# Report Title

Introduction

The research question pertains to the role of epigenetic modifications, specifically DNA methylation and histone modifications, in regulating gene expression during cellular differentiation and development, and their implications in diseases such as cancer. The study aims to elucidate the contribution of epigenetic mechanisms to the regulation of cellular gene expression, with a focus on their potential significance in disease pathogenesis and therapeutic development.

Epigenetic modifications, including DNA methylation and histone modifications, play a crucial role in regulating gene expression and have been implicated in various biological processes, encompassing development, aging, and disease pathogenesis. Understanding the significance and implications of these modifications is essential due to their regulatory influence on the gene expression patterns that influence cellular differentiation, disease progression, and therapeutic interventions.

Specifically, the study will explore the importance of investigating epigenetic modifications in the context of cellular differentiation and development, focusing on the retina as a model system. The implications of these modifications in the retina contribute to the understanding of neuronal differentiation, photoreceptor function, and retinal neurodegeneration. Epigenetic modifications are cell type-dependent and influence distinct gene expression profiles within a tissue, which is crucial for maintaining functional homeostasis.

Moreover, the significance of emerging technologies for profiling and analyzing epigenetic information, such as Single-Molecule Real-Time (SMRT) sequencing, nanopore sequencing, and computational tools, underscores the potential impact of epigenetics on understanding transcriptional regulation, disease modeling, and regeneration. By utilizing these technologies, researchers can gain deeper insights into the role of epigenetic modifications in regulating gene expression during cellular differentiation and development, as well as their implications for disease diagnosis and therapy.

The current body of knowledge regarding epigenetic modifications and their relevance to gene expression represents a critical area for research due to the potential insights into disease mechanisms and the development of novel therapeutic approaches for complex diseases like cancer.

References:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6952058/pdf/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7228806/pdf/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10251769/pdf/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8984156/pdf/

Literature

ions to maintain functional homeostasis. Furthermore, the literature highlights the involvement of histone modifications in gene regulation, particularly in the context of retinal development and aging. The study also presents evidence of changes in histone marks during aging and age-related retinal diseases, suggesting their potential role in disease pathogenesis.

In conclusion, the literature review provides a thorough overview of the experimental methods and findings related to epigenetic modifications, DNA methylation, and histone modifications in the context of cellular differentiation, development, and disease. The review highlights the importance of investigating the epigenome of specific cells and tissues, particularly in the retina, and emphasizes the role of histone modifications in regulating gene expression and disease pathogenesis.

The source url is : https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7851598/pdf/

Discussion

Based on the provided content and research question, the discussion section of the paper would encompass the following key points:

1. Role of Epigenetic Modifications: Discuss the significance of DNA methylation and histone modifications in the regulation of gene expression during cellular differentiation and development. Highlight the context- and cell type-dependent nature of these epigenetic mechanisms, particularly in specific tissues like the retina. Emphasize the importance of understanding the epigenome of specific retinal cells and how it contributes to functional homeostasis.

2. Methodological Advancements: Address the advancements in assays and sequencing technologies for studying epigenetic modifications, such as SMRT, Msp-based nanopore sequencing, ChIP-seq, PCR, BS-seq, and RRBS. These technological advancements have facilitated a deeper understanding of epigenetic mechanisms and their implications in cellular differentiation, development, and disease.

3. Therapeutic Potential: Discuss the implications of epigenomic studies in designing therapies, especially for common multifactorial diseases. Highlight the potential for developing targeted treatments based on sequence variants associated with diseases, such as cancer, located in non-coding and distal gene regulatory regions.

4. Future Research Directions: Emphasize the need for further research into the role of histone modifications in retinal development, aging, and age-related retinal diseases. Identify potential future research directions in the application of epigenetic knowledge in therapeutic development and the exploration of histone modifications in disease pathogenesis.

5. Limitations and Potential Challenges: Address the potential limitations of current methodologies for studying epigenetic modifications and discuss the challenges in translating epigenomic studies into clinical applications. Provide a critical analysis of the strengths and limitations of the methodologies discussed in the literature.

6. Comparative Analysis: Compare and contrast the research findings with those of other studies in related fields to strengthen the validity of the findings and provide a broader perspective on the significance of the results in the context of existing literature.

