

Study of Amelogenin Gene Expression in Ameloblastoma

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Abstract: Although it has been known that amelogenin gene is expressed in ameloblastoma, the precise expression pattern of X and Y amelogenin genes (AMGX, AMGY) in this tumor has not yet identified. In this study, we analyzed amelogenin gene expression in 19 samples (9 male, 10 female) of oral ameloblastomas by RT-PCR and detect the chromosomal origin of amelogenin mRNA by restriction enzyme digestion of the RT-PCR product. All tumor samples expressed amelogenin mRNA. We could detect increased level of AMGY expression in all male samples, higher than that of AMEX. It is an interesting finding as in normal male tooth development, the expression of AMGY is very much lower than that of AMGX. We postulate that epigenetic change of sex chromosomes may have some correlations with tumorigenesis of ameloblastoma. We also discuss the other possible mechanisms and points for future studies on this the change in expression pattern.

Key words: Amelogenin, AMGX, AMGY, Ameloblastoma, RT-PCR, In Situ Hybridization

Introduction

Ameloblastoma is the most common odontogenic tumor, which has close histological resemblance to the enamel organ of the developing tooth germ. In the early stages of tooth development, the internal enamel epithelial cells of the enamel organ differentiate into ameloblasts. These ameloblasts secrete a number of enamel matrix proteins. Among these enamel proteins, amelogenin is the major extracellular matrix protein of developing enamel. In normal developing tooth germ, amelogenin, secreted from the ameloblasts, accounts for 60-90% of the total enamel matrix during the first phase of mineral deposition.

Human beings has two copies of amelogenin, coded on the short arms of X and Y sex chromosomes (AMGX and AMGY). AMGX is coded on Xp21.1-22.3 region and AMGY is located at Yp11.2 region. Both amelogenin genes contain 7 exons and they shared more than 90% sequence homology in the coding regions. AMGX and AMGY transcripts are differentially spliced, resulting multiple alternative splicing variants of mRNA from each gene. In normal male developing tooth bud, the expression of amelogenin is more than 90% from the X chromosome and approximately 10% from the Y chromosome.

Materials and Methodes

Total 19 samples of ameloblastomas, 9 samples from the male patients and 10 samples from the female.

Primers

To amplify all mRNA variants from both amelogenin genes, internal primers spanning 320bp of exon 6 were used. The gene-specific primer sequences are:

[F]-5'CTCATCACCCACATCCCAGT3' and

[R]-5'GCTTGGTCTTGTCTGTTG3' respectively.

RT-PCR

Total RNA from each sample was isolated from paraffin sections using Paraffin block RNA isolation kit (Ambion, U.S.A). cDNA was synthesized using AMV Reverse Transcriptase First-strand

cDNA Synthesis Kit (Invitrogen, USA). cDNA was then amplified by PCR using gene specific primers.

In Situ Hybridization

The sections were hybridized with digoxigenin-11-UTP labeled amelogenin RNA probe at 50°C. After hybridization the sections were washed thoroughly with series of SSC. Anti-digoxigenin-AP Fab fragment (Roche, Germany) in DIG 1 buffer was applied to the sections and incubated for 30 min at room temperature. After immunoreaction, the slides were washed and equilibrated with DIG 1 and DIG 3 buffer respectively and stained with NBT/BCIP (Roche, Germany) solution in DIG 3 buffer at 37°C until the signal-noise ratio was maximum.

Restriction enzyme digestion

The PCR products were purified by Wizard SV Gel and PCR Clean-Up System (Promega, USA) and then treated with Hinf I restriction enzyme at 37°C. The restriction fragments were differentiated on 8% PAGE. The relative abundances of X and Y amelogenin transcripts of the male samples were then determined by comparing the bands densities by Image Quant software.

Results

Detection of Amelogenin Gene Expression by RT-PCR

All male and female ameloblastoma patients expressed amelogenin mRNA.

Distribution of Amelogenin mRNA in Ameloblastoma

To detect the spatial distribution of amelogenin mRNA in the tumor tissue on cell-by-cell basis, we also did the in situ hybridization with digoxigenin labeled anti-sense RNA probe. The in situ signals of the amelogenin mRNA were depicted in the cytoplasm of the peripheral epithelial cells of the tumor nest [Fig. 1].

