Comparison of immune cell infiltrate between subcutaneous melanoma and colon carcinoma mouse models

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Cancer vaccines modulate the host's anti-tumour immune response and represent an area of emerging immunotherapy research for the treatment of cancer, including colorectal cancer (CRC). Murine subcutaneous injections of tumour cell lines are used to test cancer vaccines for the treatment of CRC. We aimed to determine the baseline immune response to subcutaneous injection of a colorectal cell line, CT26, compared to a melanoma cell line, B16-OVA, to investigate whether the tumour cell type would affect local and systemic immune responses.

CT26 adenocarcinoma cells were subcutaneously injected into mice. Control mice received B16-OVA melanoma cells or saline. The immune cells: dendritic cells, macrophages, T cells, (CD4+ and CD8+) and B cells were identified via flow cytometry at the tumour site (local immune response) and in the spleen (systemic immune response).

The systemic immune response to CT26 tumours was characterised by a higher frequency of dendritic cells, a lower frequency of T cells and twice the proportion of CD4+ to CD8+ T cells, compared to mice given B16-OVA tumours (n=14 (mice given B16-OVA tumours)-15 (mice given CT26 tumours), Mann Whitney, P=0.0016, P=0.0366, P=0.001). The intra-tumoural immune response to CT26 tumours had a reduced macrophage and T cell infiltrate compared to B16-OVA tumours (n=14, Mann Whitney, P=0.0233, P=0.0185).

These data represent a baseline immune response to B16-OVA and CT26 tumours that will be used to investigate modulation caused by a therapeutic CRC vaccine. We have also identified immune cell populations likely to be involved in CRC compared to melanoma; these cells have also been shown to be important in human CRC. This work will help link animal models and human data, and help translate cancer therapeutics into treatments for human patients.

Does the subarachnoid space extend into the eye?

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Intracranial hypertension is a neurological disorder characterised by an increase in intracranial pressure (ICP) within the subarachnoid space (SAS). Considered as an extension of the brain, intraocular pressure (IOP) in the eye has been used to estimate ICP. However, recent evidence indicated that although there was a significant correlation between the IOP and ICP, changes in IOP were poor predictor of changes in ICP. The existence of physiological and pathophysiological relationships between them is still elusive. Anatomically, the optic nerve is divided into intraocular, intraorbital, intracanalicular and intracranial segments. The intracanalicular segment is the point where the SAS surrounds the optic nerve and extends into the eye, and thus may provide direct pressure transmission between the ICP and IOP. The aim of this study was to investigate the anatomical configuration of the optic nerve sheath in the optic canal.

A total of nine cadavers were examined in this study. Arachnoid mater of three cadavers were stained with haematoxylin via SAS perfusion. The specimens were prepared as sets of serial plastinated sections with a thickness of 2.5mm or 0.3mm and examined under a stereomicroscope and a confocal microscope.

The results showed that:
1. the dura mater continued the periosteum of the optic canal and joined with the tendinous fibers of the eye muscles giving rise to the optic nerve sheath (n=3), and
2. in the specimens with a SAS staining, the SAS followed the optic nerve and entered the optic canal but terminated at the midpoint within the canal (n=3). However, in specimens without staining the SAS could not be traced. Thus, the observations were not quantified.

PROCEEDINGS
This study concludes that the SAS is not continuous throughout the optic nerve sheath, suggesting that there is no anatomical basis to support mechanism of direct pressure transmission between the ICP and IOP.

**IGF-R1 pathway in crizotinib-resistance in ALK-positive non-small cell lung cancer**

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Non-small cell lung cancers (NSCLCs) account for approximately 85% of all lung cancer-related deaths worldwide. About 4% of NSCLCs contain rearrangements in the EML4 - ALK genes, which encode a fusion protein that drives cancer development. Crizotinib, an ALK inhibitor, gained fast-tracked approval from the Food and Drug Administration in 2011 on the back of unprecedented responses in clinical trials. Unfortunately, resistance to crizotinib invariably develops within two years through a variety of mechanisms. One of the major mechanisms of resistance is the activation of alternative cell signalling pathways, including the insulin-like growth factor receptor-1 (IGF-R1) pathway. This study aimed to investigate the role of the IGF-R1 pathway in crizotinib resistance and whether the combination of crizotinib with an IGF-R1 inhibitor may delay the development of resistance in vitro.

To model innate resistance, the established ALK-positive NSCLC cell line, NCI-H3122, was exposed to high dose crizotinib (10µM) for 24 hours. The drug was removed and cells were maintained for 12 days. In order to develop a crizotinib-resistant cell line (C.R-H3122), NCI-H3122 cells were maintained in crizotinib (0.8µM; the steady state plasma concentration in mice) for 114 days.

The IC50 of crizotinib in a model of innate resistance increased in treated cells (0.163µM) compared to non-treated cells (0.071µM). Chronic exposure to crizotinib led to the development of a crizotinib-resistant cell line (C.R-H3122) with an IC50 of 2.082µM, 20.8-fold higher than control. Cytotoxicity testing of an IGF-R1 inhibitor, NVP-AEW541 (NVP) revealed a lower IC50 in the C.R-H3122 cells (2.205µM) compared to control (2.994µM). Moreover, combination treatment indicated the C.R-H3122 cells are sensitised to NVP.

These results suggest the IGF-R1 pathway plays a role in two models of crizotinib resistance, suggesting the combination of crizotinib with NVP may be particularly effective in crizotinib-naïve patients.

