Stomatologija, Baltic Dental and Maxillofacial Journal, 6:73-6, 2004

The Ameloblastous Potentiality of Odontogenous Epithelium Demonstrated in Tissue Culture

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SUMMARY

Several theories have been put forward concerning the origin of the neoplastic epithelium in ameloblastoma. Because of the doubt on the possible origin of ameloblastomas from cells with odontogenic potentiality, the aim of the present was to observe the development and growth pattern of odontogenic epithelium and tooth germs in tissue culture. Fourteen molar tooth germs consisting enamel organ and dental papilla of fourteen days old mouse embryos were cultured in a medium on millipore filters. THWP 0.45 μ porosity and 25 ± 5 μ thin. Cultivation was terminated at 21st day. Explants were embedded in cellulose-paraffin for histology. After 2 days cultivation, the epithelial outgrowth of enamel organ was observed, and 2 days later was visible structure resembled the pattern of the dental lamina often seen in ameloblastomas. Histological sections of specimens showed that the outer epithelium has differentiated into a lamellar-like structure and the enamel organ has similar pattern of the ameloblastoma. Formation of microcysts and continued formation of epithelial buds was observed. Tissue culture studies of mouse embryo tooth germs and enamel organ demostrated epithelial proliferation from the borders of odontogenic epithelium and the formation of lamellar-like structure observed in ameloblastomas. This study offers additional experimental evidence that ameloblastomas orginate from epithelium with odontogenic potentiality.

Key-words: Enamel organ, tissue culture, ameloblastomatous potentiality

INTRODUCTION

Ameloblastomas are rare epithelial tumors of odontogenic origin, accounting for 1% of all tumors and cysts in the head and neck region and 11% of all odontogenic tumors (1,2). Ameloblastoma is justly considered the most unexplainable of odontogenic tumors, because of its clinical and histological features, intriguingly contradictory, paradoxal and incongruent, if its benign histological ascept and its invasive and destructive clinical behavior are considered. Histologically ameloblastoma epithelium resembles that of the enamel organ of the developing tooth. The peripherally located columnar cells in the tumor follicles resemble developing ameloblasts of the tooth germ and the centrally located cells those of stellate reticulum (3). Furthermore, some ameloblastomas share features with basal or squamous cell carcinomas, squamous odontogenic tumors, adamantinomas of the long bones or craniopharyngiomas of the central nervous system (4,5,6,7).

Several theories have been put forward concerning the origin of the neoplastic epithelium in ameloblastoma. It has been suggested to arise directly from the enamel organ of developing tooth, the remnants of odontogenic epithelium, the lining of odontogenic cyst or the basal cell layer of oral mucosa or epidermis (8, 9).

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The epithelial dental lamina signales to the mesenchyme during tooth initiation, and thereafter the mesenchymic cells regulate epithelial morphogenesis. Shape development is regulated by signals from the epithelial enamel knot and the dental papilla mesenchyme. The signal molecules (many of them are called growth factors) belong to several families and four of them have been particulary intensley studied during recent years: the hedgehodges (hh), the bone morphogenic protein, the fibroblast growth factors and the Wnt-family signaling molecules (10, 11, 12, 13).

Heikinheimo *et al*. 1989 studied the expression of cytokeratins and vimetin in various histological types of ameloblastoma and in human fetal tooth germ. Cytokeratins nos 8 and 19 were expressed in all epithelial elements of ameloblastomas and tooth germs. A similar coexpression was detected in the stellate reticulum cells of developing tooth. Ameloblastoma and human tooth germ epithelial share pattern of cytokeratin polypetides together with coexpression of vimetin. These results strongly support the theory that ameloblastomas are of odontogenic origin and not direct derivates of basal cells of oral epithelium or epidermis (14). It is accepted that defects in the craniofacial hard tissues, also in enamel and dentin in many cases results from disturbances in the extracellulare matrix molecules, such as collagens and enamel proteins (15, 16). The use of BMP in the stimulation of bone development is already being tested in clinical trial. Tooth development proceeded until cap stage when they were cultured in the presence of BMP.

