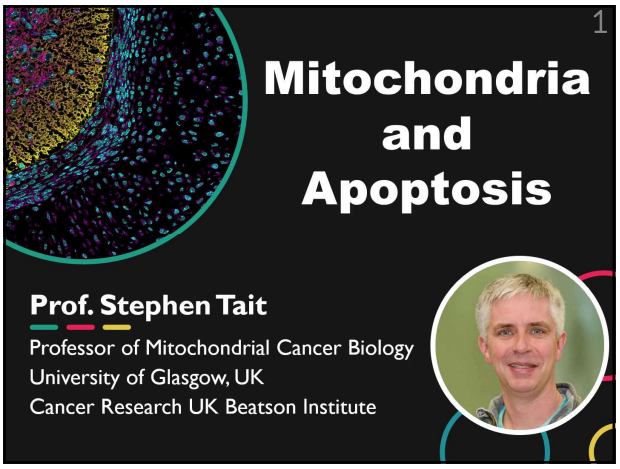
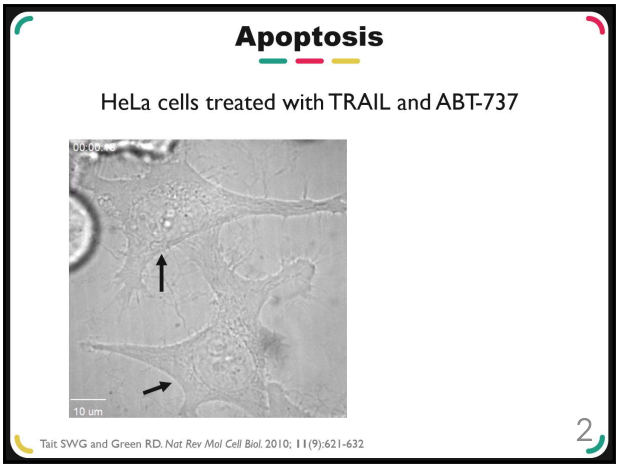
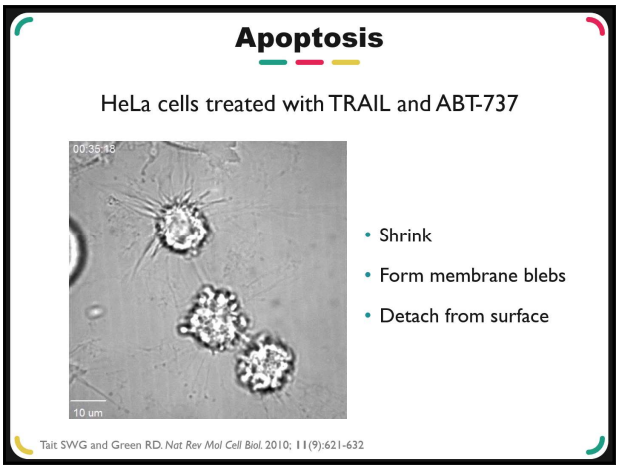
Prof. Stephen Tait – University of Glasgow, UK







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Apoptosis – what is it good for?

Preventing and treating cancer

A damaged cell or damaged DNA may trigger apoptosis
Prevents cells that have mutations from becoming cancerous
Most anti-cancer therapies work by killing cells, often through apoptosis

Immunity

Develop cells which react to self antigens
These cells are eliminated through apoptosis, so we do not develop an autoimmune response
Apoptosis is triggered by cytotoxic T-cells as they encounter infected or cancerous cells

Development

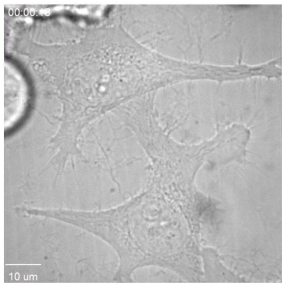
Many cells undergo apoptosis to allow correct organ development

…and a million other processes

3

How does a cell kill itself?

