The effect of tissue environments on primary tumor growth and liver metastasis in mice colon adenocarcinoma *

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The mice undifferentiated colon adenocarcinoma cells (C-26) were prepared invitro and implanted into three different sites for the comparison of primary tumor growth and liver metastasis in syngeneic Baltic mice. These tumor cells were injected in equal number into the submucosa of the stomach (group I), submucosa of the cecum (group II) and subcutaneously (group III and IV), and all the animals were observed daily. When the mice got moribund, they were sacrificed for the evaluation of primary tumor size (mm^3) and the number of macroscopic liver metastasis. Primary tumor growth rates were both higher in groups I and II than in group III when the mice were sacrificed 32 days after the tumor inoculation ($p<0.05$). Mean survival days were 22.7 and 21.5 in group I and II respectively, but the mice in group IV survived 73.3 days ($p<0.036$). The maximum liver metastases were observed in stomach group. No liver metastases were observed in group III which the mice were sacrificed before they got sick. When we waited until the animals became ill in another subcutaneous group (group IV), the rate of liver metastasis was high besides the systemic metastasis. Other studies, investigating the interactions between tissue environment and tumor cells, are necessary to explain the difference between stomach and cecum for the outcome of liver metastasis. In this study, we demonstrated that the tumor cells are greatly effected by the tissue environment and such a study can be used as a good experimental model for liver metastasis. [Turk J Med Res 1994, 14 (3):85-88]

Keywords: Neoplasm metastasis, TColon carcinoma, Mouse

Several gastrointestinal malignancies are frequently making their metastasis into the liver, such as colon and stomach cancers. The appearance of the liver metastases his a very poor prognostic sign for the patient. Although there are some difficulties to take advantage of the experimental results gained from animal models in the clinical practice, still we need a constant and natural liver metastasis model that will imitate human metastatic course. For this purpose a variety of animal models have been proposed by different methods. Recent studies showed that implanting of cancer cells to the relevant organ from which the cancer cells were derived, resulted in much higher metastatic rate (1-4). In the present study, we transplanted mouse colon adenocarcinoma cells into three different sites in singeneic mice to observe whether primary tumor growth and outcome of liver metastasis are managed by the environment. Herein, we used the same experimental model that was described to obtain liver metastasis by us previously (5).

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MATERIALS AND METHODS

Mouse transplantable adenocarcinoma C-26, which is a N-nitroso-N- methylurethane induced undifferentiated adenocarcinoma in Balb/c mice (6), was kindly provided by Dr. T. Hamura from Ajinomoto Basic Research Facility, Tokyo, Japan and maintained in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 mg/

ml) at the Chiba University, School of Medicine. The tumor cells were cultured in the condition of 37°C and 5 % CO_2 environment. After incubation, tumor cell suspensions were prepared at a concentration of 1x106 ell/0.05 ml. Almost 100% of the tumor cells were shown to be viable by the trypan-blue test.

Specific pathogen-free, female, 6-8 weeks old Balb/c mice were purchased from Shizuoka Experimental Animal Farm, Shizuoka, Japan for use in this study. All animals were maintained on a daily 12-hr light/12-hr dark cycle. All tumor implantations were carried out under nembutal anesthesia (1.25mg/25gr mouse weight, i.p.). The mice were divided into 4 groups; group I was stomach wall implantation (described below), group II was cecal wall implantation, groups Ill and IV were subcutaneous implanted mice. The mice in the third group were sacrificed 32 days after the tumor implantation for comparison with groups I and Il, where the fourth group let survive as long as they can. For stomach and cecum wall, the abdomen was sterilized with iodine and alcohol swabs. A small midline incision was made (upper abdominal for stomach, lower abdominal for cecum) and the stomach or the cecum was exteriorized. Stomach was opened with a 2-3 mm incision from the greater curvature side of the body and a 30-gauge needle attached to the tuberculin syringe was inserted into the lumen. The tumor cells were injected into the submucosal area at least 5 mm far away from that incision for preventing intraperitoneal spillage of tumor cells. Frequent trituration of cells was performed in a sterile tube to maintain uniform cell suspensions. Cells were injected so as to visibly infiltrate bullea (3-5 mm in diameter) between the mucosal and serosal layers (Fig.I). Similar approach was followed for the cecum paying attention to make the injections into the same area in all mice. After successful injection, the stomach or cecum incision was closed with 6-0 Maxon (Davis-Geck Inc.) whole layer sutures. Then the organs replaced insitu and the abdominal wall was closed with continuous 6-0 Maxon sutures. Subcutaneous injections were made into the flank. The mice were sacrificed when they became moribund except the mice in group Ill by daily observations. Those mice were sacrificed just after the first and second group mice had been killed. All organs including the stomach, cecum and liver were processed for routine histological examination after careful macroscopic examination.

RESULTS

The mean survival days in various injection sites are shown in Fig.2. While the stomach and cecum groups survived 22.7 and 21.5 days respectively, the subcutaneous group survived 73.3 days. The differences were statistically significant ($p<0.036$). All the mice in the third group were sacrificed 32 days after the tumor implantation for the comparison with groups I and Il.

