Differentiation of Gene Expression Profiles
Data for Liver and Kidney of Pigs

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I. INTRODUCTION

MICROARRAY technologies are widely used in the world for the gene expression analysis in various tissues. These technologies allow to estimate changes of a gene expression at pathological conditions as well as to identify the genes which transcription brings the defining contribution to formation of desirable economic valuable characteristics of farm animals. The analysis of a gene expression profiles in various organs using microarray technologies allows to reveal separate genes, gene ensembles, and the metabolic ways underlying the structurally functional organization of organ and its physiological function. Researches of dynamics of such profiles in ontogenesis and at pathological processes allows to reveal genes and the gene combinations which products bring defining contribution in organspecific functions. Therupon, in the present work is executed the search of such genes by comparison of a gene expression profiles in a liver and kidneys of the pigs.

One of the problems arising at the analysis of a gene expression profiles is the phenomenon of cross hybridization which can reduce reliability of a research results. Cross hybridization is one of the errors widely discussed in the literature, for example [1]. Studying of such phenomenon becomes especially actual with the recent trends of development of microarray technologies towards increase in quantity and density of probes arrangement on the carrier. In our researches to this problem it is given particular attention.

II. MATERIALS AND RESEARCH METHODS

Researches were carried out on six-monthly female pigs of Landras breed, keeping on the experimental farm of University of Minnesota, USA. Reception of the primary data of gene expression profiles was spent on experimental base of this University under the supervise of professor S.C. Fahrenkrug. Experiment was carried out with 70-mer swine protein-annotated oligonucleotide microarrays designed by request of professor S.C. Fahrenkrug, U of MN, USA.

Total RNA was isolated from liver and kidney of five pigs, for each sample separately received cDNA in RT-PCR, using special RT Primer Oligo (Cy3/Cy5). After stopping RT reaction and degrading of RNA the 1st hybridization of cDNA to microarray and 2nd hybridization of binding 3DNA Capture Reagent to cDNA on microarray was carried out.

The method is based on competitive hybridization of nucleotide sequences to probes of microarray and subsequent labeling with fluorescent dyes cy3 and cy5. Later on the relation definition cy3/cy5, carried out by microarray scanning, have been obtained the numeric datas, characterized by standard units of fluorescence for each spot.

III. RESULTS AND DISCUSSION

According to the aforesaid, the comparative analysis of intensity of a transcription in a liver and kidney of 600 genes has been made.

At the total analysis of 600 genes of a liver, with the maximum distinctions in intensity of hybridization between liver cDNA and kidney cDNA (from 1000 to 70000 standard units of a luminescence) 12 genes were found out with more than one probe in microarray. The obtained data allowed comparing separately for each animal the intensity of hybridization to microarray probes of various sites of the same gene (on the digitized signal strength).

By results of signal strength estimations two basic groups of genes among considered 12 were allocated: with the least distinctions between signal strength of hybridization of various sites cDNA of same mRNA with microarray probes (to 4500 standard units of a luminescence), and with the greatest distinctions (more than 10000 standard units of a luminescence).
At all examined individuals the first group of genes with the least differences on a signal of hybridization of various sites included following genes: Apolipoprotein A-II precursor, C4b-binding protein alpha chain precursor (C4bp), dermokine isofrom beta, Glutathione S-transferase A5-5, Glutathione S-transferase Mu 2, multiple substrate lipid kinase; the second group with the greatest differences between signals of hybridization of various sites of the same gene has been presented by two genes – Alpha-1-antichymotrypsin precursor (ACT), Fibrinogen alpha chain precursor. Observable differences were reproduced in independent experiments for all investigated animals.

Among profiles of gene expression in kidneys for 600 genes, with the maximum distinction in intensity of hybridization between kidney cdNA and liver cdNA (from 10000 to 80000 standard units of a luminescence) 9 genes were allocated with more than one probe to different sites of the same gene on the microarray. Depending on a difference in standard units of luminescence between probes after hybridization, these genes were subdivided into 3 groups: with differences in a hybridization signal between gene sites up to 4500, between 4500-10000 and above 10000. The first group (to 4500 standard units of a luminescence) included genes, with the least distinctions between probes to different sites of the same gene. This group includes CDNA FLJ12547 fis, clone NT2RM4006634 and Translationally-controlled tumor protein (TCTP) (p23). The group of genes, which internal sites of hybridization differed more than on 10000 standard units of a luminescence, consisted of the genes coding ATP synthase a chain, Chromogranin A precursor (CgA), Pituitary secretory protein I (SP-1) and Ubiquitin.

