Original Article

EDC-Protein Network Formation in Uvea Melanoma; An Analysis of Melanoma Metastasis-Associated Genes

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Abstract:

Background: Melanoma is a kind of pigment cell cancer that affects the iris, ciliary body, or choroid of the eye (collectively referred to as the uvea). Tumors arise from pigment cells located inside the uvea that stain the eye. Metastasis of melanoma in the eye can damage a number of melanoma, such as the liver. Early diagnosis and treatment of melanoma can prevent possible problems, including decreased vision or complete loss of the eye. The most common manifestations of the disease are blurred vision, diplopia, photopsia and proptosis. This study's goal is to identify melanoma-causing genes and EDCs that regulate gene expression. So we used GEO database to find genes associated with melanoma metastasis and a string database to recreate the protein-protein interaction network. Then modules are made using cytoscape and Clusterviz. To discover probable EDCs, we use the Comptox database. In this network, we add high-degree EDC. These discoveries may help researchers better understand melanoma.

Material and Methods: First, the accession number GSE22138 was used to access the Gene Expression Omnibus at the National Center for Biotechnology Information (GEO). Then, 2000 metastatic and non-metastatic melanoma genes were extracted from the NCBI database together with their P value. Then, by constructing the PPI network, we established ten modules for the genes with the highest expression levels. The comptox database was used to identify possible Endocrine Disrupting Chemicals (EDCs) for 17 high-expression genes. Cytoscape software was used to visualize the EDC-Protein network for these genes. After clustering the network, we find out the genes with adj P value less than 0.05. Finally, we analyzed GO (Gene-Ontology) and molecular pathways using the DAVID database.

Result: In melanoma, 120 potential EDCs were identified to have regulatory effects on gene expression. We present oryzalin as a very effective EDC based on a comprehensive evaluation of various EDCs for metastatic Melanoma. Furthermore, we identified 10 genes (RCHY1, RAB2A, CHMP2A, UBA2, PAFAH1B1, ID2, MARCKS, SHC1, MRPS28 and EIF1B) that their adj P value are less than 0.05 and are among the genes involved in melanoma. We also identified which biological pathways these genes are involved in.

Conclusion: Oryzalin is the EDC with the highest degree in our network. However, these results need to be experimentally confirmed to suggest improved prevention.

Key words: Uvea Melanoma; PPIN; EDC; Systems Biology.

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Introduction

Melanoma is a deadly kind of cancer that may affect the uvea, skin, and other areas [1]. Melanoma is a relatively uncommon type of tumor caused by melanocytes in a variety of anatomic locations, including the skin, ocular region (uvea, conjunctiva, eyelid, orbit), mucous membrane (nasal mucosa, oropharyngeal, pulmonary, gastrointestinal, vaginal, anal/rectal, and urinary tract), and infrequently from unknown primary sites [2]. Among ocular melanomas, 83 % originate in the uvea, 5 % in the conjunctiva, and 10 % in other locations [1,2]. The choroid is the most often seen location of uveal melanoma [2]. Annually, it is predicted that 7095 new instances of uveal melanoma occur worldwide [1]. Fair skin, difficulty to tan, light eye color, ocular or oculodermal melanocytosis, cutaneous or iris or choroidal nevus, and BRCA1-associated protein 1 mutation are all host risk factors for uveal melanoma [2]. Uveal melanomas are the most frequent kind of eye cancer. They are caused by melanocytes in the choroid, iris, or ciliary body of the eye [3, 4]. Anterior uveal melanomas are cancers that originate in the iris, while posterior uveal melanomas are tumors that originate in the ciliary body and/or choroid. Nearly half of individuals with initial posterior uveal melanoma will acquire metastatic uveal melanoma. uveal melanoma metastasizes to lymph nodes or the brain seldom [4]. The liver is the primary organ of metastasis, accounting for 71.4 percent to 87 percent of individuals with metastatic illness. In 40 % of patients, the liver is the most common location of systemic metastasis and is often the earliest metastatic site. Metastatic liver disease is the leading cause of mortality in people with uveal melanoma, with roughly 50 % acquiring liver metastases within 15 years after diagnosis [5].

The most practicable first-line treatment options for this cancer are now resection, radiation therapy, and enucleation. Radiation treatment is classified into two basic types: plaque brachytherapy (using iodine-125, ruthenium-106, palladium-103, or cobalt-60) and teletherapy (proton beam, helium ion, or stereotactic radiosurgery using cyber knife, gamma knife, or linear accelerator). Enucleation is an alternative to radiation [2].. Hepatic metastasis is a significant prognostic factor for clinical outcome and survival. Patients with hepatic metastases had a median survival of 6 to 12 months. Despite appropriate therapy, up to 50 % of individuals with primary uveal melanoma develop systemic metastases, most often in the liver [6].

An important aspect of systems biology is identifying and describing the subcellular machinery that generates the functional operational units in organs, tissues, and cell systems that lead to physiological responses [7]. Several health issues have been linked to endocrine disrupting chemicals (EDCs) in recent years. Many wonderful studies have been done on the details of EDCs in many areas, yet public anxiety persists, and the media has a hand in exaggerating it. It is thus necessary to have a broad grasp of EDCs that includes previous study results and reviews of current EDCs research trends [8-11].

