REGULATORY ROLE OF ETV4 IN EMBRYONIC STEM CELL FATE: INSIGHTS INTO MECHANOTRANSDUCTION AND LINEAGE DETERMINATION

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ABSTRACT

Conventional cell biology studies focus on cellular responses to chemical signals, but cells also react to mechanical cues like density, size, and substrate rigidity, activating specific gene expression. Embryo development leads to the formation of a gastrula, establishing body structure and germ layers (endoderm, ectoderm, mesoderm) via diverse mechanisms. In humans, gastrulation begins with the Primitive Streak (PS) and T gene expression, guiding epiblast cell migration. Self-regulation occurs in gastruloid models, derived from human embryonic stem cells, capable of differentiation. Mediators like YAP/TAZ and PIEZO1 link density to cellular responses, with ETV4 serving as a link between mechanical environment and gene expression. This research employed a systematic literature review to synthesize relevant studies. Inspired by stem cell advancements, particularly ETV4's role, searches on PubMed yielded three articles meeting inclusion criteria. ES cells maintain undifferentiated states via ETV4 and ETV5. Rapid cell growth deactivates ETV4, prompting differentiation, influenced by mechanical cues. ETV4, ETV5, and SPRY4 regulate the FGF/ERK pathway, modulating sensitivity. High density initiates neuroectodermal cell formation, impacting integrin-actomyosin and FGFR pathways, via ETV4. Fluctuations in density dictate lineage fate, with ETV4 as a key sensor, linking density shifts to lineage determination via the ERK pathway.

Keywords : ETV4; embryonic stem cell; mechanotransduction; lineage determination

INTRODUCTION

Numerous conventional studies in cell biology aim to comprehend cells reactions to chemical cues, such as diffusing signaling molecules. Nonetheless, cells also react to mechanical triggers like cell density, size, and substrate rigidity by inducing the expression of particular genes. The early stage of embryo development from a single-layered blastula that reorganizes into a structure with multiple layers, known as the gastrula, is initiated by the pre-gastrulation phase with evolutionary conservation of morphogenetic prerequisites in the embryo during the gastrulation process (Sheng, 2015). The fundamental body structure of mammalian embryos is established during gastrulation, a critical early phase that follows implantation. During this stage, the three primary layers (endoderm, ectoderm, and mesoderm) are determined through diverse cellular and spatial mechanisms (Zhai et al., 2022). In human embryo development, gastrulation commences with the emergence of the Primitive Streak (PS) in the posterior epiblast area. This PS is distinguished by the conversion of gastrulating cells into epithelial-mesenchymal cells expressing the T gene (brachyury). The PS becomes apparent during stages CS7–CS9 and serves to establish the body's midline while governing the convergent migration of epiblast cells. The presence of both the PS and the T gene signifies an asymmetrical epiblast arrangement along the anteroposterior axis (de Bree et al., 2018; Ghimire et al., 2021).

Self-regulation is a cellular process in which complex structures form spontaneously without any pre-established patterns (Sasai, 2013; Zhu & Zernicka-Goetz, 2020). Gastruloid models derived from human embryo stem cells are capable of differentiation and forming layered structures with identified patterns, where the positions of ectoderm and mesoderm have been determined (Warmflash et al., 2014). YAP/TAZ and PIEZO1 are key mediators linking cell density to cellular responses. During embryonic development, variations in mechanical stimuli drive the identification of additional factors that translate mechanical signals into gene expression (Aragona et al., 2013; Gudipaty et al., 2017).

The transcription factor ETS 4 (ETV4), known as an oncogene, acts as a molecular link between the micro-mechanical environment and gene expression. This research will discuss the crucial role of ETV4 in stem cell differentiation, particularly in the context of human embryonic stem cells.

MATERIALS AND METHODS

This research was conducted using a systematic literature review approach to collect, review, and synthesize relevant studies published within a specific period. Identification of articles relevant to the research topic was done based on scholarly databases such as PubMed, Google Scholar, and Scopus. Article selection was based on predetermined inclusion and exclusion criteria to ensure the quality and relevance of the studies included in the review. Subsequently, the key findings of previous studies were analyzed to explore trends, differences, and similarities in existing research and to provide a comprehensive understanding of the topic under discussion.

