

hypoxia may also be responsible for the loss of intrinsic function observed in many patients with RA disease, which then contributes to the development of the characteristic deformities of RA hand disease. We have taken measurements of tissue oxygen levels in the intrinsic muscles of the hand of patients with a diagnosis of RA undergoing elective surgery at Mount Vernon Hospital using the microelectrode technique established for measurement of synovial tissue oxygen. Patients undergoing elective hand surgery for conditions other than RA served as matched controls. Results so far suggest that the hand muscles of RA patients are significantly ($p < 0.05$) more hypoxic (mean oxygen tension 6.7%) than the matched controls (mean oxygen tension 10%) and there is a trend of increasing hypoxia with the RA sub-group in the radial-to-ulnar direction. Therefore, it is possible that the weakness and wasting of the intrinsic muscles of the hand observed in RA are not the result of atrophy secondary to joint disease but may be due to primary muscular involvement due to hypoxia. Ethical approval has been granted for this study.

O21. Solar radiation, carcinogenesis and radical damage: Measuring damage, susceptibility and improving protection

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The incidence of melanoma continues to rise and since 1975 has more than trebled in the UK. Melanoma is unresponsive to current radio- and chemotherapies, thus prevention remains a priority in reducing its incidence.

A central hypothesis to a free-radical theory of cancer is that susceptible individuals lack antioxidants, which would enable them to cope effectively with free-radical production and oxidative stress. Free radicals induce DNA damage, leading to mutation and carcinogenesis if not repaired. In the absence of adequate protection, such individuals experience greater oxidative stress from metabolism and environmental factors (ultraviolet-irradiation and cigarette smoke). There is increasing evidence that antioxidant enzymes are low or unbalanced in cancer compared to normal cells. UVA irradiation initiates free-radical production in human skin, increasing the risk of mutation and skin cancer. A role for UVA in skin cancer, in particular melanoma, has been controversial; however, recently evidence has converged to suggest a mechanism dependent upon melanin and oxygen radical production. A melanin-dependent mechanism may explain the disparity between animal models: UVA induces melanoma in the pigmented fish *Xiphophorus* but is ineffective in the albino mouse transgene. A higher proportion of UVA signature mutations occurring in human squamous carcinomas in the progenitor-cell-containing basal layer (compared to UVB mutations occurring predominantly in suprabasal keratinocytes) is consistent with greater skin penetration of UVA than UVB. We have investigated radical mechanisms of UVA damage to human skin and cells using electron spin resonance spectroscopy; and the role of melanin as a natural sunscreen filter or UV-photosensitiser of oxygen radicals. Understanding radical production and mechanisms of DNA damage, at different sunlight intensities, will suggest prevention strategies, both for identification of harmful UVA exposures, and improvement of sunscreen protection. This presentation reviews current findings in the context of current recommendations for sun exposure and skin cancer prevention strategies.

O22. Assessment of malignant melanoma using non-linear second harmonic generation imaging

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Introduction: Malignant melanoma causes more than three quarters of all skin cancer deaths. Current assessment and treatment is complicated by the lack of a reliable non-invasive pre-operative assessment technique. A key feature of melanoma invasion and spread is collagen re-modelling by Matrixmetalloproteinases. Thus collagen architecture can be used as a reliable marker of Melanoma invasion through the tissues, and to define melanoma invasion borders. Second Harmonic Generation (SHG) imaging can reliably demonstrate collagen architecture in human skin. SHG imaging employs infrared or longer spectrum pulsed lasers, which enables good depth penetration and no phototoxicity to tissues. Our aims were to use SHG to define collagen architecture in unstained histological sections thereby demonstrating melanoma borders, and to prove that SHG is an interchangeable with conventional techniques such as Melan-A and H&E.

