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Gene Expression and Pathway Analysis of *Wedelia chinensis* in the 22RV1 Prostate Cancer Cell Line

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ABSTRACT

Research has shown that the extract from Wedelia chinensis improves the outcomes of prostate cancer therapy. This investigation examined genes with altered expression in the 22RV1 prostate cancer cell line exposed to W. chinensis extract, utilizing data retrieved from the Gene Expression Omnibus (GEO), followed by gene ontology and pathway enrichment analyses. Expression data from the series GSE100224 were evaluated with GEO2R. Key genes showing differential expression were investigated through interaction mapping. Associated biological functions and processes linked to these genes were determined. Prominent disrupted genes and pathways were reviewed in detail. A total of seventy key differentially expressed genes, consisting of 49 upregulated and 21 downregulated, were analyzed for interactions involving inhibition, activation, expression, and binding. Cytochrome P450 and PTGS2 stood out as critical genes. The pathway primarily affected was estrogen metabolism. The evidence points to "estrogen metabolism" as the chief pathway influenced by W. chinensis in 22RV1 cells, with UGT1A1, MAOA, PTGS2, and cytochrome P450 being the main genes involved.

Keywords: Estrogen, Gene, Pathway, Prostate cancer, Wedelia

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Introduction

Globally in 2020, there were around 1 414 259 newly diagnosed cases of prostate cancer, resulting in 375 304 deaths [1]. Over the recent decade, progress in managing prostate cancer has involved novel agents, improved application of established treatments for early disease, advanced sequencing technologies, and enhanced imaging techniques [2]. The androgen receptor plays a vital role in driving the disease processes, including metabolism, cell movement, and growth in prostate cancer, making it a validated target for therapy [3]. Beyond synthetic medications, various plant-derived compounds with anticancer effects have emerged as promising options for treatment [4]. Wedelia chinensis (Osbeck) Merr is commonly included in traditional remedies for inflammation in Taiwan and southern regions of China. Evidence supports that using Wedelia chinensis extract boosts the success of prostate cancer management. Key components such as apigenin, luteolin, and wedelolactone from this plant have been found to inhibit androgen receptors [5, 6].

Cancer often involves abnormal regulation of numerous genes, making gene expression profiling an effective tool to uncover underlying molecular processes. Modulating the activity of genes central to cancer development is a core strategy in treatment approaches [7, 8]. This approach is particularly prominent in prostate cancer care [9]. Combining computational biology with genomic data has become a standard practice for exploring gene regulation in cancer detection and management [10]. Sun *et al*. presented work on profiling gene expression in prostate cancer, applying ontology and pathway analyses to pinpoint essential pathways disrupted by genetic changes. They drew data from "The Cancer Genome Atlas" and processed it with "Gene set enrichment analysis"

alongside the Kyoto Encyclopedia of Genes and Genomes (KEGG) resources [11]. Fan *et al*. described their examination of GSE55945 from GEO concerning prostate cancer. They utilized protein interaction networks, KEGG pathways, and ontology analysis, concluding that processes like cellular growth, division, cycle regulation, and junctions were most influenced by the differential genes. They highlighted RPS21, FOXO1, BIRC5, POLR2H, RPL22L1, and NPM1 as important genes linked to the disease [12]. Here, expression data for the 22RV1 prostate cancer cell line (GSE100224) from GEO were investigated via ontology and pathway methods to clarify how Wedelia chinensis impacts these cells at the molecular level, contributing to better therapeutic results.

Materials and Methods

Ethical considerations

Approval for this work was granted under code IR.SBMU.RETECH.REC.1401.427 by the ethics committee at Shahid Beheshti University of Medical Sciences.

Data collection

The dataset was sourced from GSE100224 in GEO (initial release in 2017, last update in 2021) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse100224>). Expression profiles for the human 22RV1 prostate cancer cells treated with 10 µg/mL Wedelia chinensis extract compared to untreated controls were retrieved and processed using GEO2R. The leading 250 genes by change in expression were chosen for initial querying. Genes with significant differences were selected based on a "fold change" > 2. Changes in expression were displayed in a volcano plot relating fold change to statistical significance.

Key differential genes were loaded into CluePedia, a plugin for Cytoscape, to map out regulatory connections among them. Interactions assessed included binding, expression modulation, activation, and inhibition. Networks were built with directed connections.

