օրենք՝ պայմանավորված լայնական արագության մեծացմանը զուգընթաց տեղի ունեցող ռեզոնանսային հաճախության դոպլերյան վերալարումով: Քննարկվում է հետահարման երևույթի հնարավոր դերը բարձր լուծունակության ատոմային սպեկտրասկոպիայում: Ստացված արդյունքները օգտագործվել են գազային միջավայրերի հետ լազերային ճառագայթման փոխազդեցության դեպքի վերլուծության համար: Կատարվել են համապատասխան գնահատականներ ալկալի մետաղների ատոմական գոլորշի պարունակող նանոբջիջներում լազերային սպեկտրասկոպիայի համար, որտեղ սպասվում է հետահարման երևույթի ամենամեծ ազդեցությունը:

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ЭФФЕКТ ОТДАЧИ В ЛАЗЕРНОЙ СПЕКТРОСКОПИИ АТОМОВ ЩЕЛОЧНЫХ МЕТАЛЛОВ

Проанализировано влияние механического момента, сообщенного атомам при резонансном взаимодействии с лазерным излучением (эффект атомной отдачи), в спектроскопии атомов щелочных металлов. Прямые количественные измерения проведены с использованием установки атомного пучка натрия. При мощности отклоняющего лазера 7,5 мВт на расстоянии 890 см вниз по пучку зарегистрировано отклонение атомов на 1,3 мм, что соответствует приобретенной атомами поперечной скорости ≈ 300 см/с. Зависимость отклонения от мощности лазера описывается корневым законом, что обусловлено допплеровским уходом резонансной частоты при увеличении поперечной скорости. Обсуждается возможная роль эффекта отдачи в атомной спектроскопии высокого разрешения. Полученные результаты использованы для анализа случая взаимодействия лазерного излучения с газообразными средами. Проведены соответствующие оценки для лазерной спектроскопии наноячеек, содержащих атомарные пары щелочных металлов, где ожидается наиболее сильное влияние явления отдачи.

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UDC 669.018.674

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ANALYSIS OF TRANSCRIPTOME CHANGES IN RESPONSE TO HEAVY METALS USING SELF-ORGANIZING MAPS

Abstract

In this study, we have performed self-organizing maps (SOM)-based clustering and functional annotation of gene expression in hepatoma cell lines exposed to cadmium, nickel and arsenic using publicly available microarray data. The results show that cadmium and nickel exposure is associated with overexpression of genes related to hypoxia and oxidative stress response, while arsenic causes up-regulation of

tumorigenesis related pathways. This data suggests that changes in gene expression profiles upon exposure highly depend on the type of heavy metal.

Keywords: *Self-organizing map, gene expression data, functional annotation, carcinogenesis, hepatoma cell line HepG2.*

1. Introduction

The rapid development of transcriptomics technologies started in the 1990s has resulted in the accumulation of massive amounts of gene expression data. Much of this data is stored in publicly available repositories like Gene Expression Omnibus (GEO), which encompasses more than 520,000 individual experiments and around 21,000 project submissions, most coming from microarrays [1]. These massive transcriptome data are being used to describe the expression patterns of different cell lines and under various experimental conditions. However, the categorization of these data into useful and functionally meaningful groups as well as extraction of functionally relevant information about perturbed genes and related biological processes is a current major issue.

Self-organizing map (SOM) is an artificial neural network algorithm that uses unsupervised learning to discover patterns in very large datasets. Initially introduced by Kohonen [2], SOM has found its applications in various fields including bioinformatics. It features robust clustering, dimension reduction, multidimensional scaling and visualization features that have proven advantageous over alternative methods like clustering heatmaps and negative matrix factorization when applied to transcriptomic high-throughput data [3].

In this study, we have applied the SOM algorithm to analyze the changes in gene expression in HepG2 human hepatoma cell line from a publicly available database under exposure to carcinogenic metals, metalloids, and model carcinogens. Gene expression alterations during carcinogenesis (important chronic toxicity of metals and metalloids) have been previously studied on HepG2 and some useful information has been extracted [4]. Nonetheless, the comprehensive mechanisms of carcinogens action are still uncertain. Here applying the alternative approach of investigation, we endeavour to give a broader insight into the understanding of their carcinogenic mechanisms. We have used bioinformatics pipeline that implements SOM and further integrates gene set enrichment analysis [5].