Methods: Unstained histological human skin melanoma sections ($n = 8$) were imaged using SHG imaging revealing collagen distribution, followed by single photon transmission photomicrography. Each patient's specimen consisted of three sections; one unstained and imaged using SHG, the second stained with H&E, and the third stained with Melan-A. Melanoma borders obtained with SHG imaging in unstained sections were compared for correlation with borders obtained with Melan-A staining, and histopathologist's assessment of H&E sections. Fresh pieces of ex-vivo normal human skin is currently being imaged in line with local ethical approval to determine whether the technique is transferable to whole, live human skin.

Results: All samples showed complete correlation between SHG versus H&E and Melan-A staining. *Ex-vivo*, whole, normal human skin experiments demonstrated collagen architecture throughout the whole thickness of skin ($\sim 300\mu\text{m}$).

Conclusions: We have obtained 'proof of principle' that SHG imaging can be used for determining melanoma borders in sectioned tissues. SHG is a reliable and accurate imaging method. *Ex-vivo* human skin SHG imaging shows that this imaging can be translated to humans.

O23. CYP1B1 expression in malignant melanoma: A potential molecular target

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Introduction and Aims: Melanoma is resistant to standard chemotherapies and few therapeutic measures improve patient survival in the metastatic stage of the disease. CYP1B1, a member of the cytochrome P450 enzyme family, appears to be over-expressed in a number of human malignancies whilst exhibiting little protein expression in normal tissues. CYP1B1 protein expression was investigated in primary and metastatic melanoma to assess its potential as a target for pro-drug therapy.

Methods: Tissue microarrays of primary ($n = 74$) and metastatic ($n = 104$) melanoma specimens were constructed and analysed immunohistochemically for CYP1B1 protein expression. The intensity of staining was quantified by spectral image microscopy and compared to expression in normal tissues. Melanoma cell lines A375M, A375P and SK28 were also investigated for enzyme expression both constitutively and following incubation with the dioxin TCDD.

Results: CYP1B1 protein was detected with a high degree of consistency in 86% of primary and 77% of metastatic melanoma samples analysed. Spectral imaging microscopy demonstrated significantly

higher protein expression in both primary and metastatic tumours compared to the matched normal tissues (Mann Whitney $p < 0.005$) which exhibited minimal protein expression. All three metastatic melanoma cell lines demonstrated constitutive and inducible CYP1B1 expression.

Conclusion: CYP1B1 is consistently and selectively over expressed in primary and metastatic melanoma and may represent a therapeutic target for novel CYP1 activated pro-drugs that are currently under development.

O24. Antibody-nanoparticle conjugation for hyperthermic treatment of tumours

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Immunoconjugates of antibodies with toxic agents are becoming a significant component of anticancer treatments. One ongoing challenge to the success of this approach is the exposure of healthy tissues to the cytotoxic agents employed. We propose that magnetic fluid hyperthermia, using pre-targeted antibody-iron oxide magnetic nanoparticles, can address this issue. In magnetic fluid hyperthermia an alternating magnetic field is applied to magnetic nanoparticles in target tissues causing localised heating and cell death. The externally employed magnetic field can be focused on the tumour deposit. The system has potential to provide potent and selective cancer-targeted hyperthermic therapy.

To test the hypothesis we are using the genetically engineered humanised high affinity SM3e single chain Fv (scFv) reactive with the carcinoembryonic antigen (CEA), a glycoprotein associated with epithelial cancers. We have covalently coupled SM3e to periodate-oxidised dextran coated iron oxide magnetic nanoparticles (MNP). The MNP-SM3e complex has been purified and isolated successfully from unbound SM3e by gel filtration chromatography. The complex has been shown to retain binding to the CEA protein through enzyme linked immunoabsorbent assay (ELISA) and specificity to CEA expressing cells by immunofluorescence. Further characterisation of the MNP-SM3e complex will be discussed in relation to its therapeutic potential.

O25. VEGF165 transfection of ex vivo expanded keratinocytes promotes healing and neovascularization in porcine full thickness skin wounds

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Background: A trigger to enhance Full Thickness Wound (FTW) repair is delivery of oxygen and nutrients to the wound site. After the initial inflammatory phase, platelets and keratinocytes (KC) start to secrete Vascular Endothelial Growth Factor (VEGF) to attract Endothelial Cells into the wound granulation tissue. We present a method of ex vivo expansion of autologous KC and lipid mediated gene transfer of a regulable VEGF165 plasmid for the treatment of FTW.

