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Glutamate accumulation in myelofibrosis microenvironment rewires mesenchymal stromal cells metabolic and epigenetic profiles

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Abstract

Background Myelofibrosis (MF) is a clonal myeloproliferative neoplasm characterized by bone marrow (BM) fibrosis, osteosclerosis, and a proinflammatory tumor microenvironment (TME). The interplay between malignant hematopoietic stem cells and mesenchymal stromal cells (MSCs) drives disease progression. Although glutamate has been implicated in various cancers, its contribution to MF pathogenesis remains unclear.

Methods High-performance liquid chromatography (HPLC) profiling was performed on sera from MF patients and healthy controls (HCs) to identify alterations in amino acid abundance. The proteomic profiles of MF- and HC-derived MSCs were assessed by mass spectrometry. Functional assays in glutamate- and fumarate-treated MSCs were used to investigate metabolic, epigenetic, and phenotypic changes by HPLC, real-time PCR, flow cytometry, immunofluorescence, and Azan-Mallory staining. The therapeutic potential of metabotropic glutamate receptor 5 (mGluR5) inhibition was evaluated using in vitro and in vivo models.

Results Glutamate emerged as the most enriched amino acid in MF sera. In MSCs, glutamate supplementation induced intracellular fumarate accumulation, mediated by increased expression of α -ketoglutarate dehydrogenase components and downregulation of fumarate hydratase activity. Fumarate treatment recapitulated an upregulation of oxidative phosphorylation, also enhancing mitochondrial ROS. Both fumarate and glutamate induced epigenetic remodeling, as shown by accumulation of 5-methylcytosine (5mC) and H3K36me2, which was also confirmed in

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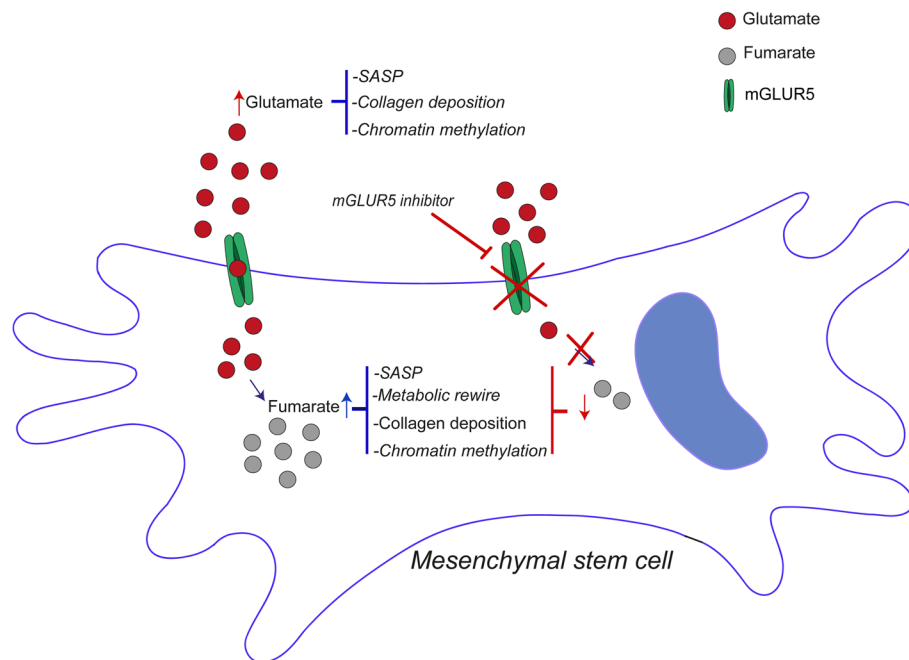
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primary MF-MSCs. Glutamate and fumarate induced a senescent phenotype in MSCs, characterized by increased β -galactosidase activity, oxidative stress and SASP-related genes induction. Both metabolites also promoted collagen deposition, supporting their involvement in fibrotic remodeling. Notably, selective inhibition of metabotropic glutamate receptor 5 (mGluR5) by UBP310 or UBP296 attenuated glutamate-driven senescence, reduced fumarate-associated epigenetic changes and limited collagen deposition in vitro and in vivo, supporting a central role for glutamate signaling in stromal dysfunction and fibrotic niche remodeling.

Conclusion This study identifies glutamate as a key metabolic and signaling factor in MF microenvironment. Glutamate rewires MSC metabolism via fumarate accumulation, drives epigenetic reprogramming, and induces senescence and fibrotic transformation. Targeting mGluR5 may therefore represent a promising therapeutic strategy to mitigate MSC dysfunction and bone marrow fibrosis in MF.

Keywords Myelofibrosis, Tumor microenvironment, Glutamate, Fumarate, Mesenchymal stromal cells

Graphical abstract



Introduction

Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) harboring clonal hematopoietic stem cells aberrant proliferation of the myeloid lineage. It might arise *de novo* as primary myelofibrosis rather than progressing from polycythemia vera or essential thrombocythemia [1]. Oncogenic driver mutations include tyrosine kinase Janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia virus proto-oncogene (MPL). In addition, 90% of MF patients harbor osteosclerosis and bone marrow (BM) fibrosis, eventually leading to extramedullary hematopoiesis [2]. To date, the usage of JAK inhibitors mitigate MF pathogenesis by controlling spleen size, alleviating symptoms, improving patients' quality of life, and prolonging overall survival [3]. However, JAK inhibitors lack the ability of resolving BM fibrosis.

MF pathogenesis relies on the interplay between hematopoietic stem cells and mesenchymal stromal

cells (MSCs). The latter are involved in fibrosis through myofibroblast differentiation, increased extracellular matrix production and aberrant degradation [4]. Interestingly, MF-MSCs exhibit unique molecular abnormalities distinct from other MPNs corroborating their participation in osteogenic deregulation independently from hematopoietic cells stimulation [5]. The complex interplay between cancer cells and MSCs involves several metabolic alterations, working in synergy to meet cancer cells metabolic demand and triggering MSC functional reprogramming. As a result, MSCs lack their hematopoiesis support ability, secreting non-collagenous extracellular matrix and several pro-inflammatory cytokines as part of senescence associated secretory phenotype (SASP) [6]. In this context, senescent MSCs boost glutaminolysis by upregulating glutaminase-1 (GLS1), in charge for glutamine conversion to glutamate. Conversely, GLS1 inhibition rescues age-related

dysfunction by eliminating senescent cells, while increased glutamate dehydrogenase (GDH) activity, allosterically regulated by glutamate, has been reported in aged mice and cellular senescence models [7, 8].

Glutamate is used as a primary source of energy in proliferating cells and can be further metabolized to α -ketoglutarate in mitochondrial matrix [9]. Several evidences highlight the role of glutamate as a functional signaling metabolite involved in transformation and progression of several tumors [10–12]. In this context, the metabotropic glutamate receptor 5 (mGluR5) has been implicated in melanoma development, oral squamous cell carcinoma and laryngeal cancers [13–15], corroborating the role of glutamate as oncogenic factor.

Table 1 Patients' cohort clinical features

	Baseline data (20 patients)
Age (years)	
Median [range]	68 [41–80]
Sex, n (%)	
Female	11 (35)
Male	20 (65)
Type of MF, n (%)	
Primary MF	14 (70)
PPV-MF	3 (15)
PET-MF	3 (15)
Constitutional symptoms, n (%)	
No	3 (10)
Yes	28 (90)
Splenomegaly, n (%)	
No	3 (10)
Yes	28 (90)
Peripheral blast count > 1%, n (%)	
No	29 (93)
Yes	2 (7)
DIPSS-MYSEC-PM, n (%)	
Intermediate-1	18 (58)
Intermediate-2	8 (26)
High	5 (16)
Fibrosis grade, n (%)	
1	17 (55)
2	8 (25)
3	6 (20)
Driver mutations, n (%)	
JAK2	21 (68)
MPL	1 (3)
CALR	5 (16)
Triple negative	4 (13)
Blood count, median (range)	
Hb, g/dL	9.3 [6.3–15.5]
WBC, 10 ³ /mmc	9.3 [1.4–149]
PLT, 10 ³ /mmc	136 [56–1014]

IPSS/MYSEC-PM International Prognostic Scoring System/MYSEC Prognostic Model Risk Calculator, Hb Hemoglobin, WBC White blood cells, PLT Platelets

Here, we aim to identify glutamate role in MF tumor microenvironment (TME), eventually defining its impact on MSC compartment.

Materials and methods

Patients and healthy donors' samples

After written informed consent (Azienda Ospedaliero-Universitaria Policlinico “G.Rodolico-San Marco”, n. 54/2022/PO), sera were collected from patients with MF at diagnosis ($n = 20$) and age-matched healthy controls (HC; $n = 13$). Patients' clinical data have been reported in Table 1. After MF diagnosis, patients were stratified by evaluation of their age, hemoglobin, leukocyte count, circulating blasts and constitutional symptoms (low, intermediate-1, intermediate-2, and high), based on the Revised International Prognostic Scoring System for Myelofibrosis [16].

HPLC analysis

Metabolic profiling was conducted following the deproteinization of serum samples from MF patients and age matched HC, as well as cell extracts, using a previously established protocol [17]. Low molecular weight metabolites associated with energy metabolism, amino acid pathways, oxidative and nitrosative stress, antioxidant status, and key compounds such as high-energy phosphates, oxidized and reduced forms of nicotinamide coenzymes, reduced glutathione were simultaneously separated using a modified version of an established ion-pairing HPLC method [18].

The chromatographic analyses were performed using a Surveyor HPLC system coupled with a PDA diode-array detector (Thermo Fisher Scientific Italia, Rodano, Milan, Italy) featuring a 5-cm flow cell and configured to detect absorbance between 200 and 400 nm. Separation was achieved on a Hypersil C18 column (250 × 4.6 mm, 5 μ m particle size) with a matching guard column, also from Thermo Fisher. Data collection and analysis were carried out using the ChromQuest® software provided by the manufacturer. Identification and quantification of metabolites in cell samples were based on retention times, UV spectra, and peak areas, with most compounds measured at 260 nm, except for GSH at 206 nm. All metabolite concentrations were normalized to cell number and reported as nmol per 10⁶ cells.

Cell cultures and treatments

Healthy immortalized mesenchymal stromal HS-5 cell line was obtained from ATCC (CRL-3611, Virginia, USA). MSCs from HC and MF patients were obtained after density gradient centrifugation on Ficoll of BM samples [17]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Milan, Italy) supplemented with 10% heat-inactivated FBS, 100 U/ml

penicillin, 100 mg/ml streptomycin and 1% l-glutamine. Cultures were maintained at 37 °C and 5% CO₂. Dimethyl fumarate (#242926), and Sodium L-glutamate monohydrate (#1.06445) were purchased from Sigma-Aldrich (Milan, Italy). UBP310 (#HY-107602) and UBP296 (#HY-107605) were obtained from MedChem (Sollentuna, Sweden).

Proteomic analysis

Cells were sonicated on ice in RIPA buffer using a micro-tip probe sonicator. Pre-chilled acetone was used to precipitate the protein content. After centrifugation, the pellet was resuspended in urea 8 M; the total amount of protein was quantified using the BCA assay (Invitrogen, Monza, Italy). Reduction and alkylation of the protein sulfhydryl groups were carried out using Dithiothreitol and Iodoacetamide, respectively. The diluted protein samples were treated with trypsin at 37 °C for 16 h.

