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RESEARCH ARTICLE

Bioinformatics Study of the Microarray Data Set of Ovarian Carcinoma in Cattle

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ABSTRACT

Ovarian tumor is one of the gynecological tumors that have a poor prognosis, especially in domestic animals. Animals frequently develop ovarian tumors, and most of these cases affect cows. It is considered the most common malignant gynecologic tumor with complex etiology. The scientific community has recently been able to conduct research on prognostic markers, prospective therapeutic targets, and ways to improve treatment outcomes thanks to the amazing advancements in bioinformatics. In the current study we used some of bioinformatics tools as Gene Expression Omnibus (GEO) database, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Cytoscape and STRING to discover the hub genes associated with the prognosis of ovarian cancers. Through the gene expression profile (GSE225981) between tumor tissue and normal tissue we found that the top upregulated hub gene involved in ovarian cancer was ESR1 gene and top downregulated hub gene was ITGA2. Complement and coagulation cascades were the most implicated pathways in ovarian tumor. Study these genes will help in reaching to know the candidate gene that responsible for ovarian cancer so found targeted treatments and lower the death rate of affected animals.

***Keywords*:** Ovarian tumor, hub genes, bioinformatics, tumors, GEO.

Introduction

Reproductive disorders and diseases are major factors that significantly impact fertility and cause the cattle business to suffer enormous economic losses. Pathological affections of the ovaries are common diseases in domestic mammals, especially cattle and buffaloes. The ovaries are unique special organs that regulate the synthesis of hormones, control the estrous cycle and fertilization [1].

The ovarian pathologies comprise ovarian developmental anomalies, such as ovarian hypoplasia and aplasia, freemartin, hermaphrodites, and white heifer disease; ovarian inflammatory conditions, such as ovarian sclerosis, oophoritis and perioophoritis, ovarian abscess, ovarobursal adhesions and encapsulation, and sub-estrum or silent heat, gestational estrus, persistent corpus luteum, ovulatory disturbances, ovarian cysts, and paraovarian cysts); and, lastly, ovarian neoplasms and associated conditions, such as epithelial tumors, germ cell tumors, sex cord–stromal tumors, and mesenchymal tumors [2].

In the past thirty years, the fight against cancer has made significant progress, and the survival rate has doubled [3]. However, finding widespread treatment is challenging. The problem is that cancer has many diverse symptoms, sometimes even within the same tumor location; it is not a singular disease.

According to systems biology, every solid tumor is an individual system that is distinguished by the heterogeneity of its cells, how it interacts with the environment in which it develops, and how it can change and adapt to it [4]. The diagnosis and treatment of cancer are being revolutionized by recent developments in our understanding of the molecular pathways underlying the disease [5].

The diverse range of cell types that make up the ovary is reflected in the histological patterns used to classify ovarian tumors, which constitute a heterogeneous group of neoplasms [6]. The World Health Organization classifies them into three main groups: germ cell cancers, sex cord-stromal tumors (such as granulosa cell tumors), and epithelial ovarian tumors, often known as common epithelial tumors [7].

Epithelial ovarian tumors usually account for 80–90% of all ovarian malignancies, the bulk of ovarian tumors are believed to be produced from the relatively pluripotent cells of the surface epithelium. Consequently, epithelial ovarian tumors can develop into multiple subtypes, each of which has a histological appearance that is similar to normal cells lining other female genital tract organs [8].

Granulosa cell tumors (GCTs) are thought to be the most prevalent ovarian sex cord tumor in domestic animals. The range of its occurrence is 0.05% to 7.14% [9]. From very old cows to virgin heifers, these tumors have been detected in every age group of cows. Although they are uncommon, granulosa cell tumors in pregnant cows have also been documented [10]. In native breeds of cows, this sort of tumor has been found both grossly and microscopically [11].

The development and demand of biological research in understanding true biology cannot be met by the traditional gene-by-gene approaches to research [12]. Bioinformatics is one of the newest fields of biological research, which is widely defined as the processing and analysis of biological data using mathematical, statistical, and computer techniques. Data management and platform integration are critical due to the vast volumes of data produced by emerging technologies like microarray chips and genomic sequencing [13]. In addition to measuring and monitoring the prognosis of the disease and the effectiveness of treatment, cancer bioinformatics is crucial in the identification and authentication of biomarkers, particularly those related to early detection and clinical phenotype [14].

