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GENE EXPRESSION DATA ANALYSIS IN RESPONSE TO NICOTINE EXPOSURE: A LITERATURE STUDY REVEALING DIFFERENTIALLY EXPRESSED GENES IN BIOLOGICAL PATHWAYS

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ABSTRACT

This research aims to deepen our understanding of the effects of nicotine usage through a descriptive literature review, with the primary focus on exploring GEO DataSets via the analysis of Differentially Expressed Genes (DEGs) in response to nicotine exposure. The GEO DataSet search was obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), a relevant biomedical data source. The results of our investigation revealed a total of 11 GEO DataSets of Homo sapiens that met the inclusion and exclusion criteria for identifying Differentially Expressed Genes (DEGs). These datasets, namely GSE125217, GSE148812, GSE125416, GSE105445, GSE56398, GSE51284, GSE71795, GSE56383, GSE40689, GSE11208, and GSE11142, contained genes that underwent altered expression in response to nicotine exposure. The results of the gene analysis were further categorized based on their functional classifications. They encompass receptor genes such as CHRNA9, nAChRs, and TLR4, regulatory genes including CDK1, CHK1, ERBB2, EGFR, and E2F1, structural genes like H-Caldesmon, L-Caldesmon, SM22, CDH1/3, BDNF/NT-3, and MLL3, immunological genes such as TNF-α, IL-1β, IL-6, IL-10, MCSF, MCP-1, and ICAM-1, metabolic genes like CYP2A6 and APOE, and enzymatic genes such as PITRM1, DDR2, DHRS7, and SLC16A7. This review search provides a comprehensive insight into the molecular-level impact of nicotine, with potential implications for the development of treatment strategies and the discovery of relevant biomarkers associated with nicotine use.

Keyword: nicotine, gene expression, differentially expressed genes.

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INTRODUCTION

Smoking is a major contributor to preventable deaths caused by non-communicable diseases (Jafari et al., 2021). Cigarettes represent the most popular form of tobacco product, accounting for approximately 80% of all tobacco products in use. Worldwide, over 1 billion individuals are active smokers, making tobacco the second most commonly used addictive substance. Tobacco comes in various forms, including combustible products like cigarettes, cigars, pipe tobacco, hookah, and non-combustible products such as electronic cigarettes (EC), heated tobacco products (HTP), and various formulations used for chewing, dipping, or snuffing (Panagiotakos et al., 2023). The World Health Organization (WHO) reports that tobacco use results in one in ten deaths globally, totaling 7 million deaths annually. If the current global tobacco consumption trend persists, it is estimated that by 2030, there will be 8 million tobacco-related deaths each year (Jafari et al., 2021). Among 159 countries with a population of over 1 million, the prevalence of tobacco use among males aged 15 and older has significantly increased in 20 countries over the past three decades, including Indonesia.

In Indonesia, there has been a significant surge in deaths attributable to tobacco use, rising from 112,800 deaths in 1990 to 246,400 deaths in 2019, representing a 118% increase. Despite various efforts to reduce smoking prevalence, significant progress has not been achieved (Dai et al., 2022).

The primary cause of tobacco addiction is nicotine dependency (Quigley & MacCabe, 2019). According to go.drugsbank.com, nicotine is a highly toxic alkaloid found in tobacco products. Nicotine is a tertiary amine that features a pyrrolidine and pyridine ring selectively binding to nicotinic cholinergic receptors at various locations in the body, including the brain, neuromuscular junctions, adrenal medulla, and ganglia (Sandhu et al., 2023). When an individual inhales nicotine through cigarettes, nicotine diffuses across the lungs into the circulation, ultimately reaching the brain. This affects the centers of activity in the limbic system and stimulates the cortex. Dopamine is released in the frontal cortex, mesolimbic area, and corpus striatum, mediating pleasurable effects (Sandhu et al., 2023).

Recent research indicates that nicotine modulates the MAO-A and MAO-B enzymes, which play a role in dopamine breakdown, primarily responsible for addiction (Tiwari et al., 2020). Furthermore, the effects of nicotine can lead to changes in nucleosome responsiveness, enhancing DNA accessibility (Brown et al., 2015). Additionally, the nicotinic acetylcholine receptor (nAChR) is one of nicotine's biological targets in the nervous system that can result in gene expression changes (Sherafat et al., 2021) and potentially cause issues in the nervous, neuromuscular, cardiovascular, respiratory, immune, and gastrointestinal systems (Lee & Fariss, 2017).

The complex effects of nicotine on various body systems underscore the importance of a profound understanding of genetic changes in biological responses to nicotine and the identification of influential biological pathways due to nicotine exposure through gene expression data analysis. Gene expression analysis is a crucial method utilized in drug discovery, biomarker research, and pathway analysis (protein-protein interactions). Essentially, gene expression analysis involves the detection of differences in two or more groups, known as Differentially Expressed Genes (DEGs). This analysis encompasses genome expression techniques such as microarrays or RNA sequencing (RNA-seq) and more specific target gene expression techniques like quantitative polymerase chain reaction (qPCR). NCBI GEO and ArrayExpress serve as public providers of gene expression data. These data repositories can contain valuable insights for new discoveries, biomarker development, and therapies (Biocompare, 2023).

The National Center for Biotechnology Information (NCBI) plays a crucial role as a provider of diverse biomedical data sources necessary for nicotine exposure-related gene expression analysis. In the year 2000, NCBI launched the Gene Expression Omnibus (GEO) database as a repository for high-throughput gene expression data. Subsequently, major journals began mandating the storage of microarray data in public repositories in 2002, which contributed to the growth of GEO. Moreover, high-throughput genomic experiments have rapidly advanced since the initial use of microarrays for gene expression analysis, prompting the GEO database to evolve to keep pace with changes in technology and applications. Currently, GEO accepts data from various technologies, including DNA microarrays, protein or tissue arrays, high-throughput nucleic acid sequencing, Serial Analysis of Gene Expression (SAGE), and reverse transcription polymerase chain reaction (RT-PCR) (Clough & Barrett, 2016).

