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Review Article

Homeopathy and Genomics: A Systematic Review of Gene Expression Studies in Experimental and Clinical Research

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Abstract

Genomics and transcriptomics have transformed biomedical sciences by enabling comprehensive investigation of molecular mechanisms underlying therapeutic interventions. In recent decades, several researchers have explored whether homeopathic medicines can influence gene expression patterns in experimental models and clinical settings. This systematic review aims to evaluate available evidence regarding genomic and transcriptomic changes associated with homeopathic interventions. Literature from in vitro studies, animal experiments, and human investigations was analyzed with emphasis on methodology, reproducibility, gene targets, and biological significance. Studies employing microarray technology, reverse transcription polymerase chain reaction (RT-PCR), RNA sequencing, and epigenetic analyses were reviewed. Experimental investigations have reported modulation of genes associated with inflammation, oxidative stress, apoptosis, immune response, and cellular signaling pathways. However, significant heterogeneity, small sample sizes, limited replication, and methodological inconsistencies remain major limitations. Current evidence suggests that molecular responses have been reported under certain experimental conditions, but definitive conclusions regarding mechanisms and clinical relevance cannot yet be established. Further multicenter studies employing standardized genomic technologies are required to clarify the biological basis and therapeutic implications of homeopathic interventions.

Keywords: *Epigenetics, Gene Expression, Genomics, Homeopathy, Precision Medicine, Systems Biology, Transcriptomics*

1. Introduction

The completion of the Human Genome Project revolutionized understanding of biological regulation and disease mechanisms. Advances in genomics have enabled identification of complex interactions between genes, proteins, and environmental influences. Gene expression profiling has become an essential tool for investigating pharmacological effects and understanding mechanisms of action of therapeutic agents. Modern omics technologies provide opportunities for exploring the molecular responses associated with complementary and integrative medicine interventions. [4]

Homeopathy, founded by Samuel Hahnemann in the eighteenth century, is based upon principles of similars and potentization. Despite its widespread global use, the molecular mechanisms underlying homeopathic preparations remain controversial. In recent decades, researchers have increasingly utilized genomic and transcriptomic approaches to investigate whether highly diluted medicines exert measurable effects on biological systems. [1,3]

The emergence of systems biology and epigenetics has generated renewed interest in understanding whether ultra-low doses can influence cellular regulatory networks. Several experimental investigations have demonstrated changes in expression of genes involved in stress responses, apoptosis, inflammatory mediators, and signal transduction pathways. Nevertheless, reproducibility and methodological rigor remain critical concerns. [1,8,11]

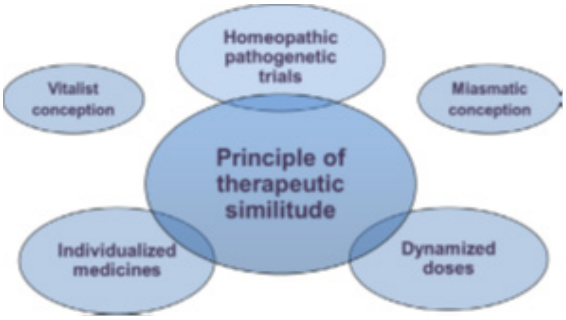

Figure 1. Scientific and philosophical assumptions of the Homoeopathic epistemological model.

2. Methodology

This systematic review was conducted according to PRISMA guidelines. Electronic databases including PubMed, Scopus, Web of Science, Embase, and Google Scholar were searched using combinations of the following keywords:

- Homeopathy
- Genomics
- Transcriptomics
- Gene expression
- RNA sequencing
- Microarray
- Epigenetics
- Systems biology

Studies published in English involving experimental, animal, or clinical investigations examining genomic responses to Homoeopathic medicines were included. Reviews, editorials, and duplicate publications were excluded. Data extraction focused on study design, model systems, intervention, genomic techniques, and principal findings.

Epigenetic Regulation

Epigenetic regulation enables changes in gene expression in response to environmental influences without altering the DNA sequence. It occurs mainly through DNA methylation, histone modification, and noncoding RNA activity. DNA methylation involves the addition of methyl groups to cytosine residues at CpG sites by DNA methyltransferase, which can suppress gene expression by preventing transcription factor binding. Hypermethylation of tumor suppressor genes such as BRCA1 may contribute to cancer development. Histone modifications, including acetylation, phosphorylation, and methylation, alter chromatin structure and influence DNA accessibility for transcription. These modifications can also recruit proteins that regulate transcriptional activity. In addition, noncoding RNAs, particularly microRNAs (miRNAs), regulate gene expression by inhibiting translation or promoting degradation of messenger RNA. The expression of miRNAs changes according to intracellular and environmental conditions, allowing dynamic regulation of cellular functions and adaptation to external stimuli.

Posttranscriptional Processes

Posttranscriptional processes are essential for converting precursor RNA into functional gene products and preparing messenger RNA (mRNA) for translation. These processes include 5′ capping, intron removal by splicing, alternative splicing, addition of a poly(A) tail, gene fusion transcript processing, and regulation of mRNA stability. Among these, intron splicing, alternative splicing, and gene fusion events significantly influence gene expression. Intron removal is mediated by small nuclear ribonucleoprotein particles (snRNPs), which contain long noncoding RNAs (U1, U2, U4, U5, and U6). Together, these components form the spliceosome, which excises introns and joins exons to produce mature mRNA. Alternative splicing allows a single gene to generate multiple mRNA transcripts through different combinations of exons, resulting in diverse protein isoforms. Gene fusion events occur when two separate genomic regions combine to form a fusion transcript. Such events may alter transcript levels and contribute to disease development. A notable example is the TMPRSS2–ERG fusion gene, which produces a chimeric protein associated with epithelial malignancies. These posttranscriptional mechanisms are important targets in gene expression studies and disease research.

