What is the Impact of *Enterococcus Faecalis* Infection on Gastric Cancer; Friend or Foe?

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Abstract

Background: *Enterococcus faecalis* (*E. faecalis*) is an important commensal microbiota member of the human gastrointestinal tract. Many studies have shown that infection rates with *E. faecalis* increase significantly in gastric cancer. The infections that developed during the cancer progress are definitive scientific evidence, but it is still not clear whether this effect is in the benefit (decrease in metastasis) or damage of the host (increase in proliferation, invasion, stem cell like phenotype) due to the infection factor. These opposed data can provide significant contrubition in the understanding of cancer progress when it is analyzed detailed.

Methods: In this study, we determined altered genes related to *E. faecalis* infection in gastric cell line MKN74 and relevant pathways to figure out whether infection slows or accelerates cancer progression. The gene expression data were retrieved from Array Express (E-MEXP-3496). Variance, t-test and linear regression analysis, hierarchical clustering, network, and pathway analysis were performed.

Results: We determined 12 genes corresponding 15 probesets expressions were downregulated following the live infection of gastric cancer cells with *E. faecalis*. We identified the network between these genes and the pathways to which they belong. Pathway analysis results have shown that these genes are mostly associated with cancer cell proliferation.

Conclusions: Almost all of the genes that we detected as significantly downregulated have an adverse effect especially on the proliferation of cancer cells in the process of infection. In this case, it seems that *E. faecalis* infection may be an important factor that slows gastric cancer proliferation.

Background

Gastric cancer has a new cases rate of 5.7% among the five common cancers with a mortality rate of 8.2% among the ten common cancers according to 2018 GLOBOCAN data (1). Risk factors include genetics, gastric ulcer, diet, smoking and alcohol use, but the main risk factor is bacterial infections (2). Infections are very important due to their effects on various mechanisms in the chemotherapeutic response. Cancer patients are more likely to get infections from flora due to weakened immune systems and various treatment procedures.

90% of gastric cancer cases are associated with bacterial infections especially *Helicobacter pylori gastritis* and it has been found that it increases the odds gastric cancer approximately 6 times within 10 years after infection. However, data on the roles of bacterial infections in cancer are definitely in two different directions. Some researchers suggest that bacterial infections have a protective role, while others emphasize the harmful effects associated with infection.

Enterococcus are a part of the normal intestinal flora of humans and animals (3). *Enterococcus faecalis* (*E. faecalis*) has a fundamental roles on the development of intestinal immunity in the early stages of life in the human gastrointestinal tract and plays as a protective agent in regulating colon homeostasis in

newborn babies (4–6). Also, due to anti-inflammatory potential, it is considered to be probiotic in the treatment of certain diseases, such as chronic sinusitis, bronchitis, or infant acute diarrhea (7–10). Besides its beneficial effects it is also known to play important roles in the formation of systemic infections and translocations (11, 12). It can easily colonized in extra-intestinal and unusual areas and can cause infections throughout certain predisposing conditions such as long-term hospitalizations and some immune compromised conditions.

It has been reported in many studies that the increase in infections caused by *E. faecalis* in various types of cancer is very common. An increase in *E. faecalis*-related infections has been noted in cancerous lesions of patients with oral and oropharyngeal squamous cell carcinoma (13). The level of *E. faecalis* infection has been shown to increase markedly in colon cancer and lead to chromosomal imbalance (11). It triggers poor prognosis by causing invasion in bladder cancer cells (14). It has been shown that cells that interact with *E. faecalis* trigger the production of reactive oxygen species (ROS) and the mitochondrial genome is impaired in gastric cancer (15).

However, uncontrolled overproduction of ROS can lead to carcinogenic, cytotoxic results. On the contrary in another study, it was found that the colorectal cancer cell line HCT-116-cocultured with *E. faecalis* downregulated the expression of the FIAF gene, which is normally associated with the development of some cancer types (16). It has been suggested that *E. faecalis* strains isolated from human breast milk may be candidates for the prevention and treatment of cancer when they inhibit the proliferation of breast cancer cells (17).

These opposed data can provide significant contrubition in the understanding of cancer progress when it is analyzed more detail whether the interaction of the *E. faecalis* will benefit or harm the host. Although there are studies reporting genetic alterations in gastric cancer caused by *E. faecalis* that may affect the chemotherapeutic response and patients' prognosis, withal their roles, related pathways and mechanisms is remains unclear.

In the current study, we aimed to determine the *E. faecalis*-related altered genes, the network relations of these genes with each other and to relevant pathways using all genome profiles of gastric cancer cells. The results can provide important information about how prognosis can be affected in the presence of infection.

