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Analysis of microglandular adenosis and acinic cell carcinoma of the breast

Acinic cell carcinoma is an indolent form of invasive breast cancer, whereas microglandular adenosis has been shown to be a neoplastic proliferation. Both entities display a triple-negative phenotype and may give rise to, as well as display, somatic genomic alterations typical of high-grade triple-negative breast cancers. The authors compared previously published data on eight carcinoma-associated microglandular adenoses and eight acinic cell carcinomas subjected to massively parallel sequencing targeting all exons of 236 genes recurrently mutated in breast cancer or DNA repair related, or both. Somatic mutations, insertions/deletions, and copy-number alterations were detected using state-of-the-art bioinformatic algorithms. All cases were of triple-negative phenotype. A median of 4.5 (one to 13) and 4.0 (one to seven) nonsynonymous somatic mutations per carcinoma-associated microglandular adenosis and acinic cell carcinoma were identified, respectively. *TP53* was the sole highly recurrently mutated gene (75 percent in microglandular adenosis versus 88 percent in acinic cell carcinoma), and *TP53* mutations were consistently coupled with loss of heterozygosity of the wild-type allele. Additional somatic mutations shared by both groups included those in *BRCA1, PIK3CA,* and *INPP4B*. Recurrent (n = 2) somatic mutations restricted to microglandular adenoses or acinic cell carcinomas included those affecting *PTEN* and *MED12* or *ERBB4*, respectively. No significant differences in the repertoire of somatic mutations were detected between microglandular adenosis and acinic cell carcinoma and between this group of lesions and 77 triple-negative carcinomas from the Cancer Genome Atlas. Microglandular adenoses and acinic cell carcinomas, however, were genetically distinct from estrogen receptor-positive or HER2-positive breast cancers, or both, from the Cancer Genome Atlas. These findings support the assertion that microglandular adenosis and acinic cell carcinoma are part of the same spectrum of lesions harboring frequent *TP53* somatic mutations. Furthermore, they likely represent low-grade forms of triple-negative disease with minimal or no metastatic potential, of which a subset has the potential to progress to high-grade triple-negative breast cancer.

Geyer FC, Berman SH, Marchiò C, et al. Genetic analysis of microglandular adenosis and acinic cell carcinomas of the breast provides evidence for the existence of a low-grade triple-negative breast neoplasia family. *Mod Pathol.* 2017;30:69–84.

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Histological and immunohistochemical assessment of oesophagitis phenotypes

The authors conducted a study on four histological-immunohistochemical oesophagitis phenotypes. Oesophageal biopsies from 311 patients were stained with H&E and the T-cell marker CD3. Additional immunohistochemical stains (n=413) were performed in 77 cases. Four histological-immunohistochemical oesophagitis phenotypes were recorded: lymphocytic oesophagitis (LyE, 40 or more CD3+ lymphocytes per high-power field [HPF] in CD3 immunostain), eosinophilic oesophagitis (EoE, 15 or more eosinophils per HPF in H&E stain), lymphocytic infiltration (39 or fewer CD3+ per HPF), and compound lymphocytic oesophagitis–eosinophilic oesophagitis (Co LyE-EoE). At index biopsy, 28.3 percent (n=88) had LyE, 21.2 percent (n=66) EoE, 10.6 percent (n=33) Co LyE-EoE, and 39.9 percent (n=124) lymphocytic infiltration. A persistent oesophagitis phenotype was found in 42.5 percent (37 of 87) in the first follow-up biopsy, 34.4 percent (21 of 61) in the second follow-up biopsy, and 48.1 percent (26 of 54) in the third follow-up biopsy. Using βF1 immunostain, two different surface T-cell receptors were detected in LyE and Co Lye-EoE: one having 40 or more βF1+ per HPF (βF1+ high) and the other having fewer than 39 βF1+ per HPF (βF1+ low). Based on literature about the significance of intraepithelial lymphocytes in the initiation of EoE, the authors submit that the intraepithelial lymphocyte phenotypes in LyE might differ from those found in EoE as they were unable to elicit the same eosinophilic response. Recent studies disclosed that group two innate lymphoid cells (ILC2s), enriched in EoE, remain undetected in CD3 immunostain as they lack surface markers for T, B, natural killer, or natural killer T cells. If ILC2s also participated in the lymphocytic infiltration of EoE, then the frequency of cases with Co LyE-EoE reported in this study might have been much higher. It is easy to recognize the four oesophagitis phenotypes described, provided the dual-staining procedure (H&E-CD3) is implemented.

Rubio CA, Ichiya T, Schmidt PT. Lymphocytic oesophagitis, eosinophilic oesophagitis and compound lymphocytic-eosinophilic oesophagitis I: histological and immunohistochemical findings. *J Clin Pathol.* 2017;70:208–216.