Common Sources of Variation in Gene Expression

Gene expression (GE) is influenced by numerous biological and environmental factors. Understanding these sources of variation is essential for designing gene expression studies and interpreting their results accurately. Researchers must consider these variables during data collection and statistical analysis to avoid confounding effects. Major factors affecting gene expression include tissue specificity, age, gender, time, environmental influences, and genetic variations.

Tissue Specificity

Gene expression differs significantly among various cell

types and tissues because different cells perform specialized functions. Each tissue expresses a unique set of genes that determine its structure and physiological role. For example, the perilipin-1 (PLIN1) gene is highly expressed in adipocytes but is absent in fibroblasts, peripheral nerves, and chondrocytes.

Embryonic stem cells exhibit a distinct pattern of gene expression compared with mature cells. Since stem cells actively divide and possess pluripotency, approximately 60% of protein-coding genes are transcribed into messenger RNA (mRNA), while only a small proportion of genes are tissue-specific.

Certain genes, known as housekeeping genes, are expressed consistently across most tissues because they are required for basic cellular functions. An example is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which serves as an internal control in gene expression studies and quality assurance procedures.

The Tissue-Specific Gene Expression and Regulation (TIGER) database provides information regarding tissue-specific expression profiles and regulatory networks. Studies involving 30 human tissues have identified more than 7,000 tissue-specific genes and approximately 9,000 tissue-specific transcription factor interactions.

Knowledge of baseline gene expression patterns is essential when studying disease-associated phenotypes. Peripheral blood provides a common example of tissue-specific expression. Various blood cell populations, including monocytes, dendritic cells, natural killer cells, CD4+ T cells, CD8+ T cells, and B lymphocytes, possess distinct expression profiles. Depending on the disease or phenotype under investigation, alterations may occur in one or several of these cell types.

For instance, studies on chronically lonely individuals demonstrated differential expression of 98 genes compared with healthy controls. Increased expression occurred mainly in dendritic cells and involved inflammatory and leukocyte activation pathways, whereas decreased expression occurred in monocytes and dendritic cells and affected type I interferon antiviral responses. Such findings provide insight into the biological mechanisms associated with psychosocial conditions.

Variation may also occur within apparently similar cell populations. Factors such as cell-cycle stage, microbial exposure, and differences in the cellular microenvironment contribute to heterogeneity in gene expression. Single-cell transcriptome sequencing enables investigators to identify variability among individual cells, classify cell populations, and discover previously unknown cell types.

Host Age

Age is another important determinant of gene expression. Numerous studies have demonstrated that gene activity changes progressively throughout life. A meta-analysis revealed that 56 genes are consistently overexpressed and 17 genes are under expressed with advancing age.

Age-related alterations are thought to result primarily from the accumulation of DNA damage, oxidative stress, and changes in epigenetic regulation. These modifications occur gradually and influence multiple biological pathways associated with cellular senescence and aging.

Because age significantly affects gene expression, researchers should record the age of participants and account for this variable during study design. When using tissue-bank specimens, samples should be matched according to age to minimize bias. If genes known to change with aging are involved, age must be included as a covariate during statistical analysis.

Understanding age-associated changes in gene expression is particularly important in studies related to cancer, neurodegenerative diseases, cardiovascular disorders, and immune system aging.

Host Gender

Gender also influences gene expression patterns. Differences in hormonal profiles, chromosomal composition, and physiological functions contribute to variations between males and females.

Studies involving healthy individuals have demonstrated significant sex-related differences in gene expression. One investigation involving 41 males and 36 females identified 46 genes exhibiting gender-associated expression differences. Among these, 35 genes showed increased expression in females and 11 genes showed increased expression in males. These differences are observed not only in sex chromosome genes but also in autosomal genes. Microarray analyses have demonstrated that many genes unrelated to reproductive function exhibit sex-dependent expression patterns.

For example, although neutrophil counts may not differ significantly between males and females, several genes are more highly expressed in female neutrophils. Such findings suggest that gene regulation can vary independently of cell numbers.

Gender-related differences are especially relevant in studies involving immune responses, autoimmune diseases, metabolic disorders, and cardiovascular conditions. Failure to account for gender may lead to inaccurate conclusions and confounded results.

Therefore, researchers should carefully document the sex of participants and consider incorporating gender as an adjustment variable during statistical analysis when studying genes known to exhibit sex-specific expression.

Importance of Controlling Sources of Variation

Variability in gene expression represents a major challenge in molecular biology and genomic research. Factors such as tissue type, age, and gender can influence baseline expres-

sion levels and may obscure disease-related changes if not properly controlled.

Appropriate study design requires careful selection of tissues, matching of samples, accurate recording of demographic variables, and application of statistical methods to minimize confounding. Advances in technologies such as single-cell RNA sequencing, transcriptomics, and bioinformatics have improved the ability to identify and characterize these sources of variation.

Understanding the biological factors that regulate gene expression provides valuable insights into disease mechanisms, personalized medicine, biomarker discovery, and therapeutic development. Recognition and control of these variables are essential for producing reliable and reproducible results in gene expression studies.

