A chemical genetics approach to investigate accelerated ageing in Werner syndrome

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Werner 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

George Martin and German Werner individual (age 36)

American Werner syndrome individual (age ~50)
What causes Werner Syndrome?

• **WS is caused by mutation in a recQ helicase (wrn)**
  – Lack of WRNp results in stalling of DNA replication, and problems with DNA repair

• **Shortened replicative lifespan of cells in culture**
  – The evidence is consistent with the premature ageing of WS patients being caused by accelerated replicative senescence
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
Telomere dependent senescence (TDS)
Telomere-independent senescence (TIS)

- TIS can be induced by extrinsic stimuli
  - e. g. oxidative stress, DNA damage

- Intrinsic signals can also induce TIS
  - e. g. activated oncogenes (Ras), genome instability
**Growth arrest pathways in senescence**

- **Short telomeres**
  - DSB
    - MKK3/6
      - p38α MAPK
        - p53
          - p21Waf1
            - Cell cycle arrest
        - p38α MAPK
          - MK2/3
            - HSP27 → pHSP27
              - F-actin stress fibres
  - Oncogenic activation
  - DNA replication stress
  - Environmental stress

- **Stress Load**
Senescence is a potential ageing mechanism
Physiological cell loss
(wear-and-tear, wounding, haemodynamic stress)

Normal

Replacement cell division

Progressive accumulation of senescent cells

Altered cellular microenvironment

Reduced division capacity

Aged tissue
Physiological cell loss
(wear-and-tear, wounding, haemodynamic stress)

Replacement cell division

Progressive accumulation of senescent cells

Altered cellular microenvironment
Reduced division capacity

Accelerated Ageing
Why do Werner Syndrome cells show premature replicative senescence?

- Have shown that WS cells do not show more rapid telomeric losses therefore mechanism is not through TDS (Baird et al, 2004, Hum Mol Genet 13, 1515)
Why do Werner 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?
Stress and WS cells

- WS cells have genome instability
- DNA replication frequently stalls
- DNA repair defects

Any of above could provide trigger for activation of stress pathways

Such stress is transduced by the p38 MAP kinase pathway
Stress pathways in WS cells

DNA replication
stress

\[ \downarrow \]

Stress Load \( \uparrow \)

\[ \downarrow \]

MKK3/6

\[ \downarrow \]

\[ \text{p38}\alpha \text{ MAPK} \rightarrow \text{MK2/3} \]

\[ \downarrow \]

\[ \text{p53} \rightarrow \text{p21}^{\text{Waf1}} \]

HSP27 \( \rightarrow \) pHSP27

\[ \downarrow \]

Growth arrest

F-actin stress
Evidence for Stressed cells in WS

young AG05229 cells

phase contrast x10; phalloidin x20
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
Effect of SB203580 on cell morphology

young control AG05229 cells

young SB203580 treated AG05229 cells
Effect of SB203580 on stress fibres

- **A** MRC5 cells
- **E** AG03141 cells
- **I** AG05229 cells
- **B** SB203580-treated young cells
- **C** Senescent cells
- **D** Senescent SB203580-treated cells
- **F** SB203580-treated young cells
- **G** Senescent cells
- **H** Senescent SB203580-treated cells
- **J** SB203580-treated young cells
- **K** Senescent cells
- **L** Senescent SB203580-treated cells
Effect of SB203580 on WS cell growth

Open circles: control

Closed circles: SB-treated

closed squares: SB-treated to point a, then drug removed

Open squares: SB-treated to point a, then drug removed: drug restored at point b
**Immunoblot analysis of p38 activity**

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Summary

With regard to cell ageing, SB203580 makes WS cells behave like normal cells.
Therapeutic opportunities for WS?

Use of p38 inhibitors to treat WS?

If accelerated replicative decline does underlie rapid ageing in WS then these inhibitors may help alleviate this and lead to longer life.

This may also be applicable to normal ageing?!!
However!