7. A Holistic Conclusion: Summarize the contributions of the discussed research to the understanding of epigenetic regulation in cellular differentiation, development, and disease. Address the need for further exploration of specific cell types and their potential therapeutic applications.

In conclusion, the discussion section should not only synthesize the information provided in the literature review but also propose future research directions and address the limitations and potential challenges in the field of epigenetic modifications in regulating gene expression during cellular differentiation and development.

Reference:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6952058/pdf/
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7228806/pdf/
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10251769/pdf/
4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8984156/pdf/

Idea

**Problem:**
Exploring the role of epigenetic modifications in regulating retinal photoreceptor regeneration and function in the context of age-related macular degeneration (AMD) and diabetic retinopathy.

**Rationale:**

The existing literature emphasizes the critical role of epigenetic factors, including DNA methylation and histone modifications, in regulating gene expression and function during retinal development, aging,

and disease progression. Additionally, evidence suggests that epigenetic regulation plays a substantial role in neuronal tissue regeneration in other species. However, the specific impact of epigenetic modifications on the regeneration and function of retinal photoreceptors in the context of AMD and diabetic retinopathy remains incompletely understood. Investigating the epigenetic regulation of retinal photoreceptor regeneration and function could provide valuable insights into the pathogenesis of these diseases and potentially lead to the development of novel therapeutic strategies aimed at modulating the epigenome to promote retinal tissue regeneration and function restoration.

Method

Method:

1. Epigenome-wide profiling:

- Perform epigenome-wide profiling of DNA methylation and histone modifications in both normal and diseased retinal tissue samples from patients with AMD and diabetic retinopathy using high-throughput sequencing techniques such as ChIP-seq and bisulfite sequencing. This will provide a comprehensive assessment of epigenetic modifications associated with retinal photoreceptor regeneration and function.

2. Correlation analysis:

- Correlate the identified epigenetic modifications with gene expression patterns in the retinal tissue to elucidate the impact of epigenetic regulation on the expression of genes involved in photoreceptor regeneration and function. This analysis will help in identifying key regulatory pathways controlled by epigenetic modifications in the context of retinal diseases.

3. Functional validation:

- Utilize in vitro and ex vivo models of retinal tissue to functionally validate the identified epigenetic modifications and their impact on photoreceptor regeneration and function. This can involve the manipulation of specific epigenetic marks followed by phenotypic assessments and gene expression analyses.

4. Integrated omics analysis:

- Integrate the epigenomic data with transcriptomic and proteomic profiles of the retinal tissue to comprehensively understand the regulatory networks governing photoreceptor regeneration and function in the context of AMD and diabetic retinopathy. This multi-omics approach will provide a holistic view of the molecular mechanisms influenced by epigenetic modifications.

Rationale:

The proposed method leverages cutting-edge epigenomic profiling techniques to comprehensively investigate the role of epigenetic modifications in regulating retinal photoreceptor regeneration and

function in the context of AMD and diabetic retinopathy. By conducting epigenome-wide profiling, correlating epigenetic modifications with gene expression patterns, and functionally validating the findings, this method aims to elucidate the specific epigenetic regulatory mechanisms underlying retinal disease pathogenesis and tissue regeneration. Furthermore, integrating epigenomic data with other omics profiles will allow for a systems-level understanding of the molecular networks affected by epigenetic regulation, providing valuable insights for the development of novel therapeutic strategies targeting the epigenome in retinal diseases.

Experiment

Experiment: Comprehensive Epigenetic Profiling and Functional Validation of Retinal Photoreceptor Regeneration in AMD and Diabetic Retinopathy

Rationale:

The objective of this experiment is to comprehensively investigate the role of epigenetic modifications in regulating retinal photoreceptor regeneration and function in the context of age-related macular degeneration (AMD) and diabetic retinopathy. The proposed scientific method and the existing literature have laid the foundation for this experiment, as it aims to address the gaps in understanding the specific impact of epigenetic regulation on the regeneration and function of retinal photoreceptors, which remains incompletely understood.

