Supplement Methods

Microarray

Using 1 µg of total RNA as template, biotinylated cRNA was synthesised by means of the GeneChip 3'IVT Express kit (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. The cRNA was fragmented to 35 to 200 nt, and hybridised to arrays. After streptavidin-phycoerythrin (Molecular Probes, Eugene, OR, USA) staining the signal was amplified by biotinylated anti-streptavidin (Vector Laboratories Inc.) and a second staining with streptavidin-phycoerythrin.

PET-imaging

The mice were fasted on average 3 h 30 min ±3 min prior to injecting 100 µl of 10.9 MBq ±1.5 MBq ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) through a tail vein catheter during a 10-15 s manual infusion. The mice were anaesthetised in an oxygen-isoflurane mixture with isoflurane concentrations of 4% for incubation and 2% for maintenance for 60 min ±5 min before ^{18}F-FDG injection. Blood plasma glucose, measured at 19 min ±11 min before injection of ^{18}F-FDG, was 5.9±2.0 mmol/l. Mice were kept under anaesthesia and positioned inside the TriumphTM LabPET-8TM small animal PET/SPECT/CT scanner (Trifoil Imaging, Northridge Tri-Modality Imaging, Inc., Chatsworth, CA). Heart rate and breathing was monitored with sensors inside a closed animal cell (Equipment Veterinaire Minerve, Esternay, France) and the temperature on the heated air was set to 35°C to prevent hypothermia. A 20 min list mode PET acquisition was started 42 min ± 3 min after ^{18}F-FDG injection. The list mode PET data was histogrammed into 1 static time frame of 20 min and reconstructed using 3-dimensional maximum likelihood expectation maximisation (MLEM) algorithm with 50 iterations. Images were corrected for

varying detector element efficiency by running a normalisation scan with a ^{68}Ge line source the day before PET, according to the manufacturer's recommendations. Corrections for radioactive decay, random coincidences, dead time losses, attenuation and scatter effects were also implemented, before a dose calibration factor was applied, to convert the output images into the unit of MBq/cm^3. Computed tomography (CT) was performed using 80 kVp, 2x2 binning, 512 projections, 1.3x magnification, immediately after PET imaging, and the raw data were reconstructed using Filtered Back Projection algorithm. Images were analyzed using PMOD (PMOD Technologies, Zürich, Swizerland). Volumes of interests (VOI) representing the primary tumor and left and right salivary glands, where lymph node metastases would overlap, were semi-automatically delineated as the inside of the 60% isodose contour of the maximum voxel value in a large enough VOI that covered the entire structure. The average of the five highest voxel values was reported for each of these regions. A VOI in the liver was delineated as a 2 mm diameter sphere in the upper right lobe of the liver and the average value in this VOI was reported for all mice. All VOI data was converted to standardised uptake value (SUV) by normalizing to injection dose per animal weight. The tumor SUV_{max} to liver SUV_{mean} ratios were calculated to further reduce individual uptake variations between animals. To investigate the kinetics of the PET tracer uptake, two of the mice underwent a 60 min PET acquisition, starting at the time of injection. This list mode PET data was histogrammed into 44 time frames (24x5s, 9x20s and 11x5min). The rest of the preparation, acquisition and analysis protocol was identical to above, except that time-activity-curves (TACs) could now be generated by extracting the average value in each VOI for all time frames.

PCR

MMP8 forward primer was 5'-GGCCATTCTTTGGGGCTCGCTCA-3' and reverse primer 5'-GGGGTCACAGGGTTTGGGTGTGC-3', product size 186 bp. β-actin (*ACTB*) [GenBank:

NM_001101.3] was measured from the same RT-reaction samples using forward primer 5'-AACTGGGACGACATGGAGAAAA-3' and reverse primer 5'-AGAGGCGTACAGGGATAGCACA-3', product size 204 bp.

qRT-PCR

CTSK forward primer used was 5'-CCTGCCCCTGACTGTGTTG-3', and reverse primer 5'-GAAAATCTCCAGCCTGTACCTGTAC-3', and *MMP8* forward primer 5'-TGCCTGACAGTGGTGGTTTT-3' and reverse primer 5'-TGTCACCGTGATCTCTTTGGT-3'. Peptidylprolyl isomerase A (*PPIA*) [GenBank: NM_021130.3] was used as the reference gene; forward primer: 5'- GCTTTGGGTCCAGGAATGG-3' and reverse primer: 5'-GTTGTCCACAGTCAGCAATGGT-3'.