HeLa cells treated with TRAIL and ABT-737

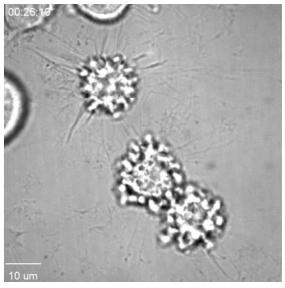


Tait SWG and Green RD. *Nat Rev Mol Cell Biol.* 2010; 11(9):621-632

4

How does a cell kill itself?

HeLa cells treated with TRAIL and ABT-737



Apoptosis is a highly regulated form of cell death

Tait SWG and Green RD. *Nat Rev Mol Cell Biol.* 2010; 11(9):621-632

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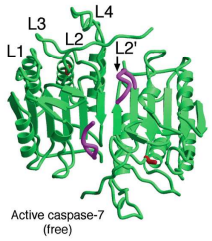
Caspase proteases are required for apoptosis

- Activated caspases cleave hundreds of different proteins in cells, bringing about rapid cellular morphological changes

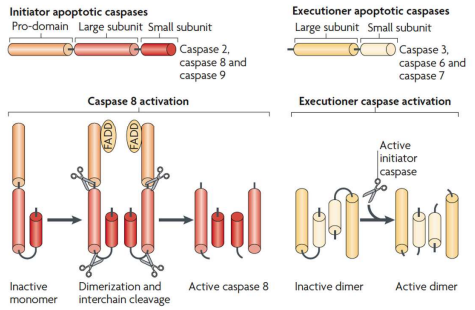


- Caspases are enzymes that are the nuts and bolts of cell death
- **C**ysteine-dependent **asp**artate-directed prote**ases**
- The active site has a cysteine, which is required for its catalytic activity and **cleave after aspartate residues**

Shi Y. *Mol Cell.* 2002; **9**:459-470

5

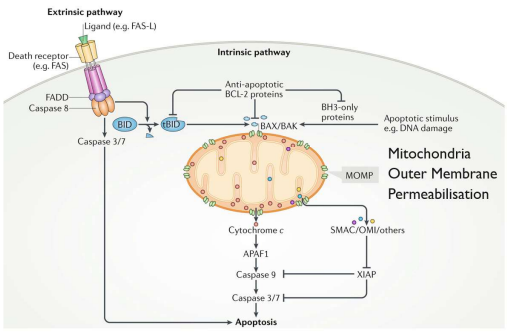
There are two types of apoptotic caspases



Tait SWG and Green RD. *Nat Rev Mol Cell Biol.* 2010; **11**(9):621-632

6

Pathways to caspase activation



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; **21**(2):85-100

7

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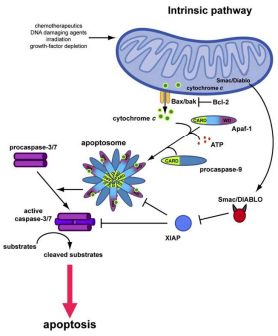
Mitochondrial/intrinsic pathway of apoptosis



Lamkanfi M. and Dixit VM. *Cell Host & Microbe*. 2010; 8(1):44-54

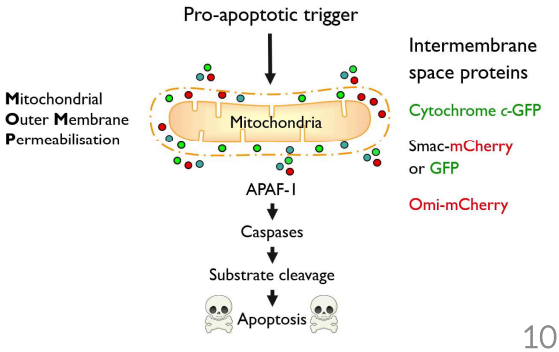
8

Point of no return!

