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Measuring the combinatorial expression of solute transporters and metalloproteinases transcripts in colorectal cancer.

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Measuring the combinatorial expression of solute transporters and metalloproteinases transcripts in colorectal cancer.

Abstract
Background It was hypothesised that colorectal cancer (CRC) could be diagnosed in biopsies by measuring the combined expression of a small set of well known genes. Genes were chosen based on their role in either the breakdown of the extracellular matrix or with changes in cellular metabolism both of which are associated with CRC progression. Findings Gene expression data derived from quantitative real-time PCR for the solute transporter carriers (SLCs) and the invasion-mediating matrix metalloproteinases (MMPs) were examined using a Linear Discriminant Analysis (LDA). The combination of MMP-7 and SLC5A8 was found to be the most predictive of CRC. Conclusion A combinatorial analysis technique is an effective method for both furthering our understanding on the molecular basis of some aspects of CRC, as well as for leveraging well defined cancer-related gene sets to identify cancer. In this instance, the combination of MMP-7 and SLC5A8 were optimal for identifying CRC.

Keywords
colorectal, transcripts, metalloproteinases, transporters, solute, cancer, expression, measuring, combinatorial

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Short Report

Measuring the combinatorial expression of solute transporters and metalloproteinases transcripts in colorectal cancer

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Abstract

**Background:** It was hypothesised that colorectal cancer (CRC) could be diagnosed in biopsies by measuring the combined expression of a small set of well known genes. Genes were chosen based on their role in either the breakdown of the extracellular matrix or with changes in cellular metabolism both of which are associated with CRC progression.

**Findings:** Gene expression data derived from quantitative real-time PCR for the solute transporter carriers (SLCs) and the invasion-mediating matrix metalloproteinases (MMPs) were examined using a Linear Discriminant Analysis (LDA). The combination of MMP-7 and SLC5A8 was found to be the most predictive of CRC.

**Conclusion:** A combinatorial analysis technique is an effective method for both furthering our understanding on the molecular basis of some aspects of CRC, as well as for leveraging well defined cancer-related gene sets to identify cancer. In this instance, the combination of MMP-7 and SLC5A8 were optimal for identifying CRC.

**Findings**

Colorectal cancer is the third-most common cancer in males and second-most common in females worldwide [1]. Its prevalence highlights a need to more deeply understand the molecular interactions that lead to its progression. Two important and well documented pathways in the progression of colorectal cancer are changes in energy source for cellular metabolism and break down of the extracellular matrix. Healthy colonocytes use short-chain monocarboxylates, in particular butyrate, as their main source of energy [2]. The solute-linked carrier (SLC) SLC5A8, a Na+-coupled transporter, and monocarboxylate transporter (MCT1) SLC16A, are possibly vehicles by which short-chain monocarboxylates are transported into the colonic epithelium [3-5]. SLC5A8 and SLC16A1 have been purported to provide a mechanism for the suppression of tumour growth in colorectal and gastric cancers [3,6] and are...
down-regulated with tumour progression [4]. As colono-
cytes become cancerous there is a shift in energy source
away from butyrate to glucose, resulting in increased lev-
els of glucose in colorectal cancer cells [7] and in carcino-
mas [8]. Associated with this is an up-regulation of the
glucose transporter SLC2A1, which has been shown in a
significant proportion of aggressive human tumours [e.g.
[9]]. Together, these changes are believed to facilitate
tumour growth and proliferation [10].

Matrix metalloproteinases (MMPs) are a family of zinc-
and calcium-dependent proteolytic enzymes that degrade
macromolecules of the extracellular matrix. Members of
this family, such as MMP-2, -9 and -7, have been shown to
be associated with the breakdown of type IV collagen
and the basement membrane. They have been implicated
in tumour progression and invasion in human cancer tis-
ues [11-13]. The proteolytic activity of some MMPs (e.g.
MMP-2, -9 and -14) can be suppressed by Reversion-
inducing cysteine-rich protein with kazal motifs (RECK)
[14]. Decreased expression of RECK is believed to result in
increased invasion, metastasis and angiogenesis [reviewed
by [15]] and is associated with poor prognosis in cancer
patients [16].

This paper investigates genes in combination from two
previous well defined processes in colorectal cancer. The
abundance of transcripts from well described candidate
genes implicated in either the tumorigenic process or met-
abolic changes associated with carcinogenesis were exam-
ined in human colorectal cancer cell lines and human
cancer and healthy colonic tissues. In particular, the
expression of the nutrient transporter genes (SLC2A1,
SLC16A1 and SLC5A8), genes encoding proteins involved
in tissue remodelling and tumour invasion (MMP-2, -7, 
and -12, and the MMP regulator RECK), were examined
in two sets of normal human colon and colorectal tumour
samples and in four human colorectal cancer cell lines.
The study used a combinatorial transcript expression bio-
informatic approach to leverage described information on
a small gene set in order to discriminate between normal
and colorectal tumour tissue and help to define interrela-
tionships between processes known to change during car-
cinogenesis.