An early diagnosis of cancer improves the prognosis, yet accepting the diagnosis at an early stage can be challenging. The significance of bioinformatics tools has increased due to the advancement of technology through the use of DNA microarrays and proteomics investigations for large-scale gene expression research [15].

The utilization of bioinformatics analysis has become a crucial method for examining etiopathogenesis and identifying hub genes associated with disease development and incidence. Hub genes are those in the gene network that interact with a large number of other genes and are frequently essential for biological functions and gene regulation [16]. In addition, Hub genes were shown to be the most strongly linked to disease [17].

High-throughput gene expression and other functional genomics data sets are archived and made publicly available by

the Gene Expression Omnibus (GEO) database, an international public repository. GEO was established in 2000 as a global resource for gene expression research, but it has since changed quickly to accommodate high-throughput data for a wide range of additional data applications, such as those that look at chromatin structure, genome methylation, and genome-protein interactions. GEO favors community-derived reporting guidelines that call for the release of raw data, processed data, and descriptive metadata, among other essential study components. In addition to giving users access to data for tens of thousands of research, the database also includes a variety of Web-based tools and techniques that let users find and find data related to their particular interests as well as view and analyze the data [18].

Small noncoding RNAs known as microRNAs (miRNAs) have been linked to the emergence of tumors. Through translation suppression or mRNA degradation, they control the expression of target genes [19, 20]. A miRNA has the ability to control hundreds of target genes [21]. Tumor suppressor genes can be inactivated, or oncogenes can be activated during the growth of tumors due to abnormal production of miRNAs [22] Since miRNA expression is tissue-specific, blood can be used to identify it [23], and correlates with clinical cancer behaviors, miRNAs are potential valuable biomarkers [24].

MicroRNAs (miRNAs) play a vital role in the development of ovarian cancer. Several studies have identified both downregulated miRNAs and potential genes in ovarian cancer samples [25] and global profiling approaches [26]. Consequently, miRNAs represent viable biological targets for drug resistance monitoring, targeted therapy, early screening, and improving prognosis in cancers like ovarian cancer [27].

This study aimed to screen the hub genes in addition to up- and down-expressed genes and most microRNA related to ovarian tumors in cattle. A gene expression profile, GSE225981 was downloaded from the GEO database. The differentially expressed genes (DEGs) between normal cells and ovarian tumors were screened using the GEO2R tool. ShinnyGo 8.0 software was applied to identify the set of possible diseases associated with the obtained DEGs and protein-protein interaction (PPI) network obtained from the String database and visualized by Cytoscape software. MicroRNAs that regulate most of DEGs are obtained from Network Analyst.

Material and methods

Microarray data

The GEO database is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles, was accessed and the gene expression dataset (GSE225981) was analyzed to determine DEGs.

Data involved two distinct groups control group (N=3 Bos taurus), and ovarian tumor group isolated from Stromal fibroblast cells (N=3 Bos taurus). The metrics commonly used to identify DEGs include fold change (log2 FC =2) and P-value < 0.05.

Gene ontology and enrichment analyses pathways

Gene Ontology (GO), a popular in silico technique, offers detailed information about the gene function of

particular genomic products based on predefined parameters. Molecular functions (MF), cellular components (CC), and biological processes (BP) make up the three sections of this investigation [28].

A database called Kyoto Encyclopedia of Genes and Genomes (KEGG) determines biological processes and tools. A range of functional annotation tools provided by the Cytoscape plugin application CLueGo enable researchers to analyze and understand the biological significance of individual gene lists. For DEGs with a false discovery rate (FDR) less than 0.05, P-value < 0.05, we look at GO and KEGG analyses.

Protein-protein interaction (PPI) network building and hub gene analysis

DEGs were uploaded to the Search Tool (STRING, https://string-db.org/) to determine interacting genes. A database called STRING is used to look into the linkages and connections between the proteins that the detected DEGs encode [29].

Detection of the expected microRNA contributes to ovarian tumors

By uploading the top 250 differentially expressed genes, the ShinnyGo tool (http://bioinformatics.sdstate.edu/go80/) is used to evaluate KEGG pathways, enrichment analysis, and gene ontology (BP, CC, and MF). The chromosomal locations of DEGs and microRNA, the molecules involved in the interaction between DEGs, can also be found using this application.

Detection of the DEGs and disease interaction

Using ShinnyGo 8.0 online program, the range of possible diseases associated with the acquired DEGs was identified. After that, PPI string analysis (http://bioinformatics.sdstate.edu/go80/) was carried out to verify the role of DEGs in ovarian tumors and related disease development.