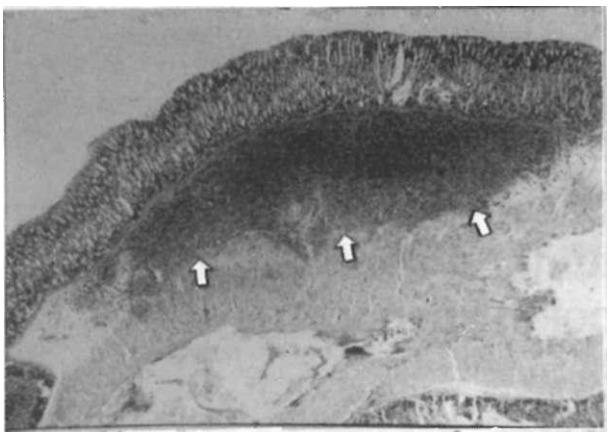

Figure 1. Submucosal implantation of C-26 tumor cells in a mouse which was sacrificed 3 days after transplantation. All the tumor injections were performed into the submucosal area.

Table 1 shows the local tumor growth, tumor volume and outcome of liver metastasis in three various sites, after the implantation of 1x106/0.05 ml of C-26 tumor cells. Local tumor growth was observed in all mice of all groups and the tumor volume was greatest in the stomach group. The mean tumor volumes in groups I and II were both bigger than the third group ($p<0.05$). When we let the subcutaneous implanted mice to survive as long as they can (group IV), the tumor reached to a huge size too.

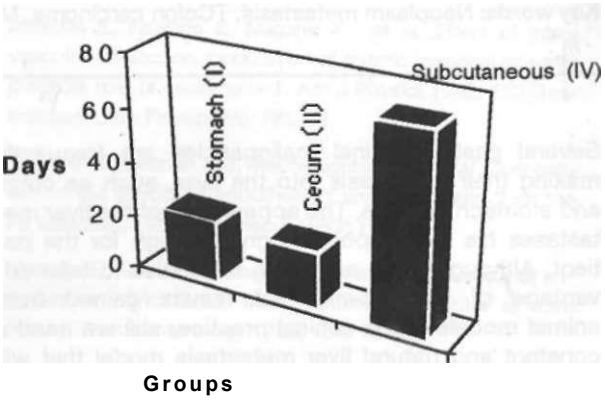

Figure 2 Mean survival days in different tumor implantation sites

While all the mice in the first group were exhibiting liver metastasis (100%), only half of the mice demonstrated liver metastasis in the second group (50%) (Fig.3). The mice in the third group had smallest

Table I.The difference of tumor growth rates and metastatic liver colonies macroscopically in various injection sites.

Groups *	Local tumor growth **	Mean tumor size(mm3)±SD	Liver metastasis ****	Incidence of liver metastasis, %a
Stomach (I)	6/6	6588±3335-p	1610 9321	100
Cecum (II)	6/6	2445±79&J	732000	50
Subcutaneous(III)	7/7	1415±947—	0000000	0
Subcutaneous (IV)	7/7	5864±2146	4332 100	71

All mice were transplanted 1x10(6)10.05 ml of Colon-26 cells.

Data are shown as number of mice which had local tumor growth per number of mice evaluated.

* Stomach implantation group had significantly better tumor growth than cecum and subcutaneous (I) groups.

• $p<0.05$, •• $p<0.005$.

•** Data are shown as number of macroscopic liver metastasis per mice.

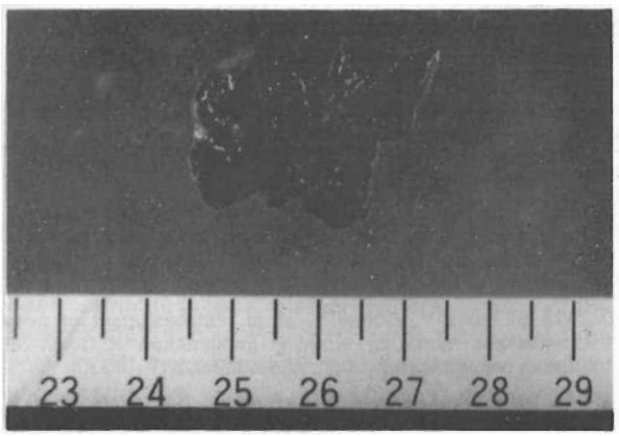

Figure 3. Noduler macro-matastasis in the liver.

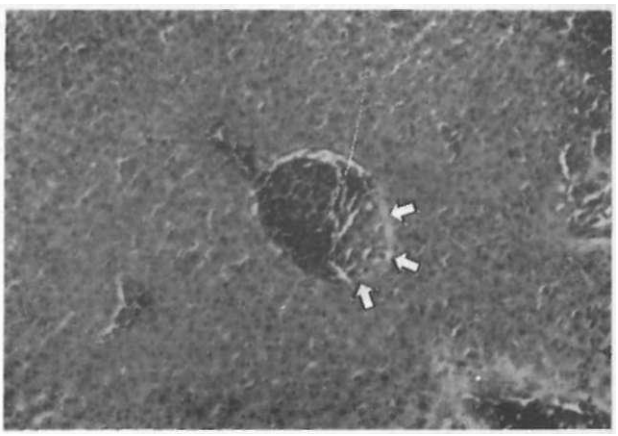

Figure 4.- Histological view of tumor cell cluster in the portal vein (x100. H.E.).

tumor volume and no liver metastasis 32 days after the tumor implantation. When the mice in the fourth group were allowed to survive as long as they can, all the mice had a plenty of lung metastasis and 5 of 7 mice (71 %) had liver metastasis.