Point of no return
in mitochondrial
apoptosis is MOMP

9

MOMP commits cells to die



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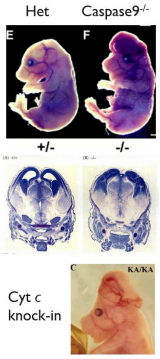
Genetic evidence for this pathway

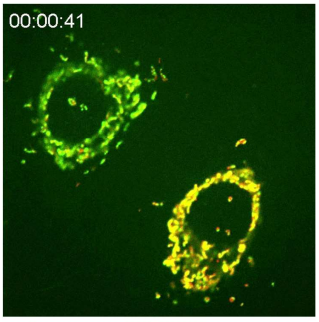


- Exencephaly
  - Delayed cell death in a subset of neurons in embryos, causing forebrain outgrowth
- Perinatal lethal
- Interdigital webbing
- Eye defects
- Apoptosis
  - Resistant to etoposide, UV, staurosporine
  - Sensitive to FasL (extrinsic trigger)

Kuida K. *et al., Cell.* 1998; **94**(3):325-337, Hao Z. *et al., Cell.* 2005; **121**(4):579-591

11

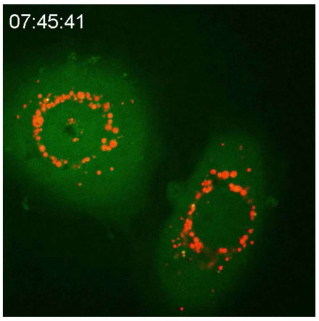
MOMP is rapid and often complete



- MCF7 cells (breast cancer cell line), which lack caspase 3 activity
- Smac: GFP
- Mitochondrial matrix: DsRed

12

MOMP is rapid and often complete



- MCF7 cells (breast cancer cell line), which lack caspase 3 activity
- Smac: GFP
- Mitochondrial matrix: DsRed

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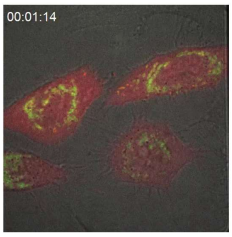
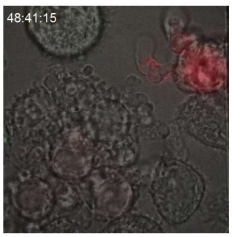
MOMP is typically the point of no return



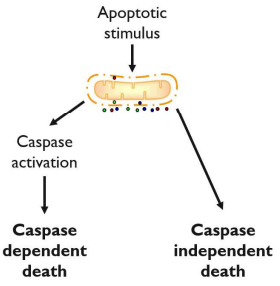
HeLa cells expressing GFP tBid, Smac mCherry and caspase inhibitor

13

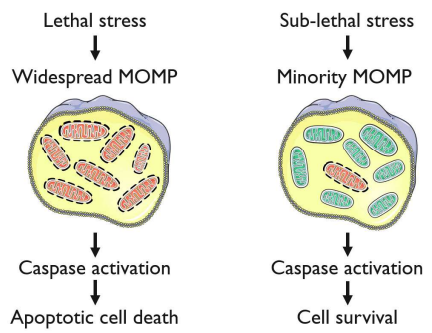
MOMP is typically the point of no return



HeLa cells expressing GFP tBid, Smac mCherry and caspase inhibitor



MOMP need not be all or nothing



Ichim G. *et al.*, *Mol Cell.* 2015; **57**(5):860-872

14

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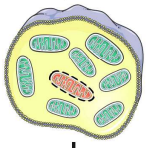
MOMP need not be all or nothing

- Survival under conditions where caspases are activated may lead to oncogenic transformation