Hydrolytic peptides were separated and detected using a UHPLC system (ThermoFisher Scientific Dionex UltiMate 3000 RSLCnano, Sunnyvale, CA, USA) coupled with an Orbitrap Fusion Tribrid mass spectrometer (Q-OT-qIT) (ThermoFisher Scientific, Bremen, Germany). The samples were diluted with a 5% aqueous formic acid (FA) solution. Peptides were eluted on a Pep-Map® RSLC C18 column (EASYSpray, 75 µm × 50 cm, 2 µm, 100 Å) and separated by elution at a flow rate of 0.250 µL/min, at 40 °C, with a linear gradient of solvent B (CH₃CN + 0.1% FA) in solvent A (H₂O + 0.1% FA). The eluted peptides were ionized by nanospray (Easy-spray ion source, Thermo Scientific) using a capillary temperature and voltage set to 275 °C and 2.0 kV, respectively. Peptide precursor scans in the m/z range of 400–1600 were performed with a resolution of 120,000 (200 m/z) with an AGC target for Orbitrap detection of 4.0 × 10⁵ and a maximum injection time of 50 ms. MS/MS spectra were acquired using a normalized collision energy (HCD) of 35. The dynamic exclusion duration was set to 60 s with a tolerance of 10 ppm around the selected precursor and its isotopes. The AGC target values and

maximum injection time (ms) for MS/MS spectra were 1.0 × 10⁴ and 100, respectively. MS data acquisition was performed using Xcalibur software v. 3.0.63 (Thermo Fisher Scientific).

Mass spectra were analyzed using MaxQuant software (version 2.5.1.0). Andromeda search engine against Homo Sapiens Uniprot sequence database was used to analyze the spectra. Quantification was performed using the integrated Label-Free Quantification (LFQ). The false discovery rate was set to 1% at the peptide level and 1% at the protein level, with a minimum peptide length of seven amino acids required. Statistical analyses were performed using Perseus software (version 2.1.3). Only proteins that were present and quantified in at least two out of three technical replicates were considered positively identified.

Real time PCR

RNA extraction was performed by TRIzol (#15596026; ThermoFisher Scientific, Milan, Italy) as previously reported [19] and High-Capacity cDNA Reverse Transcription Kit (#4368814; ThermoFisher Scientific, Milan, Italy) was used to obtain cDNA according to manufacturer's instruction. Real Time PCR (RT-PCR) was performed by using SYBR™ Green PCR Master Mix (#4309155; ThermoFisher Scientific, Milan, Italy). A list of the primers used is reported in Table 2.

Fumarate determination

Intracellular and circulating fumarate was measured by using fumarate Assay Kit (ab102516; Abcam, UK). For intracellular fumarate quantification, cells were harvested, resuspended in 0.1mL of fumarate assay buffer, and homogenized by using Dounce Homogenizer (ab286839; Abcam, UK). The lysate was transferred into 1.5mL microcentrifuge tube and centrifuged for 10 min at 13000G at 4 °C. According to manufacturer instruction, 50mL of the lysate supernatant or patient's sera were used to perform the assay. Absorbance was measured at 450 nm by using BioTek Synergy H1 Multimode Reader (Agilent, CA, USA).

Table 2 Primer's list

Primer	Forward	Reverse
Succinate dehydrogenase Subunit A	CAAACAGGAACCCGAGGTTT	CAGCTTGGTAACACATGCTGTAT
Succinate dehydrogenase Subunit B	ACAGCTCCCGTATCAAGAAA	GCATGATCTTCGGAAGGTCAA
Succinate dehydrogenase Subunit D	ATTCTTCAGGACCGACCTATCC	CAGCCTTGAGCCAGAATG
Fumarate Hydratase	GGAGGTGTGACAGAACGCAT	CATCTGCTGCCTTCATTATTGC
CoxII	TGCCCTTTCTTAACACTCACAA	CGCCGTAGTCGGTGTACTCG
CytB	TCCTCCCGTGAGGCCAAATATCAT	AAAGAATCGTGTGAGGGTGGGACT
ND4	ACAAGCTCCATCTGCCTACGACAA	TTATGAGAATGACTGCGCCGGTGA
IL-6	GAAAGCAGCAAAGAGGCACT	TTTACCAGGCAAGTCTCCT
IFN-γ	GAGTGTGGAGACCATCAAGGAAG	TGCTTTGCGTTGGACATTCAAGTC
TGF-β	TACCTGAACCGTGTTGCTCTC	GTTGCTGAGGTATCGCCAGGAA
B2M	AGCAGCATCATGGAGGTTTG	AGCCCTCTAGAGCTACCTG

Fumarate hydratase (FH) activity assay

FH activity was assessed by using Fumarase Activity Assay kit (ab196992, Abcam, UK) according to manufacturer's instruction. Briefly, mitochondria were firstly obtained by using Mitochondria Isolation Kit (ab110170, Abcam, UK). Therefore, mitochondrial proteins were extracted and placed on a plate where the colorimetric reaction was performed according to manufacturer's instruction. Absorbance was measured at 450 nm by using BioTek Synergy H1 Multimode Reader (Agilent, CA, USA).

Single cell RNA seq analysis

Single cell RNA sequencing data, obtained from BM samples of MF patients and HC, were retrieved from the GSE241825 series, publicly available on GEO datasets [20].

R package Seurat [21] was used for data managing and sample stratification, while MSCs were selected based on manual cell annotation. To investigate gene expression, single cell counts were log normalized.

Within Seurat, FindMarkers function was used to deepen the analysis on the dataset, searching for differentially expressed genes, including both gene expression and detection rate.

Flow cytometry analysis

Flow cytometry analysis was performed by using MACSQuant Analyzer 10 (Miltenyi Biotec, Bologna, Italy). Mitochondrial ROS were detected using MitoSOX™ Mitochondrial Superoxide Indicators (M36008, ThermoFisher Scientific, Milan) according to manufacturer's instruction. 5-methyl cytosine (5mC) assessment was performed as previously described [22]. 5mC was stained using 5-Methylcytosine Recombinant Rabbit Monoclonal Antibody (RM231, ThermoFisher Scientific, Milan, Italy). Donkey anti-rabbit IgG antibody (FITC) (GTX26798, CA, USA) was used as a secondary antibody. Samples incubated only with the anti-rabbit IgG antibody were used as negative controls.

For ROS detection, we used 2',7'-Dichlorodihydrofluorescein acetate (#287810, Sigma-Aldrich; Milan, Italy) to a final concentration of 5µM. Fluorescence intensity was measured according to the fluorescence detection conditions of FITC.

Immunofluorescence analysis

To assess 5mC levels, cells were fixed in paraformaldehyde and immunofluorescence assay was performed as already described [23]. 5mC was stained using 5-Methylcytosine Recombinant Rabbit Monoclonal Antibody (RM231, ThermoFisher Scientific, Milan, Italy) and Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (A-21206, ThermoFisher Scientific, Milan, Italy). For H3K36me2

detection, an anti-Histone H3 (di methyl K36) antibody was purchased from abcam (ab176921, Cambridge, UK). After incubation with anti-rabbit Alexa Fluor 647 (A32849, ThermoFisher Scientific), phalloidin staining was performed using Phalloidin-iFluor 488 (ab176753, Abcam) at dilution 1:500 and incubated for 1 h at room temperature.

Images were acquired by using a Zeiss Axio Imager Z1 Microscope with Apotome 2 system (Zeiss, Milan, Italy).

β-Galactosidase activity

β-Galactosidase activity was measured as previously reported [17]. Briefly, cells were lysed by using 500µL of M-PER mammalian protein extraction reagent (#78501; ThermoFisher Scientific, Milan, Italy) and incubated for 5 min with gentle shaking at room temperature. In a microplate, 50µL of cell lysate were mixed in equal proportion to β-Galactosidase assay reagent (#75705; ThermoFisher Scientific, Milan, Italy). Absorbance at 405 nm was measure upon 30 min incubation at 37 °C by using BioTek Synergy H1 Multimode Reader (Agilent, CA, USA).

γ-GCS activity assay

The enzymatic activity of γ-GCS was determined according to the method of Seeling and Meister [24] partially modified by Ray et al. [25].

Briefly, cells were suspended in 0.1 M Tris-HCl buffer (pH=8.2) containing 20 mM MgCl₂, 50 mM KCl and 2 mM Na₂EDTA, and lysated by sonication. The lysate was centrifuged and supernatant was used for γ-GCS activity assay by measuring the change in absorbance at λ = 340 nm. The enzymatic reaction was initiated by addition of 10 mM L-α-aminobutyrate.

The change in absorbance at λ = 340 nm was monitored spectrophotometrically (Hitachi UV 2000) and the rate of NADH oxidation was used to calculate the rate of enzymatic activity using 6.22×10^3 as value of the molar extinction coefficient.

Mallory's trichomic staining

Mallory's trichomic staining was performed as previously described [26]. Briefly, cells were firstly stained in 1% acid fuchsin solution (Sigma-Aldrich, Milan, Italy) and subsequently counterstained with a 0.5% aniline blue solution (Sigma-Aldrich, Milan, Italy), 2% orange G (Sigma-Aldrich, Milan, Italy) and 2% oxalic acid (Sigma-Aldrich, Milan, Italy) in distilled water. Mallory was imaged by using NEXCOPE NE910 Upright biological microscope (Bovenden, Germany), while Mallory quantification was performed by normalizing the blue area (collagen) on the red area (cell cytoplasm) as previously described [27].

TPO^{high} zebrafish larvae

Two-day-post-fertilization (dpf) Zebrafish larvae (*Danio rerio*) were treated for 3 days with 6 µg/mL of the thrombopoietin receptor agonist Eltrombopag Olamine (Selleckchem, Cologne, Germany) ± 100 µM UBP310. For qPCR analysis, the embryos were resuspended in TRIzol (ThermoFisher Scientific, Milan, Italy) and processed as already described [28]. For immunofluorescence analysis, the embryos were fixed in 4% PFA for 2 h. Permeabilization was performed by 15 min incubation in Proteinase K. Primary antibody against col1a1 1:500 (sc-59772, Cruz Biotechnology) and goat-anti-mouse Alexa Fluor 488 1:500 (A11001, ThermoFisher Scientific) were used for the staining. Images were acquired by Zeiss Axio Zoom V16 (Oberkochen, Germany).

Statistical analysis

All statistical analyses were conducted using GraphPad Prism (version 5.00 for Mac; GraphPad Software, San Diego, CA, USA). Data are presented as mean ± standard deviation (SD). Statistical comparisons were made using Student's t-test or analysis of variance (ANOVA), as appropriate. A p-value of less than 0.05 was considered statistically significant when comparing experimental and control groups. Bioinformatic analysis was carried out using Rstudio on Microsoft Windows (Posit team (2025). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL <http://www.posit.co/>.) in its 2024.12.1 + 563 version.

Results

Glutamate levels are increased in MF sera

Myelofibrosis TME acquires a unique metabolic landscape where tumor-surrounding cells can support or restrict cancer growth through competition for nutrients or metabolic symbiosis [29]. In this study, we used a metabolomics approach to evaluate the concentration of circulating metabolites in peripheral blood (PB) sera from MF patients. Compared to HC, multiple metabolites were differently regulated in MF patients' sera (Fig. 1A). Enrichment analysis on our dataset unveiled ascorbic acid as the most downregulated component, while glutamate was the prominent upregulated metabolite in MF patients (Fig. 1B), eventually showing a concentration of 150 µM (Fig. 1C). These results prompted us to assess the impact of this metabolite on the stromal cell compartment, with particular emphasis on MSCs, a pivotal component of the tumor microenvironment that critically contributes to the establishment and maintenance of the pathological niche in myelofibrosis.

