# THE INFLUENCE OF MARKERS IN THE DIFFERENTIATION PROCESS OF STEM CELLS INTO ENDOTHELIAL CELLS TO SUPPORT TREATMENT TESTING EXPERIMENTS

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## ABSTRACT

Research on stem cells, particularly their differentiation into endothelial cells, is highly significant in the field of biomedical science and regenerative therapy. Endothelial cells are crucial for blood vessel formation, wound healing, tissue regeneration, and the treatment of degenerative diseases. Human pluripotent stem cells can differentiate into various cell types, making them valuable for repairing or replacing damaged tissue. This study reviews the role of markers in distinguishing human stem cells into endothelial cells. A comprehensive literature search was conducted, and out of 428 screened articles, only 4 met the inclusion criteria. SOXF proteins were analyzed using scRNA-seq analysis, focusing on their role in enhancing stem cell differentiation. SOX17 was found to significantly increase the percentage of cells expressing CD34+ and Vascular Endothelial Cadherin (VEC), consistent with its known role in endoderm differentiation and endothelial cell specification. SOX17 can override pluripotency signals in human stem cells, triggering their differentiation into endothelial cells. Overexpression of SOX17 in human stem cells resulted in cells with endothelial characteristics, and combining SOX17 with FGF2 enhanced this effect, resulting in more than 90% of cells expressing endothelial stem cell markers (CD34+, VEC+, CD31+). SOXF was applied to prompt stem cell differentiation, with only SOX17 demonstrating notable effectiveness.

**Keywords : Markers; stem cells; endothelial cells**

## INTRODUCTION

Research on stem cells has become a highly interesting topic in the field of biomedicine and regenerative therapy. One very important aspect of this research is the differentiation of stem cells into endothelial cells. Endothelial cells play a crucial role in the formation of blood vessels, which is essential for wound healing, tissue regeneration, and the treatment of various degenerative diseases (Ellistasari et al., 2022).

The formation of cell type populations derived from human pluripotent stem cells is a crucial step in regenerative therapy, where these cells have the potential to differentiate into various specific cell types that can be used to repair or replace damaged tissues in the body. Stem cells, functioning as undifferentiated precursors, demonstrate extraordinary regenerative capacities. Their distribution within tissues across the human body is both diverse and specialized (Aprile et al., 2024). Historically, the differentiation of hPSCs was commonly accomplished via the creation of embryoid bodies (EBs). The blastocyst, formed after fertilization, has two types of cells: the inner cell mass (ICM) and the trophoectoderm (TE). The ICM induces fetal development and regulates the microenvironment, while the TE forms extraembryonic supportive structures like the placenta. ICM cells remain undifferentiated and have the potential to become various cell

types, including pluripotent, totipotent, multipotent, and unipotent cells. Human embryonic stem cells (hESCs) play a role in the development of the entire body and can differentiate into various cell types (Zakrzewski et al., 2019). Similar hESCs, hiPSCs have the ability to differentiate into cells forming the three germ layers. The potential of hiPSCs lies not only in modeling diseases and understanding early embryo development but also as candidates for cell replacement therapy and drug screening (Itskovitz-Eldor et al., 2000; Pettinato et al., 2014). Transcription factors (TFs) play a crucial role in directing stem cell activity, including maintaining their sustainability and directing their differentiation (Tchieu et al., 2019). The identification of heterogeneity within multiple populations that have undergone differentiation is achieved through single-cell RNA sequencing (scRNA-seq). scRNA-seq analysis has successfully identified heterogeneity within endothelial cells (ECs) and pancreatic beta cells (Paik et al., 2018; Veres et al., 2019).

The differentiation of stem cells into endothelial cells is influenced by various factors, including the molecular markers used during this process. These markers not only function to identify the stages of cell differentiation but also to guide stem cells to effectively develop into endothelial cells. A deep understanding of these markers is crucial for improving the efficiency of cell differentiation and the quality of the resulting endothelial cells.

Treatment testing experiments involving endothelial cells differentiated from stem cells require strict control and a comprehensive understanding of the biological processes involved. This research focuses on the influence of markers in the differentiation process of stem cells into endothelial cells. By understanding the impact of these markers, it is hoped that new, more effective strategies can be developed to support the development of better and safer cell-based therapies.

## MATERIALS AND METHODS

This study adopts a systematic literature review approach to investigate the influence of markers in the process of differentiating human pluripotent stem cells into endothelial cells. Detailed methodological steps are undertaken to ensure integrity and accuracy in the research process.

A research protocol is carefully developed, including defining research objectives, identifying inclusion and exclusion parameters, and devising a comprehensive literature search strategy. Literature searches are conducted through various scientific databases, including PubMed. Relevant keywords are used to retrieve articles relevant to the research topic.

Selected studies are carefully screened according to the inclusion and exclusion criteria established in the protocol. These criteria include topic relevance, study type, publication year, and methodological quality. Only studies meeting these criteria are included in the review.

