

Insights on augmenter of liver regeneration cloning and function

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Abstract

Hepatic stimulator substance (HSS) has been referred to as a liver-specific but species non-specific growth factor. Gradient purification and sequence analysis of HSS protein indicated that it contained the augmenter of liver regeneration (ALR), also known as hepatopoietin (HPO). ALR, acting as a hepatotrophic growth factor, specifically stimulated proliferation of cultured hepatocytes as well as hepatoma cells *in vitro*, promoted liver regeneration and recovery of damaged hepatocytes and rescued acute hepatic failure *in vivo*. ALR belongs to the new Erv1/Alr protein family, members of which are found in lower and higher eukaryotes from yeast to man and even in some double-stranded DNA viruses. The present review article focuses on the molecular biology of ALR, examining the ALR gene and its expression from yeast to man and the biological function of ALR protein. ALR protein seems to be non-liver-specific as was previously believed, increasing the necessity to extend research on mammalian ALR protein in different tissues, organs and developmental stages in conditions of normal and abnormal cellular growth.

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Key words: Hepatic stimulator substance; Augmenter of liver regeneration; Liver regeneration; Molecular biology

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INTRODUCTION

In 1975, La Brecque and Pesch first referred to the existence of a polypeptide, named hepatic stimulator substance (HSS), in the liver cytosol of weanling or adult partially hepatectomized rats^[1]. HSS was able to specifically stimulate hepatocyte proliferation and support liver regeneration in an organ specific but species non-specific manner^[1-2]. Since then a number of studies examined HSS levels of toxicity on animal models of liver regeneration after partial hepatectomy and post induced liver injury *in vivo*^[3,4] and *in vitro*^[5]. Subsequently, the effect of this growth factor in acute^[6-11] and chronic^[12] liver injury and decreased hepatocyte proliferative capacity^[13,14] has been reported in different animal models. A novel growth factor, obtained through progressive purification (831 000x) of the crude HSS extract, was named Augmenter of Liver Regeneration (ALR)^[15-20]. ALR, as well as HSS, did not affect quiescent hepatocytes but enhanced the liver proliferative response to hepatectomy in rats^[1,16,21] and dogs^[22] and prevented atrophy increased cell renewal caused by portacaval shunt (Eck's fistula) in dogs^[23,24]. ALR recently has been the subject of intense investigation. Several different approaches have led to the identification of the respective genes from yeast, rat, mouse, and human. This review deals with the molecular biology of ALR, examining cloning of the ALR gene and its protein product from yeast to man. The biological function of this protein product is also described.

ALR GENE AND PROTEIN FROM YEAST TO MAN

Yeast scERV1

The yeast scERV1 gene was the first and best-characterized gene of a new family found in a large number of lower and higher eukaryotes^[25-28] and in the genome of some double-stranded DNA viruses^[29-31]. This gene encodes a small protein of 189 amino acids (22 ku) having a complex influence on different aspects of mitochondrial biogenesis^[32-34] and is essential for the survival of yeast^[28]. Further studies have shown that the protein (Erv1p) was essential for normal mitochondrial morphology and for the stable maintenance of these organelles. In addition, Erv1p was identified as the first FAD-linked sulfhydryl oxidase from yeast^[34]. The enzymatic activity was located at the 15 ku carboxy-terminal domain of the Erv1p with the conserved Cys-X-X-Cys sequence motif (CXXC motif). This frag-

ment was able to bind FAD and to catalyze the formation of disulfide bonds but was no longer able to form dimers like the complete protein^[35]. Last but not least, analysis of the complete genome sequence from *S. cerevisiae* revealed a second yeast gene with homologies and structural similarities to scERV1. This gene was termed scERV2. Its protein product (Erv2p) had a length of 196 amino acids and exhibited 30% identical amino acid residues with Erv1p in the highly conserved carboxy-terminal part of the polypeptides. However, the complex expression pattern and the high degree of variability found in the amino-terminal regions of the scERV1 and scERV2 proteins, as well as the differences found between them, indicated that the gene products might have diverse functions at different locations and critical times of cellular development^[30].

