The Human Metabolome is Prognostic in Colorectal Cancer

A systematic review and metabo-meta-analysis of the prognostic value of metabolomics with genomic single nucleotide polymorphism cross-talk for colorectal cancer recurrence and five year overall survival

C.A. Fleming[1,2], H. M. Mohan[3], D. P. O'Leary[4], J Kirwan[5], H. P. Redmond[2,6]

1 Department of Colorectal Surgery, Cork University Hospital, Cork
2 Department of Academic Surgery, Cork University Hospital, Cork
3 Department of Colorectal Surgery, St Vincent's University Hospital, Dublin
4 Department of Surgical Oncology, Bon Secours Cork Cancer Centre, Cork
5 Metabolomics Facility, BIH, Max Delbrück Centre for Molecular Medicine, Berlin
6 Department of Surgical Oncology, Cork University Hospital, Cork

*Supported by a European Association for Cancer Research Travel Fellowship

Background

- Metabolomics may be defined as the comprehensive study of the metabolome, a collection of all end-points of metabolism in an organism or biologic system at a given point in time and is the end point of analysis in the omics-cascade[1]
- In its simplest form the omics-cascade proceeds from genomics to transcriptomics, proteomics and metabolomics however multi-level cross talk can also occur[1]
- Specific environmental factors impact transition along the omics cascade and metabolomics therefore most accurately represents the posttranscriptional cellular phenotype compared to upstream omics within the omics-cascade
- Cancer cells undergo metabolic transformation to sustain fast cell growth and proliferation resulting in different metabolic phenotypes in cancer cells compared with their control counterparts[2,3]
- Identifying differential metabolites has potential as diagnostic and prognostic markers in cancer and identifying therapeutic targets[4,5]

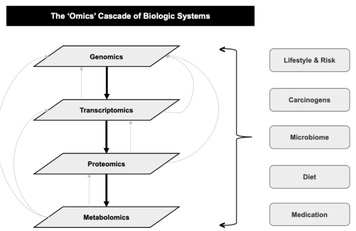


Figure 1: The 'omics' cascade of biological systems proceeds from genome to transcriptome, proteome and finally metabolome. Biologic cross-talk can occur from and within all levels and each level may be heavily influenced by environmental factors

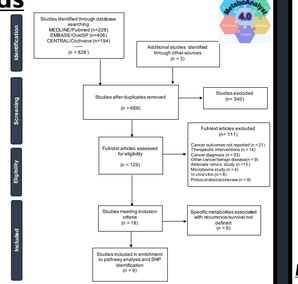
Methods

Search Strategy and Quality Assessment

- A systematic review of studies utilising metabolomics to Identify patients at risk of cancer recurrence and poor survival outcomes in CRC was performed in keeping with PRISMA guidelines to include studies on adult (>18 years old) human patients were searched that reported data on specific metabolites as prognostic indicators in colorectal cancer
- The QUADOMICS tool was used to assess study quality

Data Analysis and Multi-Omics Cross-Talk

- Author and clinicopathological data was extracted along with the following metabolomic data: biological specimen utilised and timing of sampling (tissue, serum, plasma); method of metabolome extraction and analytic platform; follow-up, recurrence and survival data. Specific metabolites significantly associated with recurrence or survival were recorded by name and whether increased, decreased or bi-directionally changed
- MetaboAnalyst software, version 4.0 was used to perform metabolomic analysis
- Metabolite set enrichment analysis (MSEA) was performed to identify consistent biologically meaningful patterns of metabolic pathway activity indicative of CRC prognosis. Metabolites significantly associated with prognosis were included in analysis with independent analyses performed for recurrence and survival. Significance was accepted as area under the curve (AUC) >0.5 and an associated p value <0.05 on diagnostic accuracy testing. reporting of results using Holm p <0.05 was used to assess for statistical significance (adjusts p value to counteract the problem of multiple comparisons) and a false discovery rate (FDR) <0.40 to further adjust for multiple comparisons and type I errors
- Pathway enrichment analysis with pathway topology analysis was then performed to identify the most active metabolites in recurrence and survival. Results were interpreted using Holm p<0.05and FDR<0.40
- Genomic single nucleotide polymorphisms (SNPs) associated with colorectal cancer prognosis and specific metabolomic patterns identified were identified through cross-referencing the following databases: Human Metabolome Database (HMDB), the Small Molecule Pathway Database (SMPDB), PubChem and Kyoto Encyclopaedia of Genes and Genomes (KEGG) Pathway Database