Glutamate overload triggers intracellular fumarate accumulation in MSCs

To test the impact of glutamate overload on the mesenchymal compartment, we supplemented HS-5 cells with monosodium glutamate using the same concentration detected in MF sera (150 µM). To characterize MSC phenotype upon exposure to glutamate, we assessed cellular proteomic profile by LC-MS-based analysis. Interestingly, up- and downregulation of several proteins was observed (Fig. 2A). Among them ATP citrate lyase (ACLY), Nicotinamide phosphoribosyltransferase (NAMPT), Carbonyl reductase 1 (CBR1), Phenylalanyl-tRNA synthetase subunit beta (FARSB), Adenylosuccinate synthetase (ADSS), Phosphoglycerate dehydrogenase (PHGDH), Dihydroliipoamide S-succinyltransferase (DLST), Fumarylacetoacetate hydrolase (FAH), Phosphoglucomutase 2 (PGM2) have been reported to have a role in cell metabolism [30–37]. Furthermore, it is known that glutamate enters the tricarboxylic acid (TCA) cycle via α-ketoglutarate (α-KG) and semialdehyde metabolism [38]. Consistently, proteomic analysis revealed an increased amount of DLST, the E2 transferase of the α-KG dehydrogenase complex, converting α-KG to succinyl-CoA in the TCA cycle [36]. Corroborating this, proteomic analysis on MF-MSCs confirmed an upregulation of DLST along with Succinate Dehydrogenase subunits A (SDH-A) compared to HC stromal cells (Supplementary Fig. 1). Therefore, we sought to perform a RT-PCR assay in MSCs exposed to glutamate for 24 h evaluating TCA-related genes expression. Our data show an upregulation of SDH-A, SDH-B, but not of SDH-D, while fumarate hydratase (FH) was downregulated (Fig. 2B). Within TCA cycle, the latter is in charge of converting fumarate into malate, therefore regulating fumarate homeostasis. Considering this, we next investigated the intracellular fumarate level upon glutamate supplementation. Interestingly, our analysis detected an increased intracellular fumarate concentration after 24 h (Fig. 2C). Therefore, we decided to assess FH activity, detecting a significant decrease only after 48 h glutamate exposure (Fig. 2D-E; Supplementary Fig. 2), while any change in FH protein level was detected (Fig. 2F).

In line with this, single cell RNA seq analysis of MSCs isolated from MF patients showed a significant FH downregulation (Fig. 2G). Concomitantly, the downstream malate dehydrogenases (MDH1 and MDH2) were also reduced, indicating an attenuated capacity for fumarate turnover within the TCA cycle. Interestingly, fumarate accumulation was confirmed in PB MF sera compared to HC (Fig. 2H). Overall, our data show that glutamate overload in MF-TME hampers TCA cycle progression, eventually triggering fumarate accumulation.

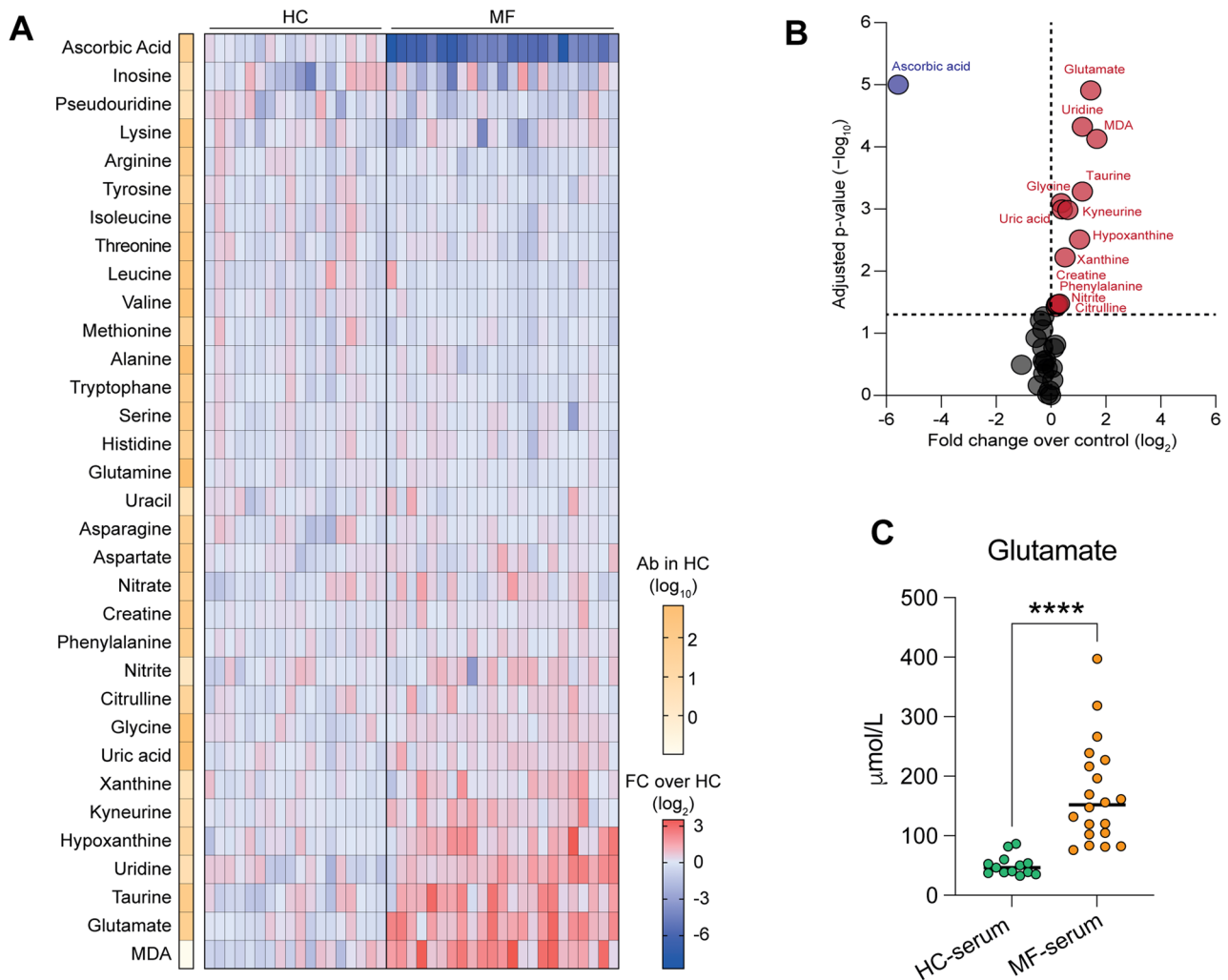


Fig. 1 Glutamate overload in MF sera. **A** Heatmap displaying the metabolic profile obtained from HPLC analysis of MF sera ($n=20$) and age matched healthy donors ($n=13$). **B** Enrichment analysis showing glutamate as the most upregulated metabolite in MF sera. **C** Glutamate quantification in MF and healthy donors' sera. Data are reported as mean $\pm$ SD (**** $P \leq 0.0001$)

Fumarate accumulation rewires MSC metabolic phenotype

As TCA cycle intermediate, fumarate is involved in the oxidation of carbohydrates, amino acids and fatty acids [39]. Therefore, we decided to investigate the impact of intracellular fumarate accumulation on MSCs energetic profile. For this purpose, MSCs were treated using a sub-lethal dose of dimethyl fumarate ($38\mu\text{M}$) for 24- and 48 h (Fig. 3A; Supplementary Fig. 3). Our data showed a significant decrease in AMP concentration following 48 h treatment, while any change in ADP profile was detected (Fig. 3B-C). Furthermore, following 24 h fumarate supplementation, we detected an increased concentration of ATP, leading to an enhancement of the ATP/ADP ratio, eventually suggesting a metabolic switch towards OXPHOS (Fig. 3D-E). Corroborating this, we found a significant increment of NAD^+ 24 h after fumarate supplementation, which was not detected after 48 h (Fig. 3F). NADH, on the other hand, was increased following

24 h fumarate supplementation, while it decreased after 48 h (Fig. 3G). As a result, fumarate treated cells showed a higher NAD^+/NADH ratio than untreated cells at 48 h (Fig. 3H). Of note, the level of electron charge potential (ECP) was consistent between the two experimental conditions (Fig. 3I).

To validate the oxidative metabolic profile of fumarate-supplemented MSCs, we investigated the expression of different OXPHOS-related genes. Interestingly, Cyclooxygenase-2 (COX-2), Cytochrome B, and NADH-ubiquinone oxidoreductase chain 4 (ND4) expression was increased 24 h post fumarate supplementation (Fig. 3J-L). Furthermore, flow cytometry analysis showed an increased amount of mitochondrial ROS after 48 h (Fig. 3M-N). Therefore, these data show that intracellular fumarate accumulation remodels stromal cell metabolism towards a more oxidative phenotype.

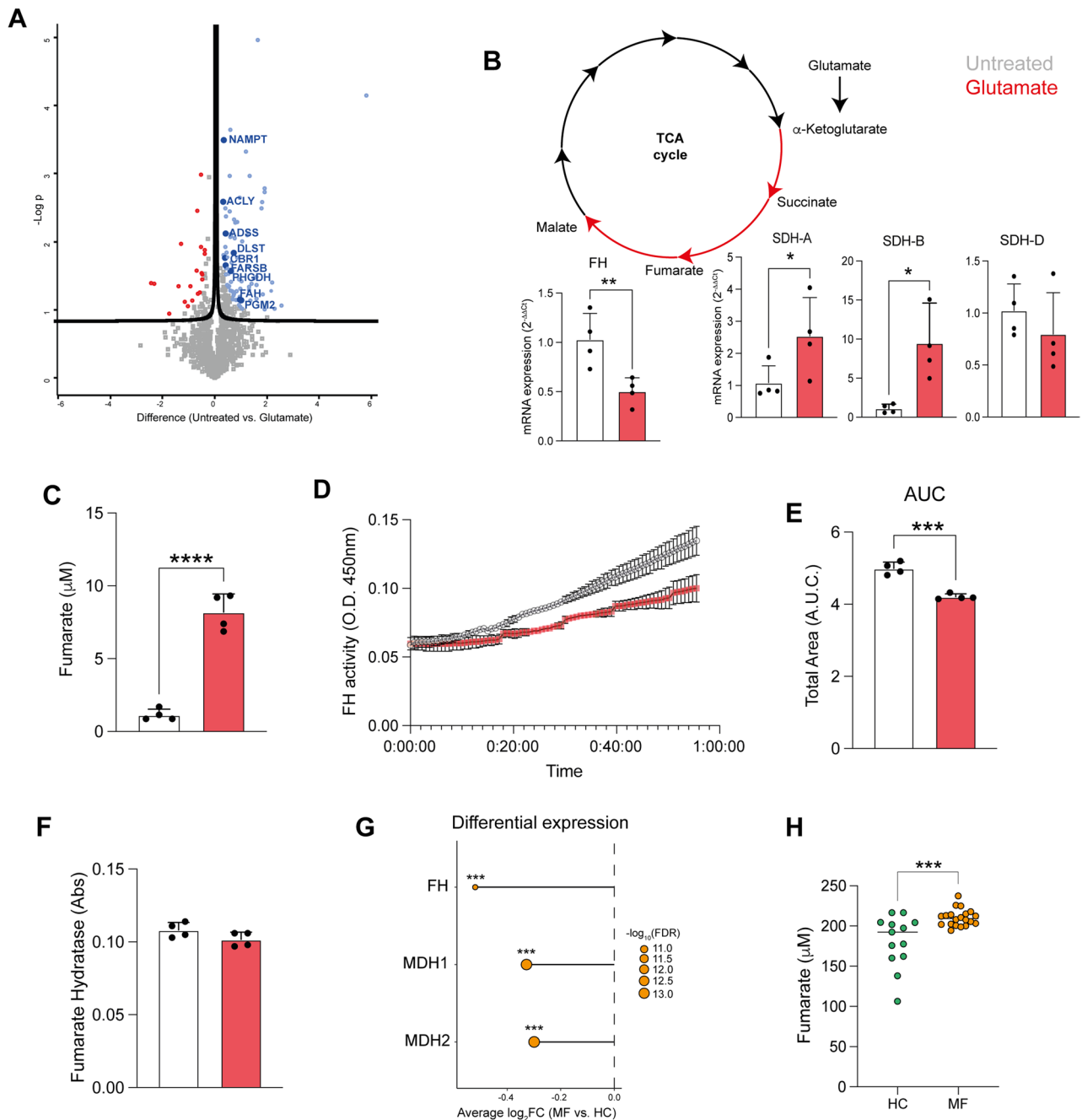


Fig. 2 Glutamate upregulation leads to intracellular fumarate accumulation. **A** Volcano plot analysis obtained on MSCs upon 72 h glutamate supplementation. **B** Quantitative PCR detecting the expression profile of SDH-A, SDH-B, SDH-D, and FH in MSCs following 24 h glutamate overload. **C** Intracellular fumarate quantification following 24 h glutamate supplementation in HS-5 cells. **D** FH activity assay performed in untreated versus glutamate supplemented healthy MSCs for 48 h. **E** Quantification of FH activity as in **(D)**. **F** FH protein quantification in glutamate-treated and untreated MSCs. **G** Lollipop plot showing FH, MDH1, and MDH2 differential expression between MF-MSCs and HC-MSCs, obtained by Seurat R package's FindMarkers function. **H** Fumarate quantification on plasma fraction obtained from peripheral blood of MF patients and age-matched healthy donors. Data are representative of four different replicates and are reported as mean $\pm$ SD (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$)

