Research Article

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Unique Osteoblast-Specific Cell-Surface Antigen Useful for Odontoblast Ontology and Dentin Regeneration

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ABSTRACT

Aim: *To obtain suitable immunological tool to identify the terminal differentiation of odontoblasts and for enrichment and purification of functional odontoblasts useful for dentin regeneration.*

Methods: *A panel of monoclonal antibodies (MAbs) recognizing cell-surface of rat osteoblastic cell line was prepared by utilizing the B cell hybridoma technology. Among MAbs recognizing osteoblasts specifically, odontoblast-reactive MAb was selected for immunofluorescence staining of cryosections of rat mandibles followed by observation by use of confocal laser scanning microscopy.*

Results: *Molecular size of the antigen recognized by A7 MAb (A7 antigen) was revealed to be around 45 KDa by western blot analysis. A7 antigen was expressed on cell-surface of unfixed osteoblasts in culture. Immunofluorescence staining and confocal laser analysis demonstrated that A7 antigen is specifically expressed in mature odontoblasts in a typical membrane expression pattern.*

Conclusions: *A7 antigen was revealed to be a unique cell-surface antigen expressed on osteoblasts and odontoblasts. This novel MAb-A7 should be a quite useful immunological tool for enrichment and purification of functional odontoblasts directly from dental pulp tissues in regenerative dental medicine.*

Keywords

Odontoblast, Dentinogenesis, Osteoblast.

Introduction

Dentin is a mineralized tissue that forms the main bulk of the tooth and is continuously deposited throughout life. Odontoblasts are specialized polarized cells bearing long cellular process, odontoblastic process, protruding in the dentinal tubules. These cells aligned as a single cell layer at the peripheral border of the dental pulp and responsible for the formation of dentin matrix and it's mineralization. These dentin-forming odontoblasts are ectomesenchymal in origin [1]. During embryogenesis, neural crest cells migrate from specified sites in the hindbrain to reside in the first branchial arch to form the ectomesenchymal cells of the dental papilla that terminally differentiate into odontoblasts [1,2]. Osteoblasts, the cells responsible for skeletal bone formation, are mesenchymal-derived cells [3]. In the craniofacial tissues, osteoblasts originate from the ectomesenchymal cells derived from neural crest cells to form jaw bones and alveolar processes [4-6]. Moreover, cultures of dental pulp stem cells maintaining characteristics of neural crest cells have the potential to differentiate into odontoblasts and osteoblasts [7]. To know the differentiation switch mechanism into odontoblasts from common ectomesenchymal cells, it is important to define a reliable molecular marker for identifying terminally differentiated odontoblasts. As osteoblasts and odontoblasts are derived from ectomesenchymal cells in the craniofacial development, it is likely that these cells share common cell surface marker molecules bearing possible

regulatory roles in their differentiation and function.

Odontoblasts have a key role in the determination of the amount of formed dentin matrices. It is well known that inductive formation of dentin occurs to form reparative dentin, in which rapid formation of regenerative dentin with irregular dentinal tubules is observed in response to the infectious stimuli affecting through dentinal tubules, to protect pulp tissues against microbial invasion [8]. To achieve successful tooth regeneration, large amount of regenerated dentin is required as dentin is the tissue that form the main bulk of the tooth. Therefore, dentin regeneration is considered to be quite important to regain lost teeth. To achieve efficient dentin regeneration, two strategies are considered. One is to utilize mesenchymal stem cells (MSCs) according to the standard methodology of regenerative medicine. Dental pulp stem cells (DPSCs) have the ability to differentiate into odontoblasts and are capable of forming dentin when cultured in the suitable differentiation conditions [9]. Although this method is well established and reliable strategy to obtain odontoblasts, it is time consuming to regenerate the whole dentin tissue after obtaining the DPSCs and inducing them to odontoblasts. Another postulated method is to utilize enriched odontoblasts and their precursors themselves which could be prepared directly from dental pulp tissues of supernumerary or wisdom teeth. If efficient enrichment of these cells become successful, it could be a very rapid methodology to regenerate dentin. However, so far almost no evidence has been reported concerning such strategy. One critical reason could be the lack of the appropriate immunological tool to achieve efficient enrichment and purification of odontoblasts.