From the selection of articles and subsequent data extraction, 4 articles met the inclusion criteria, whereas 424 articles did not and were excluded.

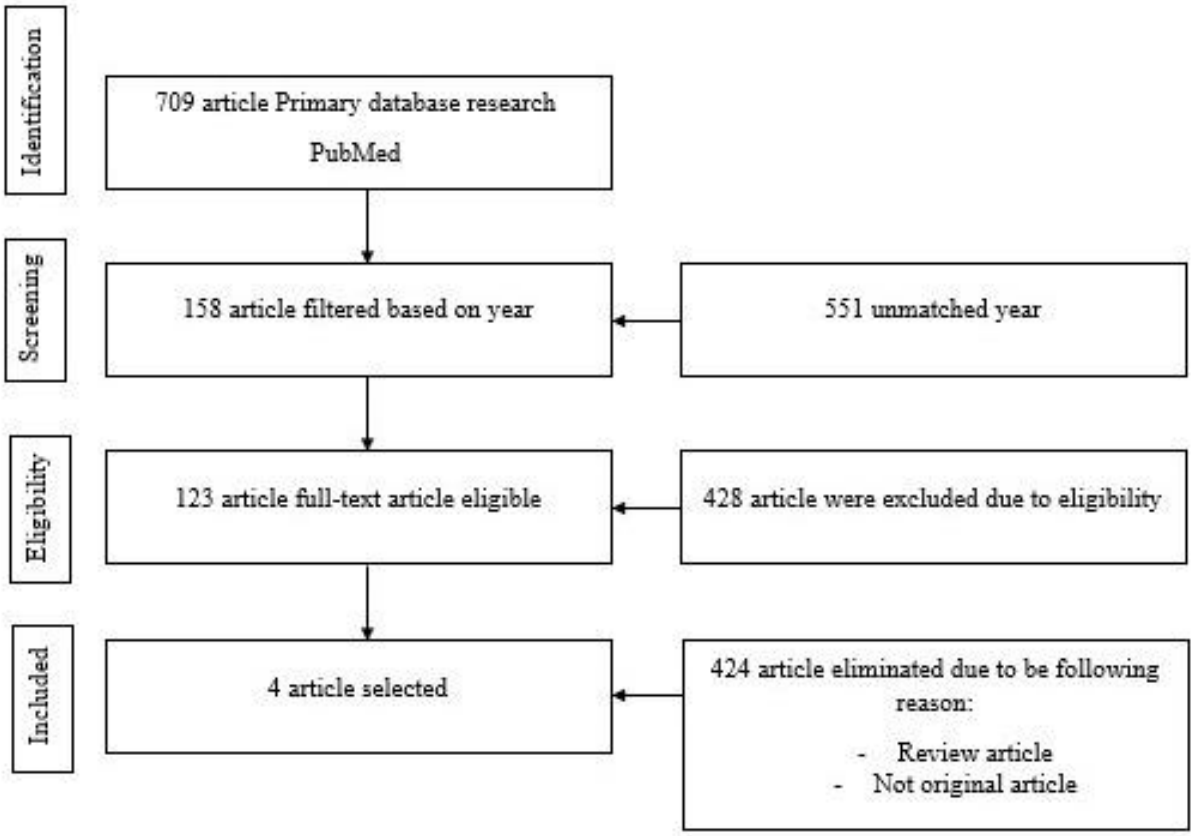


**Figure 1.** Steps of systematic literature review

## RESULT AND DISCUSSION

**Table 1.** Result of Article Review

| Refference | Result |
|---|---|
| Ream et al., 2024 | The combination of SOX17 overexpression and FGF2 treatment is highly effective in generating endothelial progenitor cells with the potential to further develop into functional endothelial cells |
| Irie et al., 2024 | The balance between SOX17 and PRDM1 is crucial for determining the fate of germ cells (cells that will develop into sperm or eggs). These two factors also play a role in regulating endoderm differentiation, the inner layer of the embryo that develops into internal organs such as the digestive tract and lungs |
| Mimura et al., 2015 | Fibroblast growth factor-2 (FGF-2) and Activin A work synergistically to regulate the initial process of differentiating human embryonic stem cells (hESCs) into neural cell pathways |
| Mossahebi-Mohammadi et al., 2010 | The FGF family comprises 22 ligands that bind to four FGF receptors (FGFRs). FGF/FGFR signaling governs a range of fundamental cellular processes, encompassing cell viability, growth, movement, specialization, embryonic growth, organ formation, tissue healing and renewal, alongside |

| Refference | Result |
|---|---|
| | metabolic functions |

## SOXF Factors and Enhanced Stem Cell Differentiation

In a recent study, scRNA-seq analysis was performed to identify several SOXF proteins. Ream and teams analyzed the SOXF factors to see if their expression could enhance stem cell differentiation. Some of the proteins analyzed included SOXF SOX7, SOX17, and SOX18. The results of the study conducted by .Ream and teams showed that only SOX17 could increase the percentage of cells that express CD34+ and Vascular Endothelial Cadherin (VEC) (Ream et al., 2024).