Result

Differentially expressed genes

Following a comparison between control and ovarian tumor GEO2R analyses, the top 250 DEGs were identified (upregulated and downregulated), (Figure 1). GEO2R revealed DEG with adjusted *P*-value < 0.05 and fold change (log2 FC > 2).

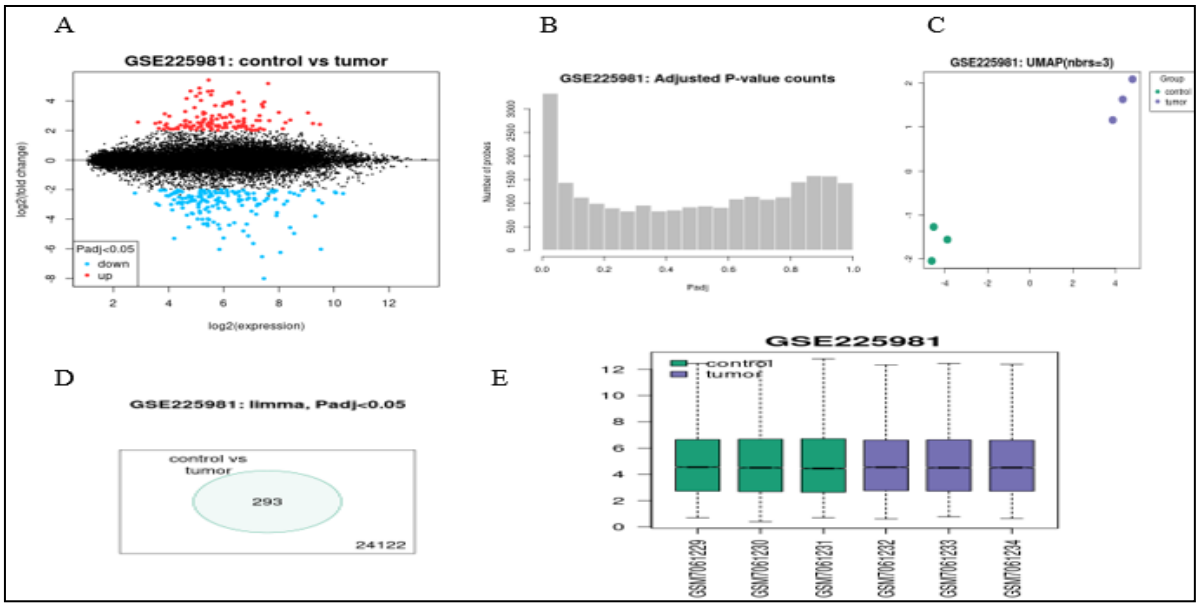

Figure 1. GEO2R reveals DEG with adjusted *P*-value < 0.05 and fold change (log2 FC > 2). A. Up and down expressed genes, B. Adjusted *P*-value for the obtained genes. C. Distribution of the samples. D. Significant genes. E. Distribution of microarray data (tumor, control).

Protein-protein interaction network building

We use database called STRING to look into the linkages and connections between the proteins that the detected DEGs encode (Figure 2).