Methods

Microarray gene expression data

The gene expression data was obtained from the Cancer Genome Project (CGP) database (http://www.cancerrxgene.org/). Transcription profile data of human MKN74 gastric epithelial cells treated with *Enterococcus faecalis* were obtained from Array Express (E-MEXP-3496) (18).

Processing And Normalization Of Data

The raw data from Array Express were normalized with the Affy package in the R software. Normalized transcription profile data consists of 23,344 different genes / 54,675 probesets. The data contains control day 1, control day 5, *E. faecalis* infected day 1 and *E. faecalis infected day 5* groups' all genome expression data and they were triplicated.

Variance, T-test And Linear Regression Analysis

Among the existing groups, significant genes with a standard deviation value above 1 were identified. In order to group the identified genes more specifically, Pearson's correlation absolute P-value based on the correlation coefficient was calculated and genes above 0.05 were selected. In addition, Pearson correlation coefficient value (R-value) was calculated and genes above 0.99 were selected. The expression values of the genes whose linear regression analysis was completed were compared with the control groups by two tail T-Test analysis and genes with a t-test p value of less than 0.01 were selected.

Hierarchical Clustering

Genes that were determined from linear regression analyzes were clustered hierarchically with mean standardized gene expression values by the Euclidean Gene Cluster 3.0 program. The data was standardized after cluster analysis and standardized data were viewed using Treeview. Hierarchical clustering was performed by clustering both genes and arrays using Euclidian distance as similarity metric and complete linkage as clustering method.

Network Analysis

To generate a network based on coexpression and genetic interactions the GeneMANIA software in Cytoscape were used and genes in similar pathways were identified (19). Out of the 138 genes/165 probesets of interest, the 12 genes that were differentially expressed were used as input. Data integrated, analyzed and visualized in order to find if sets have functionally similar genes related to each other and to determine the associated functions for different groups of genes in the network. Thus, the network relationship of these genes were determined.

Pathway Enrichment Analysis

To understand biological meaning behind our list of genes the "Database for Annotation, Visualization and Integrated Discovery" (DAVID) software was used (20, 21). The pathways to which our genes are related were determined.

Gene Set Enrichment Analysis (gsea)

The gene set enrichment analysis (GSEA) was carried out in concordance with GSEA guideline procedure (http://software.broadinstitute.org/gsea/docGSEAUserGuideFrame.html). In order to perform this analysis, E-MEXP-3496 data was used. This data includes 54,675 probe sets (23,344 different genes). In addition to this, GSEA was performed between control day 5 group and infected day 5 group so as to comprehend the *E. Faecalis* infection pattern between these groups. The fundamental purpose of this analysis is to determine which gene significantly enrich in which gene set that belongs to the GSEA as well as to understand which gene set enrich in which groups.

As considering these aims, GSEA algorithm calculate the Enrichment Score (ES), Normalized Enrichment Score (NES), Nominal p-value (NOM p-value), False Discovery Rate q-value (FDR q-value), Familywise Error Rate p-value (FWER). The ES value indicate gene's maximum deviation in gene set in other words this score helps to find most upregulated genes. NES value represents the connection or difference between gene sets and gene expression. The higher NES value shows the elevation of permutations. Hence, higher NES value increase significance of gene sets. In addition to ES and NES values, NOM p-value evaluates the importance of ES calculation. Therefore, NOM p-value directly correlated with ES as well as NES value. Increase of NOM p-value show critical role of ES. On the other hand, FWER p-value indicates false positives probability of NES and so, lower FWER p-value directly and significantly correlated with correctness of NES calculation. Moreover, FDR q-value is the most vital value of this analysis. This value needs to be lower than 0.25 and even, when this value become smaller, the enrichment of gene sets is more meaningful.

Statistical analysis

Statistical analyses were done using GraphPad Prism 5.0 (Graphpad Prism 5 Software, San Diego, CA, USA). To determine the differences between groups two tail T-test were performed. Genes with a t-test p value less than 0.01 were selected. In Pearson analysis p value less than 0.05 were selected.

Results

Time-dependent gene expression alterations in the MKN74 gastric cancer cell line treated with E. faecalis

To determine the gene expression alterations between control day 1, infected day 1and infected day 5 groups of human gastric carcinoma MKN74 cells all genome expression data analyzed by lineer regression. Based on the results, 138 genes corresponding to 165 probesets with a standard deviation value above 1.0, P-value below 0.05, and Pearson R value above 0.99 showed statistically significant expression alteration. We focused on these genes that altered variation in expression between groups for further analyzes. 10 probesets were positively correlated and upregulated and 155probesets were negatively correlated and downregulated in the time depended *E. faecalis* treated groups (Additional File 1).