Rat ALR

Hagiya *et al* in 1994, first discovered and cloned ALR in rat and then in human and mouse, proposing also its identity with HSS or hepatopoietin (HPO)^[25]. They cloned and sequenced the rat ALR gene, which was considered to encode rat HSS^[25]. They also presented evidence that ALR protein (Alrp) was acting as a homodimer and reported that the expression of the 1.2 kb gene transcript was elevated in rat testis and liver. The 1.2 kb cDNA included a 299 bp 5' untranslated region, a 375 bp coding region and a 550 bp 3' untranslated one. The rat Alrp consisted of 125 amino acids and its molecular weight was calculated to be 15 ku, which is consistent with its electrophoretically determined molecular weight under reducing conditions. The molecular weight of the purified native Alrp was calculated to be 30 ku under nonreducing conditions. The 125 amino acid sequence deduced from the rat ALR cDNA presented as 50% homologous to the polypeptide encoded by the scERV1, which is essential for oxidative phosphorylation, vegetative growth and life of the yeast *S. cerevisiae*^[25]. The same investigators, in supplementary experiments, found a single nucleotide (G) insertion at cDNA position 266, which did not alter the results of recombinant Alrp of 125 amino acids that was originally identified. On the other hand, additional G engendered two other in-frame ATG initiation sites, which were 5' to the initiation site of the Alrp they had previously reported, thus raising the possibility of additional ALR variants^[36]. Furthermore, the cloning and sequence analysis of rat genomic DNA of ALR revealed that the gene consisted of 3 exons and 2 introns and was 1508 nt long^[37]. An ALR pseudogene was also found in the rat genome, implying the existence of an ALR multigene family. The length of the pseudogene was calculated to be 442 nt^[37]. The amino acid sequence homology between the rat ALR and its pseudogene proved to be 88.8%^[38]. The crystal structure of recombinant rat Alrp has been determined to 1.8 Å. The structure revealed a unique FAD binding motif, a region containing side chain rings in a stacked parallel orientation, as has recently been reported in Erv2p, and an extensive salt bridges network being unique to ALR. The abundance of disulfide and salt bridges provides the Alrp molecule with a rigid structure and probably accounts for Alrp's thermostability and resistance to some denaturants^[39].

Mouse ALR

The mouse ALR gene was cloned and characterized by Giorda *et al* in 1996^[26]. It was shown that the protein coding portion of the mouse ALR gene contained 3 exons. The first of them consisted of the 5' untranslated sequence and the initial 18 bases after the ATG translation initiation codon. The second exon contained 198 bases and the third one consisted of the remaining portion of the protein coding sequence. Moreover, the ALR gene mapped to mouse chromosome 17, in a region syntenic with human chromosome 16^[26].

Human ALR

Giorda *et al* also published the cDNA sequence of human ALR^[26]. At the same time, Yang *et al* cloned the cDNA of human HPO by functional screening of a fetal liver cDNA library demonstrating that human HPO was identical to human ALR^[40]. The cDNA of human ALR encoding 125 amino acids presented identity of 87% with rat ALR and of 42% to yeast scERV1^[40]. The human ALR gene was located on chromosome 16, at the cytogenetic band 16p13.3-p13.12, in the interval containing the locus for polycystic kidney disease (PKD1)^[27]. The cloning and sequence analysis of human ALR genomic DNA revealed that it consisted of 3 exons and 2 introns. It was 1813 nt long, coding a protein of 125 amino acid residues. A comparison of human and mouse genomic DNA demonstrated that the 3 exons were similar in length but different in their 5'-UTR, introns and 3'-UTR in length^[41,42]. Lu *et al* isolated a variant of ALR from hepatic-derived cells with a length of 205 amino acids, characterizing a novel isoform of HPO cDNA encoding a 205 amino acid open reading frame (ORF). This was named HPO-205 to distinguish it from the previously described HPO, which lacked the N-terminal 80 amino acids^[43].

Therefore, two forms of the human Alrp were found to be present in hepatocytes. The shorter protein consisted of 125 amino acids (15 ku) and lacked the amino terminus and the longer protein had 205 amino acids (23 ku). The 15 ku of Alrp existed only in the nucleus and the 23 ku Alrp was mainly located in the cytosol, probably because they are synthesized from the same mRNA using different initiation codons^[44]. Moreover, under normal conditions, the full-length 23 ku mammalian Alrp is predominant and localized in the mitochondrial intermembrane space^[45]. As a result, ALR could be dimerized at a protein level and its gene could be alternatively spliced at the transcriptional level. Both might contribute to the existence of various Alrp complexes in hepatocytes^[44].

Recent studies reported successfully cloning the genes encoding proteins that interact with Alrp, such as metallothionein and albumin, which might be related to the transportation process of Alrp in the circulation. Selenoprotein-P, an anti-oxidant protein, might also be related to Alrp function, as well as other ALR-binding proteins, such elongation factor 1- α , transitional endoplasmic reticulum ATPase, carboxypeptidase N 83 ku chain, complete factor H related 3, and NADP dehydrogenase, which are known to play important roles in energy metabolism^[46].