**Protein modeling of nonsynonymous SNPs in apolipoprotein(a)**

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Cardiovascular disease (CVD) is New Zealand’s leading cause of death. Elevated plasma lipoprotein(a) [Lp(a)] is a strong risk factor for CVD. Lp(a) is a low-density lipoprotein (LDL) analogue comprised of Apolipoprotein B covalently linked to a unique glycoprotein apolipoprotein(a), which is transcribed from the LPA gene. Lp(a) bears significant homology to plasminogen with repeating kringle structure. This study aimed to model possible effects of nonsynonymous single nucleotide polymorphisms (SNPs) associated with altered plasma Lp(a) on 3D protein structures of apo(a) domains.

Thirty nonsynonymous SNPs were presented from a next-generation sequencing study that sequenced the LPA gene of 48 individuals. The population consisted of individuals with high, medium and low plasma Lp(a). Online protein prediction programs SIFT and PolyPhen-2 were used to indicate if SNPs were damaging to structure. There was general concordance between the two programs, and ten SNPs were strongly predicted to be damaging to protein structure, others were either possibly damaging or benign. 3D protein structures for four available kringle domains were downloaded from the Protein Databank. Four of the apo(a) kringle domains and the protease domain were unavailable and thus homology models based on the closest available apo(a) kringle structure (or the plasminogen protease domain) were generated using the FFAS Burnham server. The structures were opened in PYMOL and amino acid changes modeled using the mutagenesis wizard tool.

The SNPs R990Q and R1771C are associated with lowered Lp(a) levels and were shown to ablate polar contacts within their respective domains (KIV-4 and KVI). Superimposition of the structures showed they are analogous arginine residues in different kringle domains. This indicates that apo(a) kringle structure may be perturbed by these changes. Functional studies in tissue culture are now underway to further elucidate their effects.

**Evaluation of plasma cell-free DNA stability and preservation in Roche Cell-Free DNA Collection Tubes and Streck Cell-Free DNA BCT over a 14-day period**

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Circulating tumour DNA (ctDNA), a blood based biomarker has the potential to diagnose early stage cancer, monitor treatment response and identify metastatic relapse. This study aimed to compare two commercially available Cell Free DNA Blood Collection tubes for the stability and preservation of plasma ctDNA for 14 days post-collection.

Twenty healthy volunteers had blood drawn via venipuncture into five Streck and
five Roche Cell-Free Blood Collection tubes. Samples were stored at 22°C and processed on 0, 4, 7, 10 and 14 days post-collection. cfDNA was extracted from 4mL of plasma using the Qiagen QIAamp Circulating Nucleic Acid Kit to a final elution of 35µL. The cfDNA concentration was measured using 2µL of elution with the Quibit dsDNA HS Assay Kit (ng/mL). Pair-wise student t-test was conducted to determine whether there were any differences in the preservation capability of the two tubes.

The results indicated a significant difference of the initial cfDNA reading between the two tubes on day 0 (mean Streck 6.58ng/mL ± 3.55, Roche 5.67ng/mL ± 3.31, \(P=0.038\)). However, no significant differences were found between the two tubes for samples processed on days 4, 7, 10 and 14 (\(P>0.05\)).

The findings of the study indicate that either Streck or Roche tubes are suitable for clinical sample collection as neither were significantly better in terms of preservation as measured from day 4 onwards. The initial cfDNA reading on day 0 was significantly different, however, this may be due to sub-standard venipuncture technique resulting in cellular lysis and genomic DNA release. Hence after consideration of price (per 100 tubes) and availability in New Zealand, the Roche tubes have been selected for developing cfDNA as a cancer surveillance assay in the clinical phase.

Rotator cuff-related pain: participants’ understanding and experiences

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Persistent rotator cuff-related pain is common in the middle-aged and elderly. A psychosocial approach to treatment indicates individuals’ beliefs, and experiences need to be considered in the management of this pain. While extensive research has explored beliefs of individuals with spinal pain, less is known about individuals’ beliefs regarding shoulder pain. This qualitative study aimed to explore beliefs about the cause of pain in individuals with persistent rotator cuff-related pain and their experiences of the effect of pain on their daily lives.

Five men and five women, aged 47–68 years, with shoulder pain for more than three months were recruited from the local community via newspaper advertisements and flyers displayed at sporting facilities, physiotherapy and general practitioner clinics. Individual semi-structured interviews were audio-recorded, transcribed in verbatim and analysed using the general inductive approach.

Four key themes emerged following analysis: ‘Understanding the pain’; ‘It affects everything’; ‘Pain-associated behaviours’; and ‘Emotions, thoughts, and the future’. The cause of pain, ‘Understanding the pain’, was described in terms of anatomical factors within the context of the participants’ lives. The pain impacted all areas of life, with participants reporting, ‘It affects everything’. Participants responded to their pain by adopting certain, ‘Pain-associated behaviours’ and sought information for general management and exercise prescription, ‘Emotions, thoughts, and the future’.

The participants with persistent rotator cuff-related pain believed the cause of their pain to be local to the shoulder. Participants also described various work, sports and family related stressors in their lives. Such stressors can be pain-associated, however, the participants rarely considered these as being contributors. Rehabilitation may need to include educating individuals, expanding their understanding regarding pain mechanisms and addressing certain pain-related beliefs. The pain affected many parts of these participants’ lives and the unique experiences shared highlight the need for tailored treatment based on individual goal setting.

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