Decreased skeletal muscle necrosis and remote lung injury in c-Jun N-terminal kinase 2 knockout mice after lower-limb ischaemia–reperfusion injury

D. W. Harkin, N. Degousee, E. Stefanski, T. F. Lindsay, M. Karin and B. B. Rubin

Division of Vascular Surgery, Department of Surgery, The Toronto Hospital (General Division), Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada and Regional Vascular Surgery Unit, Royal Victoria Hospital Belfast, Belfast, UK

Background: Skeletal muscle ischaemia–reperfusion (I-R) injury initiates an inflammatory response characterized by neutrophil activation and remote acute lung injury. The purpose of this study was to define the role of c-Jun N-terminal kinase (JNK) enzymes, implicated in the regulation of the inflammatory response, in skeletal muscle and lung injury following lower-limb I-R.

Methods: JNK1- and JNK2-knockout (ko) mice or their wild-type (wt) littermates were subjected to 180 min of unilateral hindlimb tourniquet ischaemia, followed by 24 h reperfusion. Gastrocnemius muscle viability was measured by nitroblue tetrazolium staining and computerized planimetry. Myeloperoxidase (MPO) and wet-weight measured neutrophil sequestration and oedema respectively.

Results: Skeletal muscle viability following lower extremity I-R was significantly increased in JNK2-ko mice compared to JNK2-wt controls (52·8(6·9) *versus* 0·2(0·1) per cent; $P < 0·004$). Skeletal muscle oedema was significantly reduced in JNK2-ko mice compared to JNK2-wt controls (1·2(0·1) *versus* 1·6(0·1); $P < 0·04$). Skeletal muscle MPO was significantly increased in JNK2-ko mice compared to JNK2-wt controls (0·019(0·004) *versus* 0·007(0·004); $P < 0·02$). Lung tissue MPO was significantly reduced in JNK2-ko mice compared to JNK2-wt controls (0·049(0·009) *versus* 0·195(0·06); $P < 0·01$). JNK1-ko mice resembled controls. Values are mean(s.e.m.); ANOVA and Scheffé's test.

Conclusion: This is the first study to evaluate the role of a JNK isoforms in skeletal muscle injury, and clearly shows that JNK2 participates in the sequence of events that culminates in muscle necrosis and remote acute lung injury. Inhibition of JNK2 activity may decrease muscle necrosis in those patients with limb ischaemia, and protect against acute neutrophil-mediated pulmonary damage.

Type 1 tyrosine kinase receptor coexpression is a predictor of recurrence in ductal carcinoma in situ (DCIS) of the breast

N. L. P. Barnes, G. P. Boland, S. Khavari, A. Cramer, W. F. Knox and N. J. Bundred

South Manchester University Hospital and The Paterson Institute for Cancer Research, Manchester, UK

Background: Human epidermal growth factor receptor (HER) 2 overexpression is associated with endocrine resistance and early recurrence in invasive breast tumours. Expression of HER2, and other type 1 tyrosine kinase receptors, may predict risk of recurrence after surgery for DCIS; recurrences occur in up to 20 per cent of patients at 5 years, after breast-conserving surgery and radiotherapy. To determine if HER2 and HER4 expression predicted recurrence of DCIS we studied the primary DCIS of 133 women (follow-up 3–12 years), 94 who had not recurred and 39 who had recurred (27 with recurrent DCIS, 12 with invasive disease).

Methods: Tumours were compared for HER2, HER4 and oestrogen receptor (ER) expression by immunohistochemistry. HER2/4 expression was scored 0 (absent) to 3 (maximum). Scores $\geq$ 2 were taken as overexpression. ER was scored positive if $\geq$ 5 per cent of cells stained.

