Towards treatment of a human accelerated ageing disease

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Werner’s Syndrome

• Rare, autosomal recessive genetic disease
• Premature ageing of many, (but not all), tissues
• Premature development of:
  – osteoporosis
  – bilateral cataracts
  – type II diabetes
  – thymic atrophy
  – soft tissue calcification
  – ulceration
  – an overall aged appearance
• Elevated risk of atherosclerosis and some types of cancer
Why study such a genetic disease?

- **Our genes have a major influence on ageing, but ...**
  - Upwards of 3% of the total human genome has been proposed to play a role in ageing (perhaps 1200 genes)
  - This makes genetic analysis of normal ageing, even in worms, extremely complex

- **Progeroid syndromes like WS are single gene defects that mimic one or more aspects of normal ageing.**
  - They “look like ageing”.

- **We study these rare diseases in order to shed light on the normal human ageing process**
What causes Werner’s Syndrome?

• **In an WS individual** ... affected tissues are mainly those containing cells that are able to divide during life
  – Something wrong with cell proliferation?

• **In the lab** ... **WS cells have a shortened replicative lifespan**
  – All the evidence is consistent with the premature ageing of WS individuals being **caused by accelerated replicative senescence**

• **In the DNA** ... there is a mutation in a recQ helicase (wrn)
  – The WRN protein unravels DNA (e.g. when it be being replicated)
  – In WS cells without it **DNA replication stalls**, and cells have problems repairing their DNA
Replicative senescence: a cell ageing mechanism

• Finite lifespan of normal human cells in culture

• Senescence
  – is viable cell cycle arrest
  – may act as a barrier to tumour formation

• Senescent cells
  – remain metabolically active
  – display altered patterns of gene expression
Physiological cell loss
(wear-and-tear, wounding, haemodynamic stress)

Replacement cell division

Progressive accumulation of senescent cells

Altered cellular microenvironment
Reduced division capacity

Aged tissue
WS causes stalled replication forks

Lynne Cox, Oxford
WRN is found at sites of DNA replication
DNA fibre spreads to detect replication fork stalling

This shows a single DNA molecule in the process of replicating. Arrows show the direction of DNA synthesis, while red lines show where replication forks have stalled or stopped.
DNA fibre spreads

Normal cells

WS cells
Asymmetric replication forks in **WS**

**Normal**

**WS**

% replication fork symmetry
Werner's Syndrome cells show premature senescence

Why do WS cells show premature senescence?
Telomeres can count cell divisions
In WS, the telomere “clock” is still working.
Telomeres do not shorten faster

**Experimental Design Parameters**

- Primer (f): 430
- Intraclone length (KO): 11
- Reseeding: 15000
- Dropped fraction: 0.25
- Apparent fraction: 0
- Tel SD: 4
- Stems: 1
- Final pass the pool (single)
- 2x cell number: 46
- Telomere length (kb): 0

**Teloerme length**

- Mean (kb): 5.226, 5.084, 4.500
- SD (kb): 0.89, 1.00, 1.11
Why do Werner’s Syndrome cells show premature replicative senescence?

• We postulate that:

  – The shortened lifespan of WS cells results from an additional process of telomere-independent senescence (TIS)

  – This is superimposed upon normal telomere-driven senescence

  – Together, these two mechanisms together define WS cell lifespan
So what might be causing TIS?
Growth arrest pathways

- Short telomeres
- Oncogenic activation
- DNA replication stress

**Stalled replication forks in WS**

**Stress Load**

- DSB
- MKK3/6
- p53
- p38α MAPK
- p21^Waf1^
- HSP27 → pHSP27
- F-actin stress fibres

- Cell cycle arrest
- MK2/3

Environmental stress
Stress signalling in WS cells

young AG05229 cells

phase contrast x10; phalloidin x20
p38MAPK is active in WS cells
SB203580, a drug against p38MAPK
SB203580 has only a minor effect on the lifespan of normal cells ...
... but SB203580 rescues the lifespan defect in WS cells ...
... the growth fraction defect ...

AG05229C

AG03141C
... the appearance of young cells ...

5229 + DMSO  
5229 + SB203580
... and the stress fibres.
Summary

• With regard to cell ageing, SB203580 makes WS cells behave like normal cells

• What activates p38$^{\text{MAPK}}$?
  – We hypothesise that p38$^{\text{MAPK}}$ is activated by replication stress (e.g. associated with stalled replication forks)
Implications for individuals with Werner’s Syndrome
**Implications of p38MAPK activation**

- p38MAPK up-regulates the expression of many pro-inflammatory molecules (TNF, IL-1, IL6 etc.)
- These are implicated in atherosclerosis, type II diabetes, and osteoporosis

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<th>Inflammatory genes</th>
<th>Immunoregulatory</th>
<th>Growth factors</th>
<th>Oncogenes</th>
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<tr>
<td>TNF</td>
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Inflammatory genes are those induced by primary inflammatory stimuli such as microbial products (eg LPS), IL-1 or TNF. Other cytokines listed are called immunoregulatory.

A speculative unified theory for **WS**

- **Atherosclerosis, osteoporosis, diabetes**
  - p38\textsuperscript{MAPK}-driven increases in levels of circulating pro-inflammatory cytokines
  - WS patients have high circulating levels of at least one of these, TNF\textsubscript{α} (Yokote, K. et al. 2004. *Diabetes Care* 27:2562-3)

- **Cataracts, greying hair, skin changes**
  - Accumulation of senescent cells?

- **Cancer incidence**
  - Mutator phenotype
  - Chronic inflammation (c.f. *Helicobacter* and gastric cancer)
Therapeutic opportunities for WS

• **Immediate opportunity: thiazolidinedione (TZD) diabetes drugs.**
  - Pioglitazone is being investigated as a therapy for diabetes in WS (Yokote et al.).
  - It also suppresses the elevated TNFα levels in WS.
  - We propose that these drugs could also have beneficial effects on cardiovascular disease in WS, the big killer.

• **Future**
  - Some p38MAPK inhibitors are in Phase II and III clinical trials for inflammatory diseases such as Crohn’s disease, psoriasis and rheumatoid arthritis.
What about normal ageing?

- \( p38^{MAPK} \) is involved in stress signalling in normal individuals
  - Inflammation, viral infection, obesity, periodontal disease, cancer can all increase the levels of pro-inflammatory cytokines
  - These in turn will activate \( p38^{MAPK} \)

- Many diseases and stresses in our own lives will activate what we now know is a mechanism that can accelerate ageing

- Therapies designed for WS may have wider anti-ageing utility
The International WS Research Network
... and if you still don't believe that stress can accelerate ageing ...