대한악안면성형재건외과학회지 : Vol. 19, No. 3, 1997

MAKING IN VIVO MODEL TO STUDY ABOUT HUMAN ORAL CANCER (I)

Dept. of Oral and Maxillofacial Surgery, Korea Cancer Center Hospital

Hyung-Kook Park, Yong-Kack Kim

구강암 연구를 위한 동물실험모델의 개발(I)

박형국 · 김용각

원자력병원 구강악안면외과

편평상피세포암종은 악성종양 중 가장 중요한 비중을 차지하고 있는 암종이다. 하지만 편평상피세포암종의 세포주는 다른 악성종양에 비하여 아직까지 많이 개발되어지지 않았다. 또한 동물실험모델을 만들기 위한 이종이식에 있어서 편평상피세포암종은 매우 낮은 생착율을 보이고 있다. 구강암 중에서도 편평상피세포암종은 가장 많은 부분을 차지하나, 개발된 세포주는 그리 많지 않으며, 더 더욱이 동물실험 모델의 제작은 쉽지 않아, 새로운 치료 약제의 개발이나 치료 방법 개발등에 많은 제약이 있어왔다.

본 실험에서는 수종의 구강 편평상피세포암종의 세포주를 배양하였고, 특별히 고안된 사육시설을 이용하여 BALB/C nude mice를 사육하였다. 여러 농도의 구강암 세포주를 nude mice의 등에 피하로 이식하였다. 어떤 세포주는 계속적인 성장을 보였으나 어떤 세포주는 완전히 흡수되기도 하였다. 5주 이상을 관찰하였으며, 이식된 종양의 크기를 측정하고, 부피를 계산하였다. 또한 또다른 동물모델의 제작 방법으로서 특별히 고안된 cap을 nude mice의 등에 이식하고, 그 안에 구강암 세포주를 배지와 함께 이식하였으며, 1주후에 cap을 제거하였고, 4주이상을 관찰하였으며, 성장하는 종양의 모습과 크기를 관찰하였다.

본 연구는 구강암 연구에 적절한 동물실험모델을 개발하여 다른 악성종양에 비해 동물실험적으로 연구할 기회가 적었던 구강암 영역의 연구를 활발히 하며, 향후 한국인의 구강암 연구에 가장 적절한 동물실험모델을 개발하여, 보다 진보된 구강암 치료방법의 개발 및 신약 등의 개발에 이용하기 위함이다.

주요어 : 구강암, 편평상피세포암종, 동물실험모델, nude mice

I. INTRODUCTION

Squamous cell carcinoma is probably the most prevalent cancer in human being. But in vivo study about that cancer is relatviely uncommon, partly because only a few squamous cancer cell line has been developed during past decades, and partly because the taking rates of those cell lines as heterotransplantation were poor. The reason why only a few squamous cell cancer can be cultured and developed as cell lines and in vivo model is not known yet[1].

Animal model to study human oral cancer can be divided with two categories ; one is made by transplantation and another is made by induction of cancer. Some human cancer cell or tisue can be transplanted into special animal, and some carcinogen can induce cancer in certain organ of certain animal. But the model made by induction of cancer is a adequate model to study about carcinogenesis rather than to study about cancer itself, and in that model, the tissue of cancer is belong to the animal not to human. By using the model made by transplantation, we can study about more variable subjects. The therapist who wants to study about the effect of new treatment method or new chemotherapeutic drug will select the model made by transplantation method[2,3].

In our study, we maintained some kind of human oral squamous cancer cell lines, and brought up BALB/C nude mice in specially designed housing system. Various concentration of cancer cells were inoculated subcutaneously into flank area of nude mice. Some cancer cell lines were rapidly growing in nude mice, but some cancer cell line couldn't grow in nude mice and resorbed completely. And in some cacer cell line, some nude mice showed continuously growing tumor, but other didint' show any tumor growing. We observed over 5 weeks each cancer cell lines, and the size of growing tumor were measured and the volume were calculated.