Sub-lethal stress

↓

Minority MOMP



↓

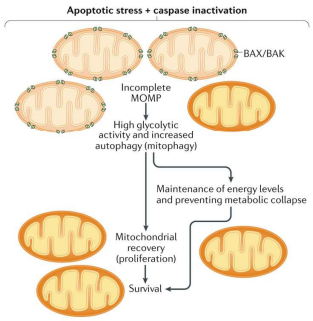
Caspase activation

↓

Cell survival

Ichim G. *et al., Mol Cell.* 2015; **57**(5):860-872

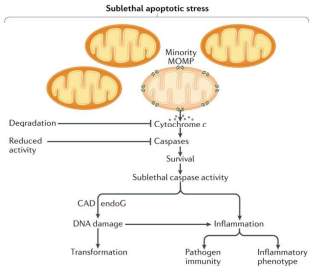
MOMP has non-lethal signalling functions



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; **21**(2):85-100

15

MOMP has non-lethal signalling functions



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; **21**(2):85-100

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Summary

Central to mitochondrial apoptosis is **Mitochondrial Outer Membrane Permeabilisation**

Mitochondrial proteins activate **caspase proteases**

MOMP is often a **point of no return**, however…

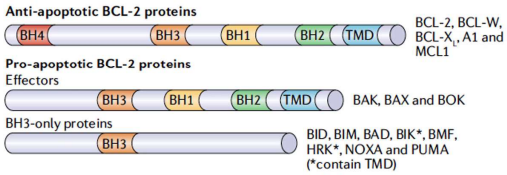
Differential levels of MOMP can occur with associated **non-lethal functions**

16

What regulates mitochondrial permeabilisation?

17

BCL-2 family regulate mitochondrial permeabilisation



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; 21(2):85–100

18

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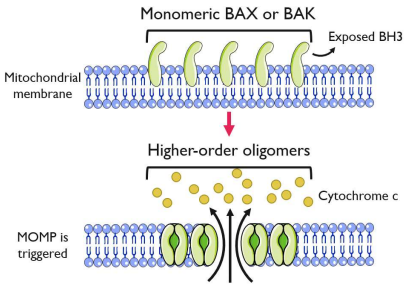
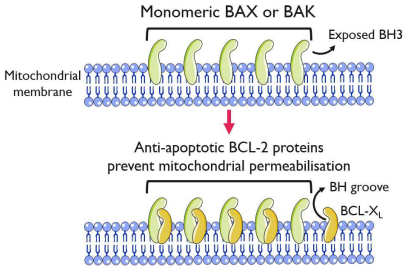
Pro-apoptotic BAK and BAX induce MOMP

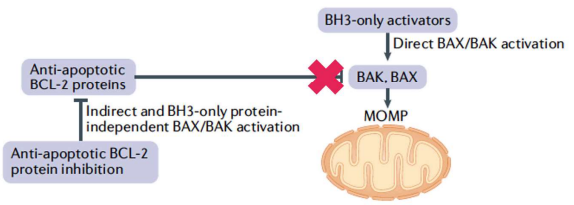


19

Pro-apoptotic BAK and BAX induce MOMP



Pro- and anti-apoptotic BCL-2 proteins dictate MOMP



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; 21(2):85–100

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BAX and BAK are typically essential for MOMP

Pro-apoptotic trigger

↓

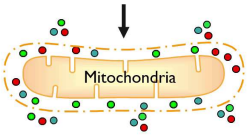
BH3 only protein

↓

BAX/BAK

↓

Mitochondrial Outer Membrane Permeabilisation



Intermembrane space proteins

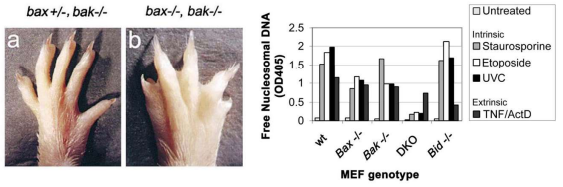
Cytochrome c-GFP

Smac-mCherry or GFP

Omi-mCherry

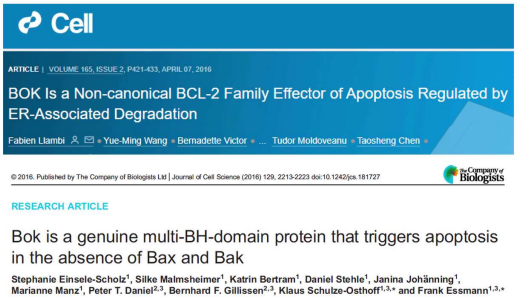
21

BAX and BAK are typically essential for MOMP



Lindsten T. *et al., Mol Cell.* 2000; **6**(6):1389-1399, Wei MC. *et al., Science.* 2001; **292**(5517): 727-730