This rescue was based on the observation that one function of the MSX I gene in the tooth buds is to stimulate the production of BMP (17).

The uncertain histogenesis of ameloblastoma has promped speculations about the possible relationship between the surface epithelium and ameloblastoma (18). It is known, that some additional stimulus is essential to initiate amelogenesis. The inner enamel epithelium ordinarily gives

rise to ameloblasts, which are enamel-forming cells. However, the ameloblastomas arises from the preameloblasts before their becoming ameloblasts in the sytage of dental proliferation (morphodifferentiation, Bell stage). This occurs early in life, although no clinical activity may become manifest until many years later (19). However, the coexpression of cytokeratins and vimetin and the expression of cytokeratins Nos 8 and 19 in most of the ameloblastomas studied speak against their gingival or epidermal origin (20).

Because of the doubt on the possible origin of ameloblastomas from cells with odontogenic potentiality, the aim of the present study was to observe the development and growth pattern of odontogenic epithelium and tooth germs in tissue culture.

MATERIAL AND METHODS

Fourteen molar tooth germs of 14 days old mice embryos were removed in aseptical conditions in a Hank´s solution. The explant consisted only of the enamel organ and the dental papilla. A stainless grid 20x20x30mm was placed in a Petri dish with the medium consisting of 80% medium 199, 15% horse serum , 4 mg/ml glucose, 0.07 mg/ml ascorbic acid, 100 u per ml penicillin and streptomycin were added. The pieces of millipore filters 0.6 cm^2 with tooth germs were placed on the platform of the grid. The specimens were placed on the surface of millipore filter resting on a metal screen so that the medium filled the culture vessel to the surface of the screen. The filter membrane used were millipore membranes THWP 0.45µ porosity and 25 ± 5 µ thick. All the explants were gassed briefly with a mixture of 50% O_2 + CO_2 gas phase in an air-tight containers and 50% N_2 and incubated at 37 °C and 100% humidity. The medium was changed after 48-72 hours. The pH was maintained at 7.6. Cultivation of the tooth germs was terminated up to 21 days. Microscopic observations were made daily on the living cultures. Bouin´s solution was used as a fixative. Tissue were embedded in cellulose-paraffin, serially sectioned at 8-10 µ and stained with hematoxylin-eosin.

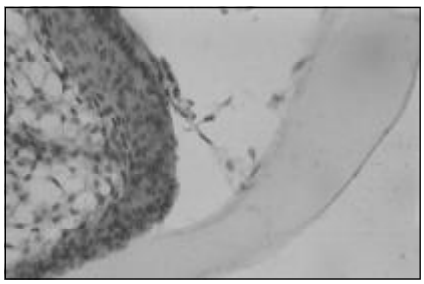


Figure 1. Initiation of the epithelial outgrowth of the mouse molar tooth enamel organ along the millipore filter after 2 days of cultivation. Note small epithelial cells, cyanophilic and polyhedral or spindle shaped. H&E x 200.

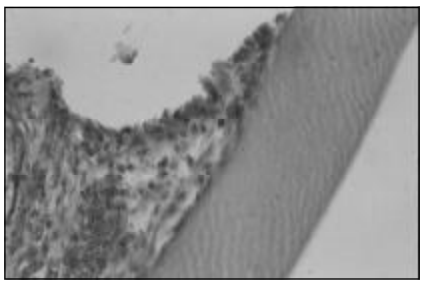


Figure 2. Mouse molar tooth germ explant cultured in vitro for 4 days continuation of more intensive epithelial outgrowth of the outer layer of enamel organ along the millipore filter. H&E x 200.