This study draws inspiration from recent advancements in stem cell research, particularly focusing on the involvement of ETV4. The author utilized Pubmed to search for articles using keywords such as “ETV4 and stem cells”, “ETV4 role in stem cell”, and “ETV4 and stem cells differentiation”. During the article collection phase, inclusion criteria were established to select relevant sources based on publication year, title, abstract, full text, Scopus ranking, and accessibility.

Based on the selection of articles and data extraction, a total of 3 articles met the inclusion criteria, while 22 articles did not meet the inclusion criteria and were excluded.

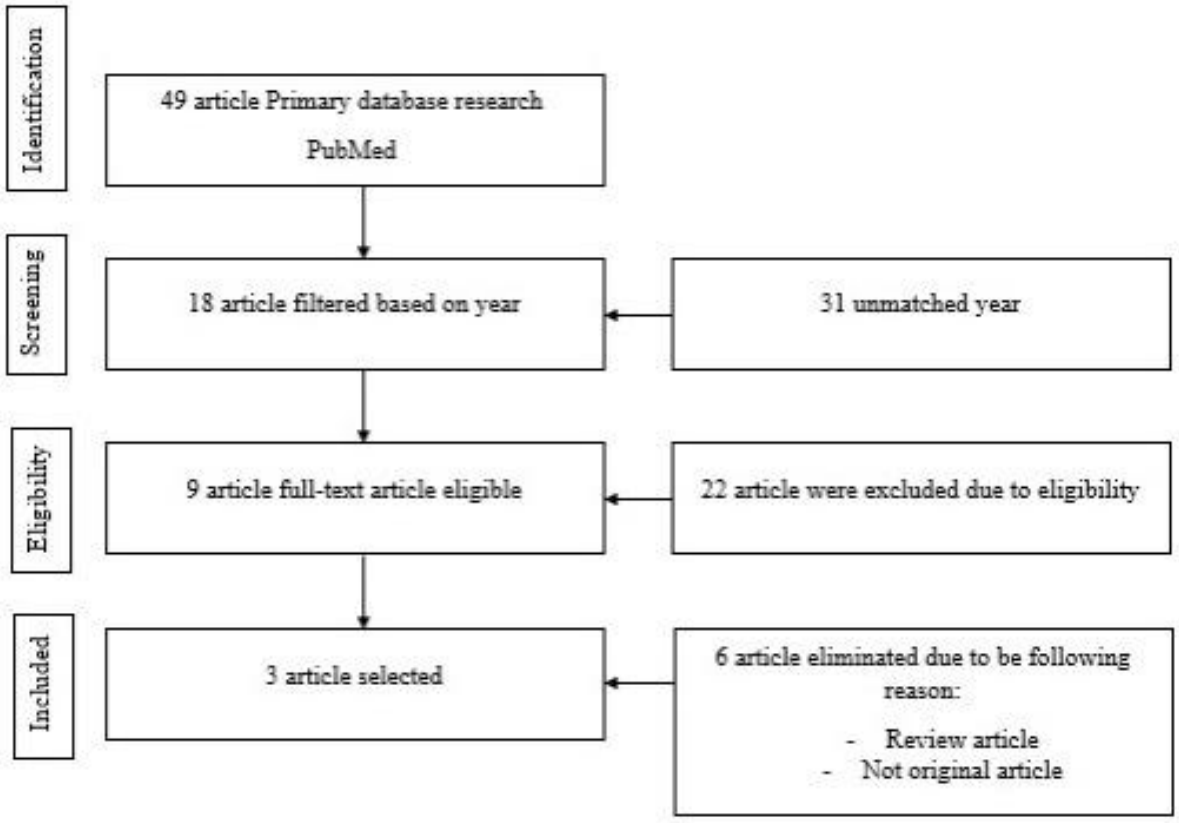

Figure 1. A graphical representation illustrating the process of conducting a literature search

RESULT AND DISCUSSION

Table 1. Several research on cell density with cellular responses and gene expression

Refference	Cell Type	Result
Akagi et al., 2015	Embryonic stem cell	ETV4 and -5 crucially determine the properties of ES cells. The results of this study conclude that the genes ETV4 and ETV5 play a role in maintaining the undifferentiated state of ES cells
Yang et al., 2024	Human embryonic stem cell	ETV4 acts as a link between mechanical conditions in the microenvironment of human embryonic stem cells and changes in gene expression, ultimately affecting the development of these cells into different cell types in the future.