BOK can also induce MOMP in some settings



Cell

ARTICLE | VOLUME 165, ISSUE 2, P421-433, APRIL 07, 2016

BOK Is a Non-canonical BCL-2 Family Effector of Apoptosis Regulated by ER-Associated Degradation

Fabien Llambi • Yue-Ming Wang • Bernadette Victor • ... Tudor Moldoveanu • Taosheng Chen •

© 2016. Published by The Company of Biologists Ltd | Journal of Cell Science (2016) 129, 2213-2223 doi:10.1242/jcs.181727

The Company of Biologists

RESEARCH ARTICLE

Bok is a genuine multi-BH-domain protein that triggers apoptosis in the absence of Bax and Bak

Stephanie Einsele-Scholz[1], Silke Malmsheimer[1], Katrin Bertram[1], Daniel Stehle[1], Janina Johänning[1], Marianne Manz[1], Peter T. Daniel[2,3], Bernhard F. Gilissen[2,3], Klaus Schulze-Osthoff[1,3,*] and Frank Essmann[1,3,*]

22

Llambi F. *et al., Cell.* 2016; **165**(2):421-433, Einsele-Scholz S. *et al., J Cell Science.* 2016; **129**(11):2213-2223

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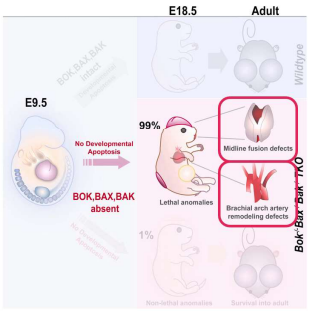
Blocking mitochondrial apoptosis affects development

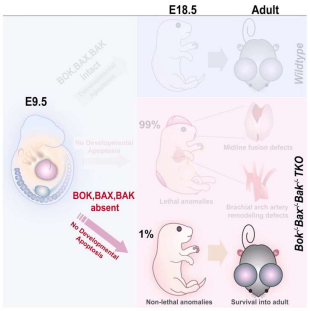


Vast majority of mice undergo embryonic lethality

Ke FFS. *et al., Cell.* 2018; **173**(5):1217-1230

23

Blocking mitochondrial apoptosis affects development

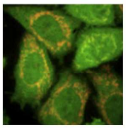


There may be other pathways that compensate for the lack of mitochondrial apoptosis, in allowing developmental cell death

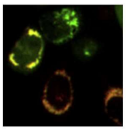
Ke FFS. *et al., Cell.* 2018; **173**(5):1217-1230

BAK/BAX localisation

- BAK is located on the mitochondria, upon activation BAX translocates to the mitochondria



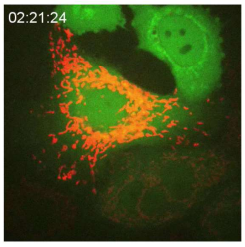
BAX is in the cytosol, the cells are healthy



Apoptosis

BAX relocates onto the mitochondria

GFP BAX
Omi mCherry



Tait SWG. *et al., Dev Cell.* 2010; **18**(5):802-813

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BAK/BAX localisation

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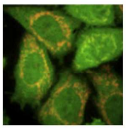

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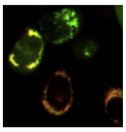
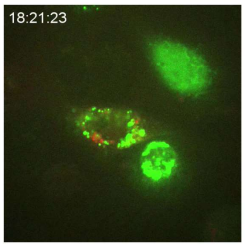


