Korean J Oral Anatomy Vol. 43, no. 1 (2022) pp.105~116

Expression patterns of high-mobility group genes during tooth development

Minjae Kyeong[1,2], Dinuka Adasooriya[1,2], Jinsun Kim[1] and Sung-Won Cho[1,2†]

[1]Division of Anatomy and Developmental Biology, Department of Oral Biology, [2]BK21 FOUR Project, Yonsei University College of Dentistry, Seoul, Korea

접수: 2022년 12월 27일/ 수정접수: 2022년 12월 29일/ 게재 승인: 2022년 12월 30일/ 출간: 2022년 12월 31일

The high-mobility group (Hmg) superfamily comprises three nuclear protein families, Hmga, Hmgb and Hmgn, which regulate cell proliferation and transcription of several genes during development. However, there are only few reports on the expression of *Hmg* genes in developing teeth. The aim of this study was to examine the expression pattern of the *Hmg* genes, including *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2* and *Hmgn1* in developing teeth at the cap stage.

The expression pattern of the *Hmg* genes, including *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2* and *Hmgn1* was examined in developing mouse teeth at the cap stage by *in situ* hybridization assay.

The strong expression of *Hmga1*, *Hmga2*, *Hmgb1* and *Hmgn1* was detected in the dental epithelium except in the enamel knot. We found a positional match among the *Hmg* gene expression, non-*Hmg* gene expression and cell proliferation. The most prominent cell proliferation in the lingual dental epithelium where the expression of *Hmg* genes overlapped the most; the total number of non-*Hmg* genes expressed was higher in the non-enamel knot than in the enamel knot.

These results suggest the possible roles of *Hmg* proteins in regulating epithelial cell proliferation and transcription of other genes during tooth development.

Keywords: Tooth development, Hmg superfamily, gene expression, cell proliferation

Introduction

Each of the three nuclear protein families in the high-mobility group (HMG) superfamily, Hmga, Hmgb and Hmgn, participates in different cellular functions by modulating chromatin dynamics [1]. *Hmga1*$^{-/-}$ mice show cardiac hypertrophy and type 2

† Corresponding author: Sung-Won Cho

Division of Anatomy and Developmental Biology, Department of Oral Biology, Yonsei University College of Dentistry, 03722, Seoul, Korea. Tel: +82-2-2228-3065

e-mail: chosome1@yuhs.ac

diabetes [2, 3], and *Hmga2*$^{-/-}$ mice show the pygmy phenotype [4]. *Hmgb1*$^{-/-}$ and *Hmgb2*$^{-/-}$ mice do not show significant morphological defects [5, 6], but the development arrests at E9.5 in *Hmgb1*$^{-/-}$; *Hmgb2*$^{-/-}$ mice and *Hmgb1*$^{-/-}$; *Hmgb2*$^{+/-}$ mice exhibit a loss of the most posterior digit in the forelimb [7]. *Hmgn1*$^{-/-}$ mice exhibit abnormalities in the development of corneal epithelium [8].

Although Hmga is a family with no transcriptional activity, this family is able to positively or negatively regulate the transcriptional activity of several genes by modulating the chromatin structure with other transcription machinery [9-11]. The expression level of *Hmga1* and *Hmga2* is the highest during embryonic development but reduces in fully differentiated cells [12]. Homozygous mutations in both *Hmga1 and Hmga2* result in the 'superpygmy' phenotype, suggesting a role of Hmga1 and Hmga2 in cell proliferation during development [13].

Hmgb1 and *Hmgb2* are ubiquitously and highly expressed during development, suggesting their essential roles in embryonic development [6, 14]. Hmgb proteins regulate numerous activities, such as transcription, replication and repair, through interactions with nucleosome and transcription factors [15-18] by bending DNA and binding preferentially to the structurally distorted DNA [19].

Proteins in the Hmgn family are highly specific transcription modulators of several genes; they act by affecting the local or global chromatin composition and structure through protein–protein interactions with specific transcription factors [20, 21].

A tooth is an organ that develops through a series of processes, such as cell proliferation and differentiation. Thus, it is expected that Hmg superfamily genes participate in tooth development. However, there are few reports on the expression of *Hmga2* and *Hmgb1* in teeth. In the dental tissues that have already developed, Hmga2/HMGA2 protein localizes in cervical loop epithelial cells of rat incisors at post-natal day (PN) 4 and PN 8 [22] and in pulp cells of human deciduous teeth [23]. The localization of HMGB1 protein was reported in adult human gingival epithelial cells and gingival fibroblasts [24]. Among developing tissues, the *Hmga2* mRNA expression has been reported in the dental epithelium of tooth germs at the bud, cap and bell stages [25], and Hmgb1 protein expression was particularly high in ameloblasts and odontoblasts at regions of mineralization [26].

Thus, information on the expression of Hmg superfamily genes during tooth development is relatively limited. Therefore, in the present study, we examined the expression pattern of *Hmg* genes, including *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2* and *Hmgn1*, in the developing tooth at the cap stage and compared their expression patterns with those of the expression of non-*Hmg* genes and cell proliferation in order to find the possible roles of Hmg proteins in regulating the cell proliferation and transcription of other genes.

Materials and methods

Animals

All animal experiments in this study were approved by the Yonsei University College of Dentistry, Intramural Animal Use and Care Committee. Embryos at E (embryonic day) 14.5 ICR mice (Koatech Co, Pyeongtaek, Korea) were used in this study.

