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백서 모델에서 수술 기구를 통한 피부악성종양의 국소 재발 가능성

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Possibility of Local Recurrence Caused by Surgical Instruments in the Mouse Skin Cancer Model

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Purpose: The goal of cancer surgery is complete removal of cancer tissue and prevention of recurrence. Surgeons can change the surgical instruments after total resection of the cancer mass. The purpose of this procedure is to prevent dissemination of the cancer cells attached to the surgical instruments. Authors hypothesize the possibility of local recurrence caused by the cancer cells attached to the surgical instruments in the skin cancer cases.

Methods: Skin cancers were induced by using DMBA-TPA two-stage carcinogenesis model in 10 of Balb/c mice. In 2-weeks, skin cancer was developed in all 10 mice. cancer cell attached surgical instruments were made by pinching the removed cancer tissue using Adson tissue forcep 10, 20, 30 times each. To count number of cancer cells in each forcep with different number of pinching was done, the forceps were washed in 30 mL of the normal saline and Cytospin preparation was done. To make recurrence models from cancer cell attached surgical instrument, three incisions were made in normal skin of each mouse, and local seeding was done by pinching subcutaneous tissue in 10, 20, 30 times each by using Adson teeth forceps mentioned above as cancer cell attached surgical instrument.

Results: All skin cancers were squamous cell carcinoma. Local recurrences were developed in 7 mice (3 in 10 times forceping site, 2 in 20 times forceping and 3 in 30 times forceping). In the cytospin test, the mean number of squamous cells in 100 microscope was 28.6 in 10 times, 47.2 in 20 times, 93.6 in 30 times, respectively. *P* value was 0.002 in Wilcoxon-Sign test.

Conclusion: The number of cell count was significantly increased as number of pinching was increased. And these cells are able to induce local recurrence by local seeding. Considering this result, authors are able to confirm that the minimal handling in cancer surgery is important factor to prevent local recurrence.

Key Words: Local recurrence, Mouse, Skin cancer, Squamous cell carcinoma, Surgical instrument

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I. INTRODUCTION

Primarily developed malignant skin cancer has relatively good prognosis than other types of cancer because it can be easily diagnosed and completely cured by surgical resection.[1] Previous studies show that incidence of squamous cell carcinoma has increased twice in number compare to 10 years ago.[2,3]

The goals of skin cancer surgery are complete removal of cancer and prevent its recurrence.[1] In cases of skin cancer with recurrence, it is caused not only by re-exposure to carcinogens but also by incomplete surgical resection or local seeding of cancer cells during operative manipulation. Surgeons should pay attention not to touch the cancer mass directly or change the surgical instruments after total resection of the cancer mass to prevent dissemination of the tumor cells which are attached to the surgical instruments.

To the best our knowledge, it has not been reported that surgical instruments can induce local recurrence in resection site by cancer cell seeding during surgical procedures of skin cancer. Therefore, authors hypothesize the possibility of local recurrence caused by the cancer cell attached to the surgical instruments in skin cancer cases.

II. MATERIALS AND METHODS

A. The induction of skin cancer from mice

Skin lesion was artificially developed in mice by incorporating DMBA (7,12-dimethyl benzanthracene, Sigma Co., U.S.A.) and TPA (tetradecanoyl-phorbol-acetate, Sigma Co, U.S.A.) two staged carcinogenesis model.[4,10] of 6 weeks old mice were used as experimental animals and kept in cage 2 week before the initiation of experiment for environmental adaptation.

First, hair of cephalic and upper back area was removed from the skin with depilatory (Nicean, thioglycol acid 80%, ILDONG phamaceutical company). Second, to initiate tumor, DMBA was applied weekly for 3 consecutive weeks and to promote cancer maturation, TPA was applied weekly for 20 weeks. As results, primary cancer masses were developed at all 10 mice and we had 2 weeks of observation period.

