Oral molecular and immunopathology

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Current information about cellular and molecular mechanisms involved in the pathogenesis of chronic oral diseases and in development and healing allows advancement of diagnostic and treatment modalities. Our group uses a range of cellular, molecular, immunological and pathological tools including cell culture, genomic and focused micro-arrays, real time PCR, laser microdissection and immunohistochemistry to investigate a range of dental and oral mucosal conditions.

Of major interest is regulation of the microenvironment in oral squamous cell carcinoma (OSCC) with respect to local and nodal immune regulation, influences on local invasion, angiogenesis and the reaction to endoplasmic stress. Exosomes, membrane bound nanovesicles released by cells into their extracellular environment, contain potential biomarkers of OSCC.

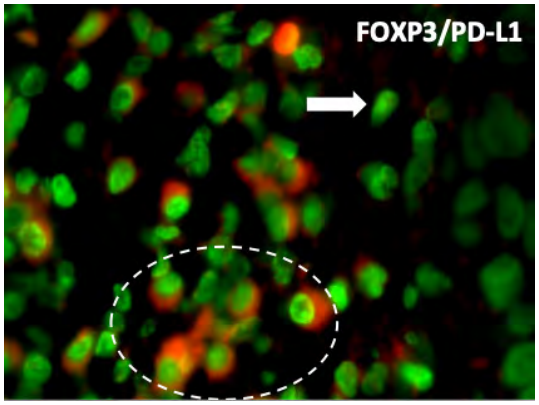
Salivary exosomes are easily accessible and we are investigating their extraction and identification. Our interest in the immune response extends to preclinical assessments of immune modulation in oral lichen planus and in the normal and inflamed pulp and associated with bone grafting materials. Pulpal responses to injury in healthy patients are being compared to responses in people with diabetes using a range of histochemical and immunohistochemical staining, tissue culture and PCR.

Current research projects

Regulation of immune responses

In oral squamous cell carcinoma-regulatory T cells and various cytokines

Oral squamous cell carcinoma develops in an immune cell-rich environment, where inflammatory cells in the tumour microenvironment establish an anti-tumour response by secreting pro-inflammatory cytokines.



Key personnel

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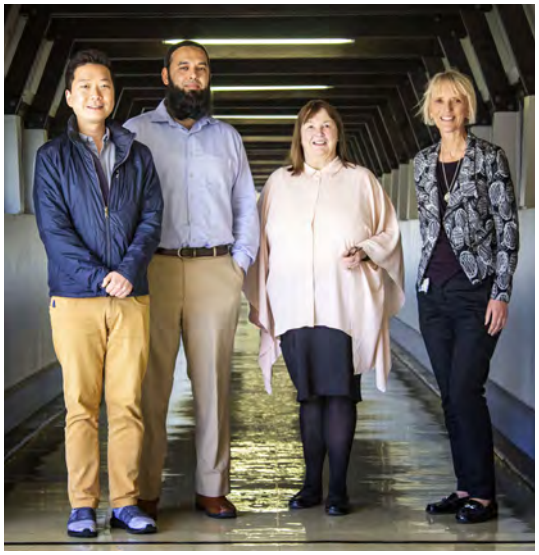
At the same time the cancer cells may induce various mechanisms suppressing the anti-tumour response such as regulating a network of suppressive cytokines and the recruitment of suppressive regulatory T Cells (Tregs). These escape mechanisms are seen at the local tumour site and similar mechanisms may also occur in regional lymph nodes. Gene analysis studies demonstrated active regulation of T cell anergy and tolerance genes in primary OSCC and in metastatic lymph nodes. Regulatory T cells and the transmembrane protein programmed cell death ligand (PD-L)-1 have been implicated in cancer development and progression. Tregs and PD-L1 have been detected in oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia. We have found an increased proportion of PD-L1-expressing Tregs, in addition to a higher proportion of Tregs, in OSCC and potentially malignant oral disorders suggesting that this specific and targeted expression may be an important mechanism in the development of OSCC.

In oral lichen planus

Regulatory T-cells and Th17 cells, which express interleukin (IL)-17, are two Th subsets that may be important in the pathogenesis of oral lichen planus (OLP). Lichen planus is a relatively common chronic dermatologic disease that often affects the oral mucosa. We have previously found that the balance between Tregs and IL-17+ cells is altered in OLP with more FoxP3+ Tregs present in OLP lesions and fewer IL-17+ than non-specific inflammatory (NSI) control cases. In light of the development of IL-17 and IL-23 inhibitors, eg tildrakizumab, and their success in the management of psoriasis, our current project is investigating the role of IL-23 in OLP and measuring the effects of its inhibition.

