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TGF-Beta Induces miR-100 and miR-125b Promoting EMT and Stemness in Pancreatic Cancer

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Aims: TGF-β induces epithelial-to-mesenchymal transition (EMT) and stemness in pancreatic ductal adenocarcinoma (PDAC). We hypothesised that uncharacterised microRNAs (miRNAs) could be playing regulatory roles, as part of TGF-β response, promoting EMT, cancer stem cell (CSC) formation and metastasis.

Methods: We used a cell line panel to examine the PDAC EMT spectrum by miRNA expression profiling and RNA-sequencing (RNA-seq), in order to discover miRNAs and transcripts implicated in the TGF-β response. SMAD2/3 chromatin immunoprecipitation sequencing (ChIP-seq) following TGF-β treatment in PANC-1 cells was then performed to identify genes regulated through it canonical pathway. To identify the targets post-transcriptionally regulated by TGF-β-induced miRNAs, we integrated miRNA overexpression with AGO2 RNA immunoprecipitation sequencing (RIP-seq) and differential expression analysis within a bioinformatics framework. Functional studies of the effects of TGF-β-induced miRNAs in vitro were performed, including assessment of cell morphology, tumour-sphere formation and growth, cell migration and invasion. The ability for TGF-β-induced miRNAs to induce metastatic colonisation was assessed in vivo. Finally, miRNA levels were measured in patient tumours (n=100) by in situ hybridisation (ISH) and correlated with clinical outcomes.

Results: We found that TGF-β transcriptionally induces miR-100 and miR-125b via SMAD2/3. Interestingly, although miR-100, miR-125b and let-7a are derived from the same primary transcript (MIR100HG), the tumour-suppressor let-7a is unchanged, as TGF-β also stimulates LIN28B which inhibits its maturation. We found that miR-100 and miR-125b each trigger EMT and stemness, and consequently reduction of their activity stunts in vivo tumourigenesis. Furthermore, inhibition of miR-125b blocks in vivo experimental metastasis. Through a genome-wide approach we identified the targets regulated by these miRNAs revealing that miR-125b represses transcripts through canonical and non-canonical base-pairing in both 3'UTRs and coding sequences, whereas miR-100 inhibits gene expression through canonical 3'UTR base-pairing. Genes targeted by these two miRNAs significantly overlap and mainly inhibit p53, DNA repair and apoptosis pathways. High miR-100 and miR-125b levels in patient tumours were both associated with poor survival after surgical resection.

Conclusions: We reveal that miR-100 and miR-125b are induced by TGF-β and may represent useful therapeutic targets to eliminate CSCs and metastatic spread in PDAC.
Attenuation of skeletal muscle Ischaemia-reperfusion injury: could insulin be the answer?

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**Aims:** Enhanced leucocyte activity is considered a critical step in the pathophysiology of skeletal muscle ischemia-reperfusion injury (IRI). We hypothesize that insulin via its anti-leucocyte activity can attenuate reperfusion injury in skeletal muscle ischemia.

**Methods:** A randomized, single-blinded, placebo-controlled clinical trial was conducted on patients undergoing vascular surgical interventions requiring clamping of aorta or patients with acute limb ischemia (ALI) requiring revascularization.

Treatment protocols between the two groups were identical except for ‘Insulin’ group who received insulin infusion at 2.5u/hr, combined with a glucose-potassium solution, until 12 hours post-reperfusion. Controls received normal saline infusion.

Endothelial-leucocyte adhesion was measured via P-Selectin and leucocyte activity via Myeloperoxidase (MPO). The temporal evolution of P-Selectin and MPO was measured in the venous effluent, via an indwelling femoral catheter.

**Results:** 24 consenting patients were randomized to ‘Insulin’ and ‘Control’ groups, 12 in each.

Elective surgery with aortic clamping was performed for 13 patients for chronic occlusive or aneurysmal disease. Remaining 11 patients presented with ALI.

