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tosan is an activator of macrophages and it is therefore suggested that chitosan, through MIP-2 production, MHC class II-CD4⁺ lymphocyte

interactions, and Fc-receptor-mediated and mannose receptor-mediated phagocytosis, may accelerate wound healing.

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Studies on the establishment of micrometastasis models using bacterial *lacZ* gene-tagged tumor cells and chemosensitivity of micrometastasis to an anti-cancer agent

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lacZ gene encodes Escherichia coli β -galactosidase, whose enzymatic activity can be simply detected by staining with the chromogenic substrate 5-bromo-4-chloro-3-indoyl- β -galactopyranoside (X-Gal), has been recently reported to greatly facilitate detection of metastasis in their earliest stages of development. In the present study, the author transfected rat differentiated adenocarcinoma cells and mouse lung carcinoma cells with a *lacZ* gene to establish micrometastasis model, and sequential events of micrometastasis formation by these cells were investigated. In addition, the therapeutic effect of anti-cancer drug UFT on micrometastasis was examined using mouse lung carcinoma cells transfected with the *lacZ* gene.

In chapter 1, a micrometastasis model was established using a rat differentiated prostatic adenocarcinoma, designated PLS301Z, transfected with the *lacZ* gene. The morphology, tumorigenicity and metastatic ability of PLS301Z were comparable to those of the parental cells. Micrometastatic foci could be specifically detected at the single cell level after X-Gal staining with a dissecting microscope. After intravenous injection, the number of X-Gal positive foci in lung

decreased progressively to a steady state level (less than 1% of injected cells) by 4-7 days, while the size of persisting positive foci started to increase from 4 days after inoculation as demonstrated by image analysis. X-Gal and BrdU double staining revealed that BrdU labeling indices of X-Gal positive cells decreased transiently at the 2 day time point and increased again from 4 days after inoculation. Type IV collagen immunostaining showed the tumor cells to be surrounded by a basement membrane intravascularly at the time point when they started new growth. Electron microscopy confirmed that, 2 days post injection, most tumor cells were degenerative or dead, but on day 4, persisting tumor cells formed multicellular clumps in contact with the vascular basement membrane inside vessels. These results indicate that PLS301Z cells begin to grow intravascularly depending upon the presence of a basement membrane before extravasation at the initial stage of micrometastasis formation.

In chapter 2, sequential events in micrometastasis formation including entry into the blood circulation and arrest, extravasation and initial growth in the lung was investigated using bacterial *lacZ* gene-tagged Lewis lung carcinoma

cells (4A1-1). Micrometastasis in the lung could thereby be specifically detected at the single cell level by X-Gal staining. After intravenous injection, X-Gal positive tumor cells appeared to extravasate within hours, the process being completed within one day, but most cells then degenerating or dying in the alveolar space by 2-3 days postinjection. A decreased BrdU labeling index to a negligible level at 2 days postinjection and reduction of X-Gal positive foci to a basal level (less than 0.1% of injected cells) by 4 days are in line with rapid clearance of tumor cells from the lung. The size and BrdU labeling indices of the persisting x-Gal positive foci, however started to increase from 4 days postinjection. Type IV collagen immunostaining demonstrated loss of pre-existing basement membranes with growth of micrometastases. When 4A1-1 cells were inoculated subcutaneously, lung micrometastasis from resulting tumors were detected as single or small numbers of X-Gal positive cells at 2 weeks postinjection. Progressive increase of lung nodules in size was noted with circulating tumor cells in blood. The results indicate that micrometastasis formation by Lewis lung carcinoma cells involves a sequence of events starting with rapid extravasation after arrest in the lung within one day, followed by death of most cells at 2-3 days and subsequent new growth and expansion of persisting tumor cells from 4 days postinjection.

In chapter 3, the chemosensitivity of micrometastasis to anticancer drug UFT was examined with micrometastasis model established in chapter 2. Spontaneous macroscopic metastasis in mice bearing primary tumors was markedly inhibited by UFT at doses of 15-20 mg/kg when it was orally administered from day 14, during the early stage of micrometastasis formation, but not when treatment was from day 21. Micrometastasis, on the other hand, was inhibited even by late stage administration of UFT and effects was observed with a lower doses (10 mg/kg). Primary tumor volume was only significantly diminished with doses of 15-20 mg/kg. Experimental metastasis was also inhibited by oral administration of UFT at a daily dose of 20 mg/kg from day 4, but not by twice daily treatment from 8 days postinjection, although in the latter case, the size of metastatic nodules were diminished substantially by the drug, as demonstrated by image analysis. Oral administration of UFT 1 hour before i.v. injection of tumor cells had no effect on tumor cell arrest in the lung. These results indicate that the initial growth phase is much more sensitive to this chemotherapeutic agent than the preceding arrest and subsequent exponential growth stages, and suggest that micrometastasis can be effectively eliminated by oral administration of anti-cancer agent at low doses.

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