Additional Sources of Variation in Gene Expression and Common Methods for Measurement of Gene Expression

Time of Sample Collection

Gene expression (GE) exhibits temporal variations that are influenced by circadian rhythms and seasonal changes. Numerous studies have demonstrated that expression patterns of many genes fluctuate over a 24-hour period as well as across different seasons. These variations are important considerations in the design and interpretation of gene expression studies.

Diurnal variation has been observed in several tissues, including whole blood, saliva, liver, and cardiac tissue. Physiological processes regulated by the biological clock influence the transcription of numerous genes, leading to fluctuations in mRNA levels throughout the day. Consequently, samples collected at different times may produce differences in gene expression that are unrelated to the phenotype under investigation.

To minimize this source of variability, researchers should collect biological specimens such as blood, saliva, and tissue samples at the same time of day whenever possible. If standardization is not feasible, the exact collection time should be recorded and incorporated into statistical analyses.

Seasonal changes also influence gene expression. Investigations have shown that more than 4,000 protein-coding messenger RNAs in white blood cells and adipose tissue display seasonal patterns of expression. Environmental factors associated with different seasons, including temperature, sunlight exposure, and infectious diseases, may contribute to these variations. Therefore, recording the date and season of sample collection is essential to ensure accurate interpretation of gene expression data.

Environmental Influences

Environmental factors play a significant role in regulating gene expression. External stimuli can activate or suppress specific genes, thereby affecting cellular functions and disease susceptibility.

Exposure to pollutants represents one example of environmentally induced changes in gene expression. Studies involving traffic-related air pollution have identified a core group of differentially expressed genes associated with pathways involved in cancer, cardiovascular disease, and chronic respiratory disorders.

Several environmental factors are capable of modifying gene expression, including:

- Psychological stress
- Dietary habits
- Physical activity
- Air pollutants
- Smoking
- Alcohol consumption
- Exposure to chemicals
- Microbial infections
- Socioeconomic conditions

These factors often exert their effects through epigenetic mechanisms such as DNA methylation, histone modification, and microRNA regulation.

For studies investigating disease susceptibility, researchers should collect information regarding relevant environmental exposures. Failure to account for these variables may introduce confounding effects and obscure genuine biological associations.

Inherited Genetic Variation

Gene expression is strongly influenced by inherited genetic factors. Genetic polymorphisms and mutations can alter transcriptional activity and contribute to interindividual differences in susceptibility to disease.

Research has demonstrated that variations in DNA sequences influence both the quantity and pattern of gene expression. One landmark study investigating obesity-related traits evaluated gene expression in adipose tissue and blood. The findings revealed strong correlations between gene expression patterns in adipose tissue and obesity-related characteristics, whereas similar associations were not observed in blood.

The investigators further demonstrated that a substantial proportion of these expression differences had a heritable basis. Genes involved in inflammatory and immune responses showed causal relationships with obesity and metabolic disturbances.

These observations emphasize that inherited genetic variation contributes significantly to differences in gene expression among individuals. Consequently, researchers may need to incorporate genetic information into their analyses to

distinguish inherited effects from environmental influences. Understanding the genetic regulation of expression is essential in personalized medicine, pharmacogenomics, and disease-risk prediction.

Relationship Between Transcript Levels and Protein Levels

Although messenger RNA levels provide information regarding gene activity, they do not necessarily reflect the amount of protein produced. Changes in transcript abundance may not correlate directly with serum protein concentrations.

Several factors contribute to this discrepancy:

- Rapid degradation of mRNA molecules.
- Variable translation efficiency.
- Degradation of protein products.
- Storage or reservoir effects.
- Post-translational modifications.
- Intracellular and extracellular environmental conditions.

Consequently, elevated mRNA expression does not always result in increased protein synthesis. Similarly, low transcript levels do not necessarily imply reduced protein concentrations.
For studies aiming to evaluate biological function or identify biomarkers, researchers should measure both gene expression and protein levels. Combining transcriptomic and proteomic analyses provides a more comprehensive understanding of cellular processes and disease mechanisms.

Common Methods for Measurement of Gene Expression

Several laboratory techniques are available for measuring gene expression. The most widely used methods include Northern blotting, quantitative polymerase chain reaction (qPCR), DNA microarrays, and RNA sequencing (RNA-Seq). Each method has unique advantages and limitations.

Northern Blotting

Northern blotting is one of the earliest and most established techniques for studying RNA molecules. It enables investigators to determine both the size and quantity of specific RNA transcripts within a sample.

Principle:

The method involves several steps:

1. RNA molecules are separated according to size using agarose or polyacrylamide gel electrophoresis.
2. The separated RNA fragments are transferred to a nitrocellulose membrane.
3. RNA becomes immobilized on the membrane through covalent interactions.
4. Specific labeled probes complementary to the target RNA are hybridized with the membrane.
5. Hybridized probes are detected using chemiluminescence or autoradiography, producing visible signals that indicate the presence of the transcript.

Northern blotting is commonly used for analyzing transfer RNA (tRNA) and specific messenger RNAs.

Advantages of Northern Blotting

Northern blot analysis possesses several benefits:

1. Simple and well-established methodology.
2. Relatively inexpensive.
3. Provides information regarding transcript size.
4. Allows detection of alternative RNA forms.
5. High specificity due to complementary probe hybridization.
6. Useful for validating results obtained by other molecular techniques.

Because of these characteristics, Northern blotting remains valuable in many molecular biology laboratories.

Limitations of Northern Blotting

Despite its advantages, Northern blotting has several drawbacks:

1. Time-consuming procedure.
2. Requires relatively large amounts of RNA.
3. Limited sensitivity.
4. Only a few samples can be analyzed simultaneously.
5. Hybridization conditions must be highly stringent.
6. Not suitable for large-scale transcriptome analysis.
7. Labor-intensive compared with modern techniques.