Refference	Cell Type	Result
Azami et al., 2019	Embryonic stem cell	Activation of the ERK signaling pathway plays a crucial role in directing cell mass differentiation within the inner cell mass (ICM) during the preimplantation development stage in mice. ETV4 and ETV5, along with SPRY4, have a crucial role in controlling the activity of the FGF/ERK pathway during embryo development. They employ a negative feedback mechanism to regulate cell sensitivity to FGF signals, thus playing a significant role in orchestrating cell differentiation during embryonic development.

Embryonic stem cells (ES) have the primary ability to self-renew and proliferate in an undifferentiated state. The genes ETV4 and ETV5 play roles in maintaining the undifferentiated state of ES cells. This was demonstrated by research conducted by Akagi and his team in 2015, which showed that the alkaline phosphatase marker, which serves as a stem cell marker, indicated no differentiation of stem cells. Akagi and his team's research concluded that the activity of alkaline phosphatase as a marker of stem cell differentiation is comparable to the activity of ETV4 cells, which can maintain the undifferentiated state (Akagi et al., 2015).

Recent research shows that rapid cell growth will support cell density, which can deactivate ETV4 activity. The absence of ETV4 activity encourages the cells to differentiate. ETV4 is a protein that functions as a transcription factor, helping to regulate gene expression. As a molecular transducer, ETV4 links signals from the mechanical environment surrounding the cell (such as pressure or density) to changes in gene expression. In the context of the epithelium (cell layer) of human embryonic stem cells, changes in cell density affect how much ETV4 is expressed. This cell density-regulated expression of ETV4 provides an initial signal that determines how these cells will develop into various cell types in the future (lineage fate) (Yang et al., 2024).

ETV4 and ETV5, along with SPRY4, play crucial roles in regulating the activity of the FGF/ERK pathway during embryonic development, using a negative feedback mechanism to control cell sensitivity to FGF signals. ETS-related molecules such as ETV4 and ETV5 are activated by the FGF (Fibroblast Growth Factor) pathway in various developmental processes and tissues. FGF/ERK signals to Etv4 and Spry4, and there is a signal response to FGF/ERK from ETV4 and SPRY4 (Aulehla et al., 2008; Morgani et al., 2018; Azami et al., 2019).

High cell density can inactivate (switch off) ETV4, allowing these stem cells to begin differentiating into neuroectodermal cells (cells that will become part of the nervous system). Cell density affects certain signaling pathways within the cell, such as the integrin–actomyosin pathway, which is crucial for maintaining cell structure integrity. Additionally, high cell density inhibits the endocytosis (absorption) of FGFRs receptors, which are important for receiving growth signals from the environment. When FGFR endocytosis is disrupted, it leads to decreased stability of the ETV4 protein. The ERK (extracellular signal-regulated kinase) pathway usually helps stabilize ETV4, so the inactivation of ERK due to disrupted FGFR

endocytosis reduces ETV4 stability. Changes in cell density influence how and when (spatiotemporally) stem cells will develop into specific cell types. ETV4 acts as a key mechanotransducer that translates these cell density changes into signals for cell lineage specification (Yang et al., 2024).

SUMMARY

Embryonic stem cells maintain their undifferentiated state through self-renewal, which is regulated by the ETV4 and ETV5 genes, as confirmed by alkaline phosphatase activity. When cell density increases due to rapid cell growth, ETV4 becomes deactivated, leading to differentiation. ETV4 is responsive to mechanical signals such as pressure and density, impacting gene expression. Variations in cell density influence ETV4 levels in epithelial cells, thereby determining lineage fate. Moreover, ETV4, ETV5, and SPRY4 collectively regulate the FGF/ERK pathway, thereby modulating sensitivity. Elevated cell density inhibits ETV4, initiating neuroectodermal cell formation and affecting integrin-actomyosin and FGFR pathways. Additionally, disrupted FGFR endocytosis reduces ETV4 stability via the ERK pathway, establishing a connection between changes in cell density and lineage determination, with ETV4 serving as a key sensor.

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