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A Review of Molecular Markers of Mature Odontoblasts and Their Role in Dentin Repair and Regeneration Research

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The terminal differentiation of odontoblasts is characterized by specific molecular markers that reflect their functional maturity. This review explores both canonical markers, such as Dentin Sialophosphoprotein (DSPP), Dentin Matrix Protein 1 (DMP1), Nestin, and Alkaline Phosphatase (ALP), and emerging markers like MAP1B, MAP Tau, and β-catenin. These markers offer valuable insights into the regulation of odontoblast differentiation and the maintenance of their polarized, dentin-secreting phenotype. The review further discusses the experimental applications of these markers in in vitro studies, dental tissue engineering, regenerative endodontics, and drug discovery. Canonical markers are utilized to confirm the maturity of odontoblasts and evaluate bioengineered tissues, while emerging markers reveal potential new targets for enhancing dentin repair and regeneration. Additionally, the role of signaling pathways, including Wnt5a, BMP, and integrin-mediated pathways, in supporting the structural and functional characteristics of mature odontoblasts is discussed. By consolidating current knowledge on these markers and pathways, this review aims to advance the understanding of odontoblast biology and contribute to the development of innovative strategies for dental tissue engineering and regenerative therapies. [J Korean Dent Sci. 2024;17(4):163-73]

Key Words: Odontoblast; Differentiation; Cell Polarity; Mineralization; Dentin

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Introduction

Odontoblasts are highly specialized cells that play a central role in the formation of dentin, the mineralized tissue underlying enamel. This hard tissue layer not only provides structural support for the tooth but also serves as a vital protective barrier for the sensitive dental pulp. Odontoblasts originate from neural crest-derived mesenchymal cells and undergo a multi-stage differentiation process. The differentiation culminates in a terminally differentiated state characterized by distinct cellular polarity, elongation, and organized cytoskeletal structures, enabling odontoblasts to secrete and mineralize the dentin matrix throughout the lifespan of the tooth (Fig. 1). These unique structural and functional adaptations are essential for the organized deposition of dentin, contributing to tooth durability, function, and integrity[1]. The process of odontoblast differentiation is regulated by a series of tightly controlled molecular and cellular events that transform progenitor cells into fully mature, dentin-producing cells. The terminal differentiation involves significant changes in gene expression, cytoskeletal organization, and cellular architecture, equipping odontoblasts with the necessary machinery to execute their specialized functions at the dentin-pulp interface[2]. Understanding the mechanisms underlying these changes is crucial, as the ability of odontoblasts to form and repair dentin directly impacts the tooth's capacity to withstand physiological wear and respond to injury.

In recent years, advances in molecular biology and dental research have enabled the identification of markers associated with the terminally differentiated state of odontoblasts[3]. These markers serve as indica-