2. Materials and Methods

Gene expression profiles were downloaded from Gene Expression Omnibus under accession number GSE8865. The data consists of 33 samples (11 treatments, three replicates per treatment) of human hepatoma cell line (HepG2) expression measures underexposure of two metals (cadmium and nickel), a metalloid (arsenic), three carcinogens (N-dimethylnitrosamine (DMN), 12-O-tetradecanoylphorbol-13-acetate (TPA) and tetrachloroethylene (TCE)) as well as under combined exposure of heavy metals with vitamin C using DNA microarray with 8795 human genes. The values of the expression matrix were transformed to a logarithmic scale.

Data analysis was performed using the SOM algorithm and downstream bioinformatics annotation implemented in oposSOM package for R [5]. After initialization, the program allocates the genes over a discrete two-dimensional quadratic grid of size 30x30 in a way that each gene expression profile is related to the most similar grid point, called metagene, measured in Euclidean distance.

Then the algorithm iteratively alters the values of each grid point following the observed profiles to effectively cover all the experimentally observed expression profiles. As a result, the genes with similar abundance values are clustered closer together within one metagene while those with divergent expression profiles are distributed in different regions of the map. Consequently, the sizable data of all gene expression profiles are translated into metagene groups (called "spots") resulting in a dimension reduction. Each grid point is colored from red to blue representing correspondingly from high to low expression profiles ensuing a colorful mosaic with smooth color transitions (Figures 1, 2). The spot-like regions of red and blue represent the groups of over- and under-expressed genes.