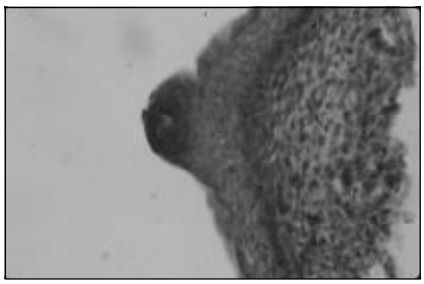


Figure 3. Mouse molar tooth germ explant after 9 days cultivation showing epithelium bud outgrowth from outer epithelial layer of enamel organ. H&E x 200.

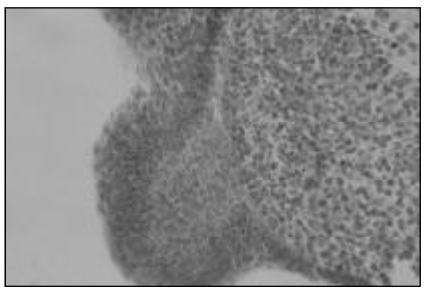


Figure 4. Mouse molar tooth germ explant after 9 days cultivation from which the epithelium of the outer layer of the enamel organ exhibited laterally disposed outgrowth bud. H&E x 400.

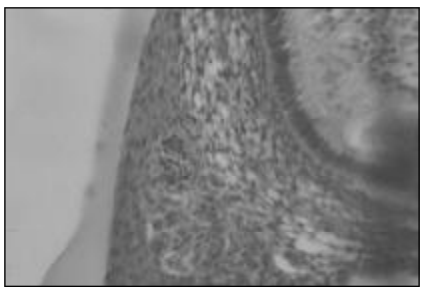


Figure 5. Mouse molar tooth explant after 12 days cultivation. Formation of microcysts in stellate reticulum. H&E x 200.

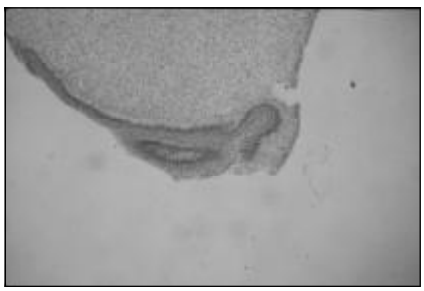
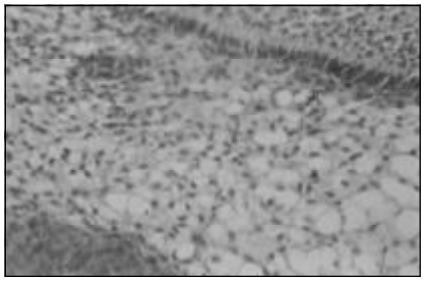


Figure 6. Mouse molar tooth germ explant after 12 days cultivation. Outer epithelium of the enamel organ has differentiated into a lamellar-like structure like the pattern of ameloblastoma. H&E x 200.



Figurre 7. Mouse molar tooth explant after 15 days cultivation. The cuboidal to columnar cells resembled internal dental epithelium and the polyhedral or spindle shape cells resembled the stellate reticulum of the enamel organ. H& x 200.

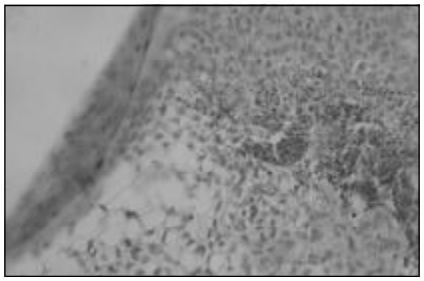


Figure 8. Mouse molar tooth germ explant after 21 days cultivation. Microcysts and penetration of epithelial sheets are noted. H&E x 200.

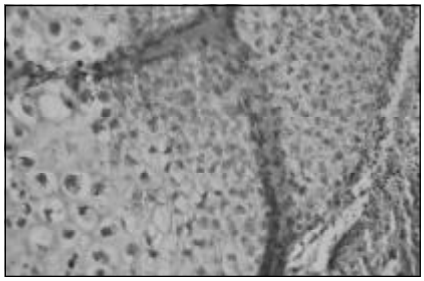


Figure 9. Mouse molar tooth germ explant after 21 days cultivation. Polyhedral or round cells with cyanophilic cytoplasm and lamellar growth and microcyst formation is seen. H&E x 200.

