# Molecular Pathology Abstracts, 3/18

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## Nonendoscopic detection of Barrett's esophagus using DNA methylation biomarkers

Esophageal adenocarcinoma is an aggressive disease, with a less than 20 percent five-year survival rate, and its incidence is rapidly increasing. Early detection of esophageal adenocarcinoma or its precursor lesion, Barrett's esophagus, would enable more effective treatment strategies and a greater chance of cure. Traditionally, Barrett's esophagus and esophageal neoplasms are diagnosed endoscopically, with direct visualization of the esophageal mucosa and tissue biopsies to assess for columnar metaplasia, dysplasia, or invasive carcinoma. However, because of its cost and invasiveness, endoscopy is not effective for population-based screening, prompting a need for other methodologies and biomarkers for detection. Aberrant methylation of cytosine residues within CpG-rich islands often occurs in neoplasia, and the methylation status of certain sequences can serve as biomarkers. For example, a recently discovered biomarker for Barrett's esophagus is methylation of the CpG island that overlaps the first exon of the vimentin gene (*VIM*), which is seen in 90 percent of affected patients. To identify additional DNA methylation markers, the authors of this study used reduced representation bisulfite sequencing, a high-throughput whole genome approach for assessing methylation of CpG-rich islands, to compare esophageal adenocarcinoma and Barrett's esophagus biopsies with matched uninvolved esophageal mucosa. After screening more than 3 million CpGs, the authors identified a methylation signature located between the promotor and the 5' untranslated region of *CCNA1. CCNA1* methylation was significantly increased in all Barrett's esophagus-related lesions. It was detected in 81 percent of nondysplastic Barrett's esophagus, 68 percent of dysplastic Barrett's esophagus, and 90 percent of esophageal adenocarcinoma, but only one percent of normal tissue. In a training set and an independent validation cohort of esophageal cytology brushings, the combination of *VIM* and *CCNA1* methylation was found to be sensitive and specific for Barrett's esophagus-related lesions, with an area under the receiver operating characteristic curve of 0.95. While DNA methylation biomarkers are promising, a screening test would require the nonendoscopic sampling of esophageal mucosa. To this end, the authors engineered an encapsulated, inflatable, surface-textured balloon for sample procurement. The device, consisting of a 16- × 9-mm capsule attached to a silicone catheter, is delivered to the stomach, where the balloon is inflated by injecting air through the catheter. The device is then gently withdrawn 3 to 6 cm back through the distal esophagus to sample the mucosa. The balloon is deflated and inverted into the capsule to protect the sample before being removed through the mouth. This device was tested on 156 patients prior to their scheduled endoscopy procedures. Although 28 patients were unable to swallow the device, those that did reported little anxiety, pain, or choking, and only mild gagging. Of the 128 patient samples received, 42 were excluded because of inadequate DNA, previous esophageal ablation, gastric intestinal metaplasia, or ultrashort Barrett's esophagus. In the 86 samples that were evaluated, esophageal pathology was detected with a sensitivity of 88 percent and specificity of 91.7 percent. The authors concluded that identification of DNA methylation biomarkers associated with esophageal pathology, coupled with a nonendoscopic esophageal mucosal sampling method that is well-tolerated and cost-effective, shows great promise as a population-screening method.

Moinova HR, LaFramboise T, Lutlerbaugh JD, et al. Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. *Sci Transl Med.* 2018. doi:10.1126/scitranslmed.aao5848.

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## Array-comparative genomic hybridization for uterine smooth muscle lesions

Uterine smooth muscle neoplasms, which include benign leiomyomas and malignant leiomyosarcomas, are distinguished histologically by cytologic atypia, mitotic count, and tumor cell necrosis. Diagnosis at the extremes of the smooth muscle neoplastic spectrum is relatively straightforward, but, occasionally, differentiation based on morphologic criteria alone is difficult or equivocal. In ambiguous cases, when the morphologic features do not fulfill the criteria for leiomyosarcoma and yet are too atypical to fit leiomyoma, a diagnosis of smooth muscle tumor of uncertain malignant potential (STUMP) is made. Such classification, however, could result in over or under treatment. Therefore, the authors conducted a study to assess whether genomic indicators could be used in the subclassification of uterine smooth muscle tumors. They collected and centrally reviewed 77 uterine smooth muscle tumors from 76 patients. The tumors comprised 19 leiomyomas, 14 STUMPs, and 44 leiomyosarcomas. The authors performed copy number analysis by array-comparative genomic hybridization analysis. For each tumor, a genomic index was calculated as A2/C, where A is the total number of alterations (segmental gains and losses) and C is the number of involved chromosomes. A genomic index score of 10 was used as a binary cutoff to split the tumors into a low genomic index group (mean genomic index, 2.3; range, 0–9.14) and a complex genomic profile group (mean genomic index, 51.8; range, 11–180). The low genomic index group included all 19 leiomyomas, two STUMPs, and no leiomyosarcomas, while the complex genomic profile group included 12 STUMPs and all 44 leiomyosarcomas. From a clinical perspective, no recurrences were seen in any of the leiomyomas or STUMPs in the low genomic index group, while seven of the 12 STUMPs and 40 of the 44 leiomyosarcomas recurred in the complex genomic profile group. Subdividing the complex genomic profile tumors, the authors found that a genomic index of greater than 35 was associated with decreased overall survival. Interestingly, while the morphologic criteria of atypia, increased mitoses, and tumor cell necrosis were significant adverse prognostic factors in univariate analysis, only a genomic index above 35 was significant in multivariate analysis. The findings suggest that the genomic index may be a more robust prognostic marker for stratifying outcomes than morphology. Other specific chromosomal alterations, including chromosome 5p gain, 1p gain, 13q loss (including *RB1*), and 17p gain (including *MYOCD*), were also associated with decreased overall survival on univariate analysis. However, on multivariate analysis that included tumor stage, only 5p gain was found to be a statistically significant adverse prognostic factor. The authors concluded that their study shows that the genomic index may be useful to subclassify STUMPs into either a group with clinical and biological behavior similar to leiomyomas or a group more similar to leiomyosarcomas. The study also demonstrates that even in histologically obvious leiomyosarcomas, the genomic index could be used to substratify patients into prognostic groups. Therefore, the study findings should be taken into account as the molecular classification of uterine smooth muscle tumors is developed.

Croce S, Ducoulombier A, Ribeiro A, et al. Genome profiling is an efficient tool to avoid the STUMP classification of uterine smooth muscle lesions: a comprehensive array-genomic hybridization analysis of 77 tumors. *Mod Pathol.* 2018. doi:10.1038/modpathol.2017.185.

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