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Prophylactic Effect of *in vitro* Transformed Autochthonous Tissue on Tumor Formation (I)

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Introduction

In 1926, it was known by Strong[60] that tumor cell receives some change on antigenicity and Lumsden[44] postulated the possibility of some kind of vaccination against cancer in 1927. However the tissue which Lumsden used as tumor antigen in his experiment was cancer cell strain originated from a xenogeneic animal and it would be said that he examined and tested general charactor of transplantation antigen.

In the years of 1950s, many workers used tumor cell or tumor tissue from inbred animal as the antigen on the experiment concerning immunity of cancer and modified this disadvantages.[11,18,26,50] However the way of challenge in these experments were transplantation of tumor tissue or inoculation of tumor cells subcultured many times. When considering on the antigenicity of tumor cell, there are differences between initial stage and after repeated subculture and subtransplantation even in same strain of the cell or tissue.[3,41] The vaccination against cancer should be effective for the inital cancer cell in nature so that these experiments carried out by these workers were not completely suited for the method to work for the development of vaccination against the occurence of cancer in nature.

A few workers in the years of 1960s used the tumor tissue of autochthonous origin as the antigen in the experiment and challenge trial was proceeded by the same tumor tisse.[36,39,61] However all the vaccines in these trials were not a vaccine preventing animal from the inital onset of cancer but post operative autovaccine intended to resist the patient against metastases of cancer after operation.

Maisin[45], in 1964, used microsome of hepatoma cells of inbred mice fed dimethylaminobenzene as antigen and challenged the mice by feeding of dimethylaminobenzene and proved the antigenicity of microsome of tumor cell upon the inital cancer without enhancing effect. This would be considered as the vaccination trial against initial cancer occured by chemical carcinogen. However, it is not possible to get antigen from an inbred animal when the vaccination trial was carried out for naturally occuring human cancer.

In 1972, Hellstrom and Hellsrtom[33] wrote about the vaccination against human cancer, "If should, indeed, be possible to build up a strong immunity to common antigens of histologically similar animal tumor, and if animals with such an immunity(But not blocking serum activity) should have an increased resistance to carcinogenesis(in the respective organ), the ground work may have been laid for a future approach to vaccination against certain human cancers."

Since the malignant transformation of normal cells in tissue culture by adding carcinogens into the media was successful by Berwald and Sachs[6] in 1963, many workers proved the discovery.[7,19,20,22,25,34,57] Furthermore even the normal cell transformed spontaneously when they were repeatedly subcultured in tissue culture media.[21] It may be possible to keep an approach to obtain the *in vitro* transformed malignantly autochthonous human tissue for the antigen of vaccination to resist the occurance of natural human cancer.

Fortunately Chen and Heidelberger[20,21] were successful *in vitro* transformation with adult animal cell; human cancer, which occurs in senescence when the immunological surveillance is weak[2,16,23,43,46,47,62,63]

and should be treated or vaccinated in adult stage, and it would be possible to obtain *in vitro* transformed autochthonous antigen of adult human.

Blocking serum activity[30,31,32] seems to be responsible by the participation of 7 S and 19 S serum protein fraction[12] and induced by some factor in the tumor cell substances.[1,4,10,13,17,23,29,37,38,40,48 51,52 55 56,58,59] In the mouse cell, this enhancing factor is concerned by H-2 locus and the antigenic substance is heat stable and extracted by alcohol and ether, lost the action at above pH 10 and below the pH 3, change the charactor by the treatment with trypsin, hyaluronidase, DNAase and neuraminidase.[4,38 49] When the antigenic tumor cell is treated to remove the enhancing factor utilizing one of above mentioned charactor, it will be possible to solve the second problem Hellström and Hellström offered on the development of human cancer vaccine.

Materials and Methods

Three months old C$_3$H male mice, the hair of about five cubic centimeters wide on back were cut with scissors and exposed the skin, were anesthetized with ether. Then the skin was washed with ethanol and phenol and about less than one cubic centimeter of the skin was removed by sharp scissors, and the wound was treated with mercurochrome dressing.

Then the skin was washed twice with PBS and cut into about two milimeter wide with sharp scissors in the PBS and cultured in Eagle medium containing 10 per cent calf serum and 100 units mycostatin per mililiter at the temperature of 37°C.

On the next day, the culture medium was replaced with newly prepared medium containing 10μg 3, 4-benzpyrene per mililiter. The benzopyrene culture was carried out for 10 days. After the benzopyrene treatment for ten days, the pieces of skin tissue were washed with PBS and treated with 0.25 per cent trypsin in PBS for 15 minutes at 37°C and continued to be cultured in normal media above mentioned. 0.25 per cent trypsin solution could not digest the skin at this time. During the benzopyrene treatment the culture tubes were covered with thick and hard black paper to screen the culture from direct artificial light. The glasswares contaminated with benzopyrene were treated with concentrated sulphuric acid, washed with water, with acetone and then with water at last. Old medium containing benzopyrene was exposed under UV lamp for four hours and discarded into sewerage.

The changes of media were carried out in every two or three days and this whole procedure of tissue culture of the skin was carried out for five weeks.

After five weeks from first mouse treatment the pieces of cultured skin were took out from media, washed with physiological saline and homogenized in the mortar. The pH of skin homogenate was adjusted to pH 10 with 0.1 N NaOH, kept for one hour at room temperature and then neutralized with 0.1 N HCl.[38]

Freund's complete adjuvant was introduced into the homogenized skin and injected intraperitoneally the half of the material to the skin donor mouse, twice weekly.

