Department of Pathology

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General Summary

The aim of our study in the Department of Pathology is to examine the causes of disease on the basis of morphology and morphological changes. We studied autopsied, surgically resected, or biopsied human body materials. These materials were examined by using several techniques, including optical microscopy, electronic microscopy, morphometry, immunohistochemical staining, and molecular pathological measurements.

Research Activities

Research on the gastrointestinal tract

1. Examination of predictive factors for lymph-node metastasis in patients with early colorectal cancer

The incidence of lymph-node metastasis (LNM) in patients with colorectal cancer invading the submucosa (pT1 colorectal cancer) is 10% to 15%. We examined the association between histopathological factors and lymph-node metastasis in 339 consecutive patients with pT1colorectal cancer who were treated in Shizuoka Cancer Center. Lesions were classified as polypoid growth type and non-polypoid growth type to clarify problems associated with submucosal invasion depth (SID) as defined by treatment guidelines for colorectal cancer. We prepared an algorithm excluding SID to predict LNM; LNM was found in 37 (10.9%) of 339 lesions. In our algorithm, LNM was found in 36 (16%) of 249 lesions with at least 1 of 3 factors, consisting of non-polypoid growth type, lymphatic invasion, and 2/3rds of sprouting. Of the 90 lesions with no factors, LNM was found in only 1 lesion (1%). These results showed that the use of our algorithm excluding SID allowed lesions to be classified into a high-risk group or a low-risk group.

2. Examination of appropriate handling of surgically resected specimens of colorectal cancer

How surgical materials of colorectal cancer should be handled is defined in detail by the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma (JCCRC). In the diagnosis of advanced pT4a stage colorectal cancer, however, some lesions cannot be detected with a method for sampling of colorectal cancer as defined in the JCCRC. In the JCCRC, pT4a is defined as tumors infiltration of serosa and extra-serosa (SE). We carefully examined the sites of SE tumors in 44 patients with a diagnosis of pT4a colorectal cancer (patients who had undergone surgery at the Shizuoka Cancer Center). The sites of the occurrence of SE tumors were classified as the mesenteric or antimesenteric side of the colon. In 44 patients, the SE tumor was identified in 80 sections: 30 sections (38%) in

the serosa of the mesentery and 50 sections (62%) of the antimesenteric side. We carefully examined the distribution of the antimesenteric side and found SE tumors around the antimesenteric side in 25 sections (50%), indicating that SE tumors more frequently occurred at such sites. Grade PT4a colorectal cancers were difficult to assess accurately with a method for splitting the long axis of the large intestine in parallel, as recommended by the JCCRC. A method for vertically splitting the long axis of the large intestine which emphasizes the positional relation between lesions and the mesentery was considered useful.

Research on the urogenital organs

1. Continuing from the previous year, we compared the prognostic ability of clinicopathological factors before surgery in patients with prostate cancer. In 2019, we compared the prognostic ability of postoperative pathological findings as clinicopathological factors in 224 patients with intermediate-risk prostate cancer whose preoperative Gleason score was 3 + 4 = 7. Consequently, the percentage of Gleason pattern 4 on biopsy was an independent predictive factor for prostate cancer with a poor postoperative prognosis.

Research on female genital organs

1. About cervical adenocarcinoma

1) We demonstrated that immunohistochemical staining of trefoil factor 2 is useful for the histological diagnosis of gastric type adenocarcinoma, a representative type of uterine cancer unrelated to human papilloma virus.

2) The new classification we proposed at the meeting in the International Endocervical Adenocarcinoma Criteria and Classification, was reflected in the World Health Organization classification, which was revised and reissued in 2020. We clarified the relation of outcomes to a microcystic, elongated, and fragmented pattern of invasion in each histologic type of cervical adenocarcinoma.

3) We summarize morphological characteristics of invasive stratified mucin-producing carcinoma, a histological type that was newly advocated by International Endocervical Adenocarcinoma Criteria and Classification as described above.

2. Regarding outcomes of clear-cell carcinoma of the ovary, the following results were obtained, and each was published in a journal

1) In patients with deletion of AT-rich interactive domain-containing protein 1A, treatment with gemcitabine might improve outcomes (Gynecol Oncol 2019, 155: 489-498).