In this research, we aim to find the genes affecting melanoma and the EDCs influencing the expression of those genes. For this reason, we discover the genes related to metastasis melanoma using GEO database and reconstruct the protein-protein interaction network using a String database. Then, modules are created using Cytoscape software and Clusterviz plugin. as a next step, we find potential EDCs using CompTox database and create EDC-GENE network. Finally, we introduce high-

degree EDC in this network. We hope these findings help researchers better understand Melanoma.

Material and Methods

Dataset and preprocessing:

The Gene Expression Omnibus at the National Center for Biotechnology Information (GEO) was used to access the required data with the accession number of GSE22138. This data compared within the 57 tumors with at least 36 months follow-up, 28 uveal melanoma from patients who developed liver metastases (meta1 group) with 29 tumors arising from patients without metastases (or later metastases, i.e. after 36 months) (meta0 group). These methods are based on the information obtained from biological databases and bioinformatics techniques. We collected a list of disease-related genes that are most often expressed during the development of metastatic melanoma from the Gene Expression Omnibus (GEO). The GEO2R was used to process raw data and find out the differentially expressed genes.

Protein-Protein Interaction network (PPIN):

We used the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/cgi/) to identify interactions between the Target Genes. Using the STRING database v.11.0, differentially expressed genes with a P value of less than 0.05 were chosen to create the protein-protein interaction network. The data was then displayed using Cytoscape v.3.8.2. Clusterviz uses a variety of techniques to do cluster analysis. A network of protein-protein interactions may be constructed by specifying an individual protein name and several IDs or amino acid sequences in the STRING database Search Tool. The network nodes are genes, and the edges represent the predicted functional associations. The data was displayed using Cytoscape v.3.8.2. Clusterviz uses a number of techniques to examine clusters.

The STRING database aims to compile a comprehensive list of all known and predicted protein-protein interactions, including physical and functional.

Cytoscape is a free and open-source bioinformatics software platform for visualizing and merging molecular interaction networks with gene expression patterns and other state information. Additional functionality is accessible through plugins.

Network of EDC-Gene Interactions:

Modules were created from the main network as sub-networks with greater expression genes. We identified effective EDCs for modular genes using the comptox dataset. Then, we plotted the EDC-Gene network using cytoscape software.

The CompTox Chemistry dashboard (https://comptox.epa.gov/dashboard) has extensive information on the toxicity of chemicals, their physicochemical properties, human exposure, and in vitro bioassay data (agonist, antagonist, up- and down-regulation) for a total of 883000 compounds (as of August, 2020).

Gene Ontology and Pathway Enrichment analysis

The DAVID online tool was used to examine the functional characteristics of the Target EDCs. The enrichment of Gene Ontology (GO) and Genomes (Reactome) pathways was conducted using adj P value less than 0.05.

Results

Melanoma associated differentially expressed genes (DEGs):

We examined the GSE22138 microarray dataset to identify differentially expressed genes associated with Melanoma's etiology. This study compared 28 uveal melanoma from patients who acquired liver metastases (meta1 group) with 29 tumors coming from individuals without metastases (or later metastases, i.e. after 36 months) among the 57 tumors having at least 36 months follow-up (meta0 group). Then, we found 2000 significant DE genes with a P value less than 0.05. Supplementary file S1 shows the list of these genes.

Analysis of these genes with GEO2R tool reveals the number of up- and down-regulated genes. According to fold changes, 1223 genes are down-regulated, and 779 genes are up-regulated.

PPI network and Clustering:

The String database was utilized to generate the 2000-gene PPI network. (Figure 1) (Supplementary file S2).

Then, we used cytoscape software to design the PPIN. Then, we used Clusterviz plugin to identify high-contrast protein groups. Finally, 10 cluster networks were found (Figure 2) (Table 1) (Supplementary file S3). Among the genes involved in module formation, RCHY1, RAB2A, CHMP2A, UBA2, PAFAH1B1, ID2, MARCKS, SHC1, MRPS28 and EIF1B genes with Adj P value less than 0.05 are involved.

EDC-Protein network:

The Comptox database was used to identify EDCs. We identified 17 genes (ACLY-CAT-CEBPB-RB1-CDK2-SKI-FGFR1-ETS1-FOXO3-PPARG-MET-FYN-KDR-STAT3-HDAC3-CSNK2A1-MAPKAPK5) that were affected by EDCs. Then, we plotted the EDC-protein network for these genes using cytoscape (Figure 3).

Then, we categorized the relationship between EDCs and genes in a table to examine the maximum impact of each EDC (Table 2).

Among the discovered EDCs, Oryzalin was recognized as the most effective drug due to its impact on 14 genes (Figure 4). The chemical FYN affected 8 genes and was, thus, identified as the least effective substance. (Oryzalin is a chemical compound with the $C_{12}H_{18}N_4O_6S$ formula and appears as yellow-orange crystals. Non-corrosive was used as an herbicide).

Gene Ontology and Pathway enrichment analysis

DAVID v.6.8 was used to analyze Go and Reactome pathways investigate the biological roles of RCHY1, RAB2A, CHMP2A, UBA2, PAFAH1B1, ID2, MARCKS, SHC1, MRPS28 and EIF1B genes in gene clusters at the cellular level. The S4 supplemental file contains the findings of the GO enrichment analysis. These genes were also studied for pathway enrichment using Reactome. Important paths are listed in the S5 file.

