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Genomic Instability in Colorectal Adenomas with and without Recurrence

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Colorectal adenomas are precursors to colorectal cancer, one of the most common types of cancer diagnosed in both men and women in the Western world. Therefore, endoscopic resection of adenomatous polyps via polypectomy performed during colonoscopy provides the opportunity to reduce cancer risk. However, recurrence of adenomas after polypectomy is a common finding and affects one-third of the patients. This fact manifests both a cost and a health burden for the affected patients. For cost reduction and to individualize endoscopic surveillance strategies, identifying epigenetic and genetic alterations which predict adenoma recurrence would be of importance. Additionally, our knowledge of understanding the evolutionary processes of recurrence needs improvement.

Here, formalin-fixed paraffin-embedded tissues of primary adenomas without recurrence (*n*=30), primary adenomas with recurrence (*n*=19), matched-pair samples (*n*=19; primary adenoma and corresponding recurrent adenoma) and normal epithelium (*n*=3) were collected. Global epigenetic analysis of the methylome of the colorectal adenomas was conducted by Illumina's HumanMethylation450K BeadChip array (HM450K) to identify differentially methylated positions (DMPs). Array validation was performed via pyrosequencing. The genetic landscape of copy number alterations (CNAs) was investigated by array-comparative genomic hybridization (aCGH) and single-cell multiplex-interphase fluorescence *in situ* hybridization (miFISH). This assay comprised fifteen probes, 14 gene probes targeting colorectal cancer-related oncogenes and tumor suppressor genes along with a centromere 10 probe.

Filtering of methylation data provided 329,573 probes. Unsupervised clustering demonstrated an association of methylation patterns with the histologic subtype (*P*=0.008). 5,094 DMPs could be revealed across the comparisons of subgroups: 2,824 (55.4%) DMPs were hypermethylated and 2,270 (44.6%) DMPs were hypomethylated. DMPs located in CpG islands were strongly hypermethylated (86%), while DMPs in open sea regions and shelves were hypomethylated (83% and 79%, respectively). DMP-associated genes were enriched in inflammatory- and cancer-related pathways such as the MAPK signaling pathway, for instance. DMP discovery unveiled panels of 35 and 347 *top gene* DMPs across recurrent adenomas versus primary adenomas with recurrence and adenomas versus normal epithelium, respectively. Despite that, no DMPs could be identified across primary adenomas with and without recurrence. Array validation via pyrosequencing used the methylation of the *GREM2* gene. The genetic landscape of adenomas unveiled by aCGH exhibited typical CNAs in colorectal tumorigenesis: chromosomes 7, 13q, 18 and 20. Most frequent focal aberrations (≤10 Mb) were located on 6p22.1-p21.33 (33.3%), 7q22.1 (31.4%) and 16q21 (29.4%). Single-cell miFISH showed frequent copy number gains within the colorectal adenomas affecting the markers *EGFR* (23.6%), *CDX2* (21.8%) and *ZNF217* (18.2%). Remarkably, copy number gains of *CDX2* were exclusively observed in primary adenomas with recurrence (25%) and recurrent adenomas (38.5%), while this CNA was absent in primary adenomas without recurrence (0%). The presence of major clone (defined as >40%) populations (average size 62.8%) accompanied by multiple minor clones was present in most adenomas (52.7%). While few adenomas were mainly composed of diploid cells (29.1%), a few adenomas were highly heterogeneous without a major clone population (16.4%) as confirmed by four quantitative diversity measures. Inferring of phylogenetic trees unraveled four distinct patterns of clonal evolution from primary adenomas towards recurrent adenomas: simplification pattern, complexity pattern, stability pattern and zero pattern. Noteworthy, copy numbers detected by aCGH and miFISH were concordant in 97.3% of observations (κ=0.861).

Collectively, the findings underpin that adenoma development and recurrence are complex processes orchestrated by genetic and epigenetic alterations. Whereas CpG methylation patterns appeared to be inconclusive for adenoma recurrence, the evaluation of CNAs affecting *CDX2* in single cells via miFISH might bear potential as a predictor of recurrence.