Supplementary material

Cell lines

The metastatic melanoma cell line A375-M2 (A375-derived cell line with higher metastatic potential) and murine fibroblasts cell line NIH-3T3 were purchased from ATCC, the bladder carcinoma cell line T24 from CLS Cell Lines Service. The metastatic melanoma cell line C8161 were kindly provided by Dr D. Constam (EPFL, Lausanne, Switzerland) and the murine lung endothelial mLuEC cells by Pr. T. Petrova (UNIL, Epalinges, Switzerland). A375-M2, C8161, and NIH-3T3 cells were maintained in DMEM/Glutamax plus 10% FBS; the T24 cells in DMEM/F12-Glutamax plus 10% FBS; mLuEC in DMEM/F12 plus 0.8% ECGM-supplements. All cultures were maintained at 37°C in a 5% CO2 humidified atmosphere.

Treatment and conditioned medium preparation

Cancer cells were treated for 24h with PPARγ agonists 5µM RGZ, 5µM Ciglitazone (*CGZ*, Focus Biomolecules, Lucerna-Chem), 5µM Pioglitazone hydrochloride *(PGZ*, MedChem Express, Lucerna-Chem), and 500nM GW1929 (Enzo Life Sciences) or PPARγ antagonist 2µM T0070907 (*T007*, Enzo Life Sciences). The $CD14^+$ monocytes were treated for 5 days. All compounds were diluted in DMSO, used as a control (minimum dilution 1:10000).

Conditioned media (CM) were prepared from WM35 and WM793 as described in the main section.

Primer sequences and references for RTqPCR

For human genes: ANGPTL4 (QT00003661) and KDR (QT00069818) are both from Qiagen. CCL2 (Fw: GCA AGT GTC CCA AAG AAG CTG, Rev: CTC CTT GGC CAC AAT GCT CT), CPT1A (Fw: CCG TAG CTG ACT CGG TAC TC, Rev: TCT AAG AGC TTC ATG GCT CAG), CSF2 (Fw: GCC CTC CAA CCC CGG AAA, Rev: CCC AGC AGT CAA AGG GGA TG), CXCL2 (Fw: TTC CAG CCC CAA CCA TGC, Rev: TGT GCG AGG AGG AGA GCT G), CXCL3 (Fw: AGC TGG AAA GGA CTT AAT GTG TT, Rev: CAG GAC TGA GCT ATG TTT GAT GA), CXCL8 (Fw: AAG GAA AAC TGG GTG CAG AG, Rev: ATT GCA TCT GGC AAC CCT AC), FABP4 (Fw: GAT GAT AAA CTG GTG GTG GAA TG, Rev: ATG CGA ACT TCA GTC CAG GT), ICAM1 (Fw: GCA ATG TGC AAG AAG ATA GCC AAC CA, Rev:

TGG AGT CCA GTA CAC GGT GAG GA), IL1A (Fw: ACC AAC GGG AAG GTT CTG AAG, Rev: CTA GGC TTG ATG ATT TCT TCC TCT G), IL1B (Fw: GCA CGA TGC ACC TGT ACG A, Rev: AGA ACA CCA CTT GTT GCT CCA TAT C), IL6 (Fw: GGA TTC AAT GAG GAG ACT TGC CTG, Rev: GAC TTT TGT ACT CAT CTG CAC AGC), PPARG (Fw: AAG GCC ATT TTC TCA AAC GA, Rev: AGG AGT GGG AGT GGT CTT CC), USP16 (Fw: TGG GCT CTG TCG CCG TGG ATT G, Rev: TGT CCG TTT CTT TCC CAT GTT GGCA C).

For murine genes: Angptl4 (QT00139748) and Kdr (QT00097020) are both from Qiagen. Fabp4 (Fw: CAT AAC CCT AGA TGG CGG GG, Rev: GCC TTT CAT AAC ACA TTC CAC CA), Icam1 (Fw: GCT CGG AGG ATC ACA AAC GA, Rev: CAG CCG AGG ACC ATA CAG C), Il1b (Fw: TGC CAC CTT TTG ACA GTG ATG AG, Rev: TCA TCT TTT GGG GTC CGT CAA C), Il6 (Fw: TAG TCC TTC CTA CCC CAA TTT CC, Rev: TTG GTC CTT AGC CAC TCC TTC), Rpl27 (Fw: CTG GCC TTG CGC TTC AA, Rev: TCA TGC CCA CAA GGT ACT CTG T).

CRISPR-Cas9 silencing

A375 cells were transfected with PPARG Human Gene Knockout Kit (OriGene, #KN201538) and puromycin-selected according to the manufacturer's instructions.

Interleukin-receptor membrane expression

A375 cells were treated for 96h in 6-well plate and collected in Cell Dissociation Buffer (Invitrogen). Cells were then incubated 35 min on ice with the following mAbs: IL-1R1, IL-1R2 and IL-6R (R&D systems). Data were acquired on the LSRII cytometer (BD Biosciences) and analyzed using FlowJo Software (Tree Star).