These limitations have resulted in the increasing use of more advanced methods such as qPCR, microarrays, and RNA sequencing.
Gene expression is influenced by numerous variables, including the time of sample collection, environmental exposures, and inherited genetic factors. Furthermore, transcript abundance does not always correspond to protein concentrations, highlighting the importance of integrating transcriptomic and proteomic analyses.
Accurate gene expression studies require careful consideration of these sources of variability during study design and statistical analysis. Among the available techniques, Northern blotting represents a classical method for measuring RNA expression, although newer technologies provide greater sensitivity and higher throughput. Understanding

these factors and methodologies is fundamental for advancing genomic research, biomarker discovery, and personalized medicine.

Method	Technique	Initial RNA Processing Step	Strengths	Limitations
Northern Blotting	Hybridization-based assay	mRNA or tRNA is labeled and immobilized on a nitrocellulose membrane	• Simple and easy to perform •Inexpensive • High specificity	• Risk of RNA degradation during electrophoresis • Low throughput • Labor-intensive and time-consuming
Quantitative Polymerase Chain Reaction (qPCR)	PCR quantification-based assay	mRNA is reverse transcribed into complementary DNA (cDNA) complementary DNA (cDNA)	• Easy to perform• Rapid assay (8–12 hours)• Highly sensitive and specific • Accurate quantification of target genes sensitive and specific • Accurate quantification of target genes	• Not suitable for exploratory studies• Requires prior knowledge of target genes • Limited number of genes analyzed simultaneously of target genes • Limited number of genes analyzed simultaneously
DNA Microarray	Multistep target preparation and hybridization-based assay	mRNA is reverse transcribed into cDNA	• Suitable for exploratory analysis• High throughput• Simultaneous analysis of thousands of genes• Useful in genome-wide association studies (GWAS)	• Total turnaround time approximately 72 hours • Specialized software required for image analysis • Limited sensitivity compared with RNA-Seq
RNA Sequencing (RNA-Seq)	Adapter ligation-, PCR amplification-, and sequencing-based assay	mRNA is reverse transcribed into cDNA; miRNA, tRNA, and rRNA may also be labeled and sequenced	• Ideal for exploratory analysis • High throughput • Lower background noise than microarrays • Superior sensitivity and detection accuracy • Useful for GWAS and transcriptome profiling • Detects novel transcripts and splice variants	• Very expensive • Turnaround time may extend up to 48 hours • Requires advanced computational analysis and large data-storage capacity

Table 1. Comparison of Techniques for RNA Measurement

Abbreviations

1. cDNA – Complementary deoxyribonucleic acid
2. GWAS – Genome-Wide Association Studies
3. miRNA – MicroRNA
4. mRNA – Messenger RNA
5. PCR – Polymerase Chain Reaction
6. qPCR – Quantitative Polymerase Chain Reaction
7. RNA – Ribonucleic Acid
8. RNA-Seq – RNA Sequencing

9. rRNA – Ribosomal RNA
10. tRNA – Transfer RNA

Northern blotting is a classical, highly specific, and inexpensive method for RNA analysis but has low throughput. qPCR is widely used for rapid and accurate quantification of known genes. DNA microarrays allow simultaneous analysis of thousands of genes and are useful for exploratory studies. RNA sequencing (RNA-Seq) is currently the most powerful transcriptomic technology, providing high sensitivity, detection of novel transcripts, and comprehensive genome-wide expression profiling, although it requires substantial financial and computational resources.

RNA-Seq is presently considered the gold standard for transcriptome analysis because of its superior sensitivity, high throughput, and ability to identify novel transcripts and alternative splice variants.

3. Genomics and Systems Biology in Homoeopathy

Systems biology considers organisms as integrated networks of genes, proteins, metabolites, and signaling pathways. Rather than focusing on single targets, genomic technologies investigate global cellular responses. Homoeopathic medicines have been proposed to act through modulation of adaptive networks rather than conventional dose-dependent receptor mechanisms. Such hypotheses have stimulated investigations using transcriptomic platforms and computational biology approaches.
Gene expression studies enable assessment of messenger RNA changes induced by external stimuli. High-throughput techniques such as DNA microarrays and RNA sequencing have facilitated identification of differentially expressed genes and molecular pathways associated with inflammatory responses, oxidative stress, apoptosis, and immune regulation.

4. Experimental Gene Expression Studies

4.1 Cell Culture Models

Several investigators have examined the effects of Homoeopathic preparations on cultured cells. Human epithelial cells, macrophages, fibroblasts, and cancer cell lines have been employed to evaluate alterations in transcriptional activity. Reported changes include modulation of cytokines, heat shock proteins, antioxidant enzymes, and genes associated with apoptosis.
Arsenicum album, Condurango, Ruta graveolens, and Carcinosinum have been investigated in experimental cancer models. Some studies have demonstrated altered expression of p53, Bcl-2, Bax, caspases, and inflammatory mediators. Such findings suggest possible effects on cellular signaling pathways involved in programmed cell death and oxidative stress responses. However, replication of these results by independent laboratories remains limited.

