Interplay between cellular senescence and plasticity at the origin of lung cancer

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This proposal combines originality, interdisciplinary synergy, and a competitive PhD position with the Early Detection Programme and in a world-class cancer research institution. We are looking for highly motivated students with an academic degree in Medicine, Biology or Biomedical Sciences. Our location in the Cambridge Biomedical Campus provides a powerful link between fundamental and translational research. Awarded applicants will benefit from extensive collaborations with our partner institutions in the Cambridge Cancer Centre, including the CRUK Cambridge Institute, clinical and research departments in the School of Clinical Medicine, other MRC laboratories and the Institute of Metabolic Sciences, as well as commercial partners such as AstraZeneca and GlaxoSmithKline. Researchers will have access to all central facilities and state-of-the-art infrastructures and technologies.

My collaborations with Drs Robert Rintoul (Aerodigestive Programme of Cambridge Cancer Centre) and Manuel Rodriguez-Justo (Consultant Pathologist at UCL Hospitals) will provide all supportive data in clinical oncology. In addition, we will interact with international centres of excellence for cancer research, including the laboratory of Dr Manuel Serrano (Spanish National Cancer Research Centre).

Project Description

Background

Identification of the cell of origin of cancer remains a challenge in medicine. Importantly, recent advances in lineage tracing are concluding in various tumour models that the cell of origin is a differentiated cell that upon damage, including the activation of oncogenes and/or the loss of tumour suppressors, undergoes de-differentiation and acquires an aberrant plastic state that initiates cancer (for review see 1). Examples can be found in glioblastomas, intestinal tumours and pancreatic cancers, where damage-induced plasticity (a “gain of cellular plasticity” in response to damage) leads to aberrant cancer initiation.
Cellular senescence is a common cell autonomous response to damage and oncogenic stress characterised by a stable proliferative arrest and an intense paracrine secretion, termed SASP, affecting nearby tissue. I have recently reported in *Cell* that cellular senescence plays an active role in orchestrating tissue remodelling (2). Besides being relevant for ageing, cellular senescence is associated with a wide variety of age-related disorders, including cancer, playing antagonistic roles (for review see 3). Cellular senescence, like apoptosis, a crucial barrier against cancer as it arrests the expansion of premalignant cells. This is well-known both in human and in mice, and gave rise to the concept of “oncogene-induced senescence” (OIS) (reviewed in 4). However, in the long term, accumulated senescent cells that are not removed by the immune system can promote malignant phenotypes by secreting pro-inflammatory and pro-tumorigenic factors (reviewed in 4). Additionally, areas enriched in senescent cells in response to chemotherapy (therapy-induced senescence), may actually contribute to the emergence of secondary cancers (therapy-induced cancers).

**Hypothesis**

During the last few years evidence is accumulating of an interconnection between cellular damage, the inflammatory microenvironment and cellular plasticity (reviewed in 5). It has been convincingly reported by several groups that the inflammatory response to damage favours cellular plasticity and reprogramming-like processes. The concept of cellular damage creating a microenvironment that favours cell de-differentiation and plasticity places senescence as an intriguing key process. On the one hand, cellular senescence is a response to damage and, on the other hand, senescent cells can increase the inflammatory milieu through the SASP.

Our **working hypothesis** is that the accumulation of senescent cells can promote, through the SASP, a “maladaptive gain of plasticity” in nearby cells favouring cancer. This negative effect may occur upon persistent damage or oncogenic stress, during ageing, in chronic pathologic disorders, and in response to chemotherapeutic drugs. In support of this we know that the conditioned medium of senescent cells strongly enhances the in vitro reprogramming efficiency of mouse embryonic fibroblasts expressing Yamanaka factors. Interestingly, we have observed that, in mouse models of bleomycin-induced pulmonary fibrosis and K-ras driven lung cancer, senescent cells coexist in close vicinity to cells expressing pluripotency markers (including Oct4).

**Objectives**

This project focuses on the processes and mechanisms that lie at the origin of lung cancer. In particular, on the role of cellular senescence and the SASP. We will use a K-ras driven lung adenoma/adenocarcinoma mouse model that recapitulates human lung cancer accurately as it has similar gene expression profiles and phenotype. Cellular senescence is a defining feature of lung adenomas (early tumours) but not of lung adenocarcinomas (advanced tumours). We may also explore models of chronic obstructive pulmonary disease (COPD), known to be associated with cellular senescence and with a higher lung cancer incidence.

Specific aims are:

(i) To address the connection between cellular senescence and plasticity in lung tumorigenesis. We will determine whether senescence-induced plasticity is an active oncogenic promoter.

(ii) To analyse the components of the secretome of senescent cells in a background of lung cancer, and to identify the specific SASP factors that may induce a “maladaptive gain of plasticity” in nearby cells.

(iii) To isolate (de-differentiated or “plastic”) tumour precursor cells and to characterise gene expression profiles and epigenetics.
Methodology

Task 1. To examine the impact of cellular senescence and the SASP-associated microenvironment on cell plasticity.

We will determine the kinetics and dynamics of the histological organisation of senescent, pluripotent, and proliferative cells in early lung lesions and tumours. To do so, we will employ both genetic (K-rasG12V) and chemical (injecting methyl-nitrosurea/urethane) models of lung adenomas/adenocarcinomas. Collected tissues and tumours at different time points will be subjected to senescence-associated β-galactosidase (SAβ-gal) assays and immunohistochemistry analyses to detect other markers of senescence (p53, p16), pluripotency (Oct4, Nanog) and proliferation (Ki67, BrdU).

To establish a causal link between cellular senescence and the induction of pluripotent features in nearby cells during lung cancer initiation we will compare wild-type mice with different senescence-altered mouse strains (including p53KO, p16ArfKO and a SASP-deficient model). Tumour progression will be monitored, and samples will be collected to determine how senescence affects tumour latency, malignancy and histology.

Task 2. Dissecting the senescence secretome in the initiation of lung cancer.

Protein arrays will be employed to identify SASP components (cytokines, metalloproteases, etc.) in the lungs at early cancer stages. Simultaneously to the proteomics approaches in vivo, the contribution of the SASP to a maladaptive gain of plasticity in nearby cells will be characterised in cultures of cell lines subjected to oncogenic stress or isolated from our cancer models. Gene expression profiles and stable isotope labelling with amino acid in cell culture (SILAC) analyses will be performed to characterise the senescence-associated secretome. These studies will be complemented by metabolomics approaches.


We will employ a conditional lineage tracing mouse model for Oct4 that activates the expression of green fluorescent protein at the cell membrane (mGFP). This model will be used to capture stable or transient events of Oct4 activation during lung cancer initiation, both in wild-type and senescence-deficient mice. The proportion of tumour cells that derive from an Oct4 positive precursor in the different genetic backgrounds will be determined. In case of evidence of cancer cells deriving from Oct4 positive cells in a senescence-dependent manner, we will take advantage of the mGFP expression to isolate these precursors by cell sorting. Tumour precursor cells will be subjected to RNA-seq analyses and also to methods applied to the study of histone modifications and DNA methylation.

Impact and Expected Outcomes

We aim to integrate into a unified model oncogene-induced senescence and oncogene-induced plasticity, and to demonstrate that senescence-induced plasticity is a potent oncogenic promoter. The proposed study will expand our knowledge of the role of cellular senescence and plasticity in tumorigenesis, and it will open new frontiers on the processes and mechanisms that lie at the origin of lung cancer.
References


Applications

To apply for this studentship please see http://www.cambridgecancercentre.org.uk/studentships

For general enquiries please contact Tina Thorn tina.thorn@cruk.cam.ac.uk

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