

Gene expression analysis of colon high-grade dysplasia revealed new molecular mechanism of disease

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ABSTRACT

Aim: The aim of this research was to find a clear molecular view of dysplasia via network analysis.

Background: There are some evidence suggest the relationship between dysplasia and colorectal cancer. Understanding of high-grade dysplasia (HGD) could be beneficial for colon cancer management.

Methods: Bioinformatics study of HGD versus healthy subjects was conducted to check the status of differentially expressed genes (DEGs). GSE31106, GPL1261, GSM770092-94 and GSM770101-6 were the sources from gene expression omnibus (GEO) that queried for protein-protein interaction (PPI) network analysis via Cytoscape and its algorithms. Hubs of network were enriched for biochemical pathways and were validated via clustering analysis.

Results: Numbers of 46 hub nodes were determined and were included in 12 pathways. A main cluster including 76 nodes was identified containing 45 hubs. 33 hubs among 46 genes were involved in biochemical pathways. IL1B, IL6, TNF, and TRL4 were the most important critical genes.

Conclusion: Many different genes as hub nodes might influence the trigger and development of advance condition and also colon cancer.

Keywords: Transcriptome, Interactome, Colon cancer, High grade dysplasia.

(Please cite as: **Malekpour H, Heidari MH, Vafae R, Moravvej Farshi H, Khodadoostan M. Gene expression analysis of colon high-grade dysplasia revealed new molecular mechanism of disease. Gastroenterol Hepatol Bed Bench 2018;11(Suppl. 1):S111-S117.**)

Introduction

Colon cancer accounts for the third cause of cancer death in the world (1). A need for early detection and management of this malignant tumor is much sensed. In this regard, pre-modifications known as precancerous conditions could be important to study for introduction of molecular targets (1, 2). High-grade dysplasia

(HGD) refers to a condition prior to cancer occurrence. High throughput molecular analysis of this condition is a way to get a better knowledge of both dysplasia condition and its progressive form known as colon cancer (1, 3). Sequential modifications occur in the genetic basis and cancers such as colorectal manifests. Oncogenes and tumor suppressor genes have a role in these changes with opposite behaviors. The series of alteration is normally from benign adenomas to colorectal carcinomas and in its way, it modifies from low-grade dysplasia to HGD. Accumulating abnormal gene expressions such as APC, p53, DCC, survivin and

Received: 7 July 2018 Accepted: 23 November 2018

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RAS have a link with this transition as the normal tissue develops precancerous and eventually cancerous stages (4). Therefore, patients with dysplastic lesions are important to be investigated for follow-up and screening in case of cancer developments. What is more about these molecular targets at the precancerous conditions, is that to find the most accurate and specific ones (2). These agents could be investigated for topological features in a whole interaction map. In this way, more support and validation can be achieved. These maps are present in organisms as a protein-protein interaction network via physical correlations (5). Functions carried out in a cell is due to the normal interactions of these molecules. When it comes to the interaction pattern of molecules such as genes and proteins, normal expression and their functions are the key. Changes in expression of any of these genes, microRNAs, and proteins could result in abnormal interactions (6). The worst part is when these genes are with high centrality and are encountered with expression changes. Furthermore, gene ontology analysis of the central elements could provide more information about the disease underlying mechanisms (7). It can show which related biological process to these central agents could be disrupted and therefore worth more evaluations. To reach this goal, there are algorithms available that could analyze the genes corresponding terms. In this research, protein-protein interaction network of differentially expressed genes of HGD in comparison with normal condition is analyzed to introduce the most promising ones for clinical applications.

Methods

Data collection

Microarray Data (GSE31106, GPL1261, GSM770092-94 and GSM770101-6) were considered to compare Gene expression profiles of normal group and treated five-week-old male mice with intraperitoneal injected with 10mg/kg Azoxymethane. The samples three cycles with Dextran sulfate sodium (2%, 1.5%, and 1.5%) were treated while controls were treated with saline injection and drinking distilled water. The extracted RNAs from colorectal tissue after 6 weeks of treatment were analyzed by Affymetrix GeneChip Mouse Genome 430 2.0 Array.

