Structured Reports for the Pathological Society Grant Awards: Career Development Fellowships (CDF), Path-Soc / J Shanks Fellowship, PhD Studentships, International Collaborator Awards, Small Grant Scheme (several types) Awards, CRUK/Path-Soc Pre-Doctoral Research Bursary & Early Career Pathology Researcher (Hodgkin & Leishman) Grants.

Recipients of grant awards from the Pathological Society of Great Britain and Ireland should submit a scientific report detailing the work undertaken with support from this award and any outputs arising from this. The reports should be set out using the following subheadings and should consist of:

Annual Reports: Final report on ICA 1019 01

Title: Thyroid Malt Lymphoma: Self-Harming to win T-Cell Help'

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Background and aims:

Mucosa associated lymphoid tissue (MALT) lymphoma originates from acquired MALT that is triggered by distinct chronic inflammatory disorders at different anatomic sites. Despite involving different aetiologies, the development of MALT lymphoma at various sites is believed to be driven by antigenic stimulations. This is best exemplified in gastric MALT lymphoma, of which majority show complete lymphoma regression after H pylori eradication by antibiotics. As tumour infiltrating T-cells are critical for H pylori mediated proliferation of malignant B-cells *in vitro* through cognate help via co-stimulation molecules (CD40/CD40L), and bystander effects via cytokines (BAFF), it is believed that H pylori eradication obliterates the T-cell help and cytokines, consequently causing tumour regression. Such T-cell help is also thought to be operational in MALT lymphoma of other sites.

In thyroid MALT lymphoma, our ongoing studies uncovered frequent mutation in *CD274* (42/76=55%) and *TNFRSF14* (39/76=51%), with a high proportion of cases harbouring mutation in both genes. The majority of mutations in these genes are deleterious alterations (frameshift indels, nonsense changes), thus most likely causing their inactivation. CD274 (PD-L1) and TNFRSF14 are ligands for PD1 and BTLA respectively, and negatively regulate the function of T-cells including T helper cells. We hypothesize that CD274 and TNFRSF14 inactivation in malignant B-cells by mutations eliminate their negative regulation on T helper cells, consequently sustaining the function of these T-cells and their help to malignant B-cells. Given thyroid MALT lymphoma invariably derives from a background of Hashimoto thyroiditis, the lymphoma cells most likely originate from an autoreactive B-cell, thus retaining their regulational (co-stimulation & repression) association with CD4+ T helper cells. We would like to investigate the role of T helper cells in the pathogenesis of thyroid MALT lymphoma and the timing of *CD274* (PD-L1) and *TNFRSF14* inactivation during the lymphoma development.

Results:

We have performed multiplex immunofluorescent staining to investigate and quantify activated T-cell subsets. We have shown that both the proportion of activated T-cells (CD4+CD69+/CD4+) within the proximity of malignant B-cells, and the level of transformed blasts were significantly higher in thyroid MALT lymphoma with *CD274*/*TNFRSF14* genetic abnormalities than those without these changes. Both *CD274* and *TNFRSF14* genetic changes were significantly associated with Hashimoto's thyroiditis (P=0.01, P=0.03 respectively), and *CD274* mutation/deletion additionally associated with increased erythrocyte sedimentation rate (ESR) (P=0.0001). Our findings suggest that *CD274*/*TNFRSF14* inactivation in thyroid MALT lymphoma B-cells may deregulate their interaction with T-cells, promoting co-stimulations and

impairing peripheral tolerance. These observations have been published in Leukemia 2021 Dec;35(12):3497-3508.

To investigate the timing of *CD274*/*TNFRSF14* inactivating mutations during thyroid MALT lymphoma development, our collaborators in Japan have identified a series of cases with early lesions showing Hashimoto thyroiditis including those also progressed into an overt lymphoma. We are in the process of investigating these early lesions by targeted sequencing, to address whether the above genetic changes occur early in reactive lesions.

Publications

Thyroid MALT lymphoma: self-harm to gain potential T-cell help.
Wu F, Watanabe N, Tzioni MM, Akarca A, Zhang C, Li Y, Chen Z, Cucco F, Carmell N, Noh JY, Ito K, Dobson R, Moody S, Yao W, Zhang W, Liu W, Liu H, Okosun J, Chott A, Bi Y, Chuang SS, Raderer M, Li JY, Marafioti T, **Du MQ.** doi: 10.1038/s41375-021-01289-z. Epub 2021 May 21.