# Proteomic and molecular analysis in colorectal cancer: validation of the biomarkers desmin, SET and CK8

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Georgia Arentz, Bachelor of Biomedical Science (Honours)

School of Molecular and Biomedical Sciences, Discipline of Physiology,
The University of Adelaide

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# Declaration

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# Abbreviations

| | |
|---|---|
| %: | percentage |
| x g: | x gravity |
| °C: | degrees Celsius |
| µg: | microgram |
| µl: | microliter |
| µM: | micromolar |
| 1°: | primary |
| 1-DE: | one-dimensional electrophoresis |
| 2°: | secondary |
| 2-DE: | two-dimensional electrophoresis |
| 2D: | two-dimensional |
| aa: | amino acid |
| ACN: | acetonitrile |
| bp: | base pair |
| BSA: | bovine serum albumin |
| BVA: | biological variation module |
| CHAPS: | 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate |
| CID: | collision-induced dissociation |
| cM: | centimetre |
| Cy: | Cyanine |
| Da: | Dalton |
| DIGE: | direct in gel analysis |
| DNA: | deoxyribonucleic acid |
| dNTP: | deoxynucleoside triphosphate |
| DTT: | dithiothreitol |
| EDTA: | ethylenediaminetetraacetic acid |
| ESI: | electro spray ionisation |
| EtOH: | ethanol |
| FA: | formic acid |
| Fig: | figure |
| GE: | General Electric |
| $H_2O$: | water |
| HCCA: | α-cyano-4-hydroxycinnamic acid |

| | |
|---|---|
| HPLC: | high performance liquid chromatography |
| IEF: | isoelectric focusing |
| IF: | immunofluorescence |
| IPG: | immobilised pH gradient |
| KCl: | potassium chloride |
| kDa: | kilodalton |
| LC: | liquid chromatography |
| LMD: | laser microdissection |
| M: | molar |
| mA: | milliampere |
| MALDI: | matrix assisted laser desorption ionisation |
| ml: | millilitre |
| mm: | millimetre |
| mM: | millimolar |
| mRNA: | messenger RNA |
| MS: | mass spectrometry |
| MS/MS: | tandem mass spectrometry |
| m/z: | mass-to-charge |
| n: | number of replicates |
| NaCl: | sodium chloride |
| NaPO4: | sodium phosphate |
| NCBI: | National Centre for Biotechnology Information |
| ng: | nanogram |
| nl: | nanolitre |
| p: | pico |
| PAGE: | polyacrylamide gel electrophoresis |
| PBS: | phosphate buffered saline |
| PCR: | polymerase chain reaction |
| pH: | hydrogen ion concentration |
| pI: | isoelectric point |
| ppm: | parts per million |
| PS: | phosphoserine |
| RNA: | ribonucleic acid |
| RO: | reverse osmosis |
| rpm: | revolutions per minute |
| RT: | reverse transcription |

| | |
|---|---|
| RT-PCR: | reverse transcription polymerase chain reaction |
| SDS: | sodium dodecyl sulphate |
| TBE: | Tris/boric acid/EDTA buffer |
| TBST: | Tris-buffered saline Tween-20 |
| TFA: | trifluoroacetic acid |
| TOF: | time of flight |
| Tris: | Tris (hydroxymethyl) aminomethane |
| U: | units |
| V: | volts |
| v/v: | volume per volume |
| w/v: | weight per volume |
| w/w: | weight per weight |

# Abstract

There is potential for significant improvements to be made in the diagnosis, staging, treatment and monitoring of colorectal cancer (CRC). The elucidation of proteins and pathways involved in CRC would aid in the development of biomarkers for detection, identify patients at risk of relapse and potentially give rise to new molecular targets of treatment. Laser microdissection (LMD) was used with minimal labelling two-dimensional gel electrophoresis (2D DIGE) and mass spectrometry to identify proteins significantly increased in eight early stage paired tumour-normal tissues. Seventeen individual proteins were identified as upregulated by >2 fold ($P$<0.05). The role of the proteins cytokeratin 8 (CK8) and SET in CRC were chosen for further analysis. A third protein, desmin, was chosen from a pilot study performed using LMD and saturation labelling 2D DIGE for further analysis.

The protein desmin was identified as significantly upregulated in a pilot study that aimed to identify proteins with altered expression in the cancerous epithelium and surrounding microenvironment. Using immunofluorescence (IF), desmin expression levels in the tissue stroma of late stage tumours compared to early stage tumours was significantly increased, $P$<0.0001. The desmin expressing cells were identified to be pericytes, formed around mature vasculature as a result of angiogenic stimulation. From this work desmin appears to have potential use as a histopathology marker for the identification of late stage patients and may help identify patients who would not benefit from the current anti-angiogenic therapies due to the presence of mature tumour microvasculature.

Three isoforms of CK8 were found to be upregulated in the DIGE study, with each isoform containing the phosphoserine (PS) residues 23, 431 and 73. Western blotting showed significantly increased phosphorylation levels at these sites in tumour compared to normal tissues. The MAP kinase ERK is known to phosphorylate the 73 and 431 residues and 50% of CRC patients have mutations in the EGFR/Ras/Raf/MEK/ERK signalling pathway (KRAS or BRAF mutations) resulting in constitutive ERK activation. Inhibition of EGFR activation in Caco2 cells showed a significant decrease ($P$<0.0001) in PS73 and PS431 levels by 59% and 65% respectively, indicating that patients with KRAS or BRAF mutations may have significantly increased PS73 and PS431 levels. Previously it has been shown that high levels of CK8 phosphorylation may help to protect cells against caspase degradation and evasion of apoptosis. This is the first report of the differential expression of phospho-CK8 isoforms in CRC.

SET is a known inhibitor of the tumour suppressor PP2A, a component of the GSK3 β complex that targets β catenin for degradation in the Wnt signalling pathway. Ninety percent of CRC patients have Wnt signalling pathway mutations resulting in constitutive pathway activation. The effect of knocking down SET via siRNA on β catenin levels was analysed in SW480 with constitutive Wnt signalling and

HEK293 with low levels of Wnt signalling. No changes in β catenin levels were observed in the SW480 cells, however a 24.5% reduction was detected in the HEK293 cells. The role of SET in other cancer associated pathways was analysed in the SET knock down cells using the $RT^2$ Profiler PCR Array 'Human Cancer Pathway Finder' plates with the expression of genes involved in apoptosis, angiogenesis and adhesion found to be altered.