



Integrative analyses of dicarbonyls and advanced glycation end-products with multiomic profiles across tissue, plasma and stool samples reveal methylglyoxal accumulation in colon cancer

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ABSTRACT

Advanced Glycation Endproducts (AGEs) arise from the reaction of proteins with highly reactive dicarbonyl compounds such as methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG), which have been implicated in inflammation and carcinogenesis. How dicarbonyls and AGEs are distributed across tumor tissue and surrogate specimens, and how they relate to systemic metabolism, AGE-related pathways, and alterations in gut microbiota in colon cancer, remains poorly understood.

An integrative multi-specimen analysis of MGO, GO, 3-DG and major AGEs was performed using targeted tandem mass spectrometry in matched tumor tissue, adjacent normal mucosa, plasma, and stool from 26 sporadic colon cancer patients. These measurements were combined with tumor RNA-sequencing, untargeted plasma metabolomics, and stool shotgun metagenomics generated from the same individuals.

A marked accumulation of MGO was observed in tumor tissue when compared with adjacent mucosa, accompanied by higher levels of the MGO-derived AGE N^ε-[5-hydro-5-methyl-4-imidazolone-2-yl]-ornithine (MG-H1). Tissue MG-H1 concentrations significantly correlated with corresponding plasma levels. Elevated tumor MGO levels were associated with up-regulation of *GLO1* (encoding for the detoxifying enzyme glyoxalase-1), *DDOST* (coding for the AGE-clearance receptor AGE-R1), and the glycolytic flux marker triose phosphate isomerase (TPI), alongside down-regulation of the AGE-scavenger receptor CD36. These findings suggest a candidate remodeling of dicarbonyl-handling pathways. The MGO/GO ratio in tumors was positively associated with the relative abundances of *Fusobacterium nucleatum* and *Parvimonas micra*, two bacterial species related to colorectal carcinogenesis, and with metagenomic signatures of oral-derived taxa colonizing the gut.

This pilot integrative analysis highlighted novel coherent associations among tissue, circulating, and stool levels of MGO-derived AGEs, the expression of AGE-related metabolic pathways, and microbial signatures in colon cancer. If confirmed in larger studies, these candidate molecular and microbial interactions may provide novel insights into the dicarbonyl stress involvement in tumor biology.

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1. Introduction

Colon cancer (CC) is among the most common malignant tumors worldwide, with high morbidity and mortality [1]. Improvements over the last two decades in health awareness and screening programs have increased the rate of CC diagnosis at earlier stages, yet this malignancy still constitutes a substantial burden for national health systems, as incidence rates continue to increase in many regions of the world [2].

Unhealthy diets and lifestyles, obesity and metabolic dysfunction have been identified as strong CC risk factors. In recent years, advanced glycation endproducts (AGEs) – which can be both diet-derived and produced endogenously – have been proposed to contribute to the development of various chronic diseases, including CC and other cancer types [3,4]. AGEs are reactive metabolites deriving from non-enzymatic reactions between sugars and proteins [5]. Dicarbonyl compounds (DCs) such as methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucose (3-DG) are key sources of AGE production. MGO is mainly derived from the glycolysis intermediates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate [6], and GO from autooxidation of sugars and lipid peroxidation [7]. 3-DG, on the other hand, is formed by the degradation of fructosamine-3-phosphate in the repair of early glycated proteins, the degradation of fructose-3-phosphate and the slow, non-enzymatic oxidative degradation of glucose and proteins glycated by glucose [8]. AGEs directly form protein- and DNA-adducts that can impact their function. They can also bind to the receptor for advanced glycation endproducts (RAGE), with the activation of pathways involved in inflammation, oxidative stress, proliferation, and angiogenesis, with potential relevance in tumor progression [9].

Dietary patterns, such as the typical Western diet, that are rich in sugars, saturated fats, and ultra-processed foods have been associated with increased risk of CC and are considerable sources of both DCs and AGEs [10,11]. AGEs are also produced endogenously in hyperglycemic and obese environments, such as in individuals with diabetes or obesity. Both conditions are associated with an increase in the body pools of DCs and AGEs [12,13], as well as with a higher risk for colorectal cancer (CRC) development [14], including at earlier-onset [15]. From this perspective, several epidemiological and experimental studies have investigated potential relationships between exposure to dietary and circulating AGEs and CRC risk [16,17] and mortality [18,19] with apparently conflicting results. Specifically, *in vitro* and *in vivo* experimental models have shown that AGEs can induce sustained colonic inflammation and promote CRC development and growth [20,21]. On the other hand, human cohort studies hamper a definitive conclusion on the role of AGEs in CRC, showing mostly an inverse risk of this type of cancer in people with both higher plasma levels and dietary consumption of AGEs [18,19,22]. These contrasting results suggest that dietary consumption estimates and measurements of circulating AGEs levels may not fully reflect exposure to DCs, nor the degree of AGEs production and accumulation in the CC milieu. Additionally, potentially important functional variation may exist among AGEs depending on their DC source.

Analyses of metabolic changes during carcinogenesis suggest that cancer cells, due to their affinity for sugars and the hypoxic microenvironment they create, may produce and accumulate AGEs [23]. Indeed, tumor hypoxia increases MGO through the formation by anaerobic glycolysis and by the down-regulation of glyoxalases I, the detoxifier of MGO [24]. Notably, recent evidence showed that a transient increase of MGO can impair the tumor-suppressor activity of the BReast CAncer 2 gene (*BRCA2*) in a mouse model of breast cancer, supporting a metabolic link between MGO production and carcinogenesis [25]. In addition, the human colon is particularly interesting to study because of its dual exposure to AGEs from both the systemic compartment and the colonic lumen via dietary AGEs. The fractions of AGEs that are not absorbed from the diet, estimated at around 70–90 % of the totality of all AGEs consumed, remain in the gastrointestinal tract and may interact with the colon mucosa and gut barrier, possibly modifying the microbiome and

inducing a pro-inflammatory/oxidative environment [26].

Although previous studies have analyzed the correlation between AGE levels in plasma and CC, there is a lack of a thorough analysis of both DCs and AGE production and metabolism in cancer cells compared to adjacent healthy cells and their impact on gene transcription, systemic metabolism, and gut microbiota. Moreover, how the levels of specific AGEs in human biofluids, including plasma and feces, may reflect alterations in tumor tissue remains largely unexplored. A multi-omic, multi-specimen analysis of DCs and AGEs is needed to better elucidate the tissue- and systemic-level impact of these compounds in CC patients. This may provide potentially useful mechanistic as well as prognostic information on the metabolic and molecular profile of cancer cells.

To investigate these aspects, analysis of the levels of AGEs and DCs was performed on four different biological sample types from the same CC patients: tumor tissue, adjacent colonic mucosa, plasma, and feces. Data were integrated with tissue transcriptional profiles, systemic metabolites, and estimation of the gut microbial population from stool shotgun metagenomic sequencing. This multi-omics analysis provides an original and comprehensive pattern between specific DCs/AGEs and the corresponding genome, metabolome, or microbiome in sporadic CC patients. It represents a potential starting point for targeted studies in larger cohorts of patients to validate specific DCs/AGEs as biomarkers of CC development.

2. Methods

2.1. Study population

The study population is part of a larger cohort of CRC patients (described in Ref. [27]) investigated for multiple omics within the European Horizon 2020 project ONCOBIOME (<https://www.oncobiome.eu>) [28,29]. Subjects were recruited in a hospital-based study at Vercelli (Italy).

All the subjects participating in the study signed a written consent, in agreement with the Helsinki Declaration. The design of the study, informed consent, and protocols were approved by the local Ethics Committees (Azienda Ospedaliera “SS. Antonio e Biagio e C. Arrigo” of Alessandria. ID: Colorectal miRNA CEC2014 and International Agency for Research on Cancer (IARC) ethical committee).

For 26 CC patients included in this study, the multiple biological specimens (plasma, stool, and tissue pair samples) available were profiled for AGEs and DCs in integration with clinical and demographic data.