Proliferation assays

A375 cells were treated for 96h in 96-well plate. All results represent three-four independent experiments done in triplicate conditions. 10µM EdU was added for 16h at 37°C. Cell proliferation was tested with the Click-it EdU microplate assay kit (Life Technology), following the manufacturer's instructions. Fluorescence (excitation 548 nm, emission 605 nm) was analyzed with a TECAN spectrophotometer (Männedorf, Switzerland).

Cell cycle analysis

A375 cells were treated for 24h in 6-well plate and collected in Cell Dissociation Buffer. Cell cycle analysis was performed with the protocol detailed in (1). Briefly,

cells were incubated in a denaturing trypsin-based solution for 10 min at RT, then denaturing RNAse A-based solution for 10 min at RT. Finally, cell DNA was stained with Propidium Iodide solution. Data were acquired within 15 min on a LSRII cytometer (BD Biosciences) and analyzed using FlowJo Software (Tree Star).

Senescence assay

72h after treatment A375 cells were analyzed with the Senescence β-galactosidase staining kit (CST, #9860) following the manufacturer's instructions. β-galactosidase Positive control was assessed with β-galactosidase staining solution at pH 4.

Proteomic analysis supplementary material

Total protein concentrations of secretome samples were determined by densitometry on whole lanes of a Coomassie blue-stained gel, compared with an externally quantified complex standard (cell lysate).

Samples were lyophilized and resuspended in 8.0M Urea in 50mM triethylammoniumbicarbonate (TEAB, ph 8.0). 60ug total protein for each sample were used as input. Cysteines were reduced and alkylated in 5mM TCEP, 20mM Chloroacetamide for 45 min at 37°C in the dark. Samples were then diluted 1:1 (v:v) with TEAB 50mM and digested for 2h with 0.5ug modified Trypsin (Promega, sequencing grade) at 37°C. After a second 1:1 dilution with TEAB buffer, a second tryptic digestion was carried out by adding 2ug Trypsin and incubating for 5h at 37°C. Digests were acidified with 0.5% TFA, desalted on micro-C18 plates (Waters), dried and redissolved in 2% acetonitrile, 0.1% formic acid. For the in-depth analysis, digests were fractionated by strong cation exchange on SCX StageTips (Thermo Fisher Scientific) into 6 fractions as described (2).

Mass Spectrometry

For initial secretome mapping, peptide mixtures were analyzed by LC-MS/MS on a Thermo Scientific Q-Exactive Plus mass spectrometer interfaced to a Dionex RSLC 3000 nano-LC system. Peptide separation was performed on an EasySpray nanocolumn (50 cm x 75 um ID) at 250nL/min from 2% to 55% MeCN in 107 min. Data dependent acquisition was used to select precursors with 2^{+}-5^{+} charges for collision induced dissociation.

For the in-depth analysis of fractionated samples, a Fusion Orbitrap trihybrid instrument (ThermoFischer) was used, operated with a "universal" acquisition method

(3). Resolution of full scan spectra was at 70'000 resolution and 120'000 on the Q-exactive and Fusion MS, respectively. MS/MS spectra were acquired at high resolution in the Q-Exactive system (17'500) and at low resolution (ion trap) on the Fusion MS.

Data analysis

LC-MS/MS data were processed with MaxQuant (4,5) to obtain label free quantitation (LFQ) values (6). Samples for the three replicates were prepared and analyzed at several weeks/months distance and this results in major differences in analytical conditions. For this reason and to avoid generating false positives and data matching artefacts, the match between runs feature (6) was applied only within samples prepared together and not between biological replicates.

Unfractionated samples

MaxQuant output data were processed with (7). LFQ values were log2 transformed and filtered to keep only proteins identified by at least 2 MS/MS spectra and with valid values in at least two replicates per condition (1699 protein groups). After normalization by median subtraction, missing values were imputed with standard MaxQuant parameters (width 0.3, downshift 1.8). A paired T-test comparing samples in each biological replicate pair was performed, with cutoff at p-value 0.05. 140 protein groups passed the test. Imputed values were thereafter removed from the data and are present as NaN in PXD009108.

In-depth analysis of replicate 3

Data analysis was similar for fractionated samples, without T-test (no replicates available). LFQ values were log2 transformed and normalization was applied by median subtraction. Missing data were substituted by zeroes. A fold-change was calculated by subtracting the obtained log2 LFQ values.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (8) partner repository with the dataset identifier PXD009108.

Statistical analyses

IL-receptor and cell cycle quantifications were compared with Tukey two-way ANOVA. Proliferation was compared with Tukey one-way ANOVA. Each *in vitro* experiment was performed in duplicate or in triplicate condition.

All statistical analyses were performed using Prism GraphPad (v7, La Jolla, CA, USA).

References

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