The differences in the groups; Insulin vs. Controls for age (years): 64.9 v 62.3, elective surgery: 7 vs. 6, emergency surgery: 5 vs. 6, mean duration of ischemia (minutes): 119.5 vs. 180.5 (p=0.078) were similar.

Mean serum insulin level was 20.9 v 29.8 mu/ml, significantly higher in the ‘Insulin’ group (p<0.01).

Clinical outcomes in Insulin v Control groups were similar. (deaths 0 vs. 1, major amputations 0 vs. 0, fasciotomy 4 vs. 4, Acute Kidney Injury 1 vs. 2, Myocardial Infarction 0 vs. 2).
Using a linear regression model the increment of P-Selectin and MPO at each time interval (0, 2, 4, 6, 12 hours) was compared between groups.

Temporal evolution of P-Selectin and MPO in the ‘Control’ group demonstrated a significant increase post-reperfusion from its’ baseline, reaching a peak 2 hours post-reperfusion. (55.04pg/ml to 99.86pg/ml for P-selectin (p<0.001) and 110.81pg/ml to 160.61pg/ml for MPO (P<0.001).

The elevation of P-Selectin was significantly diminished in the ‘Insulin’ group at the two-hour interval; 12.1%(insulin) vs. 81.2%(control) increase from baseline (p=0.001) and four-hour interval; 5.6%(insulin) vs. 73.6%(control) increase from baseline (p=0.003).

The elevation of MPO was similarly significantly diminished in the ‘Insulin’ group at the two-hour interval; 3.9%(insulin) vs. 44.9%(control) increase from baseline (p=0.001) and four-hour intervals 1.4%(insulin) vs. 34.4%(control) increase from baseline (p=0.002).

Conclusions: Serum markers of white cell activation were significantly reduced using 2.5U/hr infusion of Insulin in a clinical setting involving human skeletal muscle ischemia reperfusion.
Randomised controlled trial investigating the efficacy and mechanism of healing of two different autologous skin grafts for wound healing (EPIGRAAFT)

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Aims: Split-thickness skin grafting (SSG) is an important modality for wound closure. However, the donor site becomes a second, often painful wound, which may take more time to heal than the graft site itself. Epidermal grafting (EG) is an alternative method of autologous skin grafting that harvests only the epidermis of the skin by applying continuous negative pressure on the normal skin to raise blisters. This procedure has minimal donor site morbidity and is relatively pain-free, allowing autologous skin grafting in an outpatient setting. We compare the clinical efficacy of EG against SSG and further investigate the cellular mechanism by which each technique achieves wound healing.

Methods: This randomised controlled trial enrolled patients aged 18 years or older with healthy granulating wound bed measuring lesser than 5x5cm. The co-primary endpoints were the proportion of wounds healed in 6 weeks and the donor site healing time. The secondary endpoints include the mean time for complete wound healing, donor site morbidity, patient satisfaction, and incidence of adverse events. Analysis was by intention-to-treat and patients were followed-up for 3 months. Paired 4mm punch biopsies were taken at the wound edge and wound bed, before treatment and one week after treatment. Tissues were sectioned and stained for H&E and immunohistochemistry for gap junctional proteins and analysed using confocal microscope. This trial is registered with ClinicalTrials.gov identifier, number NCT02535481.

Results: A total of 44 patients were included, 22 in each arm. The healing outcome between EG and SSG were similar at week 6. EG had faster donor site healing (p<0.001) and lesser donor site morbidity (p<0.001). Greater downregulation of gap junctional proteins was seen in the EG group, suggesting different healing mechanism between these two treatment groups. The massive downregulation in the EG group suggests that it initiates keratinocyte migration from wound edge and activates wound edge keratinocytes into a remodelling state of wound healing. The wound bed biopsies revealed increased inflammatory cells after EG in both acute and chronic wounds, suggesting the activation of the wound bed.

Conclusions: The EPIGRAAFT trial outlines the clinical efficacy with strong mechanistic link of the healing mechanism of the autologous skin grafts. EG is a promising alternative to the more invasive conventional surgical techniques as it is less invasive and reduces the surgical burden for patients in need of wound coverage.
Omentoplasty after abdominoperineal resection for rectal cancer does not reduce perineal wound complications: a Dutch snapshot study.