In the current research, we have developed a monoclonal antibody specifically recognizing cell-surface antigen, designated as A7 antigen, expressed in mature odontoblasts and osteoblasts. In adult rats, A7 antigen is specifically expressed in odontoblasts in the dental pulp of molar and erupting incisor in a typical membrane pattern. A7 antigen was also expressed in odontoblasts observed in the developing tooth germ of neonatal rats. As A7 antigen is a typical cell-surface antigen, anti-A7 antigen monoclonal antibody could be an ideal tool for immunological enrichment and purification of cells in the odontoblast-lineage. As the expression of A7 antigen is so highly limited to mature odontoblasts in pulp tissues, it should also be a unique immunological probe to investigate the differentiation of odontoblasts from early precursors during tooth morphogenesis.

Materials and Methods

Animals

Female BALB/cN Sea mice were purchased from Kyudo (Tosu, Japan). Female LEW/SsN rats and neonatal SD rats were purchased from SLC (Fukuoka, Japan). All animal experiments were performed according to the guidelines for care and use of animals of Kyushu University.

Immunization and Hybridoma formation

The rat osteosarcoma cell line ROS17/2.8 was kindly provided by Dr. David Roodman (Indiana University School of Medicine). Original ROS17/2.8 cells [10] were cloned using the standard limiting dilution method. A clone with typical osteoblastic polygonal-shaped morphology characterized with rapid proliferation was selected, designated as ROS17/2.8 CL#7 and utilized for experiments in this paper. ROS17/2.8 CL#7 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) and harvested using 0.05% Trypsin, 0.02% EDTA. Single cell suspension was prepared and centrifuged at 1000 rpm for 10 min at 4ºC followed by intraperitoneal (i.p.) injection ($1x10^7$ cells in 500 µl of ice-chilled phosphate buffered saline (PBS)/mouse) into each isoflurane-anesthesized female BALB/cN Sea mouse using 26 gauge needle. Immunization (booster) was repeated two times every 2 weeks interval. Mice were then sacrificed 3 days after the final booster for hybridoma production. Formation of B-cell hybridoma was performed according to the basic protocol described by Köhler and Milstein (1975) [11], as modified by Reading (1982) [12]. Briefly, spleen cells obtained from the immunized mice were fused with murine myeloma cell line (P3X63-AG8-U1). Cell fusion was mediated by polyethylene glycol PEG 4000 (Sigma, St. Louis, MO, USA). The hybridomas were selected in HAT medium (IMDM supplemented with hypoxanthine, aminopterin and thymidine) containing 10% FBS (Life Technologies, Grand Island, NY, USA). After immunocytochemical screening of the supernatant of each hybridoma, the hybridoma secreting antibodies with high specificity to ROS17/2.8 cell clone and to osteoblast-lineage observed in rat whole bone marrow cultures for forming osteoclasts [13-15], was selected for sequential cloning by use of the limiting dilution technique. Monoclonal antibodies (MAbs) were produced by expanding the cloned hybridoma producing MAb A7 in the presence of 20 ng/ml of human recombinant IL-6 (Cell Signaling Technology ®, Danvers, MA, USA). The isotype of the A7 MAb was determined by use of mouse monoclonal antibody isotyping kit (IsoStrip, Roche Applied Science, Indianapolis, USA) according to the manufacturer's instructions.