And as new try, we implanted specially designed caps on the back of nude mice, and cancer cell lines were brought into the caps with media. We removed the cap after 1 week, and observed over 4 weeks. The shape and size of growing tumor were appraised. The purpose of this research is making in vivo model of oral cancer to study more briskly and actively about human oral cancer, and developing in vivo model to more similar situation with human being. We will make try to make more improved animal model of more relevantly applicable in various method of cancer study.

II. MATERIALS AND METHODS

1. Maintenance and Preparation of Cell lines

KB cell, SCC-4, SCC-9, SCC-15 and SCC-25 cell lines were thawed and mainatined, which had been purchased from the Korean distributor of ATCC. KB is a well-known cell line that was developed from squamous cell carcinoma of mouth floor of 54-year-old white man in 1954. SCC-4, SCC-9, SCC-15 and SCC-25 cell lines were developed from squamous cell carcinoma of tongue of 55-year-old, 25-year-old, 55-year-old and 70-year-old male, respectively, in 1980. KB was maintained with Eagle's minimum essential media supplemented with non-essential amino acid and 10% fetal bovine serum. SCC-4 and SCC-25 were maintained with 1 : 1 mixture of Ham's F12 and Dulbecco's modified Eagle's medium supplemented with 0.4μg/ml hydrocortisone and 10% fetal bovine serum. SCC-9 and SCC-15 were also maintaine with above mixtrure but

supported on special feeder layer that were made of irradiated human lung cells. These cell lines are incubated at 37℃ with 5% CO_2. The media were renewed every 2 or 3 days.

2. Mude Mice

Nude mice, which were BALB/c/nunu strain and five to six week old male, purchased from B&K Universal Inc. Initially they were brought up using positive rack and micro isolator cages. After we constructed a specially designed rearing system, we could bring mice in the more strict condition, which can maintain the environment of nude mice within the controlled temperature, moisture and the clean air in 26℃ 60% humidity, and uniform 12 hours of dark-light cycle. In which each micro isolator cage contained the two or three nude mice. We also used UV-sterilized water, irradiated sterilized feeding, and autoclaved sterilized bedding. All manipulations were done in sterilized condition as in clean bench.

3. Xenografting or cancer cell lines into nude mice.

Our maintaining cell lines were washed with phosphate buffered saline and added with trypsin and EDTA. After incubated for 5 minutes, the obtained cell were centrifuged with those media and resuspended with adequate volume. The cells were counted with haemocytometer and the cell viability were determined with trypan blue exclusion. The cells were adjusted to various concentrations. We injected KB cells of concentraion of 1.0×10^6 cells, 2.0×10^6 cells, 5.0×10^6 cells, and 1.0×10^7 cells in 0.1ml of medium, into flank area of six nude mice subcutaneously with 1cc tuberclin syringe and 25-gauge needle. In one mouse, we inoculated twice ; 5.0×10^6 KB cells into the upper back and lower back area. And we injected SCC-25 cells of concentration of 1.0×10^6 cells, 2.0×10^6 cells, 5.0×10^6 cells and 1.0×10^7 cells in 0.1ml of medium into flank area of six nude mice, and injected SCC-9 cells of concentration of 5.0×10^6 cells in two nude mice, and injected SCC-4 cells of concentration of 2×10^6 cells in one nude mice.

4. Growing Tumor Measurement and Histopathologic Examination

We observed each mouse more than 5 weeks after cancer cell inoculation. The growing tumor volumes after xenograft were measured with vernier celiper in half millimeters. We considered the tumor mass as hemiellipsoidal and measured the length and widith and height. The tumor volume were calculated as following formula ; Volume $(mm^3)=1/2(4\pi/3)\times(L/2)\times(W/2)\times H$(L : length, W : width, H : height in millmeters). To minimize differences, same person always measured the tumor sizes.

The tumor and various tissue were dissected out from the tumor-bearing animals, and placed buffered formalin. The tissues were than dehydrated in graded water-alcohol mixtures, and after completed dehydration, embedded in paraffin. Five-micrometer sections were then cut and stained with hematoxylin and eosin. The tisses were microscopically observed.

5. Xenografting of Cancer Cell using Implanted Plastic Cap

Specially designed plastic caps were selected and implanted on the back of nude mice after appropriate anesthesia, which were holding in subcutaneously, we brought 1.0×10^7 KB cells with 0.5ml media into the caps, The caps were remved after 1 week. After then the shape and size of growing tumor were appraised. We observed more than 4 weeks, and the tissue were histopathologically exami-

ned.