Left: Photomicrograph showing cells with green single stained nuclei (white arrow) which as FoxP3+cells. In the white circle are cells double-stained with green (FoxP3+) and red programmed cell death ligand (PD-L)-1. This is from the BDS (Hons) project undertaken by Bomi Aum (2020, 1st class) who found more double-stained FoxP3+PD-L1+ cells in oral squamous cell carcinoma than in non-specifically inflamed tissue.



(L-R) Dr Benedict Seo, Mohammad Aziz, Prof Alison Rich and Prof Merilyn Hibma, of the 'Exosomes in oral cancer' project.

Collaborations

We have international collaborative studies with:

- Oral Cancer Research and Coordinating Centre (OCRCC), University of Malaya and MAHSA University (www.malaysiaoralcancer.org) Malaysia (immune modulation in oral cancer, exosomes in oral cancer)
- Kyoungpook National University, Korea, Dr J-K Jung and colleagues (lymphangiogesis in oral lichen planus)
- University of Sri Jayewardenepura, Sri Lanka, Dr M Weerasekera and colleagues (Joint projects investigating the role of Candida in oral carcinogenesis and vascular endothelial growth factor (VEGF) in oral cancer
- School of Dentistry, University of Birmingham, UK – with Prof Mike Milward & colleagues investigating the role of phototherapies in the treatment of oral & dental diseases.

Key funding successes

$192,138. *In vitro* inhibition in oral lichen planus. Sun Pharma Global FZE, 2019. (Alison Rich, Haizal Hussaini, Benedict Seo, Qing Sun)

$15,000. Investigating biomarkers in exosomes derived from serum and saliva of patients with oral squamous cell carcinoma. New Zealand Dental Research Foundation, 2019. (Alison Rich, Mohammad Aziz, Merilyn Hibma (Pathology, OMS), Haizal Hussaini, Benedict Seo)

$206,046. Interrogating immunotherapy for dental pulp therapy and management. Health Research Council of NZ, 2020. (Haizal Hussaini, Lara Friedlander, Chuen Yen Hong, Benedict Seo, Qing Sun)

$15,000. Can immunotherapy be used in inflamed dental pulp tissue to preserve tooth vitality? New Zealand Dental Research Foundation, 2020. (Haizal Hussaini, Shelly Arora, Paul Cooper, Lara Friedlander, Alison Rich, Shakila Rizwan (Pharmacy), Benedict Seo)

$15,000. Exosomal biomarkers in blood plasma and saliva of oral cancer patients. New Zealand Research Foundation of the Australia & NZ Head and Neck Cancer Society, 2020. (Benedict Seo, Merilyn Hibma (Pathology, OMS), Haizal Hussaini, Benedict Seo, Alison Rich)

$14,980. Osteoinductive potential of bioactive glass, collagen and lyophilized platelet-rich fibrin scaffold for alveolar cleft osteoplasty. New Zealand Dental Research Foundation, 2020. (Haizal Hussaini Aida Ngah, George Subasinghe Dias (Anatomy), Darryl Tong, Jithendra Ratnayake)

In the dental pulp

Inflamed pulp produces cytokines including interleukins to defend against infection. However, if ILs are produced in excess and for a longer time, this can impede pulp healing leading to death of the tissue. Our particular interest is in the role of IL-23 as its inhibitors have been used successfully to treat chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. We propose that by blocking the IL-23 pathway, inflammation in the pulp can be controlled and tissue healing ma be facilitated. This is the subject of our current study, which uses in vitro cell culture models.

Exosomes in oral cancer

In the first part of this study we developed methodology to extract and identify exosomes from oral cancer and normal oral keratinocyte cell lines. To extract exosomes from OSCC cells grown in culture ultracentrifugation and an exosome isolation kit (Exoquick TC plus) were used. The extracted vesicles were characterised with a Zetasizer which uses using dynamic light scattering to determine the size of particles for the size range 0.6 nm to 6 μm, in addition to using Transmission electron microscopy. Having extracted an adequate number of vesicles and confirmed they were exosomes, we were then able to extract RNA from them.