2.2. Sample collection

Stool samples. Naturally evacuated fecal samples were obtained from all participants previously instructed to self-collect the specimen at home. Stool samples were collected in nucleic acid collection and transport tubes with RNA stabilizing solution (Norgen Biotek Corp) and returned to the endoscopy unit. Stool aliquots (200 μ L) were stored at -80°C until RNA and DNA extraction [30,28].

Plasma samples. Plasma samples were obtained from 8 mL of blood collected in EDTA tubes, centrifuged for 10 min at $1000\times g$ and aliquots were stored at -80°C until analysis.

Tissue samples. Paired primary tumor tissue and nonmalignant adjacent mucosa were collected during surgical resection of confirmed CC patients at Clinica S. Rita in Vercelli (Italy) and immediately immersed in RNAlater solution (Ambion). Matched, unaffected colon mucosa was taken 20 cm away in the caudal direction and was confirmed as unaffected by the pathologist. All tissue samples were stored at -80°C until use. The tumor clinical characteristics are reported in Table 1.

2.3. Extraction of nucleic acids

For DNA extraction, tissues were initially homogenized in a homogenization solution (Promega) and then processed with Maxwell® RSC Tissue DNA Kit (Promega). Before loading samples onto Maxwell® RSC Cartridges, 300 µl of Lysis Buffer and 30 µl of Proteinase K were added to the homogenized samples and further incubated for 20 min at 56 °C. DNA was quantified with Qubit™ 4 fluorometer using Qubit™ dsDNA High Sensitivity Assay Kit (Invitrogen).

For total RNA extraction, tissue samples were homogenized with ULTRATURRAX Homogenizer (IKA) and then processed using QIAzol (Qiagen), followed by phenol/chloroform extraction according to the manufacturer's standard protocol.

DNA from stool was extracted with the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions with a final elution buffer volume of 50 µl to increase DNA concentration, which was quantified with the Qubit DNA High Sensitivity Assay Kit (Invitrogen).

Total RNA from stool was extracted using the Stool Total RNA Purification Kit (Norgen Biotek Corp) as previously described [31].

Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used for dicarbonyls and AGE measurements.

Protein-bound AGEs and α-DCs were measured in EDTA plasma, feces, and colon biopsies using UPLC-MS/MS, as described in detail previously [32,33].

Colon tissue was crushed and grinded with a pestle and mortar submerged in liquid nitrogen. The obtained crushed powder was homogenized in 0.1 mol/l sodium phosphate buffer with 0.02 % Triton-X (10 % m/v). Total protein content in feces was determined using colorimetric quantification [34]. All protein-bound fractions of AGEs were expressed per mmol lysine to adjust for the protein amount in each sample. Briefly, for the measurement of protein-bound CML, CEL, MG-H1 and lysine, 25 µl of sample homogenate or plasma was mixed with 50 µl of water. After the addition of 200 µl sodium borohydride (100 mmol/L) dissolved in borate buffer (pH 9.2, 200 mmol/L), samples were incubated at room temperature for 2 h. Next, samples were deproteinized with 1000 µl of cold (4 °C) trifluoroacetic acid. After centrifugation (4300×g, 4 °C, 20 min), the supernatant was carefully removed. Samples were then hydrolyzed by adding 500 µl 6 N HCl to the protein pellet and incubated for 20 h at 90 °C. After hydrolysis, 40 µl hydrolysate and 20 µl internal standard were mixed in a reaction vial. For CML, CEL and MG-H1, this mixture was evaporated to dryness under a stream of nitrogen at 70 °C and subsequently derivatized with 100 µl 1-butanol:HCl (3:1, v/v) for 90 min at 70 °C. Samples were then evaporated to dryness under nitrogen and redissolved in 200 µl of water. For

the measurement of lysine, 10 µl hydrolysate was diluted in 800 µl water. Twenty µl of this mixture and 20 µl internal standard were diluted in 500 µl 10 mmol/l ammonia. Derivatized CML, CEL and MG-H1, and underivatized lysine were analyzed by UPLC tandem MS.

Sample levels of the dicarbonyls GO, MGO and 3-DG were measured with UPLC-MS/MS, as described previously [35]. Intra- and inter-assay coefficients of variation were 4.3 % and 14.3 % for GO, 2.9 % and 7.3 % for MGO, and 2.4 % and 12.0 % for 3-DG [33].

2.4. TruSight™ oncology 500 high-throughput genomic profiling assay

DNA libraries were prepared using the hybrid capture-based TruSight™ Oncology 500 High Throughput (TSO500-HT) Library Preparation Kit (Illumina) following the manufacturer's protocol and sequenced on Illumina® NovaSeq™ 6000 Sequencer (Illumina) (read length of 200 bp paired-end). The full procedure is described in Ref. [36]. Data were analyzed with the Illumina TSO500 Local App (v2.2), generating information on quality controls, genetic variants, MSI status, and tumor mutational burden.

2.5. Library preparation for total RNA sequencing (RNA-Seq)

Total RNA from tissue was cleaned up and DNase-treated with the RNA Clean & Concentrator™-5 kit (Zymo Research), following the manufacturer's protocol. Thereafter, the RNA quality was determined by RNA Integrity Number (RIN) measurement obtained with an Agilent Bioanalyser® RNA 6000 Nano chip (Agilent Technologies), and 500 ng of RNA was used for the library preparation. RNA-Seq libraries were prepared with the NEBNext® Ultra II Directional RNA Library Prep for Illumina® kit (New England Biolabs) after ribosomal RNA depletion, following the manufacturer's instructions. The barcoded libraries were run on an Illumina® NovaSeq™ 6000 platform (paired-end, read length of 200 bp). Among the samples, three were excluded from the analyses since they failed to meet all the quality control standards during the library preparation.

Data were pre-processed using FastP v.0.21 with default settings and then aligned on Gencode annotations (v.40) using Salmon v.1.4 with default settings and with seqBias and gcBias options to correct for hexamer priming and GC biases, respectively. Read counts were normalized in transcripts per million (TPM) with the txImport R package v.1.22. Paired differential expression analysis was performed with the DESeq2 package (Wald test). A gene was considered differentially expressed if associated with an adjusted (adj.) $p < 0.05$ and a median TMP >1. The definition of CMS was performed from RNA-Seq data using CMScaller v2.0.1.

MetaFlux v0.0.09 [37] was applied to compute a Metabolic Reaction Activity Score (MRAS) of metabolic pathways based on the expression levels of enzyme-encoding genes. The tool was applied using the gene TPM levels as input of the calculate_reaction_score function.

2.6. Untargeted plasma metabolomics

Sample preparation and analysis methods have been previously described [38]. Briefly, a 30-µl aliquot of plasma was mixed with 200 µl of cold acetonitrile, vacuum-filtered through a 0.2 µm polypropylene filter well plate, and diluted 1:1 (v/v) with ultrapure water. The samples ($n = 450$, including 22 out of 26 participants of this study) were analyzed using a liquid chromatography-mass spectrometry system consisting of a 1290 Binary LC system with a reversed phase column and a 6550 QTOF mass spectrometer with an electrospray ionization source in positive ion mode (Agilent Technologies). Details on the metabolomic data analysis are reported in the Supplementary Materials of the manuscript.

Table 1

Clinical data of the study population and molecular characteristic of the tumors.

