Expression and processing of small integrin-binding ligand N-linked glycoproteins in mouse odontoblastic cells

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Abstract

Objective—Small integrin-binding ligand N-linked glycoproteins (SIBLINGs) are expressed in dentin and believed to control dentinogenesis. Five members of SIBLING family include bone sialoprotein (BSP), osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP). These genes are clustered on chromosome 4q in humans and share similar biological features. DSPP and DMP1 are processed into given structural/functional fragments in rat and porcine. It still remains unclear whether these evidences occur in mouse and other SIBLING members are also processed into given fragments from their parent precursors. The aim of this study was to identify expression and processing of the five proteins in two mouse odontoblastic cell lines.

Design—Two mouse odontoblastic cells were used to study expression and processing of the five SIBLING proteins by immunohistochemistry and Western blot analyses.

Results—Immunohistochemistry study showed that all of the five SIBLING members were expressed within the cytoplasm and cellular processes in the mouse odontoblastic cell lines. Expression levels of DMP1 and DSPP were higher in differentiated mouse odontoblasts than undifferentiated mouse odontoblasts. Immunolabeling signal of DSP and MEPE was also detected within the nucleus in the two cell lines. Western blot assay indicated that all five members were processed into at least two fragments in these cells.

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Conclusions—These results suggest that different processed products and expression levels of the SIBLING proteins may play distinct biological functions in tooth development and mineralization.

Keywords

SIBLING; odontoblasts; dentinogenesis; dentin; teeth

1. Introduction

Dentin is one of the major mineralized tissues of tooth and originates from odontoblasts, which synthesize collageous and non-collagenous proteins (NCPs) (1-4). Among NCPs, a family of small integrin-binding ligand N-linked glycoproteins (SIBLINGs) consists of osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE, also known as OF45), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP). Genomic structures of the five SIBLING genes are located in chromosomes 4q in human and 5q in mouse (5-8). They share common gene structure features such as small non-translational first exon, start codon in exon 2 and large coding segment in the last exon (5-8). Also, these proteins contain an Arg-Gly-Asp (RGD) motif that mediates cell attachment/signaling via interaction with cell surface integrins and post-translational modification of phosphorylation and glycosylation. These SIBLING genes are highly expressed in mineralizing tissues related to tooth and bone development and mineralization (9-17).

BSP is a sulfated, phosphorylated and glycosylated protein characterized by its ability to bind to hydroxyapatite through polyglutamic acid sequences. High BSP expression is restricted to the mineralized connective tissues and seen at the onset of bone, dentin and cementum formation, indicating a role of this protein in the initial mineralization of these tissues (10,18-19). OPN is a secreted adhesive glycoprotein expressed in both mineralized and non-mineralized tissues (20). In mineralized tissues, this gene expression is relatively abundant in bone (13) but also found in dentin (9,21-22). Defective OPN gene in mice increases mineral content and crystallinity (23). MEPE was isolated from a human oncogenic hypophosphatemic osteomalacia (OHO) tumor cDNA library (24). Mouse and rat MEPE genes were also cloned (25-26). This protein is rich in aspartate, serine and glutamate residues and highly expressed in mineralized tissues (12,21,27-28). Increased expression of MEPE occurs in patients with X-linked hypophosphatemic rickets (27). Targeted disruption of MEPE gene in mice results in increased bone formation and bone mass, suggesting that MEPE inhibits mineralization (28).

DMP1 is an acidic phosphoprotein which is predominantly expressed in dentin and bone (14,29-31). Mutations of DMP1 in human and mice cause profound defects in mineralization of both dentin and bone (32-35). DSPP is highly expressed in dentin compared to bone and other tissues (13-14,16-17). DSPP protein is a precursor that is proteolytically processed into dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) in rat teeth (36) whereas DSPP protein in porcine teeth is processed into DSP, DPP and a small segment termed dentin glycoprotein (DGP), which is located between DSP and DPP (37). Either DSP or DPP domain plays distinct biological functions during tooth development (38-44). In addition, two major fragments, 37- and 57-kDa originally from DMP1 precursor were observed in rat bone (45). Although cDNA sequences from mouse DSPP and DMP1 genes have been identified, it remains unknown if DSPP and DMP1 proteins in mouse are processed into given fragments and those proteolytical fragments are the same expressional patterns as that of rat and porcine. Furthermore, analysis of other SIBLING protein sequences shows that several proteinase cleavage sites exist in those proteins (46-47). Thus,

it is not known whether OPN, MEPE and BSP proteins like DSPP and DMP1 are processed into given fragments.

Mouse is a comprehensive model for the study of tooth development and formation. Several mouse immortalized odontoblast-like cell lines have been established and used to study their biological functions responsible to intrinsic and extrinsic stimulations (17,48-53). As the processing of SIBLING proteins in mouse odontoblasts has not been documented, in this study, we used two mouse immortalized odontoblast-like cell lines to investigate expression and processing of the five SIBLING proteins by immunohistochemistry and Western blot analyses.

2. Materials and Methods

2.1. Cell lines and cell culture

Mouse differentiated odontoblast-like (MO6-G3) and undifferentiated odontoblast-like (MD10-F2) cells were obtained from dental papilla mesenchymal cells isolated from the first mandibular molars of Swiss Webster mice at embryonic day 18 and immortalized by infection with recombinant defective retrovirus containing the temperature sensitive SV-40 large T-antigen cDNA (48). Both MO6-G3 and MD10-F2 cells express several markers of the odontoblastic genotype positive in alkaline phosphatase (ALP) staining and form Von Kossa nodules *in vitro* (17,48,53). Compared to MD10-F2, MO6-G3 cells express higher levels of the NCPs (17) and are identified as differentiated odontoblast-like cells by immunocytochemical and ultrastructural studies (53). MO6-G3 and MD10-F2 cells were grown at 33°C under 5% CO_2 in alpha minimum essential medium (α-MEM) supplemented with 10% fetal calf serum, 100 units/ml penicillin/streptomycin, 50 μg/ml ascorbic acid, and 10 mM Na β-glycerophosphate (Sigma, St. Louis, MO).

