activation, activates AP1 and c-myc expression, which leads to increased ODC expression and therefore enhanced polyamine concentrations. NO, Nitric oxide; CAR, Constitutive androstane receptor.

However, due to the overlapping structure of ALR isoforms (Fig. 1), a knock-out or silencing of a specific isoform to explore isoform-related functions is -until now- not possible. Therefore, the specific knock-in of ALR isoforms is of greater importance to verify the effects of the different isoforms in their different sub-cellular compartments.

6.4. *The functional role of exogenous ALR (recombinant ALR, rALR)*

Although the current emphasis is placed on the development of “gene therapy” for human diseases, the production and use of recombinant proteins have a great impact on genetic disease due to improvements in production of these biomolecules as well as their availability [127]. Therefore in this paragraph we will summarize the effects and action of recombinant ALR (rALR) application in different injury models (Table 6).

Studies in the kidneys showed that application of rALR attenuates the injury after I/R (ischemia reperfusion) [61,83,128–130] and after gentamicin application [62]. It was suggested that the activation of PI3k/Akt (phosphoinositide 3-kinase/ Akt) by ALR reduces p53 phosphorylation and increases Bcl2/Bax ratio and therefore reduces apoptosis [129]. Furthermore, ALR signaling represses the activity of NFκB in the kidneys [128,130,131] which reduces the production of pro-inflammatory cytokines like IL6 and IL1β [128,130]. ALR was also reported to reduce renal fibrosis by modulating the TGFβ1 signaling and reducing EMT in renal tubular cells [131]. Moreover, in human derived glioma [58] and neuroblastoma cells [59] it was shown that rALR attenuates H_2O_2-induced injury by reducing reactive oxygen species (ROS) and cytochrome C release, which leads to reduced apoptosis. In addition a similar outcome of ALR is seen on concanavalin A-treated human lymphocytes [132] with reduced apoptosis. Interestingly, rALR could also contribute to survival of transplanted hepatocytes [133] and liver after transplantation [134] by modulating the inflammatory response as well as reducing the production of pro-inflammatory cytokines [133,134]. It is worth to mention that application of rALR upon liver transplantation reduces the renal tubular injury by repressing the expression of the proinflammatory TNFα [135]. In addition, Adam et al. have shown that application of rALR reverses diabetes in the pancreas of fetal rats upon transplantation [136].

Previous reports have focused on analyzing the impact of rALR on the liver and how rALR transduces its signals in liver cells (Fig. 4). The augmenting effect of rALR in liver regeneration was proposed by reduced activity (killing ability) of natural killer (NK) cells after rALR treatment [137,138] and subsequently enhanced survival of hepatocytes after injury [138]. Further, rALR treatment of Kupffer cells has been shown to activate NFκB signaling and to induce the synthesis of IL6 and TNFα [139]. The authors found an ALR specific- and cholera toxin sensitive- receptor (hetero trimeric G-protein) on Kupffer cells may be responsible for rALR effect on liver regeneration after 40% partial hepatectomy [139]. On the other hand, there have been contradictory reports about the expression of an ALR receptor (ALR-R) on hepatocytes [69,140]. Whereas on rat hepatocytes, no ALR receptor could be found so far [69], on human hepatoma cells it was shown that ALR binds to its receptor (ALR-R, approx. 75 kDa, not sequenced yet) and activates the EGF receptor (EGF-R) on the cytoplasmatic side [140]. This results in the activation of EGF-R pathways *via* MAPK activation [141] and subsequently AP1 activation [116]. Upon activation of the MAPK pathway, rALR increases polyamines synthesis, a prerequisite for liver regeneration, by increasing the expression of c-myc and ODC (ornithine-decarboxylase), the rate-limiting enzyme of polyamine synthesis [142]. Moreover, after rALR treatment, primary human hepatocytes show increased proliferation, but reduced expression and activity of various cytochrome P450 [143], which might be mediated by activated NFκB [143]. Later on, it was reported that rALR activates besides MAPK/Erk the PI3k/Akt pathway and was shown to induce

these pathways with different kinetics than EGF [144]. Interestingly, a recent study has investigated the role of rALR in modulating the IL6 signaling pathway and the acute phase response (APR) [113] by reducing the activation of STAT3 and subsequently the expression of acute phase response proteins (APP) [113].

The anti-apoptotic effect of rALR has been demonstrated in different injury models in the liver. It was shown that rALR reduces caspase 3/7 activity after free fatty acid- [64] and bile acid-induced injury (unpublished data), which may be due to reduced expression of Bax [64]. Furthermore, rALR reduces EtOH- and death ligand-induced apoptosis by reducing cytochrome C release [145]. This was further confirmed in a study showing that rALR reduces cell damage after partial hepatectomy by inducing the expression of anti-oxidative clusterin and anti-apoptotic Bcl2 and suggested that rALR boosts liver regeneration through apoptosis attenuation rather that inducing proliferation [66]. In addition, besides the immune-modulating properties, anti-apoptotic effects of rALR may account for the reduced liver injury upon IRI [146] and bile duct ligation (BDL), a common model of cholestasis [147]. Therefore, it is reasonable to anticipate that further investigations about rALR will serve in a better understanding of its mechanism of action and might also lead to modifying ALR-engineering strategies to develop a novel therapy with that would be beneficial in wide-spread pathologies like renal I/R injuries, hepatic fibrosis, cirrhosis and carcinogenesis.