B. Count number of tumor cells and local recurrence study

To remove skin cancer developed in the mice, Zolpidem was intramuscularly injected, excision was done by No.15 blade including 2 mm of normal-appearing skin surrounding it and hematoxylin and eosin staining was done for histopathologic diagnosis. To produce local recurrence model, 3 of 5 mm incisions were made on cancer free skin of mice (lower back area). With Adson forceps, we pinched tumor mass removed from mouse 10, 20, and 30 times each and with the same forceps we pinched incision site 10, 20, and 30 times with 10 mm of interval (Fig. 1).

On the other hand, to count the number of tumor cells in surgical instrument, we pinched the tumor mass with same manner done for the local seeding using Adson forceps. And we washed them with 30 mL of normal saline, centrifuged them in 1,500 rpm for 5 minutes and then abandoned the supernatant fluid and put in chamber for cyto-centrifugation and centrifuged them in 1,700 rpm for 2 minutes to smear automatically on slide and fixed in 95% alcohol. Finally, papanicolau staining[5] was done for cell counting. Tumor mass was fixed in formalin and hematoxylin and eosin staining was done for histopathologic diagnosis. Wide excisions were made on tumor cell seeding site for local recurrence after 20 weeks of observation and hematoxylin and eosin staining was done to check whether tumor had recurred or not.

All procedure explained above was done by a single surgeon to minimize bias.

III. RESULTS

After 3 weeks, skin lesion has developed in DMBA application site (Fig. 2) and after 20 weeks of TPA application, skin tumor had developed in all mouse (Fig. 3). Results of histopathologic study done for skin tumor developed in mouse were well-differenciated type of squamous cell carcinoma and average diameter of mass was 5.6 mm (Fig. 4, Table I).

In local recurrence study, recurrence developed in 7 mice (3 in 10-grasps site, 2 in 20-grasp and 3 in 30-grasp) (Table II).

In cyto-centrifugation test, the mean numbers of squamous cells calculated with a 100 microscope were 28.6 in 10-grasped forcep, 47.2 in 20-grasp forcep and 93.6 in 30-grasp forcep (Fig. 5, Table III).

p value was 0.002 in Wilcoxon-Sign test.

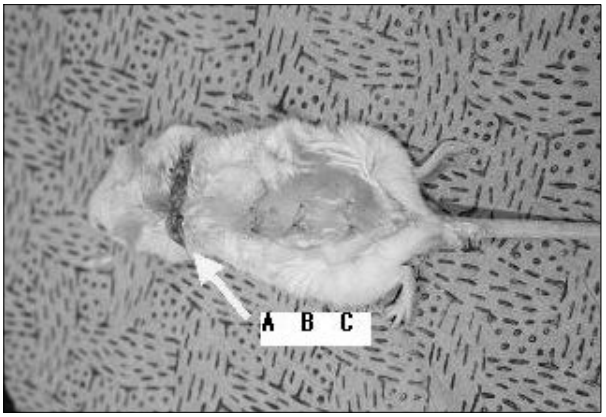

Fig. 1. Primary closure after excision of initial tumor (arrow). A is local implantation site of tumor cells with 10 times pinched forceps (B-20 times, C-30 times).

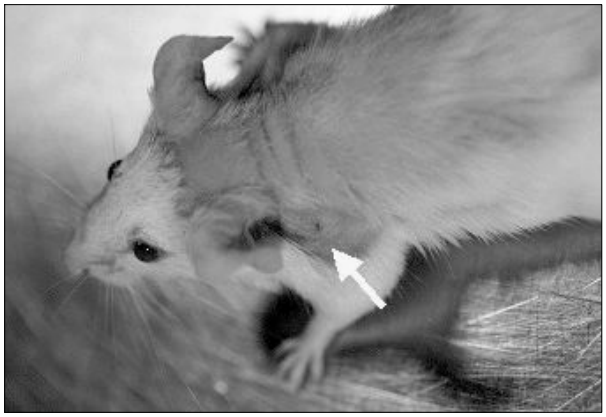

Fig. 2. Skin lesion of DMBA applied site after 3 weeks (arrow).