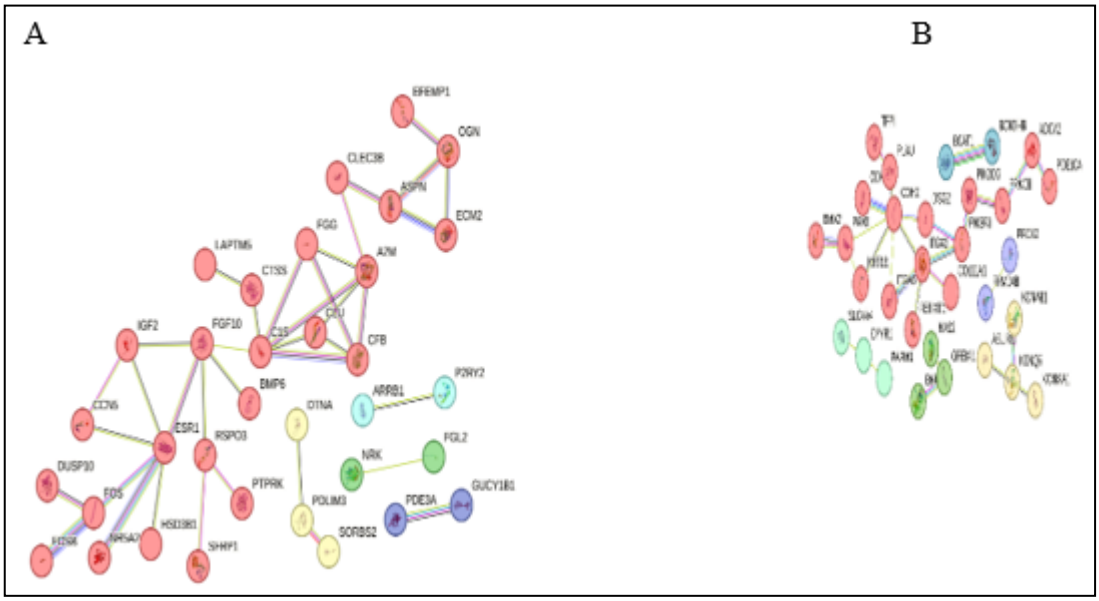

Figure 2. PPI string analysis used to illustrate the interaction between DEGs. A. Upregulated genes. B. Downregulated genes.

Functional enrichment analysis of DEGs

The KEGG pathways analysis revealed *Staphylococcus aureus* infection and complement and coagulation cascades in upregulated genes (Figure 3A) but revealed pancreatic secretion, thyroid hormone synthesis, cholinergic synapse, and dilated cardiomyopathy in downregulated genes (Figure 3B).

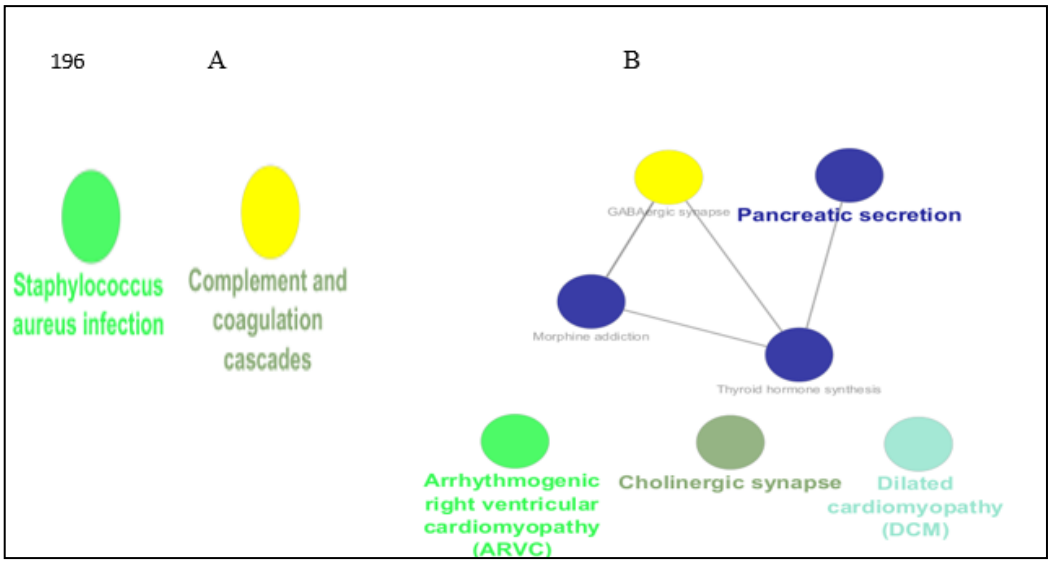

Figure 3. GO and KEGG databases identify functional enrichment analysis of DEGs. A. Upregulated pathways. B. Downregulated pathways.

In addition, the GO results for BP are cellular response to reactive oxygen species, response to vitamins, regulation of JUN kinase activity (Jun N-terminal kinases that responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock), response to calcium ions, biomineral tissue development, protein activation cascade, cellular response to transforming growth factor beta stimulus for upregulated genes, neuroepithelial cell differentiation, telencephalon regionalization, cell adhesion mediated by integrin, renal system process for downregulated genes (Figure 4).

