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Expression of isgylation related genes in regenerating rat liver

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Our recent studies have revealed the early up-regulated expression of interferon alpha (IFNα) in the liver, induced by partial hepatectomy. The role of this cytokine of innate immune response in liver regeneration is still controversial. **Aim.** To analyze expression of canonical interferon-stimulated genes *Ube1l*, *Ube2l6*, *Trim25, Usp18* and *Isg15* during the liver transition from quiescence to proliferation induced by partial hepatectomy, and acute phase response induced by laparotomy. These genes are responsible for posttranslational modification of proteins by ISGylation. The expression of genes encoding TATA binding protein (TBP) and 18S rRNA served as indirect general markers of transcriptional and translational activities. **Methods.** The abundance of investigated RNAs was assessed in total liver RNA by real time RT–qPCR. **Results.** Partial hepatecomy induced steady upregulation of the *Tbp* and *18S rRNA* genes expression during 12 hours post-surgery and downregulation or no change in expression of ISGylation-related genes during the first 3 hours followed by slight upregulation at 12 hours. The level of *Isg15* transcripts was permanently below that of the control during the prereplicative period. Laparotomy induced a continuous downregulation of *Tbp* and *18S rRNA* expression and early (1–3h) upregulation of ISGylation–related transcripts followed by a sharp drop at 6 hours and slight increase/decrease at 12 hours. The changes in the abundance of *Ifnα* and ISGylation-related mRNAs were oppositely directed at each stage of the response to partial hepatectomy and laparotomy. **Conclusion.** We suggest that the expression of ISGylation-related genes does not depend on the expression of *Ifnα* gene after both surgeries. The indirect indices of transcription and translation as well as the expression of ISGylation-relaled genes are principally different in response to partial hepatectomy and laparotomy and argue for the high specificity of innate immune response.

Keywords: Interferon α, ISGylation, liver regeneration, acute phase response

Introduction

Extensive damage of liver parenchyma induced by partial hepatectomy causes an innate immune response manifested by complement activation, cytokine production, expansion of natural killer cells [1–10], activated signaling from several toll-like receptors, and inhibitory signaling from signal transducer and activator factor (STAT) 3 preventing liver failure [11]. These processes direct quiescent liver cells to partially synchronized proliferation with eventual restoration of liver mass and function [3, 4, 12–14].

One of the active players in innate immunity is interferon α (IFNα). Its expression is upregulated during the early hours following partial hepatectomy

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in concert with specific intracellular signaling and typical target gene encoding e.g. protein kinase R [15–18]. The potential role of IFNα in liver restoration is still contentious [19, 20].

The present research is focused on the question whether, and in what manner, IFNα impacts the process of liver restoration. IFNα exerts its functions via regulation of gene expression predominantly at the transcriptional level through several pathways, among which the JAK-STAT is a primary signal transduction pathway. Binding of IFNα to its cognate receptors results in the activation of ISGF3 transcription factor (interferon stimulated gene factor 3) which specifically binds interferon-stimulated response element (ISRE) in large collection of genes [21].

In this study we address the expression of typical target genes of IFNα that are engaged in the process of ISGylation, a post-translational modification of proteins via transient attachment of small tag protein, a product of interferon stimulated gene 15 (*ISG15*) [22, 23]. ISG15, a 17 kDa member of the ubiquitin-like protein group, shows significant sequence homology with ubiquitin (Ub). The mechanism of ISGylation resembles that of ubiquitination and both modifications go through three sequential reactions operated by: activation, or E1 enzyme first forming an ATP-dependent Ub/ISG15-adenylate; conjugation, or E2 enzymes that transfer activated Ub/ISG15 to their active site cysteine; E3 ligases which transfer Ub/ISG15 from E2 to target protein forming isopeptide bond between, in most cases, carboxyl group of terminal glycine and *e*-amino groups of lysine residues of target proteins. Protein modification by ISG15 is a reversible process [22–24].

For ISGylation, only one specific E1-like ubiquitin – activating enzyme (Ube1) and three nonspecific E2 enzymes, Ube2L6 (aka UbcH8), Ube2E1 (aka UbcH6) and Ube2E2, are known. Unlike Ube1, the conjugating enzymes may also bind Ub, meaning both ISG15 and Ub conjugating pathways converge at the level of E2 enzyme [25–27]. However, the affinity of Ube2L6 with ISG15-UBE1L is much greater than the affinity with Ub-E1 and the amount of the thioester intermediate formed by Ube2E1 is significantly lower than that which is formed by Ube2L6. Thus, Ube2L6 is considered as a major E2 enzyme for ISG15 [28]. E3 ligase activities, which provide substrate specificity for the entire process of ISGylation, at the present moment, are represented in rats only by TRIM25 [29]. The deISGylation enzyme isopeptidase Usp18 (aka Ubp43) preferentially removes ISG15 from its conjugates [30].