2.2. Antibodies

Polyclonal rabbit anti-mouse DSP and anti-mouse DMP1 were purchased from Alpha Diagnostic International, San Antonio, TX, USA. The anti-DSP and anti-DMP1 antisera were raised in rabbit (Alpha Diagnostic International) against recombinant fragments representing the NH_2-terminal portion (amino acid residues Ile18-Lys371) of mouse DSPP (54) and amino acid residues 1-508 of mouse DMP1, respectively (55). High titer polyclonal antisera as measured by enzyme-linked immunosorbent assay (ELISA) were obtained and then further purified by affinity column. Polyclonal rabbit anti-mouse MEPE (kindly provided by Dr. Peter Rowe, The Kidney Institute, University of Kansas Medical Center, MS, USA), polyclonal rabbit anti-mouse BSP (a gift from Dr. Larry Fisher, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA), monoclonal mouse anti-mouse OPN, (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were used as primary antibodies. Negative control of mouse IgG I was purchased from Dakocytomation (Carpinteria, CA, USA).

2.3. Immunohistochemistry

MO6-G3 and MD10-F2 cells were cultured on glass slides, rinsed twice with 1 x cold phosphate buffer saline (PBS) and fixed for 10 min on ice with methanol/acetone (1:1). After washing once with 1x PBS, cells were treated with 10% normal goat serum (Sigma) for 60 min at room temperature and followed by washing three times for 2 min with PBS. The cells were then incubated with a dilution of 1:100 of primary antibodies specific for BSP, DMP1, DSP, MEPE and OPN in PBS containing 1% BSA and 10% goat serum. Negative control of mouse IgG I was purchased from Dakocytomation (Carpinteria). The cells were incubated at 4°C for overnight and then washed 3 x for 5 min with PBS containing 0.1% goat serum, followed by a 1 : 1,000 dilution of the secondary antibodies

(goat-anti rabbit or goat-anti mouse) with Alexa Fluo® 488 (Molecular Probes, Eugene, OR, USA) for 60 min at room temperature. Excess secondary antibody was removed by washing the cells three times with PBS. For nucleus staining, the cells were incubated with a 1: 5,000 dilution of Hoechst (Sigma) for 5 min at room temperature. After washing the cells with PBS, the sections were mounted using Vectashield mounting medium (Vector Laboratory Inc., Burlingame, CA, USA). Images of Alexa Fluo® 488 staining of the various proteins in cultures were obtained at the Core Optical Imaging Facility at UTHSCSA under the same parameters in a Nikon inverted microscope and quantitated by means of NIS-GIEMENTS software. For each experiment, all slides were simultaneously processed for a specific antibody, so that homogeneity in the staining procedure was ensured between the samples. After the capture of these images at the same magnification, the threshold was set and maintained for each slide in the experiment. The optical density was calculated by use of the morphometric analysis within the software package.

2.4. Western blot analysis

Western blotting assay was performed as described earlier (17). Briefly, MO6-G3 and MD10-F2 cells were washed with 1x cold PBS and lysed with RIPA buffer (1x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/ml phenylmethylsulfonyl fluoride, 30 μl/ml aprotinin, 100 mM sodium orthovanadate; Santa Cruz Biotechnology, Inc.). Whole cell lysates (50 μg/well) were resolved by 7 % SDS-polyacrylamide gel electrophoreses (SDS-PAGE) and transferred to Trans-Blot membranes (Bio-Rad Laboratory, Inc. Hercules, CA, USA). For the detection of mouse SIBLING proteins, the membranes were blocked with 5% non-fat milk in TBST buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Tween-20) for 60 min at room temperature. After washing, the membranes were incubated with primary antibodies against DSP, DMP1, MEPE, BSP and OPN with appropriate dilution (1:500-800) for overnight at 4°C. The secondary antibodies (horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG) were used a dilution of 1:10,000 at room temperature for 60 min. Immunoreactivity was determined using the ECL chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ, USA). As a control, goat anti-mouse β-actin antibody was used (Santa Cruz Biotechnology, Inc.).

3. Results

3.1. BSP

To assess BSP expression in mouse odontoblastic cells, we performed immunohistochemistry. The results in Figure 1 G-L showed that BSP expression was well distributed in the cytoplasm and cellular processes in both mouse odontoblastic cells. High magnification analysis demonstrated that BSP signal appeared more intense in the perinuclear basis (Fig. 1 K-L). To identify expressional patterns of BSP protein, we performed Western blot analysis. Data showed that approximately 39 and 27 kDa fragments of BSP protein were detected in the two odontoblastic cells (Fig.1Y).

3.2. OPN

Like BSP, OPN expression was predominantly detected in the cytoplasm and cellular processes in MD10-F2 and MO6-G3 cells (Fig.2 G-L). Expressional patterns of OPN in the mouse odontoblasts were analyzed by Western blot assay using anti-OPN antibody. Figure 2Y showed that three major fragments of OPN polypeptides were seen in a 7% SDS-PAGE gel.

3.3. DMP1

DMP1 signal was distributed in the cytoplasm and cellular processes in MD10-F2 and MO6-G3 cells by immunohistochemical analysis (Fig.3 G-L). Compared to MD10-F2 cells, DMP1 expression level in MO6-G3 cells was more profound (Fig.3 K-L). The same processed fragments of DMP1 protein in the two mouse odontoblastic cells were seen at molecular weight of about 53, 51 and 35 kDa, respectively in a 7 % SDS-PAGE gel using immunoblotting assay (Fig. 3Y). The processed patterns of mouse DMP1 polypeptides were similar to that of full-length mouse recombinant DMP1 protein cleaved by BMP1/tolloid-like proteinases *in vitro* (56).

3.4. MEPE

To examine MEPE expression in the mouse odontoblastic cells, we performed immunohistochemistry assay and found MEPE expression within both the cytoplasm and nucleus in MD10-F2 and MO6-G3 cells (Fig. 4G-L). Expressional patterns of MEPE were analyzed by Western blot assay. The results showed that three major fragments of MEPE protein were seen in the mouse odontoblastic cells (Fig.4Y).