4.2 Animal Studies

Animal experiments have provided additional evidence regarding molecular responses following administration of Homoeopathic medicines. Investigations involving mice and rats have reported changes in genes related to immune function, antioxidant defense, and inflammatory processes. Differential expression of NF-κB, TNF-α, IL-6, COX-2, and superoxide dismutase has been observed in some studies.
Several studies have suggested protective effects against chemically induced toxicity and carcinogenesis. These effects have been attributed to modulation of gene regulatory pathways and enhancement of antioxidant mechanisms. Nonetheless, many studies have involved relatively small sample sizes and variable experimental protocols, thereby limiting generalizability.

5. Transcriptomic Technologies

Microarray Analysis

DNA microarray technology enables simultaneous evaluation of thousands of genes. Early transcriptomic investigations in Homoeopathy utilized microarrays to identify differentially expressed genes following exposure to highly diluted preparations. Reported effects involved genes associated with immune regulation, cellular metabolism, and stress responses.

RNA Sequencing

RNA sequencing has emerged as a powerful technique for comprehensive transcriptome analysis. Compared with microarrays, RNA sequencing offers greater sensitivity and detection of novel transcripts. Future studies utilizing next-generation sequencing may provide more reliable and reproducible evidence regarding molecular effects of Homoeopathic medicines.

6. Epigenetics and Homoeopathy

Epigenetic mechanisms regulate gene activity without altering DNA sequence. DNA methylation, histone modifications, and microRNAs are important regulators of cellular adaptation and disease development. Some investigators have proposed that Homoeopathic medicines may exert effects through epigenetic modulation.
MicroRNAs have emerged as potential mediators of adaptive responses and intercellular communication. Preliminary studies suggest that ultra-low doses may influence epigenetic pathways, although the evidence remains insufficient and

requires validation using modern molecular approaches.

HOMOEOPATHIC MEDICINES ACT ON THE GENOME BY MODULATING GENE EXPRESSION (GENE REGULATORY HYPOTHESIS)

Based on experimental studies that showed the effect of Homoeopathic medicines in repairing chromosomal damage caused by toxic or radioactive stimuli, since 1997 Khuda-Bukhsh defends the hypothesis that the mechanism of action of Homoeopathic medicines occurs through the regulation of gene expression. Evidencing the experimental studies that demonstrate the action of homeopathic medicines in molecular biology, Dei and Bernardini reaffirm the hypothesis of Khuda-Bukhsh, suggesting that the action of Homoeopathic medicines "is not quenched by ultrahigh dilution and proceeds through modulation of gene expressions." Analogously describing experiments that evidence the action of Homoeopathic medicines on gene expression, Bellavite et al. suggest that "these findings support the hypothesis that homeopathic remedies could turn some important genes on or off, initiating a cascade of gene actions to correct the gene expression that has gone wrong and produced the disorder or disease." Considering that Homoeopathic medicines act in the regulation of the vital force, these experimental studies reiterate the hypothesis that the genome (exome plus epigenome) is the representation or the biological substrate of the vital principle.

Major Mechanisms of Epigenetic Regulation

DNA Methylation

DNA methylation represents one of the most extensively studied epigenetic mechanisms. It involves the addition of methyl groups to cytosine residues, primarily within CpG islands, thereby regulating transcriptional activity. Hypermethylation of promoter regions generally suppresses gene expression, whereas demethylation permits gene activation. Abnormal methylation patterns are implicated in numerous diseases, including cancer, cardiovascular disorders, autoimmune conditions, and psychiatric illnesses. Environmental stress, childhood trauma, diet, and exposure to toxins have all been shown to influence DNA methylation patterns. Thus, life experiences can leave enduring molecular signatures that affect health and disease susceptibility.

Histone Modification

DNA is packaged around histone proteins to form nucleosomes. Histones undergo various post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications alter chromatin structure and regulate gene accessibility.
Histone acetyltransferases and histone deacetylases serve as important regulators of chromatin architecture. Abnormal histone modifications have been associated with malignancies, neurodegenerative diseases, and inflammatory disorders. Several anticancer drugs currently target histone-modifying enzymes.

Chromatin Remodeling

ATP-dependent chromatin remodeling complexes dynamically regulate nucleosome positioning and influence accessibility of transcriptional machinery to DNA. Disruption of these complexes contributes to the pathogenesis of cancer, autoimmune diseases, and developmental disorders.

Non-Coding RNAs

MicroRNAs and long non-coding RNAs regulate gene expression at the post-transcriptional level. A single microRNA may simultaneously influence hundreds of target genes. Furthermore, exosome-mediated transfer of microRNAs represents a novel mechanism of intercellular communication. These discoveries have significantly expanded our understanding of biological regulation.

Miasms and Transgenerational Epigenetic Inheritance

Among the most fascinating parallels between Homoeopathy and modern biology is the relationship between Hahnemann's theory of miasms and transgenerational epigenetic inheritance.
Hahnemann proposed that chronic diseases arise from inherited miasmatic influences, namely Psora, Sycosis, and Syphilis. Later, J. H. Allen described the tubercular miasm as a combination of psoric and syphilitic tendencies.
For decades, the notion of inherited disease predisposition without genetic mutation lacked a biological explanation. However, modern epigenetics has demonstrated that environmental exposures can induce heritable epigenetic changes. Skinner and colleagues showed that exposure to endocrine-disrupting chemicals produced altered DNA methylation patterns that persisted through multiple generations.
Similarly, studies on famine exposure during the Dutch Hunger Winter revealed persistent epigenetic alterations affecting descendants. Research on Holocaust survivors and stress-related disorders has also demonstrated intergenerational transmission of epigenetic marks.
These findings provide a plausible molecular basis for inherited susceptibility and suggest striking conceptual similarities with the miasmatic theory of chronic disease.