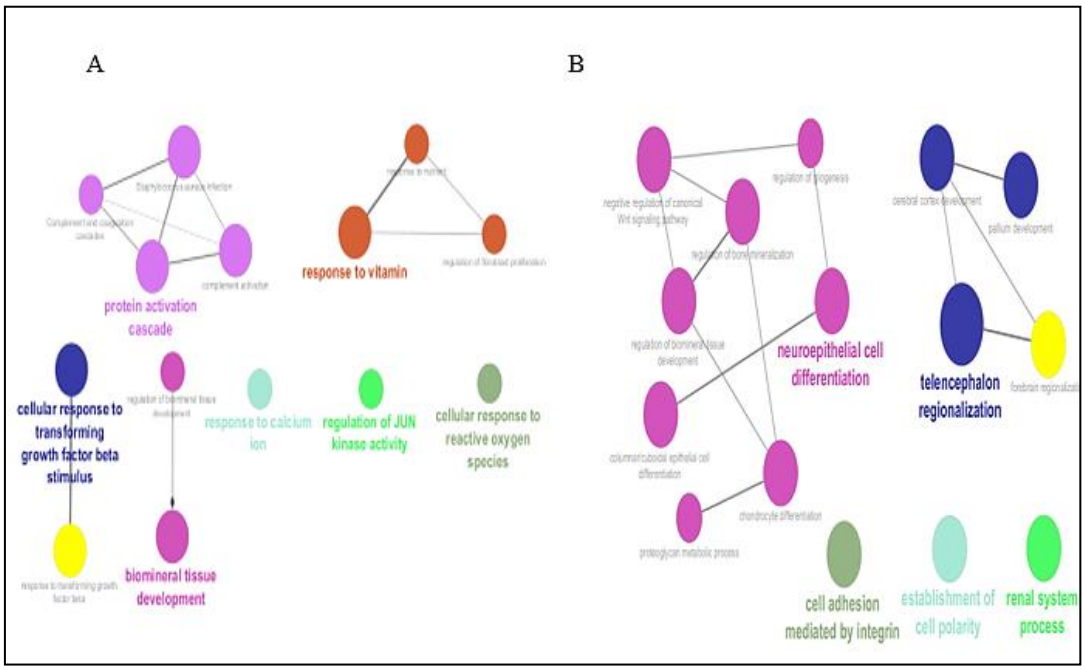

Figure 4. A. BP includes cellular response to reactive oxygen species, response to vitamin, regulation of JUN kinase activity, response to calcium ions, biomineral tissue development, protein activation cascade, cellular response to transforming growth factor beta stimulus for upregulated genes, B. neuroepithelial cell differentiation, telencephalon regionalization, cell adhesion mediated by integrin, renal system process for downregulated genes.

GO analysis for MF of upregulated genes mentioned as heparin binding, glycosaminoglycan binding and collagen binding. MF of downregulated genes were potassium channel activity, ion channel regulator activity, and voltage-gated channel activity for (Figure 5).

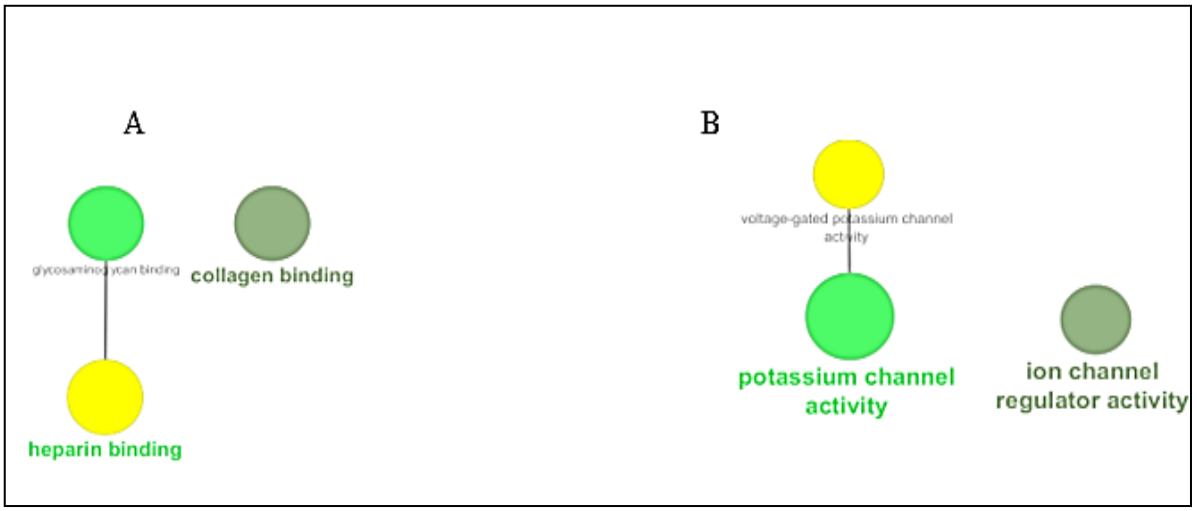

Figure 5. MF includes heparin binding, glycosaminoglycan binding and collagen binding, potassium channel activity, ion channel regulator activity, and voltage-gated channel activity for downregulated genes.