For identifying relevant biological categories, the key differential genes were processed in ClueGO. Categories were drawn from sources including WikiPathways, GO_BiologicalProcess-EBI-UniProt-GOA-ACAP-ARAP, GO_CellularComponent-EBI-UniProt-GOA-ACAP-ARAP, KEGG, REACTOME_Pathways, REACTOME_Reactions, and GO_MolecularFunction-EBI-UniProt-GOA-ACAP-ARAP (updated 08.05.2020).

Statistical analysis

Significant differentially expressed genes (DEGs) were defined using a p-value threshold of < 0.01. For biological term evaluation, thresholds of < 0.01 were applied to the term p-value, Bonferroni step-down corrected term p-value, group p-value, and Bonferroni step-down corrected group p-value.

Results and Discussion

Out of the leading 250 DEGs, 70 met the selection criteria of "fold change" > 2 combined with p-value < 0.01 and were subjected to deeper investigation. A volcano plot was created to visualise the overall expression patterns. Locations of markedly upregulated and downregulated genes are marked on this plot **(Figure 1).**

These 70 key DEGs were inputted into CluePedia to map out potential binding, expression, activation, and inhibition interactions. CluePedia recognised 58 of the 70 genes, which consisted of 36 standalone nodes, 6 pairs, one triplet, and one large interconnected cluster. The mapped genes and their connections are illustrated in **Figure 2.**

ClueGO was employed on the same 70 DEGs to uncover linked biological processes and functions. **Table 1** lists 64 dysregulated biological terms that emerged. These were organised into four main clusters: group 1 ("apoptosis-related network due to altered Notch3 in ovarian cancer"), group 2 ("oxidative stress"), group 3 ("tryptophan metabolism"), and group 4 ("estrogen metabolism"). The allocation of terms to each cluster is displayed in **Figure 3.** To better identify pivotal DEGs, links between the genes and these clusters were further explored. The GEO repository provides a rich dataset for disease research, and numerous prostate cancer studies have leveraged it as a primary data source [13, 14]. Box plots highlighting expression changes in treated cells showed clear dysregulation across many genes. In total, there were 49 upregulated and 21 downregulated DEGs. Log (fold change) values ranged from 1.0 to 5.8 for upregulation and from (-1.0) to (-2.1) for downregulation. The gene with the strongest upregulation was CYP1A1, while PMEPA1 showed the greatest downregulation.

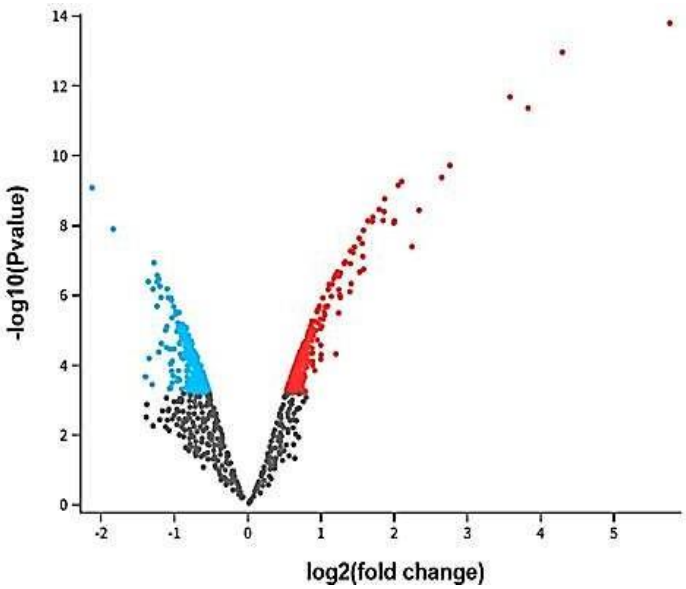

Figure 1. Volcano plot for the gene expression profiles of GSE100224; The up and down regulated spots were determined based on "fold change" > 1.5.