Immunofluorescence staining of unfixed cultured osteoblasts

ROS17/2.8 CL#7 cells were cultured at a density of $1.8x10^4$ cells/ml in 35 mm glass-bottomed dishes (MatTek Co., Ashland, MA, USA) in 2ml DMEM containing 10% FBS. After aspirating the culture medium, cells were rinsed with PBS and reacted with A7 MAb or control mouse IgG (Abcam, Cambridge, UK) for 30 min on ice. The cells were then rinsed with serum-free medium and fixed in 4% paraformaldehyde (PFA) in PBS (pH 7.4) at room temperature for 20 min. After rinsing and blocking with 3% goat serum in PBS for 30 min, cells were incubated with goat anti-mouse IgG conjugated with Alexa Fluor 488 (Biolegend, San Diego, CA, USA) at room temperature for 30 min in a dark condition. Cells were then treated with 4',6-diamidino-2-phenylindole (DAPI) (Sigma/Merk FGsA, Darmstadt, Germany) to stain nuclei for 5 min in a dark condition. After rinsing in PBS, cells were imaged using BZ-8100 fluorescence microscope (Keyence, Tokyo, Japan).

Immunofluorescence confocal laser analysis of the frozen sections

Neonatal SD rats (2-days old) were anaesthesized with isoflurane inhalation and aseptically dissected to obtain mandibles followed

by tissue fixation in 4% PFA in PBS overnight at 4ºC. Four weeks old female Lewis (LEW/SsN) rats were anaesthesized with isoflurane inhalation followed by i.p. injection of pentobarbital (1mg/Kg body weight). The rats were aseptically dissected and fixed by perfusion using 4% PFA in PBS from the left ventricle. Excised mandibular bone tissues were decalcified in 10% EDTA at 4ºC for one week (neonatal SD rats) or three weeks (Lewis rats). Decalcified bone tissues were embedded in OCT compounds to prepare frozen tissue blocks and stocked in -80ºC until use. Frozen tissue blocks were prepared using cryostat HM560MV (Zeiss, Oberkochen, Germany), then processed for immunofluorescence staining according to the standard protocol. After quenching the unreacted aldehyde groups by 10 mM glycine, sections were blocked in 5% chick albumin in PBS for 6 hrs at room temperature and 10% goat serum in PBS overnight at 4ºC. Sections were incubated with primary antibody (A7 MAb) or control IgG at room temperature for 2 hrs in a moisture chamber. After washing with PBS, sections were incubated with goat anti-mouse IgG polyclonal antibody conjugated with Alexa Fluor 488 (Biolegend, San Diego, CA, USA) diluted 300 times in 1% Bovine Serum Albumin (BSA) in dark. Nuclei were stained with DAPI for 5 min in dark. After rinsing in PBS, sections were mounted in Aqua-Poly/Mount (Polysciences Inc., Washington, PA, USA) and observed using confocal laser scanning microscopy C2si (Nikon Instech, Tokyo, Japan).

Western blotting

After rinsing with PBS, proteins were extracted from confluent ROS17/2.8 CL#7 cells using extraction buffer (RIPA buffer) (150 mM NaCl, 1% v/v NP-40, 0.5% DOC, 50 mM Tris-HCl (pH 8.0), 0.1% SDS) supplemented with protease inhibitor mixture P1860 (Sigma, St. Louis, MO, USA) on ice for 30 min. Cell lysates were centrifuged (12,000 rpm, 4ºC) and the supernatants were collected. Protein concentration of supernatants was determined using protein assay kit (Bio-Rad, Hercules, CA, USA). Cell extracts were applied to 10% SDS-PAGE (10 µg protein per lane) and transferred to PVDF membrane (GH Healthcare, Chicago, IL, USA). After being blocked with 5% non-fat dry milk, membrane was incubated with the primary antibody (A7 MAb) or control IgG overnight at 4ºC. Membrane was treated with HRP-conjugated anti-mouse IgG antibody (Cell Signaling Technology, USA) as the secondary antibody for 1 hr. Signals were detected using luminogenic reagent ECL™ Prime Western Blotting Detection Reagent (GE Healthcare).

Results

Detection of A7 antigen expressed on cell surface of unfixed osteoblasts in culture

Figure 1 shows the reactivity of monoclonal antibody A7 (A7 MAb), which recognizes cell-surface antigen (A7 antigen) expressed in osteoblasts, to unfixed osteoblastic cell line ROS17/2.8 cell clone (ROS17/2.8 CL#7). Typical cell-surface expression of A7 antigen was detected in osteoblasts at day 4 culture (Figure 1, left panel). Control IgG showed no immunofluorescence signals (Figure 1, right panel). These data indicate the expression of A7 antigen on cell surface of osteoblasts. To determine the molecular weight of A7 antigen, cell lysates of ROS17/2.8 CL#7 cells were prepared and subjected to western blotting (Figure 1b). As compared to the isotype control IgG which detected no protein band (Figure 1b, lane 1), A7 MAb detected a specific band of approximate molecular weight of 45 KDa (Figure 1b, lane 2).