7. Conclusions and future challenges

Initially, and as the name suggests, ALR was thought to be mainly involved in the complex process of liver regeneration. However, accumulating evidence has shown the ubiquitous expression of ALR in the liver as well as brain, kidney testis and others. Moreover, different functions have been attributed to different ALR isoforms which is emphasized by their differential subcellular localizations. Nevertheless, the knowledge about this protein is still insufficient. One of the most interesting aspects to address is the actual mechanism of how different isoforms of ALR are formed: (i) through “selective” mRNA expression at different ATGs or (ii) as “post translational modifications” products of the longest form (23 kDa). Furthermore, the mechanism by which ALR (most likely cytosolic/nuclear) alters gene expression still requires further exploration. Finally, exploring how ALR might be applied to augment liver regeneration in humans is of great importance, due to the significance in improving patients’ survival as well reducing the overall need of liver transplantation. To conclude, we strongly recommend to outline which isoform of ALR is addressed in studies, its sub-cellular localization as well as analyzing all isoforms if expression studies are performed. This will aid in the better understanding of ALR different functions and facilitate its possible future use as a therapy option.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cytogfr.2018.12.003.

References

[1] A. Reuben, Prometheus and Pandora–together again, Hepatology 39 (2004) 1460–1463.
[2] N. Fausto, J.S. Campbell, K.J. Riehle, Liver regeneration, Hepatology 43 (2006) S45–53.
[3] G.K. Michalopoulos, Liver regeneration, J. Cell. Physiol. 213 (2007) 286–300.
[4] G.K. Michalopoulos, Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas, Am. J. Pathol. 176 (2010) 2–13.
[5] G.K. Michalopoulos, M.C. DeFrances, Liver regeneration, Science 276 (1997) 60–66.
[6] R. Taub, Liver regeneration: from myth to mechanism, Nat. Rev. Mol. Cell Biol. 5 (2004) 836–847.
[7] F. Bohm, U.A. Kohler, T. Speicher, et al., Regulation of liver regeneration by growth factors and cytokines, EMBO Mol. Med. 2 (2010) 294–305.
[8] R. Pawlowski, J. Jura, ALR and liver regeneration, Mol. Cell. Biochem. 288 (2006) 159–169.
[9] C.R. Gandhi, Augmenter of liver regeneration, Fibrogenesis Tissue Repair 5 (2012) 10.
[10] K.H. Nguyen, A.H. Nguyen, D.V. Dabir, Clinical implications of augmenter of liver regeneration in cancer: a systematic review, Anticancer Res. 37 (2017) 3379–3383.
[11] A.J. Erdogan, J. Riemer, Mitochondrial disulfide relay and its substrates: mechanisms in health and disease, Cell Tissue Res. 367 (2017) 59–72.
[12] D. Fass, The Erv family of sulfhydryl oxidases, Biochim. Biophys. Acta 1783 (2008) 557–566.
[13] J.M. Herrmann, F. Kauff, H.E. Neuhaus, Thiol oxidation in bacteria, mitochondria and chloroplasts: common principles but three unrelated machineries? Biochim. Biophys. Acta 1793 (2009) 71–77.
[14] D.C. Wilkerson, U. Sankar, Mitochondria: a sulfhydryl oxidase and fission GTPase connect mitochondrial dynamics with pluripotency in embryonic stem cells, Int. J. Biochem. Cell Biol. 43 (2011) 1252–1256.
[15] G. Higgins, R. Anderson, Experimental pathology of liver. I. Restoration of liver of the white rat following partial surgical removal, Arch Pathol. 12 (1931) 186–202.
[16] D.R. LaBrecque, L.A. Pesch, Preparation and partial characterization of hepatic regenerative stimulator substance (SS) from rat liver, J. Physiol. 248 (1975) 273–284.
[17] A. Francavilla, P. Ove, L. Polimeno, et al., Extraction and partial purification of a hepatic stimulatory substance in rats, mice, and dogs, Cancer Res. 47 (1987) 5600–5605.
[18] D.R. LaBrecque, G. Steele, S. Fogerty, et al., Purification and physical-chemical characterization of hepatic stimulator substance, Hepatology 7 (1987) 100–106.
[19] D.R. LaBrecque, Hepatic stimulator substance. Discovery, characteristics and mechanism of action, Dig. Dis. Sci. 36 (1991) 669–673.
[20] A. Francavilla, M. Hagiya, K.A. Porter, et al., Augmenter of liver regeneration: its place in the universe of hepatic growth factors, Hepatology 20 (1994) 747–757.
[21] M. Hagiya, A. Francavilla, L. Polimeno, et al., Cloning and sequence analysis of the rat augmenter of liver regeneration (ALR) gene: expression of biologically active recombinant ALR and demonstration of tissue distribution, Proc. Natl. Acad. Sci. U. S. A 91 (1994) 8142–8146.