Discussion

In this study, 2000 effective genes in melanoma were isolated with a P value ≤ 0.05. The PPI network was designed to separate genes with different expressions. For further investigation, ten subunits that were mainly associated with melanoma metastasis were separated. The main purpose of this study was to find potential EDCs in regulating the expression of sub-networks genes. Among the sub-network genes, 10 EDCs were found for 17 genes. Diethanolamin affected eight genes and was identified as the least effective EDC,

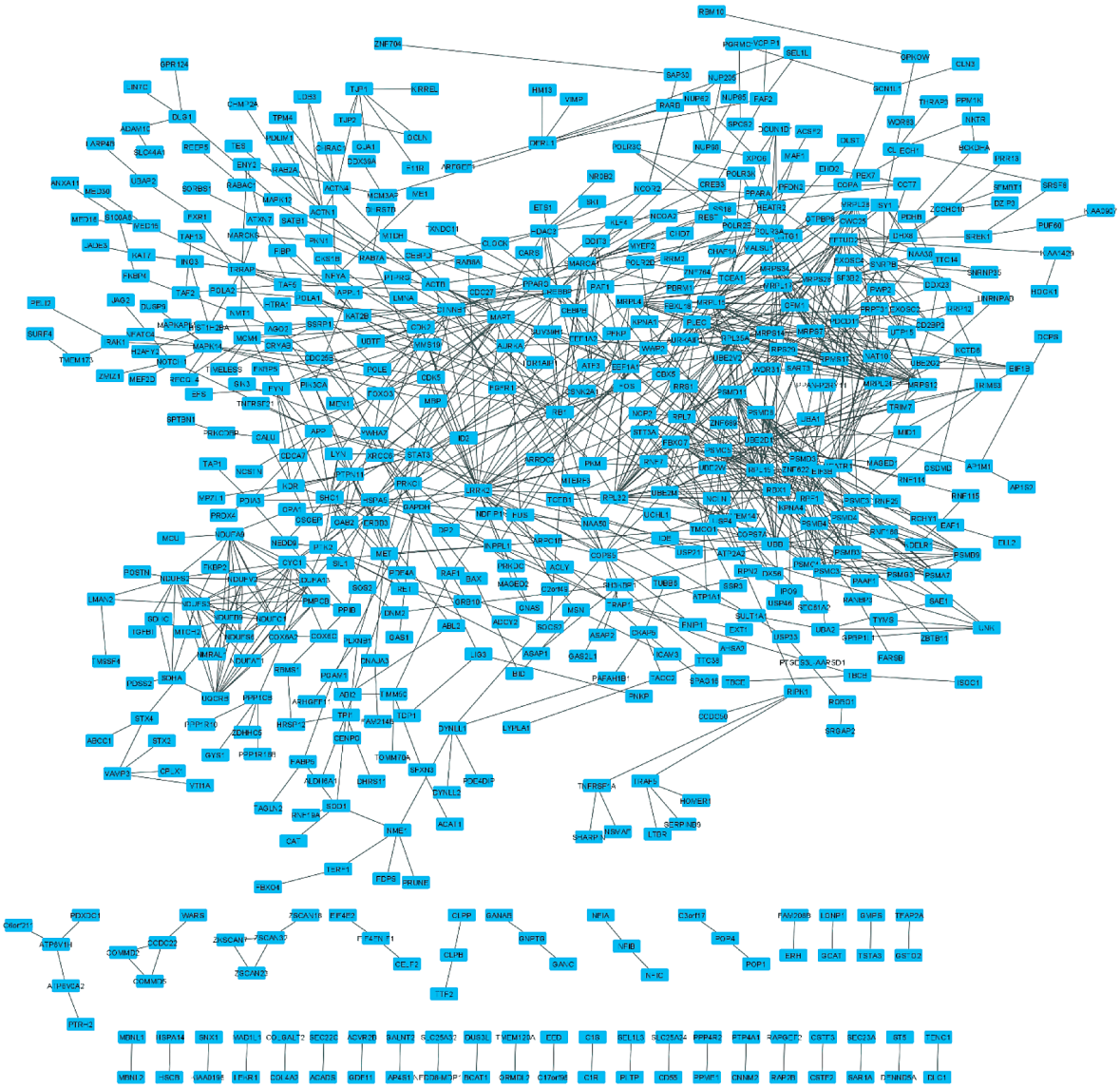

Figure 1: Protein-Protein Interaction Network of the Target-Genes. The network nodes are proteins. The edges represent the predicted functional associations

and Oryzalin showed effects on 14 genes.

The prognosis of uveal melanoma is determined by a number of clinical factors, including tumor location within the ciliary body, diffuse (flat) configuration, large tumor size, and extraocular extension, as well as histopathologic and cytogenetic factors, including epithelioid cell type, increased mitotic activity, tumor vascular networks, infiltrating lymphocytes, and chromosomal mutations of monosomy 3 and 8q addition. Numerous papers have highlighted tumor size as a critical clinical characteristic of metastasis. The multivariate analysis identified tumor thickness as a significant predictor of metastasis, with a 1.06 hazard ratio for each millimeter increase. Tumor thickness upon diagnosis was 2.7mm in the case of iris melanoma, 6.6mm in the case of ciliary body melanoma, and 5.5mm in the case of choroidal melanoma [12]. Choroidal melanoma is often identified as a pigmented (85 %) tumor located under the retina with a