III. RESULTS

1. Tumor growths in BALB/C nude mice after KB cell incoulations according to their concentrations

Seven inculations of KB cells in six nude mice were done subcutaneously. One inoculation of 1.0×10^6 cells (In1) and one inoculation of 2.0×10^6 cells (In2) were done into each two mouse. Two inoculations of 5.0×10^6 cells (In3 nd In4) and one inoculation of 1.0×10^7 cells (In5) were done into each three mouse. Two inoculations of 5.0×10^6 cells (In6 and In7) were injected into anterior and posterior back of one mouse. Among them six inoculations (In2 to In7) were developed as tumor in five nude mice. Only in one nude mouse (In1), tumor didn't grow in which 1×10^6 cell was inoculated. The growing tumors were visible between 3 and 7 days after inoculation. And after then, the tumors were continously growing as in Fig. 1 and 2. The volumes of growing tumors (In1 to In7) were graphed in Fig. 3. The growth of each tumor followed the exponential growth curve of following formula ; $Y=start.e^{K\cdot X}$. The r-squares of tumors of In2, In3, In4, In5, In6 and In7 were 0.9702, 0.9686, 0.9833, 0.9738, 0.9452 and 0.9762. The exponential growth curves of tumors after

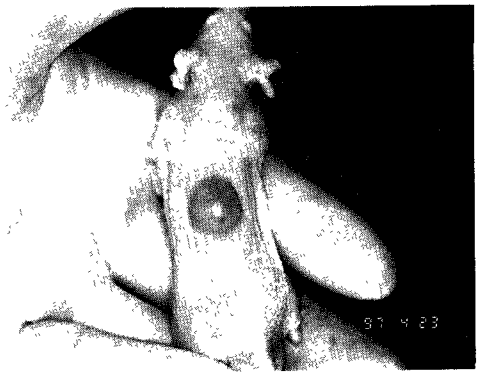

Fig. 1. Growing tumor on a back of a nude mice 16 days after inoculation of 5.0×10^6 cells of KB (In3)

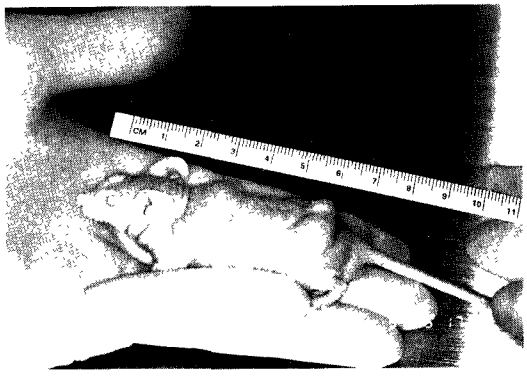

Fig. 2. Growing tumor on a back of a nude mice two weeks after double inoculations of 5.0×10^6 cells of KB (In6 and In7).

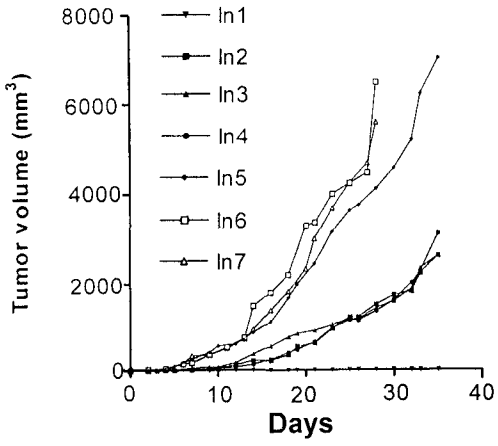

Fig. 3. Volumes of growing tumors after KB cell xenografts according to concentration of inoculations ; In1(1.0×10^6 cells/0.1mL), In2(2.0×10^6 cells), In3(5.0×10^6 cells), In4(5.0×10^6 cells), In5(1.0×10^7 cells), In6(5.0×10^6 cells), In7(5.0×10^6 cells into the same mouse of In6).