Table 1 Augmenter of liver regeneration from yeast to man

	Homo Sapiens (Human)	Mus Musculus (Mouse)	Rattus Norvegicus (Rat)	Saccharomyces cerevisiae (Yeast)
Description	Augmenter of liver regeneration (ALR)/growth factor, erv1 (<i>S. cerevisiae</i>)-like (augmenter of liver regeneration) (GFER)/hepatic regenerative stimulation substance (HSS)/hepatopoietin (HPO)	Augmenter of liver regeneration (Alr)/growth factor, erv1 (<i>S. cerevisiae</i>)-like (augmenter of liver regeneration) (Gfer)	Augmenter of liver regeneration (ALR)/growth factor, erv1 (<i>S. cerevisiae</i>)-like (augmenter of liver regeneration) (Gfer)	<i>Saccharomyces cerevisiae</i> ERV1 (scERV1)/flavin-linked sulfhydryl oxidase localized to the mitochondrial intermembrane space, has a role in the maturation of cytosolic iron-sulphur proteins; ortholog of human hepatopoietin (ALR)
Genomic Location	Chromosome: 16 Cytogenetic: 16p13.3-p13.12	Chromosome: 17 Cytogenetic: 17 A3.3	Chromosome: 10 Cytogenetic: 10q12 Genetic: 17 10.0 cM	Chromosome: VII
Peptide Length	205 aa (23 ku) 125 aa (15 ku)	198 aa	125 aa (15 ku)	189 aa (22 ku)
Protein Function	In liver regeneration and spermatogenesis	In liver regeneration and spermatogenesis	In liver regeneration and spermatogenesis	Essential for the biogenesis of mitochondria, cell viability and for vegetative growth
Protein Subunit	Homodimer/Heterodimer	Homodimer	Homodimer	Homodimer
Protein Similarity	Belongs to ERV1/ALR family	Belongs to ERV1/ALR family	Belongs to ERV1/ALR family	Belongs to ERV1/ALR family
Percentage of similarity to human		85.6% [The comparison with human is based on nucleic acid (GeneCard for GFER)] 87.20% [The comparison with human is based on amino acid sequence (UniGene)]	85.87% [The comparison with human is based on nucleic acid (GeneCard for GFER)] 85.60% [The comparison with human is based on amino acid sequence (UniGene)]	52.85% [The comparison with human is based on nucleic acid (GeneCard for GFER)] 46.85% [The comparison with human is based on amino acid sequence (UniGene)]
Subcellular Location	Nucleus (15 ku) Cytosol (23 ku) Mitochondrial intermembrane space (23 ku)	Mitochondrial intermembrane space	Cytosol Mitochondrial intermembrane space	Cytosol Mitochondrial intermembrane space

The ALR gene promoter has also been studied and might represent a novel architecture for core promoters. It was TATA-less and spanned position -54 to +42 relative to the transcriptional start point. Specifically, it did not contain the TATA box, a consensus A/T rich sequence [TATA (A/T) A (A/T)], which was located -25-30 nucleotides upstream of the transcriptional start site and was recognized by the TATA binding protein subunit of TFIID. TFIID is a transcription factor that was needed to bring about the initiation of transcription by RNA polymerase II and nucleated the formation of the pre-initiation complex. In addition, the core promoter of ALR consisted of a functional initiator (Inr) and three CTGGAGGC tandem repeat elements, which were found surrounding the Inr, with all of them participating in Inr-dependent transcription. The Inr contains a pyrimidine (Y) rich core sequence [YYA+1N (T/A) YY] encompassing the transcription site. The initiator flanking element, lying either upstream or downstream from the Inr, was found to be present in many Inr-containing genes. Both the initiator and at least one of the repeats were able to bind to specific nuclear factors^[47].

The similarities and differences between the structure and function of the ALR gene and its protein product in the four most important and closely related species (yeast, mouse, rat and human) are displayed in Table 1.