3.5. DSP

Like MEPE, DSP signal was present within the cytoplasm and nucleus in the two odontoblastic cells by immunocytotistochemistry (Fig. 5G-L). However, DSP expression level in MO6-G3 cells was more intense than that of MD10-F2 cells (Fig.5G-L). To determine expressional patterns of DSP protein analyzed by Western blot assay, we were able to detect multiple fragments of DSP polypeptides in MD10-F2 and MO6-G3 cells using anti-DSP antibody (Fig.5Y). Four major bands were seen at molecular weight of about 250, 170, 70, 15 kDa, respectively.

3.6. Control study

Negative control for SIBLING proteins examined above demonstrated no positive staining by immunohistochemistry (data not shown). β-actin as internal control was used to detect whether proteins from the two odontoblastic cells were degraded during protein isolation process (Fig.5Z).

4. Discussion

SIBLING family is highly expressed in mineralized tissues and shares similar biological features as well as plays significant roles during dentinogenesis (5-8). It is well known that DSPP and DMP1 proteins are proteolytically processed into given fragments in tooth and bone tissues (36-37,45). These processed fragments from DSPP and DMP1 precursors have their unique biological roles during formation and mineralization of teeth and bones (38-44,57-58). To determine whether the other members of SIBLING family exhibit the same phenomena as the DSPP and DMP1, we assessed expression and processing of five members of the SIBLING family; BSP, OPN, MEPE, DMP1 and DSPP in two mouse odontoblastic cells. In this study, our results for the first time showed that the five SIBLING proteins were expressed and processed into given fragments in the two mouse odontoblastic cells. In addition, sizes and patterns of processed fragments from mouse DSPP and DMP1 precursors were different from that of rat and porcine described earlier (36-37,45,59).

For BSP, immunohistochemistry study indicated that its signal was dominantly seen in cytoplasm and cellular processes in MD10-F2 and MO6-G3 cells (Fig. 1 G-L) in agreement with previous reports *in vitro* and *in vivo* (10, 18). Expressional patterns of BSP protein analyzed by Western blot assay demonstrated that two bands were detected at positions, ~39- and 27-kDa, respectively in the two mouse odontoblastic cells (Fig. 1Y). Based on our

observation, this suggests that intact BSP protein was processed within the mouse odontoblastic cells before being secreted. However, we have not known what enzymes catalyze BSP and which positions of BSP are cleaved by the enzymes as well as their biological roles.

Assessing OPN expression and processing, we found that in addition to OPN expression in the two mouse odontoblastic cells, two major fragments of OPN were detected at molecular masses of ~45- and 27-kDa by Western blot analysis (Fig. 2Y). Analysis of OPN protein sequences indicates that this protein contains a thrombin cleavage site (20). Boukpessi et al. reported that two fragments of OPN protein exist in human teeth using Western blot assay (21). However, sizes of the two OPN fragments with ~60- and 46-kDa in human teeth are different from that we observed. The differences may be due to species-species variations.

Although MEPE is an extracellular matrix protein, it is interesting that its expression was detectable in both the cytoplasm and nucleus (Fig. 4G-L). However, its role in the nucleus remains obscure. To examine expressional patterns of MEPE, we observed three major fragments that were recognized by anti-MEPE antibody in the mouse odontoblastic cells (Fig. 4Y). Guo et al. found that MEPE protein is cleaved by cathepsin B into several fragments *in vitro* (47). For its biological roles, knock-out of MEPE gene in mice increased bone formation and bone mass, indicating that MEPE inhibits mineralization process (28). However, recent studies have found that given domains of MEPE protein have different biological functions. Rowe et al. reported that a small peptide released from COOH-terminus of MEPE was able to inhibit mineralization processes *in vitro* (60) whereas another fragment from N-terminal MEPE with RGD motif accelerated mineralization (61). In this study, we identified the three major fragments of MEPE polypeptides in the mouse odontoblastic cells. Which fragment is derived from which portion of MEPE protein needs to be further investigated.

In the present study, three major fragments of DMP1 were detected in the two mouse odontoblastic cells (Fig. 3Y). The expressional patterns of the three DMP1 fragments were similar to that of a full length recombinant mouse DMP1 cleaved by BMP1/tolloid-like proteinases (56). However, the processed patterns of mouse DMP1 polyepeptides were different from that of rat and porcine (45,62). Two DMP1 fragments, ~37- and 57-kDa were detected in rat bone (45) whereas two bands of porcine DMP1 were seen at molecular masses of ~90- and 60-kDa in porcine pulp-derived cells (62). It is known that rat DSPP is processed into DSP and DPP (36) whereas porcine DSPP is processed into DSP, DGP and DPP (37). Like DSPP, whether differences of the DMP1 processed products among mouse, rat and porcine are due to species-species variation needs to be further investigated. For DMP1 biological roles, DMP1 null mice showed teeth with failure of maturation of pre-dentin into dentin, enlarged pulp chambers, increased width of pre-dentin zone with reduced dentin wall and hypomineralization (35). Recent studies have found that these two fragments from rat DMP1 play different roles during mineralization processes (57-58). A fragment from the DMP1 COOH terminus (~57 kDa) isolated from rat bone enhanced the hydroxyapatite nucleation whereas the other fragment from DMP1 NH_2-terminal domain (~37 kDa) inhibited nucleation of hydroxyapatite. Which fragment (37 kDa or 57 kDa) of DMP1 plays its dominant role during different stages of tooth and bone development and mineralization is currently unknown. We also observed that DMP1 was only expressed in the cytoplasm, but not in the nucleus in the two mouse odontoblastic cells (Fig.3G-L). Narayanan et al. (63) found that calcium is able to trigger DMP1 protein translocation from the nucleus into the cytoplasm in mouse pre-osteoblastic (MC3T3-E1) cells. However, the phenomenon occurred in MC3T3-E1 cells rather than in mouse fibroblast (NIH3T3) cells, suggesting cell-type specific. In addition, DMP1 expression in MO6-G3 cells was higher

than MD10-F2 cells (Fig.3G-L). This indicates that its expression is relevant to odontoblastic differentiation and mineralization (14,29-31).