2) The expression of hepatocyte nuclear factor 1 homeobox B may be related to outcomes after chemotherapy (Clin Cancer Res 2019, 25: 3962-3973).

Research on the respiratory system

The deletion of regions, including 3p24, 3p12, 3p22.2, 3p25.3, and 3p14.2, in the developmental process of small-cell lung cancer and its significance

1. The background and objectives were as follows: A known culprit tumor suppressor gene in the short arm of the chromosome 3 (3q) region is inactivated at an early stage, and other chromosomal changes and gene alterations occur at an advanced stage. This is the most powerful theory about the development of smoking-associated lung cancers, such as squamous-cell carcinoma and small-cell cancer. However, culprit genes associ-

ated with the development and progression of lung cancer in the 3p region remain unknown. Microsatellite instability (MSI) analysis has been known as the most powerful tool for examining the localization of target genes using microsatellite markers that exist in or near DNA sequences on a chromosome. Last year, we used this analytical technique and found that tumor suppressor genes associated with the development of lung squamous epithelium might be located in the 3p22 region. This year, we performed a study to clarify the relation between the developmental process of small-cell cancer, another smoking-associated lung cancer, and 3p chromosomal changes.
Materials and methods were as follows: ***
Cancerous tissue and noncancerous tissue were collected from unstained formalin-fixed paraffin-embedded sections using a microdissection method in 21 surgically resected specimens of small-cell lung cancer. From these tissues, DNA was extracted. In Japanese or other Asians, 18 heterozygous microsatellite markers existing in all 3p regions were selected, and an MSI analysis was performed. Furthermore, regarded as a control group were 21 patients with pulmonary large-cell neuroendocrine carcinomas that were histopathologically classified as the same category as neuroendocrine tumors. The same analysis was performed in the control group. In the control group, the mean incidence of MSI was 27% in 18 markers for large-cell neuroendocrine cancer and 54% in 18 markers for small-cell neuroendocrine cancer. Regions in which chromosome depletion frequently occurred in the developmental process of small-cell cancer were 3p24, 3p12, 3p22.2, 3p25.3, and 3p14.2. This result suggested that tumor suppressor genes associated with the development of small-cell lung cancer might be present in several 3p regions.

Research of the liver, the gallbladder, and the pancreas
1. In general, the degree of fibrosis in the portal region (such as the presence or absence of bridging) can be used as an indicator of the basic structure of liver tissue. However, information about the biliary system is not reflected. Therefore, we observed the liver to examine how the connection from the hepatic cell cord and the normal liver lobules to the narrow bile duct and interlobular bile duct in the portal region are impaired. As materials, 166 liver biopsy specimens were obtained from patients with various liver diseases, including chronic hepatitis. CD10 staining was performed to visualize the bile canaliculi, and CK7 staining was performed to visualize the narrow bile duct and the interlobular bile duct. We observed the maintenance status of the biliary system and found that the state of the bile canaliculus and the proliferation of the narrow bile duct are influenced by various illnesses and underlying diseases, leading to structural changes. At present, accumulated data are being analyzed. Finally, we will submit a paper.
2. About tumor immunity in patients with pancreatic cancer
Cases of cancer with a histologically confirmed tertiary lymphoid follicles (lymphoid follicles around cancer, LFC) are considered to have good outcomes. Pancreatic cancer generally has a poor outcome. However, LFC formation is a good prognostic factor for pancreatic cancer, although LFC where the tumor is located has been confirmed in several patients. Such LFCs are considered to control immune reactions, similar to a secondary lymphoid follicles. However, the formation and maintenance mechanism remain unclear. In the present study, we approached the mechanism of LFC formation in patients with

pancreatic cancer from the aspects of immunology and of pathology on the basis of morphological observations. We performed immunohistochemical staining with paraffin-embedded sections obtained from patients who had pancreatic cancer with histologically confirmed LFC formation. We found that CXC motif ligand 13 (CXCL13), a chemokine that induces lymphoid tissue derived cells, frequently accumulated around the blood vessels in the stroma of the lymphatic device. Recently, the role of heparan sulfate on the accumulation of chemokines has being elucidated. Particularly, heparan sulfate arrays with high affinity for CXCL13 have been reported. In patients with pancreatic cancer, specific heparan sulfate arrays are considered to be expressed around blood vessels as a background factor for CXCL13 accumulation required for LFC formation. The goal of our study was to investigate this phenomenon by examining specimens from patients with pancreatic cancer. The following 2 approaches were used to prove that specific heparan sulfate arrays are expressed in LFC: (1) a method for visualizing the expression of specific heparan sulfate arrays on tissue sections and (2) a method proving that the production of these heparan sulfate arrays are promoted at the cellular level.