1. Epigenome-wide Profiling:

a. Collection of Retinal Tissue Samples:
- Obtain normal and diseased retinal tissue samples from patients with AMD and diabetic retinopathy, ensuring proper consent and ethical considerations.
- Characterize the demographic and clinical parameters of the patients to account for potential confounding factors.
b. High-throughput Epigenomic Profiling:
- Perform ChIP-seq and bisulfite sequencing to map DNA methylation and histone modifications in the collected retinal tissue samples.
- Utilize state-of-the-art sequencing techniques to ensure high-resolution profiling of epigenetic marks, enabling a comprehensive assessment of epigenetic modifications associated with retinal photoreceptor regeneration and function.

2. Correlation Analysis:

a. Gene Expression Profiling:
- Perform transcriptomic analysis to measure gene expression patterns in the same retinal tissue samples used for epigenomic profiling.
- Apply statistical methods to identify differentially expressed genes associated with photoreceptor regeneration and function in the context of AMD and diabetic retinopathy.
b. Integration of Epigenomic and Transcriptomic Data:
- Correlate the identified epigenetic modifications with gene expression patterns to elucidate the impact of epigenetic regulation on the expression of genes involved in photoreceptor regeneration and function.

- Employ bioinformatic tools and statistical analyses to identify key regulatory pathways controlled by epigenetic modifications in the context of retinal diseases.

3. Functional Validation:

a. In Vitro and Ex Vivo Models:
- Establish in vitro and ex vivo models of retinal tissue using cell lines and primary cell cultures derived from both normal and diseased retinal tissue samples.
- Target specific epigenetic marks identified through epigenomic profiling in these models to investigate their impact on photoreceptor regeneration and function.
b. Phenotypic Assessments and Gene Expression Analyses:
- Conduct phenotypic assessments to evaluate the effects of manipulating specific epigenetic marks on photoreceptor regeneration and function in the in vitro and ex vivo models.
- Perform gene expression analyses to validate the functional impact of epigenetic modifications on the regulatory networks governing photoreceptor regeneration.

4. Integrated Omics Analysis:

a. Data Integration:
- Integrate the epigenomic data with transcriptomic and proteomic profiles of the retinal tissue to comprehensively understand the regulatory networks governing photoreceptor regeneration and function in the context of AMD and diabetic retinopathy.
- Apply systems biology approaches to delineate the molecular mechanisms influenced by epigenetic modifications, providing a holistic view of the interconnected networks underlying retinal disease pathogenesis and tissue regeneration.

Overall, this experiment design aims to provide valuable insights into the specific epigenetic regulatory mechanisms underlying retinal disease pathogenesis and tissue regeneration, thereby paving the way for the development of novel therapeutic strategies targeting the epigenome in the context of AMD and diabetic retinopathy. The experiment emphasizes the integration of cutting-edge technologies, rigorous statistical analyses, and biological validations to ensure robustness, reproducibility, and validity of the findings.

More related paper

Paper 1
Title: Epigenetics in neuronal regeneration.

Abstract: Damage to neuronal tissues in mammals leads to permanent loss of tissue function that can have major health consequences. While mammals have no inherent regenerative capacity to functionally repair neuronal tissue, other species such as amphibians and teleost fish readily replace damaged tissue. The exploration of development and native regeneration can thus inform the process of inducing regeneration in non-regenerative systems, which can be used to develop new therapeutics. Increasing evidence points to an epigenetic component in the regulation of the changes in cellular gene expression necessary for regeneration. In this review, we compare evidence of epigenetic roles in development and regeneration of neuronal tissue. We have focused on three key systems of important

clinical significance: the neural retina, the inner ear, and the spinal cord in regenerative and non-regenerative species. While evidence for epigenetic regulation of regeneration is still limited, changes in DNA accessibility, histone acetylation and DNA methylation have all emerged as key elements in this process. To date, most studies have used broadly acting experimental manipulations to establish a role for epigenetics in regeneration, but the advent of more targeted approaches to modify the epigenome will be critical to dissecting the relative contributions of these regulatory factors in this process and the development of methods to stimulate the regeneration in those organisms like ourselves where only limited regeneration occurs in these neural systems.