Besides the regional lymph node metastasis in all mice, the C-26 tumor cells made their liver metastasis by portal vein when they were inoculated into either the stomach or cecum wall (Fig.4).

DISCUSSION

The major goal of the present study was to determine whether the implantation site of C-26 cells influenced the primary tumor growth and outcome of liver metastasis in syngeneic Balb/c mice. The topic in this study had widely been studied by several authors, especially by I.J. Fidler et al (7). Our results have supported their previous reports in which also athey did not get any visceral metastasis when the human colon carcinoma cells were injected subcutaneously whereas regional lymph node and liver metastasis outcame from the cecum wall. In the present study, although plenty of regional lymph node and liver metastasis were obtained in the stomach and cecum groups, no metastases were observed in the subcutaneous group

III which the mice were sacrificed 32 days after tumor implantation. In the fourth group, when we let the mice live as long as they can survive, we observed several visceral organ metastases. When we implant the gastrointestinal tumor into a part of the alimentary tract such as colon or stomach, the tumor exhibited better tumor growth, much more liver metastases and less survival rate than the subcutaneous group for a certain period. Maybe the adaptation of the tumor into a different environment takes longer time than it needs for the same environment from where it is originated. We need furt-her studies to explain this difference. Maybe the other thing we can interpret is; the distant organ metastasis occurred in the late phase of tumor growth in the subcutaneous group. In other study of us, the liver metas-tases had occurred at least 10 days after tumor implantation into the stomach wall by sacrificing the mice 3, 7, 10 and 14 days after the tumor injection (data not shown).

The importance of orthotopic transplantation of tumors for the metastasis has been stressed recently (2,8-11). For example, human colon cancer cells were disaggregated and injected into the cecal wall of nude mice to produce tumors that eventually metastasized to the liver, demonstrating that cecal implantation can enhance the metastatic capability of human colon cancer cells in nude mice(3). We observed the similar results, but interestingly colon cancer cells (C-26) had better tumor growth and much more liver metastasis in the stomach wall than the cecum wall. Again it is hard to interpret this difference easily. In this trial, we also observed that the liver metastasis had occurred via the hematogenous route rather than the direct invasion, because all the metastatic nodules rose from the periportal space. In conclusion, we established a liver metastasis model by stomach wall implantation of C-26 cancer cells in Balb/c mice. Also we should consider more about the influence of organ environment on the tumor growth and the outcome of visceral metastasis in order to find new therapeutic modalities for cancer treatment.

Fare kolon adenokarsinomunda (C-26) farklı doku ortamlannın primer tümör büyümesi ve karaciğer metastazına etkisi

Farelerin indiferansiye kolon adenokarsinom (C-26) hücreleri in-vitro kültürlerle hazırlanarak sinjenik Balb/c farelerinde 3 farklı yere implante edildi ve primer tümör büyümesi ile karaciğere metastaz oluşumu açısından karşılaştırıldı. Eşit sayıda hazırlanan tümör hücreleri mide submukozasına (I.grup), çekum submukozasına (II.grup) ve cilt-altına (III. ve IV.grup) enjekte edilerek hayvanlar günlük olarak takip edildi. İleri derecede hastalanan fareler sakrifiye edilerek, primer tümör büyüklüğü (mm³) ve karaciğerde oluşan makro metastazlar değerlendirildi. I. ve II. grupta gözlenen primer tümör büyümesi 32. günde sakrifiye edilen III. gruba göre çok daha fazla İdi (p<0.05). I. ve II. gruptaki hayvanlann ortalama yaşam süresi sırasıyla 22.7 ve 21.5 gün iken, IV. grupta bu süre 73.3 gün idi (p<0.036). En fazla karaciğer metastaz! mide duvarı implantasyonu ile gözlendi. Hayvanların hastalanmadan sakrifiye edildiği III. grupta hiç karaciğer metastazı gözlenmedi. Ancak ciltaltı enjeksiyonundan sonra hayvanlar hastalanıncaya kadar beklenildiğinde yüksek oranda sistemik metastazlar yanında karaciğer metastazları da gözlendi. Karaciğere metastaz oluşumunda mide ile çekum arasında gözlenen farkın izahı için doku ortamı ile tümör hücreleri arasındaki etkileşimi inceleyen başka çalışmalara ihtiyaç vardır. Biz bu çalışmada tümör hücrelerinin bulunduğu ortamdan etkilendiğini ve böyle bir çalışmanın deneysel karaciğer metastaz modeli olarak kullanılabileceğini gösterdik, fjurk J Med Res 1996, 14 (3): 85-88]

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