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Aims: Perineal wound complications are often encountered following abdominoperineal resection (APR). Filling of the pelvic space using an omentoplasty might prevent presacral abscess formation and small bowel descent, but there is no conclusive evidence to support its routine application. The aim of this study was to evaluate the effect of omentoplasty on perineal wound healing and prevention of long-term complications.

Methods: All patients undergoing APR for non-locally advanced rectal cancer in 2011 were selected from a collaborative snapshot research project performed by 71 Dutch hospitals. Primary perineal closure with or without omentoplasty (OP) was evaluated regarding perineal wound healing, abscess formation, reintervention for ileus, and perineal hernia development.

Results: Of 477 patients, 172 (36%) underwent OP. Median follow-up was 41 months (IQR 22-47), with no difference between groups. Non-healing of the perineal wound at 30-days was 47% after OP and 48% without OP, with a non-healing rate at end of follow-up of 9% and 5%, respectively. Presacral abscess developed in 12% after OP and 13% without OP. Reintervention for small bowel obstruction was performed in 5% after OP and 3% without OP. Perineal hernia developed significantly more often after OP (13% vs. 7%; P=0.025), also by multivariate analysis (OR 3.16; 95% CI 1.51-6.61; P=0.002).

Conclusions: Omentoplasty after APR with primary perineal closure does not improve perineal wound healing and does not reduce reintervention for small bowel obstruction, while it increases the long-term risk of perineal hernia formation.
Does dietary nitrate supplementation improve performance in cardiopulmonary exercise testing and recovery after surgery in patients with colorectal cancer?

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Aims: Major surgery increases oxygen demand and consumption perioperatively. The inability to meet this increased demand is associated with a higher rate of complications and poorer outcomes. Enhanced recovery pathways after surgery focus on improving oxygen delivery. However, dietary nitrate supplementation with beetroot juice (BRJ) in athletes has been shown to improve the oxygen utilisation. Cardiopulmonary exercise testing (CPX) is an established method of assessing patients’ cardiopulmonary reserve prior to surgery. The anaerobic threshold (AT) is calculated during CPX and can predict postoperative outcomes and complications. Patients with colorectal cancer have a lower AT. This study aims to see nitrate loading with BRJ improves the AT in patients with colorectal cancer and therefore improves outcomes after laparoscopic colorectal surgery.

Methods: A single centre double blind randomised controlled trial (NCT02319356) was conducted between 2015-2017. Patients were randomised to receive either a 70ml BRJ shot (containing nitrate 6.5mmol) or a nitrate deplete shot (placebo). An initial CPX was performed using a standard protocol and standard measurements including AT calculated. Patients were given a supply of BRJ as determined by the randomisation code and asked to drink one a day until discharged from hospital after surgery. After 7 days’ supplementation, a second CPX was performed. Standardised laparoscopic surgery was performed and patients followed an enhanced recovery pathway. The primary outcome of the study was the change in AT before and after nitrate supplementation. Secondary outcomes including return of GI function, length of stay and complications were recorded.

Results: In our interim analysis, the first 74 of 85 patients were unblinded. There was no significant difference in patient characteristics. For the primary outcome; AT; 19 patients were excluded (n=5 each for did not reach AT, no second CPX, tests not comparable, n=4 dropouts) leaving 55, 26 in the nitrate group. There was no significant difference between the baseline ATs in both groups (p=0.46).

The mean AT significantly improved after nitrate supplementation (Before 12.1, after 12.8, p=0.04). The mean AT did not significantly change with placebo supplementation (Before 12.8; After 12.4, p=0.26). The change in AT between the nitrate and placebo groups was significantly improved (Mean +0.7 nitrate, -0.4 placebo (p=0.03)).

Conclusions: In our interim analysis dietary nitrate supplementation significantly improved the Anaerobic Threshold after only 7 days in this elderly population with colorectal cancer compared with placebo. This was achieved without an increase in physical activity.