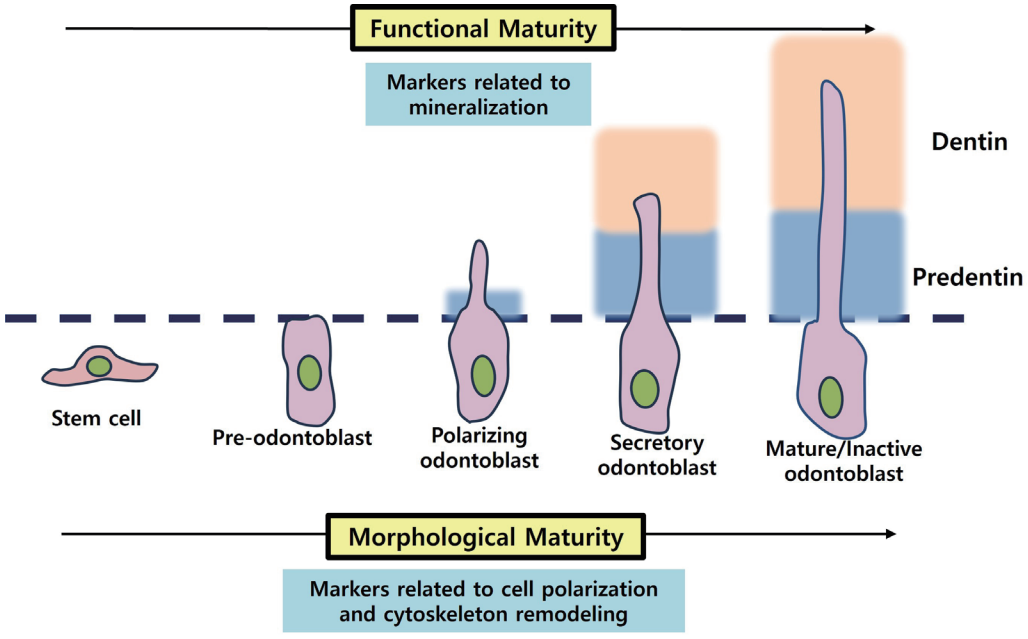

Fig. 1. The schematic illustrates the morphological and functional maturation of odontoblasts during dentinogenesis. The differentiation process begins with stem cells, progresses through pre-odontoblast and polarizing odontoblast stages, and culminates in secretory and mature/inactive odontoblasts. Polarizing odontoblasts develop distinct cellular polarity, aligning their structures for directed dentin matrix secretion. This stage involves markers associated with cell polarization and cytoskeletal remodeling. Secretory odontoblasts actively deposit the dentin matrix (predentin and mineralized dentin), guided by markers of mineralization. Mature/inactive odontoblasts maintain structural integrity and support the dentin-pulp interface.

tors of functional maturity, reflecting the distinct cellular and molecular characteristics that define mature odontoblasts. However, many markers traditionally associated with odontoblasts, such as DSPP, DMP1, and ALP, are also expressed in osteoblasts, which complicates their use in definitively distinguishing dentin from bone. This overlap highlights the need for further research to identify more specific markers or combinations of markers that can reliably differentiate odontoblast activity from that of osteoblasts. Identifying such markers has significant implications for research and clinical applications, as they allow scientists and clinicians to distinguish fully differentiated odontoblasts from precursor or less mature cell stages. This distinction is essential in fields such as developmental biology, where understanding the sequential steps of odontoblast maturation provides insight into tooth development, and regenerative dentistry, where restoring or enhancing dentin production is a primary objective. In experimental and clinical contexts, markers of terminal odontoblast differentiation are invaluable for evaluating the effectiveness of therapeutic interventions aimed at promoting dentin repair and regeneration. For instance, in tissue engineering, these markers help determine whether cells seeded on scaffolds or in bioengineered tissues have reached a level of differentiation capable of producing dentin in a manner consistent with natural odontoblasts. Additionally, understanding the regulatory pathways that govern the expression of these markers may lead to novel strategies for inducing odontoblast-like activity in stem cells or for preserving the functionality of existing odontoblasts in aging or damaged teeth.

This review will explore the molecular markers associated with terminal odontoblast differentiation, examining their roles in defining odontoblast maturity and their applications in experimental and clinical dentistry. By clarifying the cellular and molecular features that characterize mature odontoblasts, this article aims to contribute to the ongoing efforts in dental research and therapy to improve approaches for dentin regeneration, tooth preservation, and overall dental health.

Canonical Markers of Terminal Odontoblast Differentiation

Terminal differentiation in odontoblasts, the final stage of their maturation, is characterized by the expression of specific molecular markers that define their functional and structural identity. These markers reflect the odontoblast's commitment to dentinogenesis and are essential for proper dentin formation and mineralization. Canonical markers of terminal odontoblast differentiation, including Dentin Sialophosphoprotein (DSPP), Dentin Matrix Protein 1 (DMP1), Nestin, and Alkaline Phosphatase (ALP), play distinct roles in both odontoblast identity and function. This section will explore the significance of each marker in the context of terminal differentiation, explaining their roles in dentin matrix formation, structural organization, and mineralization.