Finally, GO analysis for CC includes potassium channel complex and voltage-gated channel complex for downregulated genes (Figure 6).

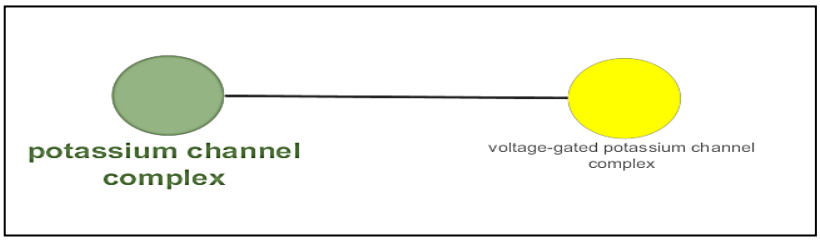

Figure 6. Cellular components (CC) include potassium channel complex and voltage-gated channel complex

Hub genes and transcription factor regulation

The top ten upregulated genes Asporin (ASPN), Corneal keratan sulfate proteoglycan 25 core protein(OGN), Estrogen receptor1(ESR1), Fibroblast growth factor (FGF10), Fibrinogen gamma-B chain (FGG), Insulin-like growth factor II(IGF2), Complement factor B Ba fragment; Factor B (CFB), Complement factor B Ba fragment; Factor B (CLU), Alpha-2-macroglobulin (A2M) and Complement C1s subcomponent heavy chain(C1S), top 10 downregulated hub genes Integrin alpha-2; Integrin alpha-2/beta-1 (ITGA2), Desmoglein 2.(DSG2), Protein kinase C beta type (PRKCB), Uncharacterized protein.(CYYR1), Potassium voltage-gated channel subfamily Q member 5 (KCNQ5), Phosphatidylinositol 3-kinase regulatory subunit gamma (PIK3R3), Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG), Adenylate cyclase type 2 (ADCY2), Paired box protein Pax-6 (PAX6) and Cadherin-2; Calcium-dependent cell adhesion protein (CDH2) were illustrated in Figure 7.

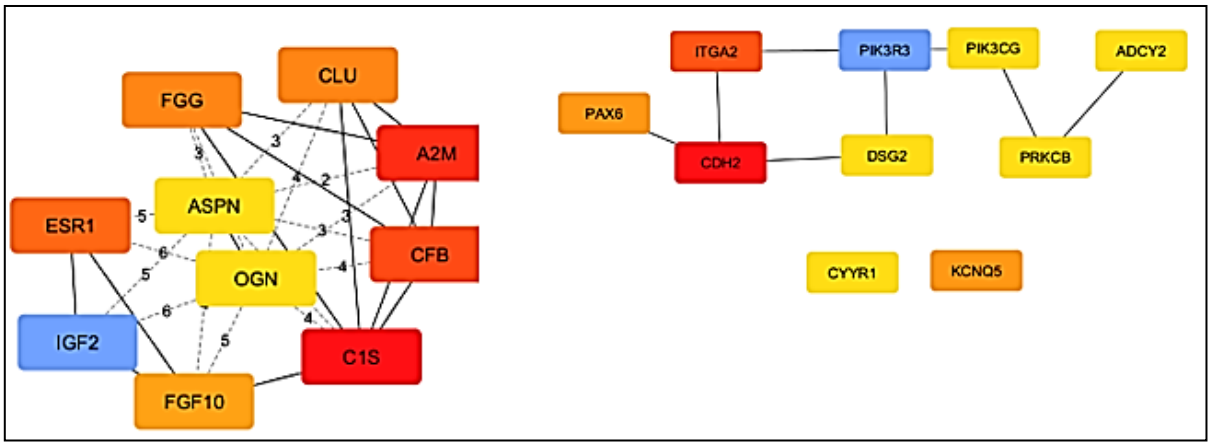

Figure 7. A. The top ten hub genes upregulated ASPN, OGN, ESR1, FGF10, FGG, IGF2, CFB, CLU, A2M and C1S, B. Top 10 downregulated hub genes ITGA2, DSG2, PRKCB, CYYR1, KCNQ5, PIK3R3, PIK3CG, ADCY2, PAX6 and CDH2.

MicroRNA regulated DEGs

Hub microRNA (Bta-miR-155) regulating the function of hub genes is illustrated in Figure8.