DOI: 10.1016/j.semcdb.2019.04.001

The impact factor: 7.499

Paper 2

Title: Genome-wide Profiling Identifies DNA Methylation Signatures of Aging in Rod Photoreceptors Associated with Alterations in Energy Metabolism.

Abstract: Aging-associated functional decline is accompanied by alterations in the epigenome. To explore DNA modifications that could influence visual function with age, we perform whole-genome bisulfite sequencing of purified mouse rod photoreceptors at four ages and identify 2,054 differentially methylated regions (DMRs). We detect many DMRs during early stages of aging and in rod regulatory regions, and some of these cluster at chromosomal hotspots, especially on chromosome 10, which includes a longevity interactome. Integration of methylome to age-related transcriptome changes, chromatin signatures,Â and first-order protein-protein interactions uncover an enrichment of DMRs in altered pathwaysÂ that are associated with rod function, aging, and energy metabolism. In concordance, we detect reduced basal mitochondrial respiration and increased fatty acid dependency with retinal age in exÂ vivo assays. Our study reveals age-dependent genomic and chromatin features susceptible to DNA methylation changes in rod photoreceptors and identifies a link between DNA methylation and energy metabolism in aging.

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The impact factor: 9.995

Paper 3

Title: The role of epigenetic methylation/demethylation in the regulation of retinal photoreceptors.

Abstract: Photoreceptors are integral and crucial for the retina, as they convert light into electrical signals. Epigenetics plays a vital role in determining the precise expression of genetic information in space and time during the development and maturation of photoreceptors, cell differentiation, degeneration, death, and various pathological processes. Epigenetic regulation has three main manifestations: histone modification, DNA methylation, and RNA-based mechanisms, where methylation is involved in two regulatory mechanisms-histone methylation and DNA methylation. DNA

methylation is the most studied form of epigenetic modification, while histone methylation is a relatively stable regulatory mechanism. Evidence suggests that normal methylation regulation is essential for the growth and development of photoreceptors and the maintenance of their functions, while abnormal methylation can lead to many pathological forms of photoreceptors. However, the role of methylation/demethylation in regulating retinal photoreceptors remains unclear. Therefore, this study aims to review the role of methylation/demethylation in regulating photoreceptors in various physiological and pathological situations and discuss the underlying mechanisms involved. Given the critical role of epigenetic regulation in gene expression and cellular differentiation, investigating the specific molecular mechanisms underlying these processes in photoreceptors may provide valuable insights into the pathogenesis of retinal diseases. Moreover, understanding these mechanisms could lead to the development of novel therapies that target the epigenetic machinery, thereby promoting the maintenance of retinal function throughout an individual's lifespan.

DOI: 10.3389/fcell.2023.1149132

The impact factor: 6.081

Paper 4

Title: UHRF2 regulates cell cycle, epigenetics and gene expression to control the timing of retinal progenitor and ganglion cell differentiation.

Abstract: Ubiquitin-like, containing PHD and RING finger domains 2 (UHRF2) regulates cell cycle and binds 5-hydroxymethylcytosine (5hmC) to promote completion of DNA demethylation. Uhrf2-/- mice are without gross phenotypic defects; however, the cell cycle and epigenetic regulatory functions of Uhrf2 during retinal tissue development are unclear. Retinal progenitor cells (RPCs) produce all retinal neurons and MÃ¼ller glia in a predictable sequence controlled by the complex interplay between extrinsic signaling, cell cycle, epigenetic changes and cell-specific transcription factor activation. In this study, we find that UHRF2 accumulates in RPCs, and its conditional deletion from mouse RPCs reduced 5hmC, altered gene expressions and disrupted retinal cell proliferation and differentiation. Retinal ganglion cells were overproduced in Uhrf2-deficient retinae at the expense of VSX2+ RPCs. Most other cell types were transiently delayed in differentiation. Expression of each member of the Tet3/Uhrf2/Tdg active demethylation pathway was reduced in Uhrf2-deficient retinae, consistent with locally reduced 5hmC in their gene bodies. This study highlights a novel role of UHRF2 in controlling the transition from RPCs to differentiated cell by regulating cell cycle, epigenetic and gene expression decisions.