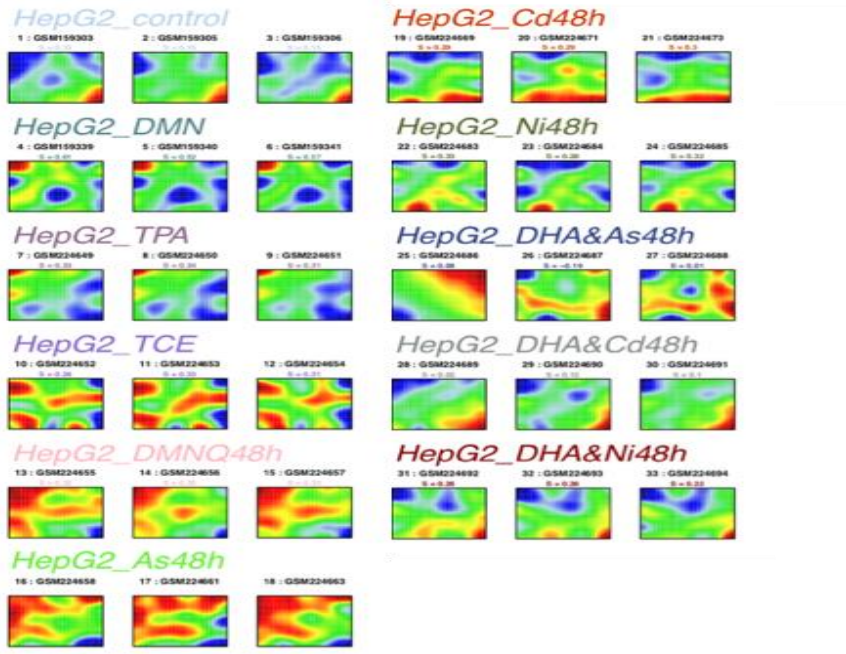


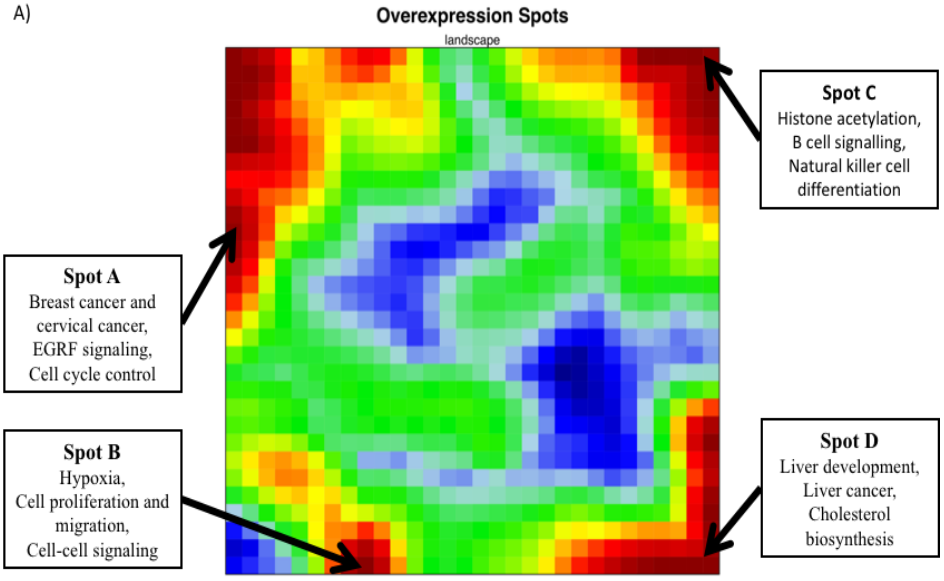
Figure 1. *SOM expression portraits of hepatoma samples exposed to heavy metal or carcinogen treatment. (HepG2_control - control, HepG2_DMN - treated with DMN, HepG2_TPA - treated with TPA, HepG2_TCE - treated with TCE, HepG2_DMNQ48h - treated with antioxidant DMNQ, HepG2_As48h - treated with arsenic, HepG2_Cd48h - treated with cadmium, HepG2_Ni48h - treated with nickel, HepG2_DHA&As48h - treated with arsenic and antioxidant, HepG2_DHA&Cd48h - treated with cadmium and antioxidant, HepG2_DHA&Ni48h - treated with nickel and antioxidant)*

The lists of genes in each spot are subsequently analyzed for possible functional annotation based on the correlation and co-expression of the gene expression profiles

within the spots. GO-gene set overrepresentation analysis using the hypergeometric test is applied to gene lists in every overexpression [6].

3. Results

SOM analysis identified 4 overexpression spots labelled A-D (Figure 2A, 2B). Spot A comprises 9 metagenes with a total of 211 genes. This expression pattern in overrepresented only in samples treated with arsenic (66.7%). Spot B consists of 6 metagenes with a total of 102 genes, that are overexpressed in the samples treated with cadmium (100%) and nickel (100%). Spot C consists of 23 metagenes with a total of 444 genes, that is overexpressed only in one sample treated with arsenic+antioxidant (33.3 %). Spot D contains 19 metagenes with a total of 364 genes. This expression pattern is overrepresented in the controls (100%) and the samples treated with cadmium+antioxidant (100%). As a result of the analysis, we have also obtained pairwise correlation maps, or PCM (Figure 3), illustrating Pearson correlation coefficients for all mutual combinations between the tissues. The metagenes clearly provide pairwise correlation map patterns of higher contrast which becomes emergent as diagonal and off-diagonal dark red/maroon and blue clusters. They refer to sample pairings with highly correlated and anti-correlated expression profiles, respectively [3]. Metagene PCM revealed a grouping consisting of control, samples treated with cadmium+antioxidant, nickel+antioxidant and arsenic+antioxidant. Furthermore, there is a comparatively higher correlation between the expression patterns of control samples and those treated with cadmium+antioxidant. Additionally, there is a grouping of samples treated with cadmium and nickel in both single gene and the metagene PCM. Finally, we observe a cluster containing samples exposed to DMN, TPA, TCE, DMNQ which also contains samples treated with arsenic.