Whole mount *in situ* hybridization

Mandibles and maxillae were isolated from E14.5 embryos and fixed overnight in 4% paraformaldehyde and dehydrated in methanol. Hybridizations were performed as described previously [27]. on these embryos with digoxigenin-labelled cRNA probes in hybridization buffer for 20 hours at 69°C. Hybridization signals were detected by alkaline-phosphatase-conjugated anti-digoxigenin antibodies plus nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, toluidine salt substrate (Roche, Mannheim, Germany). Tissue samples were embedded in OCT compound (Tissue Tek®, Sakura Finetek, Tokyo, Japan), and sectioned at a thickness of 8 μm.

Immunohistochemistry for cell proliferation

Slides with 6 μm paraffin-embedded sections were incubated in antibody against Ki67 (Thermo Fisher Scientific) and incubated with secondary antibody and streptavidin peroxidase. Results were visualized using a DAB substrate kit (Thermo Fisher Scientific).

Gene expression pattern analysis

To identify the expression patterns of previously reported genes in the dental epithelium of molars at the cap stage, we selected genes from 'Gene expression in tooth' (http://bite-it.helsinki.fi) [28], which provides large data of gene expression during tooth development. The selected genes were classified into three groups: a group of genes expressed only in the enamel knot region, a group of genes expressed only in the dental epithelium except for the enamel knot (non-enamel knot region), and a group of genes expressed in both enamel knot and non-enamel knot regions.

Results

***Hmga1, Hmga2, Hmgb1*, *Hmgb2* and *Hmgn1* were expressed in various developing organs in the oral cavity**

The mRNA expression patterns of *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* were observed in the developing palatal rugae, tongue papillae, incisors, molars and whiskers of mouse embryos at E14.5 (Fig. 1).

In the palate, all five genes were expressed. Interestingly, *Hmga2* and *Hmgb2* were expressed on palatal rugae (Fig. 1B, D), while *Hmga1* and *Hmgn1* were expressed between palatal rugae (Fig. 1A, E). *Hmgb1* was weakly expressed in palatal rugae (Fig. 1C).

Hmga1, Hmga2, Hmgb1 and *Hmgb2* were expressed in the tongue papillae. *Hmga1* was expressed only in the circumvallate papillae and foliate papillae (Fig. 1F), and *Hmga2* and *Hmgb2* were expressed only in the fungiform papillae (Fig. 1G, I). *Hmgb1* was not expressed in the fungiform papillae but was weakly expressed in the circumvallate and foliate papillae (Fig. 1H). *Hmgn1* was not observed in tongue papillae. In addition, expressions of all five *Hmg* genes were observed in developing whiskers at E14.5.

Hmga1, Hmga2, Hmgb1 and *Hmgn1* were strongly expressed in the developing molars of maxillae and mandibles, whereas *Hmgb2* was weakly expressed in molars (Fig. 1F–H). *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* were expressed in incisors in the maxilla and mandible.

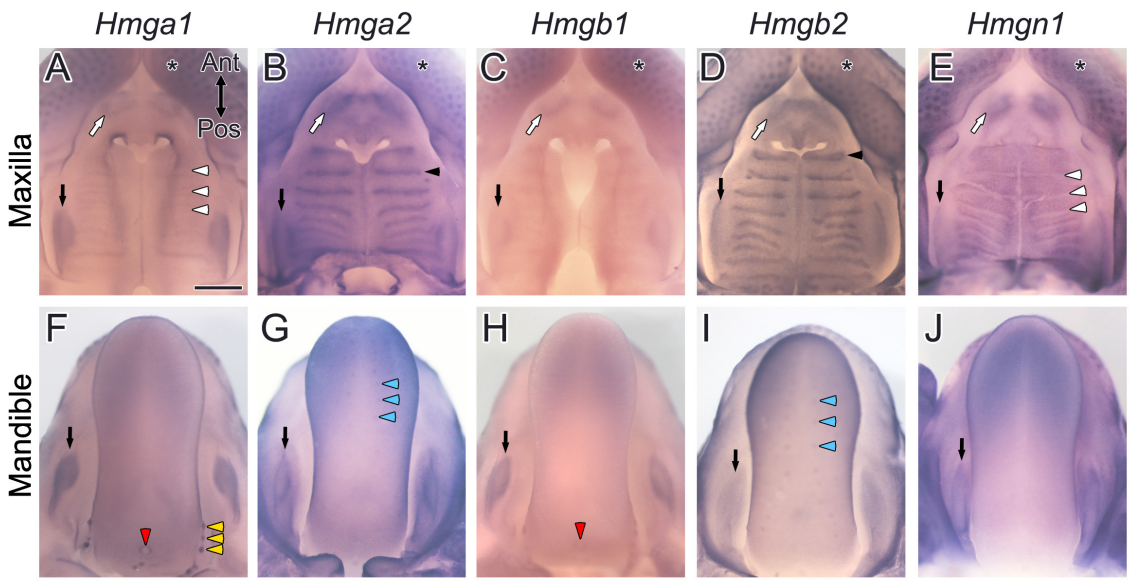

Fig. 1. Expression patterns of *Hmga1, Hmga2, Hmgb1*, *Hmgb2* and *Hmgn1* in various organs in the oral cavity of E14.5 mouse embryos. (A–E) In maxillary molars (black arrows) and incisors (white arrows), *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* are expressed. *Hmga2* and *Hmgb2* are expressed in palatal rugae (black arrowheads in B and D), while *Hmga1* and *Hmgb1* are expressed between palatal rugae (white arrowheads in A and E). Whiskers (*) show the expression of all five genes. (F–J) *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* are expressed in mandibular molars. Among tongue papillae, fungiform papillae (blue arrowheads in G and I) express *Hmga2* and *Hmgb2*; circumvallate papilla (red arrowheads in F and H) expresses *Hmga1* and *Hmgb1*; and foliate papillae (yellow arrowheads in F) express *Hmga1*. Scale bar: 500 μm

Hmga1, Hmga2, Hmgb1 and _Hmgn1_ were expressed in the dental epithelium except in the enamel knot

At E14.5 in the frontal section of the maxillary and mandibular first molars, *Hmga1, Hmga2, Hmgb1* and *Hmgn1* were strongly expressed in the dental epithelium rather than in the dental mesenchyme (Fig. 2). However, *Hmgb2* expression was not observed anywhere in the dental epithelium or the dental mesenchyme (Fig. 2D, I).