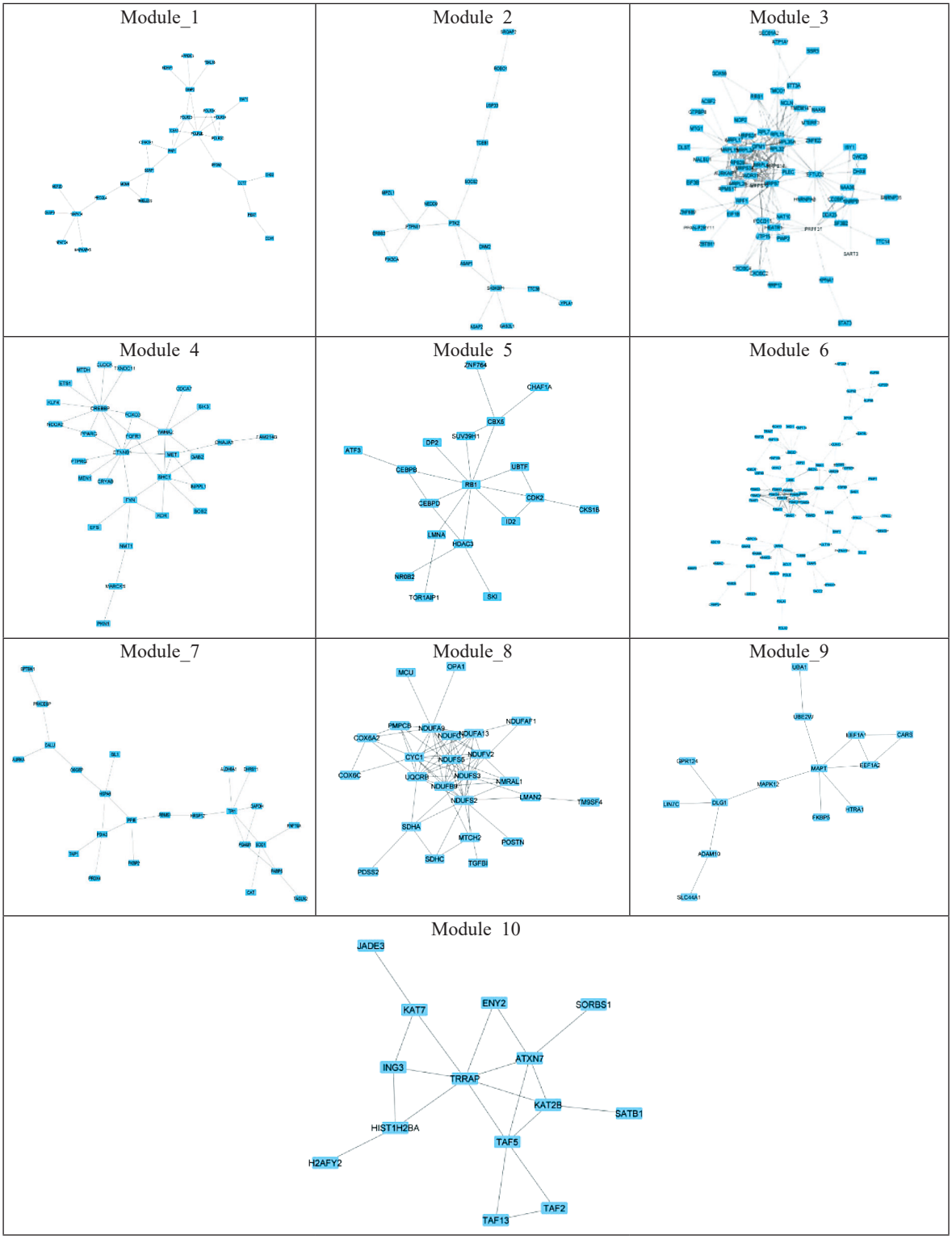

Figure 2: 10 modules obtained from network. The genes are marked

Table1: List of each sub-network and gene names

Cluster	Nodes	Edges	List of genes
1	27	36	MAPKAPK5, MEF2D, MAPK14, NFATC4, RECQL4, POLR3A, NDFIP1, MAF1, PFDN2, ECH1, EHD2, PEX7, SSRP1, CCT7, PAF1, CSNK2A1, WWP2, ARRDC3, POLR2D, POLR2E, TCEA1, DUSP9, TIMELESS, MCM4, FBXL18, POLR3C, POLR3K
2	18	20	GAS2L1, SOCS2, PTPN11, ASAP2, MPZL1, SH3KBP1, PTK2, ASAP1, SRGAP2, USP33, ROBO1, DNM2, PIK3CA, TCEB1, NEDD9, TTC38, LYPLA1, ERBB3
3	68	270	NCLN, DHX8, TTC14, DDX23, GTPBP8, SEC61A2, PDCD11, MRPL15, SNRPB, ACSF2, HEATR1, EFTUD2, EXOSC4, PRPF31, TMCO1, KPNA1, ATP1A1, UTP15, EXOSC2, CD2BP2, NOP2, MTG1, STAT3, RRS1, DDX56, RRP12, SF3B2, TMEM147, EIF3B, WDR31, EIF1B, SNRNP35, HNRNPAB, ZNF689, PLEC, ZNF622, STT3A, ZBTB11, NAA38, ISY1, CWC25, RPL7, RPL32, RPL35A, RPS29, MALSU1, NAA50, RPL15, SART3, MRPS14, MRPS12, MRPL24, GFM1, MRPL17, MTERF3, RPMS17, MRPS28, PPAN-P2RY11, MRPL4, RPF1, MRPS7, MRPL28, SSR3, MRPS34, PWP2, DLST, NAT10, AURKAIP1
4	30	40	CRYAB, SIK3, TXNDC11, PKN1, MARCKS, MTDH, CREBBP, SOS2, YWHAZ, INPPL1, EFS, KDR, GAB2, FYN, NMT1, PTPRG, MEN1, MET, PPARG, NCOA2, FOXO3, CTNNB1, FAM214B, ETS1, DNAJA3, FGFR1, CLOCK, KLF4, CDCA7, SHC1
5	18	22	ATF3, TOR1AIP1, LMNA, SKI, CDK2, NR0B2, CKS1B, SUV39H1, CHAF1A, DP2, HDAC3, UBTF, RB1, ZNF764, ID2, CBX5, CEBPB, CEBPD
