Vol 458| 9 April 2009| doi:10.1038/nature07782 (Click here to download the paper) Supplement

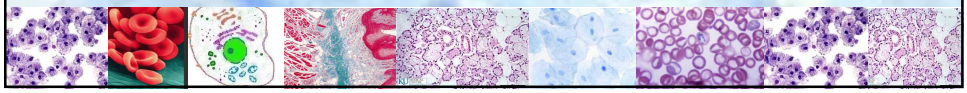
nature

# Tumours with PI3K activation are resistant to dietary restriction

Nada Y. Kalaany and David M. Sabatini.

Presented By

P.B.TIRUPATHI PICHIAH (TIRU)
Dept of Human Nutrition



**Growth**

Cell: Gradual increase in Size and mass.
Tissue: Size + mass + Number
Body: Number of growth of different tissues

**Cell Size:**

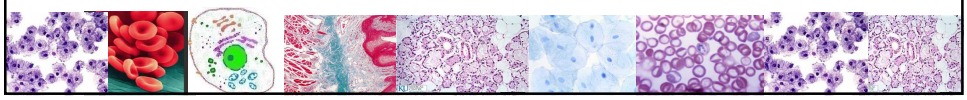
Size is a fundamental attribute impacting cellular design, fitness, and function.

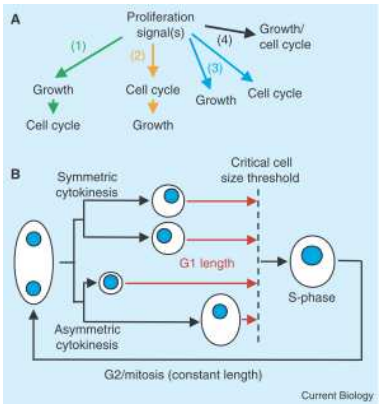
Cell growth and Cell Cycles are coupled

Maximum size of a cell after which S-phase begins.

**What are the things needed for a cell to grow ?**

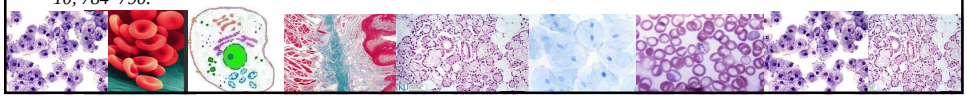
Sufficient Nutrition, Sufficient energy, Positive Signal by growth factors



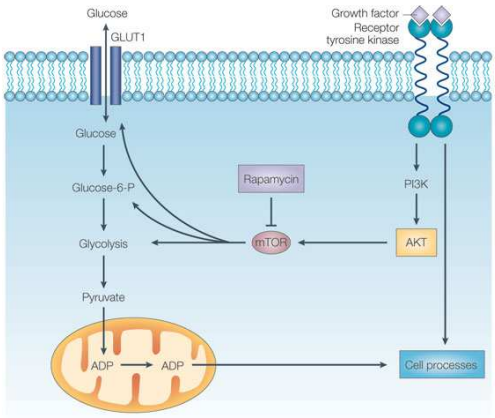



A single critical cell size requirement suffices to couple growth and division. Whether cytokinesis is symmetric or asymmetric with respect to cell mass, daughter cells must grow past a cell size threshold in order to enter S phase. The growth requirement results in variable G1 lengths while the S/G2/M phase is constant

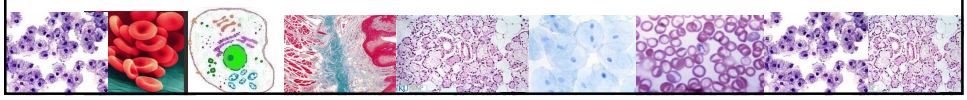
*Neufeld, T.P., and Edgar, B.A. (1998). Connections between growth and the cell cycle. Curr. Opin. Cell Biol. 10, 784–790.*

