

GENE EXPRESSION PROFILES IN LIVER AND KIDNEY OF PIG

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Using of technology of microarrays allows to carry out simultaneously monitoring of nucleotide sequences of a considerable quantity of genes and to estimate activity of their transcription (profiles of a gene expression). These methods have special importance for search of the genes which transcription brings the defining contribution to formation of desirable economic valuable characteristics, and also pathological phenotypes of some diseases of farm animals.

Profiles of a gene expression in different organs allows to reveal genes and the metabolic ways supervised by them, which changes of work can be closely connected with the formation of various phenotypic characteristics. For having possibility to systematization work of such "critical" genes for different phenotypes, it is necessary to create a database of differences between gene expression profiles in animal organs that can be related to specificity of physiological function of them. Such base can be used for consideration of interrelations between changes of "critical" genes expression and phenotypic variability of various morpho-physiological characteristics of animals.

Thereupon, the comparative analysis of profiles of a gene expression of two pigs organs, liver and kidney, having certain affinity in early stages of histogenesis and participating in the control of the biochemical homeostasis of multicellular organism was carried out in the present research.

It is important to mention that, despite wide prevalence in using of microarray technologies for the analysis of gene expression profiles, the method has some problem which can lead to erroneous results. Sources of such errors remain insufficiently investigated till now. One of such sources, which is widely discussed in the literature (for example, [1]), is a problem of cross hybridization. In our researches a particular attention was given to this question.

Materials and Research methods

Researches were carried out on four six-monthly female pigs of Landras breed, keeping on the experimental farm of University of Minnesota, USA. Reception of the primary data of profiles of a gene expression was spent on experimental base of this University under the supervise of professor S.Fahrenkrug. Experiment was carried out with 70-mer oligo microarrays designed in a lab of professor S.Fahrenkrug, U of MN, USA.

Total RNA was isolated from liver and kidney of four 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 3DNA Capture Reagent to bind to cDNA on microarray was carried out.

The method is based on competitive hybridization of nucleotide sequences, preliminary labeled with fluorescent dyes cy3 and cy5 to probes of microarray. At drawing on a matrix a mix of two samples of labeled cDNA the competition for each spot is taking place to be, creating thereby the certain relation of cDNA labeled with dye cy3 to cDNA

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labeled with dye ey5. Later on the relation definition cy3/cy5, carried out by microarray scanning, have been obtained the numeric data's, characterized by standard units for each spot.

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 50000 and more 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 isoform 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 70000 and more 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 luminescence standard units between probes after hybridization, these genes were subdivided into 3 groups: differences in a hybridization signal between gene sites were 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 NT2RM4000634 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-I) 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, that was, 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, that reproduced distinctions in signals of hybridization intensity with different probes was executed by means of program BLASTn in a sus scrofa EST databank presented in NCBI.

Alpha-1-antichymotrypsin precursor (ACT) (SERPINA3-2) - is a member of a huge family. ACT is a retarding agent of serine proteinase, which have received the name - SERine Proteinase INhibitors (Serpins). This family includes the genes coding classical retarding agents of serine proteinases, such as an alpha-1-antitrypsin and an alpha-1-antihemotrypsin, protein C depressor, *kallistatins* and non-abscopal serpins, such as globulins, connecting corticosteroids and thyroxin. Many serpins, in particular, antitrypsin and antihemotrypsin, participate in the metabolic cascades connected with coagulability of blood. Non-abscopal serpins participate in transport of hormones, and also carry out function chaperones. The length of gene SERPINA3-2 includes approximately 9.0 thousand pairs the bases, consists of five exons and four introns. The coding sequence of SERPINA3-2 cDNA has 86 % of identity with the SERPINA3-1. Gene SERPINA3-2 is charted on distal end of a short shoulder of a pigs chromosome # 7 (7q), in a serpins gene cluster, including gene P11 (SERPINA1), PI2, PI3, PI4 (parabroad gull SERPINA3), PO1A and POIB [2].

Search of a homology sites in a *Sus scrofa* EST database (refseq_ma) for 70 nucleotides, which were used as microarray probes for the analysis of gene expression profiles, has allowed to obtain the following data. The most expressed differences in a hybridization signal have been found out for three probes identifying cDNA of gene SERPINA3-2 in total cDNA of a pig's liver. The full homology for the first probe (100 %) is found out in two transcripts: LOC100153899 (positions 1096 - 1165) and LOC100156752 (positions 753 - 822), which in a database are presented as cDNA's with similarity to transcripts of a gene SERPINA3-2. The partial homology (with 14 mono- and dinucleotides discrepancies) was observed with cDNA of LOCI00156325 from a position 1096 to 1165. cDNA of a gene SERPINA3-2 found out a site of a homology from a position 1027 to 1096 with 11 mono- and dinucleotides discrepancies (84 % of a homology). The partial homology of probe (13 discrepancies, 81 % of a homology) is revealed in cDNA of a gene SERPINA3-3, from a position 1045 to 1114, and also with cDNA of SERPINA3-6 (13 discrepancies, 81 % of a homology) from a position 772 to 841. High level of coincidence (89 %) is found out for the second probe with a cDNA site of LOCI00155540 (SERPINA3-6, from a position 809 to 877) and cDNA of a gene SERPINA3-2 (from a position 1082 to 1150). For the third probe almost full homology (with one discrepancy) with a cDNA of a gene SERPINA3-2 from a position 1194 to 1263, and also a partial homology (with four discrepancies) a site of 35 nucleotides from 1295 to 1328 is observed.

The obtained data testifies that for the first probe the quantity of a homology sites both inside cDNA transcript, and between cDNA genes SERPINA3-2, SERPINA3-3, SERPINA3-6 is much more than for the second probe. Only the third probe had homology sites to cDNA of a gene SERPINA3-2. That could lead to the observable differences in hybridization signal of this cDNA with different probes.

The fibrinogen molecule consists of fibrinopeptide A and a set of subunits: alpha, beta and the gamma, connected in one molecule through sulphidic bonds. Fibrin is formed after chipping off a fibrinopeptide A from an alpha chain of fibrinogen that induces polymerisation of fibrin [3].

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 a 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 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 a gene fibrinopeptide A differed in localization on length of mRNA, and also, that 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 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 transcript plus chain, from a position 191 to 260 nucleotide, and also from 377 to 446 nucleotide, and in transcript minus chain, from a position 124 to 55 positions. For probe №2 sites of a full homology are observed in cDNA 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 in 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 tests 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/secretogranin) superfamily. Its derivatives include vasostatin 1, vasostatin II; EA-92; ES-43; pancrastatin; 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 spermatozoos 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. Ubiquitination 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 an EST database 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 (83-152); for the probe №4 - three sites of a homology in a plus chain (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 sites of a homology for cDNA of other genes, including paradens, and also on presence/absence of homologous sequences in cDNA of mRNA plus and a minus chains. The obtained data will be well co-ordinated with the assumption that such "cross" hybridization of different cDNA sites with one and different probes can be a source of errors at the comparative estimation of intensity of an expression of various genes especially essential for the genes belonging to supergene families.

The total comparative analysis of distinctions in intensity of an expression of genes in liver and kidney, differing on level of an 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 transducers 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 - dynactin 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 intracellular ionic exchange, and also mechanisms of cellular division. It would be coordinated well 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, the 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 the using of short DNA fragments (70 nanometers) for in-depth studies of genetic-biochemical mechanisms of cellular and tissue phenotype formation and to develop the experimental approaches to its control.

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