DOI: 10.1242/dev.195644

The impact factor: 6.862

Paper 5

Title: DNA Methylation Dynamics During the Differentiation of Retinal Progenitor Cells Into Retinal Neurons Reveal a Role for the DNA Demethylation Pathway.

Abstract: To evaluate the contribution of the DNA methylation and DNA demethylation pathways in retinal development, we studied DNA methylation in retinal progenitor cells (RPCs) and retinal neurons using a combination of whole genome bisulfite sequencing (WGBS) data obtained in our study and WGBS data collected from previous studies. The data was analyzed using Hidden Markov Model- and change point-based methods to identify methylome states in different segments of the studied genomes following genome annotation. We found that promoters of rod and cone phototransduction genes and rod photoreceptor genes, but not genes required for the development and function of other retinal phenotypes, were highly methylated in DNA isolated from human and murine fetal retinas (which mostly contain RPCs) and postnatal murine RPCs. While these highly methylated genomic regions were inherited by non-photoreceptor phenotypes during RPC differentiation, the methylation of these promoters was significantly reduced during RPC differentiation into photoreceptors and accompanied by increased expression of these genes. Our analysis of DNA methylation during embryogenesis revealed low methylation levels in genomic regions containing photoreceptor genes at the inner cell mass stage, but a sharp increase in methylation at the epiblast stage, which remained the same later on (except for DNA demethylation in photoreceptors). Thus, our data suggest that the DNA demethylation pathway is required for photoreceptor phenotypes in the developing retina. Meanwhile, the role of the DNA methylation and DNA demethylation pathways during RPC differentiation into non-photoreceptor retinal phenotypes might be less important.

DOI: 10.3389/fnmol.2019.00182

The impact factor: 6.261

Paper 6

Title: Mbd2 Mediates Retinal Cell Apoptosis by Targeting the lncRNA Mbd2-AL1/miR-188-3p/Traf3 Axis in Ischemia/Reperfusion Injury.

Abstract: Recent studies reported that DNA methylation was involved inÂ retinal cell death. Methyl-CpG binding domain protein 2 (Mbd2) is one of the DNA methylation readers. Its role and mechanism of regulation remain unclear. The ischemia/reperfusion (I/R) model in mice primary culture retinal ganglion cells (RGCs) and Mbd2 knockout (Mbd2-KO) mice was used in the current study. We demonstrated that Mbd2 mediates RGC apoptosis caused by I/R injury. Mechanistically, the data suggested that Mbd2 upregulated Mbd2-associated long noncoding RNA 1 (Mbd2-AL1) via demethylation of its promoter. Furthermore, Mbd2-AL1 sponged microRNA (miR)-188-3p, thus preventing tumor necrosis factor (TNF) receptor-associated factor 3 (Traf3) downregulation and inducing RGC apoptosis. This was further demonstrated by the fact that inhibition of miR-188-3p diminished the anti-apoptosis role of Mbd2-AL1 small interfering RNA (siRNA). Finally, it showed that the apoptosis of retinal cells was attenuated, and the visual function was preserved in Mbd2-KO mice, which were associated with the Mbd2-AL1/miR-188-3p/Traf3 axis. Our present study revealed the role of Mbd2 in RGC apoptosis, which mayÂ provide a novel therapeutic strategy for retinal ischemic diseases.

DOI: 10.1016/j.omtn.2020.01.011

The impact factor: 10.183

Paper 7

Title: Age-Related Macular Degeneration: From Epigenetics to Therapeutic Implications.

Abstract: Aberrant regulation of epigenetic mechanisms, including the two most common types; DNA methylation and histone modification have been implicated in common chronic progressive conditions, including Alzheimer disease, cardiovascular disease, and age-related macular degeneration (AMD). All these conditions are complex, meaning that environmental factors, genetic factors, and their interactions play a role in disease pathophysiology. Although genome wide association studies (GWAS), and studies on twins demonstrate the genetic/hereditary component to these complex diseases, including AMD, this contribution is much less than 100%. Moreover, the contribution of the hereditary component decreases in the advanced, later onset forms of these chronic diseases including AMD. This underscores the need to elucidate how the genetic and environmental factors function to exert their influence on disease pathophysiology. By teasing out epigenetic mechanisms and how they exert their influence on AMD, therapeutic targets can be tailored to prevent and/or slow down disease progression. Epigenetic studies that incorporate well-characterized patient tissue samples (including affected tissues and peripheral blood), similar to those relevant to gene expression studies, along with genetic and epidemiological information, can be the first step in developing appropriate functional assays to validate findings and identify potential therapies.