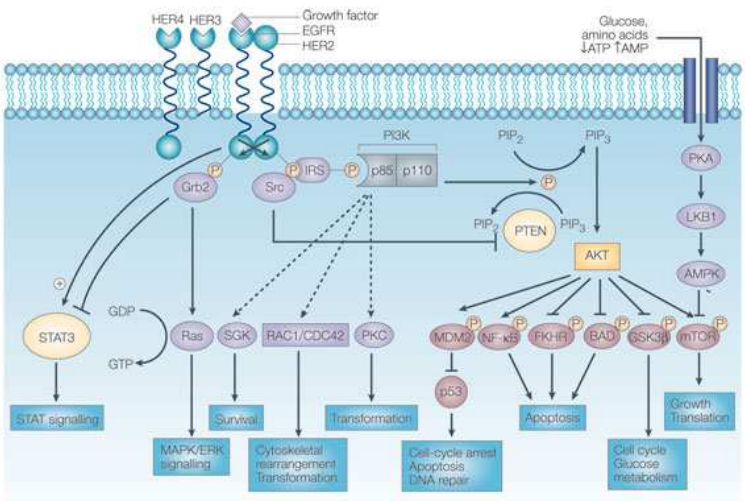


**Understanding the PI3K Pathway:**

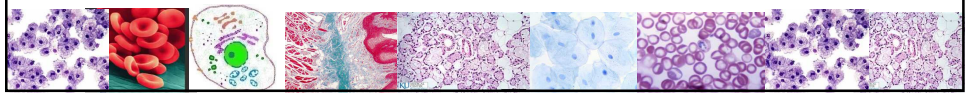


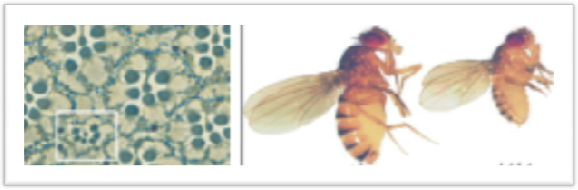




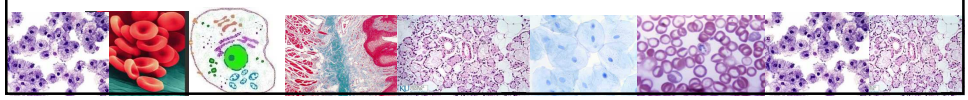

*Bryan T. Hennessy et al.Nature Reviews Drug Discovery 4, 988-1004 (December 2005)*





Loss of dAkt or dS6K in Drosophila reduces cell size.
A clone of dAkt−−/−− rhabdomeres (white box) exhibits very small cell size relative to twin-spot cells and dAkt-/+ heterozygote cells.

*Verdu, J., (1999). Cell-autonomous regulation of cell and organ growth in Drosophila by Akt/PKB. Nat. Cell Biol. 1, 500–506.*



**PI 3-kinases or PI3Ks:**

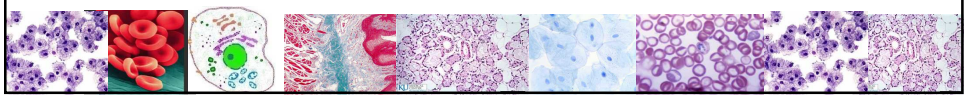
Phosphatidylinositol 3-kinases (PI 3-kinases or PI3Ks) are a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer.

**What is Akt ?**

**Akt/PKB** is a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration.

**mTOR**

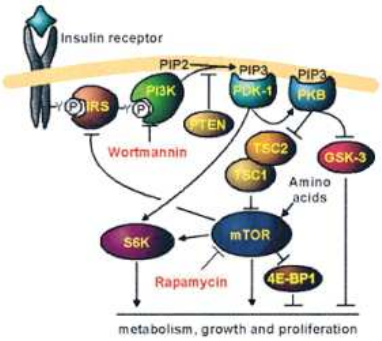
The mammalian target of rapamycin (mTOR) also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) is a protein which in humans is encoded by the FRAP1 gene. mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription.