Apoptosis

BAX relocates onto the mitochondria

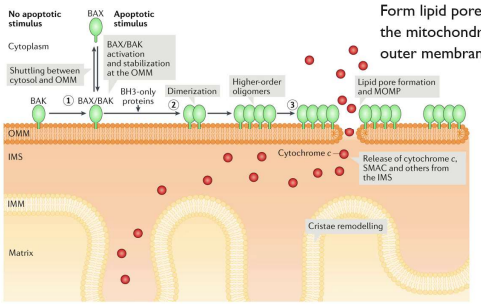
GFP BAX

Omi mCherry



Tait SWG. *et al., Dev Cell.* 2010; **18**(5):802-813

BAX and BAK oligomerize to form pores



Form lipid pores in the mitochondrial outer membrane

Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; **21**(2):85-100

Salvador-Gallego, R. *et al., EMBO J.* 2016; **35**:398-401, Großе L. *et al., EMBO J.* 2016; **35**:402-413

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Summary

BCL-2 family proteins are central to regulation of MOMP and apoptosis

Interactions between **pro-** and **anti-apoptotic BCL-2** proteins dictate whether MOMP occurs

Activate **BAX** and **BAK** cause permeabilisation of the outer membrane via **lipid pores**

Mitochondrial apoptosis is important for **development**, however viable animals can **develop in its absence**

26

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Can we target mitochondrial apoptosis in disease?

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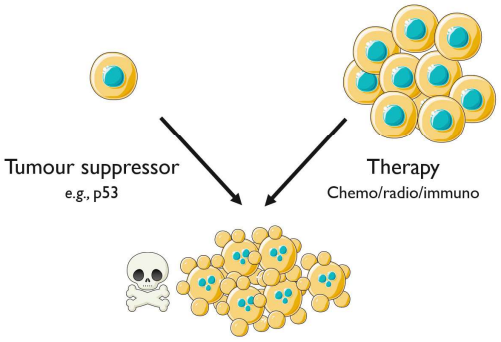
Cell death prevents and treats cancer



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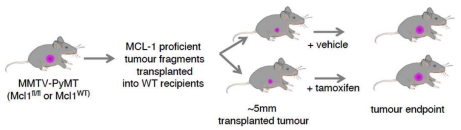
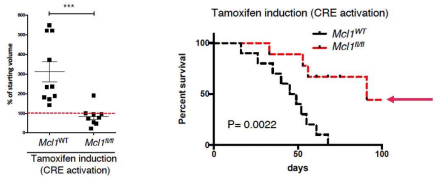
Anti-apoptotic BCL-2 proteins promote cell survival





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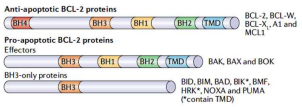
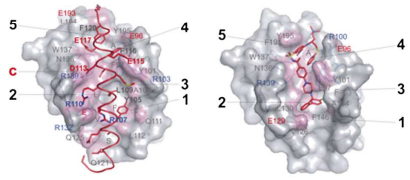
BH3-mimetics



- Develop drugs to mimic pro-apoptotic BH3-proteins '**BH3-mimetics**'



Bock FJ. and Tait SWG. *Nat Rev Mol Cell Biol.* 2020; **21**(2):85-100, Chipuk JE. *et al., Mol Cell.* 2010; **37**:299-310

30

Targeting BCL-2 to treat cancer works

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Venetoclax–Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia

J.F. Seymour, T.J. Kipps, B. Eichhorst, P. Hillmen, J. D'Rozario, S. Assouline, C. Owen, J. Gerecitano, T. Robak, J. De la Serna, U. Jaeger, G. Cartron, M. Montillo, R. Humerickhouse, E.A. Punnoose, Y. Li, M. Boyer, K. Humphrey, M. Mobasher, and A.P. Kater

Patients given BCL-2 inhibitor/rituximab for the treatment of refractory CLL showed a clear survival benefit over these only treated with rituximab

Seymour JF. *et al., N Engl J Med.* 2018; **378**:1107-1120

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Can we target mitochondrial apoptosis in other ways?