Determination of the Chromosomal Origin of Amelogenin mRNA in Ameloblastoma

When the PCR products were digested with Hinf I enzyme, X

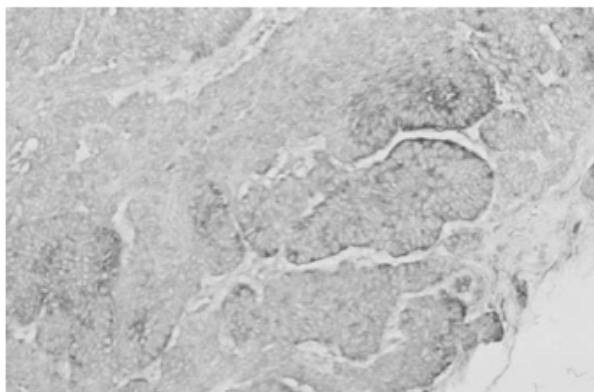


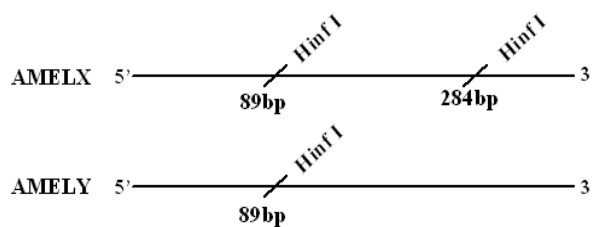
Fig.1 In Situ localization of amelogenin mRNA in ameloblastoma.

chromosomal products have 2 cutting sites resulting in 3 restriction fragments and Y chromosomal product have 1 cutting site resulting 2 restriction fragments respectively [Fig. 2].

Then, 195bp X restriction fragments and 235bp Y restriction fragments were separated by PAGE, 195bp X amelogenin fragments alone was detected in female samples but both 195bp X restriction fragments and 235bp Y restriction fragments were detected in male samples [Fig. 3]. All Y amelogenin bands were denser than that of X amelogenin bands. It means that the expression of Y amelogenin mRNA was higher than that of X amelogenin mRNA in the male ameloblastoma patients. This is a very interesting finding as this expression pattern of AMGX and AMGY in male patients is clearly different from that of normal male developing tooth germ.

Conclusion

- All ameloblastoma samples expressed amelogenin gene by RT-PCR. The expression was recognized in the peripheral cells of the tumor nests by ISH
 - The expression of AMELY was increased to the same as or more than that of AMELX in male patient unlike the expression pattern of normal male amelogenesis
- We postulate that the epigenetic change of sex chromosomes may have some correlations with tumorigenesis of ameloblastoma.



AMELX		AMELY	
Hinf I	: GATTC : 2 Sites	Hinf I	: GATTC : 1 Sites
90 - 284	: 195 base	90 - 324	: 235 base
1 - 89	: 89 base	1 - 89	: 89 base
285 - 324	: 40 base		

Fig.2 The principle used to detect the chromosomal origins (AMGX or AMGY) of amelogenin mRNA by Hinf-1 digestion of the RT-PCR products.

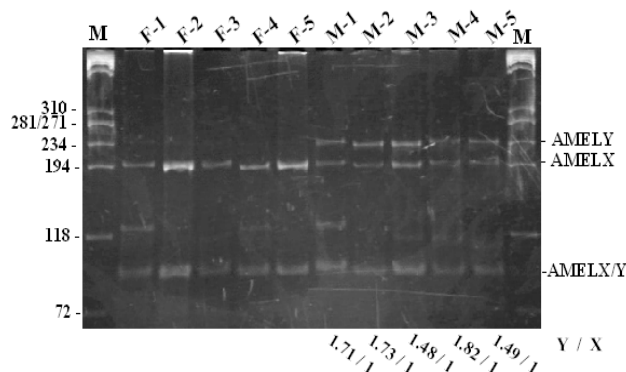


Fig.3 The representative results of Hinf-1 restriction enzyme digestion on 8% PAGE.