Results: Of non-recurrent DCIS lesions, 57 per cent were HER2 positive and 64 per cent HER4 positive, compared to 82 per cent HER2 positive ($P = 0·007^{*}$) and 36 per cent HER4 positive ($P = 0·003^{*}$) in the recurrent group. HER2/HER4 coexpression was associated with reduced recurrence compared to HER2-positive tumours lacking HER4 ($P = 0·003^{*}$). This association remained significant when stratifying for high grade ($P = 0·015^{*}$) and breast-conserving surgery ($P < 0·001^{*}$). HER4-positive DCIS was more likely to be ER positive than HER2-overexpressing DCIS (74 *versus* 51 per cent; $P = 0·048^{*}$). ER status did not influence recurrence in HER4-positive tumours ($P = 0·8^{*}$). $^{*}\chi^2$ test.

	No recurrence ($n = 94$)	Recurrence ($n = 39$)
HER2neg/HER4neg	11 (12)	5 (130)
HER2pos/HER4neg	23 (24)	20 (51)
HER2neg/HER4pos	29 (31)	2 (5)
HER2pos/HER4pos	31 (33)	12 (31)

Values in parentheses are percentages. $P = 0·003$ (χ^2 test).

Conclusion: Overexpression of HER2 in the absence of HER4 increases risk of early recurrence of DCIS.

Colorectal cancers with microsatellite instability demonstrate mRNA expression signatures characteristic of increased immunogenicity

A. Banerjea, S. Ahmed, R. Hands, F. Huang, P. M. Shaw, S. A. Bustin and S. Dorudi

Centre for Academic Surgery, The Royal London Hospital, Barts and The London Queen Mary School of Medicine and Dentistry, London, UK and Department of Pharmacogenomics, Bristol-Myers Squibb, New Jersey, USA

Background: Colorectal cancers with high degree microsatellite instability (MSI-H) have an improved prognosis compared to microsatellite stable (MSS) cancers. The observation of pronounced lymphocytic infiltrates suggests that MSI-H cancers are inherently more immunogenic.

Methods: We analysed tissue from 133 colorectal cancers with full consent and local ethics committee approval. Genomic DNA was analysed for microsatellite instability in BAT-26. High-quality RNA was used for microarray analysis on the Affymetrix HG-U133A chip (using Affymetrix protocols). Data were analysed on GeneSpring software version 6.0. Confirmatory real-time reverse transcriptase–polymerase chain reaction (RT-PCR) was performed on 28 MSI-H and 26 MSS cancers.

Results: Twenty-nine colorectal cancers (22 per cent) were identified as MSI-H. A comparison with 104 MSS cancers identified 2070 genes that were differentially expressed between the two groups ($P < 0·005$, Benjamini and Hochberg False Discovery Rate). Significantly, several key immunomodulatory genes were upregulated in MSI-H cancers. These included antigen chaperone molecules (heat shock protein (HSP) 70, HSP-110, calreticulin, gp96), proinflammatory cytokines (interleukin (IL) 18, IL-8, IL-15, IL-24, IL-7) and cytotoxic mediators (granulysin, granzyme A). Quantitative RT-PCR confirmed upregulation of HSP 70(1b) ($P = 0·016$), HSP-110 ($P = 0·002$), IL-18 ($P = 0·004$), IL-8 ($P = 0·002$) and granulysin ($P < 0·001$).

Conclusion: The novel observation of HSP upregulation in MSI-H colorectal cancer is highly significant in light of the recognized role of these proteins in innate and antigen-specific immunogenicity. Increased mRNA levels of proinflammatory cytokines and cytotoxic mediators also indicate an activated antitumour response. MSI-H colorectal cancer may be a paradigm of an inherent antigen-specific immune response and its study may significantly advance our understanding of tumour immunology and the development of immunotherapeutic strategies.