Subject characteristics	N = 26
Mean Age (range)	73 (51–86) years old
Sex	Female (11), Male (15)
Mean BMI (range)	25.6 (19.0–33.4), NA = 4
Smoking status	Never (11), Former (2), Current Smoker (10), NA (3)
Diabetes	No (18), Yes (4), NA (4)
Hypertriglyceridemia	No (20), Yes (2), NA (4)
Hypertension	No (12), Yes (10), NA (4)
Tumor localization	Proximal (15), Distal (9), Proximal/Distal (1), NA (1)
Stage	I (2), II (12), III (9), IV (2), NA (1)
Grade	G1-G2 (12), G3 (13), NA (1)
APC mutation	No (9), Yes (17)
KRAS mutation	No (17), Yes (9)
TP53 mutation	No (14), Yes (12)
MSI status	Stable (19), High (6), NA (1)
CMS	CMS1 (5), CMS1 (8), CMS1 (2), CMS1 (6), NA (5)
Mucinous component	No (16), Yes (10)

Abbreviations: APC, Adenomatous polyposis coli; BMI, body mass index; CMS, consensus molecular subtypes; KRAS, Kirsten ras oncogene homolog; MSI, microsatellite instability; TP53, Tumor protein 53. NA, Not available.

2.7. Shotgun metagenomic sequencing

Stool metagenomic sequencing library preparation was performed using the Illumina DNA Prep kit (Illumina) as previously described [39] and sequenced on the NovaSeq 6000 Sequencing System (Illumina) (read length of 300 bp paired-end). Read preprocessing and alignment on PhiX control and on the human genome were performed as previously reported [39]. Taxonomic profiling was performed with MetaPhlAn 4.1 and the ChocoPhlAn vJun23 database. MetaPhlAn was applied with default settings and with the *stat_q* option set to 0.1. Species levels were expressed as relative abundance. Low-abundant species were removed using the *nearZeroVar* function of the *caret* R package v7.0-1, and only species detected in more than 30 % of samples were selected for further analysis. Diversity metrics were computed using the *diversity* function of the *vegan* R package v2.5–6.1. An oral-to-gut introgression score was computed by counting the number of oral species detected in each sample or by computing the sum of their abundances as previously described [40].

2.8. Statistical analyses

Differences in the levels of continuous variables among study groups were evaluated by the Wilcoxon signed-rank test. Correlation analyses were performed using Spearman's method. Multiple-test correction was performed using the Benjamini-Hochberg (BH) method implemented in the *cor.test* function in R. For metabolomics and metagenomics correlation analyses, results were considered statistically significant when BH $q < 0.1$. Data analysis was performed with the R programming language v4.5.

The correlation network was designed in Cytoscape v3.10.3, with the Legend Creator v1.17 add-on used to generate the figure legend. The node shape was set to differ on feature type (DC/AGE levels = circles, metabolites = rhombi, and microbial levels = triangles). The node size was set proportional to the total node degree computed using the tool's "Analyze Network" function, while the edge color reflected the Spearman correlation coefficient. Edge thickness and transparency were set to be proportional to the absolute correlation significance. Only metabolites or microbial species correlated with at least two DCs or AGEs were reported in the network.

The R code used for the study analyses and the Cytoscape network (.cys) file are available at https://github.com/giuferrero/2025_Ferrero_et_al_AGE_CC.

3. Results

3.1. Study population

The study included 11 females and 15 males with a mean age of 73.0 ± 10.5 years (Table 1). Some of the patients presented with comorbidities, such as diabetes ($n = 4$), hypertriglyceridemia ($n = 2$), and obesity ($n = 4$), some concomitantly ($n = 2$). Tumors were classified as stage I-II ($n = 14$) and III-IV ($n = 11$), located in the proximal colon ($n = 16$) and the distal colon ($n = 10$). For all tumors, data from target sequencing of 532 genes (Illumina TSO500-HT) were available, allowing profiling of known oncogenes and tumor suppressors. From these data, *APC* was the most mutated known tumor suppressor gene ($n = 17$), followed by *TP53* ($n = 12$), and *KRAS* ($n = 9$). Nineteen tumors presented a stable microsatellite status, while six were high-MSI tumors.

Total RNA-Seq data were available for 25 tumors, of which 23 were matched with adjacent mucosa data, allowing the estimation of their consensus molecular subtype (CMS). The canonical subtype (CMS2) was the most represented ($n = 8$), followed by the mesenchymal subtype (CMS4, $n = 6$), and the MSI-immune subtype (CMS1, $n = 5$).

3.2. Characterization of AGEs and precursor DCs in the investigated biospecimens

The levels of different classes of protein-bound AGEs and their precursor DCs were analyzed in both tumor and adjacent normal mucosa biopsies of 26 CC patients (Fig. 1A and Table 1). The levels of these compounds were also evaluated in plasma ($n = 24$) and stool ($n = 19$) samples from the same subjects when biospecimens were available. The analysis was focused on MGO, GO and 3-DG and their main derived AGEs (the two MGO-derivatives N δ -[5-hydro-5-methyl-4-imidazol-2-yl]-ornithine (MG-H1) and N ϵ -[carboxy-ethyl]lysine (CEL), and the GO-derivative N ϵ -[carboxy-methyl]lysine (CML); Fig. 1A and Table 2).

Different ratios of DCs and AGEs were also evaluated to better define their chemical origin in relation to CC or adjacent mucosal metabolism. Specifically, the MGO/GO and MG-H1+CEL/CML ratios were computed to evaluate the balance between the two main AGE sources. The ratios between AGE derivatives and the corresponding precursor MG-H1+CEL/MGO were also computed to highlight differences between an early production of MGO and the accumulation of final products (Fig. 1A).

In tumor samples, a statistically significant increase in MGO was observed compared to adjacent mucosa ($p < 0.01$, Fig. 1B and Table 2). This specific accumulation of MGO and MGO-derived AGEs in tumor tissues was also confirmed at the single patient level (Fig. 1C). Indeed, for MGO, most of the patient-specific ratios were positive with more than 2-fold increases of this DC in tumor tissue (Fig. 1C). Conversely, the accumulation of the GO derivative CML was dominant in the adjacent mucosa (negative ratios in Fig. 1C). Moreover, the markedly lower ratio between the MG-H1+CEL and MGO suggested that in CC there is an increased production of MGO with relatively lower levels of its direct derivatives that are higher in the adjacent mucosa (Fig. 1B). There was no significant difference in 3-DG concentrations between tumor and adjacent mucosa (Fig. 1B).

Although the production of AGEs is enhanced in diabetes and dyslipidemia [41], no significant associations were found between DC/AGE levels and these comorbidities in our dataset. However, the assessment of their impact on DC/AGE levels in the investigated biospecimens was limited, since only four diabetic and two hyperlipidemic patients were included (Supplementary Fig. S1).

The levels of DCs, AGEs, and their ratios, with respect to patient demographic or clinical covariates were further analyzed. The most significant associations were observed in tumor samples. Among the strongest associations, MGO levels were significantly higher in CMS4 and lower in CMS3 tumors (for both comparisons, $p < 0.01$) compared to the other subtypes (Supplementary Table 1). None of the considered DCs/AGEs was associated with a specific staging, grading, MSI status, or known oncogene mutations. Similarly, a few significant results were observed considering either plasma or stool levels, probably due to the limited sample size of the cohort (Supplementary Table 1).

The analysis of the levels of DCs/AGEs between tumor and adjacent mucosa pairs stratified for CMS indicated that CMS1 tumors had significant increases in MGO and CMS2 tumors had markedly lower levels of CML, compared to the respective adjacent mucosa (Supplementary Fig. S2). There was no difference in DC/AGE levels between CC and adjacent mucosa observed for CMS3. Conversely, CMS4 tumors had markedly higher levels of MGO, paralleled by a significant reduction in CML, when compared to adjacent tissue (Supplementary Fig. S2).

3.3. Comparison of tissue DC/AGE concentrations with their plasma and stool sample levels

To assess whether the DC/AGE production in either CC or adjacent mucosa was reflected in the whole organism, AGEs were measured in plasma and stool. Only MG-H1 levels in both CC and adjacent tissues were significantly correlated to their plasma concentrations ($Rho = 0.44$ – 0.51 , $p < 0.05$, Fig. 2A and B). Conversely, no correlation was

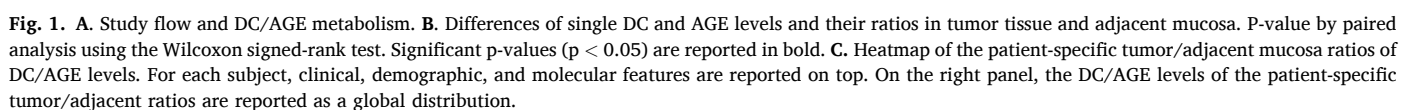


Table 2
Average levels of precursors DCs and their AGE derivatives in the biospecimens analyzed.