This study aims to analyze changes in gene expression in response to nicotine exposure. The primary focus of this investigation is the identification of Differentially Expressed Genes (DEGs) through gene expression data analysis. The required data will be obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), a relevant biomedical data source. The results of this search are expected to provide crucial insights as a foundation for the development of more effective treatment strategies for the health impacts of nicotine use based on gene expression changes.

METHOD

This study employs a descriptive literature review method with a primary focus on exploring GEO DataSets through the analysis of Differentially Expressed Genes (DEGs) in response to nicotine exposure. Samples consist of GEO DataSets of Homo sapiens obtained from the National Center for Biotechnology Information (NCBI), specifically from the Gene Expression Omnibus (GEO) DataSets, a curated collection of gene expression data, including series records and original platforms retrieved from the GEO repository. The research workflow comprises the following steps: In the first stage, a keyword search using "nicotine" was conducted to identify relevant gene expression studies related to nicotine. Subsequently, the search was narrowed down to datasets pertaining to Homo sapiens subjects. The next stage involved the filtering of gene expression series data specifically related to the effects of nicotine on Homo sapiens. The final step included screening Homo sapiens nicotine gene expression series data based on predefined inclusion and exclusion criteria to eliminate data that could not be analyzed, resulting in a refined set of data for GEO DataSets exploration. The last step involves grouping genes in each GEO DataSet based on functional classification.

The selection of GEO Datasets was conducted based on predetermined inclusion and exclusion criteria. Inclusion criteria encompassed original research GEO Datasets that generated primary data related to gene expression in response to nicotine, employing Homo sapiens as the study subjects and providing both complete and incomplete textual information. Exclusion criteria were applied to eliminate GEO Datasets that lacked specificity regarding Homo sapiens nicotine gene expression, double GEO Datasets, and those that did not exhibit genes involved in gene expression.

RESULTS AND DISCUSSION

The National Center for Biotechnology Information (NCBI) was utilized to investigate the molecular impact of nicotine and aid in the identification of potential gene targets. An initial search within the NCBI database, encompassing nicotine gene expression data across all species, yielded a total of 2,342 GEO DataSets. Subsequently, a focused search for nicotine gene expression data specific to Homo sapiens resulted in 1,048 GEO DataSets. The next stage involved the filtration of nicotine gene expression series data for Homo sapiens, resulting in 63 GEO DataSets. Further refinement of the Homo sapiens nicotine gene expression series data, limited to those directly associated with nicotine, yielded 14 GEO DataSets. In the final stage, the Homo sapiens nicotine gene expression series data underwent screening based on predefined inclusion and exclusion criteria. This process eliminated three GEO DataSets that were deemed non-analyzable, resulting in 11 GEO DataSets suitable for further GEO DataSet exploration (Figure 1). Subsequently, the analysis of these

11 GEO DataSets was performed to determine the classification of functionally expressed genes in response to nicotine exposure (Table 1). The findings from the analysis of these 11 GEO DataSets revealed the presence of potential gene candidates, classified based on functional gene categories within the body (Table 2).

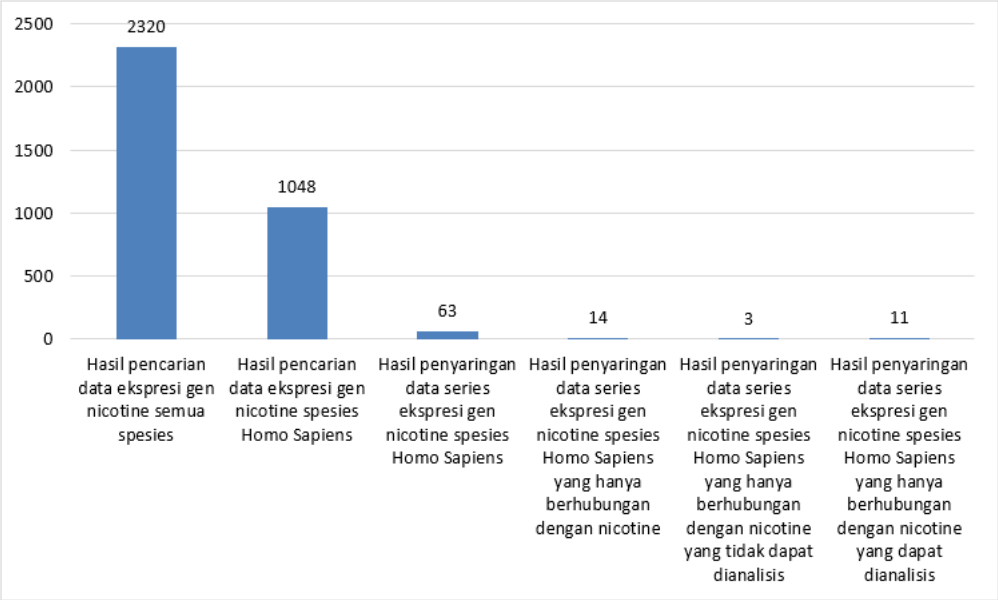

Figure 1. Graph Depicting the Quantity of Homo Sapiens Nicotine Gene Expression Data from NCBI GEO

