Signatures of mutational processes and associated risk factors in esophageal squamous cell carcinoma: a geographically-independent stratification strategy?

Maria Secrier[1] and Rebecca C. Fitzgerald

Medical Research Council Cancer Unit, Hutchison/Medical Research Council Research Centre, University of Cambridge, Cambridge, UK

[1]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK

Esophageal cancer (made up primarily of squamous and adenocarcinoma) is an understudied, yet aggressive form of cancer, ranking sixth in the world in terms of mortality[1]. While adenocarcinoma incidence has been steadily increasing in the Western world in the past decades, squamous cell carcinoma (ESCC) continues to be the major type of oesophageal cancer in Asia. Risk factors for ESCC include smoking, alcohol, drinking scalding hot beverages and poor nutrition[2]. For some of these risks, a geographical dependence can be observed; for example family history and high concentration of dietary nitrates are more prevalent risk factors in rural, high-incidence areas of China, while esophageal cancer cases in urban, low incidence areas are more frequently associated with drinking tea at high temperature[3], and hot-spots in the Kashmir Valley (India) relate to chewing nass[4]. How these factors differ in other parts of Asia, however, is less well understood.

At the genetic level, inherited risk alleles in the phospholipase C-epsilon-1 enzyme (PLCE1, hydrolyzing phospholipids into fatty acids, affecting cell growth and differentiation) and susceptibility loci in chromosomes 5, 6, 10 and 12 in Chinese

populations, as well as germline variants in alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2) in Japanese populations have been associated with ESCC, the latter increasing the risk substantially when combined with smoking and alcohol consumption[5].

With the advent of genome wide sequencing technologies a number of recent genomic studies, prevalently in Chinese populations[6-9], have started to map the somatic landscape of ESCC. These studies have highlighted tumour suppressors like TP53 and CDKN2A as common drivers, with the cell cycle (e.g. CCND1), RTK-MAPK-PI3K (e.g. PI3KCA), Notch pathways (e.g. NOTCH1), hedgehog signalling and epigenetic regulation also being frequent causes of mutation in this cancer. Inactivating mutations in genes involved in chromatin remodelling (e.g. CREBBP), cell-cell communication (e.g. FAT1), or transcriptional regulation (e.g. ZNF750), among others, have also been noted. What is lacking is a connection between the causative agents and the mutational landscape that is observed in ESCC.

In the study by Sawada et al. of 144 Japanese patients, the authors investigated the relationship between the individual patient risk factors (alcohol, smoking and variants in specific inherited loci known to alter the metabolism of these carcinogens) and the acquired pattern of mutations in the cancer itself[10]. This includes analysis of mutations in specific genes (e.g. known cancer causing genes like TP53 and TGF beta) as well as elucidating “mutational signatures”. In the past few years, large-scale analyses have revealed many mutational signatures, or characteristic imprints, across the genome of human cancers [11, 12]. These imprints are the outcome of multiple mutagenic processes that have been operating in all

cells of the human body during the lifetime of the patient. They are a combined consequence of ageing, exposures to mutagens like tobacco and UV light for example, as well as intrinsic or acquired defects in DNA repair machinery. The profile of each signature is displayed using the six possible base-substitutions: C>A, C>G, C>T, T>A, T>C, and T>G (referred to by the pyrimidine of the mutated Watson–Crick base pair). Further information is then obtained by incorporating information on the bases immediately 5' and 3' to each mutated base generating 96 possible mutation types: 6 types of substitution $*$ 4 types of 5' base $*$ 4 types of 3' base. Mutational signatures are then reported based on the observed trinucleotide frequency of the human genome. By examining these signatures in thousands of cancer genomes it is possible to start attributing causality[13].

In Japanese patients with ESCC[10] the authors identified three clusters of patients. Cluster 1 patients have a predominant APOBEC signature characterised by C>G/T substitutions with an adjacent 5' thymine. APOBEC is a cytosine deaminase enzyme which converts cytosine to uracil and may induce mutation into tumours. In keeping with this, tumours in cluster 1 have a relatively high mutation rate across their genome. Cluster 3 patients also have a high proportion of APOBEC as well as a CpG signature characterised by C>T substitutions at CpG dinucleotides. Neither of these signatures was predominant in cluster 2.