Susceptibility and Epigenetic Vulnerability

Susceptibility occupies a central position in Homoeopathic philosophy. According to Hahnemann, disease expression

depends not only on external factors but also on the individual's internal susceptibility.

Modern epigenetics supports this notion. Individuals possessing identical genetic sequences may exhibit vastly different disease patterns because of differences in their epigenomes. Monozygotic twin studies have demonstrated that epigenetic profiles diverge progressively with age and environmental influences.

Psychological stress also exerts profound effects on gene expression. Chronic stress can induce hypermethylation of glucocorticoid receptor genes, thereby altering stress responsiveness and contributing to anxiety and depression. Such findings reinforce the concept that emotional experiences can influence biological susceptibility.

Experimental Evidence Supporting Gene Regulatory Effects

Several experimental studies have investigated the molecular effects of homoeopathic preparations.

Frenkel and coworkers demonstrated cytotoxic effects of ultra-diluted Carcinosin and Phytolacca preparations on breast cancer cell lines. These remedies induced apoptosis and altered cell cycle dynamics.

Olioso and colleagues reported that Gelsemium sempervirens modified the expression of forty-nine genes involved in stress response and calcium signaling in neuronal cells. These effects were reproducible and exhibited dose dependence.

Research conducted by Sunila and associates demonstrated significant modulation of apoptotic pathways by Thuja occidentalis and Carcinosin in cancer cell cultures. Similarly, Khuda-Bukhsh and collaborators reported gene regulatory effects of potentized Arsenicum album in experimental models of arsenic toxicity.

Marzotto and colleagues observed anxiolytic effects of Gelsemium preparations in animal studies, accompanied by changes in neuronal gene expression. Collectively, these investigations suggest that Homoeopathic medicines may influence cellular processes at the molecular level.

Nanoparticles and High Dilutions

One of the major criticisms directed against Homoeopathy concerns the absence of molecules beyond Avogadro's limit. However, studies by Chikramane and colleagues challenged this assumption.

Using electron microscopy and spectroscopic techniques, they demonstrated the presence of nanoparticles derived from the original source material even in highly diluted preparations. These nanoparticles may interact with cellular signaling pathways and potentially induce biological effects.

Nanoparticles are already recognized as powerful modulators of cellular stress responses, receptor signaling, and epigenetic mechanisms. Consequently, nanoparticle research has generated renewed interest in understanding the physical basis of Homoeopathic preparations.

Vital Force and Systems Biology

The concept of Vital Force, introduced by Hahnemann, describes a dynamic, self-regulating principle maintaining homeostasis and responding to disease.

Interestingly, systems biology portrays organisms as complex adaptive networks composed of interacting genes, proteins, and metabolic pathways. These networks exhibit self-organization, resilience, and emergent properties.

The epigenome occupies a central regulatory position within these networks, coordinating responses to environmental stimuli. Therefore, some investigators have proposed that the Vital Force may be interpreted as an early conceptual representation of biological regulatory systems.

Bell and colleagues introduced the "epigenetic hypothesis of Homoeopathy," proposing that Homoeopathic medicines function as informational signals capable of restoring dysregulated gene expression patterns. Although hypothetical, this model provides a testable scientific framework.

Individualized Prescribing and Personalized Medicine

Individualization represents one of the defining characteristics of Homoeopathy. Treatment is selected according to the totality of symptoms rather than disease diagnosis alone.

Modern medicine is increasingly embracing personalized approaches based on genetic and epigenetic information. Pharmacoepigenomics investigates how epigenetic variations influence therapeutic responses.

From this perspective, constitutional prescribing in Homoeopathy resembles personalized medicine. The constitutional state of an individual may reflect a unique epigenetic profile, and appropriately selected remedies might modulate disturbed regulatory networks.

Future studies comparing constitutional types with epigenomic patterns may provide valuable insights into both personalized medicine and Homoeopathic therapeutics.

Limitations

Despite intriguing observations, substantial limitations remain. Experimental studies are heterogeneous in design and often involve small sample sizes. Reproducibility and standardization remain major challenges.

Conceptual similarities between Homoeopathy and epigenetics should not be interpreted as proof of therapeutic mechanisms. Furthermore, cellular and animal experiments cannot be directly extrapolated to clinical efficacy.

Rigorous randomized trials, advanced molecular studies, and standardized methodologies are essential before definitive conclusions can be drawn.

7. Clinical Studies

Clinical genomic investigations in Homoeopathy remain scarce. A limited number of studies have evaluated biomarkers and gene expression profiles in patients receiving individualized homeopathic treatment. Reported alterations have involved immune markers and inflammatory pathways. However, heterogeneous patient populations, individualized prescriptions, and methodological limitations have hindered reproducibility and interpretation.

Large multicenter randomized studies incorporating transcriptomic and proteomic analyses are required to establish clinical relevance and biological plausibility.

Antiproliferative Effects of *Thuja occidentalis*

The results demonstrated that Thuja occidentalis exerted significant growth-inhibitory effects on AGS gastric cancer cells in a concentration-dependent manner. The IC50 value was approximately 0.08% after 48 hours of treatment. Furthermore, treatment with Thuja markedly suppressed colony formation and spheroid development, indicating inhibition of tumorigenic potential and self-renewal characteristics associated with cancer stem cells.

These observations suggest that Thuja occidentalis possesses potent cytotoxic and antiproliferative activities against gastric cancer cells and may represent a promising complementary therapeutic agent.

Genome-Wide Molecular Alterations

Comprehensive transcriptomic analysis revealed extensive changes in gene expression following treatment with Thuja occidentalis. A total of 549 genes were significantly downregulated, while 212 genes were upregulated.