IV. DISCUSSION

A squamous cell carcinoma develops at skin has more malignancy potential than that of basal cell carcinoma, so that Moh reported that we can expect 94% full recovery rate for the primary cancer case and 84% for a recurred cancer case through micrographic surgery. According to Rowe et al.[6]'s data collected 5 years after each operations, Mohs microsurgery has much lower recurrence rate of 3.1% whereas general excision has 10.5% in a primary cancer case.

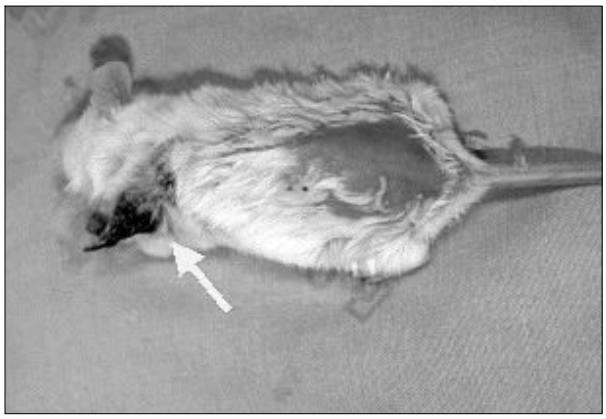

Fig. 3. Skin lesion of TPA applied site after 20 weeks (arrow).

Current domestic data does have precise statistics on the topic above, but Ahn et al.[7] has reported that general excision shows 3.9% recurrence rate after a surgery. Since this data was not collected by follow-up survey method, the researcher mentioned that the recurrence rate for the general excision could be much higher in reality.

In diagnostic and therapeutic procedures for visceral organ cancer, such as, liver, colorectal and breast, several cases of local recurrence by needle track seeding of cancer cell have been reported.[8-10]

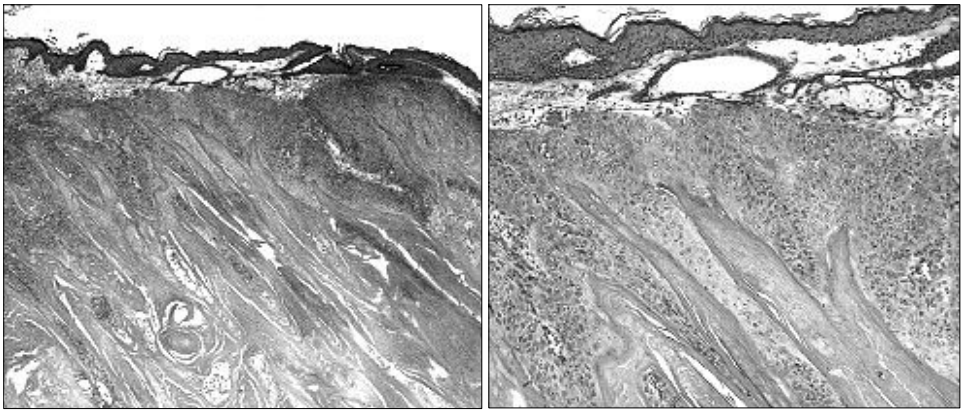

Fig. 4. (Left) (Hematoxylin and eosin stain, × 40) Histology of the initial skin cancer. (Right) (H-E stain, × 100) It looks a well differenciated squamous cell carcinoma.

Table I. Histologic Finding of Occurred Initial Skin Cancer

No	Diagnosis	Differentiation	Tumor size (m × m)
1	Squamous cell carcinoma	Well	9 × 9
2	Squamous cell carcinoma	Well	2 × 2
3	Squamous cell carcinoma	Well	4 × 3
4	Squamous cell carcinoma	Well	9 × 8
5	Squamous cell carcinoma	Well	5 × 5
6	Squamous cell carcinoma	Well	8 × 6
7	Squamous cell carcinoma	Well	5 × 5
8	Squamous cell carcinoma	Well	2 × 2
9	Squamous cell carcinoma	Well	2 × 2
10	Squamous cell carcinoma	Well	10 × 9