Methods: Autologous KC were cultured to P3 and transfected with an inducible hVEGF165 plasmid and pcDNA6/TR repression molecule. This system allows for inducible expression by addition of Tetracyclin (TC). In a porcine model 12 to 16 Full Thickness Skin Wounds (FTW) were created on the back. We designed 3 groups: The first received 1.2 million transfected KC with addition of 4ug TC (KC/VEGF-group). The second group received 1,2 million transfected KC without addition of 4 ug TC (KC-group). The third group received no KC and was treated with 2 ml saline (S-group). Wounds were covered with polyvinyl wound chambers and wound fluid (WF)

was collected daily. Biopsies were taken at day 8 and 10 for H&E staining and immunohistochemistry.

Results: At day 10, reepithelialization was significantly enhanced in the KC/VEGF- and the KC-group compared the S- group ($p < 0.01$). Moreover, the KC/VEGF- group showed enhanced revascularization ($p < 0.0001$), compared to the KC- group, as measured with lectin antibodies. However, no difference in reepithelialization was seen between KC/VEGF- and KC- group.

Conclusions: Ex vivo cultured autologous KC enhance reepithelialization in a FTW in a pig model. Overexpression of hVEGF165 by these KC in the early wound phase enhances neovascularization in the wound granulation tissue. Ex vivo culturing of autologous KC and transfection with VEGF can be useful in reconstructing large skin wounds.

O26. The influence of mmp activity on force generation by Dupuytren's fibroblasts

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Introduction: Dupuytren's disease is a common fibrocontractile disorder affecting the palmar fascia of the hand that can cause severe disability. Despite advances in surgical management, recurrence remains a common complication. The matrix metalloproteases (MMPs) are a large family of proteolytic enzymes that play an integral role in scarring in vivo and collagen contraction in vitro. The purpose of this study was to assess the influence of MMP on matrix contraction by Dupuytren's fibroblasts using the broad-spectrum MMP inhibitor, ilomastat.

Methods: Nodule and cord-derived fibroblasts were isolated by explant culture from five Dupuytren's patients, carpal ligament-derived fibroblasts acted as the control. A Culture Force Monitor (CFM) was employed as an in vitro kinetic model of fibroblast-mediated collagen lattice contraction. Fibroblast-seeded lattices were allowed to contract for 48h under basal media conditions or exposure to ilomastat. The maximum force of contraction (dynes) was recorded. The expression and activity of several MMPs (-1, -2 and MT1-MMP) and TIMPs (-1, -2) was assessed by RT-PCR and ELISA.

Results: Cord and nodule-derived fibroblasts exhibited a two-fold increase in mechanical tension (150 ± 15 , 180 ± 20 , respectively) under basal conditions compared with carpal ligament (90 ± 12 , $p < 0.01$). Ilomastat significantly ($p < 0.01$) inhibited development of mechanical tension by cord and nodule but not carpal-ligament derived fibroblasts. Treatment with ilomastat suppressed MMP-1 and -2 activity but did not affect MMP or TIMP gene expression.

Conclusions: Dupuytren's-derived fibroblasts developed greater mechanical tension in the CFM model than control cells. Ilomastat suppressed MMP-1 activity and the ability of both cord and nodule-derived fibroblasts to generate contractile force, suggesting that MMP activity may be a therapeutic target in preventing disease recurrence in patients.

O27. MRI radiological assessment of distance between metacarpal bones and flexor tendons: Significance in screw fixation of metacarpal fractures

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Introduction and Aims: There are 3 types of metacarpal shaft (MS) fractures: transverse, spiral and comminuted. The use of interfragmentary compression screw (lag screw) is well indicated for spiral fractures. However, there is a risk of potential injury to flexor tendon if the distal end of the lag screw is significantly beyond the