EURAPS Research Council 27-28th May 2015

BEST PAPER PRIZE, EURAPS RESEARCH COUNCIL 2015

In a closely contested competition, three individuals ran to a close first, second and third positions. All presented outstanding research, with clear evidence of enormous scientific insight, hard work, and supervisory support within outstanding scientific centres. The winner will be invited to present to the 2016 EURAPS meeting in Brussels.

Separating them is somewhat artefactual given their individual strength, but on the basis of five assessors' opinions, the results were:

WINNER:

Aadil A Khan[1,2], James Paget[1,2], Simon Robinson[2], Paul Harris[1] and Kevin Harrington[1]

[1] *Department of Plastic Surgery, The Royal Marsden Hospital, London, UK*

[2] *Targeted Therapy Team, Department of Cancer Biology, The Institute of Cancer Research, London, UK*

Introduction

Adjuvant radiotherapy is harmful to free flaps leading to late adverse effects (LAEs) characterized by fat necrosis, volume loss and contracture often requiring salvage surgery. Using a virally-delivered, free flap gene therapy strategy, this study aims to radioprotect free flaps from LAEs whilst maintaining the oncological efficacy of radiotherapy.

Methods: Lentiviral particles encoding the superoxide dismutase 2 gene (LVSOD2) were generated and used to infect superficial inferior epigastric artery (SIEA) flaps in Fischer (F344) male rats. LVSOD2 was delivered by intra-arterial injection, into the SIEA, performed *ex vivo*. LVSOD2-infected and control flaps were irradiated 1-month post-operatively with 50 Gy/3 fractions. Flap outcomes were measured using clinical, imaging, histological and molecular end-points. A tumour recurrence model was developed by engrafting syngeneic tumour cells into control and LVSOD2 flaps prior to irradiation with 20 Gy/5 fractions.

Results: SIEA flap irradiation with 50 Gy/3 fractions resulted in a depletion of SOD2 protein expression and biochemical activity ($p < 0.01$). LVSOD2 infection resulted in durable transgene expression *in vivo* (6 months). LVSOD2-infected flaps developed significantly less skin paddle contracture ($p<0.01$), volume loss ($p<0.001$) and less severe acute/late toxicities as scored using the RadioTherapy Oncology Group (RTOG) scoring system ($p<0.05$). They also exhibited significantly less fibrosis compared to control flaps ($p < 0.05$) and retained greater reactive oxygen species (ROS) scavenging capacity, and SOD2 protein expression, compared to controls ($p < 0.05$). Tumour recurrence studies demonstrated greater retardation of tumour growth in LVSOD2 flaps compared to controls ($p < 0.05$) and improved animal survival ($p < 0.01$) following radiotherapy.

Conclusions: We demonstrate that free flap gene therapy with LVSOD2 can protect irradiated flaps from LAEs and appears to, paradoxically, radiosensitize recurrent disease. These findings merit further evaluation of this pre-clinical concept for translation.

Meeting Supported by Malosa Medical, Cytori Therapeutics, Touch Bionics

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RUNNER UP:

Aidan Rose, Patrizia Cammareri, Owen Sansom & Gareth Inman
University of Dundee, Scotland

Solid tumors are typically considered to evolve over several years, arising from the accumulation of mutations within either stem or differentiated cells. However patients with malignant melanoma treated with inhibitors of oncogenic BRAF (vemurafenib), often present with rapid onset keratoacanthomas and/or cutaneous squamous cell carcinoma (cSCC), driven by paradoxical hyperactivation of the RAS/RAF MAPK pathway. Here, using targeted next generation sequencing, we identify frequent type-1 and type-2 TGF-b receptor (TGFBR1 and TGFBR2) mutations in skin lesions from vemurafenib treated patients. Expanding on this, we discover a similar high frequency of TGFBR1 and TGFBR2 mutations in 98 sporadic cSCC tumor samples. Functional in-vitro analysis reveals these mutations commonly ablate canonical TGF-b Smad signaling and typical TGF-b mediated tumour suppressor responses. In normal tissue, active TGF-b signalling co-localises to hair follicle bulge stem cells within both human and murine skin. Given this, we model hyperactivation of the MAPK pathway (through knockin of BRafV600E or KRASG12D) and the consequences of TGF-b signalling ablation (through the deletion of Tgfbr1) targeted to Lgr5+ve bulge stem cells. Whist BRaf or KRAS activation alone rarely led to cancer, homozygous deletion of Tgfbr1 results in rapid aggressive cSCC. Taken together, our results indicate that bulge stem cells can act as the cell of origin for cSCC, and that hyperactivation of the RAS-RAF-MAPK pathway, coupled with loss of TGF-b signalling, are driving events in skin tumorigenesis.

THIRD PLACE:

Christopher West, Tae Tawonsawatruk, Iain Murray, Bruno Peault & Hamish Simpson
The Centre for Regenerative Medicine, University of Edinburgh

Introduction: Atrophic non-union is attributed to biological failure of the fracture repair process. Pericytes are native ancestors of mesenchymal stem cells (MSC), and a promising source of bone progenitors that may provide trophic factors required for fracture healing. We aimed to evaluate whether pericytes could improve healing in an animal model of atrophic non-union and compare them to bone marrow derived MSC (BM-MSC). Methods: Pericytes and MSC were isolated from human adipose tissue and bone marrow, respectively. Seventeen Wistar rats underwent a validated procedure to induce atrophic non-union. Animals were randomly allocated to receive either pericytes (n=5), BM-MSC (n=5) or no cells (n=7). In treatment groups, 5x106 cells suspended in PBS were percutaneously injected into the fracture gap 3 weeks after operation. Controls received only PBS injection. Radiographic parameters, histology, micro-CT and biomechanical tests evaluated fracture healing at eight weeks. Results: At eight weeks, animals in cell treatment groups showed evidence of bone healing with only 1/5 in both the pericyte and BM-MSC groups progressing to non-union, whereas 6/7 in control group had developed non-unions. Radiographic parameters showed significant improvement ($p < 0.05$) over the eight-week period in cell treatment groups. Histology demonstrated bone bridges at the fracture gap in the both cell treatment groups. Bone mineral density of the fracture callus in animals injected with PSC and MSC was significantly higher than controls ($p < 0.05$). The biomechanical properties of the callus of the cell treatment groups were comparable and stiffer and stronger than the control group. Discussion: The results from this study demonstrate that pericytes have significant bone regeneration potential in an atrophic non-union model. These cells may have a role in the prevention of atrophic non-union and could enable a paradigm shift in the treatment of fractures at high risk of failing to heal and developing non-union.

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