Understanding and curing accelerated human ageing?

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Werner’s Syndrome

- Rare, autosomal recessive genetic disease
- Premature ageing of a subset of tissues
  - Premature development of osteoporosis, bilateral cataracts, type II diabetes, thymic atrophy, dysadipocytokinemia, soft tissue calcification, ulceration, and an overall aged appearance
  - Elevated risk of atherosclerosis and predominantly mesenchymal cancers
  - Affected tissues are mainly those containing cells that are division-competent during life
Why is research into ageing important and timely?

• The great biomedical success of the 20th century
  – We are all living longer, both in the UK and worldwide
  – Reduction in childhood deaths, better survival following trauma etc
  – These successes continue. Today, a 65 year old woman in the UK can expect to live > 3 years longer than in 1995

• The biomedical challenge for the 21st century
  – Pensions crisis (was David Blunkett’s job to sort)
  – Increased age-related disease and degenerations
Ageing
A major UK medical issue

• **Dementia**
  – Currently affects 750,000 people in the UK. Almost all are over 65, as are many of their carers.
  – Within the next five years there will be an additional 120,000 people in the UK with dementia.
  – By 2050 there will be 1.8 million people in the UK with dementia.

• **Bones, joints, muscles**
  – 70% of women will have an osteoporotic fracture at some time. Such fractures often lead to a hospital stay, loss of confidence, and a downward spiral to dependence.
  – An older person dies every five hours as the result of a fall at home.

• **Wound healing**
  – Chronic leg ulcers are a significant cause of disability and distress for older people.
  – They cost **£1 billion** per annum in treatment and care.

• **Immune system decline**
  – In 2000, there were 56,838 UK deaths from influenza and pneumonia. 95% were over the age of 75.
What can we do about it?

• Research that focuses on individual diseases in isolation

• Research that focuses on the underlying causes, ageing itself
Replicative senescence is one proposed ageing mechanism.
Replicative senescence *in vitro*

- 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, including secreted proteins
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
What causes Werner’s Syndrome?

• **Shortened replicative lifespan of cells in culture**
  - All the evidence is consistent with the premature ageing of WS patients being caused by accelerated replicative senescence

• **WS is caused by mutation in a recQ helicase** (*wrn*)
  - This protein unravels DNA (e.g. when it be being duplicated during cell division)
  - Without it the result is stalling of DNA replication, and problems with repairing DNA
Why do Werner’s Syndrome cells show premature senescence?
Telomeres count cell divisions
The telomere “clock” is still working
Telomeres do not shorten faster

- Conclusion based upon a combination of computational simulation (below) and highly detailed telomere length analysis using STELA (right)
Why do Werner’s Syndrome cells show premature replicative senescence?

• **Postulate that:**
  – The shortened lifespan of WS cells results from an additional process of telomere-independent senescence
  – This is superimposed upon normal telomere-driven senescence
  – 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
- Environmental stress

**Stress Load**

- DSB → MKK3/6 → p38α MAPK → p53
- p53 → p21^Waf1 → Cell cycle arrest

- MK2/3 → HSP27 → pHSP27 → F-actin stress fibres
Stress signalling in WS cells

- Some evidence for activation of the stress signalling p38$^{\text{MAPK}}$ in normal fibroblast senescence

**young AG05229 cells**

phase contrast x10;  phalloidin x20
Western analysis of p38 activity

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<th>SB</th>
<th>AG03141 cells</th>
<th>MRC5 cells</th>
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- pp38
- p38
- pHSP27
- HSP27
- p21
Blocking the p38 stress response

**SB203580**

Inhibits the $\alpha$ and $\beta$ forms of p38 MAPK (and some other targets)

4-[5-(fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine
SB203580 has only a minor effect on the lifespan of normal fibroblasts ...
... but SB203580 rescues the lifespan defect in WS cells
the growth fraction defect ...
... the morphology of young cells ...

5229 + DMSO  5229 + SB203580
... and the stress fibre phenotype.
Summary

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

• What activates p38\textsuperscript{MAPK}?
  – We hypothesise that p38\textsuperscript{MAPK} is activated by replication stress (e.g. associated with stalled replication forks)
Implications
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>TABLE 2. Genes containing destabilizing AREs in the 3’UTR</th>
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Inflammatory genes are those induced by primary inflammatory stimuli such as microbial products (e.g., LPS), IL-1, or TNF. Other cytokines listed are called immunoregulatory.

A speculative unified theory for WS

- **Atherosclerosis, osteoporosis, diabetes**
  - p38-driven increases in levels of circulating pro-inflammatory cytokines
  - WS patients have high levels of circulating TNFα (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 opportunities**
  – Thiazolidinedione (TZD) diabetes drugs are PPARγ agonists.
  – The TZD pioglitazone suppress the elevated TNFα levels in WS and are being investigated as a therapy for their diabetes (Yokote, K. et al. 2004. *Diabetes Care* 27:2562-3).
  – We propose that these would therefore also have beneficial effects on OP and CVD in WS.
  – Reducing the death rate through atherosclerosis would have a major effect on life expectancy of WS patients

• **Future**
  – Some p38MAPK inhibitors are in Phase II and III clinical trials for inflammatory diseases such as Crohn’s disease, psoriasis and RA
  – They could to target both the metabolic syndrome X phenotypes and the senescence-related symptoms
Thanks

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  – Richard Faragher (Brighton)

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... and if you still don’t believe that stress can accelerate ageing ...
1994 …

… 2004