6	73	162	PSMD8, POLA2, RAB7A, DHRS7B, LRRK2, ACLY, COPS7A, MAGED1, DYNLL1, FNIP1, SAE1, FBXO7, PSMD4, MAGED2, UBE2M, RNF115, KDELR1, MID1, RBX1, COPS5, XPO6, ARPC1B, HEATR2, DCUN1D1, PSMC3, NUP62, ARFGEF1, PSMC4, PAAF1, UBA2, NUP85, UCHL1, PAFAH1B1, PSMG3, NUP205, TACC2, SPAG16, TUBB6, UBE2V2, UBB, CKAP5, USP46, RAB2A, CHMP2A, RNF25, PSMA7, RNF168, RNF114, TRIM7, USP21, PSME3, ELL2, RCHY1, EAF1, RAB8A, GNAS, ADCY2, DYNLL2, PSMB4, PDE4DIP, PSMB3, PSMB9, SULT1A1, POLA1, UBE2D1, PSMC5, NUP98, REEP5, MMS19, RABAC1, PSMD3, POLE, PSMD11
7	24	26	RBMS1, HSPA5, PDIA3, HRSP12, SPTBN1, GAPDH, PGAM1, PRKCDBP, PPIB, TAGLN2, FKBP2, OSGEP, FABP5, DHRS11, SIL1, RNF19A, CALU, TPI1, TAP1, ALDH6A1, SOD1, PRDX4, AURKA, CAT
8	25	78	NDUFS3, NMRAL1, TM9SF4, LMAN2, CYC1, COX6C, UQCRB, NDUFA9, PMPCB, COX6A2, NDUFAF1, PDSS2, TGFBI, OPA1, POSTN, NDUFS2, SDHA, MCU, NDUFV2, NDUFA13, SDHC
9	14	15	LIN7C, MAPK12, FKBP5, EEF1A2, CARS, UBE2W, DLG1, MAPT, SLC44A1, ADAM10, EEF1A1, UBA1, HTRA1, GPR124
10	14	20	ENY2, SORBS1, TAF5, KAT2B, TAF2, TRRAP, ATXN7, TAF13, HIST1H2BA, KAT7, H2AFY2, ING3, SATB1, JADE

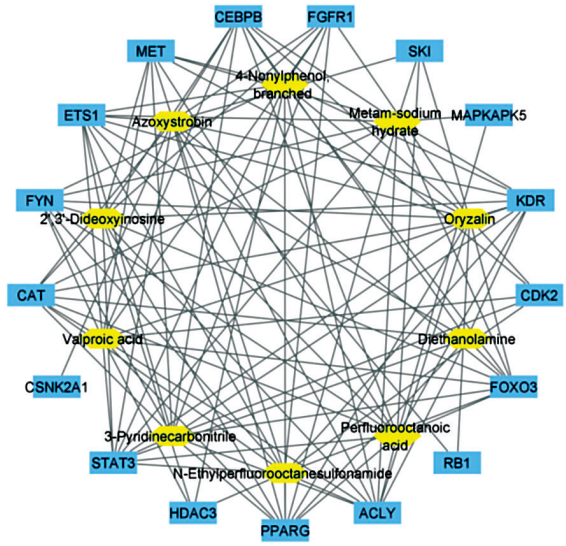

Figure 3: Edc-Gene network drawn by cytoscape. The blue rectangles represent the genes and the yellow circles the EDCs

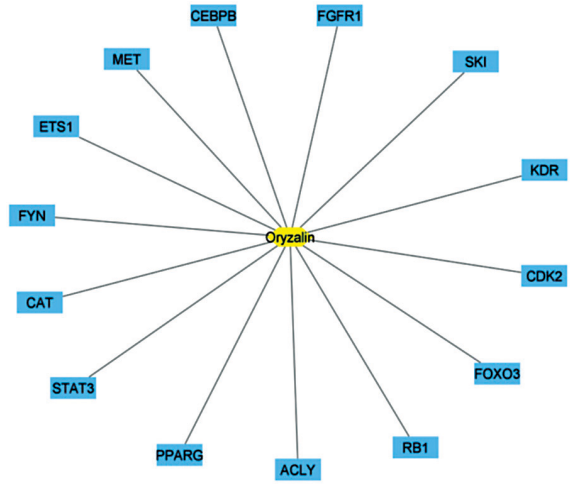

Figure 4: Oryzalin and its Target genes. blue rectangles indicate genes that are affected by the Oryzalin (yellow circle)