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P16INK4a staining of high-grade squamous intraepithelial lesions

P16INK4a tumor-suppressor protein is a biomarker of human papillomavirus oncogenic activity that has revealed a high rate of positivity in histological high-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 2 (HSIL/CIN2) lesions. However, there is a paucity of data regarding p16INK4a (p16) status as a surrogate marker of HSIL/CIN2 evolution. The authors conducted a study to evaluate the outcome of HSIL/CIN2 patients followed up without treatment for 12 months according to p16 immunohistochemical staining. Patients diagnosed with HSIL/CIN2 colposcopy-directed biopsy were recruited for the study prospectively between December 2011 and October 2013. P16 staining was performed in all HSIL/CIN2 diagnostic biopsies. Follow-up was conducted every four months by cytology, colposcopy, and biopsy if suspicion of progression and once the 12 months of follow-up was completed. Complete regression, partial regression, persistence, and progression rates of HSIL/CIN2 were defined as final outcomes. Ninety-six patients were included in the analysis. The rate of spontaneous regression was 64 percent, while 28 percent had persistent disease and eight percent progressed at 12 months of follow-up. P16 was positive in 81 (84 percent) initial HSIL/CIN2 biopsies. Regression was observed in all 15 p16-negative cases and in 46 of 81 (57 percent) p16-positive cases ($P = .001$). The authors concluded that patients with p16-negative HSIL/CIN2 biopsy had a high rate of regression during the first 12 months of follow-up. Status of p16 staining could be considered for HSIL/CIN2 management.

Miralpeix E, Genovés J, Solé-Sedeño J, et al. Usefulness of p16INK4a staining for managing histological high-grade squamous intraepithelial cervical lesions. *Mod Pathol.* 2017;30:304–310.

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Predicting lymph node metastasis in select colorectal carcinomas

Submucosally invasive colorectal carcinoma (pT1) has the potential to be cured by local excision. In the United States, surgical intervention is reserved for tumors with high-grade morphology, lymphovascular invasion, and close/positive margin. In other countries, particularly Japan, surgical therapy is also recommended for mucinous tumors, tumors with more than 1,000 µm of submucosal invasion, and those with high tumor budding. These histological features have not been evaluated thoroughly in a Western cohort of pT1 carcinomas. The authors conducted a study of a cohort of 116 surgically resected pT1 colorectal carcinomas in which high tumor budding ($P < .001$), lymphatic invasion ($P = .003$), depth of submucosal invasion greater than 1,000 µm ($P = .04$), and high-grade morphology ($P = .04$) were significantly associated with lymph node metastasis on univariate analysis. Mucinous differentiation, tumor location, tumor growth pattern, and size of invasive component were not significant. On multivariate analysis, only high tumor budding was associated with lymph node metastasis, with an odds ratio of 4.3 ($P = .004$). A subset of 48 tumors (22 node positive and 26 node negative) was analyzed for mutations in 50 oncogenes and tumor suppressors. No statistically significant molecular alterations in these 50 genes were associated with lymph node status. However, lymphatic invasion was associated with *BRAF* mutations ($P = .01$). Furthermore, high tumor budding was associated with mutations in *TP53* ($P = .03$) and inversely associated with mutations in the mTOR pathway (*PIK3CA* and *AKT;* $P = .02$). The authors concluded that this study demonstrates the importance of identifying high tumor budding in pT1 carcinomas when considering additional surgical resection.

Pai RK, Cheng Y, Jakubowski M, et al. Colorectal carcinomas with submucosal invasion (pT1): analysis of histopathological and molecular factors predicting lymph node metastasis. *Mod Pathol.* 2016;30:113–122.

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Upper tract urothelial carcinomas: MMR protein loss and link to Lynch syndrome

