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①. 調査・共同研究助成

(7) 口腔粘膜扁平上皮癌発生に関する日本と中国の比較

研究テーマ　口腔粘膜扁平上皮癌発生に関する日本と中国の比較

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要旨

口腔粘膜扁平上皮癌の発生について明らかにするために日本側は白板症の癌化の初期病変として LOH について研究を行い（研究 I)、中国側は均交系マウスを用いて腫瘍の実験発生について研究した（研究 II)。また口腔癌の病態を明らかにする為に細胞周期調節因子であるについて免疫組織化学的研究を行っている（研究 III)。

研究 I

白板症の癌化の過程でどのような遺伝学的な変化が起こっているのかを明らかにする為に、われわれは白板症とその後に発生した初期癌のパラフィン包埋ブロックを用いてマイクロディセクションし、^{32}P でラベルした３３個のマイクロサテライト・マーカーで PCR-based LOH 解析を行った。白板症で最も高い頻度で LOH がみられた染色体は 9p21 で、66.7%であった。次は 3p14-25 の 61.5%, 4q31-32 の 45.5% と 17p12-14 の 44.4%であった。白板症中の初期癌化巣で最も高い頻度で LOH がみられた染色体は 9p21 で、91.7%であった。次は 3p14-25 の 76.9%、4q31-32 の 54.5%、17p12-14 の 66.7%、 5q21-23 の 50%、6p25-27 の 44.4%、6q21-23 の 40% と 11q23 の 40%であった。

研究 II

３ケ月の均交系マウスの腎臓に 17.5 日の C3H 均交系マウスの第一大臼歯歯胚を移植した。３２例中の１３例に角化嚢胞様病変がみられ、移植後の早期に嚢胞はエナメル上皮から形成された。７例には含歯性嚢胞様病変がみられ、移植後の後期に嚢胞は網状層から形成された。角化嚢胞様病変と含歯性嚢胞様病変は違う組織学的発生経路をもつと示唆された。

研究 III

口腔病理の扁平上皮癌のパラフィン包埋資料を用いて細胞周期調節因子である MDM2、P27、Cyclin D1 抗体を免疫組織学的に染色し、現在解析中である。

KEY WORDS

初期癌化、Loss of Heterozygosity、遺伝学的変化、歯胚移植、組織学的発生

研究報告

研究I

1　目的

口腔扁平上皮癌の発生はどのような過程で起こるかは明らかでないが、多段階的な遺伝学変化を通って起こってくるものと考えられる。癌化のフロントはどんな遺伝学的な変化が起こっているのかを明らかにする為に、われわれは白板症とその後に発生した初期癌をマイクロディセクションし、LOH解析を行った。

2　材料および方法

対象は東京医科歯科大学分子病態学および検査部で、１回目の生検で白板症と診断され、２回目の生検で同じ白板症に小さな初期癌化巣が病理学的に認めた１３症例を用いた。１３症例の白板症と初期癌のパラフィン包埋ブロックを用いて、６～８μm の厚さで連続して薄切し、脱パラフィン後 H&E 染色を行った。初回の生検の白板症の部と 2 回目の生検の白板症から癌化の起こっている部を 26 ゲージ注射針を用いてマイクロディセクションした。マイクロディセクションした組織は 200μg/ml proteinase K で消化し、PCR を行った。^{32}P でラベルした３３個のマイクロサテライト・マーカーを用いた。5%ポリアクリルアミト・ゲルを用いて PCR の産物を電気泳動した。正常のアリルと比べて、バンド濃さが 50%以上の減少したものを LOH と判断した。MI は正常と比べて、病変組織で新しいアリルが発現された場が MI ありと判断した。すべてのLOH及びMI陽性症例は再実験により確認した。LOHのある染色体の腕数をinformative