Interestingly, these clusters were also shown to carry environmental and genetic associations: for example, heavy drinking in patients with the inherited ALDH2 risk allele were enriched in clusters 1 and 2, and somatic mutations in a potential tumour

suppressor ZNF750[7] and PIK3CA signalling molecule mutations were also prevalent in the first cluster (containing a more prominent APOBEC signature).

The authors also undertook a concerted effort to compare the genomic profiles of ESCC in Japanese and Chinese populations (high and low incidence areas). They found that alcohol intake and smoking behaviour were more strongly associated with the incidence of this disease in Japan compared to China - also previously reported by e.g. Lin et al.[3] - but otherwise mutational spectra, driver genes (TP53, ZNF750, NOTCH1) and pathways seemed to be conserved across Japanese and Chinese cohorts. Of note, the association between the APOBEC signature and mutations in ZNF750 was preserved among the different populations. Extending to a pan-cancer type study including head-and-neck and lung squamous cell carcinoma, similar signature profiles were observed, but different from oesophageal adenocarcinoma, emphasizing again the fundamentally different carcinogenic mechanism of the latter.

Sawada et al. also highlighted TET2 as a new mutational target in ESCC occurring in around 6% of their cohort. This gene has roles in cell invasion and mutations were related to poor prognosis. This is interesting, especially in view of recent work that relates miR-22 to TET2 in the promotion of stem cell transformation [14]. The same microRNA has also been shown to inhibit tumour growth and metastasis in gastric cancer through MMP14 and Snail targeting [15]. These studies suggest that TET2 and its regulatory (especially miRNA) network constitute targets worthy of further investigation in ESCC. At the pathway level, disruption of epigenetic regulation was highlighted as one of the dominant mechanisms in this cohort (affecting 59% of the

patients), with an important role of repressive epigenetic marks in the pathogenesis of ESCC.

In terms of what this study adds to our understanding, the mutational signature analysis performed here builds upon the work of Zhang et al[9], who were the first to document the signature related to the APOBEC family of cytidine deaminases and the CpG signature in ESCC. Importantly, however, the study by Sawada et al shows that the various contributions of mutational processes in this cancer reveal subgroups of distinct aetiology, and these are informative of the different environmental influences and genetic predispositions in this disease (Figure 1). APOBEC mRNA deregulation has been linked to the APOBEC signature in a variety of cancers and was suggested to contribute to carcinogenesis[16]. Considering that cluster 1 displays a relatively high contribution of the APOBEC signature, as well as significantly more mutations in ZNF750, it will be interesting to see future studies into the molecular mechanism of this process in this ESCC subgroup.

Furthermore, the study highlights the importance of environmental risk factors to the development of ESCC and the fact that this can differ significantly even within territories of close geographical proximity. It is thus imperative to collect detailed exposure data in large-scale genomic cancer studies such as the International Cancer Genome Consortium to enable these inferences to be made.

From a therapeutic perspective, it is encouraging to observe that this diversity in risk factors is not reflected to the same extent at the genomic level, with driver genes and pathways being overall conserved among Chinese and Japanese populations. Recent studies in other cancers have highlighted the potential of mutational

signature-based stratification in the clinic. It has been suggested, for instance, that gastric tumours with homologous recombination (BRCA-signature related) defects might benefit from platinum/PARP inhibitors therapy[17]. Further research is needed to elucidate the applicability of this type of classification for early detection, prognosis or treatment of ESCC, especially since the mutational spectra and subgroup profiles seem to be maintained throughout Chinese and Japanese populations – and thus, would offer a promising strategy universally applicable in this cancer.