Characterized DEGs with fold change (FC) less than 0.5 and more than 2 among the 250 top significant DEGs were selected to be examination via PPI network analysis.

PPI network analysis

STRING database (8) and Cytoscape software (9) version 6.3.2 were applied to construct PPI network. The network was evaluated by Network Analyzer application of Cytoscape and the hubs were selected based on degree cutoff (mean+2SD) (10). Related biological processes to the hub nodes were identified by ClueGO v 2.5.0 plugin of Cytoscape software from KEGG 20.11.2017.

Statistical analysis

Gene expression profiles were matched via boxplot analysis and p-value <0.05 was considered for significant findings. Biological processes were identified based on K-score, at least 10 genes/Term, and 10% participation of genes per term.

Results

Gene expression profiles of 6 HGD samples and 3 controls were matched via box plot analysis. As it is shown in the figure 1, the samples are comparable. It is appeared that 50% of genes are characterized in high levels of expression in both HGD and normal control samples. Numbers of 250 top significant DEGs based on p-value criterion were selected. Among 250 selected DEGs numbers of 24 individual were not characterized which excluded for more analysis. Numbers of 129 DEGs amongst 226 significant and characterized DEGs were included in PPI network analysis based on fold change less than 0.5 and above 2. STRING database recognized 98 DEGs and network was constructed via these ones and 50 added relevant genes. The network was included 34 isolated nodes and a main connected component. This component which we call it as network of HGD, contains 114 nodes and 1451 edges. Centrality analysis leads to introduce 46 hub nodes that are tabulated in the table 1. Betweenness centrality (BC) and closeness centrality (11) of the determined hubs are presented in this table. Numbers of 12 pathways related to the 46 hub nodes from KEGG were identified (Figure 2). As it is shown in figure 2, 32 hubs are involved in these pathways. Two hub nodes; IL1B and IL6 are complicated in 92% of pathways while

Table 1. Hub nodes of HGD network.