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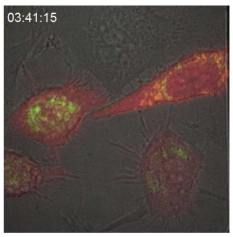
MOMP is a point of no return

Lethal stress

Widespread MOMP

Caspase -Independent Cell Death (CICD)

Apoptosis



Tait SWG and Green RD. *Nat Rev Mol Cell Biol.* 2010; **11**(9):621-632

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MOMP is a point of no return

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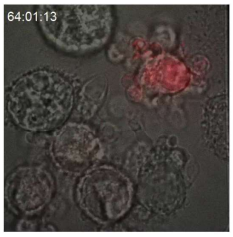

Does it matter how a cell dies?

Tait SWG and Green RD. *Nat Rev Mol Cell Biol.* 2010; **11**(9):621-632

TNF is up-regulated following MOMP

MOMP

APAF-1

Caspase-9

Caspases 3/7

Apoptosis

Giampazolias E. *et al., Nat Cell Biol.* 2017; **19**:1116-1129

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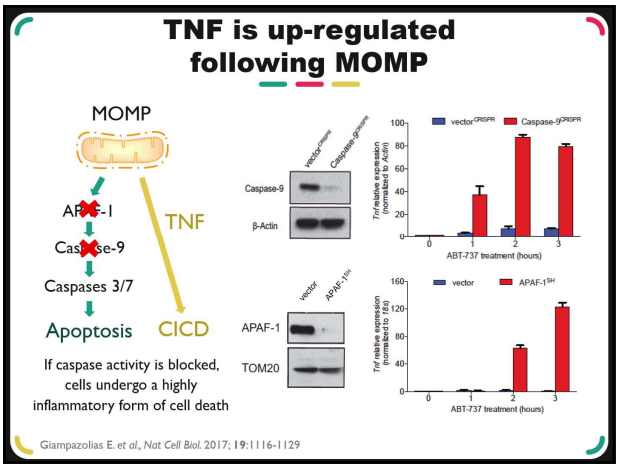

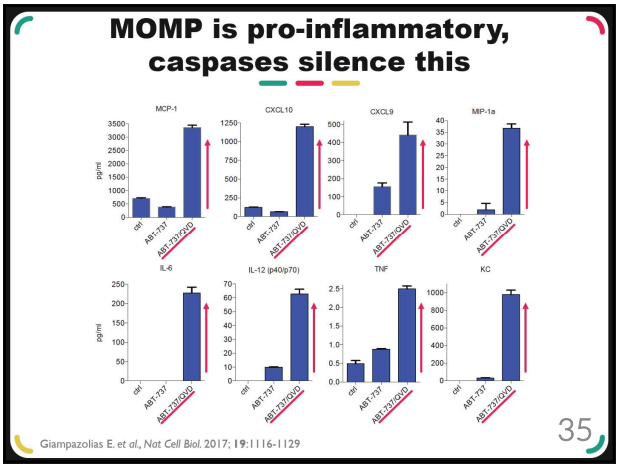

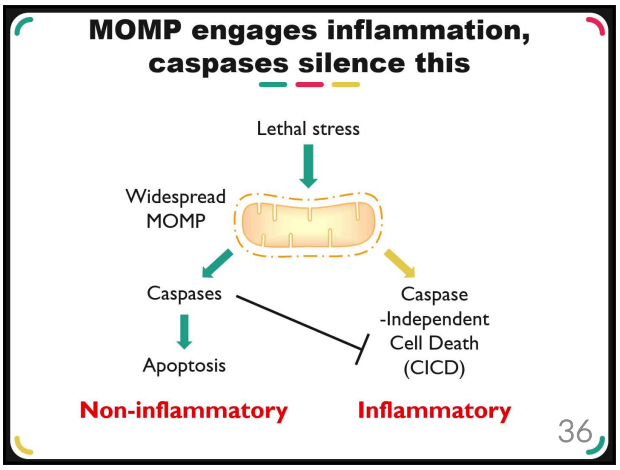

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How do mitochondria drive inflammation?