To get insight into the potential role of IFNα and posttranslational modifications by ISG15 in liver restoration we evaluate the abundance of *Ifnα* and *Isg15* mRNAs along with mRNAs encoding Ube1L, Ube2L6, Trim25 and Usp18 enzymes in the liver after partial hepatectomy and laparotomy. The abundance of mRNAs encoding TATA-binding proteins (Tbp) and 18S RNA serves as indirect, general markers of transcriptional and translational processes.

Materials and Methods

Surgical Procedures

Male Wistar rats (200–250g) were used in the study. Operations of partial hepatectomy and laparotomy were performed according to the standard procedures under ether anesthesia [31]. Three animals were used for each post-surgery time after partial hepatectomy and laparotomy, 24 animals in total. Two resected liver lobes were used as individual controls for the remnant lobes investigated at specific times after partial hepatectomy and as integrated control samples for the liver after laparotomy. After time intervals of 1, 3, 6, and 12 hours following partial hepatectomy and laparotomy, the liver was *in situ* perfused with ice-cold physiological solution, removed and stored at – 80 °C until use. The Ukrainian law "On the Protection of Animals from Cruelty" was strictly implemented.

RNA isolation

The total RNA was isolated from approximately 100 mg of liver tissue that was ground in liquid nitrogen with mortar and pestle. Homemade TRIzol (for receipt see Supplement), 1.0 ml, was added and the mixture was passed through 22 G needle five

times. All sequential procedures followed the protocol for RNA isolation with TRIzol® Reagent from manufacturer. The isolated RNA was dissolved in sterile DEPC-treated water, aliquoted and stored at – 80 °C. Before the reverse transcription, RNA was subjected to DNAse treatment according to the standard procedure [32] and its concentration was measured on a NanoDrop ND-2000 device (Thermo Scietific, USA). The purity of RNA was assessed by the standard OD ratios (A260/A280 and A260/A230 ranging from 1.8– 2.2) and its integrity was assessed according to the 28S/18S ribosomal RNA ratio (~ 2.0) determined by denaturing formaldehyde agarose electrophoresis. The DNA contamination was controlled via qPCR with primers to 18S rDNA without preceding cDNA synthesis. PCR products were visualized in 2% agarose gel electrophoresis.

Synthesis of RNA Spike

To absolutely quantify the abundance of investigated RNAs and to maximally diminish the role of potential variability of all procedures preceding qPCR, the exogenous RNA spike was synthesized. For this purpose, the fragment of firefly luciferase gene (*Luc*), irrelevant to the rat genome, was cut out from the pGL3-Basic plasmid (Promega, USA) by XbaI and HindIII restrictases and cloned into the same restriction sites of the pGEM-3Z vector (Promega, USA) according to routine procedures. The obtained product was referred to as pGEM-3ZLuc and was subjected to *in vitro* transcription. The final mix of 100 µl contained 1 µg of linearized by EcoRI pGEM-3ZLuc DNA, 2 mM NTP, 10 µl of 10x IVT Buffer, 50 U of RiboLock™ RNAse inhibitor, 30 U of SP6 polymerase (Thermo Scientific, USA). The reaction lasted for 2 hours at 37 °C and was followed by subsequent phenol/chlorophorm extraction of RNA and ethanol – 0.3 M NaAc precipitation. The integrity of spike RNA was confirmed by electrophoresis in 1 % agarose gel.

Reverse transcription

First-strand cDNA synthesis was performed in a total volume of 20 µl containing 5 µg of total RNA, 10 pg of spike RNA Luc, 100 pmol of random hexamer primers, 20 U of RiboLock™ RNase inhibitor, 1 mM dNTP mix, 10 mM each and 200 U of MMuLV reverse transcriptase. Random primers, RNAse inhibitor and reverse transcriptase were obtained from Thermo Fisher Scientific, USA. After inactivation of the enzyme by heating for 10 min at 70 °C the reaction mix was diluted with sterile DEPC-treated water to the concentration 33 ng/µl, aliquoted and stored at – 80 °C.