| R | display name | Description | Degree | BC | CC |
|----|--------------|--|--------|-------|-------|
| 1 | GAPDH | glyceraldehyde-3-phosphate dehydrogenase | 67 | 0.059 | 0.706 |
| 2 | IL6 | interleukin 6 (interferon, beta 2) | 64 | 0.021 | 0.677 |
| 3 | TNF | tumor necrosis factor | 63 | 0.025 | 0.673 |
| 4 | INS | Insulin | 63 | 0.058 | 0.681 |
| 5 | TGFB1 | transforming growth factor, beta 1 | 58 | 0.071 | 0.657 |
| 6 | IL8 | interleukin 8 | 58 | 0.026 | 0.653 |
| 7 | ALB | Albumin | 58 | 0.019 | 0.653 |
| 8 | CCL2 | chemokine (C-C motif) ligand 2 | 58 | 0.012 | 0.657 |
| 9 | AKT1 | v-akt murine thymoma viral oncogene homolog 1 | 58 | 0.046 | 0.661 |
| 10 | VEGFA | vascular endothelial growth factor A | 57 | 0.029 | 0.649 |
| 11 | IL1B | interleukin 1, beta | 56 | 0.024 | 0.646 |
| 12 | JUN | jun proto-oncogene | 56 | 0.021 | 0.661 |
| 13 | PRDM10 | PR domain containing 10 | 56 | 0.027 | 0.657 |
| 14 | IL10 | interleukin 10 | 54 | 0.009 | 0.638 |
| 15 | IL4 | interleukin 4 | 54 | 0.013 | 0.638 |
| 16 | CSF2 | colony stimulating factor 2 (granulocyte-macrophage) | 54 | 0.012 | 0.638 |
| 17 | MMP9 | matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) | 53 | 0.005 | 0.631 |
| 18 | TLR4 | toll-like receptor 4 | 52 | 0.008 | 0.631 |
| 19 | DECR1 | 2,4-dienoyl CoA reductase 1, mitochondrial | 51 | 0.046 | 0.635 |
| 20 | SRC | v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) | 51 | 0.015 | 0.631 |
| 21 | VCAM1 | vascular cell adhesion molecule 1 | 51 | 0.009 | 0.628 |
| 22 | NOS3 | nitric oxide synthase 3 (endothelial cell) | 50 | 0.028 | 0.628 |
| 23 | TP53 | tumor protein p53 | 49 | 0.051 | 0.632 |
| 24 | CXCR4 | chemokine (C-X-C motif) receptor 4 | 48 | 0.007 | 0.608 |
| 25 | ITGAM | integrin, alpha M (complement component 3 receptor 3 subunit) | 48 | 0.011 | 0.611 |
| 26 | IL13 | interleukin 13 | 48 | 0.008 | 0.611 |
| 27 | IL17A | interleukin 17A | 47 | 0.025 | 0.595 |
| 28 | TLR2 | toll-like receptor 2 | 47 | 0.014 | 0.604 |
| 29 | MAPK1 | mitogen-activated protein kinase 1 | 47 | 0.019 | 0.614 |
| 30 | EGFR | epidermal growth factor receptor | 46 | 0.047 | 0.604 |
| 31 | F2 | coagulation factor II (thrombin) | 45 | 0.015 | 0.608 |
| 32 | EGF | epidermal growth factor | 45 | 0.012 | 0.604 |
| 33 | CXCL12 | chemokine (C-X-C motif) ligand 12 | 45 | 0.003 | 0.592 |
| 34 | CD44 | CD44 molecule (Indian blood group) | 44 | 0.004 | 0.598 |
| 35 | IL18 | interleukin 18 (interferon-gamma-inducing factor) | 43 | 0.003 | 0.579 |
| 36 | CAT | Catalase | 43 | 0.040 | 0.601 |
| 37 | IGF1 | insulin-like growth factor 1 (somatomedin C) | 43 | 0.007 | 0.592 |
| 38 | NOS2 | nitric oxide synthase 2, inducible | 43 | 0.014 | 0.598 |
| 39 | SPP1 | secreted phosphoprotein 1 | 41 | 0.021 | 0.577 |
| 40 | MMP2 | matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) | 41 | 0.002 | 0.582 |
| 41 | ITGAX | integrin, alpha X (complement component 3 receptor 4 subunit) | 40 | 0.006 | 0.562 |
| 42 | MYD88 | myeloid differentiation primary response 88 | 39 | 0.032 | 0.562 |
| 43 | CCR5 | chemokine (C-C motif) receptor 5 (gene/pseudogene) | 37 | 0.002 | 0.554 |
| 44 | AGT | angiotensinogen (serpin peptidase inhibitor, clade A, member 8) | 36 | 0.008 | 0.559 |
| 45 | CCR2 | chemokine (C-C motif) receptor 2 | 35 | 0.001 | 0.549 |
| 46 | CXCR2 | chemokine (C-X-C motif) receptor 2 | 34 | 0.001 | 0.538 |

EGF, EGFR, GAPDH, IGF1, INS, MMP2, MMP9, CXCL12, SPP1 are convoluted in one term, see table 2.

The network was analyzed for protein clustering by

ClusterOne plug-in, Cytoscape as indicated in figures 3 and 4.

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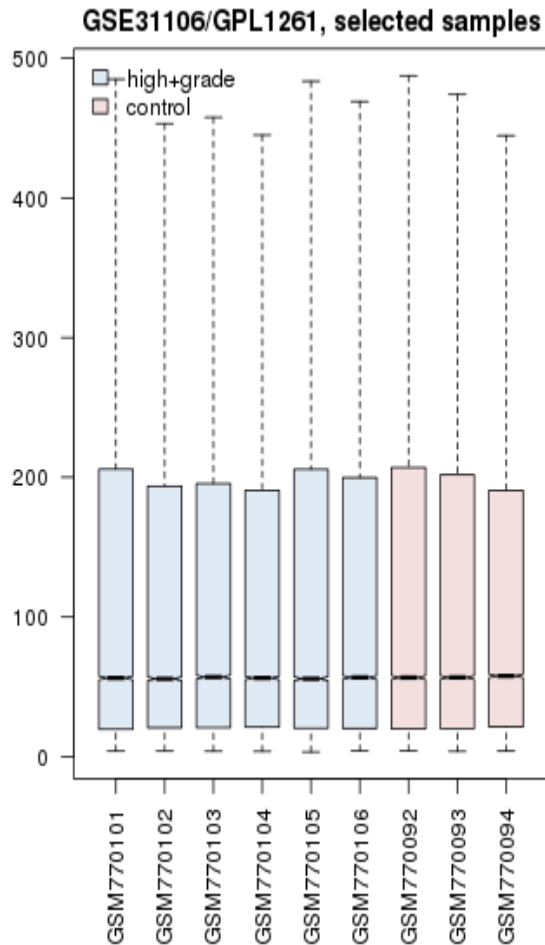