It was possible to expect, that one of the reasons of differences in intensity of hybridization of different sites cdNA of same transcript were caused by fact that nucleotide sequence of microarray probe, giving a superfluous hybridization signal in comparison with probe to other site of the same gene, complementary simultaneously to cdNA of different mRNA, transcripts of different genes. It was obvious, that possibility of such "cross" hybridization of microarray probes with homologous transcript sequences of different genes was necessary for considering at the analysis of profiles of gene expression as it could be a source of essential errors at revealing of "critical" genes in organ-specific transcriptome.

To check up this assumption, the search a homology sites to microarray probes with microarray probes with homologous transcript sequences of different genes was necessary for considering at the analysis of profiles of gene expression as it could be a source of essential errors at revealing of "critical" genes in organ-specific transcriptome.

For finding-out of activity of a fibrinopeptide A transcription in a pigs liver, the search of a homology of two probes to cdNA transcripts of this gene was executed. The
following data is obtained. In a refseq_rna database (Sus scrofa) homologous sequences for the first probe have not been revealed, but in an EST database was revealed sequences carried to cDNA mRNA of a fibrinopeptide A, on the basis of high homology degree with cDNA mRNA of this gene in other kinds of mammals, such as mice, human, cattle. cDNA of this gene was characterised by one, strongly pronounced feature - except 70 nucleotides with a full homology to the first probe, cDNA included partially blocking the same sequence in length about 55 nucleotides, from more than 80 % a homology. For example, in cDNA gb:FD606208.1 the full homology is observed from a position 535 to 604; The second site of a partial homology is located between a position 509 and 563, (44 nucleotides from 55, 80 % of a homology). Presence of such internal repetitions in cDNA to the same probe can create complexities during hybridization because of a competition for the same probe of internal (blocked) homologous cDNA sites. The homologous sequence to the second probe (100 % homology) was observed for cDNA of this gene between positions 209 - 278. That is, two probes for cDNA of a gene fibrinopeptide A differed in localisation on length of mRNA, and also, for one probe there was a site of an additional internal homology for 55 nucleotides that can cause differences in intensity of hybridization between this probes.

The analysis of homology sites for the three probes to cDNA of a gene ATP synthase a chain, that gave differences in a hybridization signal in pigs kidneys, has allowed to obtain the following data.

ATP synthase – is a membrane-connected complex enzyme combining synthesis/hydrolysis ATP with transport of protons through a membrane. There are two parts - actually complex ATP sintaza/hydrolysis including five subunits (an alpha, beta, gamma, delta, an epsilon) and transmembrane component (F0), consisting at least from three subunits (A-C) which are coded with mitochondrion DNA (A-G, F6, F8). Subunit A (or Subunit 6) is a key component of ATPase and defines transmembrane proton transport.

In connection with a key role in power supply of endocellular fundamental biochemical processes, ATPases are the most ancient enzymes which occurrence in evolution preceded occurrence of photosynthesis and cytochromes of a respiratory chain. ATPases remains highly conservative enzymes in all live organisms: ATPases founded in thylakoids of chloroplasts in eucariotic mitochondrial membranes keep high nucleotide and aminoacid similarity with ATPases in a plasmatic membrane of bacteria. Special conservatism is found out in subunits and its parts that are most essential for catalytic function of enzyme [4].

Search in a EST database has allowed to find out a considerable quantity of sequences, homologous to probe №1 to ATP synthase a chain - F0 - F6. The full homology to the same cDNA fragment for the probe №1 is found out both in transcripts of a plus chain, from a position 191 to 260, and also from 377 to 446, and in transcript of a minus chain, from a position 124 to 55 positions. For probe №2 sites of a full homology are observed in cDNA of a plus chain, both in direct sequence, and in complimentary variant, and also in a minus chain. For the probe №3 some sites of a full homology are revealed for cDNA only of a plus chain. Thus, probes to cDNA of subunit A of ATP synthase transmembrane site differ by quantity of homologous sequences in cDNA transcripts both in plus, and in a minus chains of mitochondrial DNA. It is necessary to notice that homology sites to all three probes are found out in a database of clones of sequenced nucleotide sequences of different pigs chromosomes.

The Chromogranin A precursor (CgA) concerns to granins (hromogranin/sektroegrannin) superfamilly. Its derivatives include vasostatin 1, vasostatin II; EA-92; ES-43; pancreastatin; SS-18; WA-8; WE-14; LF-19; AL-11; GV-19; GR-44; ER-37 [5]. Chromogranin A and its derivatives participate in regulation of calcium and glucose metabolism, condition of cardiovascular systems and of some other functions [6].