Dentin Sialophosphoprotein (DSPP)

One of the most widely studied markers of terminal odontoblast differentiation, DSPP, is crucial for dentin matrix secretion and mineralization[4]. DSPP is a precursor protein that undergoes proteolytic cleavage to produce two major fragments, Dentin Sialoprotein (DSP) and Dentin Phosphoprotein (DPP), both of which contribute to the structural integrity and mineralization of dentin[5]. DSP has been implicated in the regulation of mineralization initiation, whereas DPP, a highly phosphorylated protein, binds calcium ions and aids in the nucleation of hydroxyapatite crystals within the dentin matrix. The high expression of DSPP in mature odontoblasts underlines its importance in dentinogenesis, as cells lacking DSPP exhibit disrupted dentin structure, reduced mineralization, and altered dentinal tubule formation[6].

Research indicates that DSPP expression is upregulated during the final stages of odontoblast differentiation, marking cells that have fully committed to dentin

formation[7]. Due to its critical function and specific expression in terminally differentiated odontoblasts, DSPP is frequently used in both *in vitro* and *in vivo* studies to confirm the odontoblast-like phenotype of cells, especially in dental tissue engineering and regenerative studies. The specific role of DSPP in promoting mineralization and maintaining the structural organization of dentin makes it a core marker for identifying mature odontoblasts.

Dentin Matrix Protein 1 (DMP1)

DMP1 is a critical protein in the mineralization of dentin and is expressed during the early stages of odontoblast differentiation, with its expression peaking in newly differentiated odontoblasts. Initially synthesized as a precursor, DMP1 undergoes proteolytic cleavage into functional fragments that play vital roles in the regulation of dentin and bone mineralization. Unlike other proteins that remain consistently expressed throughout odontoblast life, DMP1's expression is temporally regulated, with downregulation observed as odontoblasts become fully mature and highly differentiated[8,9].

In odontoblasts, DMP1 localizes primarily at the mineralization front, particularly within the predentin and initial dentin layers, where it plays a pivotal role in hydroxyapatite crystal formation. By interacting with calcium and phosphate ions, DMP1 facilitates the nucleation and growth of hydroxyapatite, contributing to the mineralized matrix's structural integrity[10,11]. Its crucial role in early dentin mineralization underscores its involvement in establishing the mechanical properties of dentin, such as rigidity and strength. Studies on DMP1-deficient models reveal significant defects in dentinogenesis, including widened predentin zones, poor mineralization, reduced dentin thickness, and increased porosity[12]. These findings highlight the indispensable role of DMP1 in regulating the transition from predentin to dentin, ensuring proper mineral deposition and structural organization. Although its expression diminishes in highly mature odontoblasts, its earlier high-level expression serves as a reliable marker for assessing the differentiation status of odontoblasts, especially in experimental and therapeutic contexts focused on dentin repair and regeneration.

In regenerative dentistry, the role of DMP1 as a regulator of mineralization and matrix organization makes it a key target for studies aiming to enhance dentin repair. Understanding its precise functions and expression dynamics can help optimize strategies for inducing odontoblast-like activity in stem cells and for developing therapies to improve dentin-pulp complex regeneration.

Nestin

Nestin is an intermediate filament protein prominently expressed during the differentiation of odontoblasts, marking its importance as a structural marker[13]. Unlike DSPP and DMP1, which are directly involved in matrix production and mineralization, Nestin primarily supports the cytoskeletal organization necessary for odontoblast polarity and function. Its expression begins at the bell stage of tooth development, particularly in odontoblasts and pulp fibroblasts in the cusp region, and is essential for the formation of the dentin matrix[14]. In mature odontoblasts, Nestin is localized in the cellular processes extending into dentinal tubules, facilitating the directed secretion of dentin matrix proteins essential for tubular dentin formation. This structural role is critical for maintaining the polarized architecture of odontoblasts, a hallmark of their terminal differentiation. Interestingly, while Nestin expression decreases in aging teeth, it is upregulated in response to injuries, such as caries or dental procedures, suggesting its involvement in tissue repair and regeneration[14]. Studies have highlighted that the regulation of Nestin in odontoblasts may involve different enhancers than those in neural tissues, pointing to a unique regulatory mechanism in dental cells[15]. Furthermore, BMP4 has been identified as a regulator of Nestin expression, linking this intermediate filament to signaling pathways that promote odontoblast dif-