BIOLOGICAL FUNCTION OF ALRP

Characterization of human *Alrp* as a structural and functional homologue for yeast *Erv1p*

Alrp and *Erv1p* belong to a protein family, members of which are found in lower and higher eukaryotes from yeast to man^[25-28] and even on the genome of some double-stranded DNA viruses^[29-31]. They have essential functions in the biogenesis of mitochondria, the cell division cycle, and in the development of organs such as liver and testis in higher eukaryotes^[48]. The carboxy terminus of the human *Alrp* was characterized as a structural and functional homologue for yeast *Erv1p*^[27]. Especially, Lisowsky *et al* found four major similarities at the amino acid residues of carboxy termini of *Erv1p* and human *Alrp*, concluding a conservation of structure and function^[27]. However, Hofhaus *et al* showed that the conserved carboxy terminus of mammalian and yeast proteins were functionally interchangeable between distantly related species such as yeast and man^[49]. On the other hand, the amino terminal parts of the proteins displayed a high degree of variability and significant differences even among closely related species^[49]. Specifically, Hofhaus *et al* first revealed that the yeast protein contained a leader sequence for mitochondrial localization whereas the human protein is not detectable inside the mitochondria. Moreover, they noted that, in yeast only, one form of scERV1 was found

whereas in human cells two protein isoforms were present. Therefore, these two significant differences in the amino termini between Erv1p and human Alrp suggested that the human Alrp did not appear to be functional in yeast^[49].

The enzymatic activity of Alrp as sulfhydryl oxidase

The Alrp/Erv1p family belongs to flavin-linked sulfhydryl oxidase participating in disulfide bond formation^[35,50]. Lisowsky *et al* identified the enzymatic activity of rat and human Alrp as sulfhydryl oxidases by their ability to oxidize thiol groups in a protein substrate, the presence of FAD moiety in the carboxy terminal domain and the formation of dimers *in vivo*^[50]. In general, the sulfhydryl oxidase proteins contain a conserved CXXC motif in the carboxy terminal domain and a non-covalent FAD, which are vital for their catalytic activity^[51-53]. The conserved CXXC motif in the carboxy terminus most likely represents the redox-active site of the enzyme, sharing significant homologies with human growth regulator quiescin Q6^[52] and yeast Erv1p^[35]. It was also reported that the two vicinal cysteines directly interact with bound FAD for the redox reaction. Furthermore, sulfhydryl oxidases form dimers *in vivo* and catalyze the formation of disulfide bonds with the reduction of molecular oxygen to hydrogen peroxide according to the following reaction: $2RS-H + O_2 \rightarrow RSSR + H_2O_2$ ^[51-53].

An interesting new aspect for mitochondrial sulfhydryl oxidase activity and the formation of disulfide bonds was the recent finding of a direct correlation between the electron transport chain and the formation of disulfide bonds in *E. coli*^[54,55]. A very recent study on the fundamental behavior of the short form of the human Alrp suggested that this flavoenzyme might not necessarily function as sulfhydryl oxidase in the mitochondrial intermembrane space but might be coupled with the respiration chain *via* mediation of cytochrome *c* and without the generation of hydrogen peroxide observed in oxidase reactions. Thus, cytochrome *c* should be considered as a potential oxidant for ALR *in vivo*^[56].

The presence of a specific receptor for Alrp and modes of Alrp signaling

Wang *et al* showed the existence of an Alrp receptor on rat hepatocytes and human hepatoma cells with a molecular weight of about 75 ku, presenting with high affinity and specificity, reversibility and saturation^[57]. Alrp binds the receptor in cell membranes initiating a corresponding signal transduction pathway and mediating its biological function on hepatocytes. This function includes specific phosphorylation and dephosphorylation of important proteins in the process of liver regeneration^[57].

Yang *et al* showed that recombinant human HPO/Alrp (125 amino acids, 15 ku) could stimulate proliferation of hepatocytes as well as hepatoma cells *in vitro*, promoting regeneration and recovery of damaged hepatocytes and ameliorating acute hepatic failure *in vivo*. Such observations support the contention that Alrp is a hepatotropic growth factor^[58,59].

It was shown that HPO/Alrp was able to stimulate hepatocyte proliferation by two signaling pathways. In the

first pathway, extracellular HPO autocrined from hepatocytes and hepatoma cells activated the mitogen-activated protein kinase (MAPK) signaling pathway *via* binding to an ALR receptor. Specifically, Alrp triggered MAPK activation and proliferation in hepatoma cell lines through the induction of tyrosine phosphorylation of epidermal growth factor receptor (EGFR)^[60].