On examining the expressions in the dental epithelium in higher detail, two evidently common features were found. The first common feature was that *Hmga1, Hmga2, Hmgb1* and *Hmgn1* were not expressed in the enamel knot (Fig. 2A–C, E, F–H, J), which is known as the signaling center of developing tooth [29]. The second feature was that there was an unbalanced expression pattern of *Hmga2*, *Hmgb1* and *Hmgn1* in the dental epithelium. *Hmga2,* and *Hmgb1* were more strongly expressed in the lingual dental epithelium than in the buccal dental epithelium, whereas *Hmga1* was expressed uniformly in the dental epithelium (Fig. 2A, F). The imbalance was particularly severe in the *Hmga2* expression (Fig. 2B, D). This unbalanced expression of *Hmga2* was reproduced in the developing incisors of the maxilla and mandible at E14.5 (Fig. 3A–B).

In the dental mesenchyme, *Hmgn1* expression

was observed adjacent to the dental lamina in the mandible (Fig. 2J), and *Hmga2* and *Hmgn1* were expressed weakly in the dental follicles (Fig. 2B, G, E).

In the oral epithelium, *Hmga2* and *Hmgb2* were strongly expressed in the lingual oral epithelium in maxillary molars (Fig. 2B, D). Interestingly, in the buccal oral epithelium of the mandibular molar, the basal layer strongly expressed *Hmga1*, whereas surface layer cells strongly expressed *Hmga2* (Fig. 2F, G).

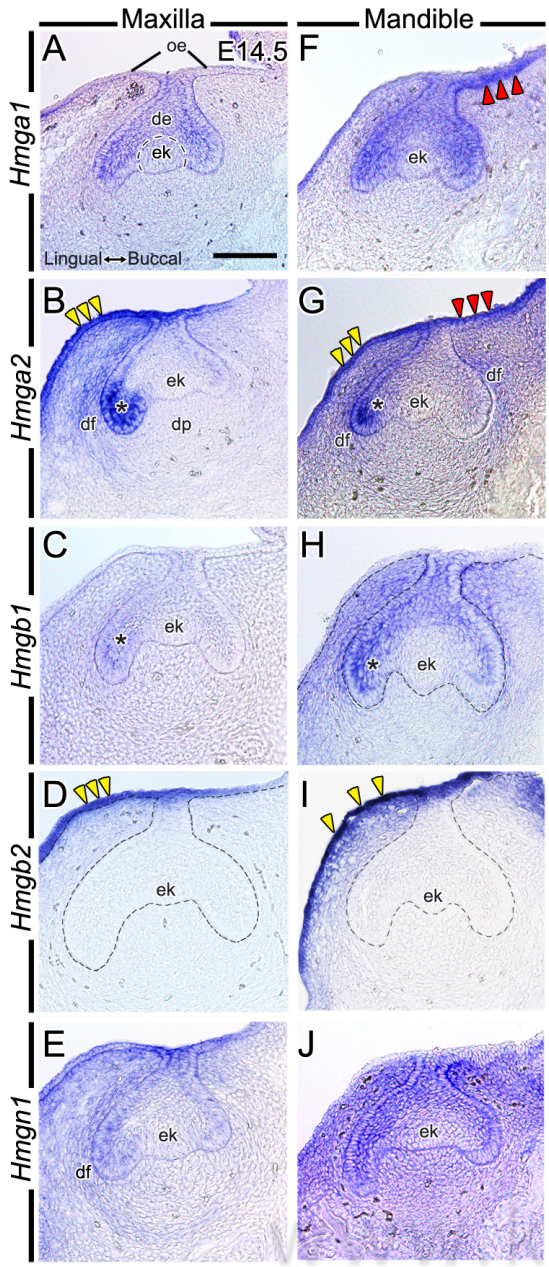

Fig. 2. Expression patterns of *Hmga1, Hmga2, Hmgb1*, *Hmgb2* and *Hmgn1* in molars of E14.5 mouse embryos. (A–J) In the frontal section of maxillary and mandibular molars, dental epithelium (de) shows the expression of *Hmga1, Hmga2, Hmgb1* and *Hmgn1* (A–C, E, F–H, J) but not of *Hmgb2* (D, I). Oral epithelium (oe) expresses *Hmga2* and *Hmgb2* (yellow arrowheads in B, D, G, I). In the buccal oral epithelium of the mandibular molar, the basal layer strongly express *Hmga1*, whereas surface layer cells strongly expresses *Hmga2* (red arrowheads in F, G). None of five genes are expressed in the enamel knot (ek in A–J). *Hmga2* and *Hmgb1* are more strongly expressed in the lingual dental epithelium than in the buccal dental epithelium (* in B–C, G–H), whereas *Hmga1* and *Hmgn1* are expressed uniformly in the dental epithelium (A, E, F, J). The dental mesenchyme shows the expression of *Hmga2* and *Hmgn1* not in the dental papilla (dp) but in the dental follicle (df in B, E, G). Scale bar: 100 μm

Cell proliferation was the most prominent where the expression of Hmg superfamily gene overlapped the most

To summarize the *Hmg* genes expressing in each part of the dental epithelium of E14.5 mandibular molar, the expression patterns of *Hmg* genes were superimposed on a schematic (Fig. 3C). The dental epithelium generally expressed several *Hmg* genes. Among them, the lingual dental epithelium expressed the largest number of *Hmg* genes, including *Hmga1, Hmga2, Hmgb1* and *Hmgn2*.

