Defining the mode of melanoma heterogeneity and drug sensitivity by real-time cell cycle imaging

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Melanoma intra-tumoural heterogeneity

Tumour heterogeneity

inter-patient  ➔  intra-patient  ➔  intra-tumoural

clonal evolution + cancer stem cells + cancer cell plasticity

- microenvironment
- invasion
- migration
- proliferation

What regulates differentially cycling tumour subpopulations?

FUCCI

Fluorescence Ubiquitination Cell Cycle Indicator

Sakaue-Sawano et al. (2008) Cell

Haass et al. (2014) PCMR; Movie S1
Melanoma spheroids are composed of differentially cycling tumor cells in a subcompartment-specific distribution

Haass et al. (2014) PCMR; Movie S2
Haass et al. (2014) PCMR; Movie S3
Haass et al. (2008) Clin Cancer Res
Haass et al. (2014) PCMR; Beaumont et al. (2015) JoVE; Beaumont et al. (2016) JID; Spoerri et al. (in press) Methods Mol Biol
Cell cycle-specific drug resistance as an escape mechanism of melanoma cells

Beaumont et al. (2016) JID; Haass & Gabrielli (under review) Exp Dermatol
Influence of MITF level on cell cycle behavior in mouse xenograft tumours

High levels of MITF decrease cell cycle compartmentalisation in xenograft tumours.
Influence of MITF level on cell cycle behaviour in spheroids

Azami Green
Kusabira Orange

G1 arrested zone (size and concentration):

- Increased in spheroids generated with MITF knock-down cell lines.
- Decreased in spheroids generated with MITF overexpressing cell lines.
- Inverse correlation between MITF level and G1 arrest.

MITF decreases G1-arrested zone in spheroids.

Loredana Spoerri, Crystal Tonnessen, Kimberley Beaumont
Effect of MITF levels on spheroid morphology

- High-MITF spheroids are larger but flatter and less compact.
- MITF overexpression decreases structural integrity.
- More permeable?

Direct relation between MITF level and spheroids size

- High-MITF spheroids showed a more irregular outline and appeared less dense.

Light sheet microscopy (green fluorescence)

Bright field microscopy (view from top)
Molecule diffusion into spheroids

- Coomassie diffused faster in spheroids generated with cell lines overexpressing MITF.

Cell adhesion

- Decreased cell-to-cell adhesion in cells overexpressing MITF.
- High MITF cells generate looser structures that allow deeper and more efficient molecule diffusion.
- This phenomenon could apply to other molecules and compounds.

Absorbance signal normalised to CTL

- 45 min incubation with 100 μM Coomassie

Sheena Daignault
Summary

1. We have established novel melanoma models to study the effects of the cell cycle on drug sensitivity in real time.

2. The discovery of tumour cell subpopulations with different cell cycle behaviour in vivo is an important finding as these populations have different responses to therapies.

3. Cell cycle phase-specific drug resistance is an escape mechanism of melanoma cells that has implications on the choice and timing of drug combination therapies.

4. Would optimized scheduling of drug combinations yield better patient outcomes? What have we missed in the past? Future clinical perspectives?
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SAVE THE DATE!

9th WORLD CONGRESS OF MELANOMA
A JOINT MEETING WITH THE SOCIETY FOR MELANOMA RESEARCH

18–21 October 2017
Brisbane Australia
www.worldmelanoma2017.com
Link between G1-arrested clusters and hypoxia in mouse xenograft tumours

- Cycling areas are located in close proximity to blood vessels, whereas arrested cluster are situated further away.
- Hypoxic areas co-located with G₁ arrested zones

G₁ arrested clusters are caused by oxygen and nutrient deprivation.

Kimberley Beaumont, David Hill
Oxygen distribution and cell cycle compartmentalisation in spheroids

- Presence of outer cycling ring and inner G1-arrested zone.

- G1 arrested zone localised where hypoxia was most severe.

- FUCCI melanoma spheroids recapitulate the cycling behavior observed in tumours in terms of cell cycle behaviour.

Haass et al. (2014) PCMR; Beaumont et al. (2014) Healthcare
MITF heterogeneity is maintained during MAPK inhibitor treatment

Overcoming heterogeneity driven therapy failure

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Smith et al. (submitted)
MITF and cell cycle behavior switch occur in different hypoxic ranges

- MITF positivity in the most external ring of the inner G1-arrested zone in spheroids where MITF has been lowered artificially.
- G1-arrested cells can express MITF
- 3-zone model when MITF is knocked-down: presence of an intermediate ring in spheroids where hypoxia is severe enough to cause cell cycle arrest but not to downregulate MITF expression.
Link between cell cycle behaviour/hypoxia and MITF expression in spheroids

MITF predominantly detected in the outer cycling zone in spheroids generated with MITF-high cell lines.

MITF-positive ring overlapped with cell cycling ring.

MITF influences cell cycle behaviour and proliferation impacts MITF expression.
Link between cell cycle behaviour/hypoxia and MITF expression in spheroids

- MITF predominantly detected in the outer cycling zone in spheroids generated with MITF-high cell lines.
- MITF-positive ring overlapped with cell cycling ring.
- MITF was not detectable in spheroids generated with MITF-low cell lines.
- MITF expression is not upregulated in the outer cycling ring (low-MITF cell line spheroids).
- MITF influences cell cycle behaviour (compartmentalisation) and cell cycle behaviour impacts MITF expression.
Molecules diffusion into spheroids

- Coomassie diffused faster in spheroids generated with cell lines overexpressing MITF.

Cell adhesion

- Decreased cell-to-cell adhesion in cells overexpressing MITF.
- Increased cell-to-fibronectin/collagen adhesion in cells overexpressing MITF.

- Faster Coomassie diffusion together with decreased cell-to-cell adhesion support looser structures that allow deeper and more efficient molecules diffusion.

Absorbance signal normalised to CTL

Contribution of Sheena Daignault