Table 1: Summary of tissue sample details^

| Site   | Normal | Tumour pathology |
|--------|--------|------------------|
|        |        | Dukes A | Dukes B | Dukes C | Dukes D |
| Study 1| Left   | 4       | 2       | 1       | 2       |
|        | Right  | 1       | 2       | -       | 2       |
| Study 2| Left   | 6       | 1       | 4       | 2       |
|        | Right  | 6       | 1       | 4       | -       |
| Transverse | 2   | -       | -       | 1       | -       |

^see Additional file 1 for more details.

Methods
Sample collection
Human colon tissue was sourced from the Division of Tis-
sue Pathology, Institute of Medical and Veterinary Sci-
ence, University of Adelaide. There were two sets of
normal and CRC tissues as outlined in Table 1 (for further
details of these samples [see Additional file 1 Tables S1
and S2].

Total RNA extraction, cDNA synthesis and real-time PCR
The human tissue samples were obtained from resections
of specimens and placed in OCT (optimal cutting temper-
ature cryopreservation medium) [17], snap-frozen in liq-
nuid nitrogen and then stored at -86°C. After histological
verification RNA was extracted by placing samples in 1 ml
of Trizol® Reagent (Invitrogen, Sydney, Australia), then
homogenised using beads (mix of 2.5 mm glass and 0.1 –
1.0 mm diameter silicon-zirconium beads) in a MiniBead-
beater-8™ (BioSpec Products Inc., Oklahoma, USA) and
extracted according to Invitrogen's instructions. Samples
were then further processed using RNAeasy mini spin col-
umns (QIAGEN, Doncaster, Australia) with contaminat-
ing DNA being removed via DNase on-column digestion
as per the manufacturer's instructions. Similarly, cultured
cells that were at least 70% confluent were extracted
directly using the RNAeasy spin columns. The integrity of
RNA samples from Study 2 and the cell lines were checked
using a Bioanalyzer 2100 (Agilent Technologies) [18]. All
of the RNA samples were then quantified using a Nano-
Drop® ND-1000 Spectrophotometer. Samples were then
diluted to100 ng/ul.

cDNA was synthesised using SuperScript II (Invitrogen)
reverse transcriptase (Invitrogen) using 1 ug of RNA per
20 ul reaction and incubated as outlined in the manufac-
turer's instructions. Real-time PCR assays were conducted
using off-the shelf optimised and guaranteed TaqMan®
Gene Expression Assays (Applied Biosystems, Foster City,
California, USA), that consisted of primers and a probe
for the specific genes (Table 2). Three housekeeping genes
were used: the conventional reference gene 18S ribosomal
RNA (18S) [e.g. [19]], as well as a ribosomal protein (large
P0, a component of the 60S subunit) and HUWE1 (see
Table 2). The latter two were identified as suitable using a
commercial database from Gene Logic (Gaithersburg, Maryland, U.S.A.). The database contains information on 44928 probe-sets (HUG-133A and B Affymetrix arrays) derived from 462 individuals covering the classes: normal 222; adenoma 29; cancer 161; and other disease 50. The dataset was normalized using the GCRMA algorithm [20] and then probe-sets were selected that had minimal variance across all arrays and had appreciable expression levels. The aim was to use three housekeeping genes of varying abundance (18s as the highest and HUWE1 as the lowest) to cover the range of target transcript differential expression. Also, the target and housekeeping gene assays were selected where possible to prime over an intron-exon boundary to avoid amplifying any contaminating genomic DNA (which could otherwise bias results). The assays were then set up in four aliquots per cDNA sample using TaqMan® Universal PCR Master Mix commensurate with the manufacture's instructions, except that 5 μl reaction volumes were used. Assays consisted of 20× mix of unlabeled PCR primers and TaqMan® MGB probe (FAM dye-labelled). Assays were run in 384-well plates on an Applied Biosystems PRISM® 7900HT real-time thermocycler and analysed using Sequence Detection System software (version 2.3) as outlined in the TaqMan® Gene Expression Assays protocol.