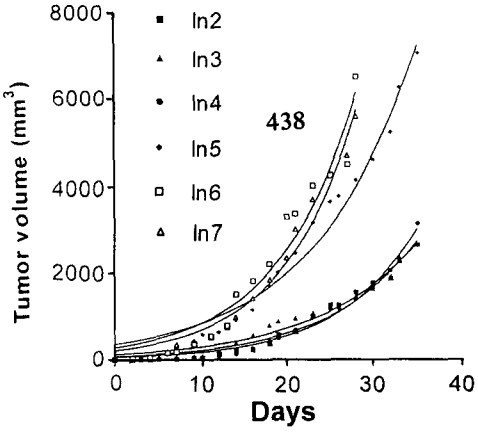

Fig. 4. Exponetial Growth curves of each tumor (In2 to In7) overlapped on their growth curves.

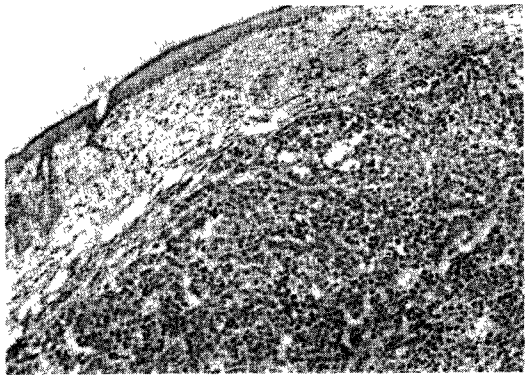

Fig. 5. Histopathologic feature of subcutaneously grown tumor (×100), which was well demarcated from surrounding tissue of the nude mouse (In2).

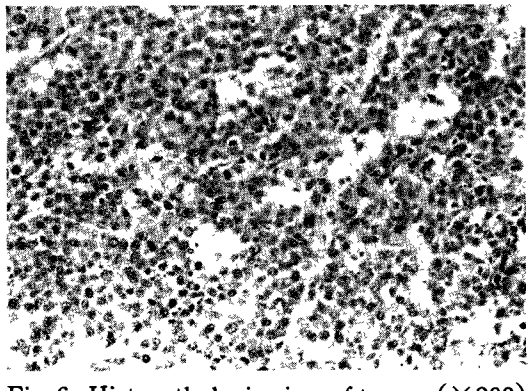

Fig. 6. Histopathologic view of tumor (×200), which was a feature of poorly differentiated carcinoma.

lnoculations (In2 to In7) were graphed in Fig. 4. The histologic views of dissected tumor were shown in Fig. 5 and Fig. 6.

2. Results after SCC-25 cell inoculation into BALB/C nude mice according to their concentration

Six inoculations of SCC-25 cells were done subcutaneously in each six nude mice. One inoculation of 1.0×10^6 cells, one inoculation of 2.0×10^6 cells, three inoculation of 5.0×10^6 cells and one inoculation of 1.0×10^7 cells were done. We observed almost 8 weeeks. But any of them were developed as tumor. the autopsies showed complete resorptions of inoculated cancer cells.

3. Results after SCC-9 cell inoculation into the BALB/C nude mice

Two inoculations of 5.0×10^6 SCC-25 cells were done subctaneously in two nude mice. In the inoculation sites, very slow enlarging, somewhat stationary mass were detected. They were observed over 4 weeks. But the autopsies showed only simple inflammations.

4. Results after SCC-4 cell inoculation into BALB/C nude mice

2×10^6 SCC-4 cells were inoculated into the back of a nude mouse. We observed over 7 weeks, but there was not any enlarging mass. The autopsy showed complete resorption.

5. Results about cell lines maintenance and nude mice bringing

Our KB cell was well growing cell line, but SCC series didnt' grow well. Especially we couldn't maintain SCC-15 cell lines, and couldn't transplant this cell line. Nude mice were well grown in specially designed housing system. No nude mouse died unintentionally during bringing. In some nude mice subcuta-

neous abscess were happened, they were excluded in experiments, but the abscesss were not lethal to nude mice. The body weight of 5-week old mouse was about 20 gram, and grew up to 30 gram at 10 weeks.