Table 1. Articles Related to Gene Expression in Response to Nicotine Exposure

GEO DataSet Code	Authors	Title	Method
GSE125217	Lee WH, Ong SG, Zhou Y, Tian L et al, 2020	RNA-seq of iPSC-ECs treated e-cig liquids with and without nicotine	Expression profiling by high throughput sequencing
GSE148812	(Jiang et al., 2019)	An Exome-Wide Association Study Identifies New Susceptibility Loci for the Risk of Nicotine Dependence in European-American Populations Nicotine Dependence in European-American Populations	Genome variation profiling by genome tiling array
GSE125416	(Guo et al., 2019)	Single-Cell RNA-Sequencing of Human Embryonic Stem Cell Differentiation Delineates Adverse Effects of Nicotine on Embryonic Development	Expression profiling by high throughput sequencing
GSE105445	(Lin et al., 2019)	Mammary tumors of xenograft mice with the treatment of nicotine and JMY117 as well as CHRNA9 and SLC19A7 knockdown in MDA-MB-231, A549 and Hep3B cells	Expression profiling by array
GSE56398	(Oni et al., 2016)	Increased response to nicotine in human dopaminergic neurons derived from iPSC carrying the risk-associated SNP rs16969968	Expression profiling by high throughput sequencing

GEO DataSet Code	Authors	Title	Method
GSE51284	(Bergen AW, Wacholder AC, Nishita DM, Michel M, Krasnow R, Javitz HS, Fugman DA, Tischfield JA, Hops H, Benowitz NL, Enoch M, 2023)	Expression data from monozygotic twins discordant for nicotine metabolism	Expression profiling by array
GSE71795	(Brown et al., 2015)	Nucleosome Repositioning: Nicotine- and Cocaine-induced Changes	Genome binding/occupancy profiling by array
GSE56383	(Yoshiyama et al., 2014)	Vascular smooth muscle cell: control vs. exposed to nicotine	Expression profiling by array
GSE40689	(Pillai et al., 2015)	B-arrestin1 associated genomic regions in nicotine induced Non-Small Cell Lung Carcinoma (NSCLC) cell line A549	Genome binding/occupancy profiling by high throughput sequencing
GSE11208	(Kuo, 2019)	Chronic nicotine exposure (kuo-affy-human-232930)	Expression profiling by array
GSE11142	(Y, 2019)	Nicotine effect on CEM model T cell line (kuo-affy-human-232861)	Expression profiling by array

Table 2. Classification of Genes Based on Their Functions

	GEO DataSet Code										
Functional Category	**GSE125217**	**GSE148812**	**GSE125416**	**GSE105445**	**GSE56398**	**GSE51284**	**GSE71795**	**GSE56383**	**GSE40689**	**GSE11208**	**GSE11142**
Metabolism Genes		*DHRS7*	APOE	IFNGR1		CYP2A					
Immunology Genes	ICAM-1, TNF-α, IL-1β, IL-6, IL-10, MCSF, MCP-1			CD40, APP							

	GEO DataSet Code						
Receptor Genes	*DDR2*	TLR4	nAChR, CHRNA9	CHRNA5	nAChRs, GPCR	nAChR	nAChR
Nervous System Genes		nAChRs	NTRK2, nAChR				
Structural Genes	*FOXN1*	HMGB1	CD44, CDH1/3, BDNF/NT-3		H-Caldesmon, L-Caldesmon, SM22	VIM, FN1, E-cadherin,	
Regulatory Genes		nAChRs, BNIP3,	CDK1, CHK1, ERBB2 (HER2, HER2+), EGFR (ErbB/HER2)	TP53, CDNK1C, LITAF, MLL3, NFKBIB, EGR1		β-arrestin-1, E2F1, SMAD, ZEB1, ZEB2	
Enzymatic Genes	*PITRM1*		SLC16A7, SLC12A9				
Signaling Pathway Genes					p38 MAPK, ERK1/2	nAChR	

The review of the 11 GEO Datasets involved an analysis of gene expression data at the molecular level in response to nicotine exposure, which met the following inclusion and exclusion criteria:

GSE125217

Research with the GEO DataSet code GSE125217, conducted by Lee et al. (2020), aimed to investigate the effects of flavored electronic cigarette (e-cigarette) liquids and serum isolated from e-cigarette users on endothelial health and endothelial-dependent macrophage activation. The GEO DataSet utilized human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) and a high-throughput screening approach to assess endothelial integrity following exposure to six different e-liquids with varying nicotine concentrations and serum from e-cigarette users. The samples included 15 individuals and were divided into three groups: flavored e-liquid (RY), Marlboro (MAR), and control (RKT). Two healthy iPSC lines (78 and 273) were differentiated into ECs, and iPSC-ECs were treated with e-cigarette liquids RY4 (RY) or Marlboro (MAR) containing 18 mg/ml nicotine (18) and nicotine-free (0).

The review of GEO DataSet GSE125217 revealed that there are seven genes that exhibit Differential Expressed Genes (DEG) in response to nicotine exposure, namely TNF-α, IL-1β, IL-6, IL-10, MCSF, MCP-1, and ICAM-1, and all of these genes are classified as "immunological genes." TNF-α plays a role in the context of tumor necrosis factor (TNF)-α cytokine stimulation related to the immune response and inflammation. IL-1β and IL-6 genes are inflammatory factors produced by M1 macrophages involved in the immune response and inflammation. IL-10 gene is an M2-related cytokine that is a component of the immune response. MCSF gene (macrophage colony-stimulating factor) is involved in stimulating macrophage growth and differentiation. MCP-1 gene (monocyte chemoattractant protein-1) plays a role in attracting monocytes to the site of inflammation, and ICAM-1 gene (intracellular adhesion molecule-1) shows increased expression after stimulation with tumor necrosis factor (TNF)-α, which is an inflammatory cytokine.

