

## Department of Pathology

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### General Summary

The objective of our research in the Department of Pathology is to morphologically investigate the causes of disease and to evaluate morphological changes. We used human tissue samples resected at autopsy and surgery or obtained at biopsy. These samples were examined by means such as light microscopy, electron microscopy, morphological measurement, immunohistochemical staining, and molecular pathological techniques.

### Outline of Education and Research

#### *Research on the gastrointestinal tract*

1. We evaluated the clinicopathological characteristics of early-stage epithelial tumors of the duodenum. The study group comprised 101 patients with intramucosal tumors (110 lesions) whose duodenal epithelial tumors were endoscopically or surgically resected. The mucin phenotype of the tumor was evaluated immunohistochemically, and the histological grade of atypia was classified according to the 2010 World Health Organization classification. In addition, the frequencies of differentiation into fundic glands in the tumors and the underlying gastric mucosa were studied immunohistochemically. The tumors were classified into 2 subgroups according to mucin phenotype: intestinal-type tumors (98 lesions, 89.1%) and gastric-type tumors (12 lesions, 10.9%). The intestinal-type tumors were subclassified into tubular type (91 lesions, 82.7%) and tubulovillous type (7 lesions, 6.4%). Gastric-type tumors were subclassified into gastric foveolar type (3 lesions, 2.7%) and pyloric type (9 lesions, 8.2%) according to mucin phenotype. The grade of atypia was significantly higher in gastric-type tumors. Pyloric gland-type tumors were characterized by the proliferation of mucous glands similar to pyloric glands and showed a high frequency of differentiation into fundic glands. Fundic gland tissue was found in 16 (14.5%) of 110 specimens of non-tumorous (normal) mucosa situated adjacent to lesions. The majority of duodenal epithelial tumors were evaluated to be intestinal-type tumors on the basis of mucin phenotype. However, about 10% of the duodenal epithelial tumors were gastric-type tumors associated with gastric-type mucin. These gastric-type tumors were large lesions with a high histological grade of atypia. Among the gastric-type tumors, 9 lesions (8.2%) were pyloric gland-type tumors, showing a high frequency of differentiation into fundic glands. The presence of fundic glands in the normal mucosa of duodenal tumors suggested that proliferating cells situated in the duodenum possess the ability to differentiate into fundic glands and participate in the develop-

ment of pyloric gland-type tumors.

2. Active inflammation associated with ulcerative colitis (UC) is generally histologically evaluated according to Matts' classification. However, there is often a divergence between Matts' classification and endoscopic findings. We analyzed biopsy specimens and studied the correlation between disease activity evaluated on the basis of endoscopic findings and disease activity evaluated on the basis of histologic findings. The study group comprised 191 patients (527 biopsy specimens) with UC who underwent lower gastrointestinal endoscopy with biopsy in our hospital from June 2015 through June 2016. Six endoscopic findings in the biopsy specimens (non-inflamed mucosa, loss of visible vascular patterns, granular mucosa, friable mucosa, spontaneous bleeding, and erosions and ulcers) were evaluated according to the main variables of Matts' classification and the Mayo classification. Histological findings of the biopsy specimens were evaluated on the basis of the presence or absence of the following 5 findings: the 4 main variables of Matts' classification (neutrophilic infiltration, cryptitis, crypt abscess, and erosions or ulcers) and the presence or absence of basal plasmacytosis (BP). In patients with highly inflamed mucosa associated with endoscopic findings of erosions or ulcers, the rates of histological findings of crypt abscess and of erosions or ulcers were 27.4% and 18.3%, respectively. However, BP was found in 58.5% of patients. BP was thus suggested to be a useful finding suggesting the presence of active inflammation.

3. Six cases of large villous adenoma (lesions consisting of  $\geq 80\%$  villous components) were selected from among the lesions that were resected surgically and endoscopically. Guanine nucleotide binding protein, alpha stimulating gene (GNAS) mutations in the villous adenomas were analyzed by real-time polymerase chain reaction (RT-PCR). The expression of GNAS mutations (601C>T) was confirmed in 4 cases. The villous gland fields obtained from these 4 cases were classified into 2 regions (high-grade atypia region, low-grade atypia region), and 3 sites including regions of non-villous glands at the tumor margin were analyzed. The experimental results suggested that GNAS mutations in villous adenomas show not only homogeneous expression, but also heterogeneous expression.

4. Serrated lesions of the colorectum, for which the diagnostic criteria remain unclear, were studied morphometrically. The lesions were classified on the basis of the maturity of crypt surface cells and the presence or absence of branched tubules. In addition, Ki-67 immunostaining was performed to compare the relations to zones of proliferating cells. Types 1 and 2 corresponded to conventional hyperplastic polyps, and the proliferative zone was located in the crypt floor. Type 3 corresponded to sessile serrated adenomas/polyps, and the proliferative zone extended to the middle of the crypts. Types 4 and 5 corresponded to serrated adenomas, and proliferating cells tended to be distributed in all layers of the crypts.

