Type I interferons and cGAS-STING pathway in cancer

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INTRODUCTION

Type I interferons (IFNs) play a central role in activating and modulating the immune response against viruses and intracellular bacteria

Most types of cells produce type I interferons upon pathogen infection. However, type I IFNs are mainly produced by dendritic cells (antigen-presenting cells).

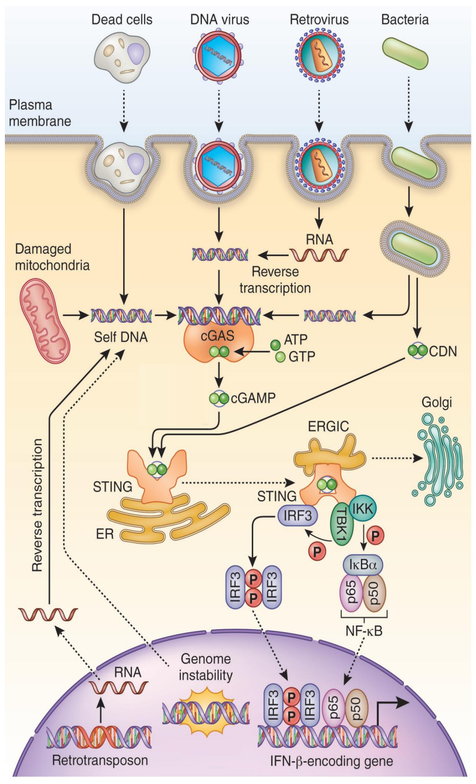
Type I IFNs act on both infected cells to limit the spread of pathogens and immune cells to trigger immune response.

Direct effects of type I IFNs on infected cells include:

- upregulation of the expression of antigens
- inhibition of cell proliferation
- induction of apoptosis

Immunomodulatory effects of type I IFNs include:

- activation of dendritic cells to cross-present antigen to T cells
- activation of effector and memory T cells



Chen et al. 2016 Nat. Immunol.

Type I interferons (IFNs) are crucial for antitumour immune response

A tumour can elicit production of type I IFNs by dendritic cells, which enhance their ability to cross-present antigens, required for activation of tumour antigen-specific T cells.

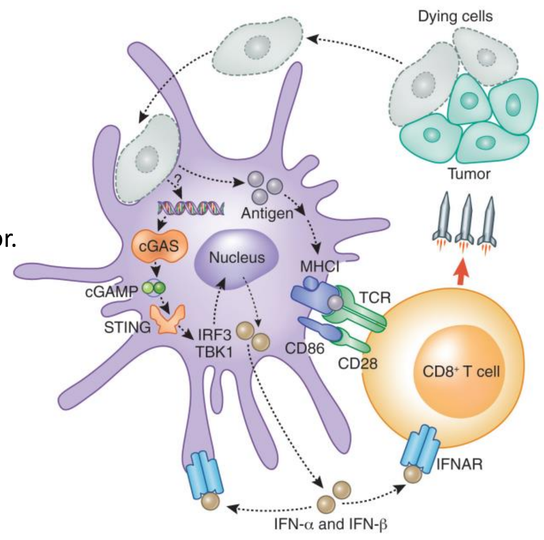
cGAS-STING pathway leads to type I interferons production in dendritic cells in response to tumour

cGAS-STING signalling pathway senses cytosolic tumour-derived DNA within the cytosol of tumour-infiltrating dendritic cells.

Upon binding cytosolic dsDNA, cGAS catalyses the synthesis of cyclic GMP-AMP (cGAMP), which in turn engages STING as a second receptor.

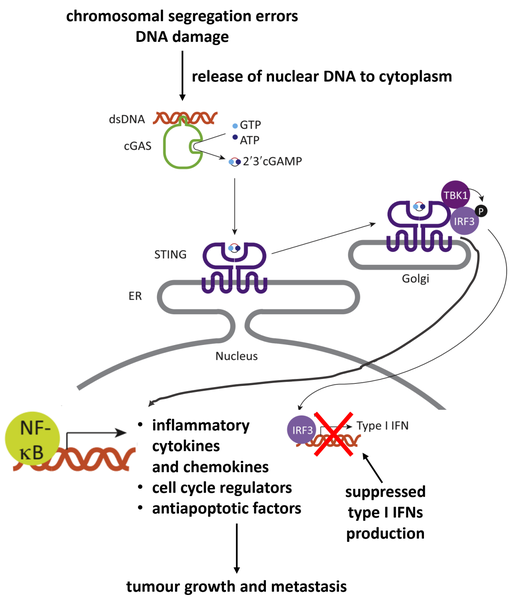
cGAS and STING signalling upregulates two distinct pathways:

1. type I IFNs production through IRF3 (interferon regulatory factor 3)
2. activation of NF-κB signalling pathway (nuclear factor kappa B)



Chen et al. 2016 Nat. Immunol.