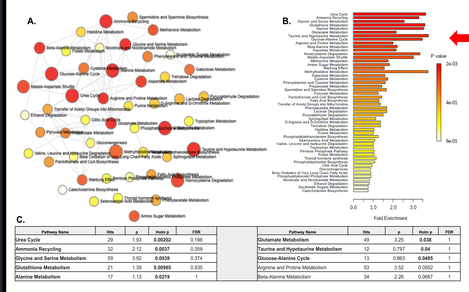
Results

Clinical Results and QA

- Nine studies were included in final analysis reporting on 1117 CRC patients
- Overall, studies were of high quality with scores ranging from 83.8-95.6% (median score 86.5%)
- Of the 1117 CRC patients studied, 52.6% (n=588) were male and 47.2% (n=529) female; median age ranged from 57-73.9
- Four studies reported on the metabolome of tumour tissue, one study reported on a combination of plasma and tumour tissue and three studies blood-based metabolome (one analysing plasma and two analysing serum)
- Metabolite detection was performed using a variety of methods including, most commonly: ultra-performance liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS); liquid chromatography-mass spectrometry (LC-MS)

AUTHOR	YEAR	COUNTRY	CRC Cohort (N)	Age	Gender	BMI	CRC Stage	Biospecimen	Metabolite Detection	Pre-Processing Analysis	Data Analysis	Follow-Up	QUADOMICS Score	Weight (%)
Cai	2020	USA	197	Mean 63.3-73.9 SD 5.8-16.1	Male 54.7% Female 45.3%	Not reported	I 23.9% II 43.7% III 32.4%	Tissue - Tumour and normal colon	UPLC-QTOF-MS	ProteoWizard MS Converter v3.06150	R CAMERA package; R v3.4.3	Five years	89.7%	17.6
Zaimenko	2019	Germany	92	Median 73 Range 45-87	Male 54% Female 46%	25	All stage II	Plasma – single pre-operative sample	LC-MS	Bruker Data Analysis; centWave	R CAMERA package	Median 65 months	94.1%	8.2
Wang	2019	China	73	Median 60.2 SD 9.4	Male 55% Female 45%	Mean 23.0-23.5 SD 3.2-2.8	I 17.8% (n=13) II 39.7% (n=29) III 37% (n=27) IV 5.5% (n=4)	Tissue - surgical specimen Plasma - pre-op D1 and post-op D7	LC-MS	ProteoWizard MS Converter v 3.06150	R package CAMERA	Five years	86.8%	6.5
de Vroome	2018	Netherlands	185	Mean 63-66.8 SD 10.6-13.4	Male 54.7% Female 45.3%	Not reported	Is 2.7% (n=5) I 18.4% (n=34) II 36.8% (n=68) III 28.1% (n=52) IV 14% (n=26)	Serum – pre-operatively and >45 days post-operative	LC-HILIC-SPE	MALDI-TOF-MS	SIMCA software v13	Five years	88.2%	16.6
Pacholczyk	2015	Poland	52	Mean 61 Range 34-86	Male 44% Female 56%	Not reported	I 19.2% (n=10) II 26.9% (n=14) III 30.8% (n=16) IV 23.1% (n=12)	Tissue - surgical specimen	HR-MAS-NMR	TopSpin 2.1	AMIX v3.9.14, STATISTICA X (StatSoft)	5.5 years	83.8%	4.7
Qiu	2014	USA/China	227	Median 57-61 Range 31-86	Male 53% Female 47%	Not reported	I 13.2% (n=30) II 32.2% (n=73) III 5.4% (n=103) IV 9.2% (n=21)	Tissue – surgical specimen	GC-TOF-MS	ChromaTOF V4.22, Leco Co.	ChromaTOF V4.22, Leco Co. SPSS (IBM v20)	Five years	86.8%	20.3
Jimenez	2013	UK	26	Median 72 Range 26-87	Male 38% Female 62%	Not reported	I 7.7% (n=2) II 38.5% (n=10) III 53.8% (n=14)	Tissue –surgical specimen	HR-MAS-NMR; GC-MS	TopSpin 2.2 Amix v3.9.9	MATLAB v. 2011a SIMCA-P+ v12.0.1	Median 49 months Range 5-64	95.6%	2.3
Bertini	2012	Italy	153	Median 63 Range 46-87	Male 60.2% Female 39.8%	Underweight 6% Normal 45.3% Overweight 33% Obese 15.1%	All stage IV	Serum - pretreatment	^{1}H-NMR	TopSpin V2.1 Amix v3.8.4	AMIX V3.8.4	Five years	85.3%	13.7
Farshidfar	2012	Canada	112	Median 63-72 SD +/- 11-13	Male 59.8% Female 40.2%	Not reported	II 18.8% (n=21) III 18.8% (n=21) IV 62.4% (n=70)	Serum - pretreatment	^{1}H NMR; GC-MS	Metabolite Detector v2.06	SIMCA-P+ v12.0	-	86.8%	10.7
			1117										Median 86.5%	