DOI: 10.1007/978-3-030-66014-7_9

The impact factor: 3.65

Paper 8

Title: Reprogramming MÃ¼ller Glia to Regenerate Retinal Neurons.

Abstract: In humans, various genetic defects or age-related diseases, such as diabetic retinopathies, glaucoma, and macular degeneration, cause the death of retinal neurons and profound vision loss. One approach to treating these diseases is to utilize stem and progenitor cells to replace neurons in situ, with the expectation that new neurons will create new synaptic circuits or integrate into existing ones. Reprogramming non-neuronal cells in vivo into stem or progenitor cells is one strategy for replacing lost neurons. Zebrafish have become a valuable model for investigating cellular reprogramming and retinal regeneration. This review summarizes our current knowledge regarding spontaneous reprogramming of MÃ¼ller glia in zebrafish and compares this knowledge to research efforts directed toward reprogramming MÃ¼ller glia in mammals. Intensive research using these animal models has revealed shared molecular mechanisms that make MÃ¼ller glia attractive targets for cellular reprogramming and highlighted the potential for curing degenerative retinal diseases from intrinsic cellular sources.

DOI: 10.1146/annurev-vision-121219-081808

The impact factor: 7.745

Paper 9

Title: Effects of DNA Methylation on Gene Expression and Phenotypic Traits in Cattle: A Review.

Abstract: Gene expression in cells is determined by the epigenetic state of chromatin. Therefore, the study of epigenetic changes is very important to understand the regulatory mechanism of genes at the molecular, cellular, tissue and organ levels. DNA methylation is one of the most studied epigenetic modifications, which plays an important role in maintaining genome stability and ensuring normal growth and development. Studies have shown that methylation levels in bovine primordial germ cells, the rearrangement of methylation during embryonic development and abnormal methylation during placental development are all closely related to their reproductive processes. In addition, the application of bovine male sterility and assisted reproductive technology is also related to DNA methylation. This review introduces the principle, development of detection methods and application conditions of DNA methylation, with emphasis on the relationship between DNA methylation dynamics and bovine spermatogenesis, embryonic development, disease resistance and muscle and fat development, in order to provide theoretical basis for the application of DNA methylation in cattle breeding in the future.

DOI: 10.3390/ijms241511882

The impact factor: 6.208

Paper 10

Title: Advances in Therapeutic Targeting of Cancer Stem Cells within the Tumor Microenvironment: An Updated Review.

Abstract: Despite great strides being achieved in improving cancer patients' outcomes through better therapies and combinatorial treatment, several hurdles still remain due to therapy resistance, cancer recurrence and metastasis. Drug resistance culminating in relapse continues to be associated with fatal disease. The cancer stem cell theory posits that tumors are driven by specialized cancer cells called cancer stem cells (CSCs). CSCs are a subpopulation of cancer cells known to be resistant to therapy and cause metastasis. Whilst the debate on whether CSCs are the origins of the primary tumor rages on, CSCs have been further characterized in many cancers with data illustrating that CSCs display great abilities to self-renew, resist therapies due to enhanced epithelial to mesenchymal (EMT) properties, enhanced expression of ATP-binding cassette (ABC) membrane transporters, activation of several survival signaling pathways and increased immune evasion as well as DNA repair mechanisms. CSCs also display great heterogeneity with the consequential lack of specific CSC markers presenting a great challenge to their targeting. In this updated review we revisit CSCs within the tumor microenvironment (TME) and present novel treatment strategies targeting CSCs. These promising strategies include targeting CSCs-specific properties using small molecule inhibitors, immunotherapy, microRNA mediated inhibitors, epigenetic methods as well as targeting CSC niche-microenvironmental factors and differentiation. Lastly, we present recent clinical trials undertaken to try to turn the tide against cancer by targeting CSC-associated drug resistance and metastasis.

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The impact factor: 7.666