Our hierarchical cluster analysis demonstrated gene alterations between 3 certain groups; control day 1, infected day 1 and infected day 5. Results defining as 155 of the probesets were negatively correlated, high expressed in the control day 1 group and expressions are decreases at infected day 1 group and so on at infected day 5 group. And conversely 10 of the probesets were positively correlated, low expressed in the control day 1 group and expressions are increases at infected day 1 and so on at infected day 5 group (Fig. 1). So, there were significantly altered genes between control and treated groups.

Gene alterations due to treatment with E. faecalis

In order to determine whether this expression change was caused by *E. faecalis* or cancer itself, varience analyzes and t-test were performed between control day 5 group and infected day 5 group 138 genes/165 probesets expression data. To better analyze gene expression alteration, long range group “infected day 5” group was selected for variance analysis and t-test. Thus, 12 statistically significant genes (NDC80, NCAPG, CENPA, KIF23, BUB1B, BUB1, CASC5, KIF2C, CENPF, SPC25, SMC4, KIF20A) corresponding 15 probesets with a standard deviation above 1.0 and a T-test p-value less than 0.01 were detected and selected (Table 1) (Additional File 2).

Table 1

The list of 12 genes (15 Probe Sets) which have the most alterations in expression. These genes have standard deviation above 1.0 and T-test value less than 0.01 between 5 days control group and 5 days treated group. These significant values indicate that the change occurred due to infection.

Probe Set	Gene Symbol	St Dev	T-Test
204162_at	NDC80	1.76016363	0.00153695
218663_at	NCAPG	1.74829872	0.0002009
204962_s_at	CENPA	1.5109911	0.00039232
218662_s_at	NCAPG	1.46733952	0.00062378
204709_s_at	KIF23	1.42573476	0.00046214
203755_at	BUB1B	1.36025723	1.7849E-05
215509_s_at	BUB1	1.30655845	0.00019534
209642_at	BUB1	1.26836983	0.0001898
228323_at	CASC5	1.19910534	4.6314E-05
211519_s_at	KIF2C	1.17437172	0.00024729
207828_s_at	CENPF	1.13683208	8.8436E-05
209891_at	SPC25	1.13377039	0.00260856
201664_at	SMC4	1.05623047	0.00770427
218755_at	KIF20A	1.02361512	0.0033544
209408_at	KIF2C	1.02269745	0.00046939

All of these 12 genes were found negatively correlated and downregulated in the linear regression analysis.

Figure 2 shows the expression alterations of these 12 genes time-based between control day 1, infected day 1 and infected day 5 (Fig. 2). Gene expressions have been shown to decrease as the infection process progresses in all these 12 genes.

Network construction and identification of key candidate genes using gene-gene interaction

Network analysis was carried out with Cytoscape to better show the biological connection of these 12 genes, which decreased in expression from day 1 to day 5 during infection. As understood from the figure, our determined 12 genes were shown to have a strong network relationship between each other and also with other candidate genes (Fig. 3). The linking line between genes illuminate the network of these genes. The thickness of the linking line determines the power of connection of the related genes. For instance,

the linking line between NCAPG and SMC4 represent one of the thickest lines. This indicates the link formed between these genes has been determined to be stronger by studying more clearly. Additionally, the black nodes indicate the target genes giving by authors. On the other hand, the gray nodes demonstrate the genes which associated genes determined by GeneMANIA application. The size of nodes reversely correlated with score. For example, NDC80 gene has virtually 0,56 score and the other gene, CENPT's score is 0,05. This directly correlated with the size of the nodes. NDC80 has higher node size than CENPT.

Functional Enrichment Of Genes And Correlations With Pathways

To reveal the relationship of these 12 genes with cellular functions, pathways and to understand the new significance biological processes pathway analysis was done by DAVID software. 4 important pathways cell cycle, pyrimidine metabolism, mismatch repair, P53 signaling pathway have been identified which associated with our 12 genes that were significant during the exposure to *E. faecalis*. The relationships of these pathways with cancer development is very clear such as importance of cell cycle in the gastric cancer cells (Table 2).

Table 2

Pathways related to the genes are linked. It is seen that important pathways in cancer progression are related to our genes. Most of these genes are linked to the cell cycle pathway from the database for annotation, visualization and integrated discovery (DAVID).