[22] M. Hagiya, A. Francavilla, L. Polimeno, et al., Cloning and sequence analysis of the rat augmenter of liver regeneration (ALR) gene: expression of biologically active recombinant ALR and demonstration of tissue distribution, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 3076.
[23] R. Giorda, M. Hagiya, T. Seki, et al., Analysis of the structure and expression of the augmenter of liver regeneration (ALR) gene, Mol. Med 2 (1996) 97–108.
[24] X.M. Yang, Z.Y. Hu, L. Xie, et al., [In vitro stimulation of HTC hepatoma cell growth by recombinant human augmenter of liver regeneration (ALR)], Sheng Li Hsueh Pao 49 (1997) 557–561.
[25] J. Cheng, Y.W. Zhong, Y. Liu, et al., Cloning and sequence analysis of human genomic DNA of augmenter of liver regeneration, World J. Gastroenterol. 6 (2000) 275–277.
[26] K.N. Tzirogiannis, G.I. Panoutsopoulos, M.D. Demonakou, et al., Effect of hepatic stimulator substance (HSS) on cadmium-induced acute hepatotoxicity in the rat liver, Dig. Dis. Sci. 49 (2004) 1019–1028.
[27] G. Gribilas, A. Zarros, A. Zira, et al., Involvement of hepatic stimulator substance in experimentally induced fibrosis and cirrhosis in the rat, Dig. Dis. Sci. (2008).
[28] X. Yi, M. Song, Y. Yuan, et al., Hepatic stimulator substance alleviates toxin-induced and immune-mediated liver injury and fibrosis in rats, Dig. Dis. Sci. 57 (2012) 2079–2087.
[29] Y. Wu, J. Zhang, L. Dong, et al., Hepatic stimulator substance mitigates hepatic cell injury through suppression of the mitochondrial permeability transition, FEBS J. 277 (2010) 1297–1309.
[30] J. Lu, W.X. Xu, Y.Q. Zhan, et al., Identification and characterization of a novel isoform of hepatopoietin, World J. Gastroenterol. 8 (2002) 353–356.
[31] L. Banci, I. Bertini, C. Cefaro, et al., An intrinsically disordered domain has a dual function coupled to compartment-dependent redox control, J. Mol. Biol. 425 (2013) 594–608.
[32] S. Allen, V. Balabanidou, D.P. Sideris, et al., Erv1 mediates the Mia40-dependent protein import pathway and provides a functional link to the respiratory chain by shuttling electrons to cytochrome c, J. Mol. Biol. 353 (2005) 937–944.
[33] L. Banci, I. Bertini, V. Calderone, et al., Molecular recognition and substrate mimicry drive the electron-transfer process between MIA40 and ALR, Proc. Natl. Acad. Sci. U. S. A 108 (2011) 4811–4816.
[34] C. Lu, Y. Li, Y. Zhao, et al., Intracrine hepatopoietin potentiates AP-1 activity through JAB1 independent of MAPK pathway, FASEB J. 16 (2002) 90–92.
[35] S.K. Ang, H. Lu, Deciphering structural and functional roles of individual disulfide bonds of the mitochondrial sulfhydryl oxidase Erv1p, J. Biol. Chem. 284 (2009) 28754–28761.

[36] Y. Li, W. Liu, G. Xing, et al., Direct association of hepatopoietin with thioredoxin constitutes a redox signal transduction in activation of AP-1/NF-kappaB, Cell. Signal. 17 (2005) 985–996.
[37] G. Hofhaus, J.E. Lee, I. Tews, et al., The N-terminal cysteine pair of yeast sulfhydryl oxidase Erv1p is essential for in vivo activity and interacts with the primary redox centre, Eur. J. Biochem. 270 (2003) 1528–1535.
[38] T. Lisowsky, D.L. Weinstat-Saslow, N. Barton, et al., A new human gene located in the PKD1 region of chromosome 16 is a functional homologue to ERV1 of yeast, Genomics 29 (1995) 690–697.
[39] Y. Zhao, F. Tang, J. Cheng, et al., An initiator and its flanking elements function as a core promoter driving transcription of the Hepatopoietin gene, FEBS Lett. 540 (2003) 58–64.
[40] L.Y. Dong, X.N. Wang, Z.G. Song, et al., Identification of human hepatic stimulator substance gene promoter and demonstration of dual regulation of AP1/AP4 cis-acting element in different cell lines, Int. J. Biochem. Cell Biol. 39 (2007) 181–196.
[41] L.Y. Dong, G. Sun, L. Jiang, et al., Epidermal growth factor down-regulates the expression of human hepatic stimulator substance via CCAAT/enhancer-binding protein beta in HepG2 cells, Biochem. J. 431 (2010) 277–287.
[42] D. Guo, L.Y. Dong, Y. Wu, et al., Down-regulation of hepatic nuclear factor 4alpha on expression of human hepatic stimulator substance via its action on the proximal promoter in HepG2 cells, Biochem. J. 415 (2008) 111–121.
[43] S. Ibrahim, R. Dayoub, M. Melter, et al., Bile acids down-regulate the expression of Augmenter of Liver Regeneration (ALR) via SHP/HNF4alpha1 and independent of Egr-1, Exp. Mol. Pathol. 105 (2018) 236–242.