The qPCR with SYBR green I

The primers for qPCR were designed with Vector *NTI AdvanceTM 9.0* and their specificity was checked with Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers were produced by Syntol*TM* (Russia, Moscow) and at the IMBG (Kyiv, Ukraine) (Table 1). For the absolute quantitation of mRNA abundance the amplicons for each type of transcript were obtained. The PCR reaction, in total volume of 50 µl, contained 120 ng of cDNA in 4 µl, 20 pmoles of each primer, 160 nM dNTP, 2 mM MgCl2, 2.5 U Hot Start Taq polymerase (Syntol, Russia) and 5.0 µl 10x Buffer (Syntol, Russia). Amplification was performed by a 2- step PCR and consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 15 sec, annealing with simultaneous elongation at 60 °C for 30 sec, and final elongation at 72 °C for 4 min. Amplicons were purified by a Genejet Purification kit (ThermoScientific, USA). The identity of amplicons was controlled by restriction analysis (Table 1). The dilutions of amplicons for the standard curves covered the range of 0.001–10.0 amoles per 25 µl of PCR reaction volume.

The estimation of individual RNA abundance was performed in triplicate with PCR in real time on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories Ltd., USA). The 25 µl of PCR mixture contained 66 ng of cDNA, 10 pmoles of each primer, 160 nM dNTP, 2 mM MgCl2, 1.25 U of Hot Start Taq polymerase (Syntol, Russia) and 2.5 µl of 10x Buffer (50 mM KCl, Tris-HCl pH 8,8) with SYBR Green I (Syntol, Russia). To reduce the dimerization of primers for *Isg15* amplification 5 µl of 5x Q-Solution (QIAGEN Inc., Valencia, USA) were

Table 1. **Characteristics of primers**

mRNA (Refseq)	Primers	Amplicon location, bp	Restriction analysis of amplicon	
			Enzyme	Restricts, bp
Tbp (NM_001004198.1)	F 5'- TCAGTCCAATGATGCCTTACG - 3' R 5'- CTGCTGCTGCTGTCTTTGTT - 3'	348–448	*Hpy 1881*	50; 51
18 S (NR_046237.1)	F 5'- GTTCCGACCATAAACGATGC-3' R5'- CGCTCCACCAACTAAGAACG -3'	1078–1341	*Hinf*I	175; 67; 44
*Ifnα**	F 5' - CTGCTGTCTAGGATGTGACCTGC -3' R 5' - TTGAGCCTTCTGGATCTGCTG - 3'	57–225	*HinfI*	81; 46; 42
Isg15 (NM_001106700.1)	F 5′ - CCTCTGAGCATCCTGGTGAG- 3' R5′ - CAGTGGCTCTTT GTCCTCCA - 3'	376–546	*PvuII*	58; 113
Ube1l (NM_001106856)	F 5′ - GGGCCTGGGAGTTAGGGATAATGG- 3'′ R 5′ - CGTCCACCCTGGAGAAGAAGTCGT - 3'	1492–1730	*Ear I*	90; 149
Ube2L6 (NM_001024755.1)	F 5′ -ACCAACTTCCCTATCGCCTCAAGG- 3' R 5′ - GAGGTCAGCTAGTTCCAAACGCACA- 3'	591–850	*Bgl II*	95; 165
Trim25 (NM_001009536.1)	F 5′ - CGCAAATGTTCCAGGCACAACC- 3' R 5′ -CATCCTCCAGTGCTTTGCTCGCT - 3'	521–725	*Rsal*	10; 195
Usp18 (NM_001014058.1)	F 5′ - ATACAACGTGC CATTGTTTGTCC- 3' R 5′ - TCGGTCCAGATTGT GAACAGATC- 3'	496–627	*EarI*	48; 84
Luc (*In vitro* transcript from PGEM-3z-Luc)	F 5'- AACCCTATTCTCCTTCTTCG - 3' R 5'- ATATCCTTGCCTGATACCTG - 3'	867–1020		

Note: For *Ifnα* indicated with an asterisk (*), the primers are common for *Ifnα1*, NM_001014786.1; I*fnα2*, NM_001271218.1; *Ifnα4*, NM_001106667.1 and *Ifnα16l*, XM_575856.1.

added to the mix. On each plate there were the samples from either partial hepatectomy or laparotomy together with the set of corresponding diluted amplicons for standard curve and 'No Template' controls. For the amplification profile, see above. After the PCR procedure, the melting of amplicon was performed with increments of 0.5 °C over a temperature range of 50 °C – 95 °C with an incubation interval of 5 sec at each step. Results of RT-qPCR were processed with a *CFX Manager*™ program software (Bio-Rad, USA) using the formula

$$\log A_0 = (C_q - Y)/S,$$

where: $\log A_0$ – logarithm of the initial amount of mRNA of interest, C_q – quantification cycle, Y – the value at the intersection of standard curve with Y axis, S – the slope of standard curve. For the example of standard curve see Fig. S1. The units of the calculated amount are the same as the units used to construct the standard curve, the attomoles per the volume of PCR reaction in our case. We transferred the obtained units to copies of target using Avogadro constant.