Table 2: EDCs and the affected genes

EDC	Gene
4-Nonylphenol,branched	MET-ETS1-FYN-CEBPB-STAT3-HDAC3-PPARG-ACLY-FGFR1-FOXO3-CDK2-KDR
Meta-Sodium hydrate	MET-ETS1-FYN-CEBPB-CAT-STAT3-PPARG-ACLY-FOXO3-MAPKAPK5-KDR-SKI
Azoxystrobin	CSNK2A1-STAT3-CAT-ACLY-PPARG-RB1-FOXO3-ETS1-KDR-SKI-CEBPB-FGFR1
Oryzalin	FGFR1-SKI-CEBPB-MET-ETS1-FYN-KDR-CAT-STAT3-CDK2-FOXO3-PPARG-RB1-ACLY
Perfluorooctanoic acid	SKI-MAPKAPK5-CEBPB-MET-KDR-ETS1-FYN-CAT-STAT3-FOXO3-PPARG-ACLY
Diethanolamin	KDR-CEBPB-ETS1-FOXO3-CAT-PPARG-STAT3-ACLY
N-Ethylperfluorooctanesulfonamide	ACLY-FOXO3-CDK2-KDR-PPARG-FGFR1-MET-ETS1-FYN-CAT-HDAC3-STAT3
3-Pyridinecarbonitrile	ACLY-PPARG-FOXO3-CDK2-STAT3-KDR-FGFR1-CEBPB-MET-ETS1-CAT-FYN
Valproic acid	HDAC3-STAT3-ACLY-PPARG-FOXO3-CDK2-KDR-CAT-FGFR1-CEBPB-FYN-MET-ETS1
2',3'-Dideoxyinosine	STAT3-HDAC3-ACLY-PPARG-FOXO3-CAT-KDR-FYN-ETS1-FGFR1-MET-CEBPB

median basal diameter of 11mm and a mean thickness of 4.5mm. Each millimeter increase in thickness results in a 5 % rise in the chance of metastasis. Choroidal melanoma has distinctive ophthalmoscopic and diagnostic imaging characteristics. The mean thickness of the tumor at the time of discovery was 5.0 mm [1].

In uveal melanoma, cells display increased levels of c-Met, IGF-IR, and CXCR4. The liver is the only organ that expresses these receptors' matching ligands at a high level (HGF, IGF, and CXCL12). These pathways may play a significant role in the development of liver-specific metastases in uveal melanoma. Previous research indicates that increasing levels of c-Met expression in patients' initial tumors considerably enhance the likelihood of eventual liver metastasis. When HGF activates c-Met, multiple downstream signaling pathways are activated, including the Ras protein kinase pathway. These pathways result in the upregulation of numerous genes and have been shown to increase cellular proliferation, motility, cell cycle progression, and invasive capacity [5]. The SKI protein represses the TGF-β tumor suppressor pathway with the help of Smad transcription factors. SKI expression is increased in human malignant melanoma tumors as the illness progresses, and its overexpression stimulates melanoma cell proliferation and migration in vitro. SKI is up-regulated in human melanoma as the illness progresses, resulting in the down-regulation of p21Waf-1 and the up-regulation of MITF and Nr-CAM (two proteins associated with melanoma cell survival, motility, growth, and transformation). Additionally, SKI may decrease TGF-induced growth inhibition by binding to and sequestering TGF-activated Smads and limiting their nuclear localization. SKI functions as a sensor and a modulator of TGF- signaling by preventing Smad2/3 from achieving complete nuclear localization, tethering C-terminally phosphorylated Smads to transcriptional repressor complexes, and promoting linker region phosphorylation of Smad3, which is associated with its activity shifting from tumor suppression to oncogenesis [13]. Fyn is a kinase of the SRC family (SFK). FYN has been shown to have a number of biological roles as a proto-oncogene, including cell proliferation, adhesion, survival, and platelet activation. Multiple cancers, including melanoma, prostate cancer, and glioblastoma, have been shown to express Fyn. Fyn is one of the most substantially changed genes in liver metastatic uveal melanoma when compared to non-metastatic uveal melanoma. Previously, the expression of Fyn in melanoma and normal tissues was compared to the GEO database's RNA-seq findings (GSE114445 and GSE29359). The data indicate that Fyn is highly expressed in melanoma and that silencing Fyn significantly decreases melanoma growth and promotes apoptosis, suggesting that Fyn plays a critical role in melanoma. Lj-1-60 (Fyn inhibitors) suppresses melanoma growth and promotes cell cycle arrest in the G2/M phase and death through reducing Stat3 phosphorylation [14]. In several malignancies and many human tumor cell lines, abnormal production of fibroblast growth factor (FGF)-2 and its receptors results in aberrant cell proliferation. FGF1 and FGF2 are abundantly expressed in primary uveal melanomas, as is their FGFR1 receptor. Four distinct genes (FGFR1–4) encode FGFRs, and the varied interactions between these growth factors and their receptors control the specificity of FGF-induced downstream signaling and biological activity. Thus, activation of FGFR1 was necessary for the proliferation and survival of uveal melanoma cells. ERK1/2