Publications

Yabuuchi Y, Hotta K, Aizawa D. An Unusual Lesion of the Colon Resembling a Submucosal Tumor. *Gastroenterology.* 2019 May; **156**(6): 1578-1579. doi: 10.1053/j.gastro.2019.01.009. Epub 2019 Jan 11. PubMed PMID: 30641056.

Fujiya K, Ohshima K, Kitagawa Y, Hatakeyama K, Nagashima T, Aizawa D, Sugino T, Urakami K, Yamaguchi K, Terashima M. Aberrant expression of Wnt/β-catenin signaling pathway genes in aggressive malignant gastric gastrointestinal stromal tumors. *Eur J Surg Oncol.* 2020 Jun; **46**(6): 1080-1087. doi: 10.1016/j.ejso.2020.02.036. Epub 2020 Feb 26. PubMed PMID: 32147424.

Takenaka M, Köbel M, Garsed DW, Fereday S, Pandey A, Etemadmoghadam D, Hendley J, Kawabata A, Noguchi D, Yanaihara N, Takahashi H, Kiyokawa T, Ikegami M, Takano H, Isonishi S, Ochiai K, Traficante N, Gadipally S, Semple T, Vassiliadis D, Amarasinghe K, Li J, Mir Arnau G, Okamoto A, Friedlander M, Bowtell DDL; Australian Ovarian Cancer Study Group. Survival Following Chemotherapy in Ovarian Clear Cell Carcinoma Is Not Associated with Pathological Misclassification of Tumor Histotype. *Clin Cancer Res.* 2019 Jul 1; **25**(13): 3962-3973. doi: 10.1158/1078-0432.CCR-18-3691. Epub 2019 Apr 9. PubMed PMID: 30967419.

Tate S, Nishikimi K, Kato K, Matsuoka A, Kambe M, Kiyokawa T, Shozu M. Microscopic diseases remain in initial disseminated sites after neoadjuvant chemotherapy for stage III/IV ovarian, tubal, and primary peritoneal cancer. *J Gynecol Oncol.* 2020 May; **31**(3): e34. doi: 10.3802/jgo.2020.31.e34. Epub 2019 Dec 9. PubMed PMID: 31912684; PubMed Central PMCID: PMC7189082.

Honda M, Kimura T, Kamata Y, Tashiro K, Kimura S, Koike Y, Sato S, Yorozu T, Furusato B, Takahashi H, Kiyota H, Egawa S. Differential expression of androgen receptor variants in hormone-sensitive prostate cancer xenografts, castration-resistant sublines, and patient specimens according to the treatment sequence. *Prostate.* 2019 Jun; **79**(9): 1043-1052. doi: 10.1002/pros.23816. Epub 2019 Apr 18. PubMed PMID: 30998834.

Yorozu T, Sato S, Kimura T, Iwatani K, Onuma H, Yanagisawa T, Miki J, Egawa S, Ikegami M, Takahashi H. HER2 Status in Molecular Subtypes of Urothelial Carcinoma of the Renal Pelvis and Ureter. *Clin Genitourin Cancer.* 2020 Aug; **18**(4): e443-e449. doi: 10.1016/j.clgc.2019.12.003. Epub 2019 Dec 13. PubMed PMID: 31983622.

Sato S, Kimura T, Yorozu T, Onuma H, Iwatani K, Egawa S, Ikegami M, Takahashi H. Cases Having a Gleason Score 3+4=7 With <5% of Gleason Pattern 4 in Prostate Needle Biopsy Show Similar Failure-free Survival and Adverse Pathology Prevalence to Gleason Score 6 Cases in a Radical Prostatectomy Cohort. *Am J Surg Pathol.* 2019 Nov; **43**(11): 1560-1565. doi: 10.1097/PAS.0000000000001345. PubMed PMID: 31436554.