### Data acquisition

Data on the expression levels of target and reference genes were obtained in the form of crossing points [21] or threshold (Ct) values. The target genes were then analysed following the delta-delta Ct value procedure [22,23] with the assumption that efficiency was 100% and amplicons doubled each cycle. Briefly, the Ct for each housekeeping gene (HK) was subtracted from each corresponding target gene such that,

\[ \Delta Ct_{\text{normalised target gene}} = Ct_{\text{target gene}} - Ct_{\text{HK gene}} \]

The mean of the normal tissues was used to create a reference tissue value. So,

\[ \Delta Ct_{\text{reference}} = 1/n \sum_{\text{all normal tissues}} (Ct_{\text{target (gene normal tissue)}} - Ct_{\text{HK gene}}) \]

and then,

\[ \Delta \Delta Ct = \Delta Ct_{\text{normalised target gene}} - \Delta Ct_{\text{reference}} \]

This process allowed for the data to be analysed for artefacts, real-time PCR repeatability and stability of HK expression. As three HK genes were used, the process was then repeated with each housekeeping gene and the median calculated; the ratio of the gene expression is 2-ΔΔCt.

The normalised ΔΔCt data sets were then combined and all subset variable selection with Linear Discriminant Analysis (LDA) was performed to ascertain the best combination of transcripts that separated tumour from normal. The error rate for the model was estimated using ‘leave-one-out estimates’ for cross validation [24].

Transcript expression from cultured colorectal cancer cell lines (HT29, HCT116, Caco2 and LM1215) was then used to further test the optimal combinations using LDA.
The effect of tissue sampling site (i.e., left, transverse or right colon), the type of 'normal' and Dukes stage was also analysed.

**Results**

The normalised data sets for all seven target genes for study 1, which consisted of randomly grouped tumour and normal, were analysed using LDA [see Additional file 1 for figure S1, Additional file 2 for the raw Ct values], resulting in a separation of the normal and tumour samples (the leave-one-out error estimate from the LDA is 0.25, [see Additional file 1 – Figure S2]. Using an all subsets variable selection procedure with LDA created a reduced model using only RECK and MMP-7 (see Figure 1), giving an improved leave-one-out error estimate of 0.06. Note, all the 'normal' samples clustered together regardless of their source (i.e. from a CRC or healthy patient). Therefore, there was no effect by the source of 'normal'.

For study 2, which consisted of paired data, the normalised data sets for all seven target genes were then analysed using LDA [see Additional file 1 – Figure S3 and Additional file 3 for raw Ct values], resulting in separation of the normal and tumour samples (the leave-one-out error estimate from the LDA is 0.178, [see Additional file 1 figure S4]. This separation was further increased using an all subsets variable selection procedure with LDA. The resultant reduced model, this time using only MMP7 and SLC5A8, had a leave-one-out error estimate from the LDA of 0.035 (see Figure 2).

When data from both the human tissue studies were combined and LDA applied to the four classes (Study 1 normal, Study 1 tumour, Study 2 normal, Study 2 tumour); the two sets of 'normals' could not be separated and the two sets of 'tumours' could not be separated. This indicates that it is feasible to combine the studies. The optimal transcript combination that separated tumour from normal was SLC5A8 and MMP-7 (see Figure 3) resulting in a leave-one-out error estimate of 0.128. There was no effect of sampling site (i.e., left, right or transverse colon) or Duke's stage on the expression of MMP-7 or SLC5A8.

Then, as an exercise to test the mathematics of this approach, the two studies plus CRC cell line data [see Additional file 1 for Figure S5 and Additional file 3 for raw Ct values] were then combined. The maximum separation between tissue types (normals, as opposed to tumour tissue plus cell lines) occurred with the transcript combination of MMP-7, RECK and SLC5A8 (see Figure 4).

**Discussion**

This communication investigated expression patterns of transcripts associated with processes involved in the development of colorectal cancer. Genes examined were the solute transporters SLC2A1, SLC5A8 and SLC16A1, which are associated with changes in the cellular import...
of energy sources, and MMP-2, MMP-7, MMP-9 and MMP-12, which are related to the breakdown of the extracellular matrix, and the MMP negative regulator, RECK. Individual differential gene expression patterns were established for normal and cancerous tissue samples. When the data were combined, a combination of MMP-7 and SLC5A8 (and, to a lesser extent, RECK) provided the greatest separation between healthy colon tissue and colorectal cancer (tissue or cell lines). One possible interpretation of these results is that the mechanisms which act to break down the extracellular matrix and promote tumour invasion also induce MMP negative regulation. Whilst in parallel, SLC5A8 levels in tumours were reduced compared to normal tissue and cell lines, which is consistent with previous studies [4] showing an association between SLC5A8 down-regulation and tumour progression.