Like DMP1, higher levels of DSP expression were detected in MO6-G3 cells and correspond to odontoblastic differentiation (13-14,17). It is interesting to note that DSP protein signal was also present within the nucleus in the two mouse odontoblastic cells (Fig. 5G-L). However, its role within the nucleus remains unknown. Furthermore, multiple processed fragments of mouse DSP polypeptides were detected by Western blot analysis (Fig.5Y). Qin et al. characterized 13 peptides of various regions of DSP peptides from rat teeth and identified major fragments ending at amino acid residues 409 and 421 (36). Yamakoshi et al. found that DSPP is processed into DSP, DPP and DGP in porcine teeth (37). DGP molecular weight on SDS-PAGE is 19 kDa and it contains an 81 amino acid segment located between the DSP and DPP domain. DGP has not been identified in rat and mouse teeth so far. DSP and DPP domains play different biological roles during tooth development and formation. Heterogeneous mutations of DSP domain in human are associated with dentinogenesis imperfecta type II (DGI-II) and dentin dysplasia type II (DDII) (38-39,41-42) whereas DPP domain mutations cause dentinogenesis imperfecta type III (DGI-III) (40). DSPP null mice showed teeth similar to human DGI-III with discolored teeth, enlarged pulp chambers, a wider pre-dentin zone with thin dentin wall and hypomineralization (43). In addition to dentinogenesis, DSP and DPP also have distinct functions in enamel formation. Paine et al. reported that overexpression of DSP in transgenic mice resulted in increased rate of enamel formation, but DPP overexpression created "pitted" and "chalky" enamel of non-uniform thickness that is more prone to wear as well as resulted in abnormal primatic enamel structures (44). Most of studies of DSPP-derived proteins have been performed in rat and porcine tissues (36-37). In the present study, we found the four major DSP fragments existed in the two mouse odontoblastic cells and expressional patterns of mouse DSP were different from that of rat and porcine (36-37). The differences among mouse, rat and porcine may be due to species-species variations. As a full-length DSPP protein has not been detected within cells so far, it is speculated that DSPP is catalyzed by given enzymes within cells before being secreted. Moreover, based on our observations, it indicates that BSP, OPN and MEPE proteins were processed into given fragments within the mouse odontoblastic cells.

In conclusions, our first approach was to detect expression of the SIBLING family; BSP, OPN, MEPE, DMP1, and DSP in the two mouse odontoblastic cells by immunohistochemistry assay. Besides their expression in cytoplasm, signal of MEPE and DSP was also seen in the nucleus. The second approach was to investigate processing of the five SIBLING proteins in the two mouse odontoblastic cells by Western blot analysis. The results showed that these five proteins were processed into given fragments within the two odontoblastic cells before being secreted.