activation plays a critical part in the FGF2/FGFR1 autocrine loop, which results in the autonomous development of uveal melanoma cells. By inhibiting either FGFR1 or FGF2, activation of ERK1/2, cell proliferation, and survival were decreased [15]. STAT3 promotes metastatic growth and antagonizes MITF in melanoma via directly increasing CEBP family member transcription. STAT3 and MITF are antagonistic and have distinct functions in the development of melanoma. MITF is involved in the regulation of cell survival, differentiation, and proliferation. This work established that STAT3 inhibits MITF expression through overexpression of members of the CAAT Box Enhancer Binding Protein (CEBP) family. ATAC-seq study demonstrated that CEBPa/b binding to the MITF enhancer region silences the MITF locus. As a consequence, STAT3 deficiency or downregulation reduces melanoma spread, and hence MITF expression is increased [16]. ETS1 is a transcription factor belonging to the ETS family, which is characterized by the presence of a winged helix-turn-helix DNA-binding domain. ETS1 expression is increased in melanoma in situ, invasive, and primary metastatic tissues and cell lines, but is absent or low in normal skin melanocytes or simple nevus structures (lentigo simplex). ETS1 promotes tumor cell survival, growth, and invasion. ETS1 may operate as a pro- or antiapoptotic factor, depending on the cell type. In melanoma, ETS1 acts as an anti-apoptotic factor. Increased ETS1 protein results in an increase in the amount of MET, while ETS1 inhibition results in a reduction in MET receptor expression. The MET promoter contains two ETS1 elements, and activation through these two elements is enhanced by a variety of processes including PAX3 or hepatic growth factor (HGF). The induction of MET by ETS1 through this second site is enhanced by activating hepatic growth factor-dependent ETS1. Additionally, ETS1 and MET protein levels are substantially [17].

In mammals, the FoxO family includes FoxO1, Foxo3, and FoxO4. FoxO3 is a transcription factor and tumor-inhibiting gene that plays a role in the ATK / PI3K / IGF-1R cellular pathways and is reduced or inactivated in most human cancers. This gene binds to the TTGTTTAC motif and regulates cellular functions like replication, differentiation, DNA repair, and apoptosis. Inhibiting this gene causes cell growth and tumor progression and angiogenesis, and its expression suppresses tumors. Poor survival leads to UM. Given the suppressive role of Foxo3 in UM, these genes and downstream genes could be an important target for developing new therapies [18, 19].

The peroxisome proliferator-activated receptor (PPARs) gene is a ligand-activated transcription factor that belongs to the nuclear receptor family. Active PPARs with retinoids form a heterodimeric complex, bind to peroxisome proliferator elements (PPREs) in DNA and initiate transcriptional genes. This family has the three isoforms of PPARα, PPAR β / δ, and PPAR γ, each of which has different ligands. PPAR γ gene is highly expressed in adipocytes and is the main regulator of differentiation in adipocytes. Recently, the expression of these genes in tumor cells was caused by the malignancies activated in various cancers. For example, in melanomas, the NFκ-B signaling pathway has been activated as the main regulator in cancer cell survival. Thus, by inhibiting this pathway, cancer cell growth in Laboratory conditions has decreased. Of course, its mechanisms are still being studied, and it is known as a molecular method for developing anticancer drugs [20].

Increased lipogenesis and mitochondrial function are important features in melanoma. However, their roles in finding targeted therapies have not been fully elucidated. ATP-citrate lyase (ACLY) is a vital lipogenic enzyme for organisms and can play a role in tumor formation. This enzyme is elevated in melanoma and is sometimes a carcinogen produced in vitro and in vivo. The tumor has done. This indicates that it can also be presented as a potent treatment by inhibiting ACLY carcinogenesis, melanoma growth, and mitochondrial biogenesis [21].

Cyclin and cyclin-dependent protein kinases are important proteins needed to regulate and express a large number of cellular functions, such as CDK2 as a major regulator of the cell cycle that transports cells from phase S to G1. It also promotes the S phase. In any cell cycle, members of the CDK family can inhibit many malignancies through cycle deactivation. For example, CDK2 can suppress cell growth and development and is used as a suitable drug treatment for melanoma [22, 23].

The CAT gene in melanoma inhibits cell proliferation by delaying the S-G2 / M stage. Experimental studies have shown that its expression in melanoma cells does not change the number of cells on consecutive days, indicating that the function of this gene is to prevent cell proliferation, rather than cell death [24].

The KDR gene product is the VEGF-A receptor, VEGFR2, promoting the growth of several cellular pathways, including ERK1 / 2, PI3Kinase-protein kinase B (PKB), p38 MAPK and CSK (Src), by affecting endothelial cells and binding to its ligands. Survival, migration and angiogenesis of the cell is a mutation of this gene known as KDR Q472H in melanoma disease and angiogenesis in tumor cells [25, 26].

The RB1 gene has 27 exons and 26 introns and is located on the long arm of chromosome 13. This gene suppresses tumors and had an important role in cell cycle differentiation, apoptosis, growth suppression and cell cycle arrest. It is also one of the genes in patients with melanoma. It increases the susceptibility to melanoma to some extent, and identifying people with such mutations can help better identify susceptible individuals [27, 28].