Double-blind randomized placebo-controlled trial of the antiplatelet effects of aspirin–clopidogrel combination *versus* aspirin alone at endovascular intervention for intermittent claudication of the lower limb

K. Cassar, P. Bachoo, I. Ford, M. Greaves and J. Brittenden

Departments of Vascular Surgery, and Medicine and Therapeutics, University of Aberdeen and Vascular Unit, Aberdeen Royal Infirmary, Aberdeen, UK

Background: Intermittent claudication is a common problem which causes significant impairment of quality of life and increased mortality. Endovascular recanalization, widely used for symptomatic relief, carries a high risk of reocclusion. Platelets play a central role in this process. Aspirin currently used in claudicants reduces the risk, and more potent antiplatelet strategies may

reduce this further. The aim of this study was to investigate the antiplatelet effect of aspirin–clopidogrel *versus* aspirin alone in patients with claudication undergoing endovascular intervention.
Methods: This was a double-blind randomized placebo-controlled trial; 132 patients were randomized to clopidogrel and aspirin or to placebo and aspirin with a loading dose 12 h before endovascular intervention. Flow cytometric measurement of platelet fibrinogen binding and P-selectin expression as measures of platelet activation status and of platelet responsiveness to stimulation at baseline, 12 h post-loading dose, 1 h, 24 h and 30 days postintervention.
Results: Platelet activation was significantly diminished in the clopidogrel group at 12 h post-loading dose compared to baseline (P-selectin: 27·3 per cent reduction, $P = 0{\cdot}017$; bound fibrinogen: 34·7 per cent reduction, $P = 0{\cdot}024$; stimulated bound fibrinogen: 49 per cent, $P < 0{\cdot}001$). No significant change was observed in the control group. Platelet function was significantly suppressed in the clopidogrel group at 1 h, 24 h and 30 days after endovascular intervention compared to the placebo group ($P < 0{\cdot}001$).
Conclusion: Clopidogrel–aspirin combination dramatically inhibits platelet function in claudicants before and after intervention. The combination treatment may help reduce reocclusion after endovascular recanalization.

Co-activator and oestrogen receptor expression may determine tamoxifen resistance in breast cancer

F. J. Fleming, A. D. K. Hill, E. W. McDermott, N. O'Higgins and L. S. Young

Department of Surgery and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Ireland

Introduction: The precise mechanism of tamoxifen resistance remains unclear. We examined the role of oestrogen receptor (ER) isoform expression and the co-activator proteins SRC-1and AIB-1 in tamoxifen resistance.

Methods: The expression of ER-α, ER-β, AIB-1 and SRC-1 was evaluated in breast cancer specimens by immunohistochemistry ($n = 52$).The ability of β-oestradiol and 4-hydroxytamoxifen (4-OHT) to modulate ER and co-regulatory protein expression in primary breast cancer cell cultures ($n = 48$) was assessed by western blotting. ER interaction with the oestrogen response element (ERE) in the presence of β-oestradiol and 4-OHT was assessed by electromobility shift assays. ER-α/ER-β–co-regulatory protein interactions were assessed by immunoprecipitation.
Results:

	No. of patients ($n = 52$)	Recurrence ($n = 14$)	No recurrence ($n = 38$)
ER-α positivity	40 (77)	10 (71)	30 (79)
ER-β positivity	28 (54)	4 (29)*	24 (63)
SRC-1 positivity	14 (26)	12 (84)‡	2 (5)
AIB-1 positivity	30 (57)	12 (84)‡	18 (47)

Values in parentheses are percentages. *$P = 0{\cdot}026$, †$P < 0{\cdot}001$, ‡$P = 0{\cdot}01$ *versus* no recurrence (χ^2 test).

β-Oestradiol upregulated ER-β and SRC-1 but not AIB-1 expression in immunoblotting experiments and increased ER-α/ER-β interaction with the ERE. 4-OHT increased ER-α and AIB-1 expression and increased AIB-1–ERE binding. ER-α and ER-β preferentially bound SRC-1 in the presence of β-oestradiol. 4-OHT potentiated ER-α–AIB-1 interaction while conversely inhibiting that of ER-β and AIB-1.
Conclusion: The ability of ER-α, but not ER-β to recruit the co-activator AIB-1 to the ER–ERE supports our clinical observation of ER-β as a positive prognostic indicator. AIB-1 may therefore be an attractive therapeutic target for endocrine-resistant tumours.