RESULTS

After 2 days of cultivation, initiation of the epithelial outgrowth of the mouse molar tooth germ enamel organ was observed (Fig.1). After 4 days of cultivation continuation of epithelial outgrowth of the enamel organ along the millipore filter was observed. Their structure resembled the pattern of the dental lamina often seen in ameloblastomas of the jaws of primitive type. The nuclei were round or oval with some distinct nucleoli and uniformly distributed fine granular chromatins (Fig.2). After 9 days of in vitro cultivation of a tooth germ, from which the epithelium of the outer layer of the enamel organ exhibited laterally disposed outgrowth buds (Fig.3; 4). A 12-day cultivation of the mouse tooth germ growth with the formation of microcysts in stellate reticulum continued. Areas which at first present the stellate reticulum-like apperance (Fig.5) later become microcysts (Fig.6). The reduced dental epithelium is surrounded by a capsular connective tissue, the follicular sac, which contains remanants of the dental lamina. A histologic section of a 15-day specimen showed that the outer epithelium of the enamel oragan has differentiated into a lamella-like structure like the pattern of the ameloblastoma. Formation of microcysts and continued formation of epithelial buds was observed. A layer of cuboidal to columnar cells formed the outline of the parenchyma. Inside this layer, polychedral or spindle cells were observed and in some cases metaplasia was seen. The cuboidal to columnar cells resembled internal dental epithelium (preameloblasts) and the polyhedral or spindle shape cells resembled the stellate reticulum of the enamel organ (Fig.7.). After 21 days formation of microcysts and penetration of epithelial sheets continued (Fig.8.). Welldefined polygonal or round squamous cells with cyanophilic cytoplasm and lamellar growth and microcyst formation were seen (Fig.9).

Observation of the growth pattern of enamel organ in tissue culture establishes the potentiality of this tissue to form ameloblastomas. It is evident from the study that normal odontogenic epithelium in vitro has a potentiality to proliferate and differentiate in a manner similar to that seen the static histologic section of ameloblastoma.

DISCUSSION

It is accepted, that the growth pattern of odontogenic epithelium in tissue culture is determined by the nutritional medium (21). However, the nutritional medium which has proved successful for normal growth and development for tooth germs in tissue culture offers verification of the concept that ameloblastomas arise from epithelium possessing the potentiality for enamel formation. Our previous studies also have shown that tooth germs continued the development in tissue culture with formation of dental tissues (22).

In this study observation of the growth pattern of enamel organ cultured in vitro establishes the potentiality of tissue to form ameloblastomas. The rapid advancement of gene technology during the last 10 years, has affected research in developmental biology in a particularly dramatic way. Increasingly sophisticated methods allow manipulation of cells and tissues (23, 24, 25).

Microscopically, in ameloblastoma, one sees proliferating epithelial islands and strands, a mature collagenous stroma, and an invasive pattern of growth. The tumor cells exhibit both palisading (alignment of the cell nuclei at one level in the cells) and polarization (migration of the nuclei to one end of the cell). Of great importance, also is the nature of the polarization; in ameloblasts, secretion of matrix occurs toward the connective tissue, with the nuclei polarized away from the connective tissue, an orientation that is almost unique to this cell type. Little or no inductive effect occurs in ameloblastoma, so we see no mesenchymal differentiation and no matrix production by the tumor cells. In normal tooth formation , we do not speak of "ameloblast" until actual enamel matrix formation is initiated; columnar cells with palisaded, polarized nuclei that are not yet producing matrix are most properly called "preameloblast" (6).