Given the power of network-based approaches in prioritising candidates, the DEGs were analysed this way [15]. **Figure 2** reveals that CYP1A1, CYP1A2, and CYP1B1—all belonging to the cytochrome P450 family—form part of the central interconnected cluster and rank among the most highly upregulated genes. Another standout gene in the network is prostaglandin-endoperoxide synthase 2 (PTGS2), shown in **Figure 2.** PTGS2 expression was modulated by ELF3, HMOX1, and NQO1, and in turn it activated CYP1B1. Ellinger *et al*. reported that circulating noncancerous PTGS2 DNA fragments in prostate cancer patients' serum could function as useful diagnostic and prognostic indicators [16]. The contribution of cytochrome P450 family members to prostate cancer onset and response to therapy was examined by Chen *et al*. [17].

Table 1 and **Figure 3** document 64 biological terms associated with the DEGs, divided into four clusters. The largest and most prominent cluster was "estrogen metabolism", which included 49 terms. Prior studies have substantiated the substantial involvement of estrogen and its derivatives in prostate cancer pathogenesis [18]. As evident in **Table 1**, cytochrome P450 genes dominate this cluster—of the 49 terms in group 4, 47 (96%) involve cytochrome P450 members. PTGS2 also features prominently, linked to eight terms in **Table 1**.

Another gene connected to terms across groups 4, 3, and 2 is monoamine oxidase A (MAOA). Lin *et al*. noted elevated MAOA expression in prostate cancer tissues [19]. The observed downregulation of MAOA following Wedelia chinensis treatment supports the plant's potential anticancer activity. Work by Xu *et al*. showed that blocking MAOA activity restricts prostate tumour growth [20].

UDP glucuronosyltransferase family 1 member A1 (UGT1A1) is a further gene emphasised in **Table 1**. Its involvement has been documented across multiple conditions, such as hepatobiliary disorders, diabetes, Gilbert's syndrome, cardiovascular conditions, leukaemia, Crigler–Najjar syndrome, neurological disorders, tumour formation, metabolic disturbances, Crohn's disease (CD), gallstones, myelosuppression, and obesity [21].

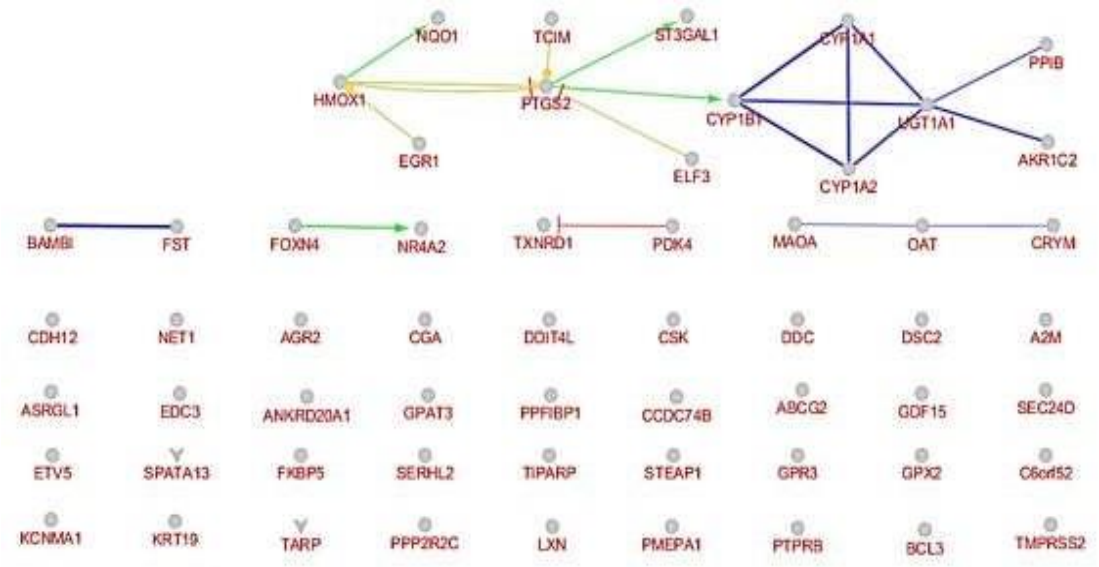

Figure 2. Interaction network among the 58 identified significant DEGs; colours blue, green, red, and yellow denote binding, activation, inhibition, and expression interactions, respectively.