To the probe №1 homology sites are observed simultaneously in different positions - at the beginning (120 - 189; 300 - 369), closer to the end (592 - 661) of plus chain, and also in cDNA of a minus chain (direct sequence 193 - 124). Blocked sites of a homology for the probe №2 are found out in a plus chain (31-100; 85-154; 120-189; 195-264), and also the partial homology with cDNA of proteins participated in maintenance of spermatozoons mobility.

The expressed differences in the intensity of hybridization signals are also found out to the probes of ubiquitin. Ubiquitin family and ubiquitin similar proteins includes the genes that provide protein proteolysis and are a part of the cascade of the co-operating proteins, that participate in consecutive stages of regulation of a cellular cycle. A number of members of this family belong to modifiers of the various proteins which are carrying out a wide spectrum of various functions. Ubiquitintion of proteins is usually accompanied by linkage final (C) domain of ubiquitin with lysine of protein-substratum [7], [8].

Searches of a homology sites to DNAprobes in a EST databases have allowed to obtain the following data. Homology sites to the probe №1 are revealed in a cDNA a minus chain (positions 399-330) and in a plus chain (positions 149-218); for the probe №2 - in three sites of a plus chain (positions 177-246; 405-474; 630-699); for the probe №3 - one site of a homology in a plus chain (positions 83-152); for the probe №4 - three sites of a homology in a plus chain (positions 138-207; 367-435; 593-661). It is visible that probes differ in quantity of homologous sites in cDNA, and some of them partially block each other.

By results of the executed analysis it is visible that all considered cases of reproduced differences between hybridization intensity of different microarray probes to cDNA genes of a liver and kidneys of a pig are typical for the genes belonging to supergene families. For all cases the expressed differences between probes are observed to cDNA of the same gene by quantity of homology fragments, on presence of homology sites for cDNA of other genes, including paradens, and also on presence/absence of
homologous sequences in cDNA of mRNA of a plus and a minus chains. The obtained data is well co-ordinated with the assumption that "cross" hybridization of different cDNA sites with one and different probes can be a source of errors at the comparative estimation of expression intensity of various genes especially essential for the genes belonging to superf gene families.

The total comparative analysis of distinctions in intensity of expression of genes in liver and kidney, differing on level of expression for more than on 20000 standard units of a luminescence, had allowed to reveal 40 genes which expression was essentially above in cages of kidney, than liver. More than half of it (26) was the genes which products were the transport proteins or the signal transductors included in plasmatic or mitochondrial membrane, representing the different part of receptor signaling pathways directly participating in ionic exchange between extracellular space and cytoplasm, and also between cytoplasm and internal mitochondrial matrix. Products of 10 genes were involved in regulation of transcription and the ubiquitin-dependent cascade, 3 genes coded proteins of ribosomes and 1 gene – dynamin complex subunit, directly participating in the control of cytokinesis. In general, the basic differences in profiles of gene expression between kidney and liver had appeared connected with the genes supervising inter- and an intracellular ionic exchange, and also mechanisms of cellular division. It would be well coordinated with dominating participation of kidneys in maintenance of ionic balance in blood of mammals, in comparison with a liver, and also with known differences of mitochondrial morphology in liver and the kidneys - lowered activity of cytokinesis in a liver (polyploidy of hepatocytes). Thus, the revealed differences in profiles of gene expression in kidney and liver corresponded to functional and histological distinguishes between these organs.

The obtained data visually show the possibilities of using of short DNA fragments (70 nanometers) for in-depth studies of genetic-biochemical mechanisms of cellular and tissue phenotype formation and it will help in developing the experimental approaches to its control.

IV. CONCLUSION

All considered genes, with essential difference in intensity of hibridization signal between different DNA probes of the same gene, belong to superf gene families. In all cases differences were observed: by quantity of homology fragments to one probe in cDNA of the same gene mRNA; on presence of a homology sites to probe in cDNA of other genes mRNA, including genes – paradens.

Differences in expression of genes are well co-ordinated with known organo-specific, physiological, biochemical, histologic differences:

Despite presence of errors (for example, cross hybridization), estimations of a gene expression profiles allow to reveal separate genes that are directly associated with organo-specific functions and to estimate organo-specific phenotypes in norm and pathology.

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REFERENCES


