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GENETICS

Driver genes are mutated early in the course of oesophageal adenocarcinoma

Contrary to expectations, a study in *Nature Genetics* has found that most recurrently mutated genes present in oesophageal adenocarcinoma (EAC) are mutated early in development of the disease, which has implications for both diagnosis and therapy.

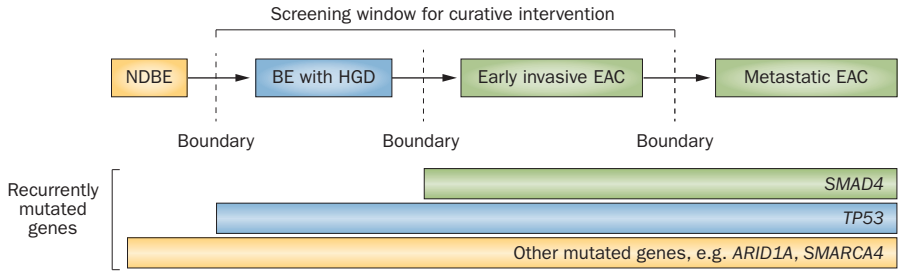
Rebecca Fitzgerald and colleagues wanted to understand at what stage in the disease process mutations occur. "If we are able to delineate when the changes occur and identify the changes that occur at the point at which Barrett oesophagus becomes malignant then that would form the basis of an ideal clinical test to find the highest risk patients who need treatment," she explains.

Initially, the team set out to find which genes were recurrently mutated in EAC by applying whole-genome sequencing to 22 EACs. In each sample in the discovery cohort, there was a median of 16,994 somatic single-nucleotide variants (SNVs) and 994 small indels (an insertion and deletion of nucleotides that alters the overall nucleotide number). 1,081 of 1,086 coding-region mutations and 23 of 25 indels were confirmed as somatic.

Unlike other cancers studied with whole-genome sequencing, and in line with the findings of others, the most frequent mutation type in EAC was T:A>G:C transversions. Using amplicon sequencing in an EAC validation cohort, the team confirmed and extended their findings. By combining data from both cohorts, 15 genes were found to contain four or more mutations, including *TP53* and *SMAD4*.

To understand the role of the recurrent mutations in the progression of EAC, the team studied the presence of the 26 most recurrently mutated genes in samples from different stages of carcinogenesis—90 EAC, 66 never dysplastic Barrett oesophagus (NDBE) and 43 Barrett oesophagus with high-grade dysplasia (HGD).

As expected, the findings confirmed that EAC is a highly mutated cancer. "What was more surprising," says Fitzgerald, "was that when we looked in NDBE cases there was also evidence of mutations in the 26 genes." Indeed, mutations were present in the Barrett oesophagus segment of 53% of individuals with NDBE; 92% of individuals with HGD had a mutation in one of the 26 genes. "It is remarkable that lesions that appear to be very benign and histologically stable over many years had mutations," she explains. Further analysis also suggests that many of the mutations may not cause invasion but be passengers, because they are selected for at multiple stages of cancer progression. Mutations in *TP53* and *SMAD4* were the only ones able to distinguish between different stages of disease. *TP53* was recurrently mutated in 72% of HGD and 69% of EAC samples, but only 2.5% of NDBE samples (a single sample). *SMAD4* was specific to EAC, but was present at a much lower frequency (13%).



Barrett oesophagus carcinogenesis—a proposed model of boundary defining mutations © Weaver, J. M. *et al.* NPG.

The final part of the study investigated how useful any recurrent mutations might be in the clinic. The team combined the use of *TP53* mutations as a biomarker for risk stratification with use of the Cytosponge —a non-endoscopic cell sampling device previously developed by Fitzgerald and colleagues. Encouragingly, *TP53* mutations had 100% specificity for differentiating HGD from no dyplasia and were identified in 86% of individuals with HGD.

"This is an important and groundbreaking study," says Stephen Meltzer, Director of the Gastroenterology Early Detection Biomarkers Laboratory at The Johns Hopkins University School of Medicine. "It would have been quite informative to have had mutation data on nondysplastic Barrett oesophagus tissue from patients with HGD or EAC. Such data might have shed more light on mutational timing during evolution of HGD from nondysplastic Barrett oesophagus, as well as on additional drivers of Barrett oesophagus-associated neoplastic progression." He also suggests that comparing nondysplastic Barrett oesophagus from patients with HGD/EAC with that from non-HGD/EAC patients may have identified differences in mutational frequency and further highlighted potential driver genes.

Meltzer agrees that the Cytosponge could become an important tool for screening and surveillance. "It is possible that with the Cytosponge, simpler, cheaper molecular assays will become more useful than high-throughput screening." Fitzgerald and colleagues are now analysing samples obtained from a trial of the Cytosponge in >1,000 patients for *TP53* mutations. "We hope that this might form the basis for an innovative clinical strategy for patients undergoing Barrett oesophagus surveillance in the future," she concludes.

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Original article Weaver, J. M. J. *et al.* Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. *Nat. Genet.* doi:10.1038/ng.3013