Prognostic Metabolome and Colorectal Cancer Recurrence



Pathway Name	Hits	p	Holm p	FDR
Urea Cycle	29	1.93	**0.00202**	0.198
Ammonia Recycling	32	2.12	**0.0037**	0.359
Glycine and Serine Metabolism	59	3.92	**0.0038**	0.374
Glutathione Metabolism	21	1.39	**0.00935**	0.935
Alanine Metabolism	17	1.13	**0.0219**	1

Pathway Name	Hits	p	Holm p	FDR
Glutamate Metabolism	49	3.25	**0.038**	1
Taurine and Hypotaurine Metabolism	12	0.797	**0.04**	1
Glucose-Alanine Cycle	13	0.863	**0.0495**	1
Arginine and Proline Metabolism	53	3.52	0.0552	1
Beta-Alanine Metabolism	34	2.26	0.0687	1

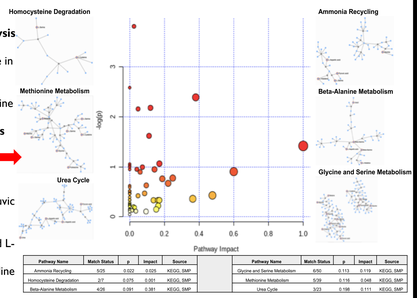
N=5 studies reported on metabolites prognostic for CRC recurrence

Pathway results from metabolite set enrichment analysis (MSEA) for CRC recurrence is summarised

Eight metabolic pathways were identified as expressively active in patients who developed CRC recurrence, three pathways demonstrated significance and an FDR<0.4: urea cycle (FDR=0.198); ammonia recycling (FDR=0.359); glycine and serine metabolism (FDR=0.374).

Pathway enrichment analysis for specific metabolites prognostic for CRC recurrence identified through pathway analysis

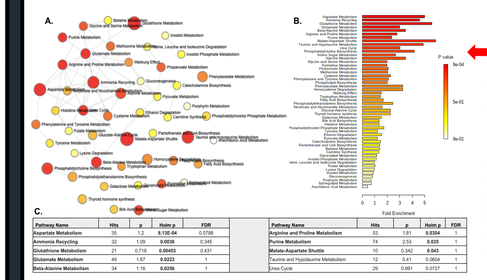
The following metabolites were particularly significant in ammonia recycling (p=0.022) with predominant flux in the following metabolites: L-aspartic acid, L-histidine, L-serine, pyruvic acid and glycine. Activity in homocysteine degradation and ß-alanine metabolism approached significance (p=0.075, p=0.091 respectively). In homocysteine degradation, flux in L-serine and L-cysteine were most significantly observed and in ß-alanine metabolism, flux in uracil, ß-alanine, L-aspartic acid and L-histidine concentrations were observed.



