Materials and Methods

Tissue preparation

Subjects diagnosed with OAC were recruited to the study (see Table S1 for clinical characteristics). Tumours were excised from resected oesophageal tissue post-operatively by pathologists and processed either for histological evaluation of tumour type and stage, or snap frozen at −80°C.

Protein extraction and digestion

Snap frozen tissue samples were briefly thawed and weighed prior to 30s of mechanical homogenization (Fisher, using disposable probes) in 4mL lysis buffer (0.02M Tris, 0.5% [w/v] IGEPAL, 0.25% [w/v] sodium deoxycholate, 0.15mM NaCl, 1mM ethylenediaminetetraacetic acid (EDTA), 0.2mM iodoacetamide supplemented with EDTA-free protease inhibitor mix). Homogenates were clarified for 10min at 2000g, 4°C and then for a further 60min at 13 500g, 4°C.

Protein concentration of tissue lysates was determined by BCA assay, and volumes equivalent to 100 mg of protein were precipitated using methanol/chloroform as previously described (1). Pellets were briefly air-dried prior to resuspension in 6 M urea/50 mM Tris-HCl (pH 8.0). Proteins were reduced by the addition of 5 mM (final concentration) DTT and incubated at 37°C for 30 min, then alkylated by the addition of 15 mM (final concentration) iodoacetamide and incubated in the dark for 30 min. 4 ug Trypsin/LysC mix (Promega) were added and the sample incubated for 4 h at 37°C, then 6 volumes of 50 mM Tris-HCl pH 8.0 were added to dilute the

22   urea to < 1 M, and the sample was incubated for a further 16 h at 37°C. Digestion was

23   terminated by the addition of 4 ml of TFA, and the sample clarified at 13,000 g for 10 min at RT.

24   The supernatant was collected and applied to Oasis Prime microelution HLB 96-well plates

25   (Waters, UK) which had been pre-equilibrated with acetonitrile. Peptides were eluted with 50 ml

26   of 70% acetonitrile and dried by vacuum centrifugation prior to resuspension in 0.1% formic

27   acid.

28   **Mass spectrometry proteomics**

29   8 µg of peptides per sample were separated by an Ultimate 3000 RSLC nano system (Thermo

30   Scientific) using a PepMap C18 EASY-Spray LC column, 2 µm particle size, 75 µm x 75 cm

31   column (Thermo Scientific) in buffer A (0.1% Formic acid) and coupled on-line to an Orbitrap

32   Fusion Tribrid Mass Spectrometer (Thermo Fisher Scientific,UK) with a nano-electrospray ion

33   source.

34   Peptides were eluted with a linear gradient of 3-30% buffer B (acetonitrile/0.1% formic acid) at a

35   flow rate of 300 mL/min over 200 min. Full scans were acquired in the Orbitrap analyser in the

36   scan range 300-1,500 m/z using the top speed data dependent mode, performing an MS scan

37   every 3 second cycle, followed by higher energy collision-induced dissociation (HCD) MS/MS

38   scans. MS spectra were acquired at a resolution of 120,000, RF lens 60% and an automatic

39   gain control (AGC) ion target value of 4.0e5 for a maximum of 100 ms. MS/MS scans were

40   performed in the ion trap, higher energy collisional dissociation (HCD) fragmentation was

41   induced at an energy setting of 32% and an AGC ion target value of 5.0e3.

42   **Proteomic data analysis**

43   Raw spectrum files were analysed using Peaks Studio 10.0 build 20190129 (2,3) and the data

44   processed to generate reduced charge state and deisotoped precursor and associated product

45   ion peak lists which were searched against the UniProt database (20,350 entries, 2020-04-07)

46   plus the corresponding mutanome for each sample (~1,000-5,000 sequences) and

47   contaminants list in unspecific digest mode. Parent mass error tolerance was set a 5ppm and

48   fragment mass error tolerance at 0.03 Da. Variable modifications were set for N-term acetylation

49   (42.01 Da), methionine oxidation (15.99 Da), carboxyamidomethylation (57.02 Da) of cysteine.

50   As previously described, carbamidomethylated cysteines were treated as variable modifications

51   due to the low concentration of 0.2 mM of iodoacetamide used in the lysis buffer to inhibit

52   cysteine proteases (4). A maximum of three variable modifications per peptide was set. The

53   false discovery rate (FDR) was estimated with decoy-fusion database searches (2) and were

54   filtered to 1% FDR.

55   **Differential protein expression**

56   Label free quantification using the Peaks Q module of Peaks Studio (2,5) yielding matrices of

57   protein identifications as quantified by their normalised top 3 peptide intensities. The resulting

58   matrices were filtered to remove any proteins for which there were more than two missing

59   values across the samples. Differential protein expression was then calculated with DEqMS

60   using the default steps (6).

61   Principal component analysis of the normalised top 3 peptide intensities was performed using

62   DESEq2 (7) and PCATools (8).

63   Results were visualised using EnhancedVolcano (9), pheatmap (10) and ggplot2 (11).

64   **Functional analysis**

65   Functional enrichment analysis was performed using g:Profiler (12) using default settings for

66   homo sapiens modified to exclude GO electronic annotations. Protein ids were used as inputs.

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