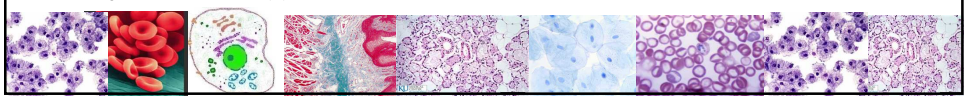
Review articles

**Regulation of cell size in growth, development and human disease: PI3K, PKB and S6K**

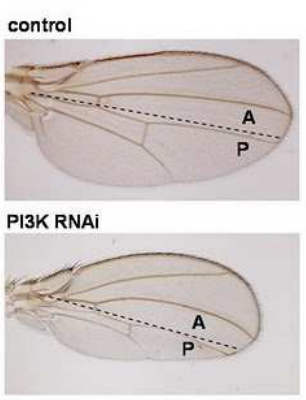
Sara C. Kozma and George Thomas*




*Bioessays. 2002 Jan;24(1):65-71.*

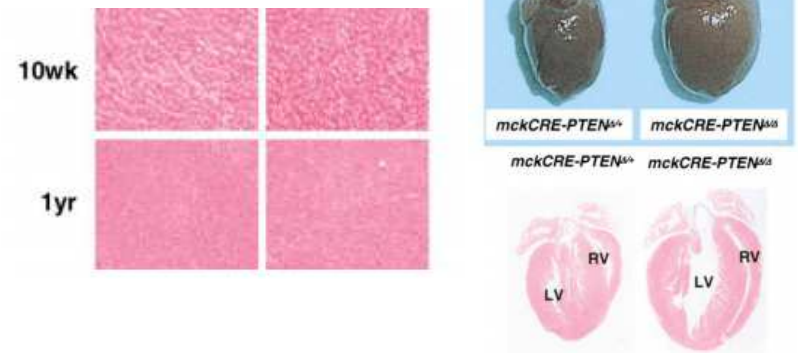


**Inhibition of PI3K decreases wings size**



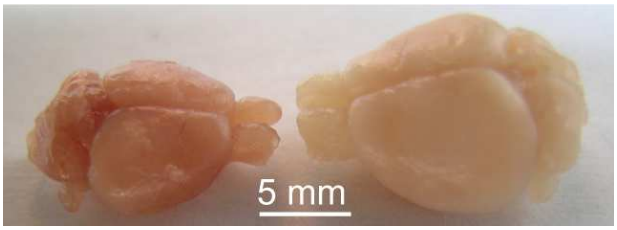

Teleman, Hietakangas *et al.*, 2008

## Regulation of Myocardial Contractility and Cell Size by Distinct PI3K-PTEN Signaling Pathways




*Michael A. Crackower et al, Cell, Vol. 110, 737–749, September 20, 2002,*

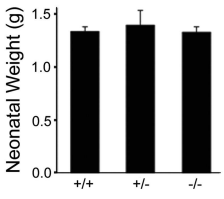
**Normal mouse brain on the left and *Pten*-knockout brain on the right**



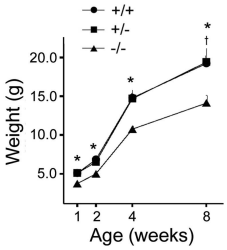

*Stéphanie A. Backman et al 2001, Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nature Genetics* **29**, 396 - 403 (2001)

**RIPcre+ Ptenfl/fl mice show growth restriction from the early postnatal period onward**

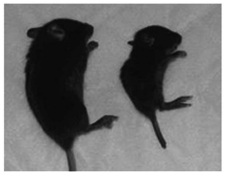
a.




b.

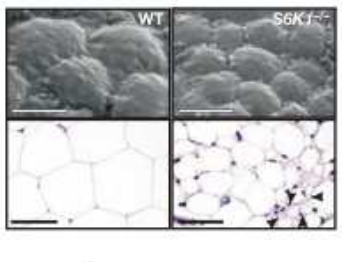


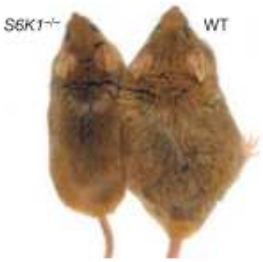

c.