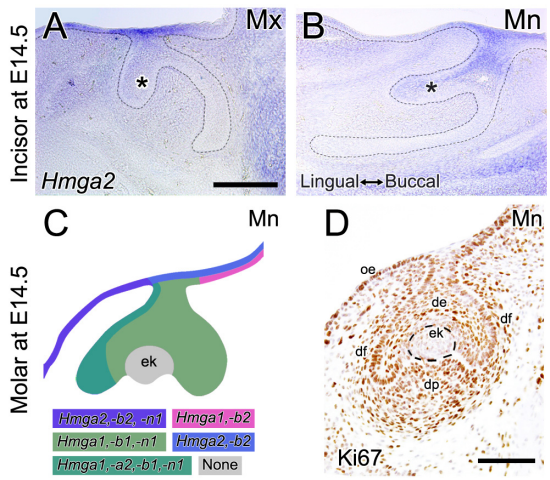

Fig. 3. Expression patterns of *Hmga2* in incisors and the localization of proliferating cells in molars at E14.5. (A–B) The expression of *Hmga2* is more pronounced in the lingual dental epithelium (* in A, B) than in the buccal dental epithelium in the developing incisors of maxilla (Mx) and mandible (Mn). (C) A schematic summarizing the patterns of *Hmg* genes expressing in the dental epithelium of E14.5 mandibular molar. (D) The Ki67-positive proliferating cells generally observed in the oral epithelium (oe), dental epithelium (de), dental papilla (dp) and dental follicle (df) but not in the enamel knot (ek). The lingual dental epithelium (*), where the expression of *Hmg* genes overlapped the most, shows a high Ki67-positivity. Scale bars: 100 μm

The fact that *Hmg* genes were not expressed in the enamel knot, where cell division is absent, led us to investigate the positional match between cell proliferation and the expression of *Hmg* genes. Ki67-positive proliferating cells were generally found in the non-enamel knot, dental papilla and dental follicle of developing teeth, but not in the enamel knot. Furthermore, the highest immunoreactivity for anti-Ki67 antibody was found in the lingual dental epithelium, where the expression of *Hmg* genes overlapped the most.

The number of genes expressed in the dental epithelium except for the enamel knot was greater than the number of genes expressed in the enamel knot

Since Hmg proteins, which are known to regulate the transcription of many genes, were expressed in the non-enamel knot region, we investigated the expression patterns of the previously reported genes in the enamel knot or non-enamel knot regions. Consequently, 132 genes expressed in the dental epithelium of molars at the cap stage were extracted from the 'Gene expression in tooth' website [28]. These genes were rearranged according to whether they were expressed in the enamel knot and/or non-enamel knot regions (Table 1). Among them, the number of genes expressed only in the enamel knot was 19, and the number of genes expressed only in the non-enamel knot was 57. The remaining 56 genes were expressed in both enamel knot and non-enamel knot regions. The enamel knot cells expressed 10 signaling molecules, whereas the non-enamel knot cells expressed 13 signaling molecules.

Discussion

The expression of *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* in various developing organs

It has been reported that *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* are ubiquitously and highly expressed in embryonic cells and play an important role in development [3, 6, 12, 14, 30-33]. Consistent with this, in the present study, *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* were expressed in various developing organs, such as palatal rugae,

Table 1. Genes expressed in the enamel knot and/or the non-enamel knot of the dental epithelium in molar at cap stage (extracted from http://bite-it.helsinki.fi)

EK only (n = 19)	non-EK only (n = 58)	EK & non-EK (n = 57)
Axin2, Bmp2, Bmp4, Bmp7, Cdkn1a, Ctgf, Edar, Edaradd, Fgf3, Fgf4, Fgf9, Jarid1b, Ndr1, Nfkbia, Shh, Slit1, Wnt10a, Wnt10b, Wnt3	Alcam, Bpag1, Cd44, Cdh1, Chuk, Col1, Col3a1, Crabp1, Creb3l1, Dlx5, Dsg, Eda, EGFR, Egr1, ErbB3, Fgf2, Fgfr1, Fgfr2, Fst, Fus, Gli1, Gli3, Hspg2, Itga6, Jag1, Krt13, Krt7, Lamc2, Lfng, Mdk, Met, Mlarp, Ngfr, Nmyc1, Notch1, Notch2, Ntf3, Ntf5, Ntrk2, Oaz1, Odc, Pcdha, Pcdhga, Ptch1, Reln, Runx1, Smo, Sostdc1, Sp6, Spry1, Spry4, Tgfb3, Timp3, Tjp1, Tjp2, Wnt4, Wnt5a, Wnt7b	Axin1, Btrc, Cdh3, Ctnnb1, Dlx2, Fadd, Fas, Fbln1, Fgf1, Gfra1, Hes1, Ikbkg, Isl1, Itgav, Itgb1, Itgb4, Itgb5, Jag2, Jup, Krt18, Krt19, Krt5, Krt8, Lama3, Lama5, Lamb3, Lef1, MFz6, Mmp14, Mmp2, Mmp9, Msx2, Ngf, Nrp2, Pace4, Pitx2, Ptch2, Rara, Robo1, Ror1, Ror2, Rxra, Rxrb, Sdc1, Sema3c, Sema3f, Spry2, Tgfb1, Tnfrsf19, Tnfsf6, Traf1, Traf2, Traf3, Traf4, Traf6, Vim, Wnt6