The data were normalized according to Luc recovery, acquired at no less than 85 %. The 100 % value corresponds to the copies of amplified *Luc* spike *per se*. The results are represented in the absolute amount of RNA copies per 1 ng of total RNA and in the ratio of abundance at definite postsurgery time to control abundance (fold change), as Mean ± SEM.

Results and Discussion

In this work we investigated the abundance of mRNAs, encoding the tag-protein ISG15 and the enzymes responsible for its transient protein attachment and detachment in the scope of general indirect characteristics of transcriptional and translational processes. The intact rat liver, the regenerating liver after 2/3 partial hepatectomy, and the liver after laparotomy were used in the experiment. The first 12 hours post-surgery correspond to the transition and prereplicative periods in the regeneration of the liver and include the liver specific, proliferation-independent acute phase response following laparotomy [3, 4, 33, 34]. The acute phase

response is initiated at the site of trauma, in this case in the areas of the laparotomy and hepatectomy, and acquires the systemic character of the location. The liver supplies the necessary components to confine local tissue destruction, clear the site of harmful agents, and aid the tissue repair [35, 36]. An acute phase response is thus an intrinsic component of the response to liver injury induced by partial hepatectomy and to laparotomy. The rate of involvement of both types of the aforementioned reactions is under strict regulation subjected to the existing requirements of the organism.

Abundance varies quite significantly within the set of investigated RNAs in the intact liver. 18S RNA is most numerous whereas other RNAs of interest, *Usp18, Trim25, Isg15, UbeH8, Tbp, Ube1L* and *Ifnα*, are present within the intact liver in descending magnitude with respect to the order of the present list (Table 2).

Table 2. **Abundance of individual RNAs in total RNA from intact liver**

Type of RNA	Copies/ng of total RNA, Mean ± SEM
Tbp	443.2 ± 41.20
18S	2 023399.7 ± 153101.78
Ifnα	19.7 ± 3.29
Isg15	1493.0 ± 330.12
Ube1L	212.8 ± 27.50
Ube2L6	1107.4 ± 117,15
Trim25	2190.0 ± 166.9
Usp18	2670.8 ± 538.13

The abundance of Tbp mRNA and 18S rRNA indirectly affects the rate of transcription and translation

TBP (TATA-binding protein), together with RNA polymerase II and general transcription factors, forms a pre-initiation complex following the reaction with the TATA box in the core promoter. A time-dependent profile of *Tbp* mRNA after partial hepatectomy reveals steady upregulation, while, following laparotomy, it is continuously downregulated (Fig. 1; Table S1). The TATA box is present in approximately 20–30 % of protein-encoding genes, particularly present in genes responsible for stress and inflammation, nucleosome and chromatin assembly, and cell proliferation and development [37–40]. So the activated and restricted transcription of corresponding genes by increased and decreased concentration of TBP may be expected after partial hepatectomy and laparotomy, respectively.

18S rRNA is a component of small ribosomal subunits and changes in the amount of cellular rRNA are associated with variation in the rate of ribosome accumulation. The profiles of 18S rRNA after both of