Patey Prize 2

Matrix metalloproteinase l and 12 transcript levels correlate with histopathological characteristics and clinical manifestations of carotid atherosclerotic plaques

N. Dastur, A. R. Morgan, K. Rerkasem, P. Gallagher, G. E. Morris, P. Eriksson, W. L. McPheat, C. P. Shearman and S. Ye

Department of Vascular Surgery and Human Genetics Division, Southampton General Hospital, Southampton, UK

Background: Previous studies have shown that atherosclerotic lesions express a number of matrix metalloproteinases (MMPs) and that increased levels are associated with histologically defined unstable plaques. We investigated whether transcript levels of MMP-l, -3, -7, -9 and -12 in carotid atherosclerotic plaques correlated with histological features and clinical events.
Methods: Atherosclerotic plaques ($n = 50$) removed from patients undergoing carotid endarterectomy were classified histologically using a system proposed by Virmani *et al.*, and MMP-l, -3, -7, -9 and -12 transcript levels in these tissues quantified by real-time reverse transcriptase–polymerase chain reaction.
Results: Comparing more stable plaques (with thick fibrous caps) to those less stable plaques (with thin caps) there was a 7·8-fold higher MMP-l transcript level ($P = 0{\cdot}006$), and 1·5–2·1-fold higher level of MMP-3, -7 and -12. MMP-12 transcript levels were significantly increased in ruptured plaques compared with lesions without cap disruption ($P = 0{\cdot}001$). MMP-l and -12 transcript levels were significantly higher in plaques from patients with amaurosis fugax than in those from asymptomatic patients ($P = 0{\cdot}029$ and $P = 0{\cdot}008$ for MMP-l and MMP-12 respectively).
Conclusion: These data support a role for MMP-l and -12 in determining atherosclerotic plaque stability and this is associated with clinical findings. We postulate that pharmacological modulation of MMPs could have a beneficial effect on clinical outcome.

Gefitinib ('Iressa', ZD1839) has activity in patients with oestrogen receptor (ER)-negative breast cancer and ER-positive breast cancer that has acquired resistance to tamoxifen: results from a phase II study

E. Gutteridge, K. L. Cheung, R. Owers, M. Koehler, L. Hamilton, J. Gee, R. I. Nicholson and J. F. R. Robertson

City Hospital, University of Nottingham, Nottingham, UK, AstraZeneca, Wilmington, Delaware, USA and Luton, UK, and Tenovus Centre for Cancer Research, Cardiff, UK

Background: Control of cell proliferation in acquired resistance may be through alternative signalling mechanisms such as the epidermal growth factor receptor (EGFR) pathway. This trial investigated in patients with tamoxifen (TAM)-resistant tumours and ER-negative breast cancer, the efficacy and safety of the oral epidermal growth factor receptor–tyrosine kinase inhibitor (EGFR-TKI) gefitinib ('Iressa', ZD1839) 500 mg/day.
Methods and results: For 33 patients the median (range) age was 61 (32–85) years. Metastases included local regional disease and distant metastases. At 6 months, using Union Internacional Contra la Cancrum (UICC) criteria, gefitinib showed antitumour activity in both groups. Of the ER-positive patients ($n = 9$), one patient had a partial response (PR) and five had stable disease (SD). Of the ER-negative patients ($n = 18$), one had a PR, one had SD and 16 patients had progressive disease. Gefitinib was generally well tolerated, with generally mild (grade 1 or 2) side-effects. Biopsies were assessed for EGFR, c-*erb*B2 and activated c-*erb*B2 using HScore analysis. In ER-positive tumours ($n = 2$), EGFR, c-*erb*B2 and activated c-*erb*B2 were coexpressed. This agrees