[44] A.R. Brooks, B.D. Blackhart, K. Haubold, et al., Characterization of tissue-specific enhancer elements in the second intron of the human apolipoprotein B gene, J. Biol. Chem. 266 (1991) 7848–7859.
[45] J.E. Gallegos, A.B. Rose, Intron DNA sequences can Be more important than the proximal promoter in determining the site of transcript initiation, Plant Cell 29 (2017) 843–853.
[46] R. Dayoub, A. Vogel, J. Schuett, et al., Nrf2 activates augmenter of liver regeneration (ALR) via antioxidant response element and links oxidative stress to liver regeneration, Mol. Med. (Cambridge, Mass.) 19 (2013) 237–244.
[47] R. Hu, C. Xu, G. Shen, et al., Gene expression profiles induced by cancer chemopreventive isothiocyanate sulforaphane in the liver of C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice, Cancer Lett. 243 (2006) 170–192.
[48] R. Hu, C. Xu, G. Shen, et al., Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray, Life Sci. 79 (2006) 1944–1955.
[49] R. Dayoub, P. Groitl, T. Dobner, et al., Foxa2 (HNF-3beta) regulates expression of hepatotrophic factor ALR in liver cells, Biochem. Biophys. Res. Commun. 395 (2010) 465–470.
[50] G. Gardian, S.E. Browne, D.K. Choi, et al., Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease, J. Biol. Chem. 280 (2005) 556–563.
[51] Y.H. Kim, J.W. Park, J.Y. Lee, et al., Sodium butyrate sensitizes TRAIL-mediated apoptosis by induction of transcription from the DR5 gene promoter through Sp1 sites in colon cancer cells, Carcinogenesis 25 (2004) 1813–1820.
[52] T. Iannitti, B. Palmieri, Clinical and experimental applications of sodium phenylbutyrate, Drugs R D 11 (2011) 227–249.
[53] Y. Li, C. Lu, G. Xing, et al., Macrophage migration inhibitory factor directly interacts with hepatopoietin and regulates the proliferation of hepatoma cell, Exp. Cell Res. 300 (2004) 379–387.
[54] J.H. Lim, M.K. Song, Y. Cho, et al., Comparative analysis of microRNA and mRNA expression profiles in cells and exosomes under toluene exposure, Toxicol. In Vitro 41 (2017) 92–101.
[55] L. Polimeno, B. Pesetti, F. Giorgio, et al., Expression and localization of augmenter of liver regeneration in human muscle tissue, Int. J. Exp. Pathol. 90 (2009) 423–430.
[56] M. Klissenbauer, S. Winters, U.A. Heinlein, et al., Accumulation of the mitochondrial form of the sulphydryl oxidase Erv1p/Alrp during the early stages of spermatogenesis, J. Exp. Biol. 205 (2002) 1979–1986.
[57] A. Tury, G. Mairet-Coello, T. Lisowsky, et al., Expression of the sulfhydryl oxidase ALR (Augmenter of Liver Regeneration) in adult rat brain, Brain Res. (2005).
[58] L. Polimeno, B. Pesetti, F. De Santis, et al., Decreased expression of the augmenter of liver regeneration results in increased apoptosis and oxidative damage in human-derived glioma cells, Cell Death Dis. 3 (2012) e289.
[59] L. Polimeno, B. Pesetti, T. Lisowsky, et al., Protective effect of augmenter of liver regeneration on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells, Free Radic. Res. 43 (2009) 865–875.
[60] L. Polimeno, R. Rossi, M. Mastrodonato, et al., Augmenter of liver regeneration, a protective factor against ROS-induced oxidative damage in muscle tissue of mitochondrial myopathy affected patients, Int. J. Biochem. Cell Biol. 45 (2013) 2410–2419.
[61] X.H. Liao, L. Zhang, Q. Liu, et al., Augmenter of liver regeneration protects kidneys from ischaemia/reperfusion injury in rats, Nephrol. Dial. Transplant. 25 (2010) 2921–2929.
[62] X.H. Liao, L. Zhang, X.P. Tang, et al., Expression of augmenter of liver regeneration in rats with gentamicin-induced acute renal failure and its protective effect on kidney, Ren. Fail. 31 (2009) 946–955.
[63] S. Kumar, J. Wang, R. Rani, et al., Hepatic deficiency of augmenter of liver regeneration exacerbates alcohol-induced liver injury and promotes fibrosis in mice, PLoS One 11 (2016) e0147864.
[64] T.S. Weiss, M. Lupke, S. Ibrahim, et al., Attenuated lipotoxicity and apoptosis is linked to exogenous and endogenous augmenter of liver regeneration by different pathways, PLoS One 12 (2017) e0184282.
[65] A. Francavilla, B. Pesetti, M. Barone, et al., Transient GFER knockdown in vivo impairs liver regeneration after partial hepatectomy, Int. J. Biochem. Cell Biol. 53 (2014) 343–351.
[66] L. Polimeno, B. Pesetti, E. Annoscia, et al., Alrp, a survival factor that controls the apoptotic process of regenerating liver after partial hepatectomy in rats, Free Radic. Res. 45 (2011) 534–549.
