Immunohistochemical expression of HBp17/FGFBP-1, FGF-1, FGF-2, CD34, p53, pRB, and Ki67 in Ameloblastomas

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Abstract

Ameloblastoma, a benign odontogenic tumor, has locally invasive behavior with high recurrence rate. Ameloblastoma are divided into 4 histological subtypes including unicystic ameloblastoma (UA), solid/multicystic ameloblastoma (SMA), desmoplastic ameloblastoma (DA) and extraosseous/peripheral ameloblastoma (EA) according to World Health Organization (W.H.O) classification. The balance between proliferation, apoptosis, and tumor angiogenesis play pivotal roles in the tumor growth and progression as well as in the molecular etiology.

Several studies have evaluated the expression of Ki67, p53, pRB, fibroblast growth factor (FGF)-1, FGF-2 as well as microvessel density (MVD) in ameloblastoma, but little is known about correlation of pRB with Ki67, p53 as well as the expression and the correlations of heparin-binding protein 17 (HBp17)/FGF binding protein (FGFBP)-1 and FGF-1, FGF-2, MVD in ameloblastomas. To elucidate the molecular roles and the correlations of these factors in ameloblastomas, the expression of pRB, p53, Ki67, HBp17/FGFBP-1, FGF-1, FGF-2, and CD34 was studied immunohistochemically and examined correlation between the each protein expression statistically.

Materials and methods:

Paraﬃn sections (4μm) from 29 primary ameloblastomas were examined immunohistochemically by using Ki67 antibody (Monoclonal Mouse, Dako,
1:100), p53 antibody (monoclonal Mouse, Dako, 1:50), pRB antibody (monoclonal Mouse, Santa Cruz, 1:100), CD34 antibody (monoclonal Mouse, Dako, ready to use), HBp17/FGFBP-1 antibody (monoclonal Mouse, R&D systems, 1:100), FGF-1 antibody (polyclonal Rabbit, Santa Cruz, 1:100) and FGF-2 antibody (monoclonal Mouse, abcam, 1:2000). For Ki67, pRB, CD34 and p53 antigen retrieval, deparaffinized sections were pretreated with heat-induced epitope retrieval method prior to blocking endogenous peroxidase activity. After blocking endogenous peroxidase activity using hydrogen peroxide, the sections were incubated with normal goat serum and then incubated overnight with the primary antibodies as described above. For double staining, the sections were incubated with first primary antibody of CD34 for 30 minutes then further incubated overnight with second primary antibody of Ki67 (or p53) followed by incubation with the corresponding secondary antibody for 30 minutes at room temperature. Immunoreactivities were then visualized using Dako envision kits.

For the evaluation of each protein expression, the following formula has been employed:

- The labeling index (LI) (%): \( LI = \frac{\text{Number of positive cells}}{\text{Total cells}} \times 100 \);

- The digital expression index (dEI) (ou/pixel): \( dEI = \frac{LI \times dISI}{100} \);

- The digital immunostaining intensity (dISI) (ou/pixel): \( dISI = \frac{255 \times (SOD - TOD)}{SOD} \);

- The microvessel density (MVD): \( MVD = \frac{\text{Total number microvessels}}{1 \text{ mm}^2 \text{ stroma}} \);

- The percentage of microvessel’s area size in stroma (pTS) (%): \( pTS = \frac{\text{Total microvessels area size}}{\text{Stroma area size}} \times 100 \);

- The estimated tumor volume (eV) (mm³) on orthopantomogram: \( eV = \frac{H \times W \times L}{2} \)
(H & L: Height & Length of tumor measured on orthopantomogram (mm)).

Several clinical factors were evaluated and studied for the correlation with the each protein expression, statistically.

**Results:**

A few scattered Ki67-positive cells were restricted to the peripheral/basal cells and occasionally seen in the stellate reticulum-like cells. The immunoreactivity of p53, FGF-1, FGF-2, HBp17/FGFBP-1, and pRB was mainly observed in the peripheral with some spreading to the stellate reticulum-like cells. The immunohistochemical intensity of these proteins in the reticulum-like cell areas was lower than that in the peripheral/basal cell areas whereas there is no significant difference in the intensity of Ki67 between these areas.

Immunoreactivity for pRB, p53, and Ki67 was mainly recognized in the nuclei while HBp17/FGFBP-1, FGF-1, and FGF-2 was mainly observed in the cytoplasm of peripheral/basal cells, squamous cells as well as in the stellate reticulum-like cells although the strongest staining intensity was seen in the squamous cells and peripheral/basal cells. The membrane of peripheral/basal cells was found to be positive with HBp17/FGFBP-1 and FGF-2. The microvessels in stroma exhibited slightly irregular shape, tortuous and dilated form.

It has been found that a pRB dEI positively correlated with p53 dEI (p<0.001), Ki67 dEI (p=0.009) and that p53 dEI with Ki67 dEI (p=0.02) whereas no correlation between p53 LI and Ki67 LI. Moreover, HBp17/FGFBP-1 dEI was also found to be correlated with Ki67 dEI (p=0.002), FGF-1 dEI (p<0.001), and FGF-2 dEI (p<0.001) as well as with MVD (p<0.001) and pTS (p<0.001). In addition, the significantly positive correlation was observed in MVD, and pTS with FGF-1 dEI (p<0.001), and FGF-2 dEI
(p<0.001), as well as the significantly positive correlation of Ki67 dEI with FGF-1 dEI (p=0.004), and FGF-2 dEI (p=0.038). In addition, the positive correlation of p53 dEI and HBp17/FGFBP-1 dEI (p<0.001), FGF-1 dEI (p<0.001), FGF-2 dEI (p<0.001), MVD (p<0.001) and pTS (p<0.001) was obtained. Furthermore, pTS, but not MVD correlated well with Ki67 LI (p=0.001), Ki67 dEI (p=0.002). Finally, an estimated tumor volume calculated from the X-ray pantomogram significantly correlated with HBp17 dEI (p=0.024), and pRB dEI (p=0.008). The significant correlation between age of patients and the expression of pRB was also found and no significant difference in the expression of these factors between SMA and UA.

**Discussion:**

In this study, it has been revealed that Ki67 dEI correlated well with p53 dEI suggesting dEI is more useful indicator than LI in evaluating the immunohistochemical expression. The positive correlation between pRB and p53, Ki67 as well as that between p53 and Ki67 suggested that pRB plays an important role in the balance between the proliferation and apoptosis and that the loss of this balance due to the loss of precise function of either pRB or p53 might be involved in the etiology of ameloblastomas. In addition, the positive correlations of p53 and HBp17/FGFBP-1, FGF-1, FGF-2 as well as tumor MVD suggested the function of p53 in tumor angiogenesis. Moreover, the positive correlation of HBp17/FGFBp-1 and FGF-1, FGF-2 with both Ki67 and MVD strongly suggested that HBp17/FGFBp-1 together with FGF-1 and FGF-2 might play important roles in the progression of the tumor growth through stimulating tumor angiogenesis.

Taken together, the pRB, p53, Ki67, HBp17/FGFBP-1, FGF-1, FGF-2 and tumor microvessels play important roles in the development and the progression of ameloblastomas.