Glutamate and fumarate MSC epigenetic profile remodeling

Besides his role as a metabolite, fumarate serves as inhibitor for ten eleven translocation enzymes (TET) and histone lysine demethylases 2 A (KDM2A), in

charge for 5mC and H3K36me2 demethylation, respectively [40]. Given the role of glutamate in mediating intracellular fumarate accumulation, we sought to assess the epigenetic profile of MSCs exposed to glutamate and fumarate. Flow cytometry analysis showed

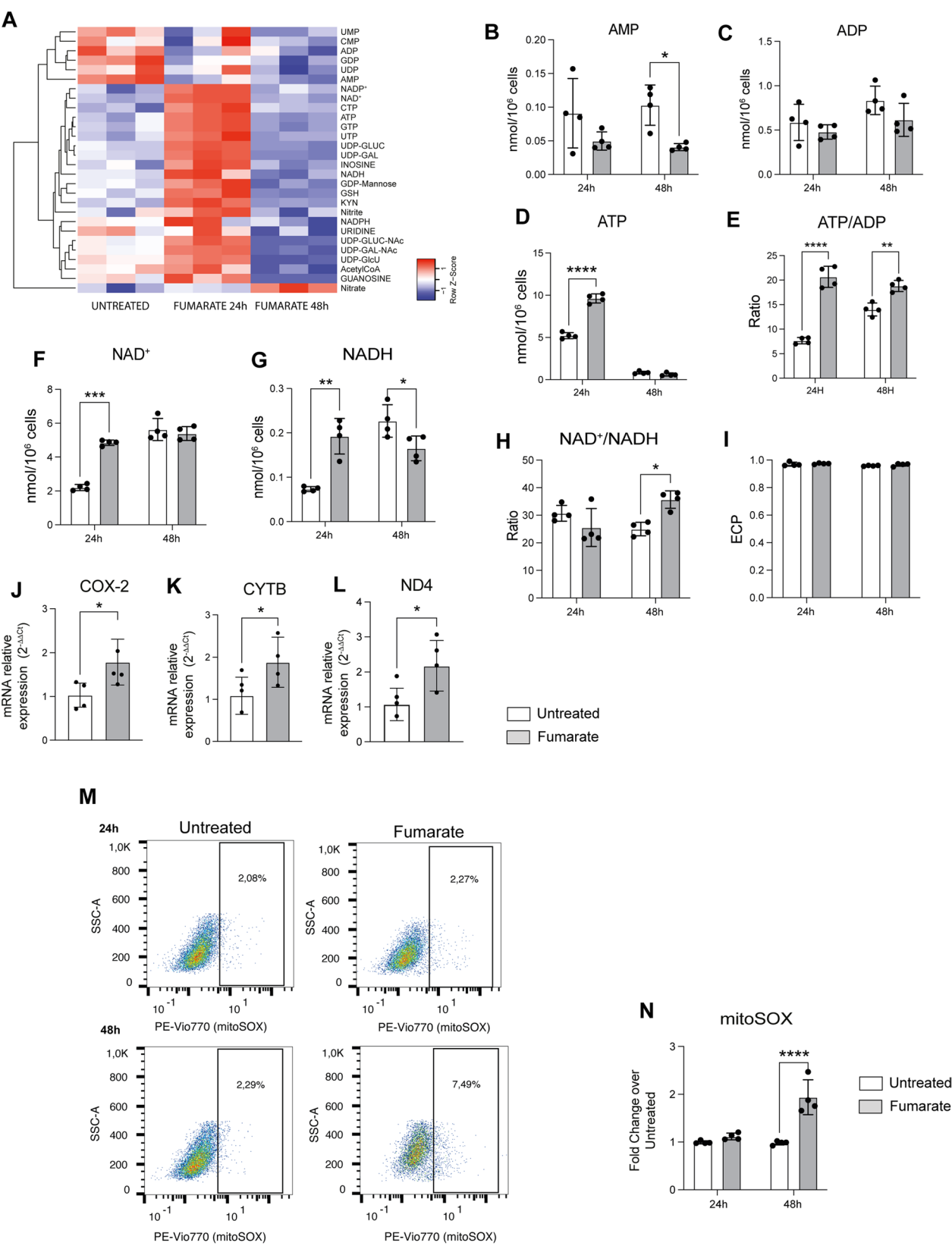


Fig. 3 (See legend on next page.)

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Fig. 3 Intracellular fumarate overload rewires MSC metabolic profile. **A** Heatmap showing the metabolic profile of HS-5 supplemented with fumarate for 24 and 48 h. Histograms representing AMP (**B**), ADP (**C**), ATP (**D**), ATP/ADP (**E**), NAD⁺ (**F**), NADH (**G**), NAD⁺/NADH (**H**), and ECP (**I**) profiles following 24- and 48 h fumarate supplementation. Quantitative PCR analysis on Cox II (**J**), Cyt B (**K**), and ND4 (**L**) genes. **M** Representative gating strategy for mitochondrial ROS investigation in MSCs supplemented with fumarate for 24- and 48 h. **N** Mitochondrial ROS quantification as in (**M**). Data are representative of four different replicates and are reported as mean $\pm$ SD (* $P \leq 0,05$; ** $P \leq 0,01$; *** $P \leq 0,001$; **** $P \leq 0,0001$)

an enhancement of 5mC level following both glutamate and fumarate 72 h exposure (Fig. 4A). Of note, immunofluorescence analysis confirmed 5mC accumulation also in MF-MSCs (Fig. 4B). Moreover, immunofluorescence analysis showed a significant H3K36me2 expression after 72 h glutamate and fumarate exposure in HS-5 cells and HC-MSCs (Fig. 4C-D). Overall, these data show that glutamate and fumarate accumulation affect MSC epigenetic profile by chromatin methylation enrichment.

Glutamate and fumarate overload drive MSC senescence

MF-MSCs are characterized by a senescent phenotype, enforcing TME inflammatory cascade through SASP [41]. Therefore, we assessed β -Galactosidase (β -Gal) activity in MSCs exposed to fumarate or glutamate overload, detecting a significant increase compared to untreated cells (Fig. 5A). Quantitative PCR also detected an enhanced expression of SASP-associated genes IL-6, IFN- γ , and TGF- β (Fig. 5B-D). Since mGluR5 inhibition has been reported to harbor a potential therapeutic effect in different cancer models [13–15], we sought to assess its potential in MF context, by using UBP310 and UBP296, two selective mGluR5 inhibitors. UBP310 was supplemented to an already established concentration of 100 μ M [42], while UBP296 was used to a sublethal dose of 70 μ M (Supplementary Fig. 4A). Interestingly, mGluR5 inhibition significantly reduced glutamate-mediated β -Gal activity (Fig. 5E, Supplementary Fig. 4B) and SASP-associated genes expression (Fig. 5F-H). Besides, mGluR5 inhibition also attenuated fumarate-mediated H3K36me2 accumulation (Fig. 5I, Supplementary Fig. 4C).

Within TME, MF-MSCs are exposed to a massive oxidative stress, eventually contributing to senescence onset [43]. Interestingly, we detected an increased concentration of ROS in cells exposed to glutamate and fumarate (Fig. 5J). Furthermore, a decreased amount of GSH was observed after 72 h (Fig. 5K). In this context, fumarate accumulation also induced a significant reduction of γ -Glutamylcysteine synthetase (γ -GCS) activity (Fig. 5L). Interestingly, glutamate-induced GSH reduction was mitigated by supplementation of UBP310 (Fig. 5M). These results show that both glutamate and fumarate accumulation activate a senescent phenotype in stromal cells, which might be recovered by mGluR5 inhibition.

Glutamate and fumarate accumulation are involved in fibrotic transformation of MF-TME

Within MF-TME, MSCs lack their hematopoietic support ability, eventually releasing collagen and fibronectin contributing to fibrosis onset [1]. We therefore sought to test MSC hematopoietic support in this context. Any significant change in CD34⁺ cell proliferation and colony forming unit was detected (Supplementary Fig. 5). Next, we evaluated glutamate and fumarate involvement in BM fibrosis onset, by performing Azan-Mallory staining. Interestingly, we observed an increase in collagen deposition after 72 h glutamate and fumarate supplementation in both HS-5 cells and HC-MSCs (Fig. 6A, Supplementary Fig. 6A). Therefore, we investigated the effectiveness of the mGluR5 inhibitors in these contexts, which were able to restrain glutamate-mediated collagen accumulation (Fig. 6B, Supplementary Fig. 6B-C).

Since glutamate is the most upregulated amino acid in MF sera, we sought to assess collagen accumulation in MSCs exposed to sera from MF patients and HC. Notably, MSCs exposed to MF sera exhibited increased collagen levels compared to those supplemented with HC sera (Fig. 6C). Interestingly, the addition of mGluR5 inhibitor UBP310 significantly reduced collagen accumulation induced by MF sera (Fig. 6C).

Overall, these data show that glutamate and fumarate accumulation contribute to fibrotic transformation of MF-TME.

mGluR5 inhibition prevents TPO-RA induced collagen accumulation in an in vivo Zebrafish model

To dissect the efficacy of mGluR5 inhibition in vivo, we induced fibrosis in 2 dpf Zebrafish larvae by 72 h administration of TPO-RA [26]. As expected, we observed an increased expression of MF hallmarks *mpl*, *cd41*, and *col1a1* upon TPO-RA supplementation (Fig. 7A-C), which was restrained by UBP310. Mirroring gene-expression data, *Col1a1* increase was also detected by immunofluorescence analysis, and eventually scavenged by mGluR5 inhibition (Fig. 7D). Therefore, this strategy attenuates megakaryocyte- and fibrosis-related gene expression along with collagen deposition in vivo.

