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理事長中島章殿

研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ 発癌性ヘテロサイクリックアミン（Trp-P-2）及び発癌物（ENU、DMBA）によるヌードマウスの実験的口腔腫瘍に関する研究

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 有 ・ (無) （学会名・内容）

(1). Effects of Various Growth Factors on Cultured Mouse Tooth-Germ. (JADR. 47th Annual Meeting, Nov. 27-28, 1999. Kobe) （ポスター発表、共同発表者）

(2). A Trial of Tumorignesis in Athymic Nude Mouse Tongue by DMBA Application. (18th Annual Meeting of Japan Society for Oral Tumors, Jan. 22-23, 2000. Nagoya). （ポスター発表、主発表者）

(3).ラットの口腔粘膜と皮膚に及ぼす Iodine Tincture 及び Trp-P-2 の影響 (第１８回日本口腔腫瘍学会総会. 2000 年 1 月２２～２３日名古屋) （ポスター発表、共同発表者）

(2) 学会誌等に発表した論文 (有) ・ 無 （雑誌名・論文名）

(1). 器官培養におけるラットの下顎頭成長におよぼす basic fibroblast growth factor (bFGF) の影響 (共著) 日顎誌、11(3) 173-181. 1999.

3．今後の研究計画

発癌物質（Trp-P-2、ENU、DMBA）がヌードマウスの口腔粘膜におよぼす影響に関する実験病理学的研究の試料である口腔組織の病理組織学切片において、Ki-67、PCNA などの免疫染色及び AgNO などの鍍銀染色を行い、口腔粘膜の実験部位での細胞増殖活性について検索を行う予定です。

4．研究指導者の意見

劉学氏は客員研究員として愛知学院大学歯学部病理学講座へ来てから研究面で真面目で勤勉であり、協調性にも優れています。今回、本研究室では研究助成金の援助の下で発ガン性ヘテロサイクリックアミン（Trp-P-2）及び発ガン物（ENU、DMBA）がヌードマウスの口腔粘膜におよぼす影響についての実験病理学的研究を行なっております。指導教授として日中医学会の援助に対して厚くお礼を申し上げます。

研究指導者氏名 亀山洋一郎 ㊞

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

論文発表に当っては、日中医学協会－日本財団補助金による旨を明記して下さい。

発癌性ヘテロサイクリックアミン（Trp-P-2）及び発癌物（ENU、DMBAによるヌードマウスの実験的口腔腫瘍に関する研究

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要旨:

本実験で胸線が欠如し、T 細胞機能欠如のある雄性ヌードマウス４週齢、４０匹を用い、発癌性物質（Trp-P-2、ENU、DMBA）を舌側縁中１／３に機械的擦過を加えて、 週３回塗布して、塗布期間が１０週間とした。病理組織学検索：Trp-P-2 群および ENU 群では舌の上皮には前癌病変である上皮性異形成が見られた。DMBA 群では強い前癌病変である上皮性異形成が認められた。

Key words:

Nude mouse; Carcinogenesis; Carcinogen; Tongue; Trp-P-2; ENU; DMBA.

Oral Tumorigenesis in Athymic Nude Mouse Tongue by Applications of DMBA, Trp-P-2 and ENU

1. Purpose:

The immune system is involved not only in defense against infections but against "spontaneously derived" aberrant cells by immune surveillance. Thus, athymic nude mice offered a new possibility as a model for testing the effects of the absence of T-cell-mediated immunity on tumor development. The present study was undertaken to examine oral tumorigenesis in athymic nude mice (Balb/c-nv/nv s/c) by applications of

Trp-P-2, ENU and DMBA.