In the second signaling pathway, intracellular Alrp interacted with Jun activation domain-binding protein 1 (JAB1), triggering the activating protein-1 (AP-1) transcriptional activity in a MAPK independent manner, for immediate early response when its intracellular levels were increased with post-partial hepatectomy or liver injury^[61]. JAB1 is the fifth subunit of COP9 signalosome (CSN), being a co-activator of c-Jun/AP-1 transcription factor, which enhances the binding capacity of c-Jun-containing AP-1 complex to their DNA consensus sites and increasing the transactivation of an AP-1 dependent promoter. It was shown that Alrp enhanced the increased phosphorylation level of c-Jun through JAB1 but had no effect on the expression of transfected c-Jun or endogenous c-Jun N-terminal kinase or on phosphorylation of c-Jun N-terminal kinase^[61]. Furthermore, recent studies elucidated a novel relationship of intracellular Alrp with the whole protein complex CSN, suggesting a possible linkage between CSN and liver regeneration^[62]. As a consequence, the CSN complex could represent an intracellular signal platform, where signals from the extracellular or intracellular environment are coordinated with transcriptional activation and with the regulation of the related functions of cells.

Chen *et al* presented a molecular link between the enzymatic redox function of HPO/Alrp and its role as a cytokine^[63]. They displayed the necessity of cysteine residues in the CXXC catalytic centre for the intracellular potentiation of AP-1 activity. As mentioned before, Alrp interacted with JAB1 to trigger AP-1 transcription activity by phosphorylation of c-Jun in a MAPK independent fashion. This effect depended on the integrity of the CXXC enzyme active site, which could provide a novel intracellular signaling pathway shortcut by redox regulation. This implied that the enzymatic activity of Alrp might be a key regulator in the intracellular mediation of the AP-1 pathway through JAB1. On the other hand, they provided evidence that the extracellular cytokine activity of Alrp did not associate with the redox CXXC motif and consequently with its sulfhydryl oxidase activity^[63].

Recently, Li *et al* demonstrated that ALR and macrophage inhibitory factor (MIF) could be considered as a pair in the course of signaling transduction^[64]. Their results showed an interaction between ALR and MIF and led to the alteration of their effects on AP-1 activity. Especially, on the one hand, the binding of MIF to JAB1 was inhibited by ALR and on the other hand the potentiation of the ALR on AP-1 activity through JAB1 was inhibited by MIF. Thus, the activity of AP-1 may be determined by the coordination and balance between ALR and MIF concentration *in vivo*. This balance is crucial for the maintenance of both homeostasis and normal development of cells and tissues. In this sense, ALR serves as physiological counter-regulatory mediator that counteracts the suppressive effect of MIF^[64].

Alrp biological activity

Previous studies have demonstrated that different forms of Alrp may be associated with different localizations and cellular functions^[26,49,65]. Specifically, a 15 ku Alrp fragment appeared to regulate mitochondrial gene expression by inducing the transcription and translation of the nuclear-encoded mitochondrial transcription factor A (mtTFA)^[66]. Polimeno *et al* found that Alrp administration induced a significant increase in mitochondrial gene expression and of nuclear-encoded mtTFA. This increase was associated with enhanced cytochrome content and oxidative phosphorylation capacity of rat liver-derived mitochondria^[66]. It has also been suggested that hepatotrophic factors, such as Alrp, play an important role in liver regeneration and might mediate their activities by regional regulation of natural killer (NK) cells. However, NK cells in liver appeared to have specific cytotoxicity against regenerating hepatocytes. A recent study revealed that *in vivo* administration of Alrp induced inhibition of hepatic NK cytotoxic activities in the population of mononuclear leukocytes infiltrating rat liver but not in those derived from spleen or peripheral blood. These data suggested that Alrp might act as an immunosuppressant agent localized to the liver^[67]. Moreover, Tanigawa *et al* suggested that Alrp production in the liver was enhanced during acute liver disease, especially in the initial stage^[68]. It was also suggested that Alrp might protect against failure of regeneration by inhibiting hepatic NK cell activity in acute liver disease^[68]. It was also demonstrated that Alrp acted as an immunoregulator by controlling, through interferon-gamma (IFN- γ) levels, the mtTFA expression and lytic activity of liver-resident NK cells. The exogenous administration Alrp in intact rats induced a reduction of IFN- γ in liver-resident NK cells, while the administration of IFN- γ in 70% of partially hepatectomized rats was followed by a significant reduction of both mtTFA expression and liver regeneration. These results demonstrated a direct link between Alrp and IFN- γ , implying IFN- γ as the main mediator of Alrp biological activity both as growth factor and immunoregulator^[69]. Recent studies discussed a constructed yeast expressive vector of ALR and this was expressed in yeast cells in order to further study the biological function and mechanism of recombinant human ALR as a regulatory factor that specifically stimulates hepatic cell regeneration. This successful expression of ALR in yeast cells may pave the way for the study of clinical use and provided a good tool for further research in this field. Their results also suggested that ALR enhanced liver regeneration not only through an indirect pathway; i.e. immune regulation, but also through a direct pathway^[70-71].