Discussion

MPN driver mutations are typically acquired early but MF usually occurs in the later decades of life [44, 45]. This long latency suggests that the cellular, biochemical and metabolic composition of the BM microenvironment

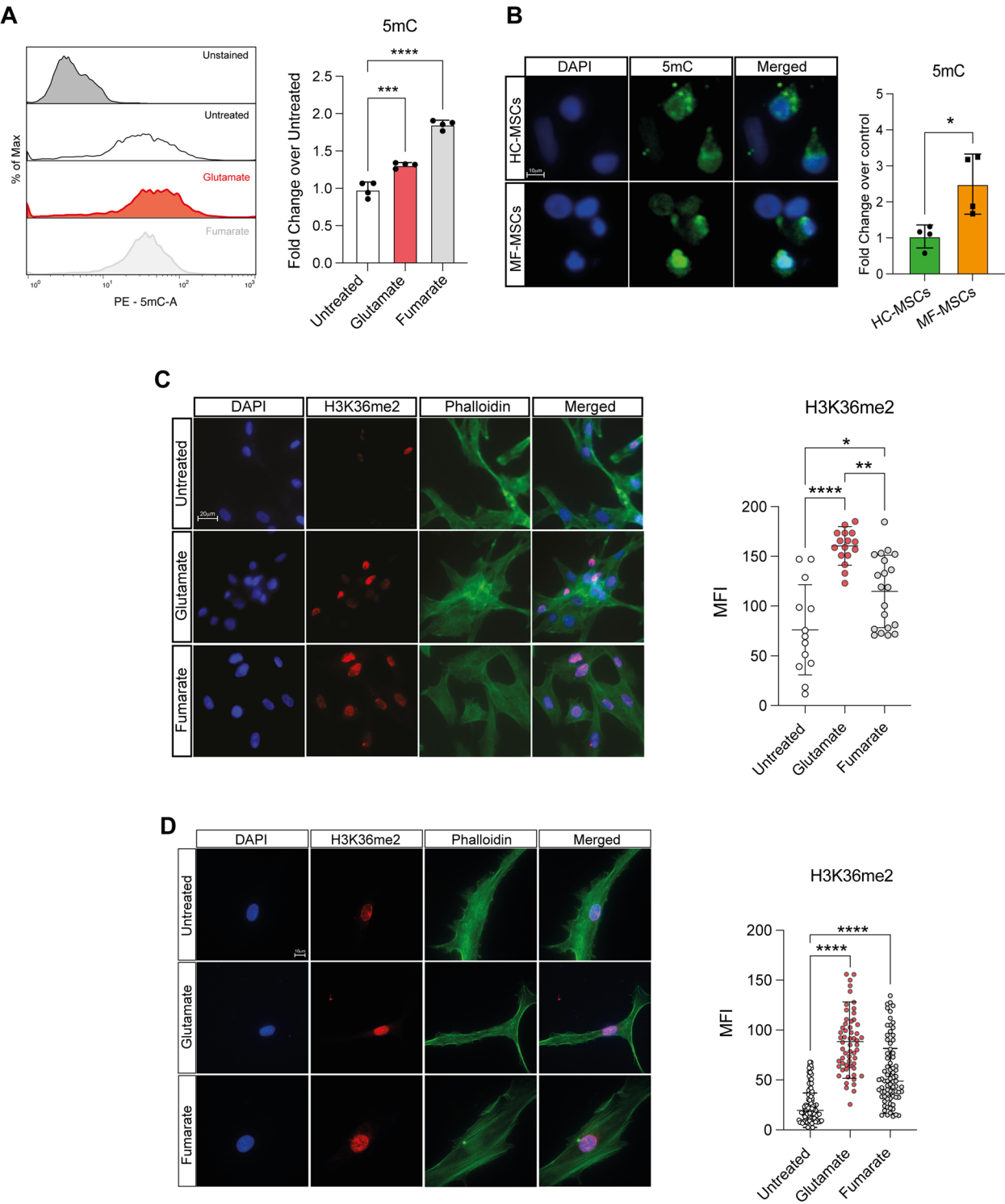


Fig. 4 Glutamate and fumarate reshape MSC epigenetic profile. **A** Flow cytometry analysis assessing 5mC in HS-5 cells supplemented with glutamate and fumarate. **B** Immunocytochemistry analysis of 5mC in HC versus MF MSCs. Immunofluorescence analysis assessing H3K36me2 in **C** HS-5 cells and **D** HC-MSCs after 72 h glutamate and fumarate supplementation. Data are representative of four different replicates and are reported as mean $\pm$ SD ($*P \leq 0,05$; $**P \leq 0,01$; $***P \leq 0,001$ $****P \leq 0,0001$)

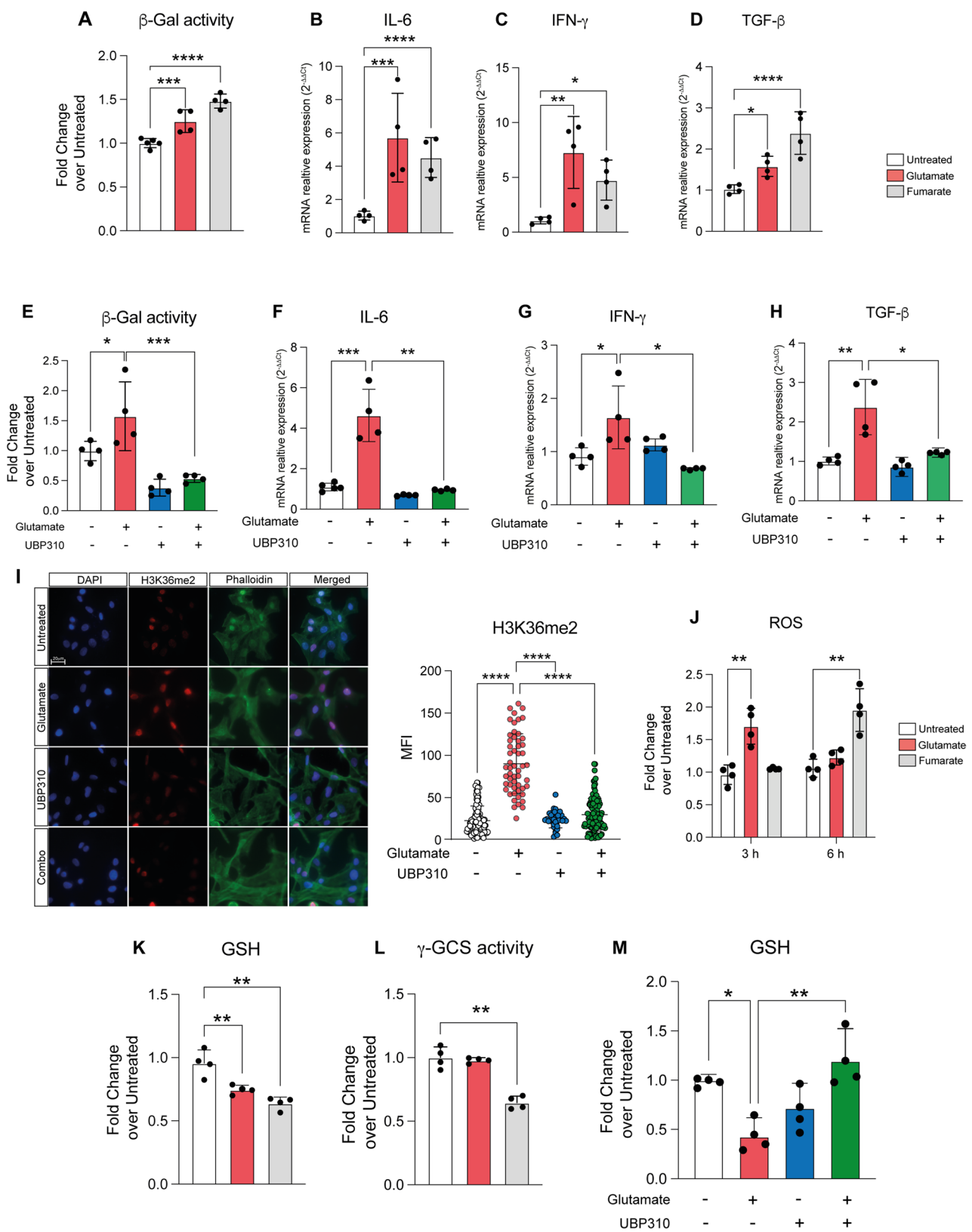


Fig. 5 (See legend on next page.)

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Fig. 5 Glutamate and fumarate trigger MSC senescence. **A** Quantification of β -Gal activity following glutamate and fumarate treatment. Quantitative PCR analyzing IL-6 (**B**), IFN- γ (**C**), and TGF- β (**D**). **E** β -Gal activity after glutamate $\pm$ UBP310 treatment. Quantitative PCR analyzing IL-6 (**F**), IFN- γ (**G**), and TGF- β (**H**) following MSC after glutamate $\pm$ UBP310. **I** Immunofluorescence analysis assessing H3K36me2 in MSCs treated with glutamate $\pm$ UBP310. **J** Flow cytometry assessing ROS in HS-0.5 cells treated with glutamate and fumarate for 3- and 6 h. **K** Graph representing HPLC quantification of GSH levels in MSCs upon 48 h treatment with glutamate or fumarate. **L** γ -GCS activity quantification following glutamate and fumarate supplementation. **M** Representative histograms of GSH concentration following MSC treatment with glutamate $\pm$ UBP310. Data are representative of four different replicates and are reported as mean $\pm$ SD (* $P \leq 0,05$; ** $P \leq 0,01$; *** $P \leq 0,001$; **** $P \leq 0,0001$)

is critical for the onset of clinically overt disease. In this context, BM-MSCs are involved in the complex alterations occurring in TME, eventually activating a pro-fibrotic and inflammatory signature with increased osteogenic potential and a matrix-producing phenotype [46]. Metabolic reprogramming in myofibroblasts is emerging as an important mechanism in the fibrotic transformation [47]. We recently demonstrated that lactate is able to activate a cancer associated phenotype in MSCs characterized by the release of osteogenic factors, mineralized matrix formation and collagen deposition [26]. Here, we screened MF patients' metabolic profile, eventually unveiling changes on the profile of several aminoacids. Among them, glutamate was the most abundant, while ascorbic acid emerged as the most downregulated metabolite in MF serum. Notably, ascorbic acid has been previously implicated in the regulation of platelets aggregation. Polymorphonuclear leukocytes are able to store high concentration of ascorbic acid and, upon its release into the bloodstream, they promote reactive nitrogen species activity eventually inhibiting platelet aggregation [48]. In MF, decreased level of ascorbic acid may contribute to the higher thrombotic tendency characterizing the disease [49]. Considerable evidence indicates glutamate as an important player in tumor development, acting as a growth factor and a signal mediator [50, 51]. Serum glutamate levels directly correlate with Gleason score and primary prostate cancer aggressiveness [52]. Here, we demonstrated that glutamate, the most abundant amino acid in MF patients' sera, alters proteomic profile of healthy stromal cell compartment by affecting expression of several proteins involved in cell metabolism. Among them, the key component of the α -ketoglutarate dehydrogenase complex, DLST, suggested glutamate conversion to α -KG supporting the TCA cycle. Gene expression analysis unveiled an upregulation of both SDH A and B subunits, while FH expression was decreased. As a consequence, we observed an accumulation of intracellular fumarate, also confirmed in MF patients' sera. Of note, the molecular mechanism underlying glutamate-mediated FH loss-of-activity remains elusive. potentially pointing to a feedback mechanism whereby impaired fumarate metabolism may further sustain its accumulation by restricting conversion to malate [53]. Evidence suggests fumarate as an oncometabolite promoting cancer cell epithelial-to-mesenchymal transition, thus promoting tumor progression

[40, 54–56]. In addition to its role as a tumor suppressor in Hereditary leiomyomatosis and Renal Cell Carcinoma [57], decreased expression of FH has been also implicated in hypertension [58] and type 2 diabetes [59]. Previously, Sarkar and colleagues reported a reduction of FH expression in BM-MSCs from patients with multiple sclerosis accompanied by downregulation of key oxidative stress regulators [60]. Fumarate accumulation can lead to metabolic consequences decreasing OXPHOS through inhibition of prolyl hydroxylases and stabilization HIF-1 α [40, 57]. Conversely, we show here that glutamate-induced fumarate accumulation increases NAD $^{+}$ /NADH and ATP/ADP ratio, along with mitochondrial ROS production, therefore promoting an oxidative metabolic shift.

Beyond its metabolic effects, fumarate may also contribute to stromal epigenetic reprogramming. Previous studies have shown that fumarate accumulation can promote histone and DNA methylation by inhibiting α -ketoglutarate-dependent dioxygenases, including histone demethylases and TET enzymes [61, 62]. Corroborating this, we observed increased H3K36me2 and 5mC levels following glutamate or fumarate exposure. Of note, the genomic targets of fumarate-associated epigenetic remodeling were not identified. Therefore, dedicated genome-wide analyses will be undertaken.

Interestingly, increased amount of 5mC was also detected in primary MSC isolated from MF patients, overall supporting the previous reports detecting an increased DNA methylation as a MF hallmark [63, 64].