Acknowledgments

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Bright	Fluorescent	Hoechst	Merge
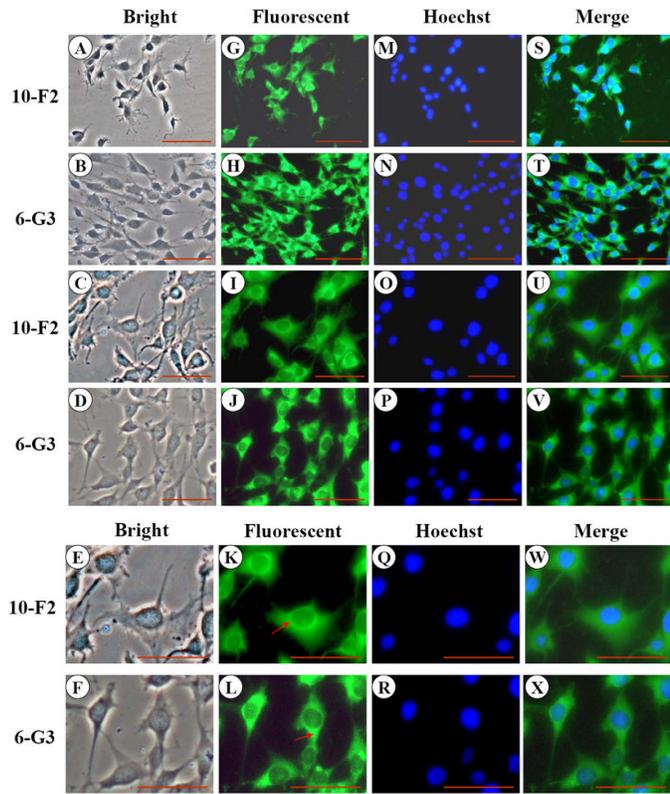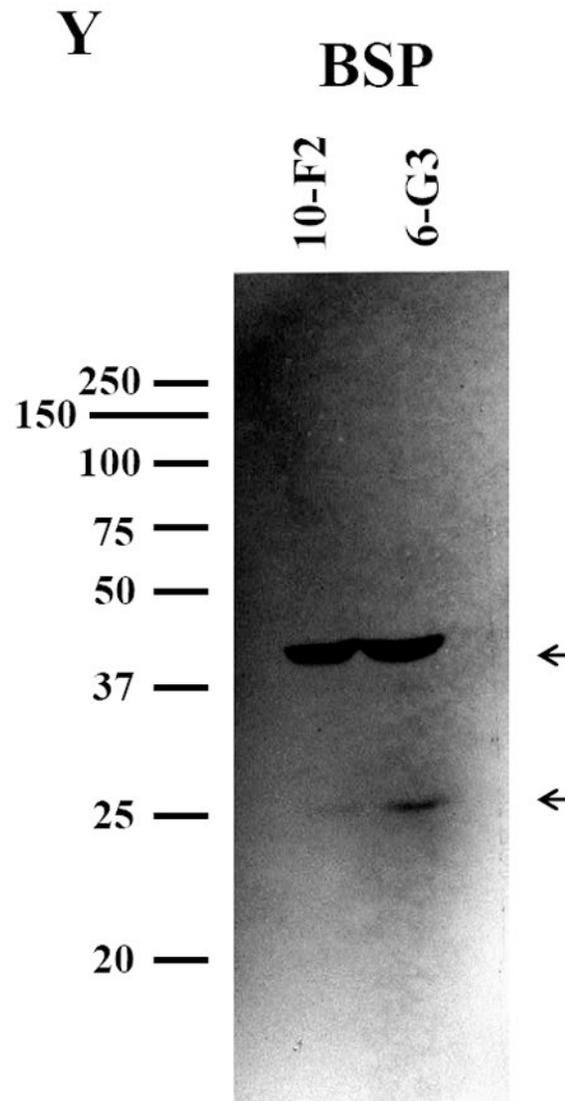
Fig. 1. BSP expression in MD10-F2 and MO6-G3 cells
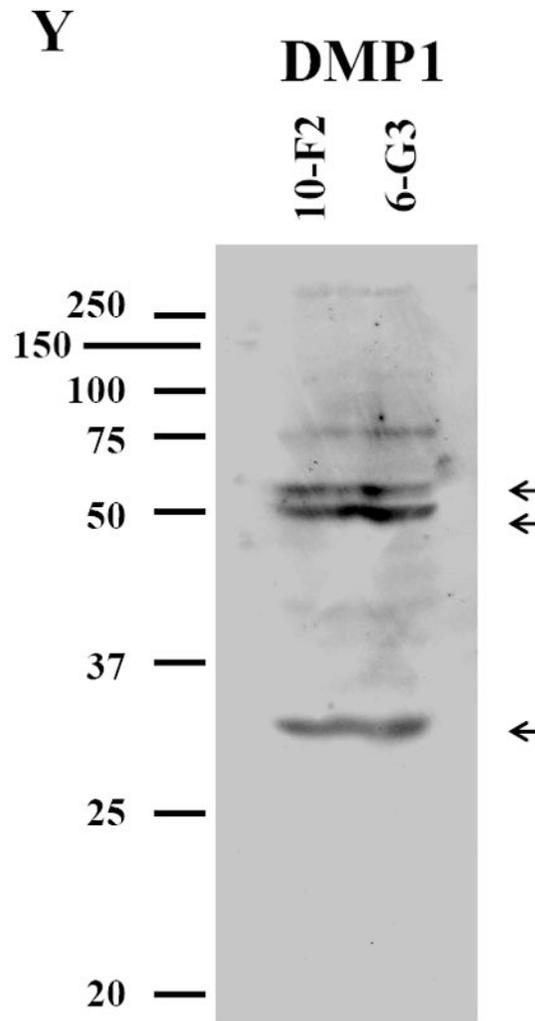**A-F**, MD10-F2 and MO6-G3 cells were photographed under a light microscope using a Nikon camera. **G-L**, The BSP expression in these two cells was analyzed by immunostaining with a specific anti-BSP antibody. **M-R**, The cells were stained with Hoechst for the nucleus. **S-X**, Images were merged. High magnification allows seeing the BSP localization to most prominent cytoplasm area. A unipolar staining of BSP is present with a triangular basis on the perinuclear area (arrows). Bar = 100 μm. **Y**, Western blot analysis of BSP expression in MD10-F2 and MO6-G3 cells. Two bands of BSP protein were detected as 39-kDa and 27-kDa on a 7% SDS-PAGE gel using anti-BSP antibody. 10-F2 and 6-G3 indicate MD10-F2 and MO6-G3 cells, respectively.
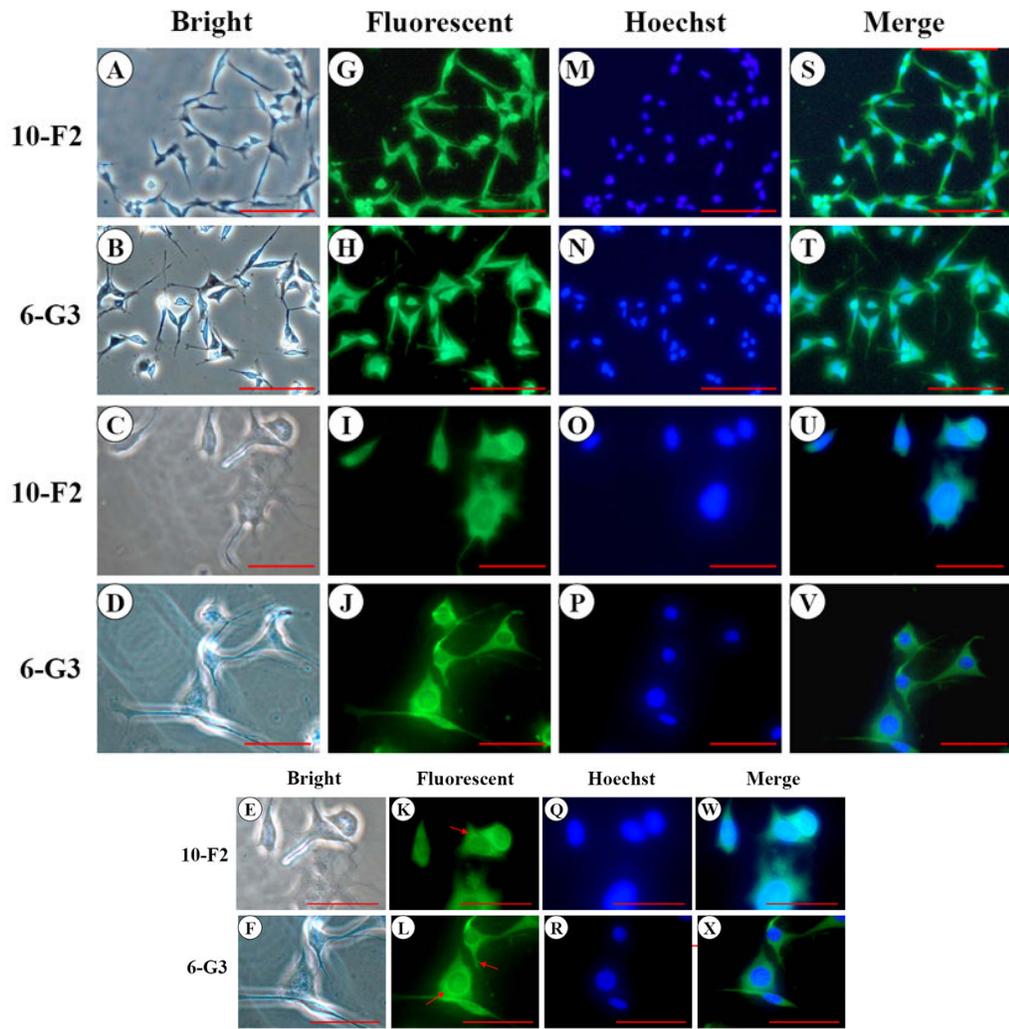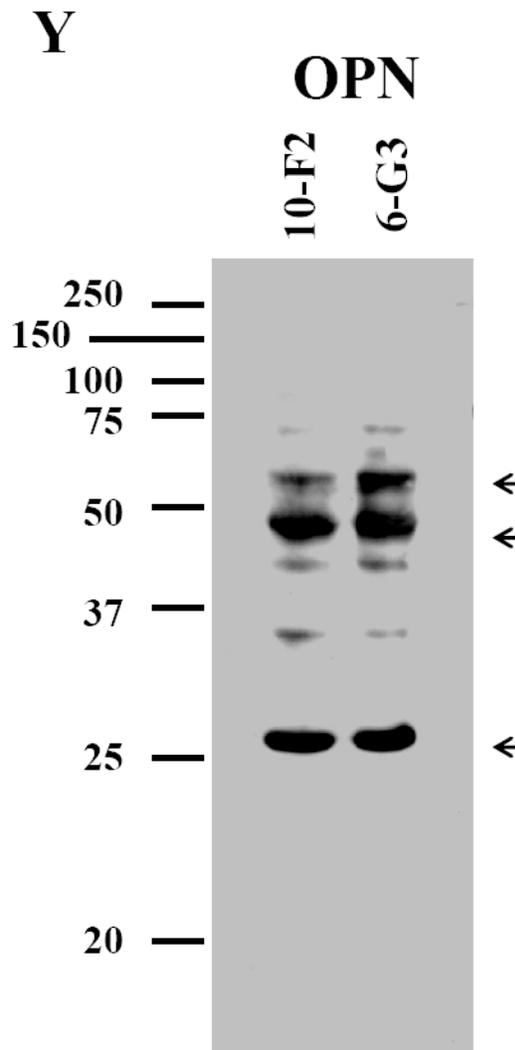
Fig. 2. OPN expression in MD10-F2 and MO6-G3 cells
