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Expression profile of polycomb group proteins in odontogenic keratocyst and ameloblastoma

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ABSTRACT

Polycomb group (PcG) proteins are repressive chromatin modifiers required for proliferation and development. PcG proteins form two large repressive complexes, namely, Polycomb Repressive Complex 1 and 2. These proteins have been shown to drive tumorigenesis by repressing cell-type specific sets of target genes. Using immunohistochemistry, we investigated the expression patterns of five human PcG proteins, including Bmi-1, Ring1b, Mel-18, Ezh2, and Suz12, in various cellular components of odontogenic keratocysts (OKCs), ameloblastomas and, pericoronal follicles (PFs). In OKCs, expression of PcG proteins were found in the majority of cases while the expression pattern was relatively different for each PcG proteins. All PcG proteins were strongly expressed in the basal cells while some proteins showed variable expression in the parabasal and luminal cell layer of OKCs. In ameloblastomas, almost all PcG proteins showed a similar expression pattern of moderate to strong staining in the peripheral ameloblast-like cells and metaplastic squamous cells. Some of the central stellate reticulum-like cells also showed positive reaction to most PcG proteins. In PFs, most PcG proteins were intensely expressed in odontogenic epithelium lining the follicles, except Mel-18 and Suz12. The present study provides the initial evidence regarding epigenetic involvement by PcG proteins in these odontogenic lesions. Although these proteins are known to be in the same repressive group proteins, differential expression patterns of these proteins in OKCs and ameloblastomas indicates that these proteins may play different roles in pathogenesis of these odontogenic lesions.

1. Introduction

Tumor development has traditionally been considered to be affected by genetic changes, however, during the past decade it has increasingly become evident that abnormal epigenetic alterations are also implicated in tumorigenesis (Hatziapostolou and Iliopoulos, 2011). Among various epigenetic regulators, polycomb group (PcG) proteins have been shown to play a pivotal role in tumor development through the control of cellular proliferation, differentiation, and invasion. These complexes modify histone tails and silence specific sets of target genes by altering higher-order chromatin structure (Gil et al., 2005; Piunti and Pasini, 2011). PcG proteins were originally identified in fruit fly as repressors of *Homeotic* genes, which are necessary for the establishment of the body plan and segmentation. PcG proteins are structurally and functionally diverse and form large multimeric complexes of two main types: Polycomb repressive complex-1 (PRC1) and PRC2. The PRC1, which is essential for maintaining transcriptional repression, contains Bmi-1, Mel-18, Ring1a, Ring1b, and others. The PRC2, which is involved in the initiation of transcriptional silencing, contains Ezh2, Suz12, and various isoforms of EED (Ringrose and Paro, 2004).

It has been shown that human PcG proteins had a tissue- and cell-type specific expression pattern and play important roles in numerous biological functions, such as control of cell differentiation and regulation of cellular proliferation (Gil et al., 2005). The pathologic role of PcG in human cancer is largely unknown but some evidences have been recognized in the literature (Kidani et al., 2009; Martinez-Romero et al., 2009; Piunti and Pasini, 2011). Bmi-1, one of the core subunits of the PRC1, is believed to promote cellular proliferation by suppressing the $p16^{Ink4a}/p19^{Arf}$ locus (Molofsky et al., 2005). Overexpression of Bmi-1 is found in several cancers such as nasopharyngeal carcinoma (Song et al., 2006), and oral squamous cell carcinoma (Kang et al., 2007). Mel-18, whose protein product is structurally highly similar to Bmi-1, is reported to act as a tumor suppressor by repressing Bmi-1 and c-Myc (Guo et al., 2007). Mel-18 overexpression inhibits proliferation of breast cancer cells (Lee et al., 2008). Ring1b or Rnf2, a critical component of the PRC1, is upregulated in pancreatic ductal adenocarcinoma (Martinez-Romero et al., 2009). Overexpression of Enhancer of zeste homolog 2 (Ezh2), a member of the PRC2, is correlated with

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malignant potential and poor prognosis in oral squamous cell carcinomas (Kidani et al., 2009). Expression of Suz12 is deregulated in several tumors, including mantle cell lymphoma and lung cancer (Liu et al., 2014; Martin-Perez et al., 2010).

Among odontogenic cysts and tumors, odontogenic keratocyst (OKC) and ameloblastoma are the two most common lesions, representing approximately 29–41% and 20–39%, respectively (Luo and Li, 2009; Osterne et al., 2011; Tawfik and Zyada, 2010). Previously, odontogenic keratocyst was named as keratocystic odontogenic tumor (KCOT) and has been moved back into the cyst category, renamed as odontogenic keratocyst in the 2017 WHO classification of head and neck tumor (El-Naggar et al., 2017). Both lesions are regarded as benign lesion, however, they show locally invasive behavior with a marked tendency to recur (Barnes et al., 2005). Even though the etiology and pathogenesis of these odontogenic lesions is still unknown, previous studies have identified various transcriptional alterations that may be responsible for their development and progression (Gomes et al., 2009; Kumamoto, 2006). However, very few studies on epigenetic alterations in these odontogenic lesions have been found in the literature (Farias et al., 2012; Kitkumthorn and Mutirangura, 2010; Moreira et al., 2009). We hypothesized that PcG proteins may be involved in the pathogenesis of these odontogenic lesions. The purpose of the present study is to investigate the immunohistochemical expression of selected PcG proteins, including Bmi-1, Mel-18, Ring1b, Ezh2, and Suz12, in OKCs, ameloblastomas and, PFs.

