Rejuvenating the aged immune system by cell reprogramming during lung carcinogenesis

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Principal supervisor's CRUK CC theme:
- Early Cancer Institute
- Thoracic Cancer Programme

Department for student registration: Oncology
Department or institute where research will take place: Early Cancer Institute

Postgraduate scheme: **Clinical Research Training Fellow (3-year PhD)**

PhD project outline:

Cellular senescence, an irreversible cell cycle arrest in response to unrepairable damage and stress, plays pivotal roles in a variety of physiological processes, but also in multiple pathologies, including cancer and age-related diseases (1). Senescent cells undergo a paracrine release of inflammatory cytokines, growth factors, and tissue-remodeling agents, termed the senescent-associated secretory phenotype (SASP), that can fuel disease progression (1, 2). Thus, clearance of senescent cells by pharmacological approaches (senotherapies) has emerged as a promising avenue for preventing cancer progression, including lung cancer, and increasing healthspan and lifespan in preclinical models (3, 4, 5).

Previous research has shown that in vivo reprogramming by expression of the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc, namely OSKM) is feasible in murine models (6). Partial reprogramming and epigenetic remodelling by expressing OSKM factors in tissues ameliorates age-associated hallmarks and prolongs lifespan in mouse models of premature aging (7).

Recently, we have identified the accumulation of senescent macrophages in premalignant lung lesions in clinical samples and murine models of KRAS-driven lung cancer, and also in lungs from normally-aged mice (8). Importantly, senescent macrophages promote lung tumorigenesis by creating and immunosuppressive microenvironment and their removal ameliorates lung tumour burden and extends mouse survival (8, 9, 10) (Figure 1). Building on these findings, we will test the central hypothesis that macrophage rejuvenation in ageing mice through partial reprogramming (de-differentiation) prevents lung tumour initiation and promotes lifespan.

This research proposal therefore stands in three specific aims:
1. To determine how the age-related immune system decline enriched in senescent macrophages impacts lung cancer initiation and progression.
2. To validate partial reprogramming as a new methodology to rejuvenate senescent/aged macrophages and immune system cells.
3. To assess whether rejuvenation of senescent/aged macrophages by partial reprogramming ameliorates lung cancer development.

PhD experimental plan:

We propose three Working Packages aligning with the specific aims stated above:

WP1. We will use young and naturally-aged mice subjected to lung carcinogenesis using

urethane and N-methylnitrosurea (NMU). Macrophage senescence will be manipulated pharmacogenetically and/or pharmacologically (e.g. by using senolytics such as navitoclax). Endpoints will include lung tumour burden and progression (by micro-CT analyses), survival rates, and phenotypic readouts (e.g. biomarkers of senescence, immune system panels, by histological studies).

WP2. We will use mouse macrophage cell lines (e.g. RAW264, RAW309CR, PU5-1R, or WR19M.1) that will be transduced with lentiviral plasmids to express the OSKM Yamanaka factors in a doxycycline-dependent manner. Macrophages will be subjected stressors promoting senescence induction (e.g. irradiation or DNA-damaging drugs) and will be subsequently exposed to culture media +/- doxycycline to induce partial reprogramming by OSKM expression. Control and senescent macrophages subjected to partial reprogramming will be tested functional assays, including chemotaxis, macrophage differentiation and polarization (M1/M2), phagocytosis, cytokine production and MDSC suppression assays.

WP3. For this purpose, we will cross an “inducible reprogramming” murine model (i4F) (6), which can express the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) in a doxycycline-dependent manner, with hCD68-rtTA mice (https://www.jax.org/strain/012418), thereby driving the OSKM expression specifically in macrophages. To induce lung carcinogenesis, we will then subject young and naturally-aged hCD68-i4F mice to carcinogens exposure by using urethane and N-methylnitrosurea (NMU), and examine the effects of macrophage partial reprogramming on tumour progression. Endpoints will include lung tumour burden and progression (by micro-CT analyses), survival rates, and phenotypic readouts (e.g. markers of senescence, reprogramming, immune system panels, by histological studies).

Altogether, the proposed experimental plan will allow us to obtain proof-of-concept that rejuvenation and/or partial reprogramming of senescent/aged immune system cells (in particular tumour-associated macrophages) impairs or ameliorates lung cancer onset and progression. If successful, this project may result in new generation therapeutic strategies to target the tumour microenvironment in lung cancer.

Main techniques:

- In vitro and in vivo reprogramming of macrophages and immune system cells by expressing Yamanaka (OSKM) factors.
- Macrophage cell-based functional assays, including tumour-associated macrophage (TAM), macrophage differentiation and polarization (M1/M2), phagocytosis, cytokine production and MDSC suppression assays.
- Induction and assessment of lung cancer (histology, tumour burden and survival) in a variety of murine models (xenografts, orthotopic transplantation of lung cancer cells/macrophages, and genetically-induced KRAS-driven lung cancer models).
- scRNAseq transcriptomics and multiomics analyses.
- Histology analyses and spatial multiplexed proteomic profiling (CODEX).

Key references:

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Key Advance: Identification of senescent macrophages as key players of the TME in lung cancer initiation. This discovery has been received
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GRAPHICAL ABSTRACT