Category	Pathways	Count	P-Value	Benjamini
KEGG_PATHWAY	Cell Cycle	6*[1]	1,3E-3	9,0E-2
KEGG_PATHWAY	Pyrimidine metabolism	2*[2]	3,1E-3	1,1E-1
KEGG_PATHWAY	p53 signaling pathway	4*[3]	1,0E-2	1,7E-1
KEGG_PATHWAY	Mismatch repair	3*[4]	9,1E-3	2,0E-1

*[1]NDC80, NCAPG, BUB1B, BUB1, CENPA, KIF23. *[2]BUB1B, BUB1. *[3]KIF2C, SPC25, KIF20A, CENPF. *[4]SMC4, CENPA, NCAPG.

The significantly enriched gene sets and their ES, NES, NOM p-value, FWER p-value, FDR q-value represented in Additional File 3. According to GSEA, the cell cycle checkpoint as well as mitotic cell cycle checkpoint gene set were found as significantly correlated with the genes that present in the data. These gene sets even correlated with our pathway analysis in Table 2 and other emphasized studies. The enriched genes in these two gene sets were NCD80, BUB1, BUB1B, CENPF. Furthermore, Fig. 4 was demonstrating the cell cycle checkpoint gene set graph. This graph showed the genes which were NCD80, BUB1, BUB1B, CENPF enriched in control day 5 group that also referred as positively correlated. On the

other hand, the same genes negatively correlated in infected day 5 group by down regulating. The same outcomes occurred for mitotic cell cycle checkpoint gene set (Fig. 5).

Discussion

In recent years, the association between intestinal flora-related microbiota and gastric cancer development has increased the interest in evaluating its role in human health, with crucial findings and effects on the host. In this context, important *E. faecalis* infections have become a major area of research in translational research, producing high expectations for science because of its potential to affect cell functions such as cell cycle that can alter the response to chemotherapy. In this study using all genome expression data of MKN74 gastric cancer cells we aimed to identify gene expression alterations for which *E. faecalis* is responsible, their network relationships and associated pathways.

Immunosuppression seen in cancer patients is the most important reason that paves the way for the emergence of infections. The range of pathogens that can lead to infection in immunocompromised cancer patients is quite wide; In addition to conventional microorganisms, factors that can lead to opportunistic infections should also be considered. *E. faecalis* is part of the normal gut flora that can pass through the gut and cause systemic infection (11). With any intestinal lesion or tumor, the barrier is violated, and the intestinal flora such as *E. faecalis* can translocate and cause infection (22).

We first examined the gene expression alterations following infection with living *E. faecalis* groups and with untreated MKN74 cells. As day increases we determined MKN74 cells treated with *E. faecalis* showed statistically significant gene expression alterations when compared to untreated cells ($p < 0.05$). As expected, these groups were hierarchically forming a very distinct cluster. Then, in order to determine whether this statistically significant gene expression alterations were due to *E. faecalis* infection, analysis was performed between control and treated groups and NDC80, NCAPG, CENPA, KIF23, BUB1B, BUB1, CASC5, KIF2C, CENPF, SPC25, SMC4, KIF20A genes were identified as downregulated in gastric cancer cell.

NDC80 is a kinetocol protein that combines with other proteins to form a microtubule binding site. Due to its properties, it has important roles in the cell cycle. An abnormality in NDC80 can lead to apoptotic cell death. NDC80 gene has a role in tumor formation in different cancers such as pancreas, breast and stomach cancer (23–25). It is highly expressed in pancreatic and breast cancer cells, and this high expression is associated with poor prognosis. In gastric cancer, as a result of loss of function in the NDC80 gene, the cell stops in the G2/M phase of the cell cycle and goes to apoptosis. NCAPG is the mitotic gene that plays a role in the cell cycle. In gastric cancer, high expression of this gene is associated with proliferation of cells (26). CENP-A is a histone H3 variant that ensures kinetochores and centromeres to form and function properly. Highly expressed levels are related with cancer progress and has an important role in cell division (27). Highly expressed CENP - A levels and the taxane-based chemotherapy response of breast cancer patients correlated strongly. High CENP - A expression can be predicted as a biomarker in the malignant progression and poor prognosis of many types of cancer. The irregularity that