2. Material and methods

2.1. Sample selection

This study received ethical approval from the Institutional Review Board, Mahidol University. A total of 40 cases of OKCs and ameloblastomas were collected from the tissue block archive of the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Mahidol University. These consisted of 20 OKCs (9 men, 11 women; mean age 30) and 20 ameloblastomas (13 men, 7 women; mean age 34.15), including 9 follicular, 11 plexiform. There were 5 cases concomitantly showing more than one histological type: follicular and acanthomatous. OKC cases associated with Nevoid basal cell carcinoma syndrome (NBCCS), ortho-keratotic, and recurrent OKCs were excluded. Recurrent ameloblastomas were also excluded from the study. Specimens of pericoronal follicles (PFs) were included as non-neoplastic odontogenic tissue. The PFs were collected from impacted mandibular third molars that were devoid of inflammation and fully covered by mucosa or bone. The immunohistochemical expression of PFs represented expression in normal odontogenic tissue.

Most OKCs occurred in the mandible (85%), with the posterior region and ramus being the most frequent anatomic site (88.2%) and 3 cases located in the maxilla region (15%). All ameloblastomas were found in the mandible, including 16 cases (80%) in the posterior mandible and ramus, and 4 cases (20%) arising in the anterior region.

2.2. Immunohistochemistry

For immunohistochemical studies, the paraffin blocks were cut into approximately 5 μm thick sections on 3-aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, Missouri, USA) coated slides. The sections were deparaffinized with xylene and rehydrated. For antigen retrieval, the sections were immersed in 0.4% pepsin (Sigma Chemical Co., St Louis, MO, USA) at 37 °C, 1 h for antibodies against Bmi-1, Ring1b, Ezh2, and Suz12. For antibody against Mel-18, the sections were microwaved in citrate buffer (pH 6.0) and cooled in room temperature. Endogenous peroxidase activity was blocked by incubating the sections with 3% H_2O_2. The slides were incubated in primary antibody against Bmi-1, Mel-18, Ezh2, Ring1b, and Suz12 (Table 1) and then incubated in the labeled polymer (Envision+ System/HRP; Dako Corporation, Carpinteria, CA, USA) for 30 min each. Diaminobenzidine (Sigma-Aldrich, St. Louis, Missouri, USA) was used as a chromogen and the sections were counterstained with hematoxylin. Negative control sections were done by omission of the relavant primary antibody. Positive controls for all antibodies were also performed (Table 1).

Table 1
Details of the primary antibodies and positive controls used in the study.

Antibody	Type of antibody	Manufacturer	Dilution	Positive control
Bmi-1	Rabbit Polyclonal	Santa Cruz, TX, USA (sc-10745)	1:100	Oral mucosa
Mel-18	Rabbit Polyclonal	Santa Cruz, TX, USA (sc-10744)	1:20	Kidney
Ezh2	Rabbit Polyclonal	Abcam, Cambridge, UK (ab84989)	1:100	Tonsil
Ring1b	Rabbit Polyclonal	Abcam Cambridge, UK (ab28629)	1:75	Kidney
Suz12	Rabbit Polyclonal	Bioss, MA, USA (LS-B5591)	1:75	Kidney

The immunohistochemical reactivity was descriptively evaluated with regard to location and intensity. Five scattered areas of each sample were selected which were rich in lesional epithelial cells and devoid of inflammation. For epithelial cells of OKCs, expression of these proteins was emphasized on (1) the basal cell layer, (2) the parabasal layer (one-to-two cell layers above the basal layer), and (3) the luminal layer (the remaining epithelial layers up to the lumen). For tumor cells of ameloblastomas, expression of these proteins was considered in 3 cell types: (1) peripheral ameloblast-like cells, (2) central stellate reticulum-like cells and (3) metaplastic squamous cells (in acanthomatous cases). For PFs, the epithelial lining cells were assessed. The expression of these proteins was also evaluated for the fibroblast-like or spindle cells adjacent to the lesional epithelial cells. The intensity of immunostaining in each cellular components of the lesions was evaluated and classified into four groups: (−), no reactivity; (+), weak reactivity; (++), moderate reactivity; (+++), strong reactivity.

3. Results

Summary of immunohistochemical findings are shown in Table 2 and illustrated in Figs. 1–4.

3.1. Polycomb group protein expression in OKCs

Immunohistochemical staining for Bmi-1, Ring1b, Mel-18, Ezh2, and Suz12 antigen was detected in 19 (95%), 20 (100%), 15 (75%), 19 (95%), and 17 (85%) cases of OKCs, respectively. Bmi-1 expression was prominently localized in the cytoplasm of the basal and the luminal cells of the epithelial lining (Fig. 1B). Weak expression of Bmi-1 was noted in the parabasal cell layer of the epithelial lining while its expression was absent in the fibroblasts beneath the epithelial lining. Intense Ring1b expression was observed in the cytoplasm and sometimes nucleus of the basal cells of the epithelial lining whereas its expression in the parabasal and luminal layer of the epithelial lining was weak to moderate (Fig. 1C). Weak to moderate immunostaining of Ring1b was detected in some fibroblasts of the subjacent connective tissue. Nuclear and sometimes cytoplasmic expression of Mel-18 was diffusely observed in both the basal and parabasal cells of the epithelial lining while weaker staining of Mel-18 was seen in the luminal layer (Fig. 1D). In the connective tissue below the lining, moderate to strong staining of Mel-18 was found in the fibroblasts of the subjacent connective tissue. The nuclear expression of Ezh2 was strongly noticed in almost all epithelial layers of the cystic lining as well as in the fibroblasts of the connective tissue below the epithelial lining (Fig. 1E). Cytoplasmic expression of Suz12 was found in all layers of the epithelial lining with a little stronger intensity in the basal layer. Weak Suz12

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