Analysis of RNASeq data of fruit flies affected by low dose gamma-irradiation, dioxin, toluene and formaldehyde

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As a result of the development of nuclear and chemical industries, the increasing incidence of man-made disasters increases the mutagenic load on human and environment. Relevant is the comparative study of the cellular mechanisms induced by different stressors of chemical and physical nature. The results of such studies can be used as the basis for the creation of new methods of bioindication and biosensing of low doses of pollutants.

It was studied the effect on the *Drosophila melanogaster* imagoes (males and females separately) of the following types of stressors: external gamma radiation from the Ra226 source (5.5h at dose rate 36 μGy/h, absorbed dose - 20 sGy), or adding to the culture medium of 2,3,7,8-tetrachlorodibenzo-p-dioxin (265 ng/ml, 3 days), or adding to the culture medium of toluene (50 μM/l, 3 days), or treatment by vapors of 7% formaldehyde solution (per day). Total RNA was isolated for deep sequencing. Quantity and quality of isolated RNA were measured using the NanoDrop 2000, Qubit 2.0 and Agilent 2100 Bioanalazer. After that study, which had given satisfactory results, sample preparation was carried out by RNAseq by Illumina GAIIx. In total, it was studied the expression of 15222 transcripts in each variant of the impact and control samples. Statistical analysis of the data was performed - using edgeR and DESeq libraries in R language environment; string-db.org, KEGG and GO databases; as well as with self-developed software. We found top-100 differently expressed genes in flies under each type of stress in comparison with control. Every top-list was clustered by distance between genes and then enriched with other fly genes, included in clusters. The distance between genes was calculated using string-db.org database. The new list of genes was clustered using KEGG-mapper and finally was obtained a list of pathways. Every pathway

was assigned enrichment-score, calculated as a proportion of number of genes from the list included in the pathway from number of genes in every pathway. We considered score reliable if it was not less than 0.5.

We found that homologs of vertebrate progesterone-mediated oocyte maturation pathway is presented in all female-pathways lists, and is not presented in male ones. Ascorbate and aldarate metabolism and Retinol metabolism pathway homologs are presented in both male and female lists in dioxin, formaldehyde and toluene lists. The correlation between all these types of stress and retinol metabolism is confirmed by literature data, but there have not been any information between correlation of these types of stress and Ascorbate and aldarate metabolism pathway yet, what makes us suppose that this is new unknown marker of dioxin, formaldehyde and toluene treatments. Notch signaling pathway, TFG-beta signaling pathway, MAPK signaling pathway, Proteasome, Basal transcription factors, Nucleotide excision repair, Jak-STAT signaling pathway, Circadian rhythm, Phototransduction, Hippo signaling pathway, mTOR signaling pathway, Ribosome, Mismatch repair, RNA polymerase, Hedgehog signaling pathway, Caffeine metabolism, DNA replication pathway homologs are presented in all pathways lists. We suppose that this means, that all this pathways are related to aging. Most of these pathways are already recognized as markers of aging. Pentose phosphate pathway homologs present only in dioxin list. It has been known, that dioxin treatment induces this pathway. It is responsible for the generation of NADPH required for the maintenance of GSH in its reduced state and, thus, the protection of cells from reactive oxygen intermediates. Drug metabolism - cytochrome P450 pathway have got enough score only in formaldehyde list. Its connection to formaldehyde stress is confirmed by literature data. We tried alternative way to cluster genes with GO by counting the percentage of genes from the list included in every GO term in three Ontology Aspects. Counting covariance between results for every type of stress showed that the greatest differences between expression of stressed fly genes are observed using KEGG mapper, so we choose this method for our research.

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