の正常細胞の染色体腕数で割って得られる値がFALの値である。

Fisher' exact testとstudent-t testを用いて解析した。

3　結果

1）　板症のLOH

白板症で最も高い頻度でLOHがみられた染色体は9p21で、66.7%であった。次は3p14-25の61.5%, 4q31-32の45.5%と17p12-14の44.4%であった。

2）　板症中の初期癌化巣のLOH

白板症中の初期癌化巣で最も高い頻度でLOHがみられた染色体は9p21で、91.7%であった。次は3p14-25の76.9%、4q31-32の54.5%、17p12-14の66.7%、5q21-23の50%、6p25-27の44.4%、6q21-23の40%と11q23の40%であった。

3）白板症と白板症中の初期癌化巣のLOHとの比較

13症例の11症例で白板症にみられたLOHがいずれも白板症中の初期癌化巣にも検出された。

4）　白板症と初期癌におけるMI

3症例では1個マイクロサテライト・マーカーにしかMがIみられなかった。症例1では白板症と白板症中の初期癌化巣ともに10qにMIがあり、症例9と11では白板症中の初期癌化巣に8pのMIがみられた。

5）　白板症と白板症中の初期癌化巣におけるFAL

FALはDNAのダメージのレベルを表するものであるが、白板症中の初期癌化巣でのFALの平均は0.46で、白板症での平均は0.27で、両者の間に有意差が認められた($p<0.05$)。

6）　アリルの異方向性

症例7では3p, 6pと9pのLOHが白板症と白板症中の初期癌化巣の両方には認められたが、17qのLOHが白板症中の初期癌化巣のみにあった。8pのLOHが白板症のみにあった。

4　考察および結論

本研究で最も高い頻度のLOHは白板症では9p21にみられ、次に3p14-25であったが、これらの遺伝子変異は白板症の癌化過程の中で早期に起こる変化と考えられる。9p21のLOHはp16の失活を起こすものと推察される。白板症及び白板症中の初期癌化巣でp53癌抑制遺伝子に関係する17p12-14のLOHは白板症で44.4%で、白板症中の初期癌化巣で66.7%で観察された。APC遺伝子を含む5q21-23のLOHは50%の白板症中の初期癌化巣で5q21-23のLOHがみられたが、白板症ではいずれの症例にもみられなかった。このことは、5q21-23があるいはその近くに白板症癌化に関与する抑制遺伝子があると推理できる。

FALは遺伝学的なダメージを表するが、本研究では白板症の平均FALは0.27で、このことは白板症が高いリスクをもつ前癌性病変であることと示す。また白板症中の初期癌化巣の平均FALは0.46で、白板症との間に有意差が認められた。これらの結果から口腔の白板症の癌化過程中では遺伝学的なダメージの蓄積が主要な変化と考えられる。

本研究ではすべて白板症のLOHが白板症中の初期癌化巣に検出できたんことから白板症と白板症中の初期癌化巣は同じクローンであることが明らかとなった。遺伝子のdivergenceがあった症例については、症例7では白板症と白板症中の初期癌化巣が同じ3p, 6q, 9pのLOHをもち、症例8では白板症と白板症中の初期癌化巣が同じ3p, 4q, 9p, 11p, 17p, 17qのLOHを示したので、これらも同じクローンからのものと考えられる。

結論としては、白板症に高い頻度のLOHがあったことから、白板症が高いリスクをもつ前癌性病変であることが示され、白板症と白板症中の初期癌化巣は同じクローンからのものと考えられる。白板症から初期癌までの一連の変化では、遺伝子のダメージが蓄積されて増えてい

くことが示された。

研究 II

1　目的

歯原性上皮は口腔粘膜から分化したものである。この上皮がどのように腫瘍をつくることが出来るかについて明らかにするためにまず歯胚の腎被膜マウスの移植実験を行った。次いでに移植歯胚に発癌剤の作用を検討しているが、今回は正常歯胚の移植までを論文とした。