DCs, AGEs, and ratios (nmol/L)	Plasma N = 24 ^a	DCs, AGEs, ratios (nmol/g protein)	Stool N = 19 ^a	Adjacent N = 26 ^a	CC N = 26 ^a	log2FC (CC/Adj.)	p-value ^b
MGO	643 ± 243	MGO	804 ± 359	222 ± 231	410 ± 546	0.89	0.003
GO	580 ± 314	GO	577 ± 499	128 ± 118	88 ± 22	−0.54	0.50
3-DG	1422 ± 462	3-DG	141 ± 386	12 ± 5	10 ± 4	−0.26	0.11
CML	2483 ± 653	CML	47 ± 25	20 ± 6	13 ± 3	−0.62	<0.001
CEL	1594 ± 1159	CEL	37 ± 42	12 ± 3	10 ± 3	−0.26	0.07
MG-H1	1645 ± 590	MG-H1	49 ± 57	17 ± 5	19 ± 9	0.16	0.50
MGO/GO	1.27 ± 0.57	MGO/GO	2.91 ± 2.98	2.63 ± 4.04	4.76 ± 5.50	0.86	0.006
MG-H1+CEL/CML	1.33 ± 0.48	MG-H1+CEL/CML	1.75 ± 1.35	1.53 ± 0.49	2.30 ± 0.59	0.59	<0.001
MG-H1+CEL/MGO	5.53 ± 2.54	MG-H1+CEL/MGO	0.12 ± 0.11	0.20 ± 0.11	0.12 ± 0.06	−0.74	0.004

^a Mean ± standard deviation.

^b Paired Wilcoxon signed-rank test with continuity correction for the CC vs Adjacent mucosa comparison.

found between AGE levels in CC or adjacent mucosa and their relative content in stool (Supplementary Fig. S3A and S3B).

Then, the increased production of MGO in CC or adjacent mucosa was investigated in relation to an increase of its derivative MG-H1 outside the tumor microenvironment (i.e., in plasma and stool). The analysis suggested that the production of MGO in tissues did not appear to influence the circulating and stool levels of MG-H1 (Supplementary Fig. S3C and S3D).

3.4. Integration analysis with tumor/adjacent mucosa transcriptomic profiles

A paired differential expression analysis of the available RNA-Seq data showed 9706 differentially expressed genes (adj. $p < 0.05$), of which 5242 and 4464 were respectively up- and down-regulated in tumor tissue (Fig. 3A and Supplementary Table 2A). As expected, the up-

regulated genes were enriched (adj. $p < 0.001$) in pathways related to cell proliferation and DNA repair, while the down-regulated genes were enriched in metabolism-related processes (Supplementary Table 2B).

Since AGE production is tightly dependent on cellular metabolism, the RNA-Seq data were further exploited to compute, for each sample, an activity score (MRAS score) of 142 metabolic pathways estimated from the expression levels of metabolic enzyme-encoding genes [37]. Initially, the pathway scores were compared between tumor and adjacent tissue, which generated 49 pathways with a statistically significant (adj. $p < 0.05$) differential activity score between the two sample types (Fig. 3B and Supplementary Table 2C). Notably, most of these pathway activities ($n = 38$) were predicted to decrease in tumor tissue, particularly those related to *oxidative phosphorylation*, *beta-oxidation of unsaturated fatty acids*, and *steroid metabolism*. Conversely, *porphyrin metabolism*, *folate metabolism*, *bile acid recycling*, and *cholesterol biosynthesis* were among the pathways with a significantly higher predicted

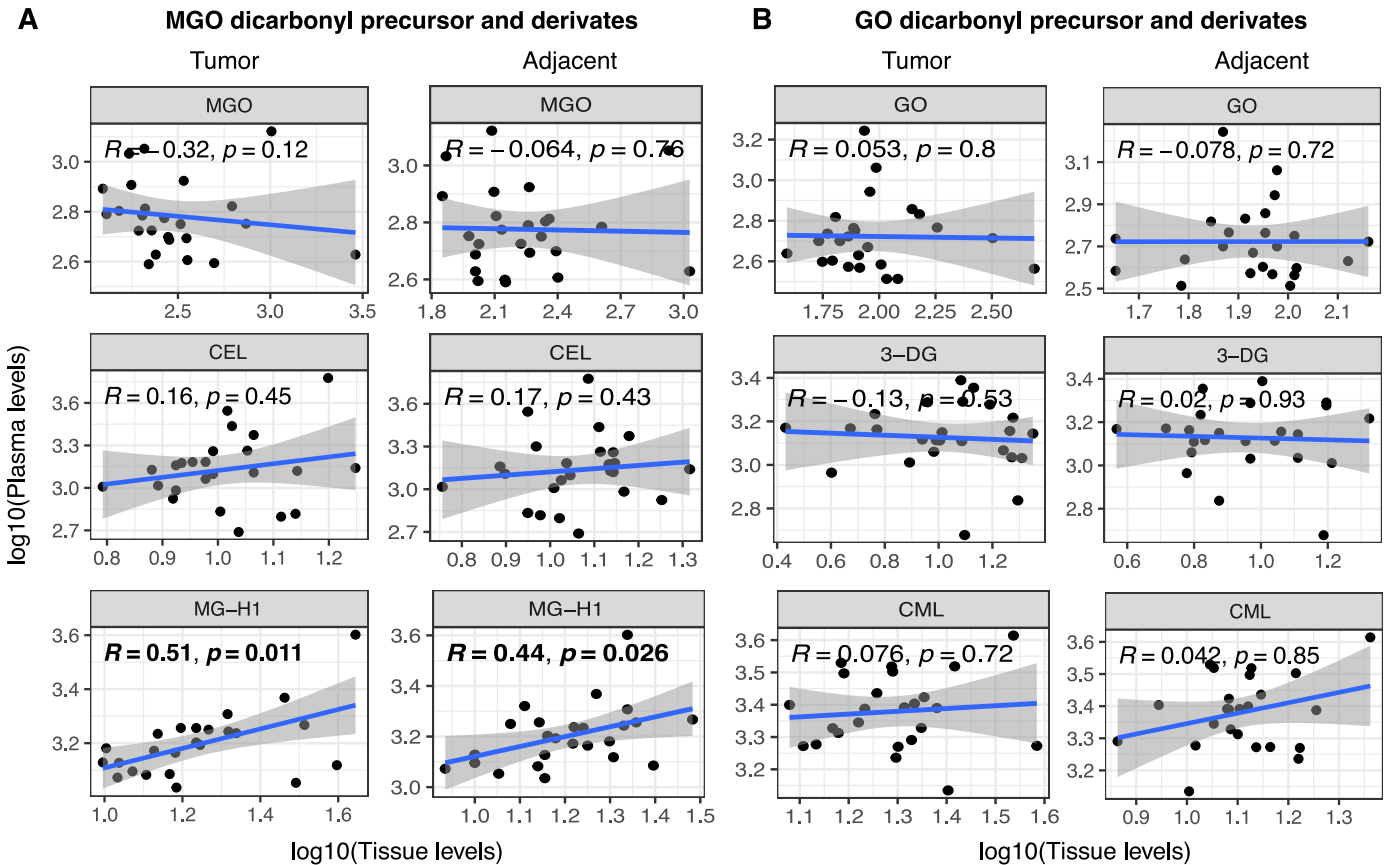


Fig. 2. MGO (A) or GO (B) and their relative derivatives in tumor and adjacent mucosa in relation to their levels in plasma samples. Results from Spearman's correlation analyses are reported.