The review of the GEO DataSet GSE125217 provides insights into the impact of nicotine exposure from flavored electronic cigarette (e-cigarette) liquids on endothelial health and endothelial-dependent macrophage activation. The analysis involved endothelial cells derived from human induced pluripotent stem cells (iPSC-ECs) and utilized an array technology-based gene expression profiling method to assess endothelial integrity after exposure to six different e-cigarette liquids with varying nicotine concentrations and serum from e-cigarette users. The findings revealed significant changes in gene expression patterns, particularly in immunological genes such as TNF-α, IL-1β, IL-6, IL-10, MCSF, MCP-1, and ICAM-1, due to nicotine exposure. These results indicate the health impact of electronic cigarette use and nicotine exposure, particularly in relation to the immune system response and endothelial integrity.

GSE148812

The research with the GEO DataSet GSE148812, conducted by Li MD (2020), aimed to uncover the molecular mechanisms underlying various phenotypes related to the risk of nicotine dependence in the European-American population, with a focus on the age at smoking initiation (ASI). This GEO DataSet involved a sample of 2,510 smokers, including individuals of African-American (AA) descent, totaling 1,654 smokers, and European-American (EA) descent, totaling 856 smokers. The participants had smoked at least 100 cigarettes in their lifetime and met the sample characteristics based on the Mid-South Tobacco Case–Control (MSTCC) study. The samples were then analyzed at the single nucleotide polymorphism (SNP) level and were separately analyzed on a gene-based basis.

The review of GEO DataSet GSE148812 yielded insights into the impact of nicotine exposure on gene expression, identifying four genes classified into distinct functional categories. Firstly, the PITRM1 gene emerged as an "enzymatic gene" with implications in enzymatic processes and the modulation of metalloendopeptidase activity, particularly associated with Alzheimer's disease. It encodes a 117-kDa mitochondrial matrix enzyme involved in amyloid beta (Aβ) degradation within mitochondria, highlighting its crucial role in Aβ digestion and mitochondrial protein transportation. Secondly, the DDR2 gene was categorized as a "receptor gene," encoding a collagen-binding tyrosine kinase receptor that regulates cell proliferation and survival, and its overexpression is associated with a poor prognosis in diseases like lung cancer. The DDR2 receptor's role in regulating cellular

responses to external signals is underscored, impacting disease development. Thirdly, the DHRS7 gene was classified as a "metabolism gene" due to its contribution to xenobiotic metabolism. It encodes an enzyme within the dehydrogenase/reductase (SDR) protein family, engaged in the metabolism of xenobiotic compounds within the body. Lastly, the FOXN1 gene, classified as a "structural gene," was associated with thymic underdevelopment, affecting organ and body structure development. These findings illuminate the molecular mechanisms and functional significance of these genes in response to nicotine exposure.

In conclusion, this review reveals the involvement of genetic analysis in exploring the impact of nicotine dependence at the molecular level. The findings shed new light on the roles of PITRM1, DDR2, DHRS7, and FOXN1 genes in responding to nicotine, providing an understanding of the biological mechanisms underlying nicotine dependence in the European-American population.

GSE125416

The study utilizing GEO DataSet GSE125416, conducted by (Guo et al., 2019), aimed to elucidate the molecular mechanisms involved in cellular responses to nicotine exposure and their effects on gene expression patterns. This GEO DataSet employed two distinct samples: Control_EB scRNA-seq [D] and nicotine-exposed_EB scRNA-seq [N]. Subsequently, they underwent analysis through single-cell RNA sequencing (scRNA-seq) involving approximately 12,500 individual cells derived from human embryoid bodies (EBs) derived from human embryonic stem cells (hESCs). These EBs were allowed to develop over a 21-day period under culture conditions containing nicotine or in a nicotine-free environment.

The review of GEO DataSet GSE125416 identified the involvement of five genes in Differentially Expressed Genes (DEG) due to nicotine exposure: APOE, HMGB1, TLR4, BNIP3, and nAChRs. These genes were then classified into five functional categories. APOE gene was categorized as a "metabolism gene," and its gene regulation has been associated with brain malformations and intellectual disabilities. HMGB1 gene was classified as a "structural gene," with increased gene expression affecting cardiac excitation-contraction and regulating Ca2+ handling and cellular contractility through Toll-like receptor 4 (TLR4) signaling. TLR4 gene falls into the "receptor gene" category, playing a crucial role in the HMGB1-TLR4 signaling (Figure 2) and contributing significantly to cardiac dysfunction pathogenesis. BNIP3 was categorized as a "regulator gene" and is involved in regulating nicotine-induced cell death and its regulatory role in cellular processes. Nicotinic Acetylcholine Receptors (nAChRs) are expressed in undifferentiated and differentiated cells and play a role in various cellular processes such as apoptosis, cell proliferation, cell differentiation, intracellular calcium regulation, oxidative stress, inflammation, and tumor development. Therefore, nAChRs genes can be classified into the categories of "regulator genes" and "nervous system genes," contributing to nicotine signaling and influencing cellular functions.

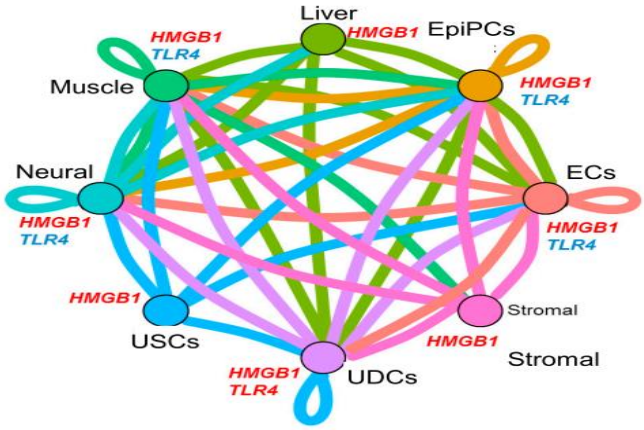

Figure 2. HMGB1-TLR4 Pathway (Guo et al., 2019)

The exploration of GSE125416 reveals that nicotine exposure can influence the expression of specific genes associated with cellular responses, involving genes APOE, HMGB1, TLR4, BNIP3, and nAChRs. The functional classification of these genes provides additional insights into the biological mechanisms underlying the impact of nicotine on gene expression patterns. This in-depth understanding may serve as a foundation for the development of more targeted intervention strategies related to the health effects of nicotine exposure.