Kuroda T, Ogiwara H, Sasaki M, Takahashi K, Yoshida H, Kiyokawa T, Sudo K, Tamura K, Kato T, Okamoto A, Kohno T. Therapeutic preferability of gemcitabine for ARID1A-deficient ovarian clear cell carcinoma. *Gynecol Oncol.* 2019 Dec; **155**(3): 489-498. doi: 10.1016/j.ygyno.2019.10.002. Epub 2019 Oct 8. PubMed PMID: 31604667.

Koide H, Kimura T, Inaba H, Sato S, Iwatani K, Yorozu T, Furusato B, Kamata Y, Miki J, Kiyota H,

Takahashi H, Egawa S. Comparison of ERG and SPINK1 expression among incidental and metastatic prostate cancer in Japanese men. *Prostate.* 2019 Jan; **79**(1): 3–8. doi: 10.1002/pros.23705. Epub 2018 Jul 26. PubMed PMID: 30051483.

Matsushima S, Shimizu T, Fukasawa N, Ojiri H. Novel Characteristic Skull Magnetic Resonance Imaging Features Associated With Meningioma. *J Comput Assist Tomogr.* 2019 Sep/Oct; **43**(5): 708–712. doi: 10.1097/RCT.0000000000000900. PubMed PMID: 31356523.

Sasaki T, Tsuboi N, Okabayashi Y, Haruhara K, Kanzaki G, Koike K, Takahashi H, Ikegami M, Shimizu A, Yokoo T. Synergistic Impact of Diabetes and Hypertension on the Progression and Distribution of Glomerular Histopathological Lesions. *Am J Hypertens.* 2019 Aug 14; **32**(9): 900–908. doi: 10.1093/ajh/hpz059. PubMed PMID: 31044221.

Okabayashi Y, Tsuboi N, Kanzaki G, Sasaki T, Haruhara K, Koike K, Takahashi H, Ikegami M, Shimizu A, Yokoo T. Aging Vs. Hypertension: An Autopsy Study of Sclerotic Renal Histopathological Lesions in Adults With Normal Renal Function. *Am J Hypertens.* 2019 Jun 11; **32**(7): 676–683. doi: 10.1093/ajh/hpz040. PubMed PMID: 31066457.

Yokoyama H, Masaki T, Inoue I, Nakamura M, Mezaki Y, Saeki C, Oikawa T, Saruta M, Takahashi H, Ikegami M, Hano H, Ikejima K, Kojima S, Matsuura T. Histological and biochemical evaluation of transforming growth factor-β activation and its clinical significance in patients with chronic liver disease. *Heliyon.* 2019 Feb 16; **5**(2): e01231. doi: 10.1016/j.heliyon.2019.e01231. eCollection 2019 Feb. PubMed PMID: 30815603; PubMed Central PMCID: PMC6378908.

Goda K, Dobashi A, Yoshimura N, Hara Y, Tamai N, Sumiyama K, Ikegami M, Tajiri H. Dye solution optimizing staining conditions for in vivo endocytoscopy for normal villi and superficial epithelial tumors in the duodenum. *Ann Gastroenterol.* 2019 Jul–Aug; **32**(4): 378–386. doi: 10.20524/aog.2019.0382. Epub 2019 May 10. PubMed PMID: 31263360; PubMed Central PMCID: PMC6595928.

Hamura R, Koyama T, Kawamura M, Kawamura T, Nakamura M, Yanaga K. Gastric calcifying fibrous tumor suspected to be complicated with immunoglobulin G4-related disease treated by laparoscopy and endoscopy cooperative surgery: a case report. *Surg Case Rep.* 2019 Oct 22; **5**(1): 150. doi: 10.1186/s40792-019-0714-6. PubMed PMID: 31641880; PubMed Central PMCID: PMC6805838.