A-F, Cells were photographed under a light microscope. **G-L**, The OPN expression in the two cells was analyzed by immunostaining with a primary anti-OPN antibody. **M-R**, Cells were stained with Hoechst for the nucleus. **S-X**, Images were merged from G-L and M-R. OPN signal was detected in the cytoplasm and cellular processes in both the two cells (arrows). Bar = 100 μm. **Y**, Western blot analysis of OPN expression in MD10-F2 and MO6-G3 cells. Three major bands of OPN protein were detected by protein blot analysis as 55-kDa, 45-kDa and 27-kDa polypeptides in MD10-F2 and MO6-G3 cells on a 7% SDS-PAGE gel. 10-F2 and 6-G3 denote MD10-F2 and MO6-G3 cells, respectively.
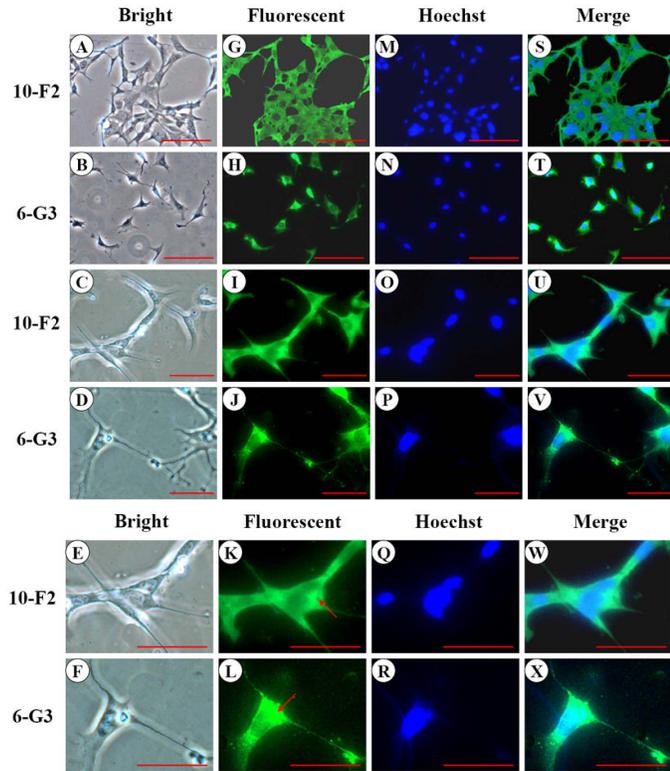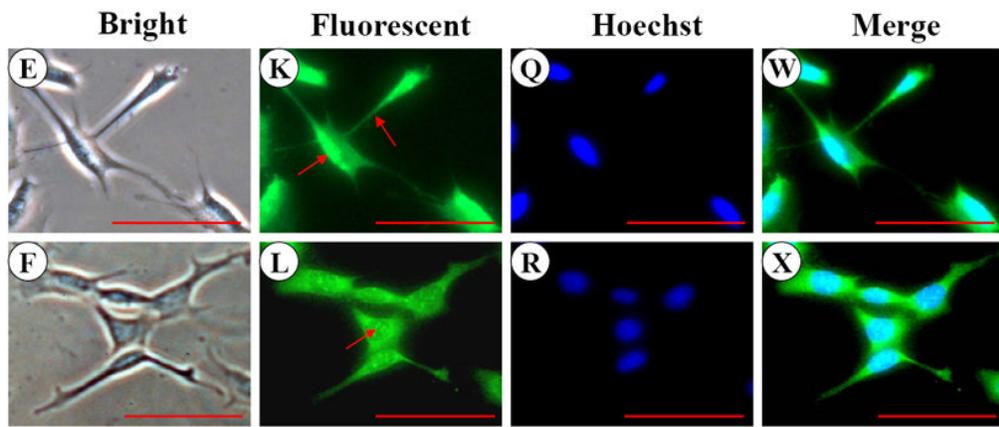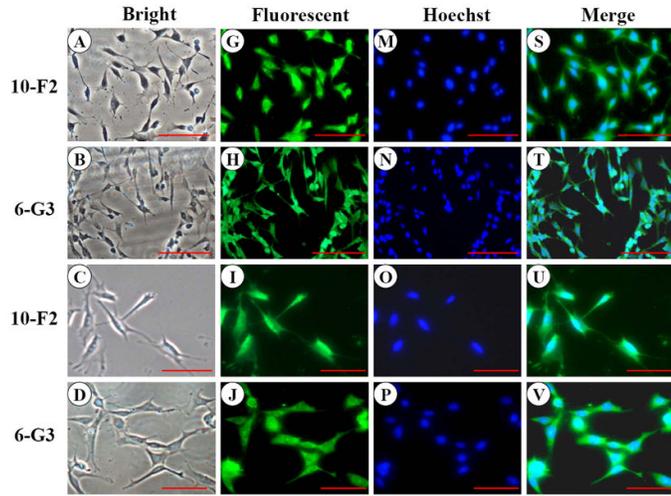
Fig. 3. DMP1 expression in MD10-F2 and MO6-G3 cells
A-F, MD10-F2 and MO6-G3 cells were photographed under a light microscope using a Nikon camera. **G-L**, The DMP1 expression in two odontoblastic cells was analyzed by immunostaining with a primary anti-DMP1 antibody. **M-R**, Cells were incubated with Hoechst dye