This study has demonstrated that it is advantageous to use a combinatorial approach to defining biomarkers of carcinogenesis processes compared to using individual candidate transcript markers. Others have used systematic approaches when analysing transcripts for cancer biomarkers (e.g. pancreatic cancer by [25]) and have shown that markers, which individually are suboptimal, can be combined to yield higher sensitivity and specificity. Even though our study uses a small patient tissue library, it demonstrates a proof-of-concept for the combinatorial approach to transcript biomarkers that now needs to be validated in larger controlled data sets [26,27]. In addition, our technique may prove useful to validate other colorectal cancer candidate transcripts, such as those defined in a recent study [28] which applied a meta-analysis or genome wide studies (e.g. microarrays) to comprehensively evaluate microarray data for biomarkers. Although using tumour-related gene expression may not be an optimal platform for colorectal cancer detection, this combinatorial approach demonstrates a method for biomarker discovery based on a priori hypotheses originating from other studies that may prove useful either in elucidating early biomarkers or in establishing auxiliary markers of prognosis. This approach could be applied in the clinical setting to increase the sensitivity and specificity of biomarkers by combining the analyses with other markers [29].

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

CK designed the study and co-authored the manuscript with RD, BMH, LC, MZ, AR, TL and RH. BMH and MZ per-
formed the molecular work. RD performed the mathematical and statistical analysis. AR provided the clinical guidance, pathology details and sourced and prepared the tissue samples. TL and RH sought the funding. All authors read and approved the final manuscript.

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**References**

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA-A Cancer J Clinicians* 2005, 55:74-108.
2. Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJ: Colonic health: Fermentation and short chain fatty acids. *J Clin Gastroenterol* 2006, 40:235-243.
3. Gupta N, Martin PM, Prasad PD, Ganapathy V: SLC5A8 (SMCT)-mediated transport of butyrate forms the basis for the tumor suppressive function of the transporter. *Life Sci* 2006, 78:2419-2425.
4. Paroder V, Spencer SR, Paroder M, Arango D, Schwartz S Jr, Mariadason JN, Augenlicht LH, Eskandari S, Carrasco N: Na(+)-monocarboxylate transport (SMCT) protein expression correlates with survival in colon cancer: Molecular characterization of SMCT. *J Proc Natl Acad Sci USA* 2006, 103:7270-7275.
5. Iwanaga T, Takebe K, Kato I, Karaki SI, Kuwahara A: Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to SLC5A8. *Biomed Res-Tokyo* 2006, 27:243-254.
6. Ganapathy V, Gopal E, Miyauchi S, Prasad PD: Biological functions of SLC5A8, a candidate tumour suppressor. *Biochem Soc T* 2005, 33:237-240.
7. He L, Li X, Luo HS, Rong H, Cai J: Possible mechanism for the regulation of glucose on proliferation, inhibition and apoptosis of colon cancer cells induced by sodium butyrate. *World J Gastroenterol* 2007, 13:4015-4018.
8. Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz S, Weiss A, Bodian C, Burstein DE: GLUT-1 glucose transporter: A marker of poorer prognosis in colon carcinoma [abstract]. *Laboratory Investigation* 1997, 76:316.
9. Younes M, Lechago LV, Lechago J: Overexpression of the human erythrocyte glucose transporter occurs as a late event in human colorectal carcinogenesis and is associated with an increased incidence of lymph node metastases. *Clin Cancer Research* 1996, 2:1151-1154.
10. Macheda ML, Rogers S, Best JD: Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol* 2005, 202:654-662.
11. Hilska M, Roberts PJ, Collan YU, Laine VJO, Kossi J, Hirsimaki P, Rahikonen O, Laato M: Prognostic significance of matrix metalloproteinases -1,-2,7 and 13 and tissue inhibitors of metalloproteinases-1,-2 and 3 in colorectal cancer. *Int J Cancer* 2007, 121:714-723.
12. Gentner B, Wein A, Croner RS, Zettitraeger I, Wirz R, Dimmler A, Dorlauke L, Hohenberger W, Hahn EG, Brueckl WM: Differences in the Gene Expression Profile of Matrix Metalloproteinases (MMPs) and their Inhibitors (TIMPs) in Primary Colorectal Tumors and their Synchronous Liver Metastases. *Anticancer Res* 2009, 29:67-74.
13. Hershzenyi L, Sipos F, Galamb O, Solyomosi N, Hritz I, Milheiler P, Berczi L, Molinar B, Tullassy Z: Matrix metalloproteinase-9 expression in the normal mucosa-adenoma-dysplasia-adenocarcinoma sequence of the colon. *Path & Oncol Res* 2008, 14:31-37.
14. Takagi S, Simizu S, Osada H: RECK Negatively Regulates Matrix Metalloproteinase-9 Transcription. *Cancer Res* 2009, 69:1502-1508.
15. Noda M, Takahashi C: Recklessness as a hallmark of aggressive cancer. *Cancer Sci* 2007, 98:1659-1665.
16. Takeuchi T, Hisanaga M, Nagao M, Ikeda N, Fuji H, Koyama F, Mukogawa T, Matsumoto H, Kondo S, Takahashi C, Noda M, Nakaijima H: The membrane-anchored matrix metalloproteinase (MMP) regulator RECK in combination with MMP-9 serves as an informative prognostic indicator for colorectal cancer. *Clin Can Res* 2006, 12:5572-5579.
17. Loken SD, Demmerick DJ: A novel method for freezing and storing research tissue bank specimens. *Hum Pathol* 2005, 36:977-980.
18. Fleige S, Walf V, Huch S, Prigomer C, Sehlm J, Paffl MW: Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. *Biotechnology Letters* 2006, 28:1601-1613.
19. Nolan T, Hands RE, Bustin SA: Quantification of mRNA using real-time RT-PCR. *Nature Protocols* 2006, 1:1559-1582.
20. Wu Z, Irizarry RA, Gentleman R, Murillo FM, Spencer F: A model based background adjustment for oligonucleotide expression arrays. *Johns Hopkins University, Dept of Biostatistics Working Papers. Working Paper 1. [serial on the internet] 2004 [http://www.bepress.com/jhubiostat/paper1/].
21. Fijal SN, Meur SC, Wittwer C, Nakagawa K, Schmittgen TD, Livak KJ: Analyzing real-time PCR data by the comparative CT method. *Techniques In Molecular Medicine* 2006, 130:111-1108.
22. Finn RS, Reed A, Chen F, Stewart CN: Statistical analysis of real-time PCR data. *BMC Bioinformatics* 2006, 7:41.
23. Hirt D: Error-rate estimation in multiple-group linear discriminant analysis. *Technometrics* 1996, 38:389-399.
24. Firpo MA, Gay DZ, Granger SR, Scaife CL, DiSario JA, Boucher KM, Mulvihill SJ: Improved diagnosis of pancreatic adenocarcinoma using haptoglobin and serum amyloid A in a panel screen. *World J Surg* 2009, 33:716-722.
25. Brenner DE, Normolle DP: Biomarkers for the cancer risk, early detection, and prognosis: The validation conundrum. *Cancer Epidemiol Biomarkers Prev* 2007, 16:1918-1920.