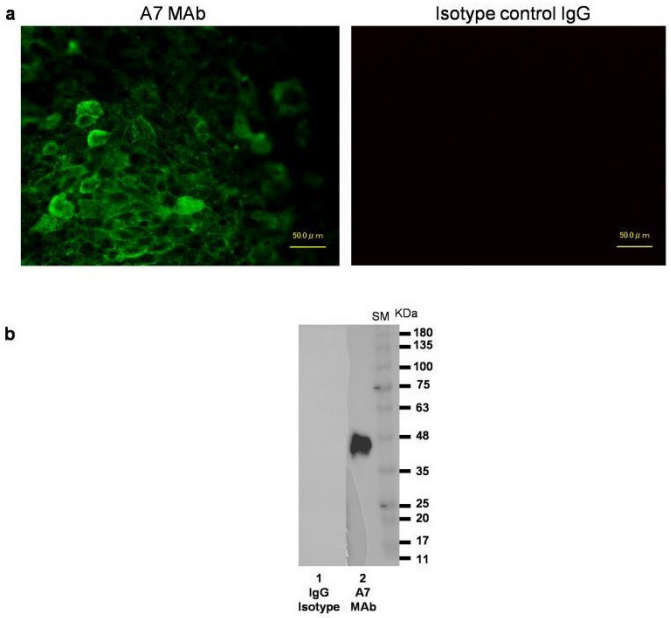

Figure 1: Immunofluorescent detection of A7 antigen on cell surface of cultured osteoblasts and western blot analysis.
(a) Detection of A7 antigen on cell surface of osteoblasts. Unfixed ROS17/2.8 CL#7 cells were stained with A7 MAb or isotype control IgG as the the primary antibody as described in Materials and Methods. Cell surface bound antibodies were detected with anti-mouse IgG conjugated with Alexa Fluor 488 (green). Left panel: A7 MAb, Right panel: Isotype control IgG. Bars, 50 µm. (b) Western blot analysis. Lysates prepared from cultured ROS17/2.8 CL#7 cells were subjected to western blot analysis by use of isotype control IgG (lane 1) or A7 MAb (lane 2). SM: size marker.

Expression of A7 antigen in mandiblar alveolar bone and teeth

Next we examined the expression of A7 antigen in vivo by utilizing cryosections of mandibles prepared from 4 weeks-old Lewis rats. Figure 2 shows the immunofluorescence staining of the alveolar bone and roots of erupted molars. In the alveolar bone, A7 MAb demonstrated intense immunofluorescence signals in subsets of bone cells covering the trabecular bone surfaces (Figure 2, arrows). These cells are likely to be cells in the osteoblast-lineage. In contrast, bone marrow tissue showed no expression of A7 antigen. Also, no immunoreactivity was observed in the periodontal ligament. A7 antigen was markedly expressed in odontoblasts of erupted molars (Figure 2, arrowheads). However, other tooth tissues as dentin, cementum and dental pulp were not stained with A7 MAb. These results suggest that A7 antigen is expressed in osteoblasts present in the alveolar bone and odontoblasts present in the most outer portion of dental pulp, the boundary of dentin and pulp, in erupted teeth. In the coronal portion of the same sections of erupted molars, coronal pulp tissue showed very weak immunofluorescence signals (Figure 3). In contrast, clear intense immunofluorescence signal was observed in the terminally differentiated odontoblasts

(Figure 3a, arrowheads). Higher magnification view of the same field showed a typical membrane expression pattern of A7 antigen in odontoblasts (Figure 3b, arrowheads; Figure 3c). No immunofluorescence signal was observed in staining control using control IgG (Figure 3d).