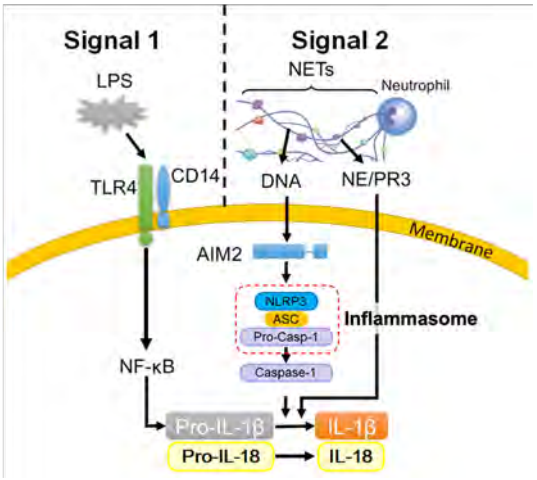
Having completed this baseline cell culture work and refined the techniques to be used going forward, we then moved on to study exosomes in saliva and blood samples from patients with OSCC and healthy controls using samples sourced from ORCCC. The mean expression level of HSPA1A was higher in both OSCC plasma and saliva exosomes compared with controls. Enzyme linked immunosorbent assay (ELISA) results showed a higher expression of FOXM1, DNMT1 and CCNB1 in OSCC plasma exosomes than matched controls.

While more precise exosome isolation methods are still being developed it was shown that saliva exosomes are very important for future research and potential clinical application as they carry genes and proteins in greater quantities when compared with plasma exosomes.

Pulpal response to insult

Effects of diabetes on the dental pulp

Histological changes were observed in normal dental pulp of participants with Type 2 diabetes (T2D) compared with healthy controls. T2D resulted in a dental pulp that was less cellular, less vascular, demonstrated thickened blood vessel walls, increased pulp calcification, increased collagen and decreased elastin deposition. More cells/unit area in T2D dental pulps were CD68+ and CD83+, while fewer were FOXP3+ compared with non-T2D samples. The cytokines IL1β, IL6, IL17 and TNF-α were more highly expressed in T2D dental pulps as was the glycation process as evidenced by increased IHC expression of AGE and RAGE.



Above: Dysregulated inflammasome signalling underpins a range of chronic inflammatory diseases. Recent studies from Paul Cooper and colleagues have indicated that this pathway is active in several dental and oral diseases. Signal 1 involves a proinflammatory stimuli such as a bacterial component, e.g. lipopolysaccharide (LPS), and signal 2 is derived from a damage associated molecular pattern (DAMP), e.g. DNA/histones present in neutrophil extracellular traps (NETs). The activated intracellular signalling leads to production of proinflammatory master regulatory cytokines [i.e. interleukins (ILs)] which are processed by caspases from their inactive (pro-) to active state prior to their cellular release.

Below: Photomicrograph showing immunohistochemistry results with anti-toll-like receptor (TLR)-4 in the pulp of normal pulps from patients without diabetes (non-T2D) and with well-controlled Type 2 diabetes. This work was part of the PhD project of Shaikha Alsamahi who found numerous differences in the histology and distribution of immune cells and cytokines in diabetic patients with ostensibly normal healthy pulps, compared to a control group of patients without diabetes.

Endoplasmic reticulum stress and the unfolded protein response

In a neoplastic model-oral squamous cell carcinoma

In this study we are investigating cellular stress pathways known as the unfolded protein response (UPR). These pathways are activated when the endoplasmic reticulum (ER), the protein-producing factory within the cell, is stressed. ER stress modulates UPR pathways, thus partially determining the cellular response to disease.

To investigate UPR in OSCC cell lines derived from normal, dysplastic and malignant oral keratinocytes were subjected to tunicamycin-induced ER stress of varying intensity and chronicity. OSCC cells maintained viability in the presence of ER stress at a significantly greater level compared with normal oral keratinocytes. Furthermore, caspase-3/7 activity and DNA fragmentation, hallmarks of cell death, were suppressed in OSCC.

It was discovered that UPR-induced apoptosis-related factors, most notably DDIT3, were significantly up-regulated in OSCC. Also, the master regulator of lipid metabolism, SREBP1, and CREB3L3, an ER-resident transcription factor closely related to ATF6, which plays an important role in linking ER stress with immune-inflammatory responses, were significantly up-regulated in OSCC.

The identified factors should be further studied and validated *ex vivo* and, eventually, *in vivo*, in view of their potential diagnostic and prognostic role in improving the diagnosis, treatment and management of oral cancer.