Pathway Name	Match Status	p	Impact	Source
Ammonia Recycling	5/25	0.022	0.025	KEGG, SMP
Homocysteine Degradation	2/7	0.075	0.091	KEGG, SMP
Beta-Alanine Metabolism	4/26	0.091	0.381	KEGG, SMP

Pathway Name	Match Status	p	Impact	Source
Glycine and Serine Metabolism	8/50	0.113	0.119	KEGG, SMP
Methionine Metabolism	5/39	0.118	0.048	KEGG, SMP
Urea Cycle	3/23	0.198	0.111	KEGG, SMP

Prognostic Metabolome and Overall Survival following Colorectal Cancer



Pathway Name	Hits	p	Holm p	FDR
Aspartate Metabolism	35	1.2	**8.13E-04**	0.0788
Ammonia Recycling	32	1.09	**0.0036**	0.345
Glutathione Metabolism	21	0.718	**0.00453**	0.431
Glutamate Metabolism	49	1.67	**0.0223**	1
Beta-Alanine Metabolism	34	1.16	**0.0256**	1

Pathway Name	Hits	p	Holm p	FDR
Arginine and Proline Metabolism	53	1.81	**0.0304**	1
Purine Metabolism	74	2.53	**0.033**	1
Malate-Aspartate Shuttle	10	0.342	**0.043**	1
Taurine and Hypotaurine Metabolism	12	0.41	0.0604	1
Urea Cycle	29	0.991	0.0727	1

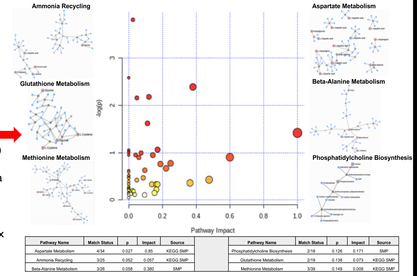
N=6 studies reported prognostic metabolites for CRC survival

Metabolite set enrichment analysis (MSEA) for CRC survival is summarised

Eight metabolic pathways were expressively active in the setting of worse CRC survival and two pathways demonstrated statistical significance and an FDR<0.4: aspartate metabolism (FDR=0.08) and ammonia recycling (FDR=0.345).

Pathway enrichment analysis for specific metabolites associated with poor CRC survival

These were particularly active in aspartate metabolism (p=0.027) with predominant flux in the following metabolites: L-aspartic acid, acetylglycine, L-asparagine and ß-alanine. Activity in ammonia and ß-alanine metabolic pathways approached significance (p=0.052, p=0.058 respectively). In ammonia recycling, flux in L-aspartic acid, L-histidine, glycine, L-serine and pyruvic acid concentrations were most active and in ß-alanine metabolism flux in uracil ß-alanine, L-aspartic acid and L-histidine concentrations were most active.



Pathway Name	Match Status	p	Impact	Source
Aspartate Metabolism	4/34	0.027	0.85	KEGG, SMP
Ammonia Recycling	3/25	0.052	0.057	KEGG, SMP
Beta-Alanine Metabolism	3/26	0.058	0.380	SMP

Pathway Name	Match Status	p	Impact	Source
Phosphatidylcholine Biosynthesis	3/18	0.135	0.173	SMP
Glutathione Metabolism	3/19	0.138	0.073	KEGG, SMP
Methionine Metabolism	3/39	0.148	0.009	KEGG, SMP

