Anatomic Pathology Abstracts, 4/17

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Proposing prognostic thresholds for lymph node yield in oral cavity cancers

Prognostic lymph node yield thresholds have been identified and incorporated into treatment guidelines for multiple cancer sites, but not for oral cancer. The authors conducted a study to identify optimal thresholds in elective and therapeutic neck dissection for oral cavity cancers. Patients with such cancers who were listed in the National Cancer Database (NCDB) were stratified into clinically lymph node-negative (cN0) and clinically lymph node-positive (cN+) cohorts to reflect the surgical management for these diseases. Univariate and multivariate analyses were performed to assess the relation between lymph node yield and overall survival, adjusting for other prognostic factors. Thresholds derived from the NCDB were validated in the Surveillance, Epidemiology, and End Results (SEER) database. In NCDB patients with cN0 cancers of the oral cavity, those who had fewer than 16 lymph nodes had significantly decreased survival rates. The proportion of positive lymph nodes was higher for patients who had 16 or more lymph nodes (27.2 percent versus 16.3 percent for fewer than 16 lymph nodes; $P<.001$). This threshold was validated in 2,715 lymph node-negative cancers from SEER, with a mortality hazard ratio of 0.825 for 16 or more lymph nodes (95 percent confidence interval [CI], 0.764–0.950; $P=.004$). In NCDB patients with cN+ oral cavity cancers, groups with fewer than 26 lymph nodes had significantly decreased survival rates. This threshold was validated in 1,903 lymph node-positive cancers from SEER, with a mortality hazard ratio of 0.791 (95 percent CI, 0.692–0.903; $P=.001$). Academic centers, higher volume centers, and geographic location predicted higher lymph node yields. The authors concluded that more extensive neck dissection (16 or more lymph nodes in cN0; 26 or more lymph nodes in cN+) was associated with better survival rates. Further evaluation of practice patterns in lymph node yield may lead to improved quality of care.

Kuo P, Mehra S, Sosa JA, et al. Proposing prognostic thresholds for lymph node yield in clinically lymph node-negative and lymph node-positive cancers of the oral cavity. *Cancer.* 2016;122:3624–3631.

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Pattern of invasion and lymphovascular invasion in SCC of floor of the mouth

Lymphovascular invasion and the histological pattern of invasion at the invasive tumor front have been reported as adverse prognosticators in oral squamous cell carcinoma (SCC). However, assessment of these parameters is hampered by variation in the criteria used for evaluation. The authors evaluated interobserver variability in the assessment of pattern of invasion and lymphovascular invasion in SCC of the floor of the mouth. They also studied the impact of pattern of invasion on clinical outcomes using varying quantitative cutoffs. Three pathologists independently evaluated 58 cases of SCC of the floor of the mouth for pattern of invasion and lymphovascular invasion. They analyzed interobserver variability using Fleiss kappa statistics. Interobserver agreement was substantial for assessing lymphovascular invasion (κ=0.64; 95 percent confidence interval [CI], 0.60–0.68). Interobserver agreement was moderate for evaluating pattern of invasion with a 50 percent cutoff (κ=0.58; 95 percent CI, 0.54–0.62), 20 percent cutoff (κ=0.58; 95 percent CI, 0.54–0.62), and worst pattern of invasion (κ=0.43; 95 percent CI, 0.39–0.46). A consensus diagnosis of the pattern of invasion was a significant predictor of locoregional recurrence, disease-specific survival, and overall survival on univariate analysis when a 50 percent cutoff was used (locoregional recurrence, *P*=.01; disease-specific survival, *P*=.01; overall survival, *P*=0.01) and when a 20 percent cutoff was used (locoregional recurrence, *P*=.02; disease-specific survival, *P*=.02; overall survival, *P*=.03), but it was not significant when worst pattern of invasion was used (locoregional recurrence, *P*=.18; disease-specific survival, *P*=.16; overall survival, *P*=.17). The authors concluded that interobserver agreement in the diagnosis of lymphovascular invasion was substantial. The pattern of invasion at the 50 percent and 20 percent cutoffs is moderately reproducible and has prognostic value in SCC of the floor of the mouth. Additional studies are necessary to establish the optimum quantitative cutoff for the pattern of invasion.

Beggan C, Fives C, O'Leary G, et al. Pattern of invasion and lymphovascular invasion in squamous cell carcinoma of the floor of the mouth: an interobserver variability study. *Histopathol.* 2016;69:914–920.