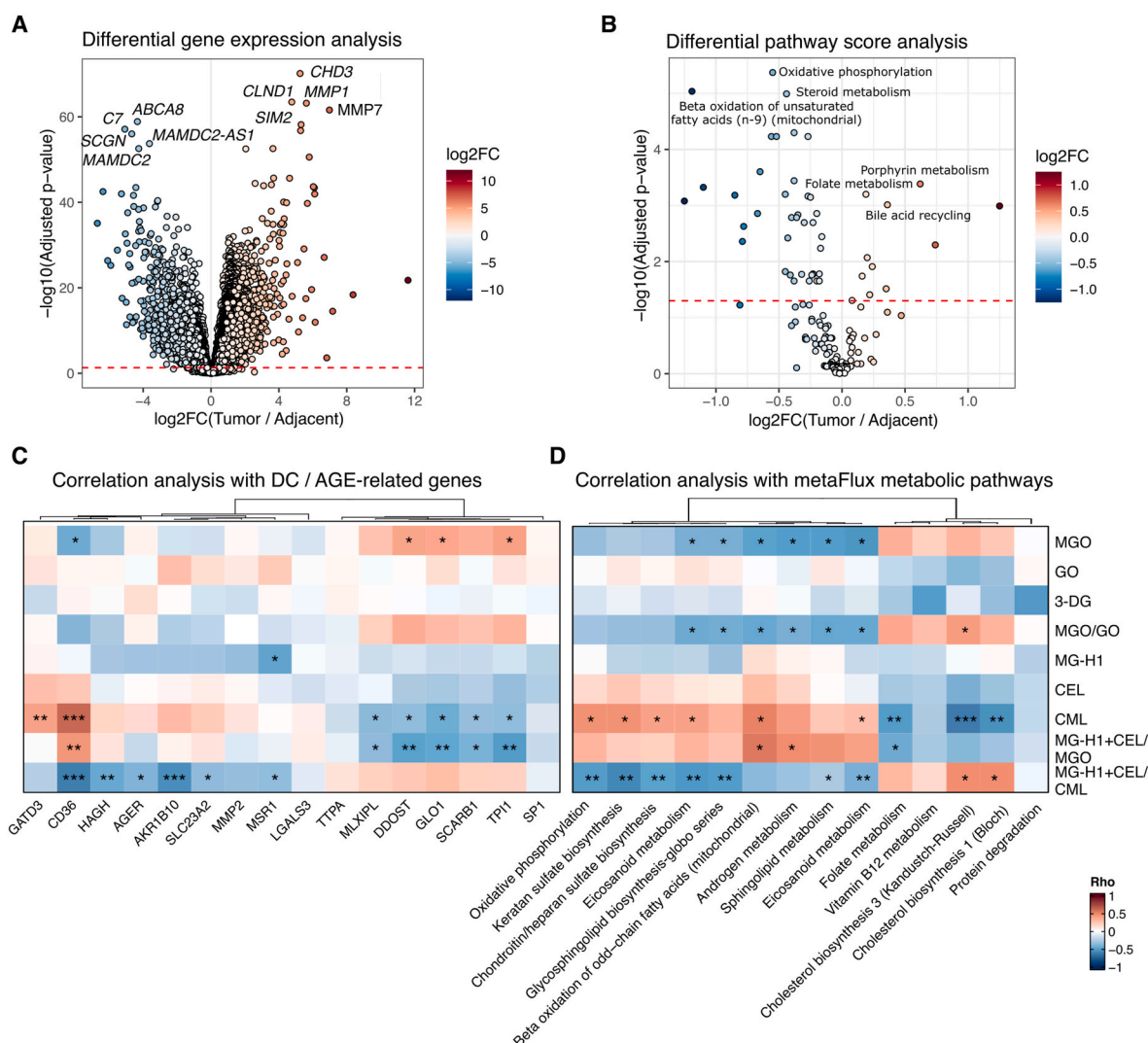


Fig. 3. **A.** Volcano plot of the paired differential gene expression analysis in the investigated comparison between tumor and adjacent mucosa samples. **B.** Volcano plot of the differential analysis of the Metaflux MRAS scores computed for tumor and adjacent mucosa samples. The red dashed line indicates the statistical threshold of 0.05. **C-D.** Heatmaps reporting the Spearman correlation analysis results obtained by comparing the DC/AGE levels with respect to selected AGE-related gene expression levels (**C**) and metabolic pathways from the Metaflux analysis (**D**). Adjusted p-value from BH correction; ***adj. $p < 0.001$, **adj. $p < 0.01$, and *adj. $p < 0.05$.

activity in tumor tissue.

Thereafter, the expression profiles of 16 genes known to be involved in AGE production and detoxification were analyzed in addition to genes involved in oxidative stress and inflammatory signaling that could be related to their altered levels in cancer (Supplementary Table 2D). Among these genes, *MLXIPL*, *GLO1*, *TPI1*, *SCARB1*, and *DDOST* were significantly up-regulated, while *CD36*, *AKR1B10*, *LGALS3*, *HAGH*, and *AGER* were significantly down-regulated in tumor tissue with respect to adjacent mucosa (Supplementary Table 2D). Analysis of the same genes in a total RNA-Seq dataset of 27 precancerous lesions matched with adjacent mucosa [42] confirmed a significant ($p < 0.05$) increase of *MLXIPL*, *GLO1*, *TPPA*, and *SCARB1* and a decrease of *CD36*, *MMP2*, *AKR1B10*, *LGALS3*, *HAGH*, and *MSR1* in lesions.

A Spearman correlation analysis was computed between the expression of the 16 genes and the DC/AGE levels detected in the same samples (Fig. 3C and Supplementary Table 2E). MGO was positively correlated ($\rho = 0.40$, adj. $p < 0.05$) to the *TPI1* gene coding for the glycolytic enzyme that interconverts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate, which is then converted to MGO. This was paralleled by positively correlated expression of the *GLO1* and *DDOST* genes encoding for the main AGEs detoxifying systems (for both

$\rho = 0.38$, adj. $p < 0.05$). In addition, MGO tumor concentrations were negatively associated with *CD36* expression ($\rho = -0.44$, adj. $p < 0.05$). Conversely, MG-H1+CEL/MGO ratio was positively correlated with *CD36* ($\rho = 0.44$, adj. $p < 0.01$) and inversely to *SCARB1* expression ($\rho = -0.41$, adj. $p < 0.05$). Moreover, AGEs were negatively correlated with *TPI1*, *GLO1* and *AGER* expression, suggesting that when DCs are converted to the advanced products, glycolysis is decreased, and AGEs detoxification is impaired. Then, the analysis was performed considering the plasma and stool DC/AGE levels with respect to AGE-related gene expression in tumor and adjacent mucosa (Supplementary Fig. S4A–D and Supplementary Table 2E). The correlations between tumor *GLO1* levels and the fecal CML and CEL concentrations were among the most statistically significant results ($\rho = 0.62$ and 0.81 , respectively; adj. $p < 0.05$; Supplementary Fig. S4C), as well as negative correlations between *MMP2* and *SLC23A2* tumor expression and MGO plasma measures ($\rho = -0.64$ and -0.69 , respectively; adj. $p < 0.01$; Supplementary Fig. S4A).

A Spearman correlation analysis was also performed between the MRAS scores and the DC/AGE levels. Interestingly, negative correlations for precursors DCs and their final AGE derivatives with metabolic pathways were observed (Fig. 3D and Supplementary Table 2E). For

instance, the higher production of MGO in tumor than in the adjacent mucosa, and the higher ratio of MGO-derived MG-H1+CEL compared to GO-derived CML in tumor were negatively correlated (adj. $p < 0.05$) to oxidative phosphorylation and fatty acid oxidation, indicating that reduced mitochondrial metabolism, as expected, is associated with increased production of MGO and its derivatives through anaerobic glycolysis. Moreover, the higher tumor/adjacent mucosa levels of MGO and its derivatives were related to lower activity of metabolic pathways modulating the synthesis of bioactive intermediates involved in inflammation, oxidative stress response and cancer progression. However, the prevalence of MGO and its derivatives in tumor was also associated with up-regulation of folate, vitamin B12, and cholesterol

synthesis, all molecules suspected to exert a role in colon carcinogenesis [43–46].