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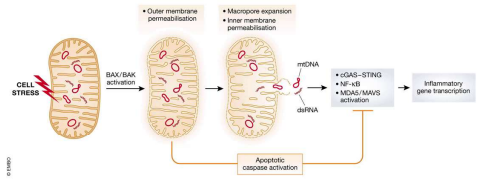
MOMP is inherently pro-inflammatory



Mitochondria DNA (mtDNA) release

Leads to the activation of the cGAS-STING signalling pathway

NK-κB

Activated independently of the cGAS-STING pathway

Double strand RNA (dsRNA) release

Activates the pathway MDA5/MAVS, leading to interferon production

Riley JS. And SWG Tait. *EMBO Rep.* 2020; **21**:e49799

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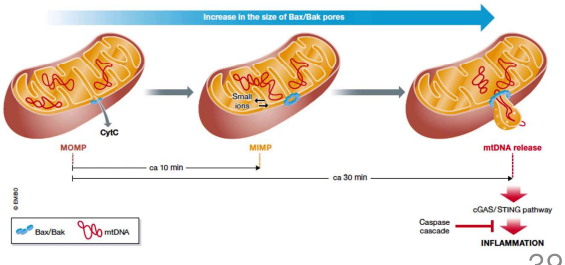
Mitochondrial inner membrane permeabilisation

- Once we get MOMP, over time we get **M**itochondria **I**nner **M**embrane **P**ermeabilisation (MIMP)



Cosentino K. and Garcia-Saez AJ. *EMBO J.* 2018; **37**(17):e100340

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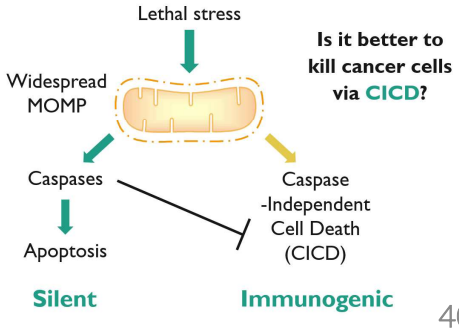
Is it better to kill cancer cells via CICD?



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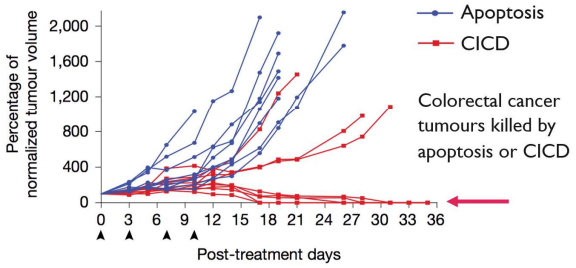
CICD effectively treats cancer



Giampazolias E. *et al., Nat Cell Biol.* 2017; **19**:1116-1129

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Summary

BH3-mimetics neutralize anti-apoptotic BCL-2 function **sensitizing tumour cells to apoptosis**

MOMP is inherently **pro-inflammatory**, caspase activity **silences inflammation**

MOMP leads to activation of various pro-inflammatory signalling pathways including **NF-κB** and **cGAS-STING**

During cell death, the **mitochondrial inner membrane is permeabilized** leading to **mtDNA release**

Inhibiting caspase function coverts apoptosis to an **immunogenic** type of cell death - potential as **anti-cancer treatment**

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HSTalks

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Acknowledgements

Evangelos Giampazolias

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Johns Hopkins

Hiromi Sesaki

Caltech

David Chan

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