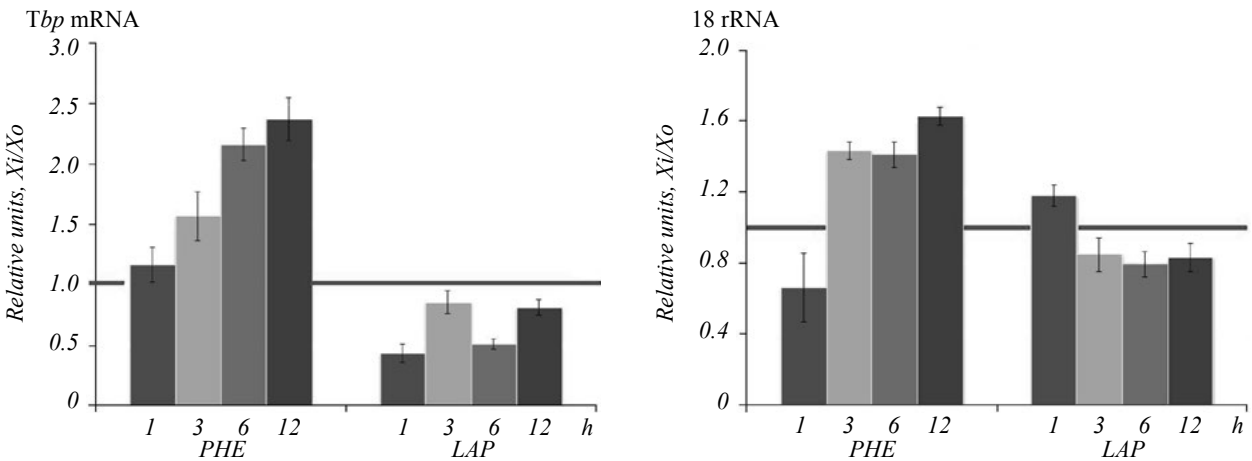

Fig.1. Abundance of *Tbp* mRNA and *18S* RNA in total liver RNA after partial hepatectomy (PHE) and after laparatomy (LAP)
Note: x_i and x_0 are the values of mRNA and rRNA abundances in the liver at the indicated post-surgery time and in control liver, correspondingly. The line marks the control level