Figure 1. Gene expression profiles of 6 HGD mice and 3 normal controls.

Discussion

HGD is important to study for colorectal cancer management. This precancerous stage could be essential for identification of factor triggering tumorigenesis. One way to reach this purpose is through molecular research. Data mining; in addition, could assist adding more information and values related to the identified molecular agents corresponding to any conditions (12). Protein-protein interaction network is one of assessment biomarkers in terms of centrality role in an interaction network. In this network analysis approach, we identify central of differentially expressed genes network in HGD via associated methods and algorithms. To do this, at first, the quality of expression profile of samples of healthy and dysplasia groups were compared in figure 1. The analysis shows that the data is suitable for comparison as the samples are median-centered.

| GO Term | Ontology Source | Term P/Value |
|--|-----------------|--------------|
| Salmonella infection | KEGG_20.11.2017 | 8.3 E-16 |
| Toll-like receptor signaling pathway | KEGG_20.11.2017 | 7.2 E-16 |
| Amoebiasis | KEGG_20.11.2017 | 2.8 E-16 |
| Pertussis | KEGG_20.11.2017 | 1.9 E-17 |
| NF-1 signaling pathway | KEGG_20.11.2017 | 8.2 E-18 |
| IL-17 signaling pathway | KEGG_20.11.2017 | 2.4 E-18 |
| Leishmaniasis | KEGG_20.11.2017 | 1.3 E-19 |
| AGE-RAGE signaling pathway in diabetic complications | KEGG_20.11.2017 | 8.4 E-20 |
| Inflammatory bowel disease (IBD) | KEGG_20.11.2017 | 2.6 E-20 |
| Chagas disease (American trypanosomiasis) | KEGG_20.11.2017 | 1.0 E-21 |
| Malaria | KEGG_20.11.2017 | 5.9 E-22 |
| Rheumatoid arthritis | KEGG_20.11.2017 | 1.6 E-22 |