Recently, an ALR recombinant plasmid was constructed and tested for therapeutic effects on rat hepatic fibrosis. Specifically, the results of this study revealed that the ALR recombinant plasmid could decrease the degrading capacity of collagen types I and III, which were regarded as important parameters reflecting the metabolism of collagen. The ALR recombinant plasmid could also decrease the deposition of the extracellular matrix and the expression of tissue inhibitors of metalloproteinase-1 (TIMP-1) in pathological liver tissue and thus reverse the hepatic fibrosis induced by porcine serum administration^[72].

In addition, the full-length mammalian Alrp (23 ku) was largely located in the mitochondrial intermembrane space and performed the export of iron/sulfur (Fe/S) clusters from the mitochondrial matrix, contributing to the biogenesis of cytosolic Fe/S proteins and to cellular iron homeostasis. Especially, Fe/S cluster components of the mitochondrial matrix preassembled and packaged Fe/S clusters, which could then be transported into the cytosol, a process possibly involving the ABC transporter Atm1p/ABC7^[45]. Cytosolic and nuclear Fe/S proteins have important regulatory functions as enzymes or transcription factors. Therefore, participation in the assembly of cytosolic Fe/S proteins appears to be the primary essential task of mitochondrial full-length Alrp^[45]. This function is crucial for all eukaryotic cell types, whereas the proposed role of 15 ku Alrp as a hepatotrophic factor is restricted to liver cells and it may be effective only after liver damage. This indicates that Alrp, like other redox-active proteins and sulfhydryl oxidases, may have diverse functions in the regulation of cell growth and differentiation^[20,48,57,60,65].

Klissenbauer *et al* first characterized full-length Alrp as intratesticular sulfhydryl oxidase, a new enzyme with expression regulated during spermatogenesis^[73]. Mitochondrial localization of full-length Alrp was of special interest for spermatogenesis because it was known that morphological and functional changes in mitochondria were associated with this highly complex cytodifferentiation process. They also observed the greatest amounts of this protein in spermatogonia and early spermatocytes. Expression levels of Alrp did not correlate with synthesis of components of the respiratory chain, indicating that the full-length Alrp in the mitochondria of spermatogonia and spermatocytes had another possible function in addition to its role in oxidative phosphorylation during sperm cell differentiation^[73].

CONCLUSION

ALR is novel growth factor, related to HSS, participating in the regulation of liver regeneration. With the availability and description of the ALR gene and gene product, a range of questions could be addressed about the cellular mechanisms in which this growth factor participates. At the molecular level, cloning of human ALR cDNA has been completed, but transcription and post-transcriptional regulation, based on the genomic structure of ALR, remains unclear. Transcription and post-transcriptional regulation are among the most important steps in the regulation of human gene expression. Therefore, it is important and essential to investigate the structure of human ALR genomic DNA. Contrary to previous belief, variable RNA expression in nonhepatic tissues suggests that ALR is not restricted to liver tissue, as was also suggested for HSS, but could be expressed in other cell types under appropriate circumstances. It is known that Alrp acts as a FAD-linked sulfhydryl oxidase belonging to the new Erv1p/Alrp family and is the only intratesticular sulfhydryl oxidase detected in the mitochondrial intermembrane space. Possible functions of sulfhydryl oxidases and other redox-active proteins in the regulation of cell growth, differentiation, changes in mitochondrial and cellular morphology, and in the formation of the

extracellular matrix have already been proposed. This indicates that Alrp, like other redox-active proteins and sulfhydryl oxidases, might have diverse functions. Additionally, limited data are available on the secreted Alrp bound to a specific receptor on the hepatocyte membrane and initiation of a signal transduction pathway and mediation of its biological effect on hepatocytes. Further studies should examine the distribution of human Alrp receptors on hepatocytes and on other cell types. The secretory pathway should also be further investigated because Alrp does not contain a typical signal peptide sequence evident of its primary structure. Moreover, Alrp production, expression regulation, secretion-transportation and importance, and also its genetic function, need to be further delineated. As a consequence, there is a necessity to extend studies on mammalian Alrp to different tissues, organs and developmental processes in conditions of normal and abnormal cellular growth. It is clear that the multiple functions of Alrp are biologically and physiologically complicated and need further study.

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