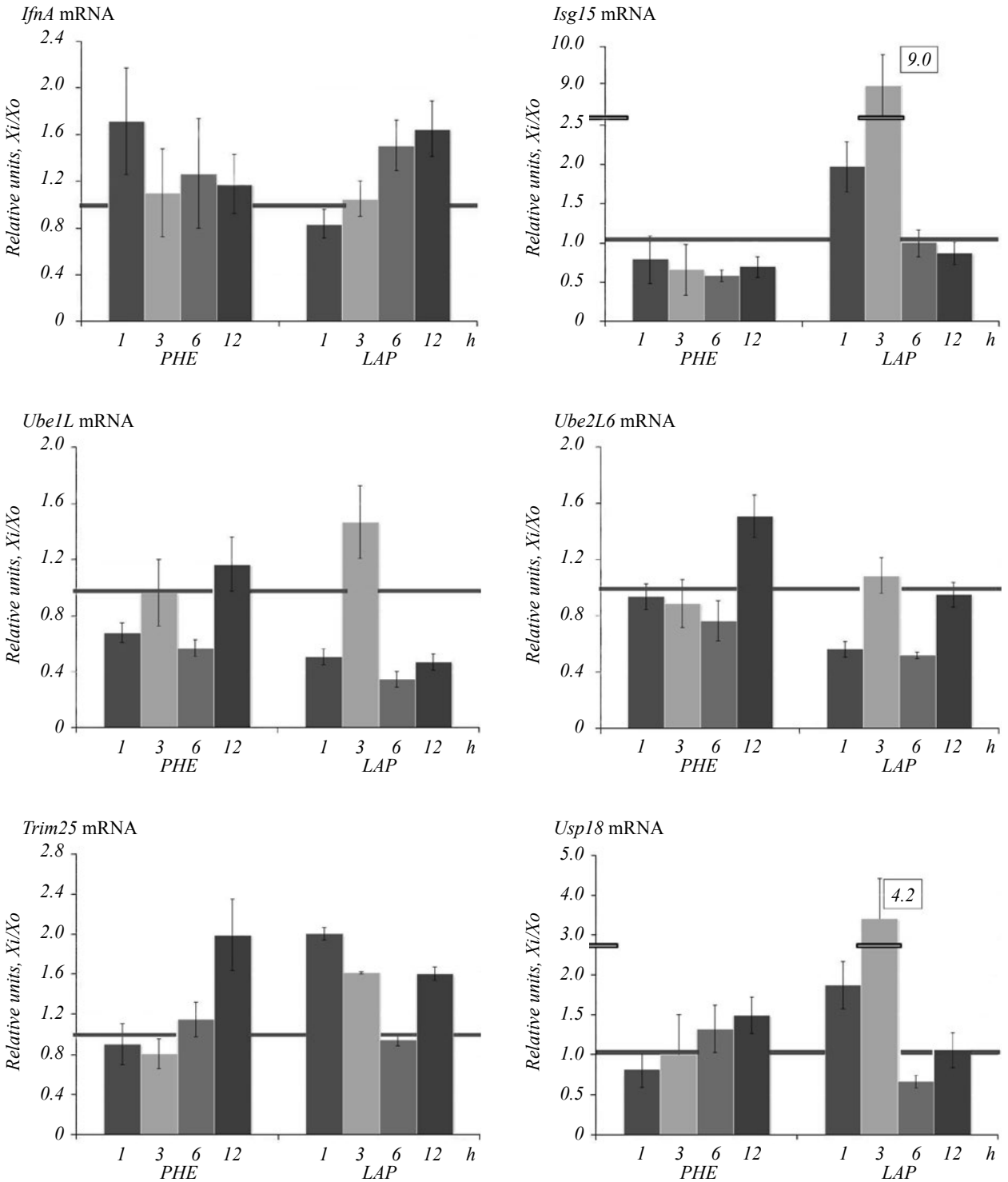

Fig.2. Abundance of individual mRNAs in total liver RNA after partial hepatectomy (PHE) and after laparatomy (LAP). The designation of x_i and x_0 is the same as in Fig.1

the surgical procedures studied resemble those of *Tbp* mRNA discussed above except decrease of its concentration at 1h after partial hepatectomy (Fig. 1; Table S1). The gradual upregulation of 18S rRNA abundance confirms the well known dynamics of ribosome synthesis during the pre-replicative period of liver regeneration, meeting the vital requirements of the intensive synthesis of the replication machinery components subsequent to the 12 hours post-surgery interval [41–43].

The decrease in *Tbp* mRNA and *18S* rRNA abundance after laparotomy is consistent with the restriction of the synthesis of the so-called negative proteins of the acute phase response (e.g. albumin, transthyretin) for the synthesis of the positive proteins [35, 36] and the reduction of translation with its highest energy consumption for a more efficient recovery of homeostasis. After partial hepatectomy the great requirement for energy resources particularly for increased transcription and translation is provided by the profound changes in the liver metabolism while the liver uses the cabohydrates and fatty acids from the muscles and adipose tissue to cover these expenses [44]. So the responses to partial hepatectomy and laparotomy by *Tbp* mRNA and 18S rRNA abundance are exactly opposite during 12 postsurgery hours.

The abundance of mRNAs is involved in the (de)ISGylation processes

Liver regeneration is a partially synchronized process that can be observed on a scale of time. The pre-replicative period extends from stimulation to the onset of DNA synthesis and is subdivided in two stages–priming, or promotion, (ca. 3 h post-surgery) and progression, or the G1 period, corresponding to the first cell cycle of hepatocytes [3, 4, 33, 34]. During the first stage, the liver cells precipitately switch from their "quiescent" program to that committed to the initiation of the cell cycle and cellular preparation for DNA replication, starting ca. 12 h post-surgery. The marked difference between the abundance of several mRNAs after laparotomy (see below) points also to the existence of specific transition period (1–3 h) during 12 h response.

The first stage after partial hepatectomy (1–3 h) is characterized by an early transient up-regulation of *Ifnα* mRNA and a concomitantly pronounced down-regulation of *Isg15* and *Ube1L* mRNAs while the level of other mRNAs remains in the range of control levels or slightly lower (Fig. 2; Table S1). The situation following the laparotomy is significantly different; the early downregulation of *Ifnα* mRNA abundance coincides with immediate (*Isg15*, *Trim25 and Usp18*) up-regulated mRNA concentrations; the abundances of *Ube1L* and *Ube2L6* mRNAs are diminished. Despite the variation in the level of mRNAs at 1h it is followed by up-regulation at 3h especially manifested by *Isg15* and *Usp18* mRNAs. So a decrease in the protein modification activity may be suggested during the transition period after partial hepatectomy and its activation during the same period after laparotomy.

During the next stage, corresponding to the pre-replicative period in hepatocytes induced by partial hepatectomy, the abundance of *Ifnα* mRNA returns to the initial levels and that of *Isg15* mRNA remains at the low level while the concentrations of all other mRNAs increase slightly during the progression of the 12 hour period. The situation following laparotomy significantly differs yet again. The abundance of *Ifnα* mRNA increases slightly while the abundance of all other mRNAs drops sharply compared to the concentrations at 3h either to control level (*Isg15* and *Trim25*) or even below it (*Ube1L, Ube2L6* and *Usp18*). At 12 h post-laparotomy the abundance of mRNAs is either at control level (*Isg15, Ube2L6 and Usp18*) or remains below it (*Ube1)*. Only the level of Trim25 mRNA exceeds control level.

Therefore the changes in the abundance of *Ifnα* mRNA are oppositely directed with the changes in the abundance of ISGylation-related transcripts at each stage of both processes. As opposed to these data the primary hepatocytes respond to the IFN alpha level recorded after liver resection by upregulation of these ISGylation-related genes expression [45]. The same type of response is recorded during viral infections [21].

