

# Intratumoral heterogeneity of esophageal squamous cell carcinoma

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The advent of large scale genome characterization led to rapidly increasing knowledge of genetic landscapes of malignancies in recent years. Besides a difference in quantity of mutations between different entities, studies revealed as well a difference in the amount of mutations in varying locations of a single neoplastic lesion: the relation of private (only present in a single sample of a tumor) and common mutations (present in all tested samples of tumor) can vary markedly (1-3). A detailed knowledge of this intratumoral heterogeneity (ITH) is of high impact for clinical management of cancer patients, since biomarker driven therapies most frequently rely on the results of a single biopsy. This especially applies to esophageal squamous cell carcinoma (ESCC), since no targeted therapies could prove a benefit in survival for this entity so far (4). Several exome sequencing studies characterized mutational landscape of ESCC, but data on ITH were lacking so far (5-8).

Hao *et al.* performed a whole exome analysis of 51 samples deriving from 13 cases with ESCC and revealed a substantial ITH with an average of 35.8% heterogeneous somatic mutations. The authors performed as well a methylation analysis of three patients and observed epigenetic phylogenetic trees to be mostly concordant with the genetic alterations, although private potential epigenetic tumor driving events were found as well.

In comparison to other solid malignancies, the amount of genetic ITH detected for ESCC can be regarded as modest: for example, the amount of ITH seems comparable to lung cancer, where 76% of common mutations were reported, but far lower than clear cell renal carcinoma, where 67% of non-synonymous somatic mutations were subclonal (3,9).

Likewise, in prostate cancer and primary breast cancer, a higher amount of ITH was reported, whereas cholangiocarcinoma seems to have a lower ITH (2,10,11). The number of samples per case analyzed by Hao *et al.* (n=3-4) is within the range of most other studies where 2-11 samples per case were analyzed. Notably, the observed degree of ITH is known to increase with the number of biopsies examined, which has to be kept in mind when comparing studies on ITH (3).

Besides genetic heterogeneity, Hao *et al.* characterized epigenetic alterations of ESCC as well: three patients were analyzed with a chip based methylation assay (Illumina HumanMethylation450 BeadChip) and the detected epigenetic changes were mostly in line with genetic mutations suggesting a parallel development. However, private hypermethylations of promoters of tumor suppressor genes were found as well. This is of interest, since most studies describing ITH focused on exome data and characteristics of epigenetic ITH in general are limited. These results point at the potential tumor driving impact of epigenetic alterations in oncogenic evolution and highlight the importance of comprehensive analyses including both genetic and epigenetic alterations. Especially in tumors with a low mutational burden (e.g., <1 mutation/mega base) such as reported for astrocytoma or neuroendocrine tumors of the small intestine, comprehensive characterization of epigenetic alterations will be important to investigate presence of potential tumor driving alterations (1,12). Of note, development of epigenetic diagnostic methods is still ongoing and new methods such as larger methylation chip arrays (e.g., Illumina Infinium MethylationEpic

Kit including >850,000 methylation sites) and RNA-seq are already available, which has to be considered in the interpretation of current epigenetic studies.

To interpret results of ITH studies, sampling methods have to be taken into account. In the study of Hao *et al.* 3–4 samples with at least 0.5 cm distance were taken after surgery of ESCC and truncal/clonal mutations and branched/subclonal were assessed as early and late events in tumorigenesis, respectively. Thereby, the authors constructed phylogenetic trees to draft the temporal order of mutations and at least 88% of mutations were compatible with this model. Notably, all samples were obtained at once and comprehensive genomic data from chronological studies of ESCC including its potential precursor lesions such as chronic esophageal inflammation and esophageal dysplasia are lacking so far. In addition to the results of Hao *et al.*, knowledge of the temporal evolution of the mutational landscape of precancerous lesions might have clinical relevance as well as it could help to perform a mutation-based risk stratification in cases with known or potential precursor lesions of ESCC. For example, a recent study suggested a correlation of inflammation-associated genomic instability with esophageal carcinogenesis (13). Moreover, a sequencing study including sequentially obtained samples on esophageal adenocarcinoma (EAC) revealed a majority of recurrently mutated genes in EAC to be as well present in metaplastic but never dysplastic Barrett's esophagus, while only mutations in *TP53* and *SMAD4* occurred stage-specific confined to high grade dysplasia and EAC (14). In addition, sequential mutational data will be interesting in correlation to clinical background such as exposition to known risk factors like abuse of alcohol and smoking as well as presence of germline polymorphisms in *ALDH2* and *CYP2A6* which could be linked to distinct mutational spectra in ESCC characterized by different proportions of CpG and APOBEC signatures (15). However, ESCC are usually diagnosed in an advanced stage and identification of high-risk groups for screening is difficult, especially in western countries where incidence of ESCC is markedly lower than in Asia (16,17). Moreover, endoscopic detection of potential pre-cursor lesions of ESCC is challenging (16,18).

In summary, Hao *et al.* report a substantial intratumoral genetic and epigenetic heterogeneity in ESCC which has to be considered in the design of biomarker driven targeted therapy trials. The future addition of comprehensive studies assessing genetic and epigenetic alterations in a chronological sequence will be of interest to chart the progress from its precursor lesions to invasive ESCC.

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## Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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