may occur in the regulation of the centromer can cause genome imbalance in cancer. This is supported by the observation that mitotic defects are caused by disruption of the CENP-A (28–30). Gastric cancer has important part in global cancer mortality with poor prognosis. Kinesin superfamily proteins (KIFs), discovered in recent years, are microtubule dependent motor proteins and function as oncogenes in cancer cells. It is known that kinesin family member KIF2A stands out for its prognostic roles in breast cancer, colorectal cancer and squamous cell carcinoma. It has been shown that MT1-MMP suppression by KIF2A downregulation can inhibit cell invasion in gastric cancer (31). In another study, the relationship between KIF14 expression was analyzed and it was shown that KIF14 was overexpressed in gastric cancer, associated with poor prognosis, and playing important roles in cancer progression and metastasis (32). The need for prognostic markers for gastric cancers has increased tremendously. Generally, it is associated with poor prognosis due to its metastasis following the detection of cancer. BUB1 is a mitotic spindle checkpoint protein (budding uninhibited by benzimidazoles 1). The high expression of BUB gene family members in gastric cancer, which encode key components of the check point regulatory pathway, has been suggested to show a strong correlation with tumor proliferation (33). On the contrary in a study, it was found that low BUB1 expression in 218 gastric cancer primary cells was associated with large tumor size (33, 34). In addition, its expression was inversely correlated with the residual tumor stage, and low BUB1 expression was associated with shorter survival ($p < 0.001$). CASC5 (cancer susceptibility candidate 5) has been shown to play important roles in various types of cancer. In a bioinformatic study, CASC5 has been shown to be a new oncogene in lung adenocarcinoma (35). Structural maintenance of chromosomes 2 (SMC2) gene encodes condensin complexes and responsible from chromosomal stability (36). Although the pathological effects of SMC2 gene changes are not fully known, some studies have shown that SMC2 gene changes may play a role in the cancer pathogenesis of gastric cancer. In another study it is showed that SMC2 gene has low expression in gastric cancer and it is a risk loci for breast and ovarian cancer (36–38). Bacterial infections as well as environmental and genetic factors play important roles in the development of gastric carcinoma (39). We identified significant expression alterations in certain genes depending on time after treatment of gastric cell MKN70 with *E. faecalis.* These genes seems often have roles in the progression of cancer by acting on cell proliferation and cell cycle. An irregularity in the elements of cell cycle pathway is an important factor that could lead gastric tumor formation. Some cyclin-related kinases with bacterial infection are known to cause upregulation in gastric cancer cells. Activation and regular functioning of these cyclin-depended kinases occur due to the progress of the cell cycle (40). Cyclin-depended kinases play an important role in the G1/S phase transition in the cell cycle by targeting E2F transcription factors. An abnormality occurring in this molecular mechanism causes damage to this connection and causes the development of gastric cancer cells.

Gene set enrichment analysis supported the results that some crucial genes function effectively in the cell cycle as a result of *E. Faecalis* infection. NDC80, BUB1B, BUB1 and CENPF genes showed a statistically significant difference in cell cycle checkpoint and mitotic cell cycle checkpoint gene sets associated with cell cycle. Genes, which are found to be statistically significant as a result of comparing the control day 5 and infected day 5, were enriched in the specified biological gene sets. This enrichment supports that

these genes have important effects on the cycles of cells. In addition, these effects trigger cancer development by causing critical changes in cell function, as noted.

Most of our identified genes are reported highly expressed in various cancers and related to poor prognosis. As a result of our bioinformatics study, we found that there was a significant decrease in these genes when they treated with *E. faecalis*. It is likely that cancer progression could also expected to decrease due to *E. faecalis* infection. The relationship of some of these genes with gastric cancer was first shown in this study. As a result of network and pathway analysis, it was determined that these genes are associated with important pathways in cancer progression. These pathways are cell cycle, pyrimidine metabolism, mismatch repair, P53 signaling pathway, endocytosis and spliceosome. It is obvious that most of these pathways are associated with cancer progression and proliferation. And this allows us to positively intervene in the progression of cancer. The limitation of this study is lack of incorporation of in vivo experiments.

Conclusion

It has been shown in this study that *E. faecalis* infection on gastric cancer can have a significant effect on proliferation of cells. The results of our study are an important indication that infections caused by *E. faecalis* directly or indirectly have an effect on the progression of cancer, and this effect could be in favor of host in battle with gastric cancer. Because most of the genes we detected have functions that can slow the rate of cancer proliferation. There is a need for more in vitro and in vivo studies to reveal which structural or functional parts are responsible for this negative effect on gastric cancer cells.

Abbreviations

E. faecalis Enterococcus faecalis

ROS Reactive Oxygen Species

CGP Cancer Genome Project

DAVID Database for Annotation, Visualization and Integrated Discovery

GSEA Gene Set Enrichment Analysis

ES Enrichment Score

NES Normalized Enrichment Score

NOM p-value Nominal p-value

FDR q-value False Discovery Rate q-value

FWER Familywise Error Rate p-value

Declarations

Ethics approval and consent to participate

Ethics approval was not necessary because the used *in silico* data were obtained from Array Express (E-MEXP-3496).