2　材料および方法

均交系 C3H マウスを使用した。17.5 日分の第一大臼歯歯胚を 32 カ月分のマウスの腎被膜下に移植をそのまま観察し、1 カ月、２カ月、３カ月、４カ月、５カ月後に経時的屠殺して移植歯胚の連続切片を作った。

3　結果および結論

移植役１～２カ月の早期には角化嚢胞様嚢胞が４、５カ月後には含歯性嚢胞様嚢胞が生じ、経過時間により発生する嚢胞に差異がみられた。また、発生母地は角化嚢胞では歯提が、含歯性嚢胞では歯乳頭の網状層からの発生が示唆された。

研究 III

1　目的

口腔粘膜の扁平上皮癌について細胞周期調節因子の染色性と癌の病態の関係を明らかにする。

2　材料および方法

上海第二医科大学口腔病理の症例を用い、日本からの抗体を染色キットで染色を行う。

3　結果

現在解析中である。

Different Histogenesis of Experimental Odontogenic Cysts by the Renal Subcapsular Transplantation of Tooth Germs of Mice

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Zhu EX, Xiao J, Liu TJ, Jiang WW and Takagi M. Different Histogeneses of Experimental Odontogenic Cysts by the Renal Subcapsular Transplantation of Tooth Germs of Mice. *Oral Med Pathol* 2000; 5: 83-86, ISSN 1342-0984.

The tooth germs of first mandibular molars of 17.5-day-old C3H mouse embryos were transplanted into the renal subcapsular spaces of 3-month-old syngenic male mice. Grafts were harvested at timed sequences from 1, 2, 3, 4 weeks and 2, 3, 4, 5 months, and were examined histopathologically by total serial sections. Of 32 cases of grafts, keratocyst-like lesions were formed in 13 cases, and dentigerous cyst-like lesions were formed in 7 cases. None of the cases developed two cysts at the same time. In the keratocyst-like lesions the cavity developed from the enamel epithelium in the early stage after transplantation, and in the dentigerous cyst-like lesion the cavity developed by cystic degeneration of stellate reticular layer of enamel organ in the late stage after transplantation. The present experiment revealed that keratocyst-like lesions and dentigerous cyst-like lesions developed through different histogeneses.

***Key words*: animal model, odontogenic cysts, tooth germ, transplantation**

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Introduction

Odontogenic cysts in human beings are not uncommon. There are many types displaying various biologic behaviors, but little is known about their histopathogenesis. It is generally considered that each of the odontogenic cysts is derived from one of the following epithelial sources: 1) enamel organ, 2) reduced enamel epithelium of a tooth crown, 3) epithelial rests of Malassez and remnants of the sheath of Hertwig, 4) remnants of the dental lamina, or 5) basal cells of oral mucosa (1, 2). However, the exact relationship between these epithelia and various types of cysts is unknown, and experimental studies in this field have been limited. In order to investigate whether enamel organ is one of the sources of odontogenic cysts, and which type of odontogenic cyst is closely connected with it, we performed the following animal experiment.

Materials and Methods

C3H/HeN mice were used. They were given mixing solid food and sterile drinking water. Two-month-old mice of both sexes mated at 9 o'clock at night and before 9 o'clock the next morning. The mice which had vaginal plugs were recognized as becoming pregnant after 0.5 day. And on day 17.5, the tooth germs of the embryos were in the bell stage, in which a specialization of cells of the enamel organ itself occur, resulting in the establishment of four distinct epithelial layers: external enamel epithelium, stellate reticulum, stratum intermedium, and internal enamel epithelium. The 17.5-day embryos were removed from pregnant females and placed in 2% bovine serum in Hanks solution. Under a dissecting microscope,