[67] C.R. Gandhi, J.R. Chaillet, M.A. Nalesnik, et al., Liver-specific deletion of augmenter of liver regeneration accelerates development of steatohepatitis and hepatocellular carcinoma in mice, Gastroenterology 148 (2015) 379–391.
[68] C. Kordes, I. Sawitza, S. Gotze, et al., Hepatic stellate cells contribute to progenitor cells and liver regeneration, J. Clin. Invest. 124 (2014) 5503–5515.
[69] C.R. Gandhi, R. Kuddus, V.M. Subbotin, et al., A fresh look at augmenter of liver regeneration in rats, Hepatology 29 (1999) 1435–1445.
[70] H.Y. Yu, D.R. Xiang, H.J. Huang, et al., Expression level of augmenter of liver regeneration in patients with hepatic failure and hepatocellular carcinoma, Hepatobiliary. Pancreat. Dis. Int 9 (2010) 492–498.
[71] W.E. Thasler, T. Schlott, P. Thelen, et al., Expression of augmenter of liver regeneration (ALR) in human liver cirrhosis and carcinoma, Histopathology 47 (2005) 57–66.
[72] R. Dayoub, H. Wagner, F. Bataille, et al., Liver regeneration associated protein (ALR) exhibits antimetastatic potential in hepatocellular carcinoma, Mol. Med. 17 (2011) 221–228.
[73] Y. Li, K. Wei, C. Lu, et al., Identification of hepatopoietin dimerization, its interacting regions and alternative splicing of its transcription, Eur. J. Biochem. 269 (2002) 3888–3893.
[74] H.Y. Yu, M.H. Zhu, D.R. Xiang, et al., High expression of 23 kDa protein of augmenter of liver regeneration (ALR) in human hepatocellular carcinoma, Onco. Targets Ther. 7 (2014) 887–893.
[75] A. Di Fonzo, D. Ronchi, T. Lodi, et al., The mitochondrial disulfide relay system protein GFER is mutated in autosomal-recessive myopathy with cataract and combined respiratory-chain deficiency, Am. J. Hum. Genet. 84 (2009) 594–604.
[76] V.N. Daithankar, S.A. Schaefer, M. Dong, et al., Structure of the human sulfhydryl oxidase augmenter of liver regeneration and characterization of a human mutation causing an autosomal recessive myopathy, Biochemistry 49 (2010) 6737–6745.
[77] E. Ceh-Pavia, S.K. Ang, M.P. Spiller, et al., The disease-associated mutation of the mitochondrial thiol oxidase Erv1 impairs cofactor binding during its catalytic reaction, Biochem. J. 464 (2014) 449–459.
[78] K. North, M.S. Korson, N. Krawiecki, et al., Oxidative phosphorylation defect associated with primary adrenal insufficiency, J. Pediatr. 128 (1996) 688–692.
[79] L. Calderwood, I.A. Holm, L.A. Teot, et al., Adrenal insufficiency in mitochondrial disease: a rare case of GFER-related mitochondrial encephalomyopathy and review of the literature, J. Child Neurol. 31 (2016) 190–194.
[80] S. Nambot, D. Gavrilov, J. Thevenon, et al., Further delineation of a rare recessive encephalomyopathy linked to mutations in GFER thanks to data sharing of whole exome sequencing data, Clin. Genet. (2017).
[81] C.C. van Diemen, W.S. Kerstjens-Frederikse, K.A. Bergman, et al., Rapid targeted genomics in critically ill newborns, Pediatrics 140 (2017).
[82] G.T. Chen, L. Zhang, X.H. Liao, et al., Augmenter of liver regeneration ameliorates renal fibrosis in rats with obstructive nephropathy, Biosci. Rep. 34 (2014).
[83] R. Yan, L. Zhang, N. Xia, et al., Knockdown of augmenter of liver regeneration in HK-2 cells inhibits inflammation response via the mitogen-activated protein kinase signaling pathway, Inflamm. Res. 64 (2015) 453–462.
[84] E. Gatzidou, M. Mantzourani, C. Giaginis, et al., Augmenter of liver regeneration gene expression in human colon cancer cell lines and clinical tissue samples, J. BUON 20 (2015) 84–91.
[85] X.W. Jia, Z.W. Li, L.Y. Dong, et al., Lack of hepatic stimulator substance expression promotes hepatocellular carcinoma metastasis partly through ERK-activated epithelial-mesenchymal transition, Lab. Invest. (2018).
[86] H. Shi, W. Han, H. Shi, et al., Augmenter of liver regeneration protects against carbon tetrachloride-induced liver injury by promoting autophagy in mice, Oncotarget 8 (2017) 12637–12648.
[87] N. Wang, H. Sun, L. Tang, et al., Establishment and primary clinical application of competitive inhibition for measurement of augmenter of liver regeneration, Exp. Ther. Med. 7 (2014) 93–96.
[88] S. Ibrahim, R. Dayoub, S. Krautbauer, et al., Bile acid-induced apoptosis and bile acid synthesis are reduced by over-expression of Augmenter of Liver Regeneration (ALR) in a STAT3-dependent mechanism, Exp. Cell Res. 374 (2019) 189–197.
