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Is post-ablation neo-squamous epithelium genomically predisposed to malignant progression?

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Radiofrequency ablation (RFA) is a frequently used treatment for dysplastic Barrett's oesophagus or early oesophageal intramucosal carcinomas.[1 2] As a treatment, it is widely considered to be safe and effective at removing these aberrant lesions. The treated areas are re-populated with stratified, squamous epithelium, much like that of the normal oesophagus. The story does not end there, though. Following ablation, Barrett's oesophagus recurs within a year in 6%–25% of patients[3] and the annual rate of neoplastic progression is 1.37%/patient-year.[4] This puts the risk of Barrett's oesophagus recurring in this neo-squamous epithelium far higher than the risk of Barrett's oesophagus in normal squamous epithelium. This raises the question of whether the neo-squamous epithelium is as normal as it looks to the endoscopic or histopathologic eye.

There can be many hypotheses as to the cause of these Barrett's oesophagus recurrences in neo-squamous epithelium, some of which have already been studied. It has already been shown that the recurrence may be facilitated by a weaker barrier function post-ablation,[5] and that recurrence does not appear to originate from undetected, residual original lesion.[6] In a new study, Akarca *et al* investigate an alternative hypothesis that ablation removes barriers to clonal expansion, and therefore allows mutations found in normal squamous epithelium to widely expand in the ablated area.[7] To take an example, *TP53* mutations, a key prognostic factor in malignant progression of Barrett's oesophagaus,[8 9] have been found frequently in normal squamous oesophagus.[10] In the normal oesophagus, *TP53* mutations tend not to have a second hit (eg, through loss of heterozygosity) and typically remain in small clones.[10 11] If ablation leads to large clonal expansions of these mutations, it is plausible that that would increase the risk of recurrence and progression. The authors sequenced post-RFA, neo-squamous epithelium and compared it with untreated, normal squamous epithelium from control patients. They detected frequent *NOTCH1* and *TP53* mutations in both the neo-squamous epithelium and normal epithelium, but no other frequently mutated cancer-associated genes. There was weak evidence for increased frequency of *TP53* mutations in neo-squamous epithelium compared with normal, native squamous. This increased frequency came from the fact there were more samples with *TP53* mutations, as well as multiple *TP53* mutations in some samples. The study also reported mutant allele fractions, defined as the frequency with which each mutation was observed in sequencing reads, which can be used as a proxy for clone size. Crucially, the allele fraction of the *NOTCH1* and *TP53* mutations was always low, and there was no difference between neo-squamous and normal epithelium: there was no evidence of large clonal expansions.

These findings should be of reassurance to clinicians and may provide useful guidance to researchers. If there is little genomically that is predisposing the tissue to malignancy, efforts can continue understanding and potentially addressing the non-genomic functional defects associated with post-ablation tissue.

This study also highlights a few interesting technical lessons. First, it is notable that these low allele fraction mutations were detectable using standard sequencing techniques. This may encourage more researchers to investigate the prevalence of these mutations in normal tissues. It should also serve as a warning about the interpretation of low allele fraction driver mutations in cancers, as they could be picked up from contamination from surrounding normal tissues that harbour inconsequential mutations as a result of ageing.

The authors recognise that the sequencing approach taken does not preclude there being other mutations being present in the tissue, either at even lower allele fractions in the sequenced sample or in part of the tissue that was not biopsied. However, this does not detract from the conclusion against the main hypothesis, of no evidence of large clonal expansions.

Finally, the sample size in this study (18 neo-squamous samples) is admittedly small, which prohibits the authors from concluding whether *TP53* mutations are increased in neo-squamous compared with normal epithelium. This is a shame, given the importance of *TP53* mutations in malignant progression, but one can empathise with the challenges of sample availability and justification for sequencing a large number of close to normal samples. A follow-up study, potentially targeted specifically on *TP53*, would be of value. However, in the meantime, we should be reassured that the common act of radiofrequency ablation is not creating a hotbed for malignant mutation takeover.

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