Metastasis is the leading cause of mortality among cancer patients. In order for tumors to overcome tissue and microenvironment obstacles to invasion and migration, the metastatic phenotype features a broad variety of flexible cellular mechanisms [29]. The capacity to remodel and destroy the extracellular matrix (ECM) and to acquire mesenchymal characteristics, often described by the expansion of actin-rich polarized protrusion and the loss or decrease in cell–cell connections, is key to one such method [30].

It is thought that the ID2 family is comprised of helix-loop-helix factors that lack a basic domain and do not directly bind with DNA. Ids are thought to behave as dominant-negative transcription factors by sequestering other factors, such as some basic helix-loop-helix, E-twenty-six, and retinoblastoma proteins. Cell cycle progression and proliferation, migration, angiogenesis, and invasion are all controlled by Ids, whereas differentiation is also inhibited [31, 32]. Many forms of cancer, including breast, pancreatic, ovaries, and head and neck, have increased Id expression, indicating that Ids are working together as oncogenes [33]. While Id2 has been linked to melanoma, nothing is known about Id3 and Id4, raising the possibility that these Ids might play a role in the illness. TGF- has been hypothesized as a mechanism to explain why melanoma development is no longer inhibited in response to TGF- in certain melanomas

but not in primary cells [34]. For the first time, retinoblastoma family members were shown to interact physically and genetically with Id2, but not with Id1 or Id3 [35, 36].

MARCKS is a membrane-bound protein that engages in various key cellular processes, such as cytoskeletal rearrangement, motility, secretion and exocytosis [37]. For instance, MARCKS has been proven to alter the actin cytoskeleton and, consequently, the amount and length of filopodia [38]. Cell mobility, membrane protrusions, and invasiveness are all increased in cancer cells that have been infected with MARCKS. A potential role for MARCKS in WNT5A-induced cell migration and invasion has not yet been studied [39]. RAB2A and RAB2B There are several similarities between mammalian orthologists and RAB2's protected evolutionary phase (from the plant to the protozoan). These proteins are normal Found and indicated on the Golgi device Role in Golgi-to-ER traffic, to govern Golgi accumulation and production Secretory granules [40, 41]. Like total protein and cells, neither Limited quantity of MT1-MMP or a5b1 are both necessary for the matrix Extinction of RAB2A has an effect on the degradation of focal invadopodium [42]. RAB2A is not simply localized Endoplasmic reticulum (ER) –Golgi ERGIC), but also participates in COPI-dependent transportation ERGIC [43]. In this context, RAB2A interacts with its Agent, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [44-46]. Mammalian mitochondrial ribosomes translate 13 proteins encoded by mitochondrial genes, all of which perform functions in the mitochondrial respiratory chain. After a lengthy time of regeneration, mitochondrial ribosomes are the most protein-rich ribosomes. Mitochondrial ribosomal proteins (MRPs) are encoded by nuclear genes, produced in the cytoplasm and then, transported to the mitochondria to be organized into mitochondrial ribosomes. MRPs not only have a function in mitochondrial oxidative phosphorylation (OXPHOS) (OXPHOS). Moreover, they engage in the control of cell state as apoptosis triggering agents. Abnormal expressions of MRPs will lead to mitochondrial metabolism problem, cell malfunction, etc. Many studies have proven the aberrant expression of MRPs in different malignancies [47]. Scientists explored the new mutations, epigenetic disorders, abnormal genes expression and protein abundance patterns based on genomics and proteomics, which establish causal relationships between MRPs abnormalities and carcinogenesis or provide diagnostic and therapeutic markers in some cases [48, 49]. Based on further experimental findings, the same MRPs may influence numerous malignancies and several MRPs anomalies may be discovered in the same cancer, which generates a complex and changing network structure. MRPs may be employed as markers for the identification of the development of certain malignancies. In the current investigation, MPRS28 was identified as one of the genes promoting the rise of ocular melanoma metastasis.

By regulating numerous tumor stimulants and suppressors, ubiquitination seems to be a key contributor and hence a possible therapeutic target for melanoma. More than 20 distinct UBDs, such as those linked to ubiquitin, have been found, and they are tiny structural entities that likely to contain ubiquitin. Domain (UBA) and Ubiquitin Interaction Motif (UIM) (50). UBDs and polyubiquitin chains interact to break ubiquitinated proteins. We found that UBA2 is a factor in the spread of ocular melanoma [51]. charged multivescular body protein 2A (CHMP2A) is a component of the endosomal sorting kit needed for transport

(ESCRT) [52]. The findings of our investigation demonstrated that the expression of this gene in uveal melanoma rises. This gene subunit's function is unknown at this time. Another gene with elevated expression in uveal melanoma was the EIF1B gene. EIF1B was one of 12 genes whose expression profile Harber et al. found to be predictive of systemic metastasis in Juve melanoma [53].

Conclusion

Our study compared 2000 DEGs associated with metastasis in melanoma and showed that a total of 17 genes with high expression had a positive effect on melanoma metastasis. Beside a certain number of EDCs also affected the expression of these genes. A network of protein-protein interactions was used to identify melanoma genes. The resultant network was constructed using genes from PPI modules and EDC-gene interactions. Due to the computational nature of our study and restricted experimental resources, the validity of these EDCs in vitro and in vivo should be further investigated in clinical and experimental trials.

However, we have seen many successful computational studies, showing potential for detecting early diereses identification, subtype stratification, stage differentiation, and other possibility in tackling the different diseases [54- 57].

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.