for the nucleus staining. **S-X**, Images are composites of G-L and M-R. DMP1 was expressed in the cellular processes and cytoplasm in the two odontoblastic cells. Arrows represent high DMP1 expression in specific areas in the cells by high magnification. Bar = 100 μm. **Y**, Western blot analysis of DMP1 expression in MD10-F2 and MO6-G3 cells. Three major fragments of DMP1 were seen as 53-kDa, 51-kDa and 35-kDa, respectively in a SDS-PAGE gel using anti-DMP1 antibody. 10-F2 and 6-G3 represent MD10-F2 and MO6-G3 cells, respectively.
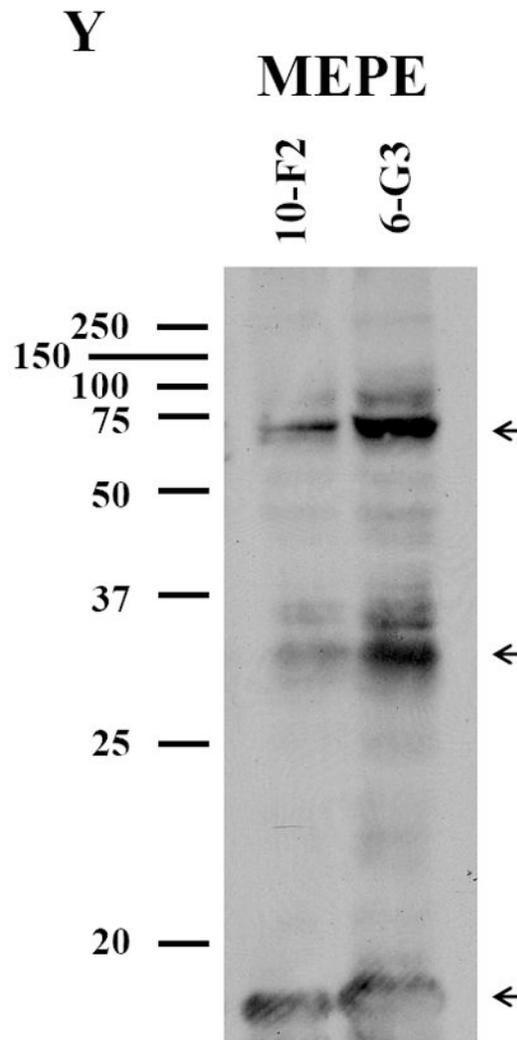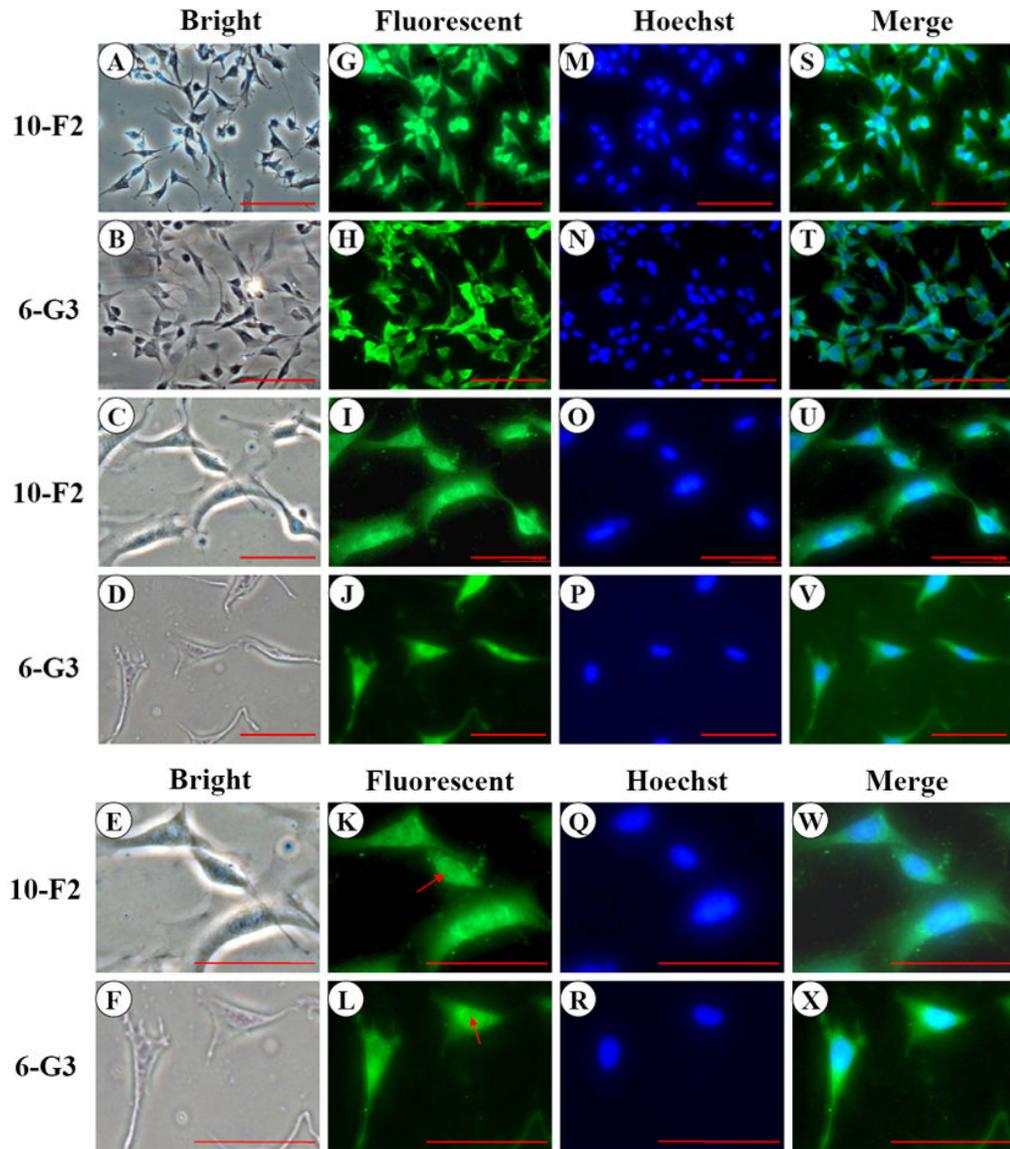
Fig. 4. MEPE expression in MD10-F2 and MO6-G3 cells
A-F, Cells were photographed under a light microscope. **G-L**, The MEPE expression in the two cells was analyzed by immunostaining with a primary anti-MEPE antibody. **M-R**, Cells were stained with Hoechst for the nucleus. **S-X**, Images were merged from G-L and M-R. MEPE signal was detected in the cytoplasm and nucleus in both mouse odontoblastic cells. Arrows show MEPE signaling within the nucleus. Bar = 100 μm. **Y**, Western blot analysis of MEPE