- SB203580 not suitable for *in vivo* use
- SB203580 inhibits several kinases other than p38
- Thus need to clarify things
However!

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Enter Mark
Clinical Inhibitors of p38 MAPK

- Applications of p38 kinase inhibitors by number of patents

- P38 kinase inhibitors in clinical development for treatment of inflammatory diseases
SCIBS: chemistry biology interface

• BBSRC *Selective Chemical Intervention into Biological Systems (SCIBS)* grant
  – A collaboration between MEDICINE and CHEMISTRY

• **Objective**
  – Design and test new chemical inhibitors of the p38<sub>MAPK</sub> signalling pathway using WS cells as model test system.

• **Goals**
  – Design of new tools for probing the pathway
  – Determine the roles played by the various members of the pathway in the accelerated senescence seen in WS cells
  – Move to pre-clinical trials with mouse WS model
Structurally Diverse Clinical Candidates

**BIRB 796 (Boehringer Ingelheim)**
- P38α: $K_d$ 76 pM, $IC_{50}$ 63 nM

**SD-06 (Pfizer)**
- P38α: $IC_{50}$ 80 nM (p38β 26000 nM)

**VX-745 (Vertex)**
- P38α: $IC_{50}$ 10 nM

**RO3201195 (Roche)**
- P38α: $IC_{50}$ 700 nM (high selectivity)
Key interactions of BIRB 796 with human p38α

- In phase III Clinical Trials for the treatment of rheumatoid arthritis and other inflammatory diseases
- Formation of the allosteric binding site requires conformational change of the activation loop (DFG-out: Asp168-Phe169-Gly170)
Disconnective Scheme for BIRB 796

BIRB 796

Synthetic needs:
• Convergent
• High purity
• Rapid
• Efficient
Synthesis of 2-aminopyrazoles

Traditional conductive heating:

\[
\text{R-C=N} + \text{H} + \text{N-R'} \xrightarrow{\text{PhMe, reflux, 18 h (88\%)}} \text{NH}_2 \\
\text{R = CMe}_3, \text{R'} = \text{Ph}
\]

Aminopyrazole component

Microwave irradiation:

\[
\text{PhMeNH}_{2} + \text{Me}_3\text{C-C=CN} \xrightarrow{\text{PhMe, AcOH, MW}} \text{NH}_2
\]

120 °C, 40 min (93%)

Amine or HCl salt

\[
\text{Me-PhN} + \text{Me}_3\text{C-C=CN} \xrightarrow{\text{MeOH, MW}} \text{NH}_3\text{Cl}
\]

120 °C, 40 min (88%)
Synthesis of aminonaphthyl ether

BIRB 796

Aminonaphthol component
Synthesis of N-pyrazole ureas

\[
\begin{align*}
\text{Me}_3\text{C} & \quad \text{PhNCO, CH}_2\text{Cl}_2 \\
\text{N} & \quad \text{Me}_3\text{C} \\
\text{NH}_2 & \quad \text{O} \\
\text{Ph} & \quad \text{NH}\text{Ph} \\
\text{Me}_3\text{C} & \quad \text{Cl} \\
\text{N} & \quad \text{Me}_3\text{C} \\
\text{NH}_3\text{Cl} & \quad \text{O} \\
\text{Me} & \quad \text{N} \\
\end{align*}
\]

- MW 80 °C, 30 min (79%)

(other carbamates are under investigation)
Synthesis of BIRB 796

MW, 100 °C
30 min, DMSO
(62%)

BIRB 796
Effect on growth rate of Werner Cells

- Control
- SB203580: $p < 0.011$
- BIRB 796: $p < 0.0081$

Chemical structures:
- SB203580
- BIRB 796
Summary

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

Other more selective inhibitors suitable for *in vivo* use can be prepared rapidly using microwave-assisted chemical synthesis.

BIRB 796 may make WS cells behave like normal cells.

Stress signals transduced by p38α MAPK may be responsible for rapid ageing in WS cells.

Pharmaceutical developments may offer clinical treatments for Werner’s syndrome.
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