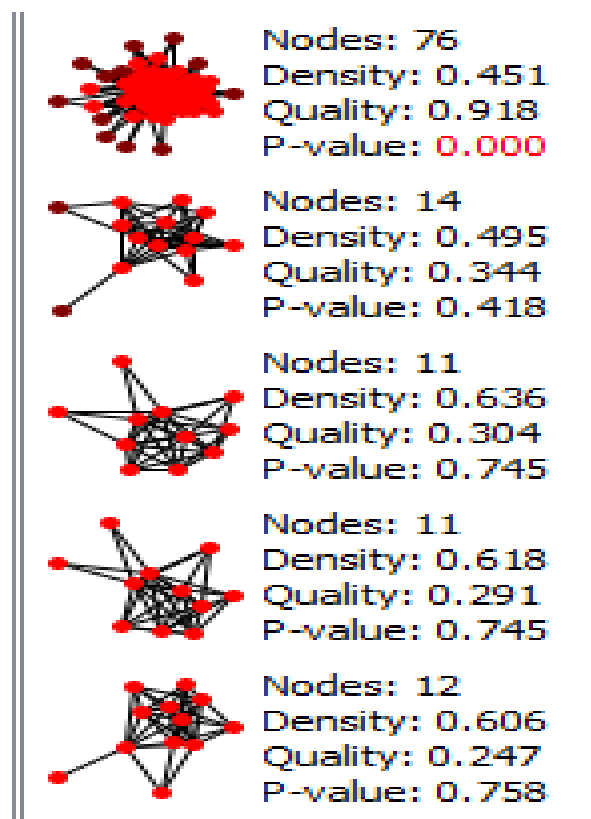
- 10 [CSF2, CXCL8, IL18, IL1B, IL6, JUN, MAPK1, MYD88, NOS2, TLR4]
- 11 [AKT1, CXCL8, IL1B, IL6, JUN, MAPK1, MYD88, SPP1, TLR2, TLR4, TNF]
- 11 [CSF2, CXCL8, IL10, IL1B, IL6, ITGAM, NOS2, TGFB1, TLR2, TLR4, TNF]
- 11 [CXCL8, IL10, IL1B, IL6, ITGAM, JUN, MAPK1, MYD88, NOS2, TLR4, TNF]
- 12 [AKT1, EGF, EGFR, GAPDH, IGF1, IL8, INS, MAPK1, NOS2, NOS3, TLR4, VEGFA]
- 12 [CCL2, CSF2, CXCL8, IL13, IL17A, IL1B, IL4, IL6, JUN, MAPK1, MMP9, TNF]
- 12 [IL10, IL1B, IL4, ITGAM, JUN, MAPK1, MYD88, NOS2, TGFB1, TLR2, TLR4, TNF]
- 13 [AKT1, CCL2, CXCL8, IL1B, IL6, JUN, MAPK1, MMP2, NOS3, TGFB1, TNF, VCAM1, VEGFA]
- 12 [IL10, IL13, IL17A, IL18, IL1B, IL4, IL6, JUN, TGFB1, TLR2, TLR4, TNF]
- 14 [AKT1, CCL2, CXCL8, IL10, IL1B, IL6, JUN, MAPK1, MYD88, NOS2, TGFB1, TLR2, TLR4, TNF]
- 12 [CCL2, CXCL8, IL10, IL18, IL1B, IL6, MYD88, TGFB1, TLR2, TLR4, TNF, VCAM1]
- 14 [CCL2, CSF2, CXCL12, CXCL8, IL17A, IL18, IL1B, IL6, JUN, TGFB1, TLR2, TLR4, TNF, VEGFA]

Figure 2. Pathways related to the hubs of dysplasia network. The involved genes in each process are presented in the bottom of figure. Except the green colored one, the other pathways are grouped in one cluster. Sequence of genes rows is corresponded with pathways up to down, respectively.

GEO2R identified genes with modified expressions and these genes were queried in Cytoscape for a network construction. The network centrality analysis introduced 46 hub genes that almost none were from DEGs of HGD. Moreover, these genes are very close in degree values and could be very important in the network integrity. Among 114 nodes, 46 individuals were identified as hubs. On the other hand, about 40% of nodes are hubs. What is more, these genes are divided in four categories consisted of immune related genes (such as ILs), oncogenes (as like AKT1, JUN, and SRC), metabolism related genes (especially INS), and other types of genes (13-15).

Table 2. Hub nodes that are involved in the 12 pathways.

| R | Gene name | Number of relevant pathways |
|----|---|-----------------------------|
| 1 | IL1B, IL6 | 11 |
| 2 | TNF, TRL4 | 10 |
| 3 | JUN | 9 |
| 4 | CXCL8, MPK1 | 8 |
| 5 | TLR2, TGFB1 | 7 |
| 6 | NOS2, MYD88, IL10 | 6 |
| 7 | CCL2 | 5 |
| 8 | CSF2, IL18, AKT1 | 4 |
| 9 | ITGAM, VEGFA, IL17A, IL4 | 3 |
| 10 | NOS3, VCAM1, IL13 | 2 |
| 11 | EGF, EGFR, GAPDH, IGF1, INS, MMP2, MMP9, CXCL12, SPP1 | 1 |

**Figure 3.** Protein cluster analysis of hub nodes via ClusterOne application is shown. The cluster was elected based on at least ten genes per cluster.

Oncogenes including AKT1, JUN, SRC are a gene set that are also prominent in colon cancer (16). The other

genes belong to metabolic pathways are GAPDH, INS, IGF1 which play significant role in proliferation and apoptosis in colon cancer (11, 17).