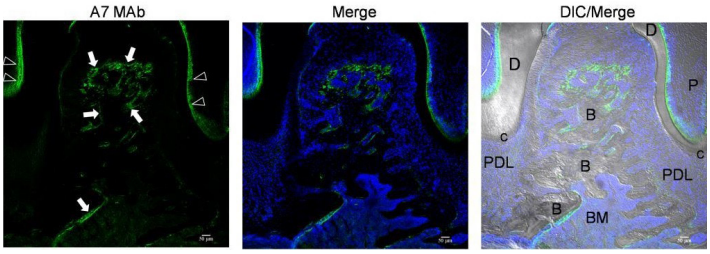

Figure 2: Expression of A7 antigen in alveolar bone surface and odontoblasts.

Sagittal frozen sections of mandible were prepared from 4-weeks-old female Lewis rats as described in Materials and Methods. Sections were processed for staining with A7 MAb and detected with anti-mouse IgG conjugated with Alexa Fluor 488 (green). Nuclei were stained with DAPI. Staining with A7 MAb shows intense expression of A7 antigen in cells of the alveolar bone surface (arrows) and odontoblasts of molars (arrow heads) (left panel); A7 MAb/DAPI merge (center panel); Merged A7 MAb/DAPI staining with DIC (right panel). D, dentin; C, cementum; PDL, periodontal ligament; B, bone; BM, bone marrow. Bars, 50 µm.

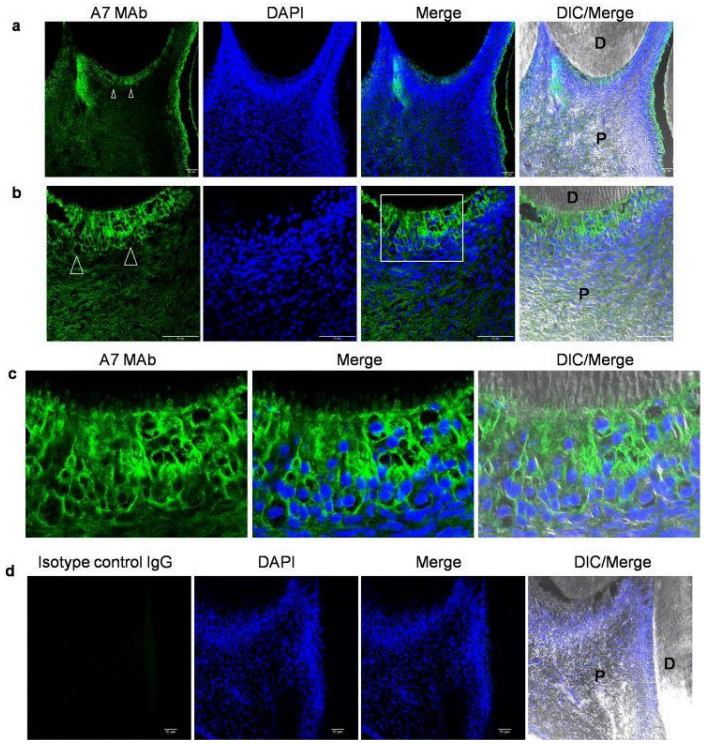

Figure 3: Expression of A7 antigen in cell membrane of odontoblasts in erupted molars.

Sagittal frozen sections of mandible were prepared from 4-weeks-old female Lewis rats as described in legends of Figure 2. (a) and (b): Expression of A7 antigen in odontoblast cell layer (arrow heads) of molars detected with A7 MAb. (b) is the higher magnification view of (a). A7 MAb staining (A7 MAb), nuclei staining with DAPI (DAPI) and A7 MAb/DAPI merge (Merge), merged A7 MAb/DAPI staining with DIC (DIC/Merge). (c) Higher magnification view of rectangle region shown in the A7 MAb/ DAPI merge of (b) showing typical mambrane expression pattern of A7 antigen in odontoblasts. A7 MAb staining (left panel), A7 MAb/ DAPI merge (center panel), merged A7 MAb/DAPI staining with DIC (right panel). (d) Isotype IgG showed no reactivity to odontoblasts or other tooth tissues. Control IgG staining (Isotype Control IgG), DAPI (DAPI), A7 MAb/DAPI merge (Merge), merged A7 MAb/DAPI staining with DIC (DIC/Merge). D, dentin; P, pulp. Bars, 50 µm.