expression in MD10-F2 and MO6-G3 cells. Three major bands of MEPE protein were detected by protein blot analysis as 70-, 30- and 10-kDa polypeptides in MD10-F2 and MO6-G3 cells on a 7% SDS-PAGE gel. 10-F2 and 6-G3 denote MD10-F2 and MO6-G3 cells, respectively.
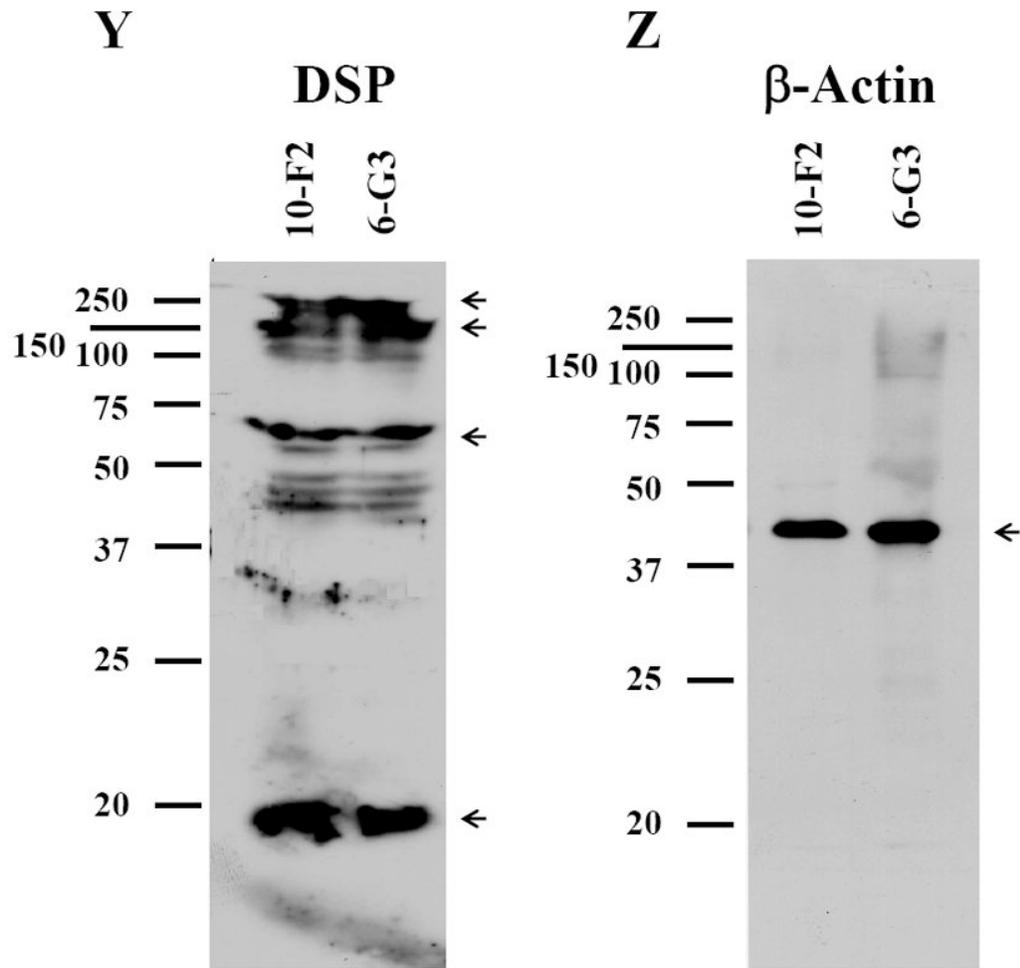
Fig. 5. DSP expression in MD10-F2 and MO6-G3 cells
A-F, Cells were photographed under a light microscope. **G-L**, The DSP expression in the two cells was analyzed by immunostaining with a primary anti-DSP antibody. **M-R**, Cells were stained with Hoechst for the nucleus. **S-X** are composites of G-L and M-R. DSP signal was seen in both the cytoplasm and nucleus in the two mouse odontoblastic cells. Arrows indicate DSP signaling within the nucleus. Bar = 100 μm. **Y**, Western blot analysis of DSP expression in MD10-F2 and MO6-G3 cells. Multiple fragments of DSP polypeptides were detected in MD10-F2 and MO6-G3 cells using anti-DSP antibody. Major bands are 260-, 170-, 70- and 15-kDa as indicated by arrows. 10F2 and 6G3 represent MD10-F2 and MO6-G3 cells, respectively. **Z**, β-actin was used as control for Western blot assay.