However, if these neoplastic cells are similar to differentiated ameloblasts, why are they unable to form a recognizable enamel matrix ? They lack some detail which transforms them in histologically active ameloblasts. This functional detail is probably the absence of a stratum intermedium adjacent to the ameloblastic layer. In normal odontogenesis, when polarization of the nuclei occurs and the epithelium passes from the inductive to the secretory phase, differentiating into active ameloblasts, profound modifications in the stellate reticulum and in the outer epithelium of the enamel organ occur. The stellate reticulum atrophies driving the outer epithelium to approximate the stratum intermedium, forming the reduced epithelium of the enamel organ. At the same time, the outer epithelium acquires a meshed aspect, becoming permeable to nutrive elements from the blood capillaries of the dental sac, which are then more conspicuous close to the reduced epithelium. protruding to the stellate reticulum. This all facilitates the arrival of nutritional elements to the stratum intermedium where they will be premetabolized, passing later to the ameloblasts (26).

In ameloblastoma although forming an ameloblastic layer in ameloblastoma, it is not able to elaborate enamel matrix, probably because it lacks the stratum intermedium. Perhaps, the explanation for the lack of stratum intermedium is in the absence of outer epithelium in the neoplastic islands, which could hinder the formation of reduced epithelium. There is no simultaneous occurrence of the cellular layers which could recall the inner and outer epithelia occurring at same neoplastic, or formation of the reduced epithelium of the enamel organ.

The stellate reticulum with the nests and cords of columnar epithelium tends to undergo degeneration, forming microscopic cysts. The microcysts then expand to form large cystic spaces within the tumor and give the ameloblastoma its multi-cystic gross and radiologic apperance. This central degeneration could have been caused by the polarization of the nuclei at the cellular end facing the stellate reticulum. This probably causes the cellular islands and not from the connective tissue facing the outher cellular extremity. This nutritive competition can cause metabolic deficiencies for the cells of the stellate reticulum, which can explain the degeneration of central cells of the island and the consequent formation of cystic cavities in its interior.

While these studies demonstrate the ameloblastomatous potentiality of odontogenic epithelium, they do not preclude the origin of ameloblastomatous from the basal cells of oral mucosa, the epithelium remnants of the dental lamina or sheath of Hertwigs rests as well as directly from enamel organ epithelium.

CONCLUSIONS

1. Tissue culture studies of mouse embryo tooth germs and enamel organ demostrated epithelial proliferation from the borders of odontogenic epithelium and the formation of lamellar-like structure observed in ameloblastomas.
2. This study offers additional experimental evidence that ameloblastomas originate from epithelium with odontogenic potentiality.