6. Tumor growth after cancer cell transplant in implanted plastic cap

Six plastic caps were implanted on the back of six nude mice after appropriate anesthesia, which were holding in subcutaneously as in Fig. 7. Sometimes we sutured the skin of nude mice to hold the caps firmly, and then we brought 1.0×10^7 KB cells with 0,5ml media into the caps. The caps were removed after 1 week. At that time there was no visible tumor. About 5 to 6 days after the caps removed, we could see the growing tumors were covered with scab. As th scab were detached from the tumor, the area was replaced with overlying growing skin and ultimately covered completely with intact skin at about 3 weeks later as in Fig. 8.

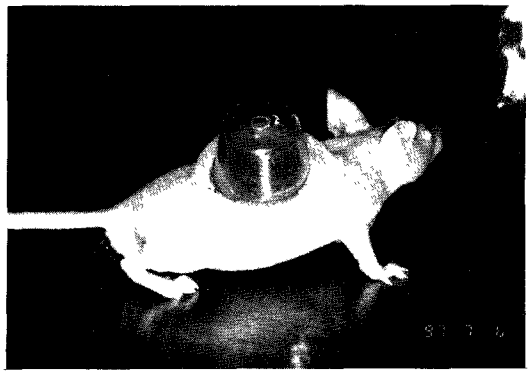

Fig. 7. A plastic cap was implanted subcutaneously on the back of a nude mouse, 1.0×10^7 cells of KB were brought into the cap.

Fig. 8. Grwoing tumor on the back of a nude mouse 12 days after the cap removal, which had been removed one week after implantation.

Ⅳ. Discussion

Squamous cell carcinoma is the most common human cancer, but this has had relatively poor take rates as heterortansplatation in animal[1]. Relatively very few human squamous cell carcinomas have been established in culture. Therefore oral squamous cell carcinoma has rarely represented by cancer cell line and in vivo model. The early successfully cultured oral squamous cancer cell line is KB cell by Eagles in 1955[4]. The cell line was developed from the poorly differentated squamous cell carcinoma involving the floor of mouth and the tognue of 54-year-old white man. In 1981, Rheinwald and Beckett[5] reported some human squamous carcinoma cell lines requirng anchorage and fibroblast support culture. Their cell lines were SCC-4, SCC-9, SCC-15, SCC-25 which were developed from squamous cell carcinoma of the tongue, and SCC-12 and SCC-13 which were developed from squamous cell carcinoma of the facial skin. They cultured these cell lines using feeder layers of the Swiss mouse embryonic fibroblast line 3T3. Krause et al[1] in 1981 and Baker[6] in 1985 reported also some human squamous carcinoma cell line, named UM-SCC-1 to UM-SCC-19. The most common cause of failure of cell line development was a lack of tumor cell growth

in culture from the tumor tissue. It could be caused by inadequated nutrient and growth factors in culture medium, and also caused by sampling error in which no viable tumor were obtained or delayed process making considerable cell death. But innate nature of tumor were probably more important. The next common cause of faillure was contamination by pre-existing or spread pathogen. We didn't use any antibiotics during cell line maintainig, therefore sometimes accidental bacterial contamination had occurred.

Early attempt to heterotransplantation of human tumor are done in privileged site where rejection phenomenon is less intense such as in anterior chamber of eye of rabbit and guinea pig, rodent brain, hamster cheek pouch. Greene[7] reported high success in transplantation of human tumor xenografts in anterior chamber of the guinea pig eye and in rodent brain. But these models are impractical to study the tumor. In the eye, the space is too small to allow the growth of the tumor, and in the brain, the observers can't detect the growth of tumor visually and can't measure serially. The hamster cheek pouch have been utillzed for human cancer research for many years. Various tumor cell line or tissue implantation into the hamster cheek pouch were attempted[8]. But the reported success rate were low as 14.7 to 16.1%. The late rejection phenomenon of the hamster cheek pouch was probably the most important cause of implant failure.