Katagi H, Louis N, Unruh D, Sasaki T, He X, Zhang A, Ma Q, Piunti A, Shimazu Y, Lamano JB, Carcaboso AM, Tian X, Seluanov A, Gorbunova V, Laurie KL, Kondo A, Wadhwani NR, Lulla R, Goldman S, Venneti S, Becher OJ, Zou L, Shilatifard A, Hashizume R. Radiosensitization by Histone H3 Demethylase Inhibition in Diffuse Intrinsic Pontine Glioma. *Clin Cancer Res.* 2019 Sep 15; **25**(18): 5572–5583. doi: 10.1158/1078-0432.CCR-18-3890. Epub 2019 Jun 21. PubMed PMID: 31227500; PubMed Central PMCID: PMC6744979.

Tsvankin V, Hashizume R, Katagi H, Herndon JE, Lascola C, Venkatraman TN, Picard D, Burrus B, Becher OJ, Thompson EM. ABC Transporter Inhibition Plus Dexamethasone Enhances the Efficacy of Convection Enhanced Delivery in H3.3K27M Mutant Diffuse Intrinsic Pontine Glioma. *Neurosurgery.* 2020 May 1; **86**(5): 742–751. doi: 10.1093/neuros/nyz212. PubMed PMID: 31225627.

Yokoyama-Mashima S, Yogosawa S, Kanegae Y, Hirooka S, Yoshida S, Horiuchi T, Ohashi T, Yanaga K, Saruta M, Oikawa T, Yoshida K. Forced expression of DYRK2 exerts anti-tumor effects via apoptotic induction in liver cancer. *Cancer Lett*. 2019 Jun 1; **451**: 100–109. doi: 10.1016/j.canlet.2019. 02. 046. Epub 2019 Mar 6. PubMed PMID: 30851422.

Mori S, Noda Y, Kato D, Hirooka S, Ohtsuka T. Desmoid-type fibromatosis arising in a bifid rib chest wall. *Gen Thorac Cardiovasc Surg*. 2019 Nov; **67**(11): 996–998. doi: 10.1007/s11748-019-01088-5. Epub 2019 Feb 21. PubMed PMID: 30790238.

Kumamoto T, Yamada K, Yoshida S, Aoki K, Hirooka S, Eto K, Yanaga K, Yoshida K. Impairment of DYRK2 by DNMT1-mediated transcription augments carcinogenesis in human colorectal cancer. *Int J Oncol*. 2020 Jun; **56**(6): 1529–1539. doi: 10.3892/ijo.2020.5020. Epub 2020 Mar 20. PubMed PMID: 32236621.

Akutsu T, Okada S, Hirooka S, Ikegami M, Ohdaira H, Suzuki Y, Urashima M. Effect of Vitamin D on Relapse-Free Survival in a Subgroup of Patients with p53 Protein-Positive Digestive Tract Cancer: A Post Hoc Analysis of the AMATERASU Trial. *Cancer Epidemiol Biomarkers Prev*. 2020 Feb; **29**(2): 406–413. doi: 10.1158/1055-9965.EPI-19-0986. Epub 2019 Dec 23. PubMed PMID: 31871108.

Ibuki E, Shiraishi A, Sofue T, Kushida Y, Kadota K, Honda K, Kang D, Joh K, Minamino T, Haba R. Characteristic electron-microscopic features of cryofibrinogen-associated glomerulonephritis: a case report. *BMC Nephrol*. 2020 Jan 29; **21**(1): 27. doi: 10.1186/s12882-020-1696-0. PubMed PMID: 31996260; PubMed Central PMCID: PMC6988214.

Sawamura M, Komatsuda A, Kaga H, Saito A, Yasuda T, Wakui H, Joh K, Takahashi N. Membranous nephropathy with solitary polyclonal IgA deposition: A case report and literature review. *Clin Nephrol Case Stud*. 2019 Oct 28; **7**: 60–65. doi: 10.5414/CNCS109807. eCollection 2019. PubMed PMID: 31673485; PubMed Central PMCID: PMC6822057.

Wester Trejo MAC, van Daalen EE, Berden AE, Wolterbeek R, van Es LA, Bos WJW, Ferrario F, Hagen EC, Jennette JC, Joh K, Neumann I, Noël LH, Pusey CD, Bruijn JA, Bajema IM. A renal risk score for ANCA-associated glomerulonephritis. *Kidney Int*. 2019 Jul; **96**(1): 245. doi: 10.1016/j.kint.2019.01.046. PubMed PMID: 31229031.