Consent for publication

Not applicable

Availability of data and materials

A majority of data generated or analyzed during this study included in this published article. The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request. The gene expression data was obtained from the Cancer Genome Project (CGP) database (http://www.cancerrxgene.org/) and transcription profile data were obtained from Array Express (E-MEXP-3496).

Competing interests

The authors declared no competing interests.

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No funding was received.

Authors' contributions

Sukru Volkan Ozguven and Can Turk conceived and conducted the study. Can Turk, Seyhan Turk, Elif Sena Temirci performed the statistical analysis. Can Turk, Seyhan Turk wrote the manuscript. Sukru Volkan Ozguven and Gulberk Ucar revised the manuscript. Sukru Volkan Ozguven and Gulberk Ucar supervised the study. All authors offered intellectual contribution for the manuscript and approved its final form.

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Figures

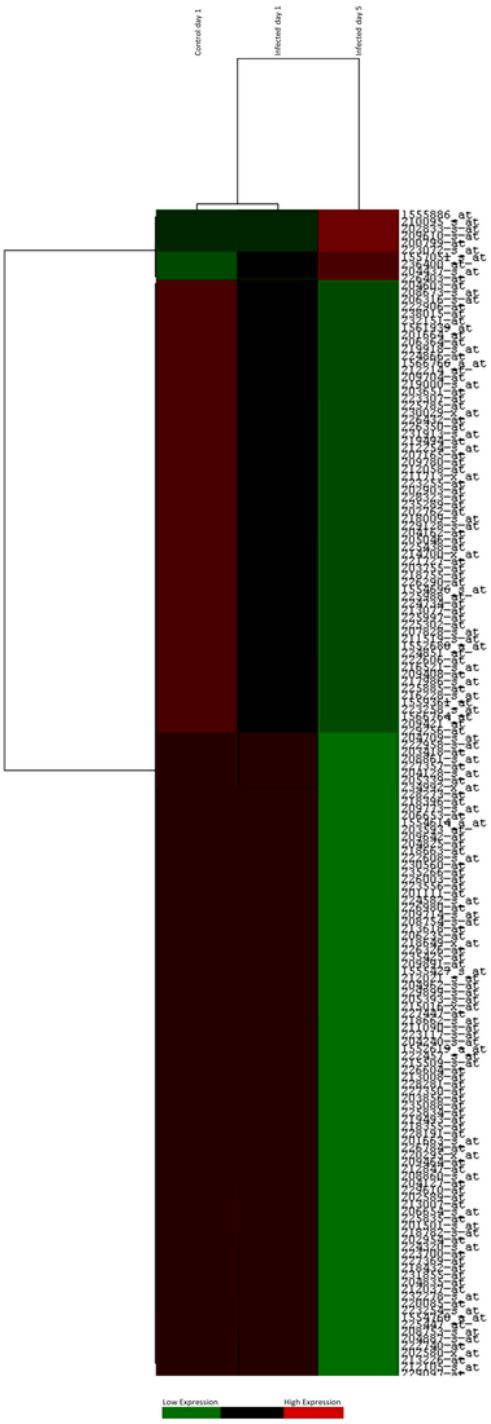

Figure 1

Hierarchical clustering of 138 statistically significant genes / 165 probesets in the three groups specified. The analysis reveals sensitive low expressions (green), intermediate (black) and high expressions (red) of 138 genes for control day 1, infected day 1 and infected day 5. Designated groups are clearly classified.