tongue papillae, incisors, molars and whiskers, at E14.5. Just as whiskers expressed all five genes, expressions of some genes overlapped among some organs in the oral cavity. However, there were no genes whose expression patterns were exactly the same. For example, the expression patterns of *Hmga2* and *Hmgb2* were similar in both palatal rugae and fungiform papillae but were different in molars.

Positional identity between cell proliferation and *Hmg* gene expression in developing teeth

A close relationship between cell proliferation and Hmg proteins has been previously reported in many types of cells and tumor tissues. HMGA1 is enriched in hematopoietic stem cells [34, 35] and is up-regulated in medulloblastoma and gastric cancer cells [36, 37]. *HMGA2* knockdown suppressed the proliferation of human lung carcinoma cells, whereas the ectopic expression of *HMGA2* in human adenocarcinomic alveolar basal epithelial cells increased cell proliferation [38]. *Hmga2*$^{+/-}$ and *Hmga2*$^{-/-}$ mice showed a pygmy phenotype with a decrease in body size of 60% and 25%, respectively, relative to the wild-type mice size [4, 39]. *HMGA2* expression is increased in acute myeloid leukemia [40]. Hmgb1 enhances embryonic neural stem cell proliferation in rats [41], and HMGB1 overexpression has been reported in melanoma [42]. *Hmgb2* knockdown by siRNA inhibited cell proliferation in rat hepatocellular carcinoma cells [43]. In hepatocellular carcinoma, HMGB2 was co-localized with a cell proliferation marker [44]. HMGN1 promotes both human B cell proliferation *in vitro* and B cell acute lymphoblastic leukemia *in vivo* [45].

In the present study, we found the same relationship between cell proliferation and *Hmg* gene expression in the epithelium of developing teeth at the cap stage. Among *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2* and *Hmgn1*, none were expressed in the enamel knot where cell division does not occur, whereas cell proliferation was the most prominent where the expression of *Hmg* genes overlapped the most.

Among the tumors in the oral cavity, the tumor most associated with human *HMG* genes is Oral squamous cell carcinoma (OSCC), which accounts for more than 90% of all oral malignancies and

approximately 40% of head and neck tumors [46]. OSCC is characterized by a high mortality because of the high incidence of nodal metastasis and high recurrence rate. HMGA2 and HMGB1 proteins were found to be highly expressed in human OSCC [47, 48], and the high expression of HMGA2 and HMGB1 induce the reduced recurrence-free Survival and poor prognosis of OSCC [49, 50]. On the other hand, HMGA1 and HMGB2 are known to be independent of OSCC [51, 52], and the relationship between HMGN1 and OSCC has not been reported. Interestingly in the present study, the lingual dental epithelium, which most strongly shows cell proliferation, is the only place where expressions of *Hmga2* and *Hmgb1* overlap. These results suggest that *Hmga2* and *Hmgb1* are more closely related to cell proliferation than other *Hmg* genes.

In the mesenchyme of developing teeth at the cap stage, their relationship was not observed. The active cell proliferation in the dental mesenchyme, where *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2* and *Hmgn1* genes were either not expressed or weakly expressed, suggests that mesenchymal cell proliferation may be regulated by other genes, which should be clarified in the future.

Possible role of *Hmg* genes in regulation of many genes in the non-enamel knot region of developing tooth

The enamel knot is a signaling center in the dental epithelium that secretes many signaling molecules during tooth development [29]. In the present study, by investigating the expression patterns of the previously reported genes in the enamel knot or non-enamel knot regions, we found that both the number of signaling molecules secreted and the total number of genes expressed are significantly higher in the non-enamel knot than in the enamel knot. This finding implies that the non-enamel knot is as important as the enamel knot as a signaling center during tooth development.

The presence of *Hmga1, Hmga2, Hmgb1* and *Hmgn1* expression in the non-enamel knot, where more genes are expressed, leaves open the possibility that the expression of genes in the non-enamel knot region may be related with Hmg proteins in developing tooth at the cap stage.

In summary, *Hmga1, Hmga2, Hmgb1, Hmgb2* and *Hmgn1* were expressed in various developing organs in the oral cavity at E14.5. *Hmga1*, *Hmga2*, *Hmgb1* and *Hmgn1* were expressed in the non-enamel knot of the dental epithelium. Cell proliferation was the most prominent where the expression of *Hmg* genes overlapped the most. The total number of genes were significantly higher in the non-enamel knot than in the enamel knot. This positional match among the *Hmg* gene expression, non-*Hmg* expression and cell proliferation suggests the possible roles of Hmg proteins in regulating the epithelial cell proliferation and the transcription of other genes during tooth development.

Acknowledgement

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT). (No.2020R1A2C2005790).