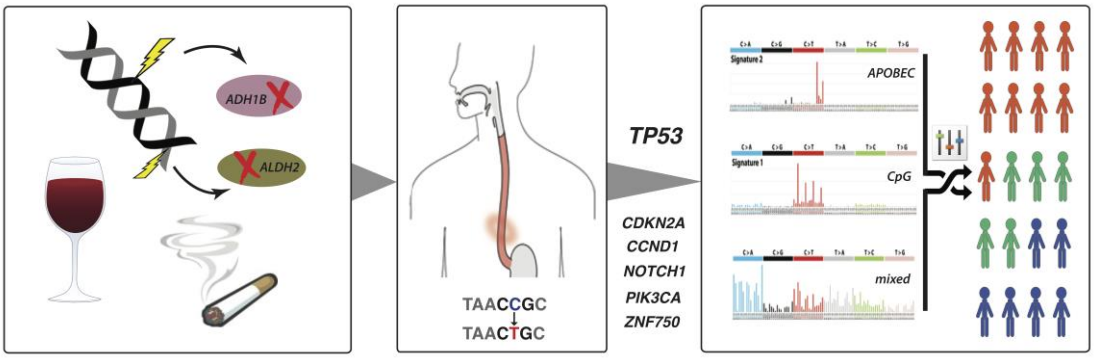

Figure 1. Aetiology and classification of ESCC in Japanese populations. Risk factors include alcohol consumption, smoking and germline variants in alcohol metabolism-related enzymes (ALDH1B, ALDH2), among others. These imprint mutational patterns in the genome that promote carcinogenesis, along with dysregulation of key driver genes: TP53, CDKN2A, CCND1, NOTCH1, PIK3CA, ZNF750 etc. The signatures of such mutational processes can be decomposed according to their trinucleotide substitution context (peaks of mutations for each substitution category, C>[A,G,T], T>[A,C,G], are shown), resulting in an APOBEC-related pattern, a CpG-predominant one, and other mixed patterns. The different contributions of

these signatures help distinguish three main subgroups of patients with ESCC in this population (denoted by different colours: orange, green, purple), and these subgroups differ in their associated risk factors and genomic landscape.

Acknowledgements

RCF and MS both work on the oesophageal adenocarcinoma International Cancer Genome Consortium project funded by Cancer Research UK with infrastructure funding from the Cambridge Biomedical Medical Research Centre and the Cambridge Experimental Cancer Medicine Centre. RCF also has funding from a National Institute for Health Research Professorship and a Medical Research Council Programme Grant.

Conflicts of interest: None to declare

References

1. Zhang Y. Epidemiology of esophageal cancer. World J Gastroenterol 2013;19:5598-606.
2. Zhang HZ, Jin GF, Shen HB. Epidemiologic differences in esophageal cancer between Asian and Western populations. Chin J Cancer 2012;31:281-6.
3. Lin Y, Totsuka Y, He Y, et al. Epidemiology of esophageal cancer in Japan and China. J Epidemiol 2013;23:233-42.
4. Dar NA, Bhat GA, Shah IA, et al. Hookah smoking, nass chewing, and oesophageal squamous cell carcinoma in Kashmir, India. Br J Cancer 2012;107:1618-23.
5. Tanaka F, Yamamoto K, Suzuki S, et al. Strong interaction between the effects of alcohol consumption and smoking on oesophageal squamous cell

carcinoma among individuals with ADH1B and/or ALDH2 risk alleles. Gut 2010;59:1457-64.
6. Gao YB, Chen ZL, Li JG, et al. Genetic landscape of esophageal squamous cell carcinoma. Nat Genet 2014;46:1097-102.
7. Lin DC, Hao JJ, Nagata Y, et al. Genomic and molecular characterization of esophageal squamous cell carcinoma. Nat Genet 2014;46:467-73.
8. Song Y, Li L, Ou Y, et al. Identification of genomic alterations in oesophageal squamous cell cancer. Nature 2014;509:91-5.
9. Zhang L, Zhou Y, Cheng C, et al. Genomic analyses reveal mutational signatures and frequently altered genes in esophageal squamous cell carcinoma. Am J Hum Genet 2015;96:597-611.
10. Sawada G, Niida A, Uchi R, et al. Genomic Landscape of Esophageal Squamous Cell Carcinoma in a Japanese Population. Gastroenterology 2016.
11. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
12. Alexandrov LB, Stratton MR. Mutational signatures: the patterns of somatic mutations hidden in cancer genomes. Curr Opin Genet Dev 2014;24:52-60.
13. Helleday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet 2014;15:585-98.
14. Song SJ, Ito K, Ala U, et al. The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation. Cell Stem Cell 2013;13:87-101.
15. Zuo QF, Cao LY, Yu T, et al. MicroRNA-22 inhibits tumor growth and metastasis in gastric cancer by directly targeting MMP14 and Snail. Cell Death Dis 2015;6:e2000.
16. Roberts SA, Lawrence MS, Klimczak LJ, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 2013;45:970-6.
17. Alexandrov LB, Nik-Zainal S, Siu HC, et al. A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun 2015;6:8683.