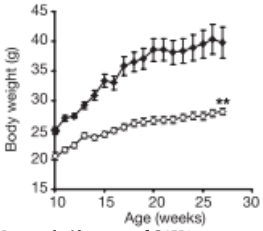


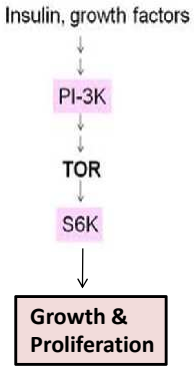
*Nguyen, K.-T. T. et al. 2006. Mol. Cell. Biol. 26(12):4511-4518*

**S6K1 $^{-/-}$ mice do not gain weight even from high calorie diet.**

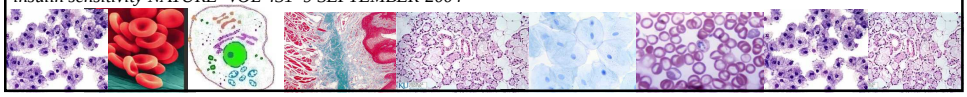


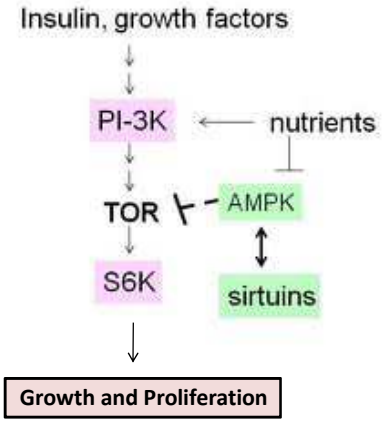



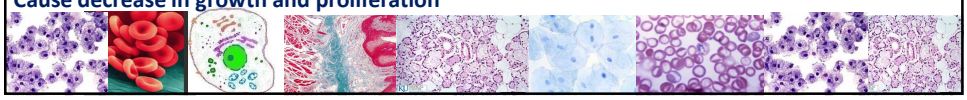

*Sung Hee Um et al, Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity NATURE VOL 431 9 SEPTEMBER 2004*



**Till now what we understand**

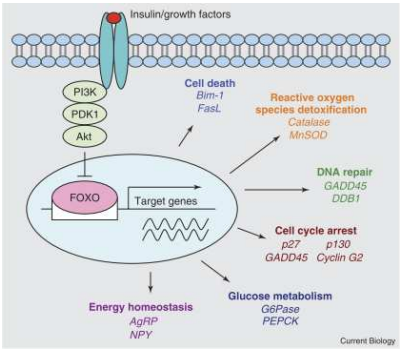


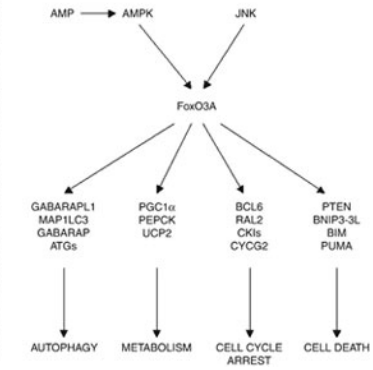

**Remember: Blocking, deleting or mutating any one of the gene downstream to PI3K Cause decrease in growth and proliferation**

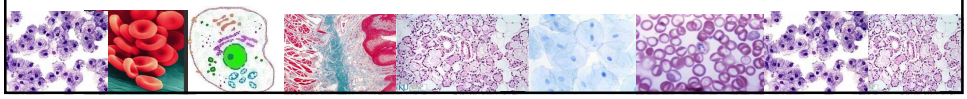


**What happens during calorie restriction or starvation ?**

Living system has a constant ratio of AMP:ADP:ATP.
When the level of ATP drops and AMP raises, causes increase in AMP/ATP ratio.
This triggers several catabolic process to meet the energy requirement.
Such as activation of AMPK.