Table II. Histologic Finding of Local Recurred Site

	10 times	20 times	30 times
1	+	+	+
2	-	-	-
3	-	-	-
4	+	-	-
5	+	-	-
6	-	-	+
7	-	-	-
8	-	-	-
9	-	-	-
10	-	+	-
Total	3	2	2

Table III. Cytologic Finding after Cytocentrifuges

	10 times	20 times	30 times
1	40	71	137
2	26	36	71
3	19	27	37
4	38	47	94
5	33	51	99
6	14	42	81
7	20	25	97
8	34	65	103
9	27	49	100
10	35	59	117
Average	28.6a	47.2b	93.6c

*a-b, a-c, b-c, Wilcoxon Sign test, p=0.002.

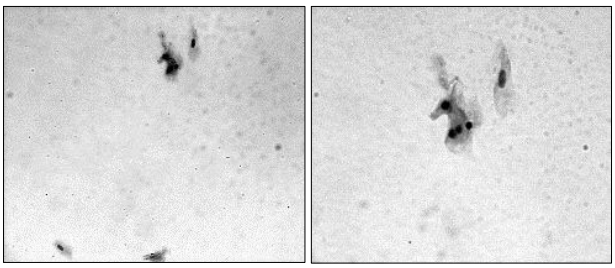

Fig. 5. (Left) (Papanicolaou staining, × 100) Cytologic findings after cytocentrifuges. (Right) (Papanicolaou staining, × 200) Atypical cell with multiple nucleuses was observed.

Hoffman et al.[11] once reported about recurrence and metastasis process of a caner the experiment transplanting human colorectal cancer cell into nude mouse, there have not been any experimental report on the possibility of local recurrence of cancer by surgical instruments. Therefore, we have decided to study the possibility of local recurrence of a cancer through the mouse skin cancer model transplanting autologous cancer cell. Harris et al.[13] had observed abstergent, which was collected after surgical operations of head and neck cancer patients, centrifuged and dyed by Papanicolau's method, and they said that the recurrence rate was 38%, if they could detect the cancer cell, but if they could not, the recurrence rate was 35%. As the percentage does not show a significant difference, the researchers reported that there had to be more research on the topic of local recurrence in aspects of other mechanisms. Harris et al.[13]'s experiment did not take the size of cancer into consideration, but this study took that as a changing factor and we could observe the relations between the cancer size and the possibility of recurrence.

Rowe et al.[6] had reported that if the size of squamous cell carcinoma is larger than 2 cm, the recurrence rate

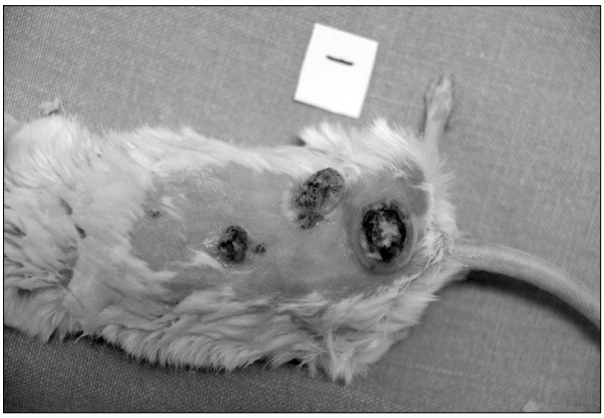

Fig. 6. Recurrence of the skin cancer at the induced site. There is no recur on the initial site.