Functional categorization of hub genes indicated 12 associated pathways in figure 2 that except one of the pathway, Hif-1 signaling pathway which highlighted in green color, other terms are presented in the same group. Distribution of hub genes in the related pathways was analyzed in table 2. The findings indicate that IL1B and IL6 are mostly involved in the all biological terms similarly TNF and TRL4 as next rank participate in 10 biological terms. These elements of these two rows are all linked to immune system category.

Furthermore, clustering analysis leads to introduce one significant cluster which contains approximately all the hubs except for NOS3. This clustering could validate the importance of these identified hubs in the HGD network. To get a better understanding, a literature review of the hub genes that are present in the most pathways (the first two rows) as well as the first two top ones among 46 genes is conducted for possible relationship with colon cancer. GAPDH, glyceraldehyde-3-phosphate dehydrogenase as a pleiotropic enzyme (18) has the highest degree, betweenness, and closeness amounts in this network and it is active in apoptosis (19). This gene has been referred with highlighted moonlighting effect in cancer development (20). It shows it is a possible key role in transition from dysplasia to cancer states. In addition, it's up-regulation has been reported for colorectal cancer (17). IL 6 the next gene that is very important in cancer, its increment has been also associated with colorectal cancer progression (21). The higher the level of IL 6 in human serum, the more developed the tumor (22). This gene is also ranked as the first group in table 2. In this grouping, IL1B as another inflammatory system gene that is famous in gastrointestinal system and promotes invasion in colorectal tumor as well (23). TNF in the next group, high signaling levels could be important in colon cancer (13). TRL4 is also reported for colon metastasis. In fact, multiple roles has been identified for this gene (24).