2. **Materials and Methods:**

Forty male athymic nude mice (Balb/c-nv/nv s/c), aged 4 weeks, were divided into five groups (groups A-E). In Group A (5 mice), the acetone solution was applied three times weekly to the right lateral border of the middle third of the tongue after scratching the area with a No.2 pulp canal cleaner. The treatment was continued for 10 days. Group B (5 mice) was treated in the same manner as Group A but received application of DMSO (dimethyl sulfoxide) instead of acetone. Groups C to E were treated as follows: Group C (10 mice), scratching plus 0.5% Trp-P-2 (3-amino-1-methyl-5H-pyrido(3,4-b) indole) in DMSO; Group D, scratching plus 0.5% ENU (N-ethyl-N-nitrosourea) in distilled water; Group E (10 mice), scratching plus painting with 0.5% DMBA (9,10-dimethyl-1,2-benzanthracene) in acetone. All animals were killed at the end of 10 weeks. The tongues were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 um, and stained with hematoxilin and eosin (H&E).

3. **Results**

3.1. Groups A and B

The mucosa of the tongue in the treated region gradually became rough, but no neoplastic lesions developed. The epithelium showed a slight hyperplasia with hyperkeratosis and the basement membranes were seen clearly.

3.2. Groups D and E

The similar lesions were found in groups D and E. The ulceration was formed in any of the nude mice. Moderate dysplasia developed in the experimental sites. Most of the basement memberanes were distinct.

3.3. Group E

A localized ulcer was produced in the treated region and this ulceration

persisted until the end of the experiment. Histological examination revealed that the epithelium exhibited a severe dysplasia in which disorientation of cells, cellular pleomorphism, nuclear hyperchromatism, proliferation of basal cells, abnormal keratinization and elongation of epithelia projection were observed. The basement membranes were not distinct.

4. **Discussion**

There has been some reports showed that applications of DMBA combined with mechanical trauma could induce carcinomas in the middle third of the lateral border of the tongues in hamasters(1-3).

In our department, Maeda & Kameyama (4) reported that carcinomas of the tongue in hamaster could be produced in less than 10 weeks by excisional wounding and DMBA, and Fujita & Kameyama (5) indicated, using the same method as Maeda's, epithelial dysplasia could be induced by application of Trp-P-2 instead of DMBA.

ENU has been shown to produce tumors of many organs when administered as a single dose to adult rats (5,6), but there have no reports concerned with lingual tumor induction by application of ENU.

Engel, A.-M.(7) reported that tumor induction with a shorter time and a higher incidence in the subcutaneous tissue was seen in nude mice (Balb/c/A/Fib/Bom) compared with immunocompetent mice after treatment with low doses of a carcinogen-Methylcholanthrene (MCA). However, his result differed from the observation by Stutman, O.(8), who found that MCA tumors developed with the same induction time and incidence in nude mice (CBA/H strain) as in normal mice.

From above-mentioned studies, it was possibly predicted that the production of a high incidence lingual carcinomas in a short time could be achieved in T-cell-mediated immunodeficiency animals by applications of DMBA combined with mechanical trauma. Our results did not completely support the prediction. In the present study, Group E, received DMBA treatment, developed severe dysplasia or premalignant lesion, although the previous study showed that carcinomas of the tongue in

hamaster could be produced in less than 10 weeks by excisional wounding and DMBA(4). The evidence for an immune response to developing tumors is quite definitive (9, 10), but there is not any evidence to indicate that athymic nude Balb/c-nv/nv s/c mice apparently have a lower resistance to the employed carcinogens in the present study. One of the reasons may be that different strains of nude mice could lead to different results of tumor development even if the same experimental sites and carcinogen are used (7,8). Both Groups C (Trp-p-2) and Group E (ENU) gave rise to moderate epithelial dysplasia. This is probably due to weaker carcinogenity of Trp-P-2 and ENU, and the other reason may be that the induction time using Trp-P-2 and ENU as carcinogens is too short to produce carcinoma. In the groups A and B, the epithelia in the experimental site appeared slight hyperplasia with hyperkeratosis. This may be a normal respondence of tissue reconstruction to the mechanical trauma .

5. References

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