The following is noteworthy – many ISGs can be induced by a number of alternate signaling pathways

activated not only by IFNs [46]. IRF9, a component of transcription factor ISGF3, is a member of the large IFN regulatory factor (IRF) family of proteins, all of which can bind to ISRE or ISRE -like elements. Signaling pathways leading to the induction of ISGs may start at different points but converge at various nodes and all cause the activation of IRF-3 or IRF-7, the transcription factors that are the common denominators of these pathways [46]. The gene *ISG15* has the simple promoter driven by ISRE only, and as a result, all agents that can activate transcription factors containing IRF proteins can efficiently induce this gene [46]. So *ISG15* may be used as a pure marker of (in)activating IRF-containing transcription factors.

Unlike the common tendencies in the changes of ISGylation-related transcripts the expression patterns of different genes at each stage of both responses are not completely similar, e.g. the abundance of *Isg15* mRNA vs. other mRNAs during 12 hours after partial hepatectomy or *Isg15* and *Usp18* mRNAs vs. other mRNAs during the acute phase response. This differential regulation of IFN response genes suggests that different regulatory pathways and/or involvement of different cell types are operative over time. The mechanisms regulating these distinct expression patterns are not understood, but evidence suggests that the IFN*α*/β response and that of interferon-stimulated genes are certainly complex.

Altogether there is a principle difference in the expression of *Tbp*, *18S* and ISGylation-related genes during transition of quiescent liver cells to the proliferation and highly tissue-specific acute phase response. This fact argues for the high specificity of innate immune response.

The ISGylation-related mRNAs, Tbp mRNA and 18S rRNA are differentially involved in liver transition from quiescence to proliferation and acute phase response

IFNα and interferon-stimulated genes products take on a number of diverse roles both enhancing and inhibiting different compounds of innate immunity. Here we addressed the expression of *IFNα* gene and a number of ISGs coined as ISGylation-related genes, the products of which are engaged in post-translational modifications of proteins. The expressions of *Tbp* mRNA and *18S* rRNA genes were used as the general indirect markers of transcription and translation during the early stages of two pathophysiological settings – liver regeneration and acute phase response.

The various levels of mRNAs encoding the enzymes involved in ISGylation/ ubiquitination can generate profound biological effects. For example, ISGylation of IRF3 increases its stability by preventing polyubiquitination, which leads to sustained transcription factor activity with subsequent activation of ISGs transcription [47]. Similarly, ISGylation of cyclin D1 leads to protein destabilization, reduced activity, and cell cycle inhibition [48]. ISGylation or ubiqitination of 14-3-3s protein in the absence of ISG15, changes its ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors. Trim 25 may directly downregulate 14-3-3s levels through a proteasome-dependent mechanism and in such way may regulate the cell cycle as 14-3-3s is a negative regulator of the cell cycle progression and is important for the G1 and G2 arrest after the DNA damage [49]. 4EHP is an mRNA 5′cap structure-binding protein and acts as a translation suppressor by competing with eIF4E for binding to the cap structure. The ISGylation of 4EHP substantially increases its affinity for binding, thereby enhancing its ability to block the translation initiation [50]. Intracellular ISG15 is a key negative regulator of IFN-α/β immunity. It serves not only as a tag protein but ensures the USP18-dependent negative regulation of IFN-α/β and prevention of IFN-α/β-dependent autoinflammation. USP 18 binds to the intracellular domain of IFNαR2, prevents the binding of JAK1, and inhibits IFN signaling [51, 52].

We may speculate that transitorial elevated abundance of ISGylation-related transcripts during the acute phase response may be accompanied by the ISGylation of: 4EHP protein that would support the restricted translation; cyclin D – to prevent prolifera-

tion; and by the ISGylation-independetnt activity of USP18 to prevent inflammation. Correspondingly the downregulated expression of ISGylation-related genes during response to partial hepatectomy may be an indispensable prerequisite for production of proinflammatory cytokines (IL-1, TNF alpha, IL-6) that are crucial for progression of regenerating process, and for initiation of replication via G1 check point. The biological effects of posttranslational modification by ISG15 *in vivo* are still obscure and at the very beginning of their investigation. The new challenges loom in perspective.

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Author Disclosure Statement

All authors declare that no competing financial interests exist.

