SUPPLEMENTARY METHODS

Whole-exome sequencing analysis of primary and matched metastatic ECs

Whole-exome sequencing (WES) data in form of Binary Sequence Alignment Map format (BAM) files from primary ECs and matched metastatic lesions from 26 EC patients described by Gibson et al. [1] were downloaded from dbGaP with accession phs001127.v1.p1. The SRA formatted files were converted to standard FASTQ by means of a fastq-dump program as part of the SRA toolkit [2]. FASTQ files were then mapped onto the reference human genome GRCh37 using the Burrows-Wheeler Aligner (BWA, v0.7.15) [3], and sequencing data analysis performed as previously described [4]. Local realignment, duplicate removal and base quality score recalibration was performed using the Genome Analysis Toolkit (GATK, v3.1.1) [5]. Somatic single nucleotide variants (SNVs) were identified using MuTect (v1.0) [6], small insertions and deletions (indels) using Strelka (v2.0.15) [7], VarScan2 (v2.3.7) [8], Lancet (v1.0.0) [9] and Scalpel (v0.5.3) [10], and further curated by manual inspection. SNVs and indels outside of the target regions were filtered out, as were SNVs and indels for which the variant allele fraction (VAF) in the tumor sample was <5 times that of the paired normal VAF, and SNVs and indels found at >5% global minor allele frequency of dbSNP (build 137), as previously described [4]. In addition to the SNV and indel identification described above, mutations that were identified in the primary or metastatic tumor sample from a given patient were subsequently interrogated in the matched respective primary or metastatic sample by manual inspection of BAM files using mpileup files generated from SAMtools mpileup (version 1.2 htslib 1.2.1)[11]. Only somatic mutations with a depth ≥20 reads in the respective normal samples were considered. Somatic copy number alterations and loss of heterozygosity (LOH) were obtained using FACETS [12], as previously described [4]. The cancer cell fractions (CCFs) of all mutations were computed using ABSOLUTE (v1.0.6)[13], as previously described [4]. A combination of mutation function predictors was employed to define the potential functional impact of each missense SNV, as previously described [14]. Mutational hotspots were assigned according to Chang *et al*. [15].

SUPPEMENTARY REFERENCES

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