[89] S. Hongbo, C. Yu, K. Ming, et al., Augmenter of liver regeneration may be a candidate for prognosis of HBV related acute-on-chronic liver failure as a regenerative marker, Hepatogastroenterology 59 (2012) 1933–1938.
[90] M. Carvajal-Yepes, K. Himmelsbach, S. Schaedler, et al., Hepatitis C virus impairs the induction of cytoprotective Nrf2 target genes by delocalization of small Maf proteins, J. Biol. Chem. 286 (2011) 8941–8951.
[91] Y. Vodovotz, J. Prelich, C. Lagoa, et al., Augmenter of liver regeneration (ALR) is a novel biomarker of hepatocellular stress/inflammation: in vitro, in vivo and in silico studies, Mol. Med. 18 (2013) 1421–1429.
[92] Y.Y. Guo, Y. Wu, X.W. Jia, et al., Augmenter of liver regeneration potentiates doxorubicin anticancer efficacy by reducing the expression of ABCB1 and ABCG2 in hepatocellular carcinoma, Lab. Invest. (2017).
[93] J. Weng, W. Li, X. Jia, et al., Alleviation of ischemia-reperfusion injury in liver steatosis by augmenter of liver regeneration is attributed to antioxidation and preservation of mitochondria, Transplantation 101 (2017) 2340–2348.
[94] Y. Li, M. Farooq, D. Sheng, et al., Augmenter of liver regeneration (alr) promotes liver outgrowth during zebrafish hepatogenesis, PLoS One 7 (2012) e30835.
[95] Y. Cao, Y.L. Fu, C.H. Ge, et al., Mice overexpression of human augmenter of liver

regeneration (hALR) in male germ cells shows abnormal spermatogenesis and reduced fertility, Endocr. J. 59 (2012) 989–999.
[96] D. Milenkovic, K. Gabriel, B. Guiard, et al., Biogenesis of the essential Tim9-Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermembrane space receptor Mia40, J. Biol. Chem. 282 (2007) 22472–22480.
[97] D.P. Sideris, K. Tokatlidis, Oxidative folding of small Tims is mediated by site-specific docking onto Mia40 in the mitochondrial intermembrane space, Mol. Microbiol. 65 (2007) 1360–1373.
[98] S.R. Farrell, C. Thorpe, Augmenter of liver regeneration: a flavin-dependent sulfhydryl oxidase with cytochrome c reductase activity, Biochemistry 44 (2005) 1532–1541.
[99] E. Kallergi, E. Kalef-Ezra, K. Karagouni-Dalakoura, et al., Common players in mitochondria biogenesis and neuronal protection against stress-induced apoptosis, Neurochem. Res. 39 (2014) 546–555.
[100] A. Sheftel, O. Stehling, R. Lill, Iron-sulfur proteins in health and disease, Trends Endocrinol. Metab. 21 (2010) 302–314.
[101] H. Lange, T. Lisowsky, J. Gerber, et al., An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins, EMBO Rep. 2 (2001) 715–720.
[102] H.K. Ozer, A.C. Dlouhy, J.D. Thornton, et al., Cytosolic Fe-S cluster protein maturation and Iron regulation are independent of the mitochondrial Erv1/Mia40 import system, J. Biol. Chem. 290 (2015) 27829–27840.
[103] I. Ferecatu, S. Goncalves, M.P. Golinelli-Cohen, et al., The diabetes drug target MitoNEET governs a novel trafficking pathway to rebuild an Fe-S cluster into cytosolic aconitase/iron regulatory protein 1, J. Biol. Chem. 289 (2014) 28070–28086.
[104] Q. Li, D.W. Liu, L.M. Zhang, et al., Effects of augmentation of liver regeneration recombinant plasmid on rat hepatic fibrosis, World J. Gastroenterol. 11 (2005) 2438–2443.
[105] X. Wu, G. Liu, M. Mu, et al., Augmenter of liver regeneration gene therapy using a novel minicircle DNA vector alleviates liver fibrosis in rats, Hum. Gene Ther. 27 (2016) 880–891.
[106] Y. Cao, Y.L. Fu, M. Yu, et al., Human augmenter of liver regeneration is important for hepatoma cell viability and resistance to radiation-induced oxidative stress, Free Radic. Biol. Med. 47 (2009) 1057–1066.
[107] T. Hu, H. Sun, W.Y. Deng, et al., Augmenter of liver regeneration protects against acetaminophen-induced acute liver injury in mice by promoting autophagy, Shock (2018).
[108] W.C. Xiao, J. Zhang, S.L. Chen, et al., Alleviation of palmitic acid-induced endoplasmic reticulum stress by augmenter of liver regeneration through IP3R-controlled Ca(2+) release, J. Cell. Physiol. (2018).
[109] L.R. Todd, R. Gomathinayagam, U. Sankar, A novel Gfer-Drp1 link in preserving mitochondrial dynamics and function in pluripotent stem cells, Autophagy 6 (2010) 821–822.
[110] E.C. Teng, L.R. Todd, T.J. Ribar, et al., Gfer inhibits Jab1-mediated degradation of p27kip1 to restrict proliferation of hematopoietic stem cells, Mol. Biol. Cell 22 (2011) 1312–1320.