3.5. AGE and DC levels in relation to plasma untargeted metabolomic profiles

The DC/AGE levels and ratios were also explored with respect to circulating metabolites in plasma.

From untargeted metabolomic profiles, a total of 602 features were detected in all samples. Four hundred fifty-five significant correlations among plasma metabolites and DC/AGE levels ($p < 0.01$, $n = 196$ with $q < 0.1$) measured in plasma ($n = 216$), tumor ($n = 125$), adjacent mucosa

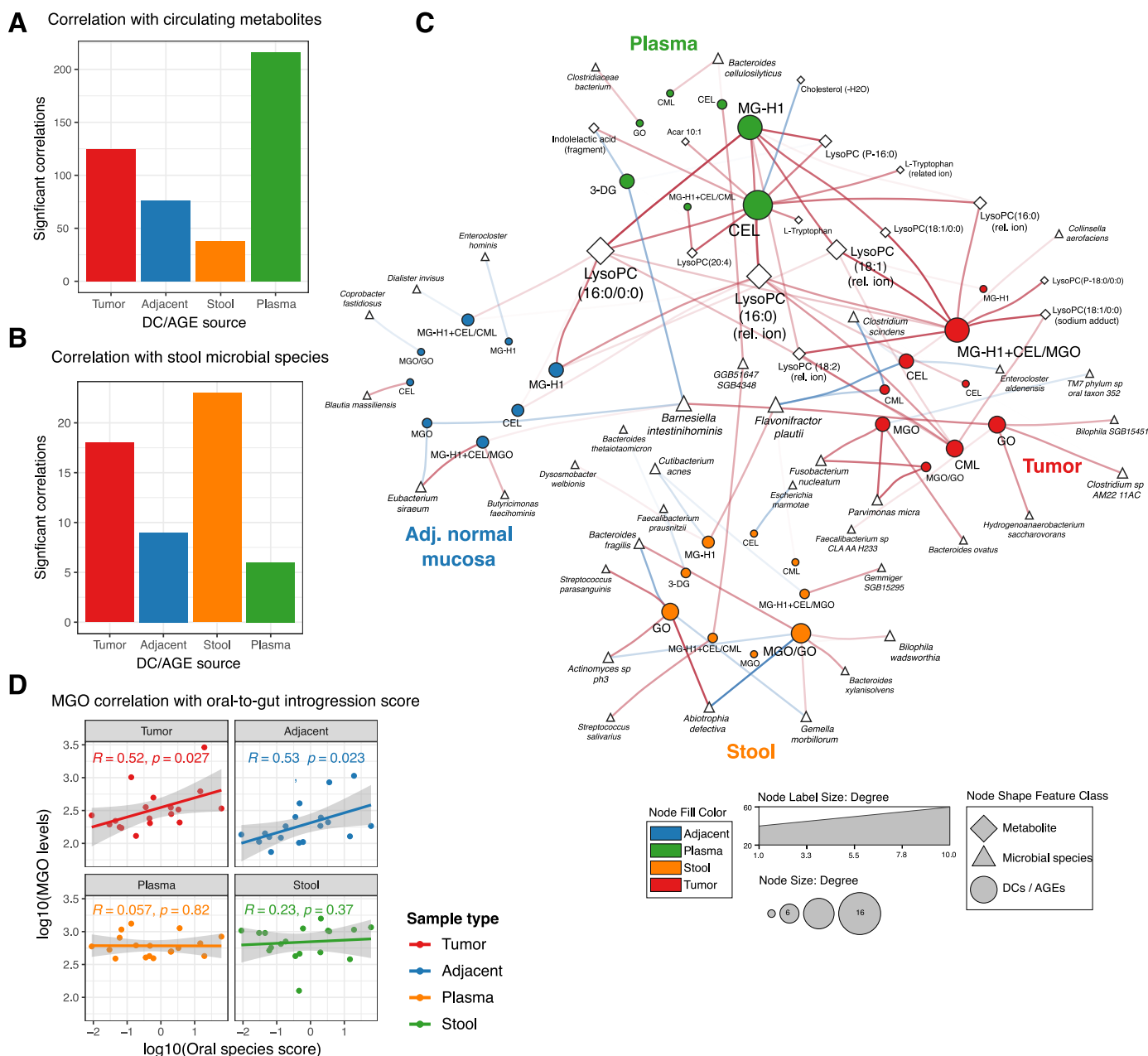


Fig. 4. A–B. Bar plot of the number of significant correlations (Spearman method, $p < 0.01$) between the DC/AGE levels measured in the four biospecimens and the levels of circulating metabolites (A) or the fecal abundance of microbial species (B). C. Network representation of significant correlations obtained with Cytoscape with the add-on Legend Creator. In the analysis of DC/AGE levels (circles) with respect to circulating metabolite (rhombus) and fecal microbial levels (triangles). The node size is proportional to the node degree while the edge color code reflects the computed Spearman correlation coefficient. Edge thickness and transparency are proportional to the correlation significance. Only metabolites/species correlated with at least two DCs or AGEs are reported. D. Scatterplot showing the MGO levels in different biospecimens with respect to the oral-to-gut introgression score computed as the sum of oral microbial species abundances. Results from the Spearman correlation analysis are reported.

($n = 76$), and stool ($n = 38$) were observed (Fig. 4A and Supplementary Table 2E). In tumor, the MG-H1+CEL/MGO ratio and CML levels were associated with the highest number of significant correlations (respectively 55 and 24), while in plasma, levels of CEL and MG-H1 were those related with the highest number of metabolites (respectively 88 and 40).

Out of the 73 metabolic features involved in the most significant correlations ($q < 0.05$), 27 were annotated, corresponding to nine individual metabolites (Supplementary Table 2E). Different lysophosphatidylcholines (LysoPCs 16:0, 18:1, 20:4), as well as two plasmalogen LysoPCs (P-16:0 and P-18:0), were among the metabolites characterized by the most significant associations, all being positively associated with the DC/AGE level in tumor tissue. Other annotated metabolites included tryptophan and indolelactic acid, a gut microbial metabolite of tryptophan, both of which significantly correlated with circulating CEL levels ($\rho = 0.66$ – 0.77). In addition, with an opposite trend, decenoylcarnitine (acylcarnitine C10:1) and cholesterol were also found to be related to CEL plasma levels ($\rho = 0.66$ and -0.68 , respectively).

3.6. AGE and DC levels and the gut microbiome composition

Fecal metagenomic profiling yielded an average of 171 ± 106 detected species per sample. No statistically significant results were obtained by the correlation analysis between DC/AGE levels and microbial alpha diversity metrics (Richness, Shannon, and Inverse-Simpson indexes) (Supplementary Table 2E). Thereafter, correlations were assessed between the relative abundance of each detected microbial species and the DC/AGE levels. The analysis identified 56 significant associations ($p < 0.01$; $n = 8$, $q < 0.1$) involving DC/AGE levels measured in stool ($n = 23$), tumor tissue ($n = 18$), adjacent mucosa ($n = 9$), and plasma ($n = 6$) (Fig. 4B and C, and Supplementary Table 2E). The most significant association was between GO levels in stool and the abundance of the bacterium *Abiotrophia defectiva* ($\rho = 0.83$, $q < 0.01$), which was also inversely related to the MGO/GO ratio ($\rho = -0.83$, $q < 0.01$). The most significant correlations with AGEs in tumor tissue included well-known species implicated in colorectal carcinogenesis [47] like *Parvimonas micra* and *Fusobacterium nucleatum*, all significantly correlated with the MGO/GO ratio ($\rho = 0.71$ and 0.73 , respectively; $q < 0.1$). Consistently, tumor MGO levels positively correlated with the relative abundance of *P. micra* ($\rho = 0.74$, $q < 0.1$) and *F. nucleatum* ($\rho = 0.71$, $q < 0.2$).