ferentiation and response to injury. Given its dynamic expression and functional relevance, Nestin remains a valuable marker for studying the structural aspects of odontoblast maturation and their response to dental tissue damage, providing insights into the organization of the dentin-pulp interface and the mechanisms underlying dentin repair.

Alkaline Phosphatase (ALP)

Alkaline Phosphatase (ALP) is widely recognized as a general marker for mineralization in various cell types, including osteoblasts and odontoblasts[16]. In odontoblasts, ALP is associated with the mineralization phase of dentinogenesis and is highly expressed in the later stages of differentiation. Its primary function is to hydrolyze phosphate esters, releasing inorganic phosphate that contributes to hydroxyapatite crystal formation within the dentin matrix. Its activity is therefore crucial for the mineralization of dentin, ensuring that mature odontoblasts can produce the highly mineralized tissue characteristic of functional dentin[17,18].

In dental research, ALP is often used as an early indicator of mineralization potential in odontoblasts and odontoblast-like cells. Elevated ALP activity reflects cellular activation towards the mineralization phase, making it a suitable marker for studies that focus on dentin repair or regeneration. Such a consistent association with the mineralization phase of odontoblast differentiation further establishes its relevance as a marker for mature odontoblasts, particularly when evaluating dentin-producing capabilities in tissue-engineered models.

Emerging Markers and Molecular Pathways

In addition to established markers, recent research has identified several emerging markers and molecular pathways that contribute to our understanding of terminal odontoblast differentiation. These emerging markers, such as MAP1B, MAP Tau, and β-catenin, are primarily involved in maintaining the cytoskeletal organization and polarity essential for odontoblast functionality. Along with associated signaling pathways, they highlight the complexity of odontoblast maturation and reveal potential targets for dental tissue engineering and regenerative therapies.

Microtubule-associated proteins (MAP) 1B and Tau

Microtubule-associated proteins, such as MAP1B and MAP Tau, have been recognized for their roles in neuronal cell differentiation. MAP1B is a critical microtubule-associated protein primarily known for its role in stabilizing and organizing microtubules in the cytoskeleton. It is highly expressed during neuronal development, where it facilitates axonal growth and guidance by regulating microtubule dynamics. In non-neuronal contexts, such as odontoblast differentiation, it supports cytoskeletal integrity, which is essential for cellular elongation, polarization, and the maintenance of odontoblastic processes extending into dentinal tubules. By supporting cytoskeletal stability within the odontoblastic process, MAP1B enables the polarized structure that is essential for the directed secretion of dentin matrix[19]. Its role in cytoskeletal organization makes it particularly relevant for odontoblasts, as the elongation of the odontoblastic process requires microtubule integrity. The presence of MAP1B in odontoblasts suggests its utility as an emerging marker for identifying terminally differentiated odontoblasts with functional dentin-producing capabilities.

MAP Tau, another protein typically associated with neural tissues, has been identified in odontoblasts, where it appears to stabilize microtubules within the cell processes[20]. It binds to tubulin dimers, enhancing microtubule polymerization and preventing depolymerization. This stabilization is critical for the structural support of mature odontoblasts, which rely on a well-organized cytoskeleton for the sustained secretion of dentin components. The expression of MAP Tau in odontoblasts nearing full differentiation highlights

its potential as an emerging marker for mature odontoblasts, particularly in studies focused on cytoskeletal dynamics and cell process formation.