GSE105445

The study conducted using GEO DataSet GSE105445 by Lin et al. (2019) aimed to evaluate the potential of JMY117 (Bupropion) as an inhibitor of the metastatic effects of cancer caused by nicotine exposure. This GEO DataSet investigated the roles of CHRNA9 and SLC16A7 in breast cancer cell lines (MDA-MB-231), lung cancer cell lines (A549), and liver cancer cell lines (Hep3B). The experiments involved 30 samples using a xenograft mouse model for breast tumors, which received various treatments, including nicotine exposure and the administration of JMY117 in two different doses. Nicotine was added to drinking water at a concentration of 10 µg/ml. Additionally, knockdown experiments of CHRNA9/SLC19A7 were conducted on MDA-MB-231, A549, and Hep3B cells to understand their impact on cancer metastasis behavior.

The review of GEO DataSet GSE105445 revealed the involvement of 15 genes that experienced Differential Expressed Genes (DEG) due to nicotine exposure. These genes were categorized into 7 functional gene classifications. IFNGR1 was classified as a "metabolism gene." It plays a role in regulating cellular responses to growth factors and cytokines that can influence cellular metabolic aspects. SLC16A7 and SLC12A9 were classified as "enzymatic genes." These genes are involved in metabolite transport and ion regulation through the cell membrane, involving enzymatic reactions. Nicotinic Acetylcholine Receptors (nAChR) were classified as "receptor genes" and "nervous system genes." These genes are receptors involved in cellular responses to nicotine and signal transduction in the nervous system. CHRNA9 is one of the subunits of the nicotinic receptor. CDK1, CHK1, ERBB2 (HER2, HER2+), EGFR (ErbB/HER2) were classified as "regulator genes." Each of these genes plays a role in controlling the cell cycle and cell proliferation, is involved in cell cycle regulation and responses to DNA damage, participates in cell signaling activation regulating cell growth, and is involved in signaling pathways that control proliferation and cell growth. CD44, CDH1/3, BDNF/NT-3 were classified as "structural genes." Each of these genes contributes to changes in the expression

of differentiation markers, is involved in cell adhesion, participates in the growth and differentiation of nerve cells, which can affect the structure and function of nerve cells. NTRK2 was classified as a "nervous system gene." This gene is involved in nerve cell responses to growth factors. CD40 and APP were classified as "immunology genes." Each of these genes is involved in the immune system, participates in signal transduction mechanisms, and has roles in cellular responses.

The analysis of the GEO DataSet GSE105445 reveals that 15 genes undergo Differential Expressed Genes (DEG) in response to nicotine exposure, with functional classifications encompassing genes related to metabolism (IFNGR1), enzymes (SLC16A7 and SLC12A9), receptors and the nervous system (nAChR and CHRNA9), regulators (CDK1, CHK1, ERBB2, EGFR), structural components (CD44, CDH1/3, BDNF/NT-3), the nervous system (NTRK2), and immunology (CD40 and APP). These findings provide profound insights into the diverse roles of genes in responding to nicotine, creating potential avenues for more targeted therapies and prevention strategies related to the health impacts of nicotine exposure.

GSE56398

The study with the GEO DataSet GSE56398, conducted by (Oni et al., 2016), aimed to understand the impact of nicotine receptor variants in humans by distinguishing Induced Pluripotent Stem Cells (iPSC) into dopaminergic (DA) or glutamatergic neurons and testing their functional properties and response to nicotine. This GEO DataSet utilized iPSCs derived from donors homozygous for the major (D398) and minor (N398) alleles of the single nucleotide polymorphism (SNP) rs16969968 in CHRNA5. A total of 10 samples were used, consisting of three culture samples, namely, midbrain dopaminergic (mDA) samples derived from iPSCs, totaling 3 samples. These iPSCs carried the homozygous major allele for rs16969968 (D398). There were also 3 samples from iPSC lines carrying the homozygous minor allele (N398). Additionally, there were 4 control differentiation samples of iPSCs derived from CD4+ T cells and reprogrammed using Sendai virus vectors, created from two different cell lines in several parts.

The review of GEO DataSet GSE56398 identified the Nicotinic Acetylcholine Receptor (nAChR) gene as a Differentially Expressed Gene (DEG) due to nicotine exposure. This gene is classified into the "receptor gene" category and plays a role as a receptor that responds to nicotine in the brain and nervous system. In this context, single nucleotide polymorphism (SNP) changes in the CHRNA5 gene that encodes the α5 subunit of nAChR, including the SNP rs16969668, which changes the amino acid at position 398 from aspartic acid (D) to asparagine (N), affect receptor activity and can contribute to nicotine-related addictive behavior.

The exploration of the GEO DataSet GSE56398 concludes that the Nicotinic Acetylcholine Receptor (nAChR) gene undergoes expression changes due to nicotine exposure, particularly at the SNP rs16969668, influencing receptor activity. These findings contribute to understanding the biological basis of nicotine-related addictive behavior and illustrate the potential impact of genetic alterations on the function of nicotine receptors in the human nervous system.

GSE51284

The review of GEO DataSet GSE51284 identified the gene CYP2A6 as a Differentially Expressed Gene (DEG) due to nicotine exposure. This gene is classified in the "metabolism gene" category and plays a role in the metabolic process of nicotine, including the conversion of nicotine into metabolites such as cotinine. The study aimed to identify genetic factors that may influence nicotine metabolism rate (NMR) and nicotine consumption patterns at the genetic level. The dataset included 24 samples from 8 individuals, including four pairs of monozygotic twins, each with three replicates. Gene expression profiles were created using array-based methods.

