Melanoma

- Melanocytes are specialised pigment cells
  - Skin, eyes, ear, brain, heart
  - Skin/hair tone
  - Protection from UV radiation (tanning response)
  - Precursors of melanoma
- Melanoma: the most deadly skin cancer
  - 10% of all skin cancers, but 80% of skin cancer deaths
  - Europe: over 62,000 cases pa; ~12,000 deaths
  - 80% are cured by surgery; 20% die of metastatic disease
  - Median survival: 6-9 months; 5 year survival: 5-10%
  - Risk factors: UV light/genetics

ERK signalling in melanoma

- CKIT: 5% (mucosal, acryl)
- NRAS: 22%
- KRAS: 2%
- HRAS: 1% (cutaneous)
- GNAQ: 5% (uveal)
- GPCR
- RAS
- BRAF: 44% (cutaneous)
- MEK
- ERK
- Hyper-activated in over 90%
RAS and RAF Signalling in Melanoma: Biology and Therapies
Professor Richard Marais

V600EBRAF transforms melanocytes
ERK signalling
Soft agar growth

Wellbrock et al., 2004 Cancer research

Mouse models of melanoma

• Express oncogene in a tissue specific manner—i.e., melanocytes
• Express oncogene at normal physiological levels
• Expression should be regulated to mimic its acquisition in humans

V600E BRAF inducible mouse

The screen versions of these slides have full details of copyright and acknowledgements
**V600E BRAF induces skin hyper-pigmentation**

- Image A: Braf^{WT}
- Image B: Braf^{V600E}

**V600E BRAF induces senescence in melanocytes**

- Images showing senescent cells (SA-β-Gal)
- Ki-67 staining
- p16 expression
- Gapdh control

**V600E BRAF induces melanoma**

- Image of melanoma induction
- Graph showing tumor growth over time
- Gene expression changes

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Tumour cells have metastatic potential

Cells grow as tumours in lungs

Role of p16INK4a?

Over 50% of human tumours have lost the tumour suppressor p16INK4a

BRAF mutations in cancer

Wan et al., 2004
Most mutants are active

Impaired activity mutants: activate ERK through CRAF

Kinase-dead mutants don’t appear to signal

Wan et al., 2004
Garnett et al., 2005
BRAF mutants have different modes of action

- Impaired activity mutants
  - BRAF
  - CRAF

- Activated mutants (high/intermediate)
  - BRAF* → MEK → ERK

- Kinase-dead mutants
  - BRAF* → ?

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BRAF mutations in cancer

- ~11,000 V600E mutations in BRAF in cancer
  - No coincidence with RAS mutations
- 32 D594 mutations in BRAF in cancer
  - 3 are coincident with KRAS mutations (G12/G13)
  - 1 is coincident with an NRAS mutation (Q61)
- Highly significant enrichment p < 10^-7
- Suggests a functional interaction between kinase-dead BRAF and oncogenic RAS?

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Conditional kinase-dead BRAF and oncogenic KRAS mice

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D594 and G12V KRAS cooperate to induce melanoma

- PD184352 (PD): MEK inhibitor (CI1040)
- Sorafenib (SF): BRAF, CRAF and other kinases
- 885-A: BRAF selective (analogue or SB590885)
- PLX4720 (PLX): BRAF selective
**BRAF is inert in RAS mutant melanoma cells**

- (NRAS) WM1781c
- (BRAF) Colo828

BRAF, CRAF, ppERK, ERK2

Dumaz et al., 2005

**CRAF and RAS are required**

D04 (NRAS mutant cells)

**RAF inhibitors induce CRAF binding to BRAF in RAS mutant cells**

WM852 (NRAS)

A375 (V600EBRAF)
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**RAS binding is required**

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**BRAF inhibitors recruit BRAF to the membrane**

**Growth factors induce BRAF-CRAF complexes and sustained signalling**
Gatekeeper mutants

Kinase-dead BRAF binds to CRAF & activates MEK

• Similar results with K46M BRAF and D594V BRAF
Sorafenib induced paradoxical CRAF activation

- Sorafenib is a pan-RAF inhibitor that may inhibit CRAF

T421NCRAF converts Sorafenib to a pathway activator

Pan-RAF inhibitors induce paradoxical CRAF activity
BRAF mutants have different modes of action

- Impaired activity mutants
  - BRAF
  - CRAF

- Activated mutants (high/intermediate)
  - BRAF
  - MEK
  - ERK

- Kinase-dead mutants
  - RAS
  - BRAF
  - CRAF

Wan et al., 2004
Garnett et al., 2005
Adapted from Garnett and Marais, 2004
Conclusions

- V600E BRAF can be a founder event in melanomagenesis
- BRAF is a validated therapeutic target
- BRAF drugs are effective in patients
- BRAF inhibition activates MEK through CRAF when RAS is mutated
- Kinase-dead BRAF and oncogenic RAS cooperate to induce melanoma
  - What of other tumours; other kinases; pseudo-kinases
- BRAF-selective drugs:
  - Should not be used in RAS mutant tumours
    (use pan-RAF, CRAF drugs or combinations)
  - Theoretical risk of tumour progression if RAS mutations are acquired
  - Theoretical risk of tumour promotion in other tissues
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