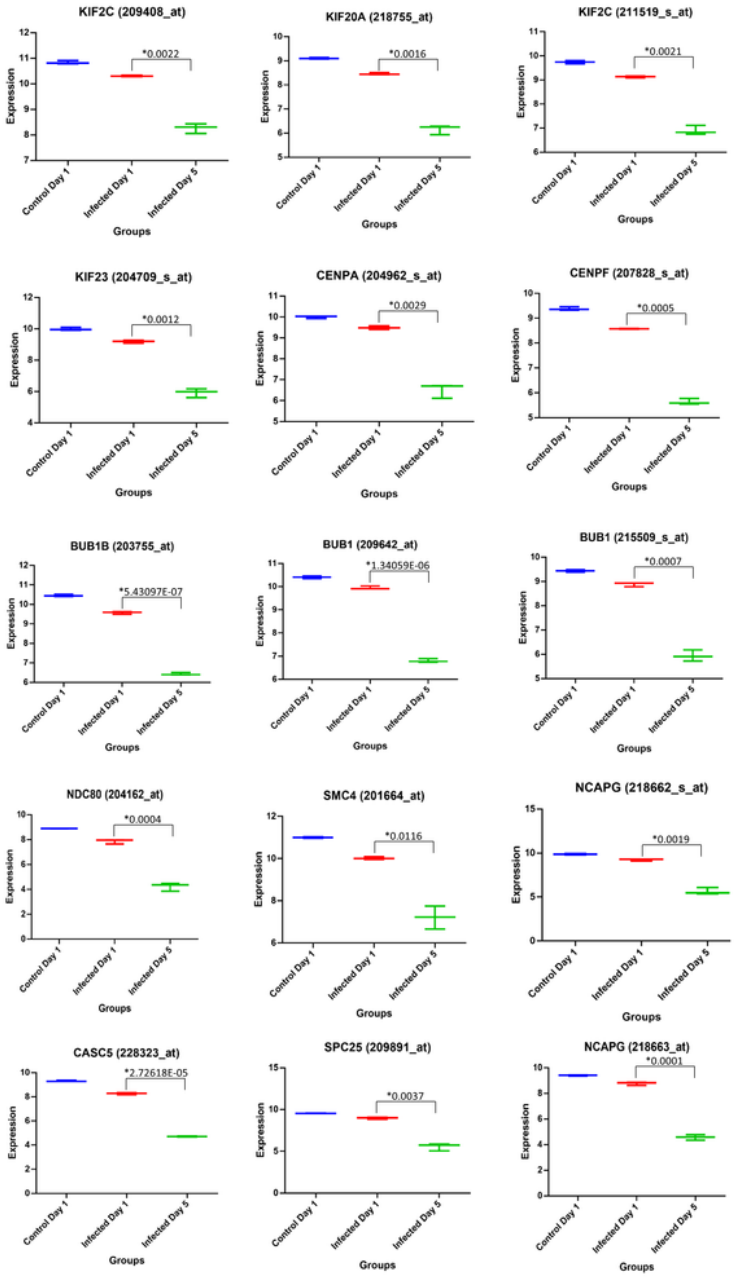

Figure 2

12 genes / 15 probesets that show significant down-regulation after treatment with live infection. A statistically significant alterations were detected between control day 1, infected day 1 and infected day 5 for all probesets.