Among the downregulated genes, several important families associated with cellular proliferation and transcriptional regulation were identified, including:

- Histone family genes
- Zinc finger (ZNF) genes
- DNA polymerase genes
- ATP-related genes

Histones are essential components of chromatin structure and play critical roles in epigenetic regulation. Their downregulation may interfere with DNA replication and cell-cycle progression, thereby suppressing cancer growth. Zinc finger proteins are transcription factors involved in cellular proliferation, differentiation, and apoptosis. Suppression of these genes may contribute to inhibition of oncogenic signaling pathways.

Similarly, decreased expression of ATP-associated genes suggests impaired cellular energy metabolism, whereas inhibition of DNA polymerase genes indicates disruption of DNA synthesis and replication processes. Collectively, these molecular changes contribute to growth inhibition and apoptosis in gastric cancer cells.

Suppression of Oncogenic Signaling Pathways

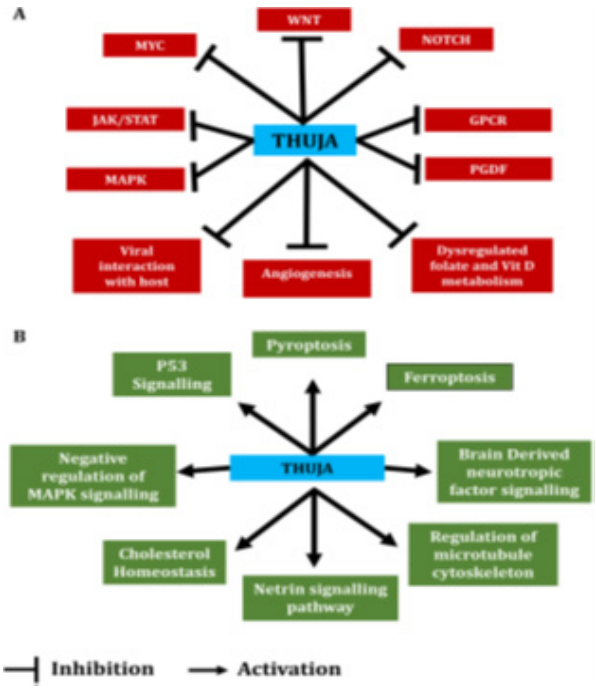

Figure 2: Illustrates the signaling pathways regulated by *Thuja occidentalis* identified through gene set enrichment analysis.

Treatment with Thuja resulted in downregulation of several oncogenic pathways, including Wnt, MAPK, NOTCH, JAK/STAT, GPCR, and PDGF signaling, along with suppression of angiogenesis and dysregulated folate metabolism. Conversely, genes upregulated by Thuja were enriched in pathways associated with pyroptosis, ferroptosis, p53 signaling, microtubule cytoskeleton regulation, cholesterol homeostasis, netrin signaling, and neurotrophic signaling. These findings suggest that Thuja occidentalis exerts anticancer effects by simultaneously inhibiting tumor-promoting pathways and activating cell death and tumor-suppressive mechanisms.

Gene set enrichment analysis demonstrated that Thuja occidentalis suppresses several major signaling pathways involved in carcinogenesis. These include:

1. Mitogen-Activated Protein Kinase (MAPK) pathway
2. MYC signaling pathway
3. Wnt/β-catenin pathway
4. NOTCH signaling pathway
5. G-protein coupled receptor (GPCR) pathway
6. Transforming Growth Factor-beta (TGF-β) pathway
7. Platelet-Derived Growth Factor (PDGF) pathway

8. Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway

These pathways regulate cell proliferation, survival, angiogenesis, invasion, and metastasis. Their simultaneous inhibition suggests that Homoeopathic Medicine Thuja occidentalis acts through a multi-targeted mechanism rather than influencing a single molecular target.
Moreover, the study revealed significant downregulation of several oncogenes, including **TGFBR2, LGR5, PDGFRA, FGFR2,** and **MYB**, providing further evidence for the anticancer potential of Thuja.

Validation of Gene and Protein Expression

Quantitative RT-PCR analysis confirmed the transcriptomic findings. Significant reductions in the expression of TGFBR2, LGR5, and NOTCH1 genes were observed following treatment with Thuja occidentalis. Conversely, the expression of KRTAP2-3 was markedly increased.

Western blot analysis further demonstrated decreased expression of key proteins involved in oncogenic signaling, including:

1. β-catenin
2. c-Myc
3. Smad4
4. STAT3
5. HIF-1α

Immunofluorescence studies also showed dose-dependent reductions in β-catenin and Smad4 protein levels. These findings validate the microarray data and confirm the suppression of Wnt and TGF-β signaling pathways.

Activation of Programmed Cell Death Mechanisms

Interestingly, Thuja occidentalis activated genes associated with multiple forms of programmed cell death. Pathway analysis revealed upregulation of:

- Ferroptosis
- Pyroptosis
- p53 signaling pathway

Ferroptosis is an iron-dependent form of cell death characterized by lipid peroxidation, while pyroptosis represents an inflammatory form of programmed cell death. Activation of these pathways indicates that Thuja induces cancer cell destruction through diverse mechanisms, thereby reducing the probability of therapeutic resistance.
The activation of p53 signaling further highlights the pro-apoptotic potential of Thuja occidentalis and supports its role in tumor suppression.