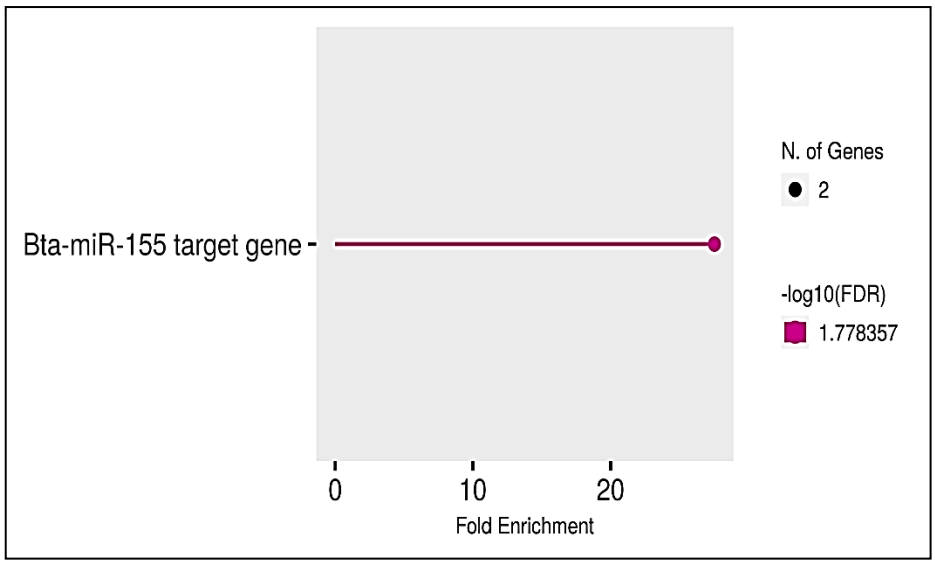

Figure 8. Bta-miR-155 is the hub microRNA that regulate the function of hub genes.

Relation between hub genes and microRNA

Figure 9 exposed the Hub genes and microRNA interaction were .

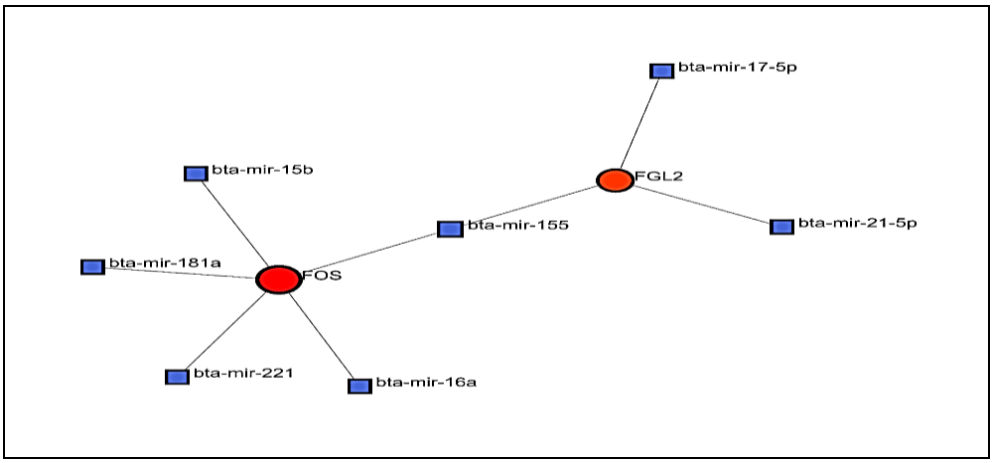

Figure 9. correlation between microRNA and hub genes that included in ovarian cancer.

Discussion

Ovarian tumors are common in domesticated animals, the majority occurring in bitches and cows. They are considered the most frequent malignant gynecologic tumors with very complicated pathogenesis. There is little information about molecular pathways and key genes contributing to ovarian tumor occurrence. Therefore, understanding the biological process is urgently needed, with the aim of early diagnosis of disease and developing specific therapies.