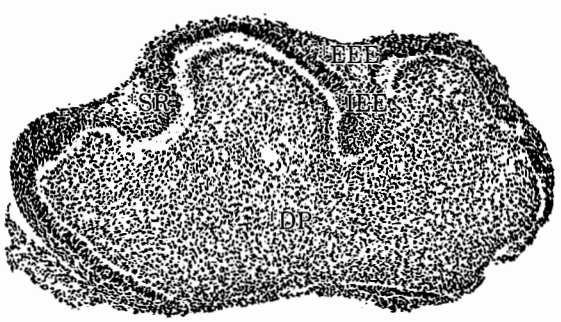

Fig. 1: First mandibular tooth germ as removed from a 17.5-day-old mouse. IEE: internal enamel epithelium, EEE: external enamel epithelium, DP: dental papilla SR: stellate reticulum. H.E. ×20

the first mandibular molar tooth germs of these embryos were dissected from the surrounding connective tissues, dental lamina and oral epithelium. The tooth germs which were just in the bell stage, had only components of enamel organ, dental papilla and dental follicle, and not other tissues, such as dental lamina and oral epithelium (Fig. 1). Three-month-old syngenic male mice were anaesthetized by the intraperitioneal injection of pentobarbital sodium (Sommonpentyl), and 32 dental germs were transplanted under the left renal subcapsular spaces of the mice at the rate of one graft per capita. The grafts were harvested 1, 2, 3, 4 weeks and 2, 3, 4, 5 months after transplantation. For histological examination, the grafts were fixed in 10% neutral buffered formalin and demineralized in 5% formic acid at 4℃ for 12 hours. Routinely the paraffin-embedded specimens were serially sectioned 4 μm thick and stained with hematoxylin-eosin.

For determining the relationship between duration of transplantation and formation of experimental odontogenic cysts, pearson chi-squared test (χ^2 test) was applied.

Results

1) Histopathological Findings

(1) One-week group

At 1 week after transplantation, small amounts of dentin and enamel appeared in the bell stage, tooth germs and stellate cells of enamel organs were still abundant. External enamel epithelium proliferated partially and the serial sections showed that these proliferations were sticking out of the enamel organs and were still connected with enamel organs by epithelial strands (Fig. 2). Formation of the inner space with hyperkeratinization was observed in these epithelial nest (Fig. 3)

(2) Two-week group

At 2 weeks after transplantation, the enamel and dentin of the tooth crown were almost formed and the stellate reticulum layer disappeared. The size of epithelial nests enlarged and keratocyst-like lesions were filled with keratin. The inner surface of cysts was lined by with keratinized or parakeratinized stratified squamous epithelium. Basal layer of stratified squamous epithelium was smooth without rete pegs (Fig. 4).

(3) Three-week group

At 3 weeks after transplantation, the crown was formed and the root began to develop. Keratocyst-like lesions continuously increased in size and daughter-cysts were sometimes seen. Alveolar bone developed and bone marrow appeared.

(4) Four-week group

At 4 weeks after transplantation, parts of enamel organs enlarged and bulged outwards and a cystic cavity was formed. There were dentigeous cyst-like lesions, and the epithelium of the cystic lining was composed of 2 to 4 layers of non-keratinized stratified squamous epithelium. Basal layer of the epithelium was flat and rete pegs were not formed (Fig. 5). The cystic cavity was filled with a fluid, desquamated epithelial cells and foamy cells.

(5) Two-to-three-month group

Dentigerous cyst-like lesions were lined with 2 to 4 layers of flattened non-keratinized stratified squamous epithelium. The crown of the tooth was enclosed in a cys-

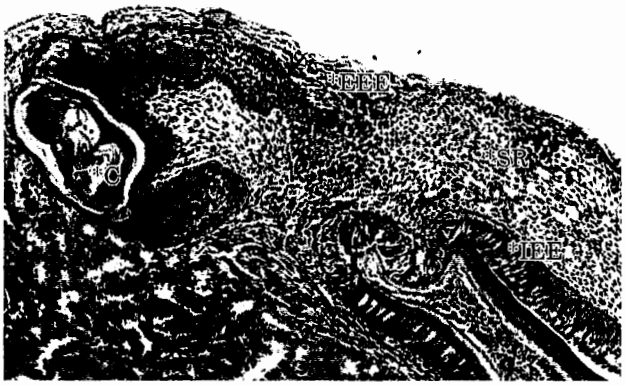

Fig. 2: one-week transplantation. The cyst was connected with enamel organ by epithelial strand. IEE: internal enamel epithelium, EEE: external enamel epithelium SR: stellate reticulum, C: cyst. H.E. ×10