Implications for Wart Treatment

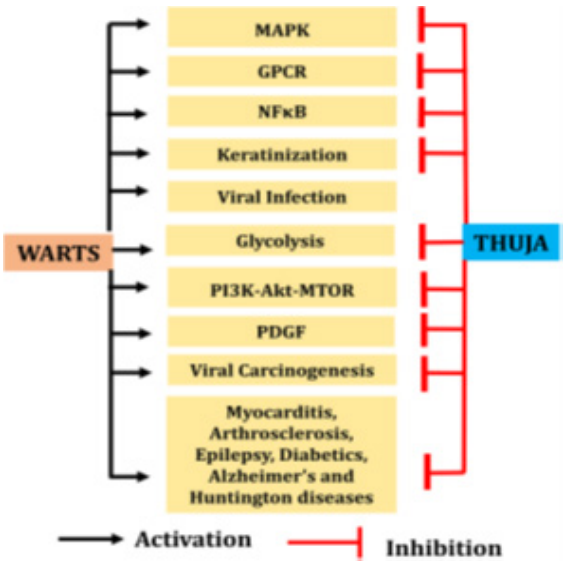

Figure 3: Demonstrates that signaling pathways abnormally activated in warts are suppressed by *Thuja occidentalis.*

Analysis of the **GEO** dataset **GSE140662** showed increased **PDGF, JAK/STAT, MAPK,** and **GPCR** signaling in wart tissues. Treatment with Thuja downregulated these pathways, supporting its traditional therapeutic role in managing warts.
Thuja occidentalis has long been prescribed in Homoeopathic practice for the management of warts. The present investigation provides molecular evidence supporting this traditional application.
Analysis of wart-associated gene expression profiles demonstrated that several pathways activated in warts—including **PDGF, GPCR, MAPK, JAK/STAT,** and viral infection pathways—were effectively suppressed by Thuja treatment. These findings indicate that Thuja occidentalis may inhibit cellular proliferation, inflammation, and viral-mediated mechanisms involved in wart formation.
Thus, the study scientifically validates the traditional use of Thuja in dermatological conditions, particularly viral warts.
Identification of Bioactive Constituents
Gas chromatography-mass spectrometry analysis identified thujone as the major constituent of Thuja occidentalis mother tincture, accounting for approximately 46.8% of the detected compounds. Fractionation studies demonstrated that fractions containing thujone exhibited significant cytotoxic effects, whereas fractions lacking thujone showed minimal activity.

Additional Bioactive Compounds Identified Included:

- α-Pinene
- Caryophyllene
- Terpinen-4-ol

- Ferruginol

These compounds possess documented antioxidant, anti-inflammatory, and anticancer properties and may act synergistically with thujone to enhance the therapeutic efficacy of Thuja occidentalis.

8. Limitations

Major Limitations Identified in the Reviewed Literature include:

1. Small sample sizes.
2. Lack of standardized protocols.
3. Inadequate blinding and randomization.
4. Insufficient independent replication.
5. Variability in potencies and preparation methods.
6. Limited application of next-generation sequencing.
7. Difficulty distinguishing biological effects from experimental artifacts.

These methodological concerns have been highlighted in systematic analyses of Homoeopathic basic research.

9. Future Perspectives

Integration of genomics, transcriptomics, proteomics, metabolomics, and systems biology offers opportunities for advancing understanding of Homoeopathic mechanisms. Artificial intelligence and machine learning approaches may facilitate identification of gene regulatory networks and biomarkers associated with therapeutic responses. Standardized multicenter collaborations employing RNA sequencing, epigenetic profiling, and bioinformatics are necessary to establish reproducible molecular evidence.

Discussion

The findings of the present review indicate that genomic and transcriptomic investigations have opened new avenues for exploring the biological effects of Homoeopathic medicines beyond traditional pharmacological paradigms. Experimental studies have reported alterations in genes associated with inflammation, oxidative stress, apoptosis, immune regulation, and cellular signaling, suggesting that Homoeopathic preparations may interact with complex adaptive networks. Such observations are consistent with systems biology and epigenetic hypotheses that emphasize regulatory rather than direct receptor-mediated mechanisms. However, considerable variability exists among studies with respect to experimental models, potency selection, analytical techniques, and outcome measures, making comparisons difficult and limiting reproducibility. Furthermore, the scarcity of large-scale clinical investigations and independent validations remains a significant challenge. Therefore, while preliminary findings are intriguing, robust evidence supporting definitive molecular mechanisms is still lacking. Future research employing standardized methodologies and advanced multi-omics approaches will be crucial for establishing biological plausibility and clinical relevance.

10. Conclusion

The emerging field of genomics has provided new opportunities to investigate the molecular basis of homeopathic interventions through gene expression studies conducted in cellular, animal, and limited clinical models. Available evidence suggests that Homoeopathic preparations may influence genes involved in inflammatory regulation, oxidative stress responses, apoptosis, immune modulation, and cellular signaling pathways. These findings support the possibility that biological systems may exhibit measurable molecular responses under certain experimental conditions. However, substantial challenges remain, including small sample sizes, methodological heterogeneity, inadequate replication, and lack of standardized protocols, which limit the interpretation and generalizability of current findings. Consequently, the existing evidence is insufficient to establish definitive mechanisms of action or clinical significance. Future investigations should emphasize rigorous experimental design, multicenter collaborations, reproducibility studies, and integration of advanced omics technologies, including transcriptomics, epigenomics, proteomics, and systems biology approaches, to provide more robust and scientifically validated evidence.

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I respectfully dedicate this review article to my most esteemed mentor, **Prof. Dr. J. Ashok** as a humble expression

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