Single-nucleotide polymorphism (SNP) Crosstalk

- Eleven SNPs demonstrated significant association with prognostic activity in identified metabolic pathways (n=3 for CRC recurrence: Rs2300701, Rs313408, Rs7018169) had previously shown clinical association in cancer and n=1 SNP prognostic for CRC survival: Rs12106696)
- RS2300701 represents an intron variant SNV on chromosome two at position 2p12, the position of the SRD5A2 gene. SRD5A2 has a controversial role in prostate cancer
- Rs313408 is also an intron variant SNV on chromosome 11 which encodes gene DYNC2H1 mutations in this gene are associated with diffuse-type gastric cancer
- Rs7018169 represents an intron variant SNV on chromosome and encodes the ST3GAL1 gene with a role in breast and ovarian cancer most notably ovarian cancer but also breast cancer through promotion of cell migration and invasion. and may be linked to development of peritoneal disease, particularly in ovarian cancer
- Rs12106696 was associated with worse survival in CRC. It is an SNV (intron variant) on chromosome 3 at position 3p13 in the position of protein-coding gene zinc finger protein 385D (ZNF385D) gene which has been observed in liver, prostate, cervical and ovarian cancer

Figure: Single nucleotide polymorphisms identified as prognostic in colorectal cancer based on metabolic signatures.
p-value is encoded by intensity of red colour of circles and size of the circles represent the diversity of genomic interactions based on the number of single nucleotide polymorphisms (SNPs) identified. Rs=reference SNP

Recurrence				
SNP	Hits	p	Holm p	FDR
Rs2194980	6	1.08E-8	**0.0001**	8.27E-5
Rs1392680	4	8.4E-7	**0.0037**	9.26E-4
Rs2567397 | Rs7664292	4	8.4E-7	**0.0037**	9.26E-4
Rs715	4	8.4E-7	**0.0037**	9.26E-4
Rs169712	5	4.1E-6	**0.0181**	0.002
Rs2300701	5	4.1E-6	**0.0181**	0.002
Rs313408	5	4.1E-6	**0.0181**	0.002
Rs7018169	5	4.1E-6	**0.0181**	0.002

Survival				
SNP	Hits	p	Holm p	FDR
Rs2194980	6	4.13E-8	**1.82E-4**	1.82E-4
Rs169712	5	2.67E-7	**0.001**	5.88E-4
Rs12106696	3	3.7E-6	**0.016**	0.005
Rs17822850	4	1.47E-5	0.065	0.008
Rs17543807	4	1.47E-5	0.065	0.008
Rs2567397 | Rs7664292	4	1.47E-5	0.065	0.008
Rs2593760	4	1.47E-5	0.065	0.008
Rs715	4	1.47E-5	0.065	0.008

Conclusion

- Recent advances in biomolecular techniques and bioinformatic technology have allowed unravelling of metabolic pathway information with real clinical application
- Herein we identified flux in specific oncometabolites and activity in specific metabolic pathways that are significantly associated with CRC recurrence and five-year survival through cross-reference of synthesised published data and publicly-available metabolite databases
- Specific metabolites and metabolic pathways are dysregulated in the setting of poor prognostic colorectal cancers and such metabolic signatures are associated with specific genomic SNPs
- These findings provide a platform for prognostic biomarker discovery and development in colorectal cancer as well as identify potential for therapeutic targeting in a retrograde approach from the clinical onco-phenotype
- This approach may contribute to better understanding the phenomenon of non-hereditary CRC in young adults as an assessment of environmental effect

Key References

1. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: The roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387-426. doi:10.1039/b906712b
2. Romero-Garcia S, Lopez-Gonzalez JS, B´ez-Viveros JL, Aguilar-Cazares D, Prado-Garcia H. Tumor cell metabolism. Cancer Biol Ther. 2014;12(11):939-948. doi:10.4161/cbt.12.11.18140
3. Janke R, Dodson A, Rine J. Metabolism and epigenetics. Annu Rev Cell Dev Biol. 2015;31:473e496.
4. Santhanama S, Alvaradoa DM, Ciorba MA. Therapeutic targeting of inflammation and tryptophan metabolism in colon and gastrointestinal cancer. Transl Res. 2016;167(1):67-69. doi:10.1016/j.physbeh.2017.03.040
5. Bettencourt IA, Powell JD. Targeting Metabolism as a Novel Therapeutic Approach to Autoimmunity, Inflammation, and Transplantation. J Immunol. 2017;198(3):999-1005. doi:10.4049/jimmunol.1601318