References

1. Hock R, Furusawa T, Ueda T, Bustin M. HMG chromosomal proteins in development and disease. Trends Cell Biol. 2007;17(2):72-9. Epub 2006/12/16. doi: 10.1016/j.tcb.2006.12.001.
2. Foti D, Chiefari E, Fedele M, Iuliano R, Brunetti L, Paonessa F, et al. Lack of the architectural factor HMGA1 causes insulin resistance and diabetes in humans and mice. Nat Med. 2005;11(7):765-73. Epub 2005/06/01. doi: 10.1038/nm1254.
3. Fedele M, Fidanza V, Battista S, Pentimalli F, Klein-Szanto AJ, Visone R, et al. Haploinsufficiency of the Hmga1 gene causes cardiac hypertrophy and myelo-lymphoproliferative disorders in mice. Cancer Res. 2006;66(5):2536-43. Epub 2006/03/03. doi: 10.1158/0008-5472.
4. Zhou X, Benson KF, Ashar HR, Chada K. Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. Nature. 1995;376(6543):771-4. Epub 1995/08/31. doi: 10.1038/376771a0.
5. Calogero S, Grassi F, Aguzzi A, Voigtlander T, Ferrier P, Ferrari S, et al. The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. Nat Genet. 1999;22(3):276-80. Epub 1999/07/03. doi: 10.1038/10338.
6. Ronfani L, Ferraguti M, Croci L, Ovitt CE, Scholer HR, Consalez GG, et al. Reduced fertility and spermatogenesis defects in mice lacking chromosomal protein Hmgb2. Development. 2001;128(8):1265-73.
7. Itou J, Taniguchi N, Oishi I, Kawakami H, Lotz M, Kawakami Y. HMGB factors are required for posterior digit development through integrating signaling pathway activities. Dev Dyn. 2011;240(5):1151-62. Epub 2011/03/09. doi: 10.1002/dvdy.22598.
8. Birger Y, Davis J, Furusawa T, Rand E, Piatigorsky J, Bustin M. A role for chromosomal protein HMGN1 in corneal maturation. Differentiation. 2006;74(1):19-29. Epub 2006/02/10. doi: 10.1111/j.1432-0436.2006.00054.x.
9. Johnson KR, Lehn DA, Reeves R. Alternative processing of mRNAs encoding mammalian chromosomal high-mobility-group proteins HMG-I and HMG-Y. Mol Cell Biol. 1989;9(5):2114-23. Epub 1989/05/01.
10. Reeves R, Nissen MS. The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. J Biol Chem. 1990;265(15):8573-82.
11. Thanos D, Du W, Maniatis T. The High-Mobility Group Protein Hmg I(Y) Is an Essential Structural Component of a Virus-Inducible Enhancer Complex. Cold Spring Harbor Symposia on Quantitative Biology. 1993;58:73-81. doi: Doi 10.1101/Sqb.1993.058.01.011.
12. Sgarra R, Rustighi A, Tessari MA, Di Bernardo J, Altamura S, Fusco A, et al. Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. Febs Letters. 2004;574(1-3):1-8. doi: 10.1016/j.febslet.2004.08.013.
13. Federico A, Forzati F, Esposito F, Arra C, Palma G, Barbieri A, et al. Hmga1/Hmga2 double knock-out mice display a "superpygmy" phenotype. Biology Open. 2014;3(5):372-8. doi: 10.1242/bio.20146759.
14. Pauken CM, Nagle DL, Bucan M, Lo CW. Molecular cloning, expression analysis, and chromosomal localization of mouse Hmg1-containing sequences. Mamm Genome. 1994;5(2):91-9.
15. Bianchi ME, Agresti A. HMG proteins: dynamic players in gene regulation and differentiation. Curr Opin Genet Dev. 2005;15(5):496-506. Epub 2005/08/17. doi: 10.1016/j.gde.2005.08.007.
16. Agresti A, Bianchi ME. HMGB proteins and gene expression. Current Opinion in Genetics & Development. 2003;13(2):170-8. doi: 10.1016/s0959-437x(03)00023-6.
17. Bonaldi T, Langst G, Strohner R, Becker PB, Bianchi ME. The DNA chaperone HMGB1 facilitates ACF/CHRAC-dependent nucleosome sliding. EMBO J. 2002;21(24):6865-73. Epub 2002/12/18. PubMed PMID: 12486007; PubMed Central PMCID: PMCPMC139112.
18. Travers AA. Priming the nucleosome: a role for HMGB proteins? EMBO Rep. 2003;4(2):131-6. Epub 2003/03/04. doi: 10.1038/sj.embor.embor741.

19. Thomas JO, Travers AA. HMG1 and 2, and related 'architectural' DNA-binding proteins. Trends in Biochemical Sciences. 2001;26(3):167-74. doi: 10.1016/s0968-0004(01)01801-1.

20. Zhu N, Hansen U. HMGN1 modulates estrogen-mediated transcriptional activation through interactions with specific DNA-binding transcription factors. Molecular and Cellular Biology. 2007;27(24):8859-73. doi: 10.1128/Mcb.01724-07.

21. Zhu N, Hansen U. Transcriptional regulation by HMGN proteins. Biochim Biophys Acta. 2010;1799(1-2):74-9. Epub 2010/02/04. doi: 10.1016/j.bbagrm.2009.11.006.

22. Ge Y, Kong Z, Guo Y, Tang W, Guo W, Tian W. The role of odontogenic genes and proteins in tooth epithelial cells and their niche cells during rat tooth root development. Arch Oral Biol. 2013;58(2):151-9. Epub 2012/05/23. doi: 10.1016/j.archoralbio.2012.04.017.

23. Kaukua N, Chen M, Guarnieri P, Dahl M, Lim ML, Yucel-Lindberg T, et al. Molecular differences between stromal cell populations from deciduous and permanent human teeth. Stem Cell Research & Therapy. 2015;6. doi: ARTN 5910.1186/s13287-015-0056-7.