#### *Research on the liver*

1. Patients with autoimmune hepatitis (AIH) underwent several biopsies to study whether histopathological findings such as interface hepatitis, activity findings associated with intralobular focal necrosis, and the status of fibrosis correlate with changes in biochemical data. The results showed that biochemical data such as ALT correlated to some extent

with inflammatory activity, but no correlations were obtained for the status of fibrosis. Therefore, the status of AIH can be ascertained to some extent on the basis of biochemical data, whereas an invasive liver biopsy must be performed to assess the status of fibrosis.

#### *Research on the kidney*

1. Molecular pathological studies related to glomerular lesions and tonsillitis in IgA nephropathy: Total RNA in frozen specimens of tonsils from patients with IgA nephropathy (5 specimens) and in tonsils affected by chronic tonsillitis (4 specimens) was amplified by quantitative RT-PCR, and Human Genome U133 Plus 2.0 Array (Affymetrix, Inc.) was used to perform transcriptome analysis (38,500 genes). Genes that showed RNA expression patterns in the tonsils of patients with IgA nephropathy that differed from the RNA expression patterns in the tonsils affected by chronic tonsillitis in the control group were identified, and 17 genes related to conditions such as cancer and lymphoma were focused on. Genes related to increased numbers of T-cell nodules in the tonsils and genes related to the bipolarization of the reticulation of the crypt epithelium were investigated, and a total of 167 genes were identified. In association with chronic stimulation of the tonsils in patients with IgA nephropathy, toll-like receptor 9 induces the overexpression of a proliferation-inducing ligand (APRIL), a member of the tumor necrosis factor (TNF) family, via B cells located primarily in lymph follicles of the tonsils and thereby contributes to the overproduction of galactose-deficient IgA1 (Muto M et al. JASN 2017). In addition, thymic stromal lymphopoietin (TSLP) expressed by dendritic cells of lymph follicles has been reported to promote IgA class switching by increasing IgA plasma cells (Meng H, et al. Transl Res. 2016).

2. Three-dimensional structure of glomerulonephritis on scanning electron microscopy (SEM): Glomeruli of 14 patients with glomerulonephritis were photographed on serial block-face scanning electron microscopy (SBE) and SEM.

In patients with IgA nephropathy (4 patients with acute active disease and 2 patients with chronic advanced disease and lupus nephritis), the cellular composition was colored and segmentation was performed to analyze the 3-dimensional structure. First, in IgA nephropathy and lupus nephritis, podocytes were found to penetrate the glomerular basement membrane and impact the mesangial matrix; contact with mesangial cells was confirmed. On the other hand, mesangial cells were found to enter the subendothelial space under the lamina densa of the glomerular basement membrane, and the findings suggested an increase in the mesangial matrix. To our knowledge, this is the first time in the world to demonstrate contact between podocytes and mesangial cells at the time of infolding into the mesangial matrix. Second, when we followed up whether the destruction of the glomerular basement membrane occurred in association with segmental thin basement membrane lesions, podocytic invasion into the intraglomerular space beside the endothelium was confirmed in the glomerular basement membrane in the region near the mesangial matrix. It has previously been suggested that some of the cells considered to be endothelial cells are podocytes.

*Research on the urogenital system*

1. The urothelial cancers underwent immunostaining (HER2, CK5/6, CK20, CD44) and HER2 fluorescence in situ hybridization (FISH). On FISH 17% of the patients had HER2 IHC 3+ tumors, and gene amplification was found in 13 patients with IHC 2+ tumors. Most of the patients with HER2-positive tumors had luminal-type tumors.
2. Since last year, we have studied the anatomic, developmental, and clinicopathological characteristics of prostate cancer. In 2016, intraductal carcinoma of the prostate (IDC-P) and its outcomes were mainly studied. The incidence of IDC-P is lower in transitional zone cancer than in peripheral zone cancer, and relatively good outcomes will most likely be obtained hereafter. The presence of IDC-P was a significant prognostic factor for poor outcomes in patients who underwent total prostatectomy.

*Research on gynecologic pathology*

1. Most cases of high-grade serous carcinoma (HGSC) of the female genital organs are serous tubal intraepithelial carcinomas (STIC) arising in the fallopian tubes including the fimbriae and causing infiltrating cancer, metastases to the ovary, and peritoneal seeding. We investigated the incidence of STIC and studied the pathological findings according to the type of primary HGSC (fallopian tube, ovary, endometrium, peritoneum).

*Research on molecular pathology*

1. To investigate the locations of disease genes related to the development of lung cancer, we studied a total of 306 patients with adenocarcinomas, squamous-cell carcinomas, and neuroendocrine tumors. Microsatellite instability (MSI) analysis was performed 19 DNA markers on chromosome 8p. The rate of MSI at 8p23.2, 8p23.1, 8p22, and 8p21 was 20%, 51%, 24%, and 15%, respectively. The rate of MSI at 8p23.1 was significantly higher than that at the other regions. In particular, a high frequency of MSI was found at the D8S1819 locus for every tissue type, suggesting that a disease gene related to the development of lung cancer is situated at 8p23.1.

**Publications**

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