The next attempt was immunosuppression of experimental animals by radiation, cortisone, or both, but these affected the tumor also, and imparied long-term viability of recipient animal was inappropriate for long term studies. Neonatal thymectomy and/or treatment of antilymphocytic serum were successfully removing the reject phenomenon in selected case, but only little data were available. All of above method were less practical system for the study of human tumor in vivo model than the immunosuppressed mutant mice model in which human tumor can be growth serially and cultured cell line can produce invasive and metastasized neoplasm[2,3]. But not all tmors grow in nude mice, the possibility and frequency of tumor growth in nude mice varies according to tumor type and origin, inoculation condition, mice age, sex, strain type, and a lot of other factors[9,10].

In 1985, Baker[6] reported the results of implantation of UM-SCC cell lines and other nonsquamous cell lines into nude mice. When they inoculated 21 SCC lines into 112 nude mice, 56% of mice developed progressively enlarging tumors, 31% of mice manifested subcutaneous tumor nodules but remained stable in size over the observation period, and 12.5% of nude mice didn't permit any tumor growth. 43% of SCC cell lines demonstrated progressive growth in at least some of the mice, 33% remained as stable non-enlarging subcutaneous nodule and 24% regressed.

Another type of in vivo model of cancer is directly inducing the cancer in animal using some carcinogens. The most frequently studied chemical carcinogen for oral mucosal cancer were DMBA(9,10-dimethyl-1, 2-benzanthracene) and recently 4 NQO (4-nITROQUINOLINE-1 OXIDE)[11]. Oral cancer model by inducing epidermoid carcinoma by continuing applicationof DMBA to hamster buccal pouch was demonstrated from 1954 by Salley[12]. After then the model have been serially refined and developed[13,14,15]. But these models are used and adequate to study about carcinogenesis rather than to study about cancer therapy or cancer itself[16,17,18,19].

After the discovery of hairless mutant "nu" gene by Flanagan in 1968 and the resultant

athymic "nu/nu" nude mice by Pantelouris in 1969, the nude mice have had very importan role in biomedical research. And after the early success of heterotranslantation of human malignant tumors to nude mice in 1969 by Pygaard et al., and the development of breeding and maintaining method of the nude mice by Givoanella et al. in 1973, these are most widely used mutant mice to study the growth of tumor or infectious agent and to study basic immunologic mechanisms and carcinogeneses [2,20]. Furthermore the nu gene can be bred into mice togethere with other mutant genes associated with immune deficiencies, makes double mutant mice.

In general, athymic nude mice lack functional T cells, although small numbers of cells carrying phenotypic T-cell marker have been detected, T-cell mediated functions are deficient or absent, and non-T-cell mediated cellular immune mechanisms may be altered. Therefore rejection of organ allograts and resistance to certain infection are absent or deficient. But nude mice are particulary susceptible to viruses commonly found in colonies of euthymic mice, which are not pathogenic in ethymic mice. And nude mice are also vulnerable to a variety of bacterial, fungal and parastic infections. Nude mice are also vulnerable to dehydration and loss of body heat because of lack of hair. To minimize the loss of water and heat through the hairless skin, they must be maintained in an atmosphere of about 60% humidity, and must be maintained in room at 26 to 28℃ temperature[21].

In our study, we initially brought up nude mice using positive rack, but later we constructed a specially designed housing system for nude mice. After we used the rearing system, we could bring mice in the more strict condition, which can maintain the environment of nude mice within the controlled temperature, moisture and filtered clean air in 26℃ 60% humidity, and uniform 12 hours of dark-light cycle. In which each micro isolator cage contained the two or three nude mice. We also used UV-sterilized water, specially sterillzed feeding and bedding. All manipulations were done in sterilized condition using clean bench. No nude mouse died during experiment except intentional killing. But some nude mice exhibited subcutaneous abscess and ascite, and they were excluded in experiments. We could bring up nude mice more than 10 weeks without special problems. As like other male animal bring, guide not the hurt each other was very important in male nude mice bringing. We purchased the homzygote athymic BALB/C nude mice of 5 to 6 weeks old from B&K Universal Inc. in U.S.A. The body weight of 5-week old mouse was about 20 grams, and grew up to 30 grams at 10 weeks.