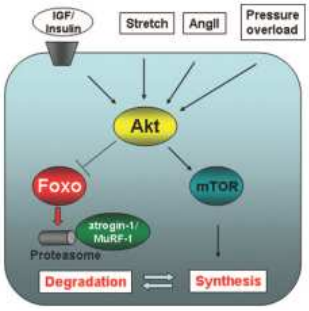



**The FOXO3a Transcription Factor Regulates Cardiac Myocyte Size Downstream of AKT Signaling***

Received for publication, January 14, 2005, and in revised form, March 11, 2005
Published, JBC Papers in Press, March 21, 2005, DOI 10.1074/jbc.M500528200

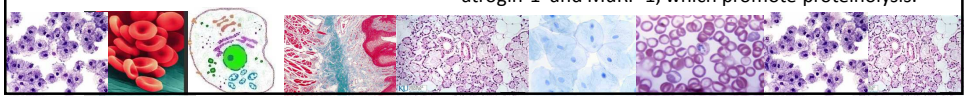
**Carsten Skurk‡, Yasuhiro Izumiya‡, Henrike Maatz‡, Peter Razeghi§, Ichiro Shiojima‡, Marco Sandri¶, Kaori Sato‡, Ling Zeng‡, Stephan Schiekofer‡, David Pimentel‡, Stewart Lecker‖, Heinrich Taegtmeyer§, Alfred L. Goldberg¶, and Kenneth Walsh‡****

*From ‡Boston University School of Medicine, Whitaker Cardiovascular Institute, Boston, Massachusetts 02118, the ¶Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, the ‖Renal Unit, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02115, and the §University of Texas Houston Medical School, Houston, Texas 77030*




**Proposed scheme for Akt/FOXO-mediated regulation of cardiac myocyte size**.

Multiple inputs including growth factors, angiotensin II, stretch, and pressure overload lead to Akt phosphorylation. Akt induces hypertrophy in cardiac myocytes by increasing protein synthesis through the mammalian target of rapamycin-dependent pathways. Akt activation also leads to inactivation of forkhead transcription factors through phosphorylation. FOXO transcription factors activate atrogenes, including the ubiquitin ligases atrogin-1 and MuRF-1, which promote proteinolysis.

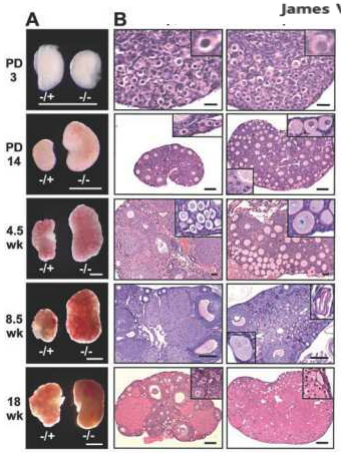


Science, 301 , 215 (2003);

Science
AAAS

# Suppression of Ovarian Follicle Activation in Mice by the Transcription Factor Foxo3a

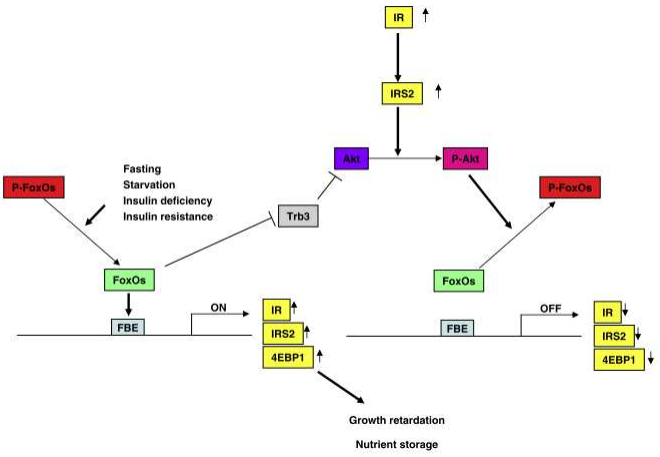
Diego H. Castrillon,[1,2]* Lili Miao,[1] Ramya Kollipara,[1] James W. Horner,[1] Ronald A. DePinho[1]†