Given the positive correlations between tumor MGO levels and these oral species (*P. micra* and *F. nucleatum*), an oral-to-gut introgression score was computed for each sample considering the number and abundance of detected oral species. Notably, significant correlations were observed between the tumor and adjacent tissue levels of MGO ($q < 0.1$) and of the MGO/GO ratio ($q < 0.01$) with respect to this parameter (Fig. 4D).

4. Discussion

In the present study, we set out to investigate whether patterns of DCs and the corresponding AGEs formed from them vary in CC compared with adjacent tissues, and whether this is reflected in plasma or stool collected from the same CC patient. We focused our investigation on the most abundant and well-characterized protein-bound AGEs in human tissues and biofluids: MG-H1 and CEL, which are the main derivatives of MGO, plus CML, the main AGE derived from GO.

A higher MGO concentration was among the most significant differences observed between tumor tissue and matched adjacent mucosa. Integrated multiomics analyses of the cancer transcriptome, gut microbiota, and systemic metabolome support MGO as a potentially important metabolite in colonic carcinogenesis and warrant further exploration.

Higher levels of MGO in tumor tissue are not surprising considering that its chemical origin is mainly from the degradation of triosephosphates from the glycolytic flux [48], which is known to be enhanced in cancer cells due to the Warburg effect [49]. In contrast, GO,

mainly produced from lipid peroxidation, was equally abundant in both tumor and adjacent tissues. The lack of significant differences in GO levels between tumor and adjacent mucosa likely reflects the fact that both tissues are exposed to similar oxidative and inflammatory conditions, fueling lipid peroxidation in the context of CC. The higher accumulation of CML in adjacent mucosa may be due to increased GO production and/or differences in detoxification or clearance mechanisms. These findings underscore the importance of considering the distinct metabolic origins and regulatory pathways of different dicarbonyls and AGEs when interpreting their roles in CC biology.

Previous studies have investigated the higher production rate of MGO by tumor cells and reported a controversial role in cancer progression and malignancy [50]. For instance, a recent study found that MGO levels were higher in peritumor tissue than in tumor tissue and reported that MGO levels gradually increased in both tissue types from low-grade dysplasia to invasive carcinoma [51]. Accordingly, a study on human CRC biopsies and disease progression observed high levels of MGO-derived AGEs, specifically MG-H1, and low GLO1 activity in high-stage and highly glycolytic tumors compared to low-stage lesions. Authors also found that the MGO scavenging reduced tumor growth in *in vivo* models [23]. Previously, in tumor with *KRAS* mutations, an enhanced accumulation of MGO had been observed due to higher glycolytic flux in these mutated cells, and the targeting of MGO production restored the *KRAS*-related drug sensitivity in the cancer cell lines [52].

In the present investigation, high heterogeneity in CC was also observed for DC/AGE levels when tumors were stratified by CMS. Interestingly, MGO levels were higher in CMS4 and CMS1 subtypes. These subtypes are respectively associated with epithelial-to-mesenchymal transition features (i.e., activation of TGF- β signaling, angiogenesis, matrix remodeling pathways) and high expression levels of genes involved in activation of innate and adaptive immune response [53]. Accordingly, studies have suggested that an increased flux of aerobic glycolysis could induce a “mesenchymal phenotype” in CRC cells, promoting lactate accumulation and consequent acidification of the tumor microenvironment [54].

Our findings also highlight a prevailing accumulation of the arginine-derived MG-H1, rather than the lysine-derived CEL, among the main MGO-related AGEs. However, the precursor DCs were more prevalent in the tumor compared to the adjacent mucosa than the corresponding AGE derivatives. These observations may suggest a temporal sequential regulation with the early production of DCs involved firstly in cancer onset and inflammation, and the subsequent accumulation of the AGE derivatives later in cancer progression. Moreover, it is not possible to date to discriminate whether tumor also uptakes DCs or AGEs from the circulation or the colonic environment. This indicates that measuring only circulating AGE concentrations is only partially informative, requiring consideration of their chemical origins and presence in other relevant biosamples [55]. The present study addresses this gap.

The role of DCs and AGEs in carcinogenesis, cancer progression, and metastasis has been extensively debated, with results from *in vitro* and clinical studies suggesting a role for generic AGE production and activation of the AGE-RAGE axis [20]. However, the complexity of molecules and chemical pathways leading to glycation and the enormous range of metabolic and genetic features of the different cancer diseases have impeded definitive conclusions. The peculiar production of DCs/AGEs in tumor tissue does not seem to correlate directly with circulating or stool levels, except for the plasma concentrations of MG-H1, which reflect those in CC tissue. Notably, the production and accumulation of the MGO-derived AGE MG-H1 is not exclusively a local phenomenon within the tumor microenvironment but may instead reflect a systemic glycation status. Even though MG-H1 is not tumor-specific, its presence in plasma may serve as a marker of systemic metabolic alterations that, in turn, can influence the intestinal microenvironment and tumor progression. Unfortunately, in the present study, we could not compare plasma and stool levels of MG-H1 in colon

cancer patients and cancer-free subjects. However, our study demonstrates that while local production of MGO, the precursor of MG-H1, is increased in tumor tissue, MG-H1 levels are distributed both locally and systemically.

DCs and free AGEs are highly reactive molecules and, whenever released from tumor cells in the gut lumen, they can be rapidly metabolized by other cells of the gut microenvironment, including specific microbial species [56]. Consistently, we found that tumor MGO levels were related to the abundance of two microbial species, namely *P. micra* and *F. nucleatum*, which are known to be increased in CC patient samples [47]. Although a definitive causal role of these bacteria in CRC initiation and progression is still a matter of debate, an enrichment of *F. nucleatum* in feces from CRC patients has been related to poor prognosis, chemoresistance, and recurrence [57]. Moreover, colonization of colorectal carcinomas by *P. micra* has been correlated with decreased CRC patients' survival by enhancing tumorigenesis activity through epigenetic reprogramming of intestinal cells and alteration of the Th17-mediated immune response [58]. *P. micra* and *F. nucleatum* are oral bacteria previously found to be more abundant in gut microbiota of CRC patients, mostly associated with tumors classified as CMS1 [59]. Here, the positive correlation between tumor MGO/GO ratio with these microbial species, and with the oral-to-gut introgression score, revealed novel insights into the mechanisms by which tumor metabolism (i.e., increased MGO production) might affect microbial colonization with the selection of specific species that influence cancer characteristics. Notably, a recent study showed that the chronic exposure of mice models to MGO promotes an alteration of the intestinal morphology and inflammation levels with effects on the gut microbiota composition [60]. Vice versa, the gut colonization of these bacteria can promote a glycolytic tumor metabolic phenotype as previously observed [61], which might be the cause of the observed MGO increase.

In addition, DC/AGE levels in stool were correlated to specific microbial abundances, like for instance, fecal GO levels and *Abiotrophia defectiva*. *A. defectiva* is an oral and gut nutritionally Gram + streptococcus and an etiologic agent of infective endocarditis, harboring relevant pro-inflammatory enzymes. As far as we are aware, the relationship between *A. defectiva* and glycation or CC has never been reported. However, other streptococci, such as *Streptococcus gallolyticus*, have been associated with both endocarditis and CRC [62]. Thus, GO, as well as the other DC/AGE molecules in stool, might also be byproducts reflecting the metabolism of specific microbial species.