β-Catenin and the Wnt Signaling Pathway

β-catenin, a multifunctional protein involved in cell adhesion and transcriptional regulation, plays a significant role in odontoblast differentiation through the Wnt signaling pathway[21]. The Wnt/β-catenin pathway is essential for cellular polarization and cytoskeletal arrangement in many cell types, and in odontoblasts, it regulates key processes related to differentiation and polarity. When activated, β-catenin translocates to the nucleus, where it influences the expression of genes involved in cellular orientation and cytoskeletal organization. This regulatory function is critical in odontoblasts as they establish the apico-basal polarity necessary for functional dentin secretion. High β-catenin expression in mature odontoblasts further supports its potential as a differentiation marker and highlights its role in promoting the structural and functional characteristics of terminal odontoblasts[22].

Intraflagellar Transport Protein 80 (IFT80)

The primary cilium, a cellular organelle associated with sensory and signaling functions, has recently been shown to influence odontoblast differentiation. IFT80, a constituent protein of the intraflagellar transport (IFT) complex, is essential for maintaining odontoblast polarity and process elongation[23]. Studies indicate that IFT80 deletion disrupts the spatial organization of odontoblasts, leading to impaired dentin formation. As a component of the primary cilium, it supports the orientation and signaling processes needed for mature odontoblast function. Its involvement in ciliary function suggests that IFT80 is an emerging marker of terminal differentiation, especially for research focused on cellular orientation and polarity in dentin formation.

CRMP1 and Cytoskeletal Stability

Collapsin Response Mediator Proteins (CRMPs) are a family of cytosolic phosphoproteins initially identified for their role in axon guidance and neuronal differentiation. They modulate cytoskeletal dynamics by interacting with microtubules and actin filaments, influencing cellular shape and motility. Initially studied in the context of neuronal development, CRMP1 has been identified in odontoblasts, where it may support cellular elongation and the structural arrangement of dentin-secreting processes[24]. The presence of CRMP1 in odontoblasts at advanced differentiation stages suggests its role in maintaining cytoskeletal stability, essential for organized dentin production. The function of CRMP1 as a cytoskeletal regulator makes it a promising marker for identifying terminally differentiated odontoblasts and offers potential insight into maintaining odontoblast structure in regenerative therapies.

Summary of Emerging Markers and Their Applications

These emerging markers–MAP1B, MAP Tau, β-catenin, IFT80, and CRMP1–along with their associated signaling pathways, offer valuable insights into the cellular mechanisms that define terminal odontoblast differentiation. Each of these markers contributes to the unique structural and functional characteristics of mature odontoblasts, from cytoskeletal organization to cellular polarity and matrix secretion. Incorporating them into experimental models and regenerative approaches could enhance our ability to restore functional dentin, supporting the development of novel therapies aimed at improving dental health and repair.

Additional Signaling Molecules and Pathways Supporting Terminal Differentiation

The maturation of odontoblasts into their terminally differentiated state is a complex, tightly regulated

process requiring multiple signaling pathways and molecular players. Beyond the canonical pathways, additional signaling molecules, such as Wnt5a and key transcription factors, are integral to supporting odontoblast polarity, cytoskeletal organization, and mineralization. Together, these signaling pathways ensure that odontoblasts maintain the structural and functional characteristics necessary for continuous dentin formation and repair.

Wnt5a Pathway

The Wnt5a signaling pathway, a non-canonical Wnt pathway, plays a pivotal role in regulating cytoskeletal organization and reinforcing odontoblast polarity[25]. Wnt5a acts through downstream effectors like Cdc42, a small GTPase that influences cytoskeletal dynamics essential for process elongation and cellular polarization. This pathway supports the structural framework of odontoblasts, allowing them to orient their processes effectively along the dentin-pulp interface, a hallmark feature of mature, terminally differentiated odontoblasts. Research has shown that Wnt5a activity enhances cytoskeletal stability, which is essential for maintaining odontoblast morphology and function in the context of dentin matrix production and secretion.

