4-6.
Investigation of the immune microenvironment around curatively resected colon cancer

（大学院博士課程 3 年東京医科大学病院　消化器・小児外科）
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Infiltration of inflammatory cells as a host immune response in colon cancer has been reported to be associated with survival independent of pathological stage, and the presence of tumor-infiltrating lymphocytes in particular is considered to be an indication of an effective immune response.

Most of the previous reports have focused only on the localized cancer foci, and there have been no reports that comprehensively examined the tumor environment surrounding the cancer.

We conducted a retrospective study of resected specimens from 106 patients with stage 2 or stage 3 colon cancer who were resected at our hospital between 2000 and 2014. Fluorescent multiplex immunohistochemistry were performed to the resected specimens of primary lesions, and the specimens were observed and photographed under a fluorescence microscope. The obtained images were mechanically classified into cancer foci and stroma by software, and the distribution, traits, and frequency of various immune cells were comprehensively examined by combining the expressed surface markers and transcription factors. In this study, we investigated the distribution and function of immune cells derived from tumor tissues of colon cancer patients.

4-7.
Comparison of cell-free DNA release mechanism between normal cells and tumor cells using in vitro model

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【Introduction】 Cell-free DNA（cfDNA）has emerged as a valuable diagnostic tool of liquid biopsy analysis in cancer management. In this study, we attempted to construct an in vitro model for detecting cfDNA from the culture supernatant of tumor cells.

【Methods & Results】 Human normal dermal fibroblast（NHDFs）and human cervical cancer cell lines SiHa were maintained using DMEM（Gibco）supplemented with 10% fetal bovine serum at 37°C. To induce apoptosis in normal and tumor cells, UV irradiation（0-80 J/cm2）was performed using a UV cross-linker（UVP）. Comparing the apoptotic activity, it increased as the UV irradiation intensity in both NHDF and SiHa.

CfDNA was collected from the culture supernatant of each UV-irradiated cell and its fragment size and concentration were compared by TapeStation. NHDF released cfDNA even without UV irradiation, and the concentration of cfDNA increased as the UV irradiation intensity increased. In tumor cells, Irradiation with 60 J/cm2 of UV drastically increased the amount of cfDNA released from SiHa. The cfDNA derived from Siha was a smear-like band, and smaller than thatof NHDF with an apex of 80-100 bp size.

【Discussion】 Interestingly, when tumor cells were exposed to 80 J/cm2 of UV, the amount of cfDNA was significantly increased despite the decreased apoptotic activity, and the size of the cfDNA was also different from that of normal cells. It suggested that tumor cells release cfDNA by different mechanisms from that of normal cells.