To unravel the influence of the specific tumor DC/AGE content on CC cell gene expression, genes implicated in DC/AGE metabolism were investigated. The highest correlation found between tissue DC levels and gene expression involves the glycolysis-derived MGO rather than the lipid peroxidation-derived GO. MGO correlates with genes encoding for proteins involved in DC/AGE production and detoxification, such as Triosephosphate Isomerase 1 (*TPI1*), AGE-receptor-1 (*DDOST*) and glyoxalase-1 (*GLO1*), and negatively correlates with the expression of the scavenger receptor *CD36*. This is in line with the significant upregulation of the first three genes and the downregulation of the latter in our tumor RNA-Seq data. Notably, *GLO1* up-regulation has been detected in aggressive tumors, as a defense mechanism against MGO toxicity [63]. Similarly, the prevalence of the final products of both MGO and GO (CML and MG-H1+CEL/MGO) strongly correlates with the expression of the same genes (*TPI1*, *DDOST*, *GLO1*, *CD36*), but in the opposite direction compared to MGO, suggesting a kind of dysregulation or exhaustion of the systems responsible for DC metabolism and degradation throughout the progression of the glycation process. In addition, a slightly significant downregulation (adj. $p = 0.046$) of *AGER* was observed in tumor tissue compared to adjacent mucosa. Despite this being counterintuitive with respect to previous findings [20], in our samples, the expression levels do not appear to reflect a substantial involvement of this gene in the observed DC level differences and the associated alterations in the metabolome and microbiome we reported.

In addition, both MGO and protein-bound AGEs were strongly

correlated to alterations of specific metabolic and stress-responsive pathways in the CC microenvironment. Specifically, the prevalence of MGO in CC compared to adjacent mucosa might be linked to the down-regulation of metabolic pathways involved in the activation of the inflammatory response, i.e., eicosanoid and sphingolipid metabolism, that are instead up-regulated by protein-bound AGEs, either deriving from MGO or GO. It has also been suggested that MGO, and not its derivatives, is involved in the regulation of immune cell recruitment and activation [64], which represents a crucial aspect of cancer immunity. In this perspective, the modulation of MGO production by cancer cells has been proposed as a potential target for potentiating immune surveillance against cancer [63]. Interestingly, an opposite direction in the relationships between MGO or AGE derivatives with many of the altered pathways was detected. This observation further strengthens the consideration that the impact of DC/AGE metabolism in CC may differ depending on the chemical origin of the prevailing molecules and the progression of the glycation process.

We thus explored the correlations between DC/AGE levels assessed in different compartments and plasma metabolites to identify potential biomarkers of glycation related to CC. Plasma levels of DCs/AGEs were highly correlated with circulating metabolite levels, and one of the most significant correlations was found between LysoPCs and the plasmatic CEL and MG-H1 levels, and adjacent mucosa levels of this AGE were also correlated. This may be the result of the increased tumor production of MGO-derived AGEs accumulating in adjacent colonic tissue and in the blood. LysoPCs are components of oxidized LDL involved in the atherosclerotic process [65]. They have been found to be increased in the plasma of diabetic patients and correlated with glycation and risk of atherosclerosis [66]. Notably, among the identified LysoPCs, two (P-16:0 and P-18:0) are classified as plasmalogens, a lipid class whose circulating levels were associated with CRC [67]. In addition, in plasma, a positive correlation for both tryptophan and its gut microbial metabolite indolelactic acid with CEL was observed. Notably, although tryptophan was previously reported to influence the AGE production [68], we did not find evidence for the role of indolelactic acid in this process. These data support an active influence of microbial products on AGE metabolisms, which definitely deserve further investigations.

This study presents some limitations. The main issue is the limited number of CC patients in which the DC/AGE profiles were analyzed, considering the high tumor heterogeneity and the potential interactions with the available omics data. However, access to different biospecimens from the same CC patients gives the study a unique relevance to the results. In this respect, rectal cancers were not analyzed in this study. Taking this aspect into account, the results obtained by multi-specimen and multi-omics analyses were more homogeneous. Thus, the several strongly significant correlations detected between MGO and MGO-derived AGEs across the different specimens, as well as the transcriptome, metabolism, and microbiota alterations, need to be further validated in a larger cohort of subjects. In addition, the analysis of the matched adjacent mucosa from cancer patients may underestimate the extent of MGO and other DCs/AGEs, as metabolic and molecular alterations also characterize the tissue surrounding a neoplastic lesion. However, we considered the adjacent mucosa taken from cancer patients as a potential good surrogate for non-cancer tissue with the additional advantage that the genetic background and dietary habits are not potential confounders since the two tissue types come from the same subject. However, analyzing DCs and AGEs in the mucosa of healthy subjects and in precancerous lesions, despite not being trivial to collect, would be critical to better understand the extent of alteration in these compounds during cancer onset and progression.

Although dietary AGEs may be potential confounding factors that may partially influence the interpretation of plasma and fecal MG-H1, CML, and CEL levels, the contribution of dietary AGEs to the body pool is estimated to be limited due to the low rate of intestinal absorption and substantial excretion with urine [26,69]. In the present study, dietary patterns were not available for most of the subjects; therefore, it

is difficult to evaluate the extent to which biofluid AGE concentrations reflect tumor-related processes versus dietary exposure. In addition, the effects of comorbidities, including diabetes, hypertriglyceridemia, and obesity, remain to be better explored in this context since our cohort did not provide enough statistical power to investigate their impact on DC/AGE levels in tumor tissue and patient biofluids.

In any case, data from an independent validation set will be required to assess whether the observed results can be generalized to patients with other CC types and, obviously, to rectal cancer patients as well. On the other hand, it must be clear that we measured correlations between MGO/AGEs and specific aspects that may be due to several other factors and thus may not correspond to actual MGO effects. In this perspective, more focused investigations are needed in both animal models and humans.

This study depicts a complex network of interrelationships among the pattern of DC/AGE in different body compartments with alterations in gene expression, metabolic pathways, immune response, and gut microbiota composition. This suggests that a specific DC/AGE profile in CC tissues may provide useful information for diagnosis, classification and prognosis. Finally, more targeted investigations, prioritizing MGO metabolism and performed on larger patient cohorts, should better elucidate the potential role of DCs and their derivatives in CC.

CRediT authorship contribution statement

Giulio Ferrero: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Raffaella Mastrocola:** Writing – original draft. **Sonia Tarallo:** Writing – review & editing, Investigation, Data curation. **Barbara Pardini:** Writing – original draft, Investigation, Data curation. **Jean Scheijen:** Investigation. **Marjo van de Waarenburg:** Investigation. **Gaetano Gallo:** Data curation. **Anastasia Chrysouvalantou Chatziioannou:** Investigation, Data curation. **Nivonirina Robinot:** Investigation. **Pekka Keski-Rahkonen:** Resources, Investigation. **Gianmarco Piccinno:** Supervision. **Nicola Segata:** Supervision, Resources. **Elom K. Aglago:** Supervision, Investigation. **David J. Hughes:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Mazda Jenab:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Casper G. Schalkwijk:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Alessio Naccarati:** Supervision, Funding acquisition, Data curation, Conceptualization.

Ethics approval and consent to participate

Subjects participating in the study signed a written consent, in agreement with the Helsinki Declaration. The design of the study, informed consent, and protocols were approved by the local Ethics Committees (Azienda Ospedaliera “SS. Antonio e Biagio e C. Arrigo” of Alessandria. ID: Colorectal miRNA CEC2014).

Consent for publication

Not applicable.

Availability of data and materials

Data are available upon reasonable request. All data associated with this study and its supplementary data files are available within the article.

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this presentation and they do not necessarily represent the decisions, policy or views of the

International Agency for Research on Cancer/World Health Organization.

No relevant conflicts of interest exist for all the authors.

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Competing interests

This publication reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains.

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Abbreviations

3-DG	3-deoxyglucosone
AGE	Advanced Glycation End-products
BH	Benjamini Hochberg
CC	Colon cancer
CEL	Nε-[carboxy-ethyl]lysine
CML	Nε-[carboxy-methyl]lysine
CMS	Consensus Molecular Subtype
CRC	Colorectal cancer
DC	Dicarbonyl Compound
GO	Glyoxal
LysoPC	Lysophosphatidylcholines
MGO	Methylglyoxal
MG-H1	N8-[5-hydro-5-methyl-4-imidazol-2-yl]-ornithine

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.freeradbiomed.2026.01.034>.

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