Runx2 and Osterix (Sp7)

Runx2 and Osterix (Sp7) are transcription factors that play essential roles in odontoblast differentiation, though their roles are distinct at different stages of maturation[26]. Runx2 is primarily active in the early stages of odontoblast differentiation, where it initiates the commitment of dental pulp progenitors to the odontoblastic lineage. As differentiation progresses, Osterix (Sp7) becomes more prominent, supporting cell maturation and expression of dentin-specific proteins[27]. Interestingly, downregulation of both Runx2 and Osterix is crucial as odontoblasts approach terminal differentiation, as their persistent expression may inhibit the formation of fully mature odontoblasts[28]. This downregulation aligns with the need for odontoblasts to transition into a stable, mineralization-ready state, emphasizing the importance of these transcription factors in controlling the timeline and quality of odontoblast maturation.

Bone Morphogenetic Protein (BMP) Pathway

The BMP signaling pathway, particularly through BMP2 and BMP4, is well-known for promoting differentiation in various cell types, including odontoblasts[29,30]. BMPs interact with receptors on odontoblasts to activate SMAD proteins, which then translocate to the nucleus to regulate gene expression involved in cellular maturation and mineralization[31,32]. In mature odontoblasts, BMP signaling is critical for the production of dentin matrix proteins and the initiation of mineral deposition, which are hallmarks of terminally differentiated odontoblasts. BMP signaling supports the cellular changes needed for dentin formation, making it an important pathway for maintaining odontoblast functionality.

Fibroblast Growth Factor (FGF) Pathway

The FGF pathway, especially involving FGF2 and FGF8, plays a regulatory role in both early and late stages of odontoblast differentiation. FGFs bind to specific receptors on odontoblasts, activating pathways such as MAPK/ERK, which contribute to cell proliferation and cytoskeletal organization as cells mature[33-35]. In terminally differentiated odontoblasts, FGF signaling supports cell polarity and the formation of a stable cytoskeleton that sustains dentin matrix deposition. By promoting these structural adaptations, FGF signaling enhances the longevity and reparative potential of mature odontoblasts.

Integrin-Mediated Pathways

Integrins are transmembrane receptors that play a significant role in odontoblast adhesion to the extracellular matrix, promoting cellular polarity and stability[36]. By interacting with matrix components like collagen, integrins support the mechanical anchorage necessary

for maintaining odontoblast alignment along the dentin-pulp interface. This interaction not only stabilizes the elongated odontoblastic process but also reinforces cellular organization, supporting the integrity of mature odontoblasts and enabling sustained dentin production.

Experimental Applications of Terminal Differentiation Markers

The identification and application of markers specific to terminally differentiated odontoblasts have advanced research in dental development, tissue engineering, and regenerative medicine. These markers serve as reliable indicators of cell maturity, making them essential tools in experimental studies aimed at understanding odontoblast behavior and enhancing dental repair. Through these markers, researchers can distinguish mature odontoblasts from precursor cells, assess the efficacy of differentiation protocols, and evaluate the potential of stem cells for dentin regeneration. This section explores key experimental applications of these markers across *in vitro* studies, dental tissue engineering, and regenerative therapies.

In Vitro Studies on Odontoblast Differentiation

In vitro studies on odontoblast differentiation rely on specific markers to confirm the maturation of cells cultured under experimental conditions. By analyzing the expression of established odontoblast markers, such as DSPP, DMP1, and ALP, researchers can monitor the progression of progenitor cells toward terminal differentiation. These markers help validate the success of differentiation protocols, particularly in studies that employ growth factors or other stimulatory agents to induce odontoblastic phenotypes in dental pulp stem cells or other progenitor cells. For example, DSPP and DMP1 expression levels are commonly assessed to verify that cells cultured *in vitro* have achieved a dentin-producing phenotype. ALP activity, another hallmark of mature odontoblasts, is frequently used as an early indicator of mineralization capacity. The reliable detection of these markers enables researchers to evaluate the effects of experimental treatments or genetic modifications on odontoblast differentiation, offering insights into molecular mechanisms that govern cellular maturation.