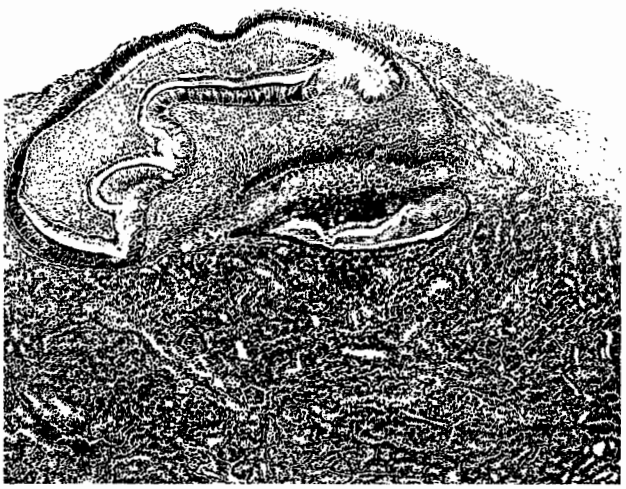

Fig. 3: One-week transplantation. Formation of inner space with hyperkeratinization is observed in the epithelial nest (*). H.E. ×80

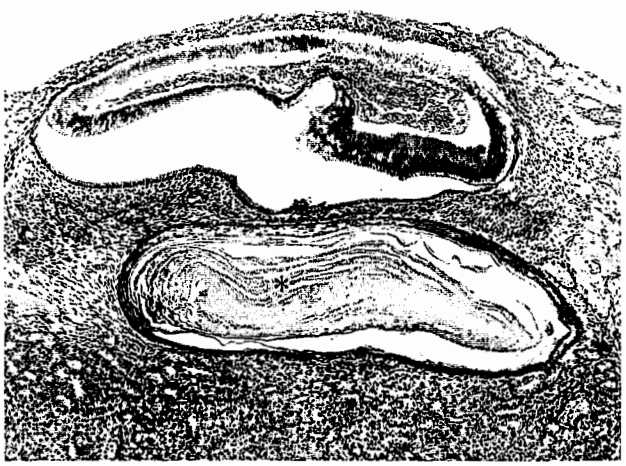

Fig. 4: Two-week transplantation. Keratocyst-like lesion is formed outside of tooth germ and filled with keratin (*). H.E. × 160

tic cavity and the cavity was filled with a fluid.

(6) Four-to-five-month group

The dentigerous cyst-like lesions continued to develop in size (Fig. 6). The lining epithelium was thin non-keratinized stratified squamous epithelium without rete pegs and was attached to the cementoenamel junction of the unerupted tooth (Fig.7) In some tooth germs, large amounts of cementum-like hard tissue was formed. The cystic cavity was filled with a faintly eosinophilic fluid and scanty cells. In the reduced enamel epithelium, a cuticle-like eosinophilic substance and irregular calcification were seen. Large amounts of alveolar bone formation and bone marrow tissue were seen.

2) Relationship between duration of transplantation and formation of odontogenic cyst

The findings in experimental transplants are summarized in Table 1.Thirteen cases of 32 transplants developed keratocyst-like lesions during 1 to 4 weeks, and 7 cases developed dentigerous cyst-like lesions during 2 to 5 months. There was also a tendency for keratocyst-like lesions to develop in the early stage and the dentigerous cyst-like lesions to develop in the late stage ($P<0.05$).