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ARID1A and ARID1B inactivation in ovarian and endometrial dedifferentiated cancers

Dedifferentiated carcinoma of the endometrium or the ovary is an aggressive epithelial malignancy that comprises an endometrioid carcinoma and an undifferentiated carcinoma. The authors recently reported that inactivation of BRG1 or INI1, core subunits of the switch/sucrose nonfermenting (SWI/SNF) complex, was the molecular event likely underlying dedifferentiation in about half of dedifferentiated carcinomas. They conducted a study in which they performed a genomic screen that included other members of the SWI/SNF complex to better delineate the molecular basis in the remainder of these tumors. The authors identified concurrent inactivating mutations involving ARID1A and ARID1B in 12 of 24 BRG1/INI1-intact, zero of three INI1-deficient, and zero of 16 BRG1-deficient dedifferentiated carcinomas. All ARID1A and ARID1B comutated tumors displayed loss of ARID1A expression in the undifferentiated component, with 11 of 12 tumors also displaying an absence of staining in the endometrioid component. ARID1B expression was absent in the undifferentiated component in all 12 tumors, whereas the corresponding endometrioid component showed intact expression. Clinically, ARID1A/ARID1B co-inactivated tumors showed aggressive behavior similar to BRG1- or INI1-inactivated tumors. Given that ARID1A and ARID1B are the only known DNA-binding subunits of the SWI/SNF-A complex, additional inactivation of ARID1B in an ARID1A-deficient background appears to represent an alternative mechanism of disruption of SWI/SNF-mediated transcriptional regulation, resulting in arrested cellular differentiation in endometrial and ovarian endometrioid cancers.

Coatham M, Li X, Karnezis AN, et al. Concurrent ARID1A and ARID1B inactivation in endometrial and ovarian dedifferentiated carcinomas. *Mod Pathol.* 2016;29:1586–1593.

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Analysis of architectural patterns for grading of prostatic adenocarcinoma

Histologic grading remains the gold standard for prognosis in prostate cancer, and assessment of Gleason score plays a critical role in active surveillance management. The authors sought to optimize the prognostic stratification of grading and developed a method of recording and studying individual architectural patterns by light microscopic evaluation that is independent of standard Gleason grade. Some of the patterns that were evaluated are not assessed by Gleason grading—for example, reactive stromal response. Individual histologic patterns were correlated with recurrence-free survival in a retrospective postradical prostatectomy cohort of 1,275 patients represented by the highest-grade foci of carcinoma in tissue microarrays. In univariable analysis, fibromucinous rupture with varied epithelial complexity had a significantly lower relative risk of recurrence-free survival in cases graded as 3+4=7. Cases having focal poorly formed glands, which could be designated as pattern 3+4=7, had lower risk than cribriform patterns with small cribriform glands or expansile cribriform growth. In separate multivariable Cox proportional hazard analyses of Gleason score 3+3=6 and 3+4=7 carcinomas, reactive stromal patterns were associated with worse recurrence-free survival rates. Decision tree models demonstrated potential regrouping of architectural patterns into categories with similar risk. The authors concluded that their data suggest that focal poorly formed glands and cribriform patterns, classified as Gleason pattern 4, should be in separate prognostic groups because the latter is associated with worse outcome. Patterns with extravasated mucin are likely overgraded in a subset of cases with more complex epithelial bridges, whereas stromogenic cancers have a worse outcome than conveyed by Gleason grade alone. These findings facilitate optimization of histologic grading and strongly support incorporating reactive stroma into routine assessment.

McKenney JK, Wei W, Hawley S, et al. Histologic grading of prostatic adenocarcinoma can be further optimized: analysis of the relative prognostic strength of individual architectural patterns in 1275 patients from the Canary retrospective cohort. *Am J Surg Pathol.* 2016;40:1439–1456.