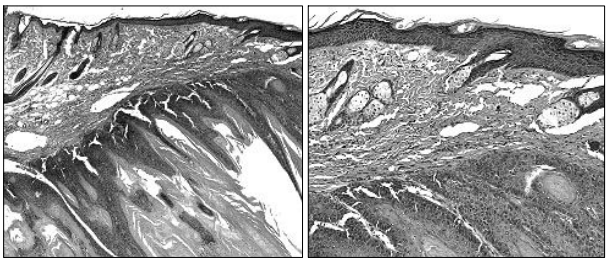

Fig. 7. (Left) (Hematoxylin and eosin stain, × 40) Histologic findings of the recurred skin tumor. (Right) (H-E stain, ×100) Squamous cell carcinoma developed at hypodermis. The cases at this study were more of hypodermal development types by artificial seeding, than that of recurrence by long distance epidermal metastasis.

is twice more than if the size is less than 2 cm. And the ratio is about 15%. Also, if a cancer size is bigger than 2 cm, the possibility of spread goes up 3 times more than that of the cancers with the size less than 2 cm. And the ratio is about 30%. Thus, we could confirm that the bigger the size of tumor is, the higher of the recurrence rate by surgical instruments through the experiment we have done. At the experiment we observed the artificial local recurrence cases and noticed that the average diameter of the tumor is 8.2 mm which is much bigger than the average diameter 3.0 mm of primary tumor that has not recurred, as it is known that the size of tumor is proportional to the recurrence and spread level.

At most cases, when we surgically remove skin cancer tumor, we also eliminate 2 mm of normal-appearing skin surrounding the cancer mass. Even if this is the case, often times the surgical instruments get direct contact with the tumor cells at ordinary clinical situations and we suture the surgical area with new instruments after the tumor is removed under the assumption that the contaminated instruments would lead us to the local recurrence cases. Through the research, we could examine the possibilities of local recurrence by local seeding of tumor cells with surgical instruments during a surgery.

The analysis of the experiment results helped us to identify the fact that as the number of Adson forceps grabs goes bigger, the number of squamous cells is increased. And the increase was statistically meaningful. At the artificial local seeding experiment, local recurrence results came up as these; when the number of grab is 10, the rate is 30%, 20 times of grab, the rate is 20%, and when the grab is 30 times, 20% of recurrence cases. In The possible reasons for the results which show the inverse proportion relation between the number of forceps and the number of local recurrence case are these. First, it would be differences in metastatic potentiality of the cancer cells and the process of extinction of tumor cells by immunity. According to Fidler,[11] even though the number of forceps is big, if the cells are mostly low in metastatic potentiality, then the local recurrence may not happen for each cell's metastatic potentiality is different and the metastatic potentiality decides the development of tumor cells in other areas. The other reason could be the heterocytotrophic apoptosis though the transplant was autograft type. One another reason is that, especially in primary cancer masses with smaller size, as number of pinches grow bigger, cancer tissue and cells could be destructed and not be able to induce local recurrence. The recurred tumor cells were formed at hypoderm and grew out to skin at the experiment. In general cases of squamous cell carcinoma develop at epidermis and grow to downward14, but the cases at this study were more of hypodermal development types by artificial seeding, than that of recurrence by long distance epidermal metastasis (Fig. 7).

The initial tumor cells were pathologically well differentiated type of squamous cell. When a tumor cell is pathologically well differentiated, then it is difficult to distinguish with normal cells. Since microscopic observations have a limit in distinguishing tumor cells from normal epithelial cells through centrifuged method, we experimented under the assumption that if the number of epithelial cells is greater, then the number of tumor cells would also be greater.

Though the research has its limits in validity and credibility since the number of mice is small as 10, we could reenact the surgical process of clinical situations with animal model, and could prove the local recurrence possibility in a skin cancer surgery.

V. CONCLUSION

A minimal handling is well known common sense in surgical removal of cancer mass and local recurrence should largely be preventable by strict adherence to this simple surgical principle. We could confirm that when the more often we use the surgical instruments in a surgery, the greater is the number of cells attached to the instruments through this study. Also, we could observe that the attached tumor cells at the surgical instruments can cause a local recurrence of skin cancer. So, we are assured that minimal handling is essential in a cancer removing surgery by this study. When we handle a big size of tumor, an "isolation technique" becomes more important since recurrence possibility has a direct proportional relationship to the size of tumor and number of forceps contacting tumor cells.

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