Table 1: Relationship between duration of transplantation and formation of odontogenic cysts

Duration of transplantation	Transplanted cases	Keratocyst-like lesions	Dentigerous cyst-like lesions	Total
1-2 weeks	8	8	0	8
3-4 weeks	8	1	2	3
2-3 months	8	0	2	2
4-5 months	8	4	3	7
Total	32	13	7	20

Discussion

It is well known that tooth germs continue to develop even in transplanted conditions (3-6). Thus it is reasonable to study histogenesis of odontogenic cysts by using transplanted tooth germs. In human beings, each of the odontogenic cysts have different histological features and biological behaviors, and it can be supposed that they have different epithelial origins. According to the development of keratocysts, two sources of the epithelium have been considered; remnants of the dental lamina and extensions of basal cells from the overlying oral epithelium (7, 8). In animal experiments, it has been demonstrated that the cervical loop and dental lamina were the sources of odontogenic keratocyst (9). Experimental odontogenic keratocysts by transplantation have been reported by Bartlett *et al*. and Soskolne *et al*. (10, 11). The histology of the cysts in these experiment was the similar to those seen in ours. In the present experiment, the serial sections from 13 cases of keratocyst-like lesions showed that the epithelial nests proliferated and finally keratocyst-like lesions were formed. Formation of keratocyst-like lesion was not seen within enamel organs. In those lesions, epithelial lining of the cysts were connected with external enamel epithelium which developed toward surrounding connective tissues. Therefore, it is suggested that exter-

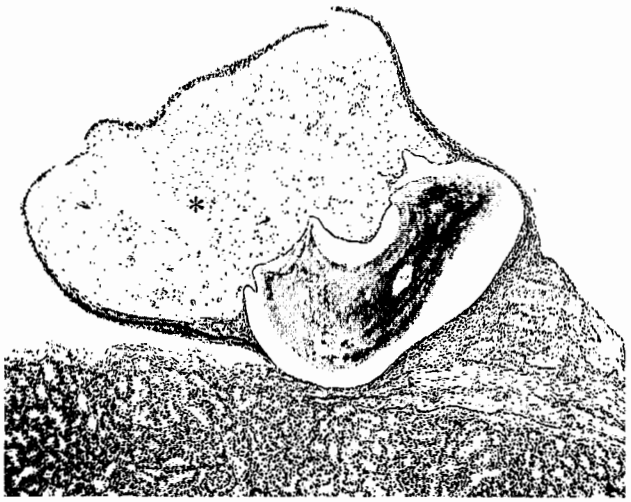

Fig. 5: Four-week transplantation. Dentigerous cyst-like lesion is lined with non-keratinized stratified squamous epithelium (*). The cystic cavity is filled with liquid, desquamated epithelial cells and infiltrating cells. H.E. ×72

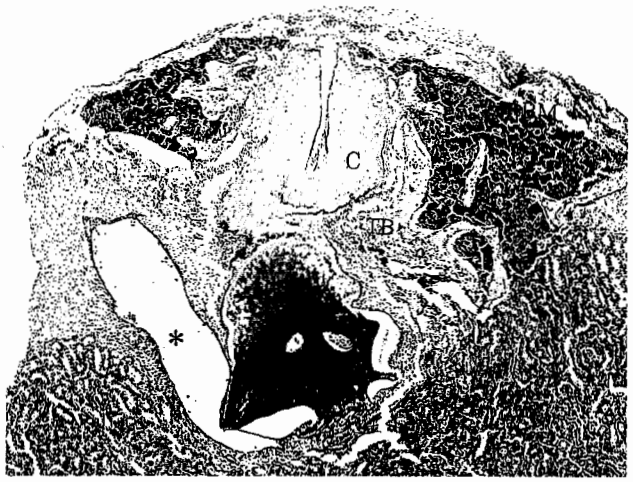

Fig. 6: Five-month transplantation. Dentigerous cyst is attached to amelocemental junction of the tooth (*). Large amount of cementum-like (C) hard tissue is formed. TB: trabecular bone, BM: bone marrow H.E. ×50