24. Morimoto Y, Kawahara KI, Tancharoen S, Kikuchi K, Matsuyama T, Hashiguchi T, et al. Tumor necrosis factor-alpha stimulates gingival epithelial cells to release high mobility-group box 1. J Periodontal Res. 2008;43(1):76-83. Epub 2007/12/11. doi: 10.1111/j.1600-0765.2007.00996.x.

25. Uchibe K, Shimizu H, Yokoyama S, Kuboki T, Asahara H. Identification of novel transcription-regulating genes expressed during murine molar development. Dev Dyn. 2012;241(7):1217-26. Epub 2012/05/29. doi: 10.1002/dvdy.23808.

26. Sugars R, Karlstrom E, Christersson C, Olsson ML, Wendel M, Fried K. Expression of HMGB1 during tooth development. Cell Tissue Res. 2007;327(3):511-9. Epub 2006/10/18. doi: 10.1007/s00441-006-0293-2. PubMed PMID: 17043795.

27. Kim JS, Seo HJ, Cho SW. Gene expression patterns of Etv5 and Etv6 in molar tooth germs at the cap stage. Kor J oral Anat. 2017;38(1):49-54. doi: 10.35607/kjoa.38.1.201712.005.

28. Nieminen P, Pekkanen M, Åberg T, Thesleff I. A graphical WWW-database on gene expression in tooth. European Journal of Oral Sciences. 1998;106(S1):7-11. doi: 10.1111/j.1600-0722.1998.tb02146.x.

29. Thesleff I. Epithelial-mesenchymal signalling regulating tooth morphogenesis. Journal of Cell Science. 2003;116(9):1647-8. doi: 10.1242/jcs.00410.

30. Liu J, Schiltz JF, Ashar HR, Chada KK. Hmga1 is required for normal sperm development. Mol Reprod Dev. 2003;66(1):81-9. Epub 2003/07/23. doi: 10.1002/mrd.10323.

31. Caron L, Bost F, Prot M, Hofman P, Binetruy B. A new role for the oncogenic high-mobility group A2 transcription factor in myogenesis of embryonic stem cells. Oncogene. 2005;24(41):6281-91. Epub 2005/07/12. doi: 10.1038/sj.onc.1208781. PubMed PMID: 16007198.

32. Furusawa T, Lim JH, Catez F, Birger Y, Mackem S, Bustin M. Down-regulation of nucleosomal binding protein HMGN1 expression during embryogenesis modulates Sox9 expression in chondrocytes. Mol Cell Biol. 2006;26(2):592-604. Epub 2005/12/31. doi: 10.1128/MCB.26.2.592-604.2006.

33. Lehtonen S, Lehtonen E. HMG-17 is an early marker of inductive interactions in the developing mouse kidney. Differentiation. 2001;67(4-5):154-63. Epub 2001/10/31. doi: 10.1046/j.1432-0436.2001.670407.x.

34. Nelson DM, Joseph B, Hillion J, Segal J, Karp JE, Resar LM. Flavopiridol induces BCL-2 expression and represses oncogenic transcription factors in leukemic blasts from adults with refractory acute myeloid leukemia. Leuk Lymphoma. 2011;52(10):1999-2006. Epub 2011/07/07. doi: 10.3109/10428194.2011.591012.

35. Chou BK, Mali P, Huang X, Ye Z, Dowey SN, Resar LM, et al. Efficient human iPS cell derivation by a non-integrating plasmid from blood cells with unique epigenetic and gene expression signatures. Cell Res. 2011;21(3):518-29. Epub 2011/01/19. doi: 10.1038/cr.2011.12.

36. Lau KM, Chan QK, Pang JC, Ma FM, Li KK, Yeung WW, et al. Overexpression of HMGA1 deregulates tumor growth via cdc25A and alters migration/invasion through a cdc25A-independent pathway in medul-

loblastoma. Acta Neuropathol. 2012;123(4):553-71. Epub 2012/01/18. doi: 10.1007/s00401-011-0934-8.