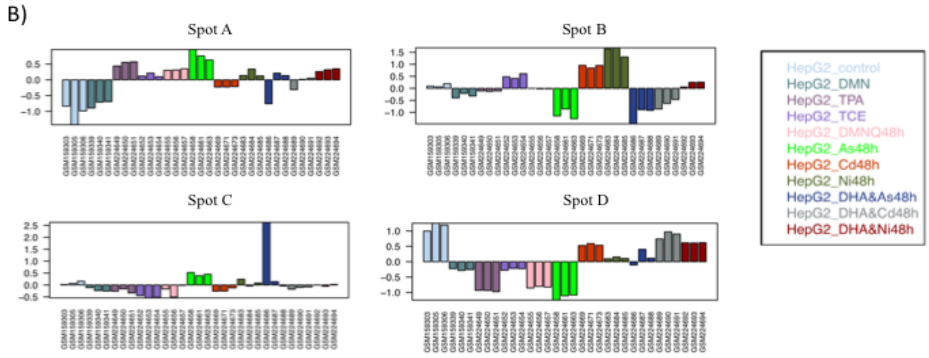


Figure 2. *Transcriptome landscape in response to heavy metal and carcinogen treatment. A) SOM summary map. Co-regulated functional gene clusters form red spot-like patterns. B) Sample-wise spot expression profiles.*

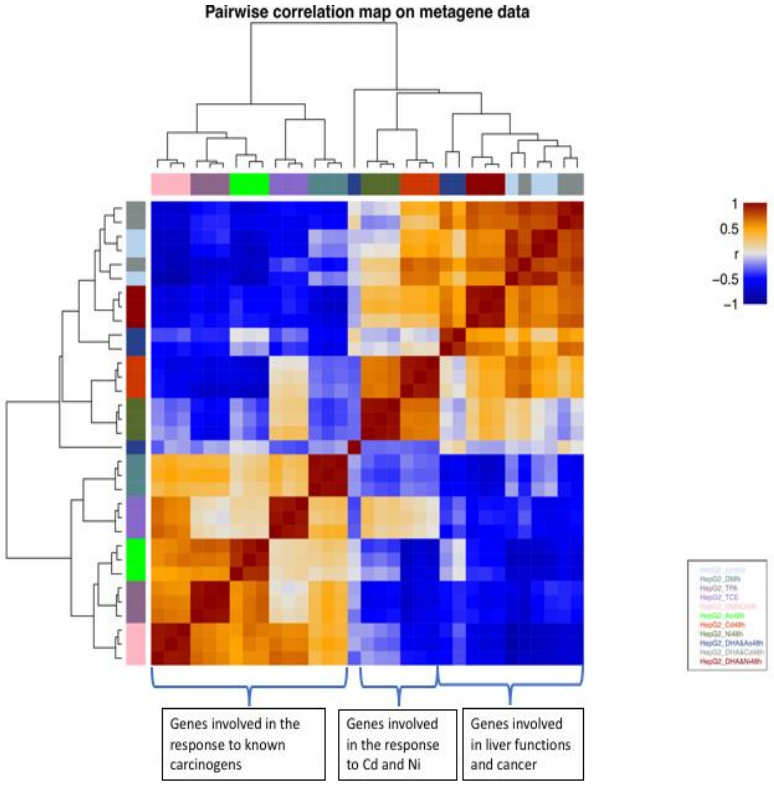


Figure 3. *Pairwise correlation of samples based on metagene data.*

4. Discussion

Several databases of gene expression patterns induced by heavy metals in human cell line experiments have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO;