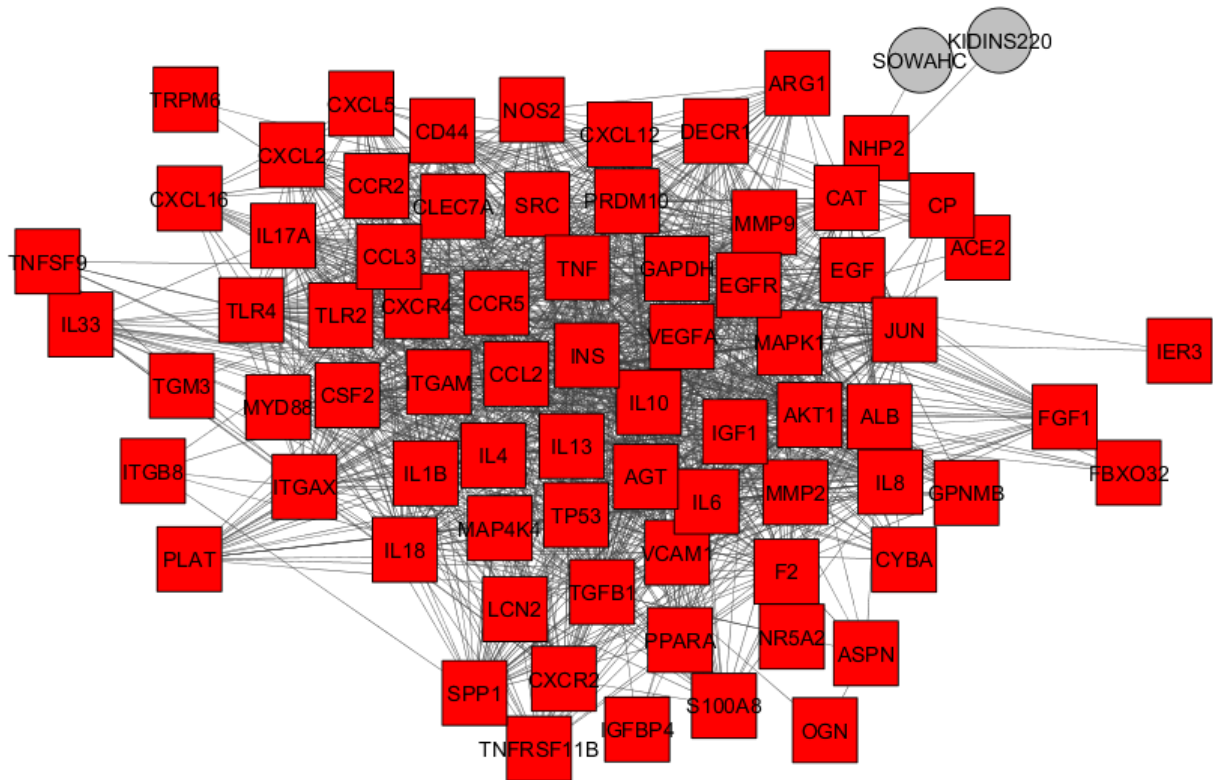


Figure 4. Cluster 1 PPI network. The two out layer nodes are displayed in gray color

In this research, it was confirmed that advance dysplasia is accompanied with vast alterations in gene expression algorithm of human body. In this regard immune system, metabolic pathways, and oncogenes are affected. In addition, deregulation of immune system and inflammation is prominent in HGD. This complexe condition in HGD may led to onset of colorectal cancer (25).

A further comprehensive knowledge of colorectal cancer and its prediction are interpreted by identification of crucial genes, which are involved in HGD (26, 27). This set of possible biomarkers and the related biological processes may play critical roles in transition between HGD and colon cancer. However, the exact participation of these genes will require more in-depth research for clinical setting.

Conflict of interests

The authors declare that they have no conflict of interest.

References

1. Hartmans E, Orian-Rousseau V, Matzke-Ogi A, Karrenbeld A, de Groot DJA, de Jong S, et al. Functional genomic mRNA profiling of colorectal adenomas: identification and in vivo validation of CD44 and splice variant CD44v6 as molecular imaging targets. *Theranostics* 2017;7:482.
2. Bashizadeh-Fakhar H, Rezaie-Tavirani M, Zali H, Faraji R, Nejad EK, Aghazadeh M. The Diagnostic Value of Serum CEA, CA-125, and ROMA Index in Low-Grade Serous Ovarian Cancer. *Int J Cancer Manag* 2018;11:e63397.
3. Larki P, Gharib E, Yaghoob Taleghani M, Khorshidi F, Nazemalhosseini-Mojarad E, Asadzadeh Aghdaei H. Coexistence of KRAS and BRAF Mutations in Colorectal Cancer: A Case Report Supporting The Concept of Tumoral Heterogeneity. *Cell J.* 2017;19:113-117.
4. Sharifian A, Pourhoseingholi MA, Emadedin M, Rostami Nejad M, Ashtari S, Hajizadeh N, et al. Burden of Breast Cancer in Iranian Women is Increasing. *Asian Pac J Cancer Prev.* 2015;16:5049-52.
5. Mansouri V, Zadeh-Esmaeel M-M, Rostami-Nejad M, Rezaei-Tavirani M. Comparative study of gastric cancer and chronic gastritis via network analysis. *Gastroenterol Hepatol Bed Bench* 2018;11:343-51.