Figure 4a shows the expression of A7 antigen in continuously growing incisor. Intense immunofluorescence signal indicating the expression of A7 antigen was detected in odontoblasts observed in the pulp-dentin boundary of rat incisor (arrowheads) with no reactivity to the pulp tissue. Higher magnification view of the same field also showed a clear membrane localization pattern of A7 antigen in the cell body of odontoblasts (Figure 4b, arrowheads) and basal part of the odontoblastic processes present in the pulp tissue just before the entrance of the dentinal tubules. However, A7 antigen was not detected in odontoblastic processes fully extended in the dentinal tubules. These data suggest that A7 antigen is a unique odontoblastic cell membrane molecule specifically expressed in mature odontoblasts in the pulp tissue.

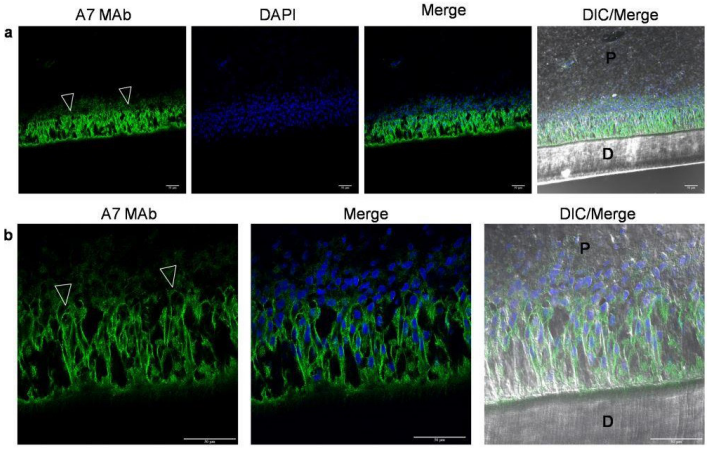

Figure 4: Expression of A7 antigen in membrane of odontoblasts present in continuously growing incisor.

Sagittal sections of incisor tooth germ were prepared from 4-weeks-old female Lewis rats as described in Materials and Methods. (a) Expression of A7 antigen in odontoblast cell layer (arrow heads) of continuously growing incisor detected with A7 MAb. A7 MAb staining (A7 MAb), nuclei staining with DAPI (DAPI), A7 MAb/DAPI merge (Merge), merged A7 MAb/DAPI staining with DIC (DIC/Merge). (b) Higher magnification of (a) showing typical membrane expression of A7 antigen in the cell body of odontoblasts (arrow heads) and only a basal part of the odontoblastic process in pulp tissue. A7 MAb staining (left panel), A7 MAb/DAPI merge (center panel), merged A7 MAb/DAPI staining with DIC (right panel). D, dentin; P, pulp. Bars, 50 µm.

Expression of A7 antigen in odontoblasts of developing tooth germs

To assess the expression of A7 antigen in odontoblast and other tissues in the developing tooth germ. Cryosections of mandibles obtained from 2-days-old rats were stained with A7 MAb or with control IgG (Figure 5). A7 MAb demonstrated a marked and specific A7 antigen expression in the cell layer of odontoblasts at the advanced bell stage of tooth development. In contrast,

no significant immunoreactivity of A7 MAb was detected in other cells of different portions of the tooth germ, inner enamel epithelium, stellate reticulum, outer enamel epithelium and dental follicle (Figure 5a). Figure 5b showing a high magnification view of the cusp tip of developing molar tooth germ revealed a typical membrane localization pattern of A7 antigen in cell body of odontoblasts (arrowheads) and basal part of odontoblastic processes that lie within the pulp and extending for short distance within the predentin layer. Inner dental (enamel) epithelium (arrows) showed very faint staining. Dentin matrix was not stained at all. No signals were detected in all tissues of the tooth germ in staining control using control IgG (Figure 5c). These data suggest that A7 antigen is a unique cell-surface antigen specifically expressed in differentiated odontoblasts during dentin formation.