GSE71795

The study with GEO DataSet GSE71795, conducted by (Brown et al., 2015), aimed to understand the molecular mechanisms underlying drug-induced changes in chromatin structure. This GEO dataset utilized 16 samples derived from human neuroblastoma cells (SH-SY5Y) and conducted detailed analyses on SH-SY5Y cells induced by nicotine. The observations were made at 10, 60, and 90 minutes with two replicates each. SH-SY5Y cells induced by cocaine were also examined at 5, 20, 40, and 60 minutes with two replicates each. Two SH-SY5Y cells served as controls. Subsequently, an examination of the interaction between DNA and nucleosomes in the promoter regions of 858 genes within nicotine- or cocaine-exposed SH-SY5Y cells was performed using a microarray method, aiming to investigate nucleosome changes.

The review of GEO DataSet GSE71795 identified 6 genes (NFKBIB, EGR1, TP53, CDKN1C, LITAF, and MLL3) that experienced Differential Expressed Genes (DEG) and were classified as "regulator genes," meaning they play a role in controlling the expression of other genes. All of these genes are involved in regulating gene transcription by influencing nucleosome movement, which can modulate gene expression.

The exploration of this GSE71795 review provides insights into the role of regulatory genes in controlling gene expression through nucleosome changes, depicting the fundamental mechanisms involved in cellular responses to nicotine or cocaine exposure at the molecular level. This knowledge may contribute to a deeper understanding of the drug's impact on chromatin structure and lay the foundation for further research in the development of targeted therapies.

GSE56383

The study with the GEO DataSet GSE56383 conducted by (Yoshiyama et al., 2014)used human aortic smooth muscle cells (HSMC) samples, with some cells exposed to nicotine treatment, and others serving as control, using array-based expression profiling methods. Generally, differentiated VSMCs can exhibit a contractile phenotype, which is a prerequisite for vasoconstriction but not for active migration. The hypothesis behind this GEO dataset is that exposure to nicotine for 48 hours will transform differentiated VSMCs from a contractile phenotype into a synthetic-like phenotype, characterized by increased proliferation and migration. It is speculated that nicotine exerts this effect through Nicotinic Acetylcholine Receptors (nAChRs). The GEO DataSet used primary human aortic smooth muscle cells (HuAoSMCs) treated with Transforming Growth Factor-B (TGF-B). Initially, the dataset identified nicotine receptors and then investigated changes in the expression levels of several differentiation markers in the cells using DNA microarray, real-time PCR, western blot analysis, and finally, it examined the activity of Mitogen-Activated Protein Kinases (MAPKs).

The review of GEO DataSet GSE56383 identified 7 genes (nAChRs, GPCR, H-Caldesmon, L-Caldesmon, SM22, p38 MAPK, and ERK1/2) that underwent Differential Expression due to nicotine exposure. They were then classified into 3 functional categories. nAChRs were classified as "receptor genes," playing a role in responding to nicotine and being involved in changes in the smooth muscle cell phenotype. GPCR genes were associated with signaling pathways stimulated by nicotine. H-Caldesmon, L-Caldesmon, and SM22 were classified as "structural genes" and played a role in indicating changes in the phenotype of human aortic smooth muscle cells (HuAoSMCs) following nicotine exposure. These genes were influenced by nicotine exposure and played a role in describing changes in the cells, especially concerning smooth muscle phenotype changes (see Figure 3). p38 MAPK and ERK1/2 were classified as "signaling pathway genes." The MAPK signaling pathway involves several protein kinases, including p38 MAPK and ERK1/2, which are responsible for controlling cellular responses to external stimuli.

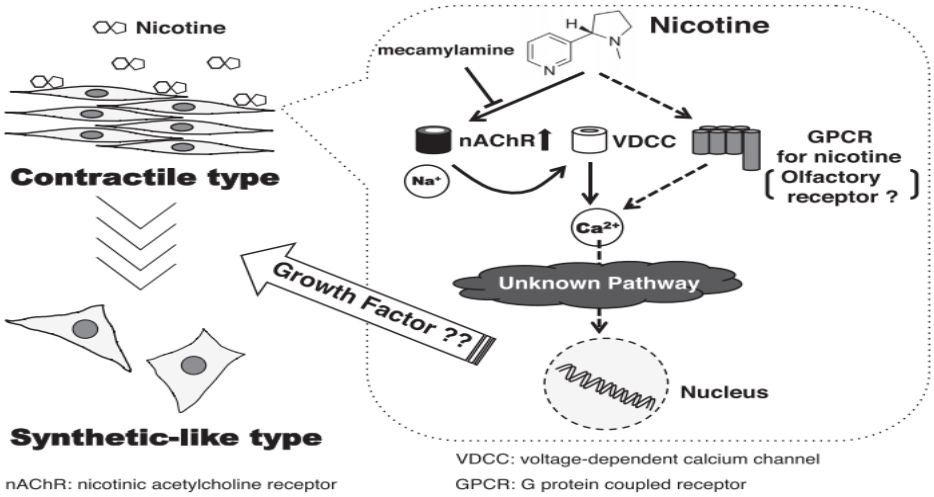

Figure 3. Schematic Diagram of the Relationship between Nicotine Exposure and Phenotypic Changes in HuAoSMCs (Yoshiyama et al., 2014)

The GSE56383 review successfully unveiled the molecular impact of nicotine exposure on human aortic smooth muscle cells (HuAoSMCs). The research outcomes highlighted the transformation of cell phenotypes following nicotine exposure, involving changes in gene expression such as nAChRs, GPCR, H-Caldesmon, L-Caldesmon, SM22, p38 MAPK, and ERK1/2. The classification of genes into different functional categories aided in understanding the role of each gene in cellular