The human oral squamous cancer cell lines used in this study were purchased from Korean distributor of ATCC. We are now planning to make our cell lines and apply our cell lines into nude mice. But developing of cancer cell lines takes so much time, so we used KB cell and SCC series made by Eagle in 1954, and Rehinwald and Beckett in 1980. About cell lines maintenance, the KB cell was well growing cell line, and SCC-25 was also relatively well grown. But SCC-4, SCC-9, SCC-15 series didnt' grow well. Especially we couldn't maintain affluent amount of SCC-15 cell lines adequate to inoculate into nude mice, even if we had used special feeder layer made by irradiated human lung cell. Therefore we couldnt' transplant SCC-15 cell line into nue mice.

Seven inoculations of KB cells in six nude mice were done subcutaneously. Various concentrations of cance rcells from 1.0×10^6 cells to 1.0×10^7 cells were tried into nude mice. And two inoculations of 5.0×10^6 cells were

injected into anterior and posterior back of one mouse, tumor didn't grow in which 1×10^6 cell was inoculated. The growing tumors were visible between 3 and 7 days after inoculation. The growths of tumor were graphed in figure. The higher concentration show rapid tmor growth in single inoculated mice, but more rapid tumor growth can be seen in double inoculated mouse. Therefore we though that the amouth of cancer cells in inoculation was important but the growing tumor were affected by indvidual characters of nude mice more than concentraion of nude mice. The growth of each tumor followed the exponential growth curve. The R-squares of the exponetial growth curves were relatively high.

We tried 9 inoculation of SCC-series cancer into the back of nude mice, but anything couldn't develop as growing tumor, and we failed in making animal study model using these cell lines. Six inoculations of SCC-25 cells were done subcutaneously in each six nude mice. One inoculation of 1.0×10^6 cells, one inoculation of 2.0×10^6 cells, three inoculations of 5.0×10^6 cells and one inoculation of 1.0×10^7 cells were done.We observed almost 8 weeks. But any of them were developed as tumor. The autopsies showed complete resorptions of inoculated cancer cells. And two inoculations of 5.0×10^6 SCC-25 cells were done subctaneously into two nude mice. In the inoculations of 5.0×10^6 SCC-25 cells were done subcutaneously into two nude mice. In the inoculation sites, very slow enarging, somewhat siationary mass were detected. They were obseved over 4 weeks. But the autopsies showed only simple inflammations. And one inoculation of 2×10^6 SCC-4 cells was tried into the back of a nude mouse. We observed over 7 weeks, but there was not any enlarging mass. The autopsy showed complete resorption.

And as a new try, we implanted specially designed caps on the back of nude mice, and cancer cell lines were brought into the caps with media. We removed the cap after 1 week, and observed over 4 weeks. When removed the cap from nude mice, there were no visible tumors on back of nude mice. After 2 to 3 days, the denuded areas of each mouse were covered with a kind of scab. And about 5 to 6days after the caps removed, we could see the growing tumor under the scab and its periphery, As the scab were detached from the tumor, the area was replaced with overlying growing skin and ultimately covered completely with intact skin.

The purpose of this research is making in vivo model of oral cancer to study more briskly and actively about thuman oral cancer, and developing in vivo model to more simlilar situation with human being. We are now planning to make our cell lines and apply our cell lines into nude mice. And we will try more various to make more improved animal model of more relevantly applicable in various method of cancer study.

V. SUMMARY

In order to make in vivo model of human oral squamous cell cancer, we brought up BALB/C nude mice in specially designed housing system, and maintained some kind of human oral squamous cancer cell lines ; KB, SCC-4, SCC-9, SCC-15, SCC-25. Various concentration of cancer cells were inoculated subcutaneouly into flank area of nude mice. We observed each nude mouse more than 5 weeks after tumor inoculation. We appraised the results, measured the tumor size, and calculated the growing tumor volumes after tumor inoculation according to cancer cell line and concentration of cancer cells in media. Some can-

cer cell lines were rapidly growing in nude mice, but some cancer cell line couldn't grow in nude mice and resorbed completely. And in some cancer cell line, some nude mice showed continuously growing tumor, but other didn't show any tumor growing. And as a new try, we implanted specially disigned caps on the back of nude mice, and cancer cell lines were brought into the caps with media. We removed the cap after 1 week, and observed over 4 weeks. The shape and size of growing tumor were observed.