The challenge injection of 1 mg 3,4-benzpyrene

Table 1. Results of Attempts to Produce Autochthonous Immunity, against Subcutaneous B.P. Challenge by Inoculation of Extransplanted Mouse Skin Treated with B.P. *in vitro*

Exp. Group	No. of Mice Taken	Treatments					No. of Mice with Tumor	Ratio of Tumorigenesis	Tumor Regression	Tumor Regression Ratio
		Skin Uptake	B.P. in Media	Alkali treatment	Immunization	Challenge with B.P.				
I	10	NT	NT	NT	NT	T	10	10/10	0	0/10
II	6	T	NT	T	T	T	6	6/ 6	0	0/ 6
III	19	T	NT	T	T	NT	19	0/19		
IV	14	T	T	T	T	T	4	4/14	4	4/ 4

B.P.-3. 4-Benzpyrene
NT-Not treated
T-Treated

./0.25 ml tricapryline took place subcutaneously at area of left scapula,[8,9] two weeks after last intraperitoneal inoculation of the antigen.

For 120 days after challenge inoculation, the mice were observed daily and checked the tumor formation.

Results

The results obtained are summarized in table 1.

Discussion

Extransplanted mouse skin tissue treated with 3,4-benzpyrene *in vitro* and homogenized, then treated at the pH 10 for one hour at room temperature then neutralyzed with 0.1 N HCl, and injected with adjuvant protects autochthonous mouse from tumorigenation by subcutaneous injection of 3,4-benzpyrene.

Burnet[14] reviewed in 1964 that most benzopyrene tumor are loss of antigenicity. However Ko'dovsky's experiment[41] revealed in 1961 that benzopyrene tumor killed with alcohol carried antigenicity not only against transplantation of benzopyrene tumor but also against other polycyclic hydrocarbon induced tumors or spontaneous tumors in inbred mice in early age of the cell. His success seems to depend upon the killing of cells with alcohol and the action of enhancing factor was removed.

In many experiments[27,35,49,53,54] which lead us to believe as if the antigenicity of chemically induced TSTA were different one another even in the same individual, one could find all of those works had been done without the consideration of elimination of the action of enhancing factor from the antigenic tumor tissue.

In this experiment, attention should be called to this point that mouse tumor cell induced *in vitro* with B.P. would carry the antigenicity when its enhancing factor was modified by treatment of high hydrogen ion concentration. And the opening of new era of vaccination against cancer would be expected.

When we assume the antigenicity of tumor: If it were reliable the operon concept, judging from the result[5] in which the antigenicity of some hepatoma cell lost its oragnospecificity of liver cell, it is possible that in the same individual the operon holding same activity would be equal on the action and the allogenic antigens induced by same carcinogen would be equal on the action and the allogenic antigens induced by same carcinogen would be equal; if it were immunological attack by somatic mutation,[15,64] when refering to the nature of DNA which has no difference between various tissues in same individual[42], and when same carcinogen react to same part of DNA in same way, at same individual, the antigens in same individual, would be of same nature.

Polycyclic hydrocarbon, aromatic amines, azocompounds and alkylated carcinogens are the classification of chemical carcinogens depending upon the chemical structures, by Daudel and Datdel[24] in 1966. The definite area on the structure works carcinogenically in the same carcinogen class, or definite metabolites works at definite organ which keeps in touch with the metabolites of the carcinogen of same class.

According to the results of Koldovsky[41] it would be suggested that if one intend to protect animal from the tumorigenesis by chemical carcinogen, autochthonous antigen would be prepared by treatment of four representative chemicals *in vitro* and eliminate the enhancing factor and utilize as vaccine.

Considering carcinogenesis by the chemical carcinogen as the only cause of tumorigenesis, environmental control should be effective. When we consider oncogenesis according to the virus as the main cause of human cancer, the virus isolation which should be successful in the future would be effective to solve the problem.

When the hypothesis of hormone theory concerning the misformulation of steroid hormone is significant for the cause of cancer, it would be not easy to cast out the cause of problem. But since structure of steroid hormone is very resemble to the that of carcinogenic polycyclic hydrocarbon, it would not be too obtrude to say that "This experiment brought us to open the door of entry of studing cancer."

Summary

Intraperitoneal injection of extransplanted C_3H male mouse skin treated with 3,4-benzpyrene *in vitro* was effective to prevent tumorigenesis of autochthonous

mice from subcutaneous injection of 3, 4-benzpyrene.

The possibility of the development of autovaccine against tumor formation was discussed.

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組織培養液中에서 轉化시킨 自己組織의 發癌防止効果 (第 I 報)

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抄　　錄

C_3H 생쥐의 皮膚를 조금 떼어내서 組織培養을 하며 培養液中에 3,4-benzpyrene을 添加하여 腫瘍組織으로 轉化시킨 다음, 알카리로 處理하여 enhancing factor를 除去한 후에 adjuvant와 함께 元來 皮膚를 提供했던 생쥐의 腹腔內에 注射하면, 3,4-banzpyrene의 皮下注射에 의한 發癌作用을 마리 防止할 수 있었다.

上記의 實驗成績을 土臺로 하여 著者는 癌에 對한 vaccine의 開發이 可能한 것이라고 考察한다.