ACKNOWLEDGEMENT

This study was supported by the Estonian Science Foundation Grant nr.5692

REFERENCES

1. Jackson IT, Callan PP, Forte RA. An anatomical classification of maxillary ameloblastoma as an aid surgical treatment. *J Craniomaxillofac Surg* 1996; 24: 230-36.
2. Regezi JA, Sciubba J. Odontogenic tumors. In: Oral Pathology: clinical-pathological correlations. 2nd ed. Philadelphia: 1993. p. 363-71.
3. Kramer IRH, Pindborg JJ, Shear M. Histological typing of odontogenic tumors. 2nd ed. Berlin: Springer-Verlag; 1992.
4. Rosai J. Tumors and tumorlike conditions of bone. In: Anderson WAD, Kissane JM, editors.Pathology. St. Louis; 1977. p.1978-83.
5. Shafer WG, Hine MK, Levy BM. Cyst and tumors of odontogenic origin. In: Shafer WG, Hine MK, Levy BM editors. A textbook of oral pathology. Philadephia: 1983. p.258-67.
6. Gallagher GT, Shklar G. Odontogenic tumors. In: Holland JF, Frei E, editors. Cancer Medicine. 5th ed London: 2000. p. 1221-26.
7. Baker WR, Swift JO. Ameloblastic fibroodontoma of the anterior maxilla. Report of a case. *Oral Surg Oral Med Oral Pathol*. 1993; 76: 294-97.
8. Eversole LR, Tomich CE, Cherrick HM. Histogenesis of odontogenic tumors. *Oral Surg Oral Med Oral Pathol* 1971;32: 569-81.
9. Gold L. Biologic behavior of ameloblastoma. *Oral Maxillofac Surg ClinNorth Am*. 1991; 3: 21-30.
10. Cam Y, Neumann MR, Oliver L, Raulais D, Janet T, Ruch JV. Immunolocalization of acidic and basic fibroblast growth factors during mouse odontogenesis. *Int J Dev Biol* 1992;36: 381-89.
11. Koyama E, Wu CS, Shimo T, et al. Development of stratum intermedium and its role as a sonic hedgehog-signaling structure during odontogenesis. *Dev Dyn 2*001; 222: 178-91.
12. Kumamoto H, Ohki K, Ooya K. Expression of Sonic hedgehog (SHH) signaling molecules in ameloblastomas. *J Oral Pathol Med* 2004; 33: 185-90.
13. Bei M, Maas R. FGF-s and BMP 4 induce both MSX 1 - independent and MSX 1 dependent signaling pathways in early tooth development. *Development 1*998; 125: 4325-33.
14. Heikinheimo K, Hormia M, Stenman G, Virtanen I, Happonen RP. Patterns of expression of intermediate filaments in ameloblastoma and human fetal tooth germ. *J Oral Pathol Med* 1989;18:264-73.
15. Forsman K, Lind L, Backman B, et al. Localization of a gene for autosomal dominant amelogenesis imperfecta (ADAI) to chromosomal 4q. *Hum Mol Genet* 1994; 3: 1621-25.
16. Thesleff I. The genetic basis of normal and abnormal craniofacial development. *Acta Odontol Scand* 1998; 56: 321-25.
17. Ducy P, Zhang R, Geoffroy V, et al. Osf2/ Cbfa1 : a transcriptional activator of osteoblast differentation. *Cell* 1997; 89: 747-54.
18. Buchner A, Sciubba JJ. Peripherial epithelial odontogenic tumors: a review. *Oral Surg* 1987; 63: 688-97.
19. Sarnat G. Basic science and clinical experimental primate studies in craniofacial biology: a personal historical review. *J Oral Maxillofac Surg* 1999;57: 714-24.
20. Van Muijen GNP, Warnaar SO, Ponec M. Differentiation-related changes of cytokeratin expression in cultured keratinocytes and in fetal, newborn and adult epidermis. *Exp Cell Res* 1987; 171: 331-45.
21. Ten Cate R. Oral histoloy: development, structure and function. 5th ed. St. Louis;1998. p. 83.
22. Leibur E. The development of tooth germ in tissue culture. *Acta et Commentationes Universitatis Tartuensis* 1978; 478: 50-7.
23. Gao YH, Yang LJ, Yamaguchi A. Immunohistochemical demonstration of bone morphogenic protein in odontogenic tumors. *J Oral Pathol Med* 1997; 26: 273-77.
24. Heikinheimo K, Jee KJ, Niini T, et al. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res* 2002; 81: 525-30.
25. Dodds AP, Cannon RE, Suggs CA,Wright JT. mRNA expression and phenotype of odontogenic tumours in v-Ha -ras transgenic mouse. *Arch Oral Biol* 2003; 48: 843-50.
26. Sandra F, Harada H, Nakamura N, Ohishi M. Midkine induced growth of ameloblastoma through MAPK a Akt pathways. *Oral Oncol* 2004; 40: 274-80.
27. Sakakura Y, Fujiwara N, Nawa T. Epithelial cytodifferentiation and extracellulare matrix formation in enamel free areas of the occlusal cusp during development of mouse molars: light and electron microscopic studies. *Am J Anat* 1989;184: 287-97.

Received: 09 06 2004
Accepted for publishing: 20 09 2004