In cancer cells chromosomal segregation errors and DNA damage lead to release of nuclear DNA to cytosol, and thus activation of cGAS-STING pathway

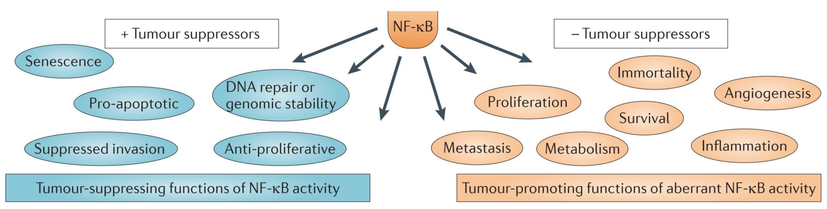


Bakhoum et al. (2018) revealed that activation of cGAS and STING in cancer is not synonymous with induction of type I interferon signalling. Cancer cells might suppress type I IFNs production while keeping cGAS-STING and NF-κB pathway upregulated.

NF-κB is a transcription factor controlling expression of a large number of genes involved in various biological processes including immune response, cell proliferation and survival.

Effects of NF-κB activity can be context-dependent, either promoting or suppressing the tumour growth.

Loss of key tumour suppressors (e.g. p53) can drive NF-κB towards tumour-promoting activity.



Perkins 2012 Nat. Rev. Cancer

Suppressing type I IFN signalling and instead upregulating NF-κB pathway may lead to STING-mediated metastasis.

References:

1. Chen Q., Sun L. and Chen Z.J. (2016) Regulation and function of the cGAS–STING pathway of cytosolic DNA sensing. *Nat. Immunol.* 17 (10): 1142-1149
2. Bakhoum S.F. et al. (2018) Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* 553 (7689): 467-472.
3. Perkins N.D. (2012) The diverse and complex roles of NF-κB subunits in cancer. *Nat. Rev. Cancer* 2 (2): 121-132.
4. Campbell J.D. et al. (2016) Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nature* 48 (6): 607-616.

RESEARCH QUESTIONS

1. **What are the mechanisms resulting in suppressing type I interferons production in tumours?**
2. **How does suppression of type I interferons production result in cancer growth and metastasis?**

AIM

The objective of this research project is to verify the following hypothesis:

Type I interferon genes cluster deletion is a potential mechanism leading to tumour growth and metastasis.

Deletion of type I IFNs enables cancer cells to supress type I IFNs signalling, while maintaining cGAS-STING pathway and NF-κB upregulated. This leads to:

1. Inhibition of apoptosis and/or increase of proliferation of cancer cells
2. Decrease of antigen expression (MHC class I expression) by cancer cells
3. Reduced stimulation and tumour infiltration by T cells

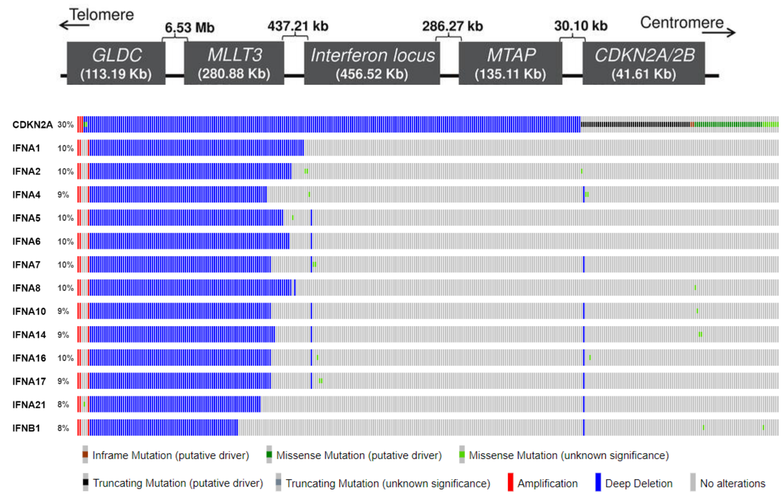
RESEARCH DESIGN AND METHODS

1. Type I IFN genes copy-number alterations in lung cancer - the Cancer Genome Atlas (TCGA) data analysis

Type I IFN gene cluster is located in the same chromosomal region as CDKN2A gene, which encodes tumour suppressor p16.