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Експресія генів, продукти яких задіяні в ІСГілюванні, в печінці щурів, що регенерує

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Наші нещодавні дослідження показали, що на ранньому етапі відновлювального процесу активується синтез інтерферону α (IFNα), цитокина вродженого імунітету. Роль IFNα в процесі відновлення печінки поки що не з'ясована. **Мета.** Проаналізувати

експресію класичних інтерферон-стимульованих генів (ІСГ), *Ube1l*, *Ube2l6*, *Trim25, Usp18* і *Isg15,* в печінці під час її переходу від стану спокою до проліферації у відповідь на часткову гепатектомію й під час реакції гострої фази після лапаротомії. Ці гени відповідають за посттрансляційну модифікацію білків шляхом ІСГілювання. Рівень експресії генів, які кодують 18S рРНК і транскрипційний фактор TBP, що зв'язується з TATA-боксом, використали в якості непрямого показника інтенсивності трансляції і транскрипції. **Методи.** Концентрацію індивідуальних РНК визначали в тотальній РНК печінки методом зворотної транскрипції і ланцюгової полімеризації в реальному часі. **Результати.** Часткова гепатектомія викликає поступове підвищення експресії генів *Tbp i 18S rRNA* впродовж 12 год. після операції і зниження експресії генів ІСГілювання впродовж перших трьох годин з наступним незначним підвищенням до 12 год. Рівень *Isg15* транскриптів залишається зниженим впродовж всього періоду дослідження. Лапаротомія викликає поступове зниження експресії генів *Tbp* і *18S rRNA* і виражене підвищення концентрації транскриптів генів ІСГілювання на першому етапі (1–3 год.), що змінюється різким зниженням до 6-ої год. з наступним незначним підвищенням/зниженням до 12-ої год. Зміни в рівні транскриптів гена *Ifnα* і генів системи ІСГілювання носять протилежний характер на кожній із стадій відповіді печінки на часткову гепатектомію і лапаротомію. **Висновки.** Припускаємо, що експресія генів, які задіяні в процесі ІСГілювання, не залежить від експресії гена *Ifnα.* Використані «показники» активності транскрипції, трансляції і посттрансляційної модифікації білків шляхом ІСГілювання принципово відрізняються між двома реакціями відповіді печінки на часткову гепатектомію і лапаротомію, що свідчить про специфічність реакцій вродженого імунітету.

Ключові слова: Інтерферон α, ІСГілювання, регенерація печінки, реакція гострої фази

Экспрессия генов, продукты которых участвуют в ИСГилировании, в регенерирующей печени крыс

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Наши недавние исследования показали, что после частичной гепатэктомии на раннем этапе восстановительного процесса активируется синтез интерферона альфа (IFNα), цитокина в системе врожденного иммунитета. Роль IFNα в процессе регенерации пока не ясна. **Цель.** Проанализировать экспрессию классических интерферон-стимулируемых генов *Ube1l*, *Ube2l6*, *Trim25, Usp18* and *Isg15* в процессе перехода печени из состояния покоя к пролиферации в ответ на частичную гепатектомию и во время реакции острой фазы после лапаротомии. Эти гены ответственны за посттрансляционную модификацию белков путем ИСГилирования. Уровень экспрессии генов, которые кодируют транскрипционный фактор TBP и 18S рРНК, использовали в качестве непрямого показателя интенсивности транскрипции и трансляции. **Методы.** Концентрацию исследуемых РНК определяли в тотальной РНК печени методом обратной транскрипции и цепной полимеризации в реальном времени. **Результаты.** Частичная гепатэктомия вызывает постепенное повышение экспрессии генов *Tbp* и *18S rRNA* в течение 12 час после операции и снижение экспрессии генов ИСГилирования в период 1 – 3 ч с последующим незначительным повышением к 12 ч. Уровень *Isg15* транскриптов неизменно снижен в течении всего исследуемого периода. Лапаротомия вызывает постепенное снижение экспрессии генов *Tbp* и *18S rRNA* и выраженное повышение концентрации транскриптов генов, участвующих в ИСГилировании (1–3 ч), которое сменяется резким снижением к 6 ч и последующим незначительным повышением/снижением к 12 ч. Изменения в уровнях транскриптов *Ifnα* и транскриптов генов системы ИСГилирования носят разнонаправленный характер на каждой из стадий ответа печени на частичную гепатэктомию и лапаротомию. **Выводы.** Предполагаем, что экспрессия генов, определяющих ИСГилирование, не зависит от экспрессии гена *Ifnα.* Использованные «показатели» активности процессов транскрипции, трансляции и посттрансляционной модификации белков путем ИСГилирования в ответ на частичную гепатэктомию и лапаротомию принципиально различны и свидетельствуют в пользу специфичности реакций врожденного иммунитета.

Ключевые слова: Интерферон α, ИСГилирование, регенрация печени, реакция острой фазы

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