http://www.ncbi.nlm.nih.gov/geo) and the results have been published [11]. However, until now, the contributions of biological pathways in gene expression alterations have been poorly discussed. We have analyzed expression data of heavy metals *in vitro* exposure on HepG2 cell line (accessible through GEO series accession number GSE8865) using self-organizing maps (SOM) machine learning. SOM portrayal approach has several advantages compared to other methods because it allows strong clustering, reduces data dimensionality and provides two-dimensional data images which enable visual evaluation of gene expression changes in different exposed groups [3], [6]. SOM analysis defined expression portraits specific to each investigated group with the spot clusters of co-regulated genes. According to the results, the common spot cluster was found for the groups which were treated with cadmium and nickel. The overexpressed genes of this spot are involved in response to hypoxia, cell proliferation, migration and cell-cell signalling. Further, the unique spot with differentially expressed genes cluster was revealed for the group exposed to arsenic, these genes are related to breast cancer and cervical cancer, EGRF signalling, cell cycle control, DNA replication, positive regulation of T and B cell proliferation. This results state that the carcinogenic effect of exposure is specific for all three heavy metals. Cadmium and nickel change the gene expression in the same way and a high number of hypoxia-related overexpressed genes might be due to ROS generation inducing oxidative stress, which is concordant with previous studies [7]. According to our results, there are no differentially expressed hypoxia-related genes in arsenic-treated samples and it seems that arsenic has a different mechanism of carcinogenic effect [8]. Moreover, our analysis revealed that gene expression patterns induced by cadmium and nickel treated with vitamin C are similar to those obtained in the untreated control group. Thus, SOM analysis revealed a common spot for mentioned three groups which include genes related to liver development, liver cancer, cholesterol biosynthesis, lipid metabolism, complement and coagulation cascades. Meanwhile, the expression patterns obtained in the group treated with arsenic and antioxidant were similar to arsenic; the overexpressed genes are involved in histone acetylation, B cell signalling, natural killer cell differentiation, colon cancer and breast cancer.

Interestingly, tested two carcinogen chemicals as well as the ROS generating substance and the third chemical show similar differentially expressed gene profiles, but none of them was comparable with heavy metals induced gene expression changes. In conclusion, changes in gene expression profile and molecular mechanisms of heavy metal exposure highly depend on the type of heavy metal.

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ԱՐՄԱՆ ՍԻՄՈՆՅԱՆ
Հայաստանի ամերիկյան համալսարանի 4-րդ կուրսի ուսանող
ԱՆԻ ՍՏԵՓԱՆՅԱՆ
կենսաբանական գիտությունների թեկնածու

ԾԱՆՐ ՄԵՏԱՂՆԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ՏԱԿ ՏՐԱՆՍԿՐԻՊՏՈՄԱՅԻՆ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐԻ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆ ԻՆՔՆԱԿԱԶՄԱՎԱՐՎՈՂ ՔԱՐՏԵԶՆԵՐԻ ՕԳՆՈՒԹՅԱՄԲ

Տվյալ աշխատանքում իրականացվել է հեպատոմայի բջջային կուլտուրաներում նիկելի, կադմիումի և արսենի ազդեցության ուսումնասիրություն: Այս նպատակով առկա կենսաչիպերով չափված գեների էքսպրեսիայի տվյալները խմբավորվել և ֆունկցիոնալ վերլուծության ենթարկվել են ինքնակառավարվող քարտեզների ալգորիթմի օգնությամբ: Արդյունքները ցույց են տվել, որ նիկելը և կադմիումը առաջացնում են հիպօքսիայի և օքսիդատիվ սթրեսի հետ ասոցացված գեների էքսպրեսիայի մակարդակի բարձրացում, մինչդեռ արսենը ակտիվացնում է քաղցկեղածին պրոցեսները: Այսպիսով՝ ստացված արդյունքները վկայում են, որ գեների էքսպրեսիայի փոփոխությունները խիստ կերպով կախված են ծանր մետաղի տեսակից:

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ИССЛЕДОВАНИЕ ТРАНСКРИПТОМНЫХ ИЗМЕНЕНИЙ ПОД ВОЗДЕЙСТВИЕМ ТЯЖЕЛЫХ МЕТАЛЛОВ С ПОМОЩЬЮ САМООРГАНИЗУЮЩИХСЯ КАРТ

В данной работе было проведено исследование влияния никеля, кадмия и мышьяка на экспрессию генов в культуре клеток гепатомы. С этой целью была проведена кластеризация и функциональная аннотация данных глобальной экспрессии генов с помощью алгоритма самоорганизующихся карт. Результаты показали, что под действием никеля и кадмия наблюдается гиперэкспрессия генов, связанных с гипоксией и оксидативным стрессом, тогда как под действием мышьяка происходит активирование путей, связанных с опухолеобразованием. Таким образом, полученные данные указывают на специфичность изменений экспрессии генов в зависимости от природы тяжелого металла.