[111] G.U. Denk, R. Wimmer, T. Vennegeerts, et al., Glycochenodeoxycholate-induced apoptosis is not reduced by augmenter of liver regeneration in the human hepatoma cell line HuH-7, Digestion 82 (2010) 262–264.
[112] M. Fischer, S. Horn, A. Belkacemi, et al., Protein import and oxidative folding in the mitochondrial intermembrane space of intact mammalian cells, Mol. Biol. Cell 24 (2013) 2160–2170.
[113] R. Dayoub, L. Buerger, S. Ibrahim, et al., Augmenter of liver regeneration (ALR) exhibits a dual signaling impact on hepatic acute-phase response, Exp. Mol. Pathol. 102 (2017) 428–433.
[114] J. Kouhara, T. Yoshida, S. Nakata, et al., Fenretinide up-regulates DR5/TRAIL-R2 expression via the induction of the transcription factor CHOP and combined treatment with fenretinide and TRAIL induces synergistic apoptosis in colon cancer cell lines, Int. J. Oncol. 30 (2007) 679–687.
[115] H. Xu, H. Wang, N. Zhao, et al., Lipid deposition in liver cells: the influence of short form augmenter of liver regeneration, Clin. Res. Hepatol. Gastroenterol. 40 (2016) 186–194.
[116] X. Chen, Y. Li, K. Wei, et al., The potentiation role of hepatopoietin on activator protein-1 is dependent on its sulfhydryl oxidase activity, J. Biol. Chem. 278 (2003) 49022–49030.
[117] Y. Wang, C. Lu, H. Wei, et al., Hepatopoietin interacts directly with COP9 signalosome and regulates AP-1 activity, FEBS Lett. 572 (2004) 85–91.
[118] M. Leech, D. Lacey, J.R. Xue, et al., Regulation of p53 by macrophage migration inhibitory factor in inflammatory arthritis, Arthritis Rheum. 48 (2003) 1881–1889.
[119] T.G. Senkevich, C.L. White, E.V. Koonin, et al., A viral member of the ERV1/ALR protein family participates in a cytoplasmic pathway of disulfide bond formation [in process citation], Proc. Natl. Acad. Sci. U. S. A 97 (2000) 12068–12073.
[120] L. Shen, J. Hu, H. Lu, et al., The apoptosis-associated protein BNIPL interacts with two cell proliferation-related proteins, MIF and GFER, FEBS Lett. 540 (2003) 86–90.
[121] T. Pu, X.H. Liao, H. Sun, et al., Augmenter of liver regeneration regulates autophagy in renal ischemia-reperfusion injury via the AMPK/mTOR pathway, Apoptosis (2017).
[122] C. Thirunavukkarasu, L.F. Wang, S.A. Harvey, et al., Augmenter of liver regeneration: an important intracellular survival factor for hepatocytes, J. Hepatol. 48 (2008) 578–588.
[123] L. Tang, H. Sun, L. Zhang, et al., Effects of the augmenter of liver regeneration on the biological behavior of hepatocellular carcinoma, Saudi Med. J. 30 (2009) 1001–1009.
[124] H.Q. Zeng, Y. Luo, S.F. Lou, et al., Silencing of augmenter of liver regeneration inhibited cell proliferation and triggered apoptosis in U266 human multiple myeloma cells, Braz. J. Med. Biol. Res. 50 (2017) e6139.
[125] L.R. Todd, M.N. Damin, R. Gomathinayagam, et al., Growth factor erv1-like modulates Drp1 to preserve mitochondrial dynamics and function in mouse embryonic stem cells, Mol. Biol. Cell 21 (2010) 1225–1236.
[126] D.V. Dabir, S.A. Hasson, K. Setoguchi, et al., A small molecule inhibitor of redox-regulated protein translocation into mitochondria, Dev. Cell 25 (2013) 81–92.
[127] C.S. Russell, L.A. Clarke, Recombinant proteins for genetic disease, Clin. Genet. 55 (1999) 389–394.
[128] Y. Li, L. Zhang, Q. Liu, et al., Exogenous augmenter of liver regeneration (ALR) attenuates inflammatory response in renal hypoxia re-oxygenation injury, Ren. Fail. 36 (2014) 432–436.
[129] X.H. Liao, G.T. Chen, Y. Li, et al., Augmenter of liver regeneration attenuates tubular cell apoptosis in acute kidney injury in rats: the possible mechanisms, Ren. Fail. 34 (2012) 590–599.
[130] R. Yan, Y. Li, L. Zhang, et al., Augmenter of liver regeneration attenuates inflammation of renal ischemia/reperfusion injury through the NF-kappa B pathway in rats, Int. Urol. Nephrol. 47 (2015) 861–868.
[131] X.H. Liao, L. Zhang, G.T. Chen, et al., Augmenter of liver regeneration inhibits TGF-beta1-induced renal tubular epithelial-to-mesenchymal transition via suppressing TbetaR II expression in vitro, Exp. Cell Res. 327 (2014) 287–296.