Dental Tissue Engineering and Scaffold Design

Terminal differentiation markers are essential for assessing the quality and functionality of bioengineered dental tissues. In dental tissue engineering, the goal is to create scaffolds or constructs that can support the differentiation of progenitor cells into mature, dentin-producing odontoblasts. By incorporating differentiation markers, such as DSPP and DMP1, researchers can verify that cells within these scaffolds have reached a functional state capable of producing and organizing dentin matrix components[37]. Markers like Nestin and MAP1B, which indicate structural maturity and cytoskeletal organization, are also valuable in evaluating how well cells have polarized and established a functional odontoblastic morphology on three-dimensional scaffolds. This approach is particularly useful when testing biomaterials designed to support the formation of dentin-pulp-like structures[38]. Integrating terminal differentiation markers into tissue engineering protocols thus helps ensure that engineered tissues have the structural and functional characteristics necessary for effective dentin regeneration.

Dentin Regeneration and Repair

Terminal differentiation markers play a pivotal role in evaluating the regenerative potential of therapies aimed at repairing or regenerating damaged dentin. Markers such as DSPP, DMP1, and ALP are used to assess whether stem cells introduced into the pulp chamber or dentin matrix have successfully differentiated into odontoblast-like cells and are contributing to the formation of new dentin. This is particularly relevant in therapies involving stem cell transplantation or growth factor delivery, where the goal is to stimulate

the natural dentinogenic capacity of cells in situ[39]. The application of terminal differentiation markers provides a means to monitor the success of regenerative endodontic treatments, offering insight into both the extent of cellular differentiation and the quality of newly formed dentin. These markers also aid in identifying which signaling pathways or biomaterials are most effective in promoting odontoblast-like differentiation, providing a basis for optimizing protocols for clinical application.

Drug Screening and Biomaterial Testing

Terminal differentiation markers are increasingly being used in drug discovery and biomaterial testing aimed at enhancing odontoblast function and dentin regeneration[40,41]. By employing markers that indicate mature odontoblast differentiation, researchers can screen for drugs or biomaterials that promote dentin formation or enhance cellular viability and function in the dentin-pulp complex. For instance, markers such as DSPP, DMP1, and ALP can be used to evaluate the efficacy of biomaterials intended to support dentin repair by assessing their ability to sustain or stimulate odontoblast-like cell differentiation and activity. Additionally, these markers allow for the testing of pharmacological agents or natural compounds that may stimulate odontoblast differentiation or support dentin regeneration in cases of pulp exposure or trauma. Using differentiation markers as endpoints, researchers can determine which compounds are most effective in promoting dentinogenic activity, thereby contributing to the development of novel treatments for tooth repair and preservation.

Conclusion

The study of terminal differentiation markers in odontoblasts has significantly enhanced our understanding of dentinogenesis and provided essential tools for advancing research in dental development, regenerative medicine, and tissue engineering. Established markers, such as DSPP, DMP1, ALP, and Nestin, define the unique structural and functional characteristics of mature odontoblasts, enabling researchers to accurately identify and study these cells in various experimental contexts. These markers not only confirm the maturity of odontoblasts in *in vitro* differentiation studies but also facilitate the evaluation of bioengineered tissues and regenerative therapies aimed at restoring dentin function.

Emerging markers and signaling pathways, including Wnt5a, Runx2, MAP1B, and integrin-mediated signaling, offer promising avenues for further research into the mechanisms governing odontoblast differentiation and polarity. By expanding the repertoire of markers and understanding their roles in odontoblast maturation, researchers can gain deeper insights into the cellular and molecular processes that sustain dentin formation throughout the life cycle of a tooth. The application of these markers extends beyond basic research, providing practical tools for drug screening, biomaterial testing, and the development of regenerative endodontic therapies. With continued exploration into both canonical and emerging markers, future research holds the potential to develop innovative strategies for dental repair and regeneration, ultimately contributing to improved outcomes in oral health and tissue preservation. As research progresses, these markers will remain invaluable for guiding the development of therapies that not only restore the dentin-pulp complex but also improve the quality of life for patients facing dental tissue loss or injury.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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