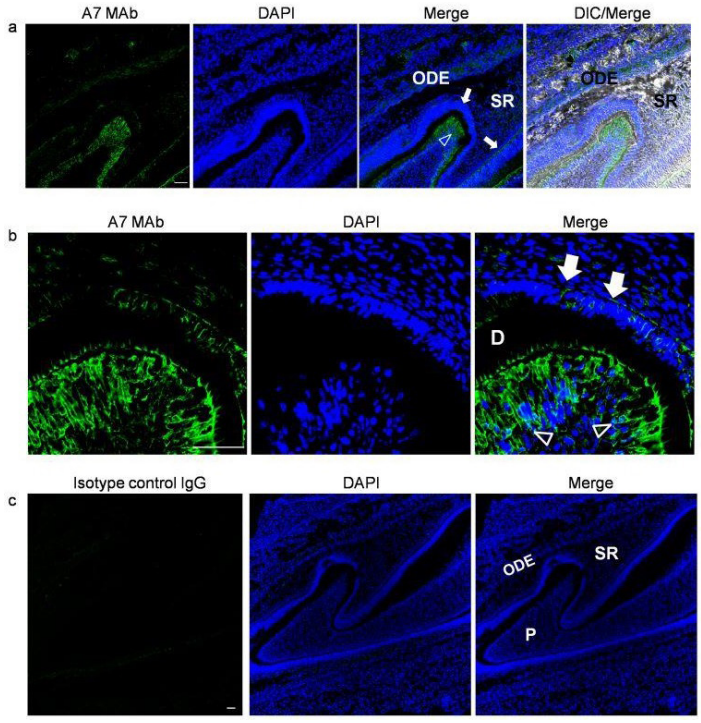

Figure 5: Expression of A7 antigen in odontoblasts of developing tooth germ.

Sagittal sections of mandible were prepared from 2-days-old neonatal rats as described in Materials and Methods. Frozen tissue sections were processed for staining with A7 MAb or isotype IgG and detected with anti-mouse IgG conjugated with Alexa Fluor 488 (green). Nuclei were stained with DAPI.

(a) A7 antigen expressed in odontoblastic cell layer (arrow head) at the cusp tip of a developing tooth germ (advanced bell stage) detected with A7 MAb. A7 MAb staining (A7 MAb), nuclei staining with DAPI (DAPI) , A7 MAb/DAPI merge (Merge), merged A7 MAb/DAPI staining with DIC (DIC/Merge). ODE, outer dental (enamel) epithelium; SR, stellate reticulum. (b) Extremely high expression of A7 antigen in odontoblasts (arrow heads) at the cusp tip of tooth germ in the late bell stage of development. Arrows show the inner dental (enamel) epithelium. A7 MAb staining (left panel), nuclear staining with DAPI (center panel), A7 MAb/DAPI merge (right panel). D, dentin. (c) Reactivity of isotype IgG to developing tooth germ. ODE, outer dental (enamel) epithelium; SR, stellate reticulum; P, Pulp. Bars, 50 μm.

Discussion

In the current paper we observed marked expression of A7 antigen in the coronal and radicular odontoblasts of erupted molars and in continuously growing incisor. Completely negative staining of dentin matrices with clear membrane staining pattern indicates that this antigen is not a secretory extracellular matrix protein but a membrane molecule expressed on cell surface of odontoblasts. Weakly stained pulp tissues in adult and negatively stained dental papilla in neonatal mandibles suggest that A7 antigen is not expressed in the early stages of odontoblast differentiation from dental pulp stem cells to the pre-odontoblastic stage and is preferentially expressed in the terminally differentiated mature odontoblasts.