Besides its role as a metabolite, glutamate is sensed by ionotropic glutamate receptors, which are ligand-gated ion channels, and mGluRs, which are G protein-coupled receptors [65]. The extracellular domain of mGluRs including a binding site for glutamate, is composed of eight groups, divided into three sub-groups: (i) mGluR1 and mGluR5, coupled to phospholipase C; (ii) mGluR2 and mGluR3, negatively coupled to adenylate cyclase, and (iii) Group III mGluRs expressed in glioblastoma cells where their antagonist suppress cell growth [66]. Interestingly, mGluRs have been involved in both physiological and pathological responses as cell proliferation, metabolic reprogramming, upregulation of the immune response and communication in the central nervous system [67]. Several evidence suggests mGluR5 as one of the members of mGluR family subunits involved in cancer progression [68–70]. Its depletion showed promising results in a glioblastoma model, by reducing cells growth,

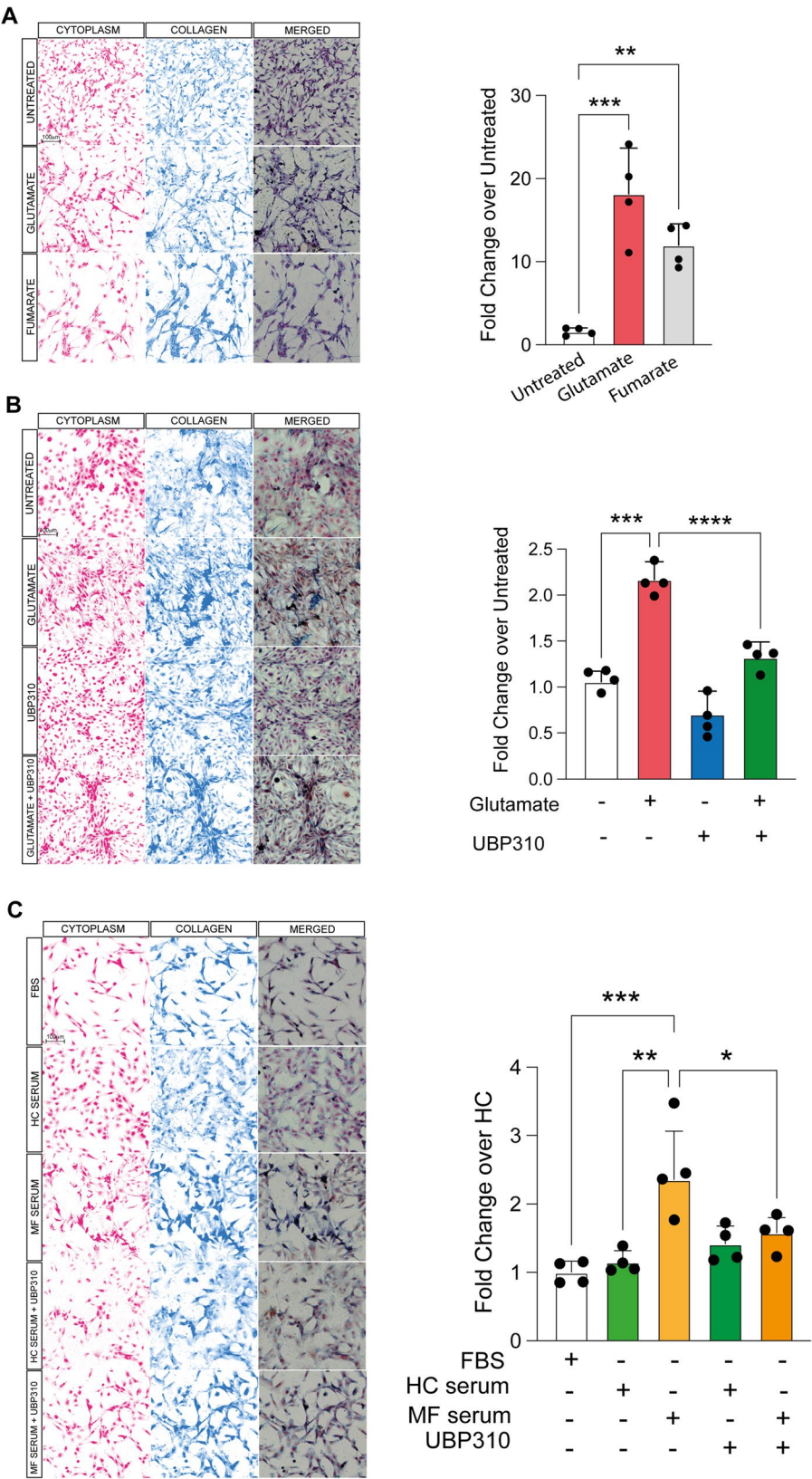


Fig. 6 Glutamate and fumarate overload promotes fibrosis onset. **A** Representative images and area quantification of Azan-Mallory stain in HS-5 cells post 72 h treatment with glutamate and fumarate. **B** Representative images and quantification of Azan-Mallory stain in MSCs post 72 h treatment with glutamate ± UBP310. **C** Representative images and quantification of Azan-Mallory stain on MSCs exposed to MF and HC sera ± UBP310. Data are representative of four different replicates and are reported as mean ± SD (** $P \leq 0,01$; *** $P \leq 0,001$; **** $P \leq 0,0001$)

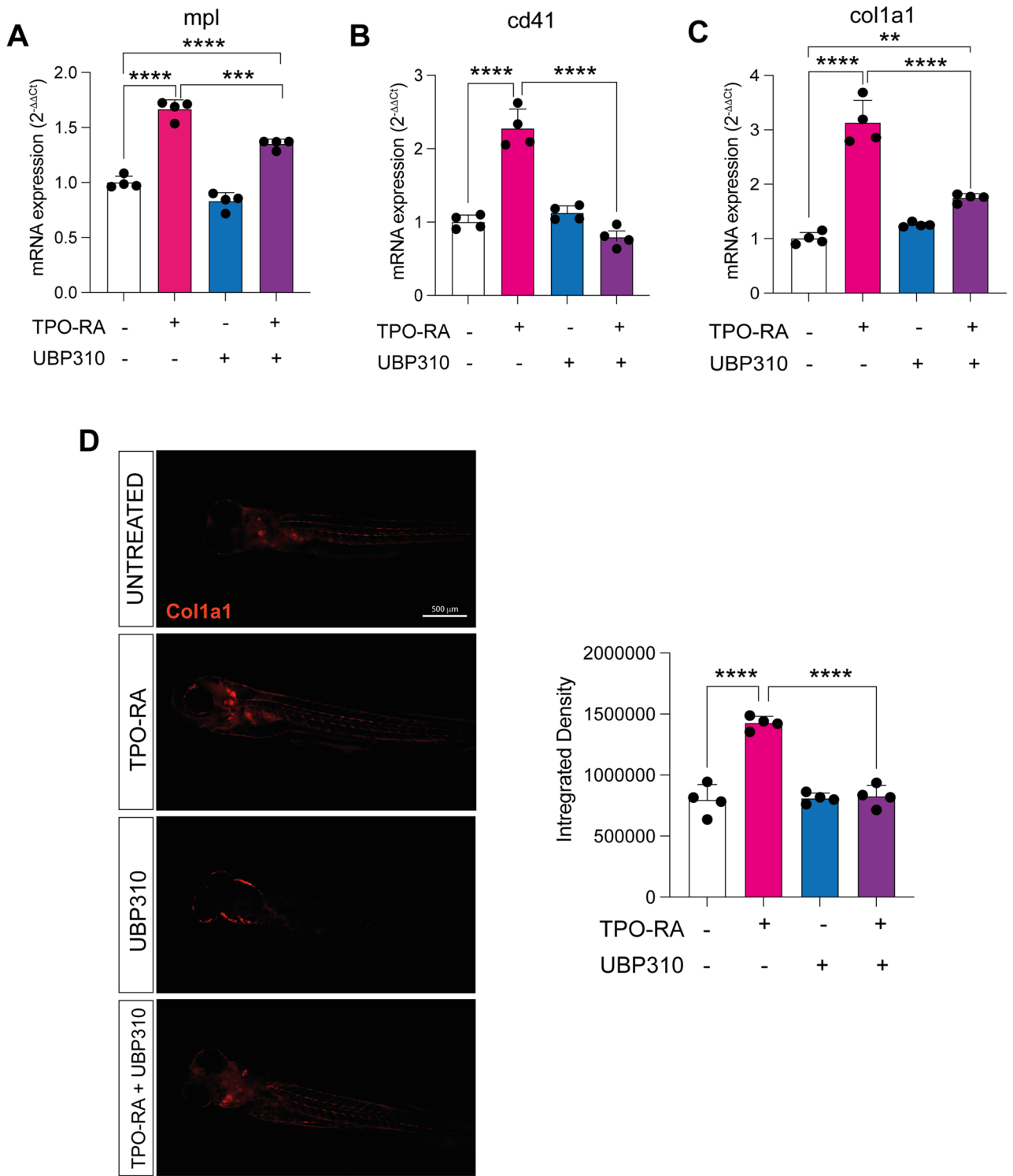


Fig. 7 mGluR-5 inhibition avoids TPO-RA induced collagen accumulation in Zebrafish larvae. Histograms showing qPCR analysis of *mpl* (**A**), *cd41* (**B**) and *col1a1* (**C**) in Zebrafish larvae treated with TPO-RA ± UBP310. **D** Representative images of Col1a1 immunostaining on Zebrafish larvae treated with TPO-RA ± UBP310. Data are representative of four different replicates and are reported as mean ± SD (** $P \leq 0,01$; *** $P \leq 0,001$; **** $P \leq 0,0001$)

migration and invasion [68]. mGluR5 has been reported to be upregulated in MSCs derived from oral cancer [14], therefore providing a hint towards its targeting in MF context. Interestingly, our findings show that selective inhibition of mGluR5 by UB310 or UB296 reduced fumarate-induced H3K36me2 levels, further supporting

a mechanistic link between glutamate signaling, fumarate accumulation, and epigenetic remodeling in stromal cells.

Glutamate and fumarate may contribute to the establishment of a senescent-associated program. Glutamate accumulation has been previously linked to senescence, mainly through its rapid conversion to α -KG upon GDH upregulation, thereby coupling glutamate homeostasis to changes in citrate cycle rate [8]. Similarly, fumarate excess resulting from FH inactivation has been reported to induce oxidative stress through GSH depletion, ultimately triggering cell senescence [71]. Considering the pivotal role of GSH in preserving redox balance, its reduction represents a key mechanistic node linking these metabolic alterations to oxidative stress. By coupling reactive oxygen species scavenging to its reversible oxidation and NADPH-dependent recycling, GSH maintains redox homeostasis and limits lipid and protein oxidation. In addition, GSH modulates redox signaling through reversible protein S-glutathionylation and supports antioxidant networks, including vitamin E regeneration, highlighting its central role in coordinating oxidative stress responses [72]. In this context, our data depicts a scenario in which both glutamate and fumarate weaken antioxidant defenses, promote oxidative stress and sustain the pro-inflammatory secretory program characteristic of senescent MSCs. Notably, mGluR5 blockade attenuated the glutamate-driven senescent phenotype and the associated SASP.

Accumulation of senescent cells is proved as a key factor to the development of fibrosis [66]. In MF, tumor cytokines stimulate stromal cells to deposit an excess of collagens and other extracellular matrix proteins destroying the hematopoietic microenvironment [73]. In this regard, GLS is crucial to maintain mitochondrial activity and supply metabolic intermediates for active biosynthesis in osteogenesis [74]. Indeed, its deletion in BM-MSCs resulted in reduction of overall osteoblast numbers and capability of bone formation. Our data point out that glutamate and fumarate accumulation contribute to collagen deposition mirroring their role in reshaping TME. This is consistent with a recent study highlighting the contribution of glutamate to the development of idiopathic pulmonary fibrosis [47]. Similarly, fumarate has been described as a driver of diabetic renal fibrosis through TGF- β signaling pathway [75]. Importantly, mGluR5 inhibition restrained glutamate-induced collagen deposition in stromal cells. Coherently, similar effects were obtained in patients' sera and UBP310 treated MSCs. Extending these in vitro observations, the antifibrotic effect of mGluR5 inhibition was also recapitulated in vivo, where UBP310 restrained TPO-RA-induced collagen accumulation in the zebrafish model, reinforcing the notion that glutamate signaling contributes to TME fibrotic remodeling.