**Additional file 1**

Further breakdown and details of tissue and cell expression data. Further details on the methods and results used in this study. Contains Tables S1 and S2 and Figures S1–S5.

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**Additional file 2**

Table of PCR Ct values for the house keeper genes 18s, HUWE1 and RP0 and target genes MMP2, MMP12, MMP7, MMP9, RECK, SLC2A1, SLC5A8 and SLC16A1 for study 1 and Cell lines (Caco2, HT29, HCT116 and LIMH1215). Worksheets containing raw Ct values for house keeper and target genes for study 1 (unpaired data) and cell lines.

Click here for file [http://www.biomedcentral.com/content/full/1756-0500-2-164-S2.xls](http://www.biomedcentral.com/content/full/1756-0500-2-164-S2.xls)

**Additional file 3**

Table of PCR Ct values for the house keeper genes 18s, HUWE1 and RP0 and target genes MMP2, MMP12, MMP7, MMP9, RECK, SLC2A1, SLC5A8 and SLC16A1, for Study 2. Worksheets containing raw Ct values for house keeper and target genes for study 2 (paired data).

Click here for file [http://www.biomedcentral.com/content/full/1756-0500-2-164-S3.xls](http://www.biomedcentral.com/content/full/1756-0500-2-164-S3.xls)
27. Vineis P, Perera F: Molecular epidemiology and biomarkers in etiologic cancer research: The new in light of the old. Cancer Epidemiol Biomarkers Prev 2007, 16:1954-1965.

28. Chan SK, Griffith OL, Tai IT, Jones SJM: Meta-analysis of colorectal cancer gene expression profiling studies identifies consistently reported candidate biomarkers. Cancer Epidemiol Biomarkers Prev 2008, 17:543-552.

29. Gupta AK, Brenner DE, Turgeon DK: Early detection of colon cancer – New tests on the horizon. Mole Diagnosis & Therapy 2008, 12:77-85.