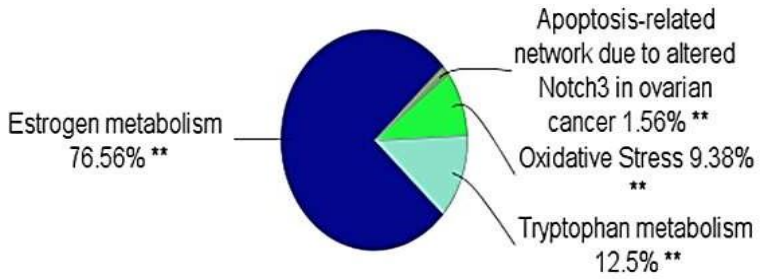

Figure 3. Distribution of gene ontology terms across groups (shown as percentages)

Table 1. The 64 biological terms associated with the significant DEGs

Rank (R)	Enriched Biological Process / Pathway	% AG	Identified Associated Genes
1	Notch3-altered apoptosis network in ovarian carcinoma	5.56	BCL3, NET1, NQO1
	Dopaminergic metabolic pathway	21.43	DDC, MAOA, NQO1
	NRF2-mediated antioxidant signaling	4.11	EGR1, GPX2, HMOX1, TXNRD1, UGT1A1
	NFE2L2 (NRF2) survival signaling triggered by photodynamic therapy	12.50	ABCG2, HMOX1, NQO1
2	Cellular response to oxidative stress	14.71	CYP1A1, HMOX1, MAOA, NQO1, TXNRD1
	Biological excretory processes	4.05	ABCG2, HMOX1, KCNMA1
	Caveola-associated cellular components	4.30	HMOX1, KCNMA1, NR4A2, PTGS2
	Tryptophan metabolic pathway	11.90	CYP1A1, CYP1A2, CYP1B1, DDC, MAOA
	Dopamine metabolism	21.43	DDC, MAOA, NQO1
	Amino acid metabolic processes	4.40	DDC, MAOA, OAT, PDK4
3	Oxidative stress–related pathways	14.71	CYP1A1, HMOX1, MAOA, NQO1, TXNRD1
	Metabolism of phenol-containing compounds	4.03	CGA, CRYM, DDC, MAOA, NR4A2
	Catecholamine metabolic activity	5.17	DDC, MAOA, NR4A2
	Catechol-containing compound metabolism	5.17	DDC, MAOA, NR4A2
	Dopamine metabolic process	7.32	DDC, MAOA, NR4A2