Increased risk for upper tract urothelial carcinoma is described in patients with Lynch syndrome, caused by germline mutations in mismatch repair genes. The authors conducted a study to identify the frequency of mismatch repair protein loss in upper tract urothelial carcinoma and its potential for identifying an association with Lynch syndrome. They queried their database to identify upper tract urothelial carcinomas. Patients were cross-referenced for history of colorectal carcinoma or other common Lynch syndrome-associated neoplasms to enrich for potential Lynch syndrome cases. Tumor histopathologic characteristics were reviewed and each case was analyzed for loss of the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2 by immunohistochemistry. Of 444 patients with upper tract urothelial carcinoma, a subset of 215 (encompassing 30 with upper tract urothelial carcinoma and another common Lynch syndrome-associated neoplasm) was analyzed for loss of mismatch repair protein expression. Of 30 patients with Lynch syndrome-associated neoplasms, six had documented Lynch syndrome, including two with Muir–Torre syndrome. Mismatch repair protein loss was identified in seven percent of total upper tract urothelial carcinomas and 30 percent of patients with Lynch syndrome-associated neoplasms, including all patients with Lynch syndrome/Muir–Torre syndrome. Of patients without a history of Lynch syndrome-associated neoplasms, five of 184 (2.7 percent) had a loss of mismatch repair protein expression. Twelve cases with mismatch repair protein loss demonstrated loss of MSH2 and MSH6, and two had isolated loss of MSH6. MLH1 and PMS2 expression were consistently retained. Although increased intratumoral lymphocytes, inverted growth, pushing tumor-stromal interface, and lack of nuclear pleomorphism were more commonly seen in cases with mismatch repair protein loss, only intratumoral lymphocytes and presence of pushing borders were statistically significant. MLH1 and PMS2 testing appear to have little utility in upper tract urothelial carcinoma. However, mismatch repair protein loss of MSH2 or MSH6 by immunohistochemistry seems relatively sensitive and specific for

identifying patients with potential Lynch syndrome.

Harper HL, McKenney JK, Heald B, et al. Upper tract urothelial carcinomas: frequency of association with mismatch repair protein loss and Lynch syndrome. *Mod Pathol.* 2017;30:146–156.

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Improving and evaluating the value of Ki-67 in endometrial cancer studies

Ki-67, a marker of cellular proliferation, is increasingly being used in presurgical window studies of endometrial cancer as a primary outcome measure. Unlike in breast cancer, however, there are no guidelines standardizing its measurement, and its clinical relevance as a response biomarker is undetermined. Therefore, it is imperative that Ki-67 scoring protocols be optimized and that the association of Ki-67 with patient survival be rigorously evaluated in order to clinically interpret the results of these studies. Using the International Ki-67 in Breast Cancer Working Group guidelines as a basis, the authors evaluated whole slide, hot spot, and invasive edge scoring protocols using endometrial biopsies and hysterectomy specimens from 179 women. Whole section and tissue microarray manual and semi-automated scoring using Definiens Developer software were also compared. Ki-67 scores were related to clinicopathological variables and cancer-specific survival in uni- and multivariate analysis. Against the criteria of time efficiency, as well as intra- and inter-observer variability and consistency, semi-automated hot spot scoring was the preferred method. Ki-67 scores positively correlated with grade, stage, and depth of myometrial invasion (P values, all $<.03$). By univariate analysis, higher Ki-67 scores were associated with a significant reduction in cancer-specific survival ($P \leq .05$). However, this effect was substantially attenuated in the multivariate model. The authors concluded that hot spot scoring of whole sections using Definiens is an optimal method to quantify Ki-67 in endometrial cancer window study specimens. Measured this way, it is a clinically relevant marker, although further work is required to determine whether reductions in Ki-67 in neoadjuvant intervention studies translate into improved patient outcome.

Kitson S, Sivalingam VN, Bolton J, et al. Ki-67 in endometrial cancer: scoring optimization and prognostic relevance for window studies. *Mod Pathol.* 2017;30:459–468.

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Benefits of genetic analysis of uterine aspirates to endometrial cancers

Endometrial cancer is the most common cancer of the female genital tract in developed countries. Although the majority of endometrial cancers are diagnosed at early stages and the five-year overall survival rate is approximately 80 percent, early detection of these tumors is crucial to improve the survival of patients given that the advanced tumors are associated with a poor outcome. Furthermore, correct assessment of the preclinical diagnosis is critical in guiding surgical treatment and management of the patient. In this regard, the potential of targeted genetic sequencing of uterine aspirates has been assessed as a preoperative tool to obtain reliable information regarding the mutational profile of a given tumor, even in samples that cannot be classified histologically. This study sequenced 83 paired samples (uterine aspirates and hysterectomy specimens): 62 endometrioid and nonendometrioid tumors, 10 cases of atypical hyperplasia, and 11 noncancerous endometrial disorders. Even though diagnosing endometrial cancer based exclusively on genetic alterations is not feasible, the study primarily found mutations in uterine aspirates from malignant disorders, suggesting its potential in the near future for supporting the standard histologic diagnosis. Moreover, this approach provides the first evidence of the high intra-tumor genetic heterogeneity associated with endometrial cancer, evident when multiple regions of tumors from an individual hysterectomy are analyzed. Notably, the genetic analysis of uterine aspirates captures

this heterogeneity, solving the potential problem of incomplete genetic characterization when analyzing a single tumor biopsy.

Mota A, Colás E, García-Sanz P, et al. Genetic analysis of uterine aspirates improves the diagnostic value and captures the intra-tumor heterogeneity of endometrial cancers. *Mod Pathol.* 2017;30:134–145.

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