**Gross and histologic analysis of Foxo3a –/– and control ovaries.**

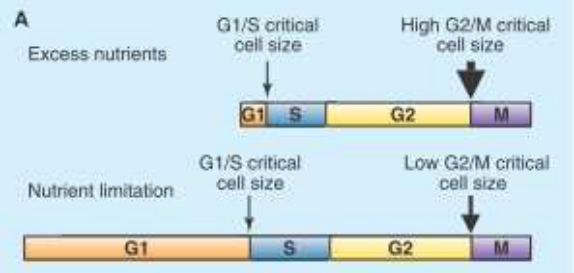
(A) Size differences in Foxo3a –/ and Foxo3a –/– ovaries; scale bar, 1 mm. (B) Histology of Foxo3a –/ and Foxo3a –/– ovaries at different ages. Scale bar 100 for all panels, except that at PD3, scale bar 20 . All sections were stained with hema- toxylin and eosin except for those at 8.5 weeks and the inset sections at 4.5 weeks, which were stained with periodic acid–Schiff. For each time point, insets are at the same magnification.

**Positive feedback loop of FoxOs on insulin signaling.**




*Jun Nakae et al, 2008, The FoxO transcription factors and metabolic regulation . FEBS Letters 582 (2008) 54–67*

Nutrient and Growth.




The Paper:

**Aim:1. To investigate the responsiveness of different types of tumours to dietary restriction.**

Six different tumors: brain (U87-MG), colon (SW620), prostate (PC3)
and breast (MDA-MB-231, MDA-MB-435, and MCF10DCIS

mice.
MDA-MB-231 and MCF10DCIS cells
(Breast cancer cell line )

**Subcutaneously**

**Orthotopically into the mammary fat pads**

NOD/ SCID mice

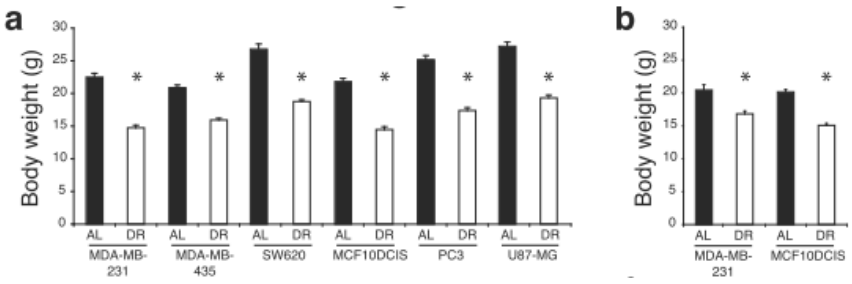
ad libitum diet for 3–4 days

Ad libitum

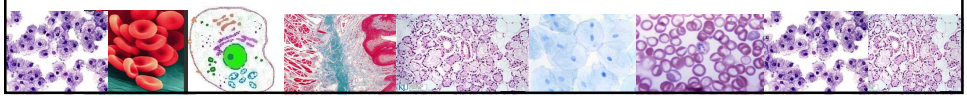
2-3 weeks

Diet Restricted (DR) 40%

**Body Weight**



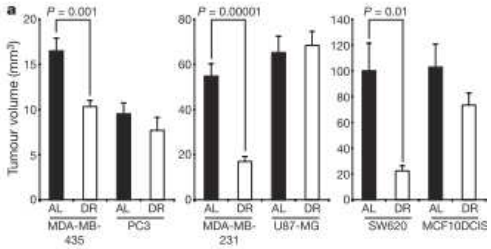

Supp fig 1a and b



**Tumour Volume**

**DR-sensitive tumours**

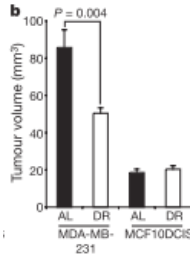
(MDA-MB-435, MDA-MB-231 and SW620)



