

Pathology Division

The research activities at the Pathology Division of the Research Center for Innovative Oncology are currently focused both on the application of the morphological study of cancer tissue to the clinical course of the patient to elucidate the biological activity of cancer and on establishing new methods of selection for treatment of patients and for clarifying the mechanism of cancer metastasis. Prognostic factors and clinicopathological characteristics of various cancers have been studied in collaboration with the Diagnostic Pathology Section of the Clinical Laboratory Division at National Cancer Center Hospital East.

The Cancer Microenvironment and Cancer Biology

The cancer microenvironment that is generated by cancer-stromal interactions plays an important role not only in carcinogenesis but also in cancer progression. There are several studies, including those being carried out at our own division, indicating that metastatic cancer cells can use the milieu of the metastatic organs to generate organ-specific metastases such as bone metastasis by prostate cancer, breast cancer and multiple myeloma. To confirm the importance of bone-derived or bone microenvironment-derived growth factors, an animal model in which human multiple myeloma (MM) cells are allowed to proliferate in human adult bone (HAB) implanted in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice was established and anti-human IGFs were applied to the model. The growth of MM was regulated by anti-human IGFs antibody both in vitro and in vivo, indicating that the growth of MM cells was predominantly stimulated not by serum-derived IGFs, but by bone-derived IGFs,

and the targeting of bone-derived IGFs might offer a new therapeutic strategy for MM (76). Prostate cancer is also frequently associated with bone metastases with marked osteoblastic changes. To elucidate the osteoblastic bone metastasis of prostate cancer cells, the effect of prostate-specific antigen (PSA), a tumor marker of prostate cancer that acts as a serine protease on osteoclast genesis, was investigated. PSA-producing prostate cancer cells induced apoptosis of osteoclasts both in vitro and in vivo. The induction of apoptosis by PSA was observed in osteoclast precursors but not in mature osteoclasts. This is consistent with previous results indicating that prostate cancer requires bone-derived IGF released by mature osteoclasts (77).

Recent reports, including our own, have revealed that bone marrow-derived cells can become constituents in a number of organs, such as the prostate, salivary glands and the liver, especially in remodeled tissue. The recruitment of bone marrow-derived cells into the mammary glands due to stimulation by estrogen was investigated using a bone marrow transplantation technique. The bone marrow-derived myoepithelial cells and periductal fibroblasts were recruited by hormonal stimulation into the mammary gland context (78).

Evaluation of Cancer Phenotypes in the Treatment of Cancer

Because chemoradiotherapy (CRT) can achieve similar survival rates to surgery or surgery+CRT, patients' individual characteristics and tumor histological features that favor chemoradiotherapy should be carefully assessed before choosing a treatment. Our previous studies have demonstrated

that hotspot microvessel density (MVD) in biopsy specimen has strong prognostic significance for patients with laryngeal squamous cell carcinomas and with hypopharyngeal cancers treated with radiation. The ratio of total microvessel perimeter (TP) to total tumor area (TA) of biopsy specimens, the ratio of total microvessel number (TN) to TA, and the tumor hypoxic ratio calculated from microvessel distribution in biopsy specimens have been further proved to be good prognostic factors for patients with early stages of laryngeal cancer treated with radiation. To confirm whether image analyses of tumor microvessels in biopsy specimens can predict a patient's prognosis, biopsy specimens of 51 esophageal cancers treated by CRT were examined by computer-assisted image analyses. The TN/TA and TP/TA ratios correlated significantly with disease-free survival and combining the computer-counted MVD or TN/TA ratio with TP/TA ratio proved more predictive than any single factor. These data indicate that computer-assisted tumor microvessel analysis is a powerful tool for predicting outcomes in patients with CRT-treated esophageal cancer (79).

To explore the underlying mechanisms of CRT responses and to contribute to the development of tumor markers which aid in selecting the best therapeutic modality prior to initiation of therapy, gene expression patterns of pretreatment biopsy specimens from 33 patients with CRT alone with long-term survivors, and short-term survivors were examined using microarrays. The expression patterns of 12,600 genes were used and 57 genes correlating with short-term survival and 120 genes correlating with long-term survival were identified. These genes were involved in the immune response for long-term survival and drug-resistant genes were involved in short-term survival. The list of these genes may contribute to the elucidation of the mechanisms of CRT response and contain useful markers for predicting the prognosis of individual esophageal cancer patients treated with CRT (25).