6. Koushki M, Atan NAD, Omidi-Ardali H, Tavirani MR. Assessment of Correlation Between miR-210 Expression and Pre-Eclampsia Risk: A Meta-Analysis. *Rep Biochem Mol Biol* 2018;7:94-101.
7. Rezaei-Tavirani M, Tavirani SR, Rostami FT. Biochemical pathway analysis of gastric atrophy. *Gastroenterol Hepatol Bed Bench* 2018;11:118-24.
8. Eesteghamati A, Gouya M, Keshtkar A, Najafi L, Zali MR, Sanaei M, et al. Sentinel hospital-based surveillance of rotavirus diarrhea in iran. *J Infect Dis.* 2009;200:S244-47.
9. Zhi J, Sun J, Wang Z, Ding W. Support vector machine classifier for prediction of the metastasis of colorectal cancer. *Int J Mol Med* 2018;41:1419-26.
10. Safari-Alighiarloo N, Rezaei-Tavirani M, Taghizadeh M, Tabatabaei SM, Namaki S. Network-based analysis of differentially expressed genes in cerebrospinal fluid (CSF) and blood reveals new candidate genes for multiple sclerosis. *Peer J* 2016;4:e2775.
11. Nazemalhosseini Mojarad E, Kuppen PJ, Aghdaei HA, Zali MR. The CpG island methylator phenotype (CIMP) in colorectal cancer. *Gastroenterol Hepatol Bed Bench.* 201;6:120-8.
12. Azodi MZ, Rezaei-Tavirani M, Nejad MR, Rezaei-Tavirani M. Human Prolactinoma: A View of Protein-Protein Interaction Pattern. *Int J Endocrinol Metab* 2018;16:e67332
13. Kraus S, Arber N. Inflammation and colorectal cancer. *Current opinion in pharmacology* 2009;9:405-10.
14. Watt SK, Hasselbalch HC, Skov V, Kjær L, Thomassen M, Kruse TA, et al. Whole Blood Gene Expression Profiling in patients undergoing colon cancer surgery identifies differential expression of genes involved in immune surveillance, inflammation and carcinogenesis. *Surg Oncol* 2018;27:208-15.
15. Principe DR, DeCant B, Mangan RJ, Wayne EA, Diaz AM, Vitello D, et al. TGF β signaling deficiency enhances tumor associated inflammation in colon cancer *AACR*; 2015.
16. Rostami Nejad M, Rostami K, Cheraghpour K, Nazemalhosseini Mojarad E, Volta U, Al Dulaimi D, et al. Celiac disease increases the risk of *Toxoplasma gondii* infection in a large cohort of pregnant women. *Am J Gastroenterol.* 2011;106:548-9.
17. Tang Z, Yuan S, Hu Y, Zhang H, Wu W, Zeng Z, et al. Over-expression of GAPDH in human colorectal carcinoma as a preferred target of 3-bromopyruvate propyl ester. *J Bioenerg Biomembr* 2012;44:117-25.
18. Tarze A, Deniaud A, Le Bras M, Maillier E, Mollé D, Larochette N, et al. GAPDH, a novel regulator of the proapoptotic mitochondrial membrane permeabilization. *Oncogene* 2007;26:2606.
19. Sirover MA. GAPDH: A Multifunctional Moonlighting Protein in Eukaryotes and Prokaryotes. *Moonlighting Proteins: Novel Virulence Factors in Bacterial Infections* 2017:147-67.
20. Sirover MA. Pleiotropic effects of moonlighting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cancer progression, invasiveness, and metastases. *Cancer Metastasis Rev* 2018:1-12.
21. Tajbakhsh M, García Migura L, Rahbar M, Svendsen CA, Mohammadzadeh M, et al. Antimicrobial-resistant *Shigella* infections from Iran: an overlooked problem? *J Antimicrob Chemother.* 2012;67:1128-33.
22. Knüpfer H, Preiss R. Serum interleukin-6 levels in colorectal cancer patients—a summary of published results. *Int J Colorectal Dis* 2010;25:135-40.
23. Li Y, Wang L, Pappan L, Gallier-Beckley A, Shi J. IL-1 β promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. *Molecular cancer* 2012;11:87.
24. Yesudhas D, Gosu V, Anwar MA, Choi S. Multiple roles of toll-like receptor 4 in colorectal cancer. *Front Immunol* 2014;5:334.
25. Nazemalhosseini Mojarad E, Farahani RK, Haghghi MM, Aghdaei HA, Kuppen PJ, Zali MR. Clinical implications of BRAF mutation test in colorectal cancer. *Gastroenterol Hepatol Bed Bench.* 2013;6:6-13.
26. Norouzinia M, Asadzadeh H, Shalmani HM, Al Dulaimi D, Zali MR. Clinical and histological indicators of proximal and distal gastric cancer in eight provinces of Iran. *Asian Pac J Cancer Prev.* 2012;13:5677-79.
27. Zamanian Azodi M, Peyvandi H, Rostami-Nejad M, Safaei A, Rostami K, Vafaei Ret al. Protein-protein interaction network of celiac disease. *Gastroenterol Hepatol Bed Bench.* 2016;9:268-277.