[132] N. Wang, H. Sun, Y. Shen, et al., Augmenter of liver regeneration inhibits apoptosis of activated human peripheral blood lymphocytes in vitro, Immunopharmacol. Immunotoxicol. 35 (2013) 257–263.
[133] N. Wang, Z. Wang, H. Sun, et al., Augmenter of liver regeneration improves therapeutic effect of hepatocyte homotransplantation in acute liver failure rats, Int. Immunopharmacol. 15 (2013) 325–332.
[134] Y. Chen, S. Liang, F. Long, et al., Augmenter of liver regeneration attenuates acute rejection after rat liver transplantation, Am. J. Surg. 212 (2016) 128–137.
[135] F.W. Long, S.Y. Liang, Z.J. Liu, et al., Augmentor of liver regeneration ameliorates renal tubular epithelial cell injury after rat liver transplantation, Transplant. Proc. 40 (2008) 2696–2699.
[136] G.A. Adams, M. Maestri, E.C. Squiers, et al., Augmenter of liver regeneration enhances the success rate of fetal pancreas transplantation in rodents, Transplantation 65 (1998) 32–36.
[137] A. Francavilla, N.L. Vujanovic, L. Polimeno, et al., The in vivo effect of hepatotrophic factors augmenter of liver regeneration, hepatocyte growth factor, and insulin-like growth factor- II on liver natural killer cell functions, Hepatology 25 (1997) 411–415.
[138] K. Tanigawa, I. Sakaida, M. Masuhara, et al., Augmenter of liver regeneration (ALR) may promote liver regeneration by reducing natural killer (NK) cell activity in human liver diseases, J. Gastroenterol. 35 (2000) 112–119.
[139] C.R. Gandhi, N. Murase, T.E. Starzl, Cholera toxin-sensitive GTP-binding protein-coupled activation of augmenter of liver regeneration (ALR) receptor and its function in rat kupffer cells, J. Cell. Physiol. 222 (2010) 365–373.
[140] G. Wang, X. Yang, Y. Zhang, et al., Identification and characterization of receptor for mammalian hepatopoietin that is homologous to yeast ERV1, J. Biol. Chem. 274 (1999) 11469–11472.
[141] Y. Li, M. Li, G. Xing, et al., Stimulation of the mitogen-activated protein kinase cascade and tyrosine phosphorylation of the epidermal growth factor receptor by hepatopoietin, J. Biol. Chem. 275 (2000) 37443–37447.
[142] R. Dayoub, W.E. Thasler, A.K. Bosserhoff, et al., Regulation of polyamine synthesis in human hepatocytes by hepatotrophic factor augmenter of liver regeneration, Biochem. Biophys. Res. Commun. 345 (2006) 181–187.
[143] W.E. Thasler, R. Dayoub, M. Muhlbauer, et al., Repression of cytochrome P450 activity in human hepatocytes in vitro by a novel hepatotrophic factor, augmenter of liver regeneration, J. Pharmacol. Exp. Ther. 316 (2006) 822–829.
[144] M. Ilowski, C. Putz, T.S. Weiss, et al., Augmenter of liver regeneration causes different kinetics of ERK1/2 and Akt/PKB phosphorylation than EGF and induces hepatocyte proliferation in an EGF receptor independent and liver specific manner, Biochem. Biophys. Res. Commun. 394 (2010) 915–920.
[145] M. Ilowski, A. Kleespies, E.N. de Toni, et al., Augmenter of liver regeneration (ALR) protects human hepatocytes against apoptosis, Biochem. Biophys. Res. Commun. 404 (2011) 148–152.
[146] A. Khandoga, K. Mende, E. Iskandarov, et al., Augmenter of liver regeneration attenuates inflammatory response in the postischemic mouse liver in vivo, J. Surg. Res. 192 (2014) 187–194.
[147] C. Tang, H. Lin, Q. Wu, et al., Recombinant human augmenter of liver regeneration protects hepatocyte mitochondrial DNA in rats with obstructive jaundice, J. Surg. Res. 196 (2015) 90–101.
[148] Y. Shen, Q. Liu, H. Sun, et al., Protective effect of augmenter of liver regeneration on vincristine-induced cell death in Jurkat T leukemia cells, Int. Immunopharmacol. 17 (2013) 162–167.
[149] S. Rebouissou, J. Zucman-Rossi, R. Moreau, et al., Note of caution: contaminations of hepatocellular cell lines, J. Hepatol. 67 (2017) 896–897.
[150] L.M. Zhang, D.W. Liu, J.B. Liu, et al., Effect of naked eukaryotic expression plasmid encoding rat augmenter of liver regeneration on acute hepatic injury and hepatic failure in rats, World J. Gastroenterol. 11 (2005) 3680–3685.
[151] L. Polimeno, F. Capuano, L.C. Marangi, et al., The augmenter of liver regeneration induces mitochondrial gene expression in rat liver and enhances oxidative phosphorylation capacity of liver mitochondria, Dig. Liver Dis. 32 (2000) 510–517.
[152] L. Polimeno, M. Margiotta, L. Marangi, et al., Molecular mechanisms of augmenter of liver regeneration as immunoregulator: its effect on interferon-gamma expression in rat liver, Dig. Liver Dis. 32 (2000) 217–225.

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