REFERENCES

1. Krause CJ, Carey TE, Ott RW, Hurbis C, McClatchey KD, Regezi JA : Human sqyamous cell carcinoma. Arch Otolaryngol 107 : 703−710, 1981.
2. Giovanella BC, Stehiin JS, Williams LJ : Heterotransplantation of human malignant tumors in nude thymusless mice. II. Malignant tumors induced by injection of cell cultures derived from human solid tumors. J Natl Cancer Inst 52 : 921−930, 1974
3. Dinesman a, Haughey B, Gates GA, Aufdemorte T, von Hoff DD : Development of a new in vivo model for head and neck cancer. Otolarynogl Head Neck Surg 103 : 766−774, 1990.
4. Eagle H : Propagation in a fluid medium of a human epidrmoid carcinoma, strain KB. Proc Soc Exp Biol Med 89 : 362−364, 1955.
5. Rheinwald JG, Beckett A : Tumorigenic kerationcyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinoma. Cancer Res 41 : 1657−1663, 1981.
6. Baker SR : An in vivo mdoel for squamous cell carcinoma of the head and neck. laryngoscope 95 : 45−56, 1985.
7. Green HSN : Signifcance of heterologous transplantability of human cancer. Cacer 5 : 24−44, 1952
8. Foley GE, Handler AH : Differentiation of "normal" and neoplastic cells maintained in tissue culture by implantation into normal hamster. Proc Soc Exp Biol Med 94 : 661−664, 1957.
9. Giovanella BC, Sstehlin JS : Influence of the host's sex on the growth of huan tumors heterotransplanted in nude thymusless mice. Proc Am Assoc Cacner Res 15 : 23, 1974.
10. Sharkey FE, Gogh J : Considerations in the use of nude mice for cancer research. Cancer Metastasis Rev 3 : 341−360, 1984.
11. Hawkins BL, Heniford Bw, Ackermann DM, Leonberger M, Martinez SA, Hendler FJ : 4NQO carcinogenesis : A mose model of oral cavity squamous cell carcinoma. Head Neck 16 : 424−432, 1994.
12. Salley JJ : Experimental carcinogenesis in the cheek pouch of the syrian hamster. J Dent Res 33 : 253−262, 1954.
13. Morris AL : Factors influencing experimental carcinogenesis in the hamster cheek pouch. J Dent Res 40 : 3−15, 1961.
14. Silverman S, Shklar G : The effect of a carcinogen(DMBA) applied to the hamster's buccal pouch in combination with croton oil. Oral Surg 16 : 1344−1365, 1963.
15. Santis H. Shklar G, Chauncey HH : Histochemistry of experimentally induced leukoplakia and carcinoma of the hamster buccal pouch. Oral Surg 17 : 207−218, 1964.
16. Odukoya O : Initiation and promotion in experimental oral carcinogenesis. Oral Srg 58 : 315−320, 1984.
17. Odukoya O, Shklar G : Two-phase carci-

nogenesis in hamster buccal pouch. Oral Surg 54 : 547−552, 1982.
18. Wong DTW, Biswas DK : Expression of c-erbB proto-oncogene during dimethylbenzanthraceneinduced tumorigenesis in hamster cheek pouch. Oncogene 2 : 67−72, 1987.
19. Shin K, Gimenez IB, Lee JS, Nishioka K, Wargovich MJ, Thacher S, Lotan R, Slaga TJ, Hong WK : Expression of epidermal growth factor receptro, polyamine levels, ornithine decarboxylase activity, micronuclei, and transglutaminase 1 in a 7,12-dimethylbenzanthracene-induced hamster buccal pouch carcinogenesis model. Cancer Res 50 : 2505−2510, 1990.
20. Giovanella BC, Stehiin JS : Heterotransplantation of human malignant tumors in nude thymusless mice 1. Breathing and maintenance of nude mice. J Natl Cancer Inst 51 : 615−619, 1973.
21. McDermott-lancaster RD, lto T, Kohaka K, Colston MJ : the nude mouse-characteristics, breeding and husbandary. Int J Leprosy 55 : 885−888, 1987.