Overall, in the current study we documented for the first time that glutamate is a critical metabolite involved in remodeling composition of BM *milieu* in MF. It exerts a dual effect on MSCs by affecting their metabolism, leading to fumarate accumulation and by acting as a signaling molecule that ultimately induces a senescent and pro-fibrotic phenotype. Moreover, our data establish a link between glutamate and fumarate overload, epigenetic remodeling and activation of a SASP with an inflammatory and pro-fibrotic signature. Therefore, our work shed a light on the importance of integrating metabolic targeting approaches to prevent BM microenvironment transformation and disruption of hematopoietic niche in MF patients. However, several mechanistic aspects warrant further investigation. In particular, future studies will address the genome-wide targets of fumarate-associated epigenetic remodeling, the molecular basis of glutamate-dependent FH deregulation, and the translational potential of interfering with the glutamate–mGluR5 axis to limit BM niche disruption and fibrotic progression.

Abbreviations

MF	Myelofibrosis
MPN	Myeloproliferative Neoplasm
JAK2	Janus Kinase 2
CALR	Calreticulin
MPL	Myeloproliferative Leukemia Virus Proto-Oncogene
BM	Bone Marrow
MSCs	Mesenchymal Stromal Cells
SASP	Senescence-Associated Secretory Phenotype
GLS1	Glutaminase 1
GDH	Glutamate Dehydrogenase
TME	Tumor Microenvironment
mGluR5	Metabotropic Glutamate Receptor 5
HC	Healthy Controls
PB	Peripheral Blood
HPLC	High-Performance Liquid Chromatography
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
NAD ⁺	Nicotinamide Adenine Dinucleotide (oxidized form)
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
ECP	Electron Charge Potential
COX-2	Cyclooxygenase-2
ND4	NADH-ubiquinone Oxidoreductase Chain 4
TET	Ten-Eleven Translocation enzyme
5mC	5-Methylcytosine
KDM2A	Histone Lysine Demethylase 2 A
IL-6	Interleukin 6
IFN- γ	Interferon gamma
TGF- β	Transforming Growth Factor Beta
GSH	Reduced Glutathione
γ -GCS	Gamma-Glutamylcysteine Synthetase
DLST	Dihydrolipoamide S-succinyltransferase
SDH-A	Succinate Dehydrogenase Subunit A
SDH-B	Succinate Dehydrogenase Subunit B
SDH-D	Succinate Dehydrogenase Subunit D
FH	Fumarate Hydratase
DPF	days post fertilization
ACLY	ATP Citrate Lyase
NAMPT	Nicotinamide Phosphoribosyltransferase
CBR1	Carbonyl Reductase 1
FARSB	Phenylalanyl-tRNA Synthetase Subunit Beta
ADSS	Adenylosuccinate Synthetase
PHGDH	Phosphoglycerate Dehydrogenase

FAH	Fumarylacetoacetate Hydrolase
PGM2	Phosphoglucomutase 2
RT-PCR	Real-Time Polymerase Chain Reaction
LC-MS	Liquid Chromatography–Mass Spectrometry
UHPLC	Ultra-High-Performance Liquid Chromatography
MS/MS	Tandem Mass Spectrometry
MS	Mass Spectrometry
PDA	Photodiode Array Detector
FA	Formic Acid
SDS	Sodium Dodecyl Sulfate
Hb	Hemoglobin
WBC	White Blood Cells
PLT	Platelets

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12964-026-03040-6>.

Supplementary Material 1.

Authors' contributions

SG, JF, CG, DT and VDF were involved in the conception and design of the work. LL, ELS, FB, AMA, and CDG provided support in performing the experiments related to mass spectrometry, HPLC and γ -GCS detection. AD and GP provided the biological samples included in the study. NV, IAB, GLV, and DT provided supervision for the work as well as funding acquisition. All authors reviewed and approved the final manuscript draft.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. La Spina E, Giallongo S, Giallongo C, Vicario N, Duminuco A, Parenti R, Giuffrida R, Longhitano L, Li Volti G, Cambria D, et al. Mesenchymal stromal cells in tumor microenvironment remodeling of BCR-ABL negative myeloproliferative diseases. *Front Oncol*. 2023;13:1141610.
2. Holl K, Chatain N, Krapp S, Baumeister J, Maie T, Schmitz S, Scheufen A, Brock N, Koschmieder S, Moreno-Andres D. Calreticulin and JAK2V617F driver mutations induce distinct mitotic defects in myeloproliferative neoplasms. *Sci Rep*. 2024;14:2810.
3. Sastow D, Tremblay D. Emerging Treatment Options for Myelofibrosis: Focus on Anemia. *Ther Clin Risk Manag*. 2023;19:535–47.
4. Vukotic M, Kapor S, Dragojevic T, Dikic D, Mitrovic Ajtic O, Diklic M, Suboticki T, Zivkovic E, Beleslin Cokic B, Vojvodic A, et al. Inhibition of proinflammatory signaling impairs fibrosis of bone marrow mesenchymal stromal cells in myeloproliferative neoplasms. *Exp Mol Med*. 2022;54:273–84.
5. Martinaud C, Desterke C, Konopacki J, Pieri L, Torossian F, Golub R, Schmutz S, Anginot A, Guerton B, Rochet N, et al. Osteogenic Potential of Mesenchymal Stromal Cells Contributes to Primary Myelofibrosis. *Cancer Res*. 2015;75:4753–65.
6. Zhang F, Guo J, Yu S, Zheng Y, Duan M, Zhao L, Wang Y, Yang Z, Jiang X. Cellular senescence and metabolic reprogramming: Unraveling the intricate crosstalk in the immunosuppressive tumor microenvironment. *Cancer Commun (Lond)*. 2024;44:929–66.
7. Choudhury D, Rong N, Ikhopoh I, Rajabian N, Tseropoulos G, Wu Y, Mehrotra P, Thiagarajan R, Shahini A, Seldeen KL, et al. Inhibition of glutaminolysis restores mitochondrial function in senescent stem cells. *Cell Rep*. 2022;41:111744.
8. Sun X, Li Q, Tang Y, Hu W, Chen G, An H, Huang D, Tong T, Zhang Y. Epigenetic activation of secretory phenotypes in senescence by the FOXQ1-SIRT4-GDH signaling. *Cell Death Dis*. 2023;14:481.
9. Yu LJ, Wall BA, Wangari-Talbot J, Chen S. Metabotropic glutamate receptors in cancer. *Neuropharmacology*. 2017;115:193–202.
10. Prickett TD, Samuels Y. Molecular pathways: dysregulated glutamatergic signaling pathways in cancer. *Clin Cancer Res*. 2012;18:4240–6.
11. Ribeiro FM, Paquet M, Cregan SP, Ferguson SS. Group I metabotropic glutamate receptor signalling and its implication in neurological disease. *CNS Neurol Disord Drug Targets*. 2010;9:574–95.
12. Willard SS, Koochekpour S. Glutamate, glutamate receptors, and downstream signaling pathways. *Int J Biol Sci*. 2013;9:948–59.
13. Park SY, Lee SA, Han IH, Yoo BC, Lee SH, Park JY, Cha IH, Kim J, Choi SW. Clinical significance of metabotropic glutamate receptor 5 expression in oral squamous cell carcinoma. *Oncol Rep*. 2007;17:81–7.
14. Kuribayashi N, Uchida D, Kinouchi M, Takamaru N, Tamatani T, Nagai H, Miyamoto Y. The role of metabotropic glutamate receptor 5 on the stromal cell-derived factor-1/CXCR4 system in oral cancer. *PLoS ONE*. 2013;8:e80773.
15. Stepulak A, Luksch H, Uckermann O, Siffringer M, Rzeski W, Polberg K, Kupisz K, Klatka J, Kielbus M, Grabarska A, et al. Glutamate receptors in laryngeal cancer cells. *Anticancer Res*. 2011;31:565–73.
16. Tefferi A, Guglielmelli P, Pardanani A, Vannucchi AM. Myelofibrosis Treatment Algorithm 2018. *Blood Cancer J*. 2018;8:72.
17. Giallongo C, Dulcamare I, Giallongo S, Duminuco A, Pieragostino D, Cufaro MC, Amorini AM, Lazzarino G, Romano A, Parrinello N, et al. MacroH2A1.1 as a crossroad between epigenetics, inflammation and metabolism of mesenchymal stromal cells in myelodysplastic syndromes. *Cell Death Dis*. 2023;14:686.
18. Tavazzi B, Lazzarino G, Leone P, Amorini AM, Bellia F, Janson CG, Di Pietro V, Ceccarelli L, Donzelli S, Francis JS, Giardina B. Simultaneous high performance liquid chromatographic separation of purines, pyrimidines, N-acetylated amino acids, and dicarboxylic acids for the chemical diagnosis of inborn errors of metabolism. *Clin Biochem*. 2005;38:997–1008.
19. Spampinato M, Zuppelli T, Dulcamare I, Longhitano L, Sambataro D, Santisi A, Alanazi AM, Barbagallo IA, Vicario N, Parenti R et al. Enhanced Antitumor Activity by the Combination of Dasatinib and Selinexor in Chronic Myeloid Leukemia. *Pharmaceuticals (Basel)* 2024;17.
20. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, et al. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res*. 2013;41:D991–995.
21. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, Srivastava A, Molla G, Madad S, Fernandez-Granda C, Satija R. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol*. 2024;42:293–304.
22. Longhitano L, Giallongo S, Orlando L, Broggi G, Longo A, Russo A, Caltabiano R, Giallongo C, Barbagallo I, Di Rosa M et al. Lactate Rewrites the Metabolic