European Colorectal Congress

28 November – 1 December 2022, St.Gallen, Switzerland

Monday, 28 November 2022

09.50
Opening and welcome
Jochen Lange, St.Gallen, CH

10.00
It is leaking! Approaches to salvaging an anastomosis
Willem Bemelman, Amsterdam, NL

10.30
Predictive and diagnostic markers of anastomotic leak
Andre D'Hoore, Leuven, BE

11.00
SATELLITE SYMPOSIUM
ETHICON
PART OF THE Johnson&Johnson FAMILY OF COMPANIES

11.45
Of microbes and men – the unspoken story of anastomotic leakage
James Kinross, London, UK

12.15
LUNCH

13.45
Operative techniques to reduce anastomotic recurrence in Crohn's disease
Laura Hancock, Manchester, UK

14.15
Innovative approaches in the treatment of complex Crohn Diseases perianal fistula
Christianne Buskens, Amsterdam, NL

14.45
To divert or not to divert in Crohn surgery – technical aspects and patient factors
Pär Myrelid, Linköping, SE

15.15
COFFEE BREAK

15.45
Appendiceal neoplasia – when to opt for a minimal approach, when and how to go for a maximal treatment
Tom Cecil, Basingstoke, Hampshire, UK

16.15
SATELLITE SYMPOSIUM
Medtronic
Further,Together

17.00
Outcomes of modern induction therapies and Wait and Watch strategies, Hope or Hype
Antonino Spinelli, Milano, IT

17.30
EAES Presidential Lecture - Use of ICG in colorectal surgery: beyond bowel perfusion
Salvador Morales-Conde, Sevilla, ES

18.00
Get-Together with your colleagues
Industrial Exhibition

Tuesday, 29 November 2022

9.00
CONSULTANT'S CORNER
Michel Adamina, Winterthur, CH

10.30
COFFEE BREAK

11.00
SATELLITE SYMPOSIUM
INTUITIVE

11.45
Trends in colorectal oncology and clinical insights for the near future
Rob Glynne-Jones, London, UK

12.15
LUNCH

13.45
VIDEO SESSION

14.15
SATELLITE SYMPOSIUM
BD

15.00
COFFEE BREAK

15.30
The unsolved issue of TME: open, robotic, transanal, or laparoscopic – shining light on evidence and practice
Des Winter, Dublin, IE
Jim Khan, London, UK
Brendan Moran, Basingstoke, UK

16.30
SATELLITE SYMPOSIUM
Takeda

17.15
Lars Pahlman lecture
Søren Laurberg, Aarhus, DK

Thursday, 1 December 2022
Masterclass in Colorectal Surgery
Proctology Day

Wednesday, 30 November 2022

9.00
Advanced risk stratification in colorectal cancer – choosing wisely surgery and adjuvant therapy
Philip Quirke, Leeds, UK

09.30
Predictors for Postoperative Complications and Mortality
Ronan O'Connell, Dublin, IE

10.00
Segmental colectomy versus extended colectomy for complex cancer
Quentin Denost, Bordeaux, FR

10.30
COFFEE BREAK

11.00
Incidental cancer in polyp - completion surgery or endoscopy treatment alone?
Laura Beyer-Berjot, Marseille, FR

11.30
SATELLITE SYMPOSIUM

12.00
Less is more – pushing the boundaries of full-thickness rectal resection
Xavier Serra-Aracil, Barcelona, ES

12.30
LUNCH

14.00
Management of intestinal neuroendocrine neoplasia
Frédéric Ris, Geneva, CH

14.30
Poster Presentation & Best Poster Award
Michel Adamina, Winterthur, CH

15.00
SATELLITE SYMPOSIUM
OLYMPUS

15.45
COFFEE BREAK

16.15
Reoperative pelvic floor surgery – dealing with perineal hernia, reoperations, and complex reconstructions
Guillaume Meurette, Nantes, FR

16.45
Salvage strategies for rectal neoplasia
Roel Hompes, Amsterdam, NL

17.15
Beyond TME – technique and results of pelvic exenteration and sacrectomy
Paris Tekkis, London, UK

19.30
FESTIVE EVENING

Information & Registration **www.colorectalsurgery.eu**