We have demonstrated that cell bodies of polarized odontoblasts were clearly and specifically labeled with A7 MAb with typical odontoblast morphology. In the pathway of odontoblast differentiation, pre-odontoblast appear to be oval or rounded in morphology with large nucleus and little polarization when cells are at the end of the mitotic cycle [16]. As cells differentiate into typical odontoblasts, they become columnar in shape with highly polarized cell bodies, large eccentric nucleus and extend their odontoblastic processes within the dentinal tubules [17]. Odontoblasts finally change into flattened morphology with tooth aging [18]. Our observation confirm the expression of A7 antigen in typical polarized odontoblasts.

As dentin formation progresses, odontoblasts retract in a direction towards the center of the pulp tissue with elongation of their processes in a step known as odontoblastic emigration [19,20]. The expression of A7 antigen on the membrane of odontoblastic processes that lies in pulp tissue or on a small portion of the processes within predentin, suggest a regulatory role of this antigen in the initial formation of odontoblastic processes and in the odontoblastic emigration that requires cytoskeletal reorganization and elongation of the process during active dentin formation, a role similar to that of the odontoblast membrane integrins [21]. Transmembrane proteins known as αvβ3 integrins were reported to be involved in stabilizing odontoblastic processes and in continuous reorganization of actin microfilaments of odontoblastic processes during their elongation in human teeth [21]. Although αvβ3 integrin is also abundantly expressed in cells in the osteoclast lineage [22]. A7 antigen is not expressed in cells in osteoclast-lineage (unpublished data).

Torres-da-Silva et al. [23] reported a novel transmembrane protein known as Teneurins-2 expressed in odontoblasts of rat developing and erupted molars during physiological dentin deposition and in response to dentin injury. However, Teneurins-2 was also expressed in active apical cementoblasts and periapical alveolar bone in response to injury [23]. In the current paper, we have demonstrated the expression of A7 antigen in normal physiological conditions. As Teneurins-2 is a transmembrane glycoprotein with approximately 2800 aminoacids (MW is around 200 KDa) [24], molecular size of this molecule is completely different from that of A7 antigen. Further studies are required to examine the induction

of A7 antigen in response to various external stimuli. It is also important to examine the induction of A7 antigen in response to inflammatory stimuli.

Our in vivo observations in frozen sections of developing tooth germs of neonatal rats revealed that A7 antigen was only expressed in odontoblasts with a typical membrane pattern without being expressed in other tissues of the tooth germ. E11 is a cell membrane protein mainly localized in osteoblasts and osteocytes [25] and was reported to be expressed on cell membrane of odontoblasts and developing alveolar bone at early bell stage of tooth development [26]. Although E11 was not localized in pulp tissues, expression of E11 antigen was not specific to odontoblasts as other tissues of the tooth germ (inner and outer enamel epithelium) also showed intense positive immunostaining. E11 protein was reported to play a non-significant role during dentinogenesis [26]. In contrast to E11 antigen, A7 antigen is highly specific to odontoblasts with no significant expression in other tissues of the tooth germ. Furthermore, molecular weight of E11 is 30 KDa [25], which is different from that of A7 antigen.

It is known that dentinogenesis starts at the cusp tips then progresses cervically [17]. In the current study there was no observable difference in A7 staining intensity across the entire odontoblastic cell layer. Odontoblasts at cusp tips demonstrated almost the same intensity as compared to those cells located in the roots or cervically at the Hertwig epithelial root sheath. This observation strongly suggest that A7 antigen is expressed equally in secretory and newly differentiated odontoblasts even before dentin deposition and that it could elicit a signal denoting that cells are in a specific morpho-differentiation stage and capable of dentin formation and that A7 antigen continues to be expressed throughout primary and secondary dentin formation. As we have shown A7 antigen expression in late bell stage of tooth development, further studies are required to investigate A7 antigen in earlier (bud, cap and early bell) stages.

Conclusion

Although A7 antigen is highly expressed also in osteoblasts, specific expression of A7 antigen in odontoblasts in pulp tissues would be quite useful in the enrichment and purification of the functional mature odontoblasts directly from pulp tissues of supernumerary and wisdom teeth to regenerate dentin. Such immunological strategy should open one promising research field in regenerative dentistry in near future.

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