The research findings of Ream and colleagues align with those of a study conducted by Irie et al., 2024, where the presence of SOX17 is crucial as the SOX17 factor regulates endoderm differentiation (Irie at al., 2024).

## Mechanism of hPSC Conversion without EP with the Assistance of SOX17 Expression

SOX17 plays a crucial role in regulating the specification (determination of identity) of endothelial cell lines (cells that form the inner layer of blood vessels) as well as in the process of endothelial cell regeneration. This indicates that SOX17 has a significant influence in controlling how endothelial cells develop and in the process of endothelial tissue healing or regeneration (Trinh et al., 2023).

The use of the positive control line XLone ETV2 in the context of programming human pluripotent stem cells (hPSCs). ETV2 can facilitate the programming of endothelial progenitor (EP) from hPSCs (Wang et al., 2020).

The advanced programming ability possessed by SOX17 can override signals supporting the maintenance of pluripotent status (the ability to develop into various cell types) in human pluripotent stem cell (hPSC) culture media. This leads to their differentiation, characterized by changes in their morphology (shape and structure) indicating the loss of pluripotent characteristics. In other words, SOX17 expression can trigger the differentiation process of hPSCs from a pluripotent state into more specific forms (Ream et al., 2024).

## The Combination of SOX17 and FGF2 Enhances the Production of CD34+ VEC+ and CD31+ Cells, Supporting Endothelial Progenitor (EP) Differentiation

When SOX17 is overexpressed, it is sufficient to produce cells expressing the CD34+ and VEC+ markers (indicating endothelial cell properties) in those hPSCs. However, when FGF2 is introduced during the programming process mediated by SOX17, it results in a more specific cell generation, with over 90% of these cells expressing the CD34+ VEC+ CD31+ marker, indicating that they are endothelial progenitor cells (EPs). This suggests that the combination of SOX17 overexpression and concurrent administration of FGF2 can yield a stronger effect in directing the differentiation of hPSCs into endothelial cells (Ream et al., 2024). In line with previous studies demonstrating that FGF2 also regulates stem cell differentiation (Mimura et al., 2015).

FGF influences hematopoietic stem cells, nerve cells, spermatogonia, prostate cells, and mesenchymal stem cells derived from bone marrow (Huang et al., 2015; Tian et al., 2019; Mossahebi-Mohammadi et al., 2020). Several members of the FGF family, such as FGF2, FGF4, FGF6, FGF7, FGF8, and FGF9, have

been reported to have significant impacts on pluripotent stem cells (PSCs). This indicates that FGF can play a crucial role in regulating the differentiation pathways and growth of pluripotent stem cells into various specific cell types in the body. Fibroblast growth factor 2 (FGF2) plays a crucial role in maintaining the pluripotent state (the ability to develop into various cell types) of induced human pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) (Mossahebi-Mohammadi et al., 2020).

## CD34+, VEC+ and CD31+ as markers of progenitor stem cell differentiation

The vascular endothelium, which is the inner layer of blood vessels, is activated by treating pluripotent stem cells that have been programmed to become mesoderm cells using VEGF-A (vascular endothelial growth factor) and forskolin (a substance that stimulates protein kinase A activity). Subsequently, these vascular endothelial cells are identified using the CD144 protein. These cells also exhibit positivity for markers such as SOX17 and several endothelial markers like CD31, CD34, von Willebrand factor (vWF), and PECAM1. In other words, these cells demonstrate characteristics of vascular endothelial cells after induction and identification (Kennedy et al., 2021).

The vascular endothelial cells are then selected or identified using CD144 protein as a marker. The protein CD34, identified almost four decades ago, serves as a biomarker for hematopoietic stem cell precursors. CD34 plays a crucial role in various cellular aspects, including growth regulation, cell differentiation, adhesion, and morphogenesis (Radu et al., 2023). The expression of CD34 is observed in various types of cells, including hematopoietic stem/progenitor cells, multipotent stromal cells (MSCs), muscle stem cells, interstitial cells, fibrocytes, and endothelial stem cells (Baumheter et al., 1993; Nielsen & McNagny, 2008; Radu et al., 2023). The CD34 protein has the ability to enhance the growth of progenitor cells, which are immature cells with the potential to develop into various specialized cell types in the body (Sidney et al., 2014; Radu et al., 2023).

## SUMMARY

Markers used to observe the presence of stem cell differentiation activity include CD34+, VEC+, and CD31+. These three markers were observed through scRNA-seq analysis. SOXF was administered to stimulate stem cell differentiation. The stem cells were treated with SOXF, and only SOX17 was proven to produce significant results. SOX17 has an important role in supporting endoderm differentiation. The administration of SOX17 combined with FGF2 was shown to enhance stem cell differentiation activity.

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