- Reprogramming of Uveal Melanoma Cells and Induces Quiescence Phenotype. *Int J Mol Sci* 2022;24.
23. Zhong S, Li Z, Jiang T, Li X, Wang H. Immunofluorescence Imaging Strategy for Evaluation of the Accessibility of DNA 5-Hydroxymethylcytosine in Chromatins. *Anal Chem*. 2017;89:5702–6.
24. Seelig GF, Meister A. Gamma-glutamylcysteine synthetase. Interactions of an essential sulfhydryl group. *J Biol Chem*. 1984;259:3534–8.
25. Ray S, Misso NL, Lenzo JC, Robinson C, Thompson PJ. Gamma-glutamylcysteine synthetase activity in human lung epithelial (A549) cells: factors influencing its measurement. *Free Radic Biol Med*. 1999;27:1346–56.
26. Spampinato M, Giallongo C, Giallongo S, Spina E, Duminuco A, Longhitano L, Caltabiano R, Salvatorelli L, Broggi G, Pricoco EP, et al. Lactate accumulation promotes immunosuppression and fibrotic transformation of bone marrow microenvironment in myelofibrosis. *J Transl Med*. 2025;23:69.
27. Wolun-Cholewa M, Szymanowski K, Andrusiewicz M, Szczerba A, Warchoł JB. Trichrome Mallory's stain may indicate differential rates of RNA synthesis in eutopic and ectopic endometrium. *Folia Histochem Cytobiol*. 2010;48:148–52.
28. Scalia F, Capparucci F, Amico MD, Marino M, Lamparelli EP, Longhitano L, Giallongo S, Falletti R, Rappa F, Iaria C, et al. Toxic effects of biodegradable polylactic acid nanoplastics on developing zebrafish (*Danio rerio*). *Sci Rep*. 2025;15:38145.
29. Gao F, Shah R, Xin G, Wang R. Metabolic Dialogue Shapes Immune Response in the Tumor Microenvironment. *Eur J Immunol*. 2025;55:e202451102.
30. Feng X, Zhang L, Xu S, Shen AZ. ATP-citrate lyase (ACLY) in lipid metabolism and atherosclerosis: An updated review. *Prog Lipid Res*. 2020;77:101006.
31. Garten A, Petzold S, Korner A, Imai S, Kiess W. Nampt: linking NAD biology, metabolism and cancer. *Trends Endocrinol Metab*. 2009;20:130–8.
32. Hara A, Endo S, Matsunaga T, El-Kabbani O, Miura T, Nishinaka T, Terada T. Human carbonyl reductase 1 participating in intestinal first-pass drug metabolism is inhibited by fatty acids and acyl-CoAs. *Biochem Pharmacol*. 2017;138:185–92.
33. Wang Y, Wang G, Hu S, Yin C, Zhao P, Zhou X, Shao S, Liu R, Hu W, Liu GL et al. FARSF Facilitates Hepatocellular Carcinoma Progression by Activating the mTORC1 Signaling Pathway. *Int J Mol Sci* 2023; 24.
34. Gooding JR, Jensen MV, Dai X, Wenner BR, Lu D, Arumugam R, Ferdaoussi M, MacDonald PE, Newgard CB. Adenylosuccinate Is an Insulin Secretagogue Derived from Glucose-Induced Purine Metabolism. *Cell Rep*. 2015;13:157–67.
35. Rathore R, Schutt CR, Van Tine BA. PHGDH as a mechanism for resistance in metabolically-driven cancers. *Cancer Drug Resist*. 2020;3:762–74.
36. Shen N, Korm S, Karantanos T, Li D, Zhang X, Ritou E, Xu H, Lam A, English J, Zong WX, et al. DLST-dependence dictates metabolic heterogeneity in TCA-cycle usage among triple-negative breast cancer. *Commun Biol*. 2021;4:1289.
37. Weiss AKH, Loeffler JR, Liedl KR, Gstach H, Jansen-Durr P. The fumarylacetoacetate hydrolase (FAH) superfamily of enzymes: multifunctional enzymes from microbes to mitochondria. *Biochem Soc Trans*. 2018;46:295–309.
38. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, Rodrigues F, Silvestre R, Cheng SC, Wang SY, et al. Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. *Cell Metab*. 2016;24:807–19.
39. Schatton D, Frezza C. Fumarate. *Trends Endocrinol Metab*; 2025.
40. Giallongo S, Costa F, Longhitano L, Giallongo C, Ferrigno J, Tropea E, Vicario N, Li Volti G, Parenti R, Barbagallo I et al. The Pleiotropic Effects of Fumarate: From Mitochondrial Respiration to Epigenetic Rewiring and DNA Repair Mechanisms. *Metabolites* : 2023;13.
41. Lyamina S, Baranovskii D, Kozhevnikova E, Ivanova T, Kalish S, Sadekov T, Klabukov I, Maev I, Govorun V. Mesenchymal Stromal Cells as a Driver of Inflammation. *Int J Mol Sci* 2023; 24.
42. Pinheiro PS, Lanore F, Veran J, Artinian J, Blanchet C, Crepel V, Perrais D, Mülle C. Selective block of postsynaptic kainate receptors reveals their function at hippocampal mossy fiber synapses. *Cereb Cortex*. 2013;23:323–31.
43. Nouis L, Kanavars P, Barbouti A. Oxidative Stress-Induced Cellular Senescence: Is Labile Iron the Connecting Link? *Antioxidants (Basel)* 2023; 12.
44. Williams N, Lee J, Mitchell E, Moore L, Baxter EJ, Hewinson J, Dawson KJ, Menzies A, Godfrey AL, Green AR, et al. Life histories of myeloproliferative neoplasms inferred from phylogenies. *Nature*. 2022;602:162–8.
45. Van Egeren D, Escabi J, Nguyen M, Liu S, Reilly CR, Patel S, Kamaz B, Kalyva M, DeAngelo DJ, Galinsky I, et al. Reconstructing the Lineage Histories and Differentiation Trajectories of Individual Cancer Cells in Myeloproliferative Neoplasms. *Cell Stem Cell*. 2021;28:514–e523519.
46. Giallongo S, Duminuco A, Dulcamare I, Zuppelli T, La Spina E, Scandura G, Santisi A, Romano A, Di Raimondo F, Tibullo D et al. Engagement of Mesenchymal Stromal Cells in the Remodeling of the Bone Marrow Microenvironment in Hematological Cancers. *Biomolecules* : 2023; 13.
47. Hamanaka RB, O'Leary EM, Witt LJ, Tian Y, Gokalp GA, Meliton AY, Dulin NO, Mutlu GM. Glutamine Metabolism Is Required for Collagen Protein Synthesis in Lung Fibroblasts. *Am J Respir Cell Mol Biol*. 2019;61:597–606.
48. Raghavan SA, Sharma P, Dikshit M. Role of ascorbic acid in the modulation of inhibition of platelet aggregation by polymorphonuclear leukocytes. *Thromb Res*. 2003;110:117–26.
49. Kc D, Falchi L, Verstovsek S. The underappreciated risk of thrombosis and bleeding in patients with myelofibrosis: a review. *Ann Hematol*. 2017;96:1595–604.
50. Stepulak A, Siffringer M, Rzeski W, Brocke K, Gratopp A, Pohl EE, Turski L, Ikonomidou C. AMPA antagonists inhibit the extracellular signal regulated kinase pathway and suppress lung cancer growth. *Cancer Biol Ther*. 2007;6:1908–15.
51. Herner A, Sauliunaite D, Michalski CW, Erkan M, De Oliveira T, Abiatari I, Kong B, Esposito I, Friess H, Kleeff J. Glutamate increases pancreatic cancer cell invasion and migration via AMPA receptor activation and Kras-MAPK signaling. *Int J Cancer*. 2011;129:2349–59.
52. Koochekpour S. Glutamate, a metabolic biomarker of aggressiveness and a potential therapeutic target for prostate cancer. *Asian J Androl*. 2013;15:212–3.
53. Rajender S, McColloch RJ. A kinetic investigation of fumarate reaction at high substrate concentrations. *Arch Biochem Biophys*. 1967;118:279–83.
54. Yang M, Soga T, Pollard PJ, Adam J. The emerging role of fumarate as an oncometabolite. *Front Oncol*. 2012;2:85.
55. Sciacovelli M, Goncalves E, Johnson TI, Zecchini VR, da Costa AS, Gaude E, Drubbel AV, Theobald SJ, Abbo SR, Tran MG, et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature*. 2016;537:544–7.
56. Kulkarni RA, Bak DW, Wei D, Bergholtz SE, Briney CA, Shrimp JH, Alpsoy A, Thorpe AL, Bavari AE, Crooks DR, et al. A chemoproteomic portrait of the oncometabolite fumarate. *Nat Chem Biol*. 2019;15:391–400.
57. Valcarcel-Jimenez L, Frezza C. Fumarate hydratase (FH) and cancer: a paradigm of oncometabolism. *Br J Cancer*. 2023;129:1546–57.
58. Tian Z, Liu Y, Usa K, Mladinov D, Fang Y, Ding X, Greene AS, Cowley AW Jr, Liang M. Novel role of fumarate metabolism in dahl-salt sensitive hypertension. *Hypertension*. 2009;54:255–60.
59. Adam J, Ramacheya R, Chibalina MV, Ternette N, Hamilton A, Tarasov AI, Zhang Q, Rebelato E, Rorsman NJG, Martin-Del-Rio R, et al. Fumarate Hydratase Deletion in Pancreatic beta Cells Leads to Progressive Diabetes. *Cell Rep*. 2017;20:3135–48.
60. Sarkar P, Redondo J, Hares K, Bailey S, Georgievskaya A, Heesom K, Kemp KC, Scolding NJ, Rice CM. Reduced expression of mitochondrial fumarate hydratase in progressive multiple sclerosis contributes to impaired in vitro mesenchymal stromal cell-mediated neuroprotection. *Mult Scler*. 2022;28:1179–88.
61. Jiang Y, Qian X, Shen J, Wang Y, Li X, Liu R, Xia Y, Chen Q, Peng G, Lin SY, Lu Z. Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation. *Nat Cell Biol*. 2015;17:1158–68.
62. Laukka T, Mariani CJ, Ihtantola T, Cao JZ, Hokkanen J, Kaelin WG Jr, Godley LA, Koivunen P. Fumarate and Succinate Regulate Expression of Hypoxia-inducible Genes via TET Enzymes. *J Biol Chem*. 2016;291:4256–65.
63. Dunbar A, Park Y, Levine R. Epigenetic Dysregulation of Myeloproliferative Neoplasms. *Hematol Oncol Clin North Am*. 2021;35:237–51.
64. Martinez-Calle N, Pascual M, Ordenez R, Eneriz ESJ, Kulis M, Miranda E, Guruceaga E, Segura V, Larrayoz MJ, Bellosillo B, et al. Epigenomic profiling of myelofibrosis reveals widespread DNA methylation changes in enhancer elements and ZFP36L1 as a potential tumor suppressor gene that is epigenetically regulated. *Haematologica*. 2019;104:1572–9.
65. Reiner A, Levitz J, Isacoff EY. Controlling ionotropic and metabotropic glutamate receptors with light: principles and potential. *Curr Opin Pharmacol*. 2015;20:135–43.
66. Mebratu YA, Soni S, Rosas L, Rojas M, Horowitz JC, Nho R. The aged extracellular matrix and the profibrotic role of senescence-associated secretory phenotype. *Am J Physiol Cell Physiol*. 2023;325:C565–79.
67. Vafabakhsh R, Levitz J, Isacoff EY. Conformational dynamics of a class C G-protein-coupled receptor. *Nature*. 2015;524:497–501.
68. Hou G, Xu X, Hu W. GRIK1 promotes glioblastoma malignancy and is a novel prognostic factor of poor prognosis. *Oncol Res*. 2024;32:727–36.
69. Koda S, Hu J, Xu X, Sun G, Shao S, Tang RX, Zheng KY, Yan J. The role of glutamate receptors in the regulation of the tumor microenvironment. *Front Immunol*. 2023;14:1123841.

70. Stepulak A, Luksch H, Gebhardt C, Uckermann O, Marzahn J, Siffringer M, Rzeski W, Staufner C, Brocke KS, Turski L, Ikonomidou C. Expression of glutamate receptor subunits in human cancers. *Histochem Cell Biol*. 2009;132:435–45.
71. Zheng L, Cardaci S, Jerby L, MacKenzie ED, Sciacovelli M, Johnson TI, Gaude E, King A, Leach JD, Edrada-Ebel R, et al. Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat Commun*. 2015;6:6001.
72. Averill-Bates DA. The antioxidant glutathione. *Vitam Horm*. 2023;121:109–41.
73. Li R, Colombo M, Wang G, Rodriguez-Romera A, Benlabiod C, Jooss NJ, O'Sullivan J, Brierley CK, Clark SA, Perez Saez JM, et al. A proinflammatory stem cell niche drives myelofibrosis through a targetable galectin-1 axis. *Sci Transl Med*. 2024;16:eadj7552.
74. Zhou T, Yang Y, Chen Q, Xie L. Glutamine Metabolism Is Essential for Stemness of Bone Marrow Mesenchymal Stem Cells and Bone Homeostasis. *Stem Cells Int* 2019, 2019:8928934.
75. Miura Y, Hayakawa A, Kikuchi S, Tsumoto H, Umezawa K, Chiba Y, Soejima Y, Sawabe M, Fukui K, Akimoto Y, Endo T. Fumarate accumulation involved in renal diabetic fibrosis in Goto-Kakizaki rats. *Arch Biochem Biophys*. 2019;678:108167.

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