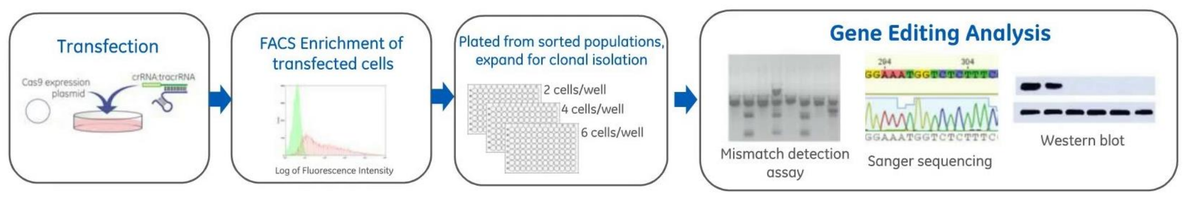
p16 plays a critical role in cell cycle regulation. It is frequently deleted/mutated in lung cancer patients.

Type I IFN genes are co-deleted with p16 in a significant number of patients with lung cancer.



Pan-Lung Cancer (TCGA, Campbell et al. 2016 Nat. Genet.)

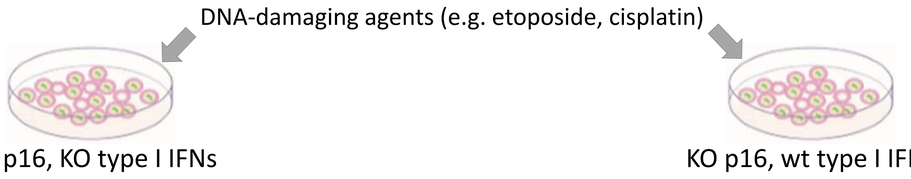
2. Generation of p16 and type I IFNs knockout (KO) lung cancer cell lines (CRISPR/Cas9 method)



3. Treatment of lung cancer cell lines with STING inducing factors

STING agonists (e.g. synthetic analogs of cyclic dinucleotides)

DNA-damaging agents (e.g. etoposide, cisplatin)



KO p16, KO type I IFNs

KO p16, wt type I IFNs

4. Analysis of STING and NF-κB activation in cancer cells

western-blotting using antibodies recognizing:

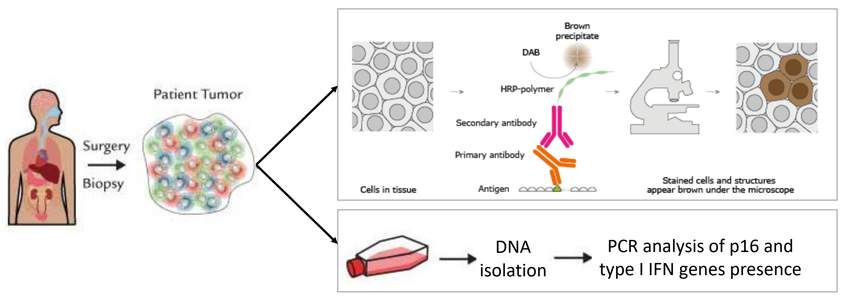
- STING and phospho-STING
- p65 phospho-p65 subunit of NF-κB

real-time qPCR using primers specific for IL-6 (cytokine induced by NF-κB)

5. Analysis of biological effects on cancer cells

- proliferation (cell viability and proliferation assay)
- apoptosis (assay using Annexin V)
- MHC class I expression (western-blotting using anti-MHC class I antibodies)

6. Immunohistochemical analysis of T cell presence in lung cancer samples



verification whether deletion of type I IFNs is linked to reduced T cell infiltration

SIGNIFICANCE

Presented research project will allow for better understanding the mechanisms leading to tumour progression and metastasis.