GSE11208

The study with GEO DataSet GSE11208, conducted by Kuo Y. (2019), aims to investigate the long-term effects of chronic nicotine exposure on the nervous system's function. GEO DataSet GSE11208 analyzed 11 samples involving gene expression profiling based on array technology as the biological target of nicotine's action. Nicotinic Acetylcholine Receptors (nAChR) are members of the ion channel superfamily with a crucial role in chemical signal transduction throughout the human nervous system. GEO DataSet GSE11208 holds significant relevance in the context of nicotine addiction, which is a serious public health and economic issue. Its primary objective is to identify changes in gene expression triggered by nicotine exposure in human neuroblastoma cells SH-SY5Y,

revealing at least two subtypes of nicotinic receptors. Experiments were conducted to provide evidence of nicotine exposure's impact on the nervous system function through alterations in gene expression. This was achieved by exposing human neuroblastoma SH-SY5Y cells to effective doses of nicotine or control media during two different time periods.

The review of GEO DataSet GSE11208 identified the involvement of nAChR as experiencing Differentially Expressed Genes (DEG) due to nicotine exposure, thus classifying them as "receptor genes." The role of nAChR in this context focuses on the biological target of nicotine action and plays a vital role in chemical signaling throughout the brain and body. These receptors are responsible for regulating various nervous system functions and are involved in nicotine addiction.

The exploration from GSE11208 review provides a comprehensive understanding of the long-term impact of nicotine exposure on the nervous system function. Through gene expression analysis, with a particular focus on the role of Nicotinic Acetylcholine Receptors (nAChR), this research successfully reveals alterations in gene patterns resulting from nicotine exposure in human neuroblastoma cells SH-SY5Y. The findings underscore the crucial role of nAChR in chemical signal transduction throughout the human nervous system, particularly concerning nicotine addiction. The experimental results also offer tangible evidence that nicotine exposure can influence the function of the nervous system through changes in gene expression, serving as a crucial foundation for more specific research and therapeutic development related to the health impacts of nicotine addiction. These findings bear significant implications in the context of public health and further strengthen our understanding of the biological mechanisms underlying the molecular-level effects of nicotine.

GSE40689

The study with GEO DataSet GSE40689, conducted by (Pillai et al., 2015), aimed to investigate the crucial role of the protein β-arrestin-1 in signaling transduction through Nicotinic Acetylcholine Receptors (nAChR), which are responsible for nicotine-induced proliferative effects. GEO DataSet GSE40689 used three samples: A549 serum, A549 nicotine, and A549 control. The analytical method involved log transformation of mRNA expression data for four genes: β-arrestin1, vimentin (VIM), fibronectin (FN1), and 18S rRNA to approach normal distribution. Assessment of gene-gene correlations was conducted using Pearson rank correlation (r), while the relationship between the mRNA expression of these genes and pathological stage was evaluated using the appropriate normal score test. Furthermore, a simplified monotonic regression model was used to assess the correlation between mRNA expression of these genes and the number of years of smoking in the population. The optimal cutoff point for disease-free survival analysis, defined as the time between surgical resection and disease recurrence or death, was evaluated using the maximal chi-square test. All statistical analyses were performed using SAS software (Version 9.2; SAS Institute; Cary, NC).

The review of GEO DataSet GSE40689 identified 9 genes (nAChRs, VIM, FN1, β-arrestin-1, E-cadherin, E2F1, SMAD, ZEB1, and ZEB2) that experienced Differentially Expressed Genes (DEG) due to exposure to nicotine. These genes were further classified into two functional categories. The nAChRs were categorized as "signal pathway genes" because they are involved in the signaling pathways that regulate nicotine's effects on the Epithelial-Mesenchymal Transition (EMT) and metastasis processes. The genes VIM, FN1, β-arrestin-1, E-cadherin, E2F1, SMAD, ZEB1, and ZEB2 were classified as "regulator genes." These genes play a role in the response to nAChR stimulation

and nicotine-induced EMT. Several reasons support this classification. The significant role of β-arrestin-1 in regulating the expression of these genes strongly indicates that the regulation of VIM and FN1 genes is related to the signaling pathway involving β-arrestin-1. Transcription-level analysis results confirmed the key role of β-arrestin-1 in regulating gene expression and emphasized the regulation at the transcription level. Furthermore, the response of VIM and FN1 genes to nicotine indicates their involvement in the signaling pathway regulated by nAChR. In addition, β-arrestin-1 does not play a role in gene induction mediated by TGF-β, indicating the unique role of β-arrestin-1 in regulating the expression of VIM and FN1 genes in response to nAChR stimulation. Lastly, the E2F1 gene was described as having a significant role in regulating genes involved in the Epithelial-Mesenchymal Transition (EMT), which is a crucial process in tumor development, invasion, and metastasis regulation.

As a whole, the exploration of the GEO DataSet GSE40689 provides a comprehensive understanding of the role of β-arrestin-1 in signal transduction through Nicotinic Acetylcholine Receptors (nAChR) and its impact on gene expression. The research findings identify changes in gene expression patterns, including nAChR, VIM, FN1, β-arrestin-1, E-cadherin, E2F1, SMAD, ZEB1, and ZEB2, in response to nicotine exposure. By classifying these genes into "signal pathway genes" and "regulator genes," the study highlights the involvement of nAChR in regulating signaling pathways that influence the Epithelial-Mesenchymal Transition (EMT) and metastasis processes. These findings lay a crucial foundation for understanding the relationship between nicotine exposure and cellular responses, particularly in the context of developing more specific therapies related to nicotine addiction and its impact on public health as a whole.