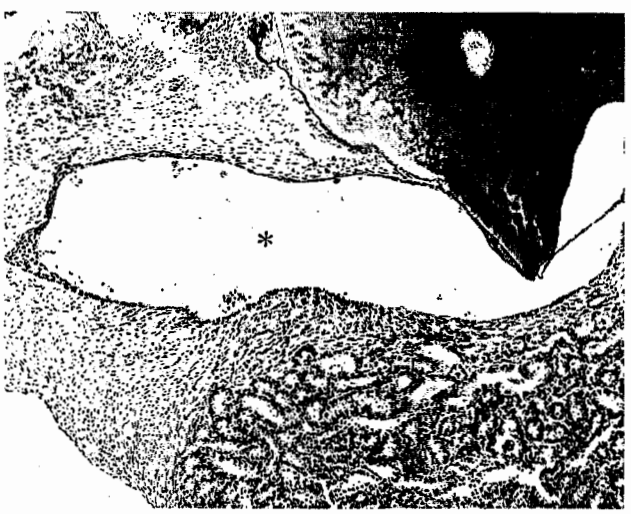

Fig. 7: Five-month transplantation. Higher magnification of Fig. 6. Cyst is lined by non-keratinizing stratified squamous epithelium. H.E. ×160

nal enamel epithelium is the sources of keratocyst-like lesions. However the biological behaviors of the keratocyst-like lesions were considered to be different from odontogenic keratocysts of humans. The keratocyst-like lesion in the present experiment did not show aggressive growth.

Dentigerous cysts may develop by accumulation of fluid either between the reduced enamel epithelium and the enamel or within the enamel organ itself. Atkinson described the formation of cysts derived from the enamel organ around the crowns of mouse molar teeth transplanted subcutaneously into an inbred strain of mice (12). He also examined proliferative populations of cells by ^{3}H labelling method in the experimental odontogenic cysts (13). In the present experiment, the dentigerous cyst-like lesions occurred in the stellate reticular layer. At first cellular degeneration and necrosis appeared within the stellate reticular layer, and a fluid accumulated. The cysts were attached to cementoenamel junctions, showing a tooth-containing or half-tooth-containing appearance. In those cases proliferation of external enamel epithelium was not observed. It is suggested that the enamel organ is one of the sources of dentigerous cyst-like lesions. Experimental dentigerous cysts have been reported by Riviere *et al*., Al-talabani *et al*. and Alini *et al*. (14-16), and they suggested the same histogenesis.

In the present experiment, most of keratocyst-like lesions were observed during 1 to 4 weeks, and dentigerous cyst-like lesions developed during 2 to 5 months. None of the cases developed the two cysts at the same time. It is supposed that keratocyst-like lesions were formed in the early stage of tooth development, and dentigerous cyst-like lesion were formed in the late stage ($P< 0.05$).

Atkinson described that the initial loss of blood supply and subsequent establishment of vascular connection between host and graft influenced the pattern and types of degeneration and repair (12). Formation of experimental odontogenic cysts by transplantation without any specific pretreatment has been reported by several authors (11-16). Maeda *et al*. reported that mechanical injury was an important factor in the production of odontogenic carcinoma by N-ethyl-N-nitrourea (17). However Miura *et al*. considered that in vitro 4-nitroquinoline-1-oxide (4 NQO) treatment is necessary for induction of experimental odontogenic cysts (18). In the present experiment, the injury factors might have three sources. The first is the procedure of removing the tooth germ from pregnant females and dissecting it from the surrounding tissues. The second might come from the pressure of the renal capsule. The third factor may be connected with the orientation of the tooth germ under the renal capsule since the orientation has something to do with the resistance of the surrounding tissues to the tooth germ.

Acknowledgment

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