37. Akaboshi S, Watanabe S, Hino Y, Sekita Y, Xi Y, Araki K, et al. HMGA1 is induced by Wnt/beta-catenin pathway and maintains cell proliferation in gastric cancer. Am J Pathol. 2009;175(4):1675-85. Epub 2009/09/05. doi: 10.2353/ajpath.2009.090069.
38. Gao X, Dai M, Li Q, Wang Z, Lu Y, Song Z. HMGA2 regulates lung cancer proliferation and metastasis. Thorac Cancer. 2017;8(5):501-10. Epub 2017/07/29. doi: 10.1111/1759-7714.12476.
39. Anand A, Chada K. In vivo modulation of Hmgic reduces obesity. Nat Genet. 2000;24(4):377-80. Epub 2000/03/31. doi: 10.1038/74207.
40. Tan L, Wei X, Zheng L, Zeng J, Liu H, Yang S, et al. Amplified HMGA2 promotes cell growth by regulating Akt pathway in AML. J Cancer Res Clin Oncol. 2016;142(2):389-99. Epub 2015/09/01. doi: 10.1007/s00432-015-2036-9. PubMed PMID: 26319392.
41. Wang L, Yu L, Zhang T, Wang L, Leng Z, Guan Y, et al. HMGB1 enhances embryonic neural stem cell proliferation by activating the MAPK signaling pathway. Biotechnol Lett. 2014;36(8):1631-9. Epub 2014/04/22. doi: 10.1007/s10529-014-1525-2.
42. Pardo M, Garcia A, Thomas B, Pineiro A, Akoulitchev A, Dwek RA, et al. The characterization of the invasion phenotype of uveal melanoma tumour cells shows the presence of MUC18 and HMG-1 metastasis markers and leads to the identification of DJ-1 as a potential serum biomarker. Int J Cancer. 2006;119(5):1014-22. Epub 2006/03/30. doi: 10.1002/ijc.21942.
43. Kwon JH, Kim J, Park JY, Hong SM, Park CW, Hong SJ, et al. Overexpression of high-mobility group box 2 is associated with tumor aggressiveness and prognosis of hepatocellular carcinoma. Clin Cancer Res. 2010;16(22):5511-21. Epub 2010/09/21. doi: 10.1158/1078-0432.CCR-10-0825.
44. Stros M, Ozaki T, Bacikova A, Kageyama H, Nakagawara A. HMGB1 and HMGB2 cell-specifically down-regulate the p53- and p73-dependent sequence-specific transactivation from the human Bax gene promoter. J Biol Chem. 2002;277(9):7157-64. Epub 2001/12/19. doi: 10.1074/jbc.M110233200.
45. Lane AA, Chapuy B, Lin CY, Tivey T, Li H, Townsend EC, et al. Triplication of a 21q22 region contributes to B cell transformation through HMGN1 overexpression and loss of histone H3 Lys27 trimethylation. Nat Genet. 2014;46(6):618-23. Epub 2014/04/22. doi: 10.1038/ng.2949.
46. Beenken SW, Urist MM. Head and neck tumors 11th ed. Way LWD, G. M., editor. New York: Medical Books/McGraw-Hill; 2003.
47. Miyazawa J, Mitoro A, Kawashiri S, Chada KK, Imai K. Expression of Mesenchyme-Specific Gene HMGA2 in Squamous Cell Carcinomas of the Oral Cavity. Cancer Research. 2004;64(6):2024-9. doi: 10.1158/0008-5472.Can-03-1855.
48. Sasahira T, Kirita T, Oue N, Bhawal UK, Yamamoto K, Fujii K, et al. High mobility group box-1-inducible melanoma inhibitory activity is associated with nodal metastasis and lymphangiogenesis in oral squamous cell carcinoma. Cancer Sci. 2008;99(9):1806-12. Epub 2008/07/12. doi: 10.1111/j.1349-7006.2008.00894.x.
49. Fang CY, Liew PL, Chen CL, Lin YH, Fang CL, Chen WY. High HMGA2 Expression Correlates with Reduced Recurrence-free Survival and Poor Overall Survival in Oral Squamous Cell Carcinoma. Anticancer Res. 2017;37(4):1891-9. Epub 2017/04/05. doi: 10.21873/anticanres.11527.
50. Supic G, Kozomara R, Zeljic K, Stanimirovic D, Magic M, Surbatovic M, et al. HMGB1 genetic polymorphisms in oral squamous cell carcinoma and oral lichen planus patients. Oral Dis. 2015;21(4):536-43. Epub 2015/02/03. doi: 10.1111/odi.12318.
51. Sterenczak KA, Eckardt A, Kampmann A, Willenbrock S, Eberle N, Langer F, et al. HMGA1 and HMGA2 expression and comparative analyses of HMGA2, Lin28 and let-7 miRNAs in oral squamous cell carcinoma. BMC Cancer. 2014;14:694. Epub 2014/09/24. doi: 10.1186/1471-2407-14-694.
52. Syed N, Chavan S, Sahasrabuddhe NA, Renuse S, Sathe G, Nanjappa V, et al. Silencing of high-mobility group box 2 (HMGB2) modulates cisplatin and 5-fluorouracil sensitivity in head and neck squamous cell carcinoma. Proteomics. 2015;15(2-3):383-93. Epub 2014/10/21. doi: 10.1002/pmic.201400338.

한글초록

치아발생 중 *Hmg* 유전자의 발현 패턴

경민재[1,2], Dinuka Adasooriya[1,2], 김진선[1], 조성원[1,2]

연세대학교 치과대학 [1]구강생물학교실, [2]BK21 창의치의학융합 교육연구단

High-mobility group (Hmg) 유전자 집단은 발생 동안 세포 증식 및 여러 유전자의 전사를 조절하는 세 가지 핵-단백질 유전자인 Hmga, Hmgb 및 Hmgn으로 구성된다. 지금까지 치아발생 과정동안 *Hmg* 유전자의 발현에 대한 보고는 없었다. 본 연구의 목적은 모자기 시기의 치배에서 *Hmga1, Hmga2, Hmgb1, Hmgb2* 및 *Hmgn1*을 포함하는 *Hmg* 유전자의 발현 패턴을 조사하는 것이다.

Hmga1, Hmga2, Hmgb1 및 *Hmgn1*의 강한 발현은 법랑질결절(enamel knot)을 제외한 치아상피에서 발현되었다. Hmg 유전자의 발현이 가장 많이 중첩되는 설측 치아 상피에서 가장 두드러진 세포 증식이 관찰된 것으로 보아, *Hmg* 유전자 발현과 세포 증식 간의 긴밀한 상관관계가 있는 것으로 판단된다. 또한 치배에서 발현되는 많은 *non-Hmg* 유전자들이 법랑질결절 외부에서 발현되는 것을 확인하였다. 이러한 결과는 치아발달 동안 상피 세포 증식 및 다른 유전자의 전사를 조절하는 Hmg 단백질의 가능한 역할을 시사한다.

주제어: 치아발생, Hmg 유전자 그룹, 유전자 발현, 세포 증식