GSE11142

The research conducted by Kuo Y. (2019) with the GEO DataSet GSE11142 aimed at a long-term investigation of the role of Nicotinic Acetylcholine Receptors (nAChR) in the development and regulation of the immune system, particularly in the context of nicotine exposure found in tobacco. GEO DataSet GSE11142 involved the analysis of 18 samples and utilized a gene expression profiling method based on array technology. The analysis was designed to enable the evaluation of the impact of nicotine exposure on gene expression in a T-cell model.

A review of the GEO DataSet GSE11142 identified the presence of nAChR (Nicotinic Acetylcholine Receptors) as Differentially Expressed Genes (DEG) due to nicotine exposure. nAChR is classified as a "receptor gene," serving as a biological target for nicotine action. This receptor plays a role in regulating the immune system's functions by linking nicotine exposure to changes in gene expression within the T-cell model.

The exploration of the GEO DataSet GSE11142 provides insights into the role of Nicotinic Acetylcholine Receptors (nAChR) in the regulation and development of the immune system, particularly in the context of nicotine exposure found in tobacco. The analysis of 18 samples, utilizing gene expression profiling based on array technology in T cells, successfully revealed significant changes in gene expression due to nicotine exposure, with nAChR identified as a Differentially Expressed Gene (DEG). Classified as a "receptor gene," nAChR proved to be a biological target in

response to nicotine action, associating the exposure with alterations in gene expression within the T cell model. The scrutiny of the GEO DataSet provides a deeper understanding of the molecular-level impact of nicotine on the immune system. This study contributes to the existing knowledge of nicotine's influence on the immune system and lays the foundation for further research in understanding the intricate interactions at the molecular level.

The review conducted through an analysis of 11 GEO datasets revealed a strong relationship in each GEO dataset with functional gene classifications that indicated changes in gene expression due to nicotine exposure. The relationships identified in the review of GEO DataSets are reflected in an illustrative diagram, demonstrating coherence in the behavior of genes related to their functional classifications when responding to nicotine (figure 4). The review of GEO DataSets provides a deeper understanding of the impact of nicotine at the molecular level and opens up opportunities for the development of new therapies and the identification of biomarkers related to the effects of nicotine on human health.

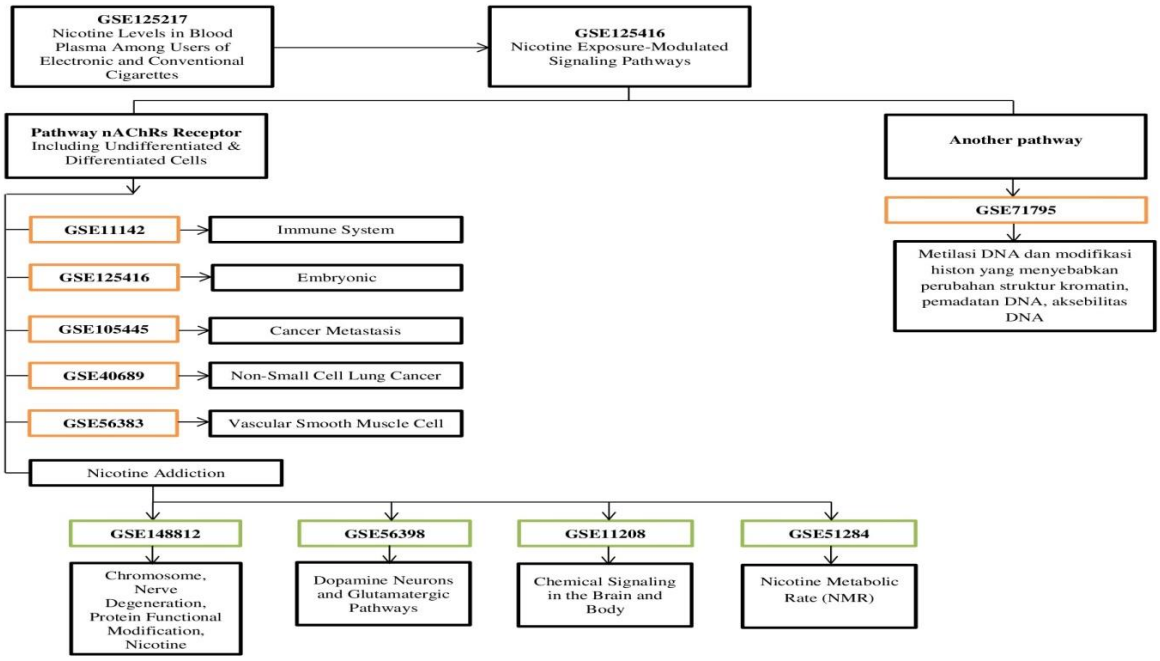

Figure 4. The interconnection of the 11 GEO DataSets

CONCLUSION

The review of GEO DataSets has yielded interesting findings regarding gene expression in response to nicotine exposure, through the analysis of 11 GEO DataSets. The review identified Differentially Expressed Genes (DEG), which are genes that undergo changes in expression in response to nicotine exposure. These DEGs identified in the review can be classified into several functional categories, including receptor genes (such as CHRNA9, nAChRs, and TLR4), regulatory genes (such as CDK1, CHK1, ERBB2, EGFR, and E2F1), structural genes (such as H-Caldesmon, L-Caldesmon, SM22, CDH1/3, BDNF/NT-3, and MLL3), immunological genes (including TNF-α, IL-1β, IL-6, IL-10, MCSF, MCP-1, and ICAM-1), metabolic genes (such as CYP2A6 and APOE), and enzymatic genes (including PITRM1, DDR2, DHRS7, and SLC16A7). The results of the review provide a deeper

understanding of the impact of nicotine exposure at the molecular level, shedding light on the gene expression involved in the response to nicotine. This is expected to have potential applications in drug discovery and the search for biomarkers related to the health effects of nicotine exposure in humans.

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