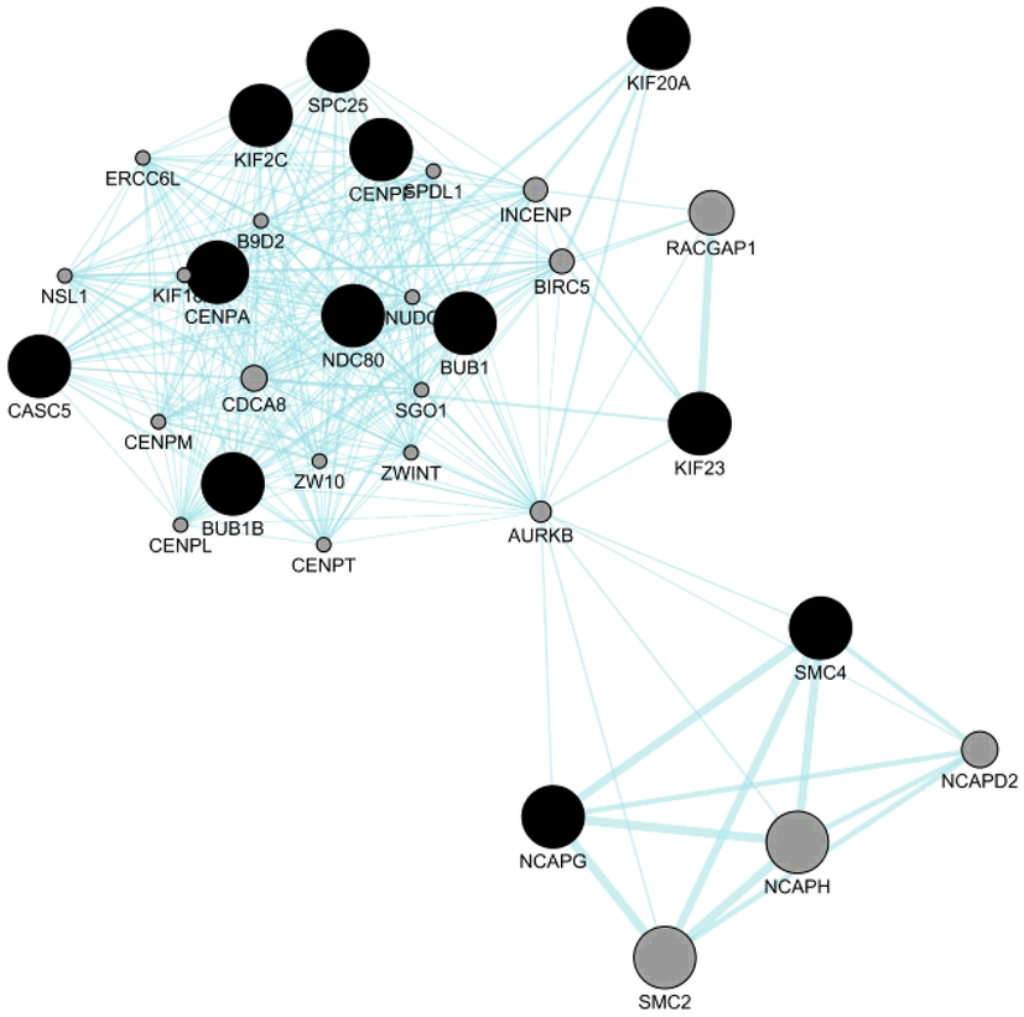

Figure 3

Network analysis of 12 genes that are statistically significant. The figure shows that these 12 genes have a strong network connection (Cytoscape). The linking line between genes illuminate the network of the genes. The thickness of the linking line determines the power of connection of the related genes. This indicates the link formed between these genes has been determined to be stronger by studying more clearly. Additionally, the black nodes indicate the target genes giving by authors. On the other hand, the gray nodes demonstrate the genes which associated genes determined by GeneMANIA application. The size of nodes reversely correlated with score. For example, NDC80 gene has virtually 0.56 score and the other gene, CENPT's score is 0.05. This directly correlated with the size of the nodes. NDC80 has higher node size than CENPT.

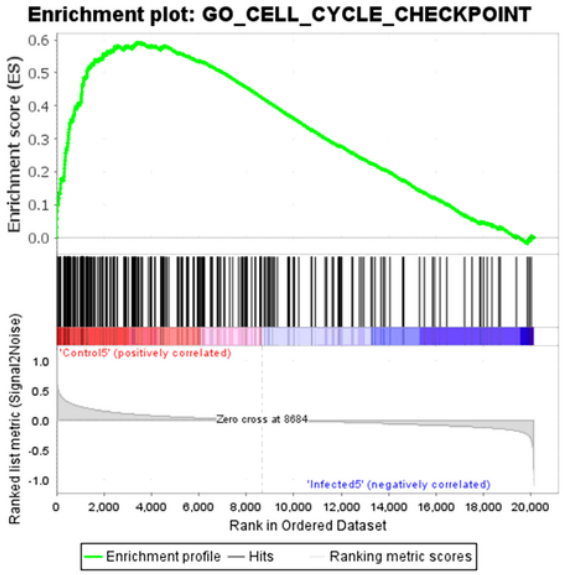

Figure 4

The cell cycle checkpoint enrichment plot was represented. The black straight line refers the enriched genes in the groups. Red part contains the genes that positively correlated in control day 5 group, were upregulated in control day 5. On contemporarily, blue line includes downregulated or in other words negatively regulated genes that belongs to infected day 5.

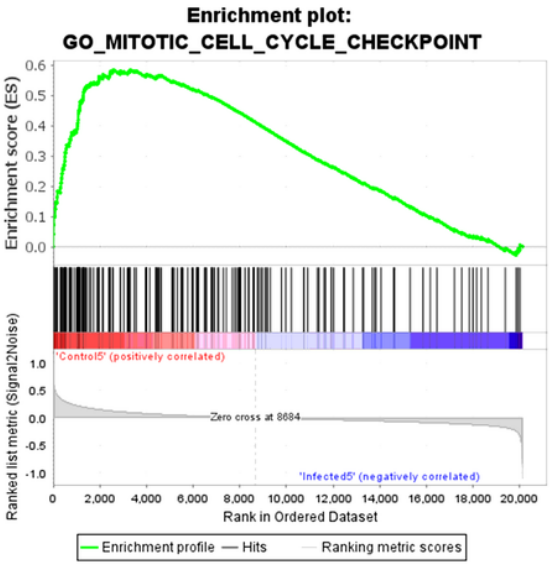

Figure 5

The mitotic cell cycle checkpoint enrichment plot was represented. The black straight line refers the enriched genes in the groups. Red part contains the genes that positively correlated in control day 5 group, were upregulated in control day 5. On contemporarily, blue line includes downregulated or in other words negatively regulated genes that belongs to infected day 5.

Supplementary Files

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- AdditionalFile3.docx
- AdditionalFile2.tif
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