Clinicopathological Studies on Various Cancer in Collaboration with the Diagnostic Pathology Section

Clinicopathological Studies on Gastrointestinal Tract and Lung Tumors were Performed in Close Collaboration with the Clinical Divisions of the National Cancer Center Hospital East and Other Hospitals.

1. Gastrointestinal tract

1-1. The expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was immunohistochemically examined in colorectal cancer tissues. A significant increase of iNOS and COX-2 levels in tumors was observed in 52%. iNOS expression was correlated with tumor differentiation, but COX-2 expression increased according to tumor development. These data indicate that iNOS and COX-2 have separate roles in colon carcinogenesis (80).

1-2. HER2 gene amplification assessed by fluorescence in situ hybridization and HER 2 protein expression by immunohistochemistry was compared both in surgical sections and biopsy specimens of gastric cancer. The concordance between IHC and FISH was 86.9% and that of IHC between surgical sections and the biopsy specimens was 88.7%. These data indicate that HER2 expression can be assessed in biopsy specimens of gastric cancer (81).

1-3. Gastrointestinal neuroendocrine cell carcinoma is a highly aggressive tumor with poor prognosis. Immunohistochemical and mutational analysis of c-kit in gastrointestinal neuroendocrine cell carcinoma was performed in collaboration with Saitama Cancer Center. 26% of gastrointestinal neuroendocrine cell carcinoma expressed c-kit, but no c-kit mutations were detected. These data will

provide new insights into targeting therapy for c-kit in gastrointestinal neuroendocrine cell tumors (82).

- 1-4. Primary gastric diffuse large B-cell lymphomas are generally well controlled by combination chemotherapy, with some exceptions. To select patients who are resistant to chemotherapy, the relationship between the condition of Epstein-Barr virus (EBV) infection and clinical outcome was investigated. A correlation was observed between resistance to chemotherapy and EBV-associated gastric diffuse B-cell lymphomas (83).

2. Lung

- 2-1. To confirm the prognostic factors after surgery for pulmonary metastases of colorectal carcinoma, immunohistochemical prognostic factors were examined in 86 surgically resected pulmonary metastases. Reduced E-cadherin expression, aerogenous spread with floating cancer cell clusters and vascular invasion proved to be independent prognostic factors (84). These data indicate that these limited but useful indicators should be taken into account as prognostic factors when planning the patient's treatment (85).
- 2-2. Morphological differential diagnosis between largecell neuroendocrine carcinoma (LCNEC) and small-cell lung carcinoma (SCLC) is sometimes difficult. To clarify the immunohistochemical differences between LCNEC and SCLC, 48 antibodies were immunohistochemically examined using tissue microarray. The expression of CK7, CK18, E-cadherin and beta-catenin proved to be more a characteristic finding in LCNEC than in SCLC (86).
- 2-3. Squamous cell carcinoma of the lung (SCC) accounts for about 30% of all lung cancers. Clinicopathological and biological features were compared between central-type and peripheral-type SCC of the lung. Central-

type SCC shows more frequent squamous metaplasia and CK7 expression than the peripheral type. These results suggest that the two types of SCC have different clinicopathological and biological features (87).

- 2-4. The immunohistochemical differences among mucin-producing carcinoma of the lung, including signet ring cell carcinoma (SRCC), adenocarcinoma with mucin production (SA), mucinous bronchioloalveolar carcinoma (m-BAC), mucinous carcinoma and mucoepidermoid carcinoma were examined using tissue microarray with various antibodies. The results showed that SRCC and SA belong to the same category as alveolar lining cells, whereas m-BAC proved to belong to another category, indicating that SRCC is a separate clinicopathological feature, as reported previously (88).
- 2-5. The expression level of EG5, an inhibitor of mitotic kinesin, was compared with the response to chemotherapy in advanced non-small cell lung cancer (NSCLC). Eg5 expression was a predictor of response to antimitotic agents combined with platinum chemotherapy in advanced NSCLS (89).

● A. Ochiai ●