	Steroid hormone biosynthetic pathway	8.20	AKR1C2, CYP1A1, CYP1A2, CYP1B1, UGT1A1
	Tryptophan metabolism	11.90	CYP1A1, CYP1A2, CYP1B1, DDC, MAOA
	Retinoid metabolic pathway	4.48	CYP1A1, CYP1A2, UGT1A1
	Xenobiotic metabolism via cytochrome P450	5.19	CYP1A1, CYP1A2, CYP1B1, UGT1A1
	Drug metabolic processes	4.17	CYP1A2, MAOA, UGT1A1
	Ovarian steroid biosynthesis	7.84	CGA, CYP19A1, CYP1B1, PTGS2
	Chemical carcinogenesis pathways	7.23	AKR1C2, CYP1A1, CYP1A2, CYP1B1, PTGS2, UGT1A1
	Cytochrome P450 pathways categorized by substrate	4.55	CYP1A1, CYP1A2, CYP1B1
	Formation of epoxy- and dihydroxyeicosatrienoic acids (EET/DHET)	37.50	CYP1A1, CYP1A2, CYP1B1
	Arachidonic acid metabolic pathway	8.47	CYP1A1, CYP1A2, CYP1B1, GPX2, PTGS2
	Biosynthesis of 16–20 HETE derivatives	33.33	CYP1A1, CYP1A2, CYP1B1
	Hydroxylation of arachidonic acid to 16/17/18-HETE by CYP (1)	100.00	CYP1A1, CYP1A2, CYP1B1
	Hydroxylation of arachidonic acid to 19-HETE by CYP (2)	37.50	CYP1A1, CYP1A2, CYP1B1
	Epoxidation of arachidonic acid to 5,6-EET by CYP (4)	75.00	CYP1A1, CYP1A2, CYP1B1
	Epoxidation of arachidonic acid to 8,9/11,12/14,15-EET by CYP (5)	42.86	CYP1A1, CYP1A2, CYP1B1
4	Hydroxylation of arachidonic acid to 20-HETE by CYP (3)	50.00	CYP1A1, CYP1A2, CYP1B1
	Disorders related to biological oxidation enzymes	8.33	CYP1B1, MAOA, UGT1A1
	Biosynthesis of DHA-derived specialized pro-resolving mediators	17.65	CYP1A1, CYP1A2, PTGS2
	Production of specialized pro-resolving mediators (SPMs)	15.79	CYP1A1, CYP1A2, PTGS2
	Dopamine metabolic pathway	21.43	DDC, MAOA, NQO1
	Aryl hydrocarbon receptor signaling pathway	10.42	CYP1A1, CYP1A2, CYP1B1, NQO1, PTGS2
	Estrogen receptor–associated signaling	30.77	CYP1A1, CYP1A2, CYP1B1, PDK4
	Melatonin metabolism and biological effects	9.52	CYP1A1, CYP1A2, CYP1B1, MAOA
	Cytochrome P450–mediated oxidation	4.76	CYP1A1, CYP1A2, CYP1B1
	Tryptophan metabolic process	9.52	CYP1A1, CYP1A2, CYP1B1, DDC
	Tamoxifen metabolic pathway	14.29	CYP1A1, CYP1A2, CYP1B1
	Benzo(a)pyrene metabolic process	33.33	AKR1C2, CYP1A1, CYP1A2, CYP1B1
	Estrogen metabolism	26.32	CYP1A1, CYP1A2, CYP1B1, NQO1, UGT1A1
	Cellular toxin metabolic process	12.90	CYP1A1, CYP1A2, CYP1B1, DDC
	Hydro-lyase enzymatic activity	4.23	CYP1A1, CYP1A2, CYP1B1
	Tetrapyrrole metabolism	5.63	CYP1A1, HMOX1, UGT1A1
	Hormone metabolic processes at the cellular level	4.76	AKR1C2, CYP1A1, CYP1A2, CYP1B1, EGR1, TIPARP, UGT1A1
	Porphyrin-containing compound metabolism	8.70	CYP1A1, CYP1A2, HMOX1, UGT1A1
	Primary alcohol metabolic process	4.08	AKR1C2, CYP1A1, CYP1A2, CYP1B1
	Catecholamine metabolic process	5.17	DDC, MAOA, NR4A2
	Estrogen metabolic process	11.63	CYP1A1, CYP1A2, CYP1B1, TIPARP, UGT1A1
	Steroid hydroxylase enzymatic activity	7.50	CYP1A1, CYP1A2, CYP1B1
	Catechol-containing compound metabolism	5.17	DDC, MAOA, NR4A2
	Oxidoreductase activity incorporating molecular oxygen	8.11	CYP1A1, CYP1A2, CYP1B1
	Hydroperoxy-icosatetraenoate dehydratase activity	50.00	CYP1A1, CYP1A2, CYP1B1
	Dopamine metabolic process	7.32	DDC, MAOA, NR4A2

Estrogen 16-alpha-hydroxylase activity	33.33	CYP1A1, CYP1A2, CYP1B1
Aromatase enzymatic activity	10.71	CYP1A1, CYP1A2, CYP1B1
Retinol metabolic process	5.77	CYP1A1, CYP1A2, CYP1B1
Arachidonic acid metabolic process	6.35	CYP1A1, CYP1A2, CYP1B1, PTGS2
Long-chain fatty acid biosynthesis	8.33	CYP1A1, CYP1A2, PTGS2
Epoxygenase cytochrome P450 pathway	15.00	CYP1A1, CYP1A2, CYP1B1
Omega-hydroxylase cytochrome P450 pathway	33.33	CYP1A1, CYP1A2, CYP1B1

AG: associated genes; R: group number.

Research has shown that the UGT1A1 enzyme contributes to the estrogen detoxification pathway in the prostate [22]. These results provide new opportunities for examining the therapeutic potential of Wedelia chinensis.

Conclusion

Overall, “estrogen metabolism” emerges as the primary pathway influenced by W. chinensis in the 22RV1 prostate cancer cell line. Members of the cytochrome P450 family were identified as the principal targeted genes. Key contributions of UGT1A1, MAOA, and PTGS2 in the cellular response to W. chinensis exposure were observed. Further studies exploring the impact of individual compounds from Wedelia chinensis on cancer cells could prove valuable for advancing cancer treatment strategies.

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Conflict of Interest: None

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Ethics Statement: None

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