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Genomic profiling of malignant phyllodes tumors: pathogenesis and progression

Malignant phyllodes tumors of the breast are poorly understood rare neoplasms with potential for aggressive behavior. Few efficacious treatments exist for progressed or metastatic disease. The molecular features of malignant phyllodes tumors are poorly defined, and a deeper understanding of the genetics of these tumors may shed light on pathogenesis and progression and potentially identify novel treatment approaches. The authors sequenced 510 cancer-related genes in 10 malignant phyllodes tumors, including five tumors with liposarcomatous differentiation and one with myxoid chondrosarcoma-like differentiation. They assessed intratumoral heterogeneity by sequencing two separate areas in seven tumors, including nonheterologous and heterologous components of tumors with heterologous differentiation. Activating hotspot mutations in *FGFR1* were identified in two tumors. Additional recurrently mutated genes included *TERT* promoter (six of 10), *TP53* (four of 10), *PIK3CA* (three of 10), *MED12* (three of 10), *SETD2* (two of 10), and *KMT2D* (two of 10). Together, genomic aberrations in FGFR/EGFR PI-3 kinase and RAS pathways were identified in eight (80 percent) tumors and included mutually exclusive and potentially actionable activating *FGFR1*, *PIK3CA*, and *BRAF* V600E mutations, inactivating *TSC2* mutation, *EGFR* amplification, and *PTEN* loss. Seven (70 percent) malignant phyllodes tumors harbored *TERT* aberrations (six promoter mutations and one amplification). For comparison, *TERT* promoter mutations were identified by Sanger sequencing in 33 percent borderline (n=12) and no (zero; n=8) benign phyllodes tumors (*P*=.391 and *P*=.013

versus malignant tumors, respectively). Genetic features specific to liposarcoma, including *CDK4/MDM2* amplification, were not identified. Copy number analysis revealed intratumoral heterogeneity and evidence for divergent tumor evolution in malignant phyllodes tumors with and without heterologous differentiation. Tumors with liposarcomatous differentiation revealed more chromosomal aberrations in nonheterologous components compared with liposarcomatous components. EGFR amplification was heterogeneous and present only in the nonheterologous component of one tumor with liposarcomatous differentiation. The authors concluded that the results identify novel pathways involved in the pathogenesis of malignant phyllodes tumors, which increases understanding of tumor biology and has potential clinical impact.

Liu SY, Joseph NM, Ravindranathan A, et al. Genomic profiling of malignant phyllodes tumors reveals aberrations in FGFR1 and PI-3 kinase/RAS signaling pathways and provides insights into intratumoral heterogeneity. *Mod Pathol.* 2016;29:1012–1027.

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Influence of decalcification procedures in breast cancer

Distant breast cancer metastases are routinely biopsied to reassess receptor status and isolate DNA for sequencing of druggable targets. Bone metastases are the most frequent subgroup. Decalcification procedures may negatively affect antigenicity and DNA quality. The authors evaluated the effect of several decalcification procedures on receptor status and DNA/RNA quality. In 23 breast tumors collected prospectively, they compared estrogen receptor alpha, progesterone receptor, and HER2 status by immunohistochemistry in nondecalcified tissue routinely processed for diagnostic purposes and in parallel tissue decalcified in Christensen's buffer with and without microwave, EDTA, and Formical-4. They also assessed HER2 FISH and DNA/RNA quantity and quality. The authors found that the percentage of ERα-positive cells was on average lower in EDTA (P=.049) and Formical-4 (P=.047) treated cases than in controls, and progesterone receptor expression showed decreased antigenicity after Christensen's buffer treatment (P=.041). Overall, a good concordance (weighted kappa) was seen for ERα, progesterone receptor, and HER2 immunohistochemistry when comparing the nondecalcified control tissues with the decalcified tissues. For two patients (nine percent), there was a potential influence on therapeutic decision-making with regard to hormonal therapy or HER2-targeted therapy. HER2 FISH interpretation was seriously hampered by Christensen's buffer and Formical-4, and DNA/RNA quantity and quality were decreased after all four decalcification procedures. Validation on paired primary breast tumor specimens and EDTA-treated bone metastases showed that immunohistochemistry and FISH were well assessable and DNA and RNA yield and quality were sufficient. The authors concluded that common decalcification procedures have only a modest negative influence on hormone and HER2 receptor immunohistochemistry in breast cancer. However, they may seriously affect DNA/RNA-based diagnostic procedures. Overall, EDTA-based decalcification, therefore, is preferrable as it best allows FISH and DNA/RNA isolation.

Schrijver WA, van der Groep P, Hoefnagel LD, et al. Influence of decalcification procedures on immunohistochemistry and molecular pathology in breast cancer. *Mod Pathol.* 2016;29:1460–1470.

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