Several molecular pathways were implicated in the occurrence and metastasis of ovarian tumors; however, the exact molecular pathways are still unknown. Thus, this study was designed to illustrate DEGs, hub genes, and hub miRNA contributing to ovarian tumor occurrence. The DEGs between tumor tissue isolated from Stromal fibroblast cells and normal tissue were screened using the GEO2R tool. The (GSE225981) profile involved two distinct groups tumor group isolated from Stromal fibroblast cells (N=3 Bos taurus) and normal group (N=3 Bos taurus). The standard parameters for detecting DEGs are P-value < 0.05 and fold change (log2 FC >2), about 250 DEGs were obtained, and the top ten upregulated genes are ASPN, OGN, ESR1, FGF10, FGG, IGF2, CFB, CLU, A2M and C1S, top 10 downregulated hub genes were ITGA2, DSG2, PRKCB, CYYR1, KCNQ5, PIK3R3, PIK3CG, ADCY2, PAX6, and CDH2. This result disagrees with a previous study that showed that there are 879 common DEGs and a high expression of structural maintenance of chromosome protein 4 (SMC4) was revealed in the Kaplan-Meier plotter analysis to be meaningful for the prognosis of OC and was verified at both the mRNA and protein levels [30].

The results of the current in silico study revealed that the most important KEEG pathways involved in the development of ovarian tumors are Staphylococcus aureus infection and complement and coagulation cascades in upregulated genes but revealed pancreatic secretion, thyroid hormone synthesis, cholinergic synapse and dilated cardiomyopathy in downregulated genes. This result did not correspond with

previous study showed that a number of DEGs were enriched in extracellular matrix organization, pathways in cancer, focal adhesion, and ECM-receptor interaction [31].

Lastly, this study proposes that the primary hub miRNA regulating DEGs is Bta-mir-155. This disagrees with previous study that indicate microRNAs (miR-9) play a vital role in the development of ovarian cancer [32].

Conclusion

Based on bioinformatical analysis in the current study, the top ten upregulated genes are ASPN, OGN, ESR1, FGF10, FGG, IGF2, CFB, CLU, A2M, and C1S; the top 10 downregulated hub genes were ITGA2, DSG2, PRKCB, CYYR1, KCNQ5, PIK3R3, PIK3CG, ADCY2, PAX6, and CDH2, moreover complement and coagulation cascades were the most often identified pathways in the pathogenesis of tumor ovarian stromal cells. Furthermore, the 10 hub genes comprising the pattern could be significant as diagnostic indicators of ovarian cancer.

. Our in-silico study discovered that Bta-mir-155 is the most miRNA-controlling DEG, which will aid in the development of targeted treatments and lower the death rate of affected animals. Clearly, understanding genetics and the genome as a whole and its variation are integral to understanding disease processes, and this understanding provides the foundation for curative therapies, beneficial treatments, and preventative measures. However, additional investigations are necessary to validate these findings and ascertain the biological importance of these hub genes in the genesis and advancement of ovarian cancer. Also, experimental studies should be conducted to ensure these results.

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الملخص العربي

دراسة المعلوماتية الحيوية لمجموعة بيانات ميكروأري لسرطان المبيض في الماشية

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ورم المبيض هو أحد الأورام التى تصيب الاناث و التي يكون تشخيصها سيئًا خاصة في حيوانات المزرعة. حيث تصاب الحيوانات بشكل متكرر بأورام المبيض، وأغلب هذه الحالات تصيب الأبقار. وهو يعتبر الورم الخبيث الأكثر شيوعًا في الأمراض التى تصيب المبيض، والذي له مسببات معقدة حقًا. حيث تمكن المجتمع العلمي مؤخرًا من إجراء أبحاث حول علامات التشخيص، والأهداف العلاجية المحتملة، وطرق تحسين نتائج العلاج بفضل التقدم المذهل في المعلوماتية الحيوية. استخدمنا في الدراسة الحالية بعض أدوات المعلوماتية الحيوية مثل قاعدة بيانات Gene Expression Omnibus (GEO)، وعلم الجيناتGO، وموسوعة كيوتو للجينات والجينوم KEGG، وCytoscape، وSTRING لاكتشاف الجينات المحورية المرتبطة بتشخيص سرطانات المبيض. من خلال ملف تعريف التعبير الجيني GSE225981 بين أنسجة الورم والأنسجة الطبيعية وجدنا أن الجين المحوري الأعلى المنظم في سرطان المبيض هو جين ESR1 وكان الجين المحوري العلوي المنظم ه .ITGA2 وكانت شلالات التكميل والتخثر هي المسارات الأكثر تورطًا في ورم المبيض. دراسة هذه الجينات ستساعد في الوصول إلى معرفة الجين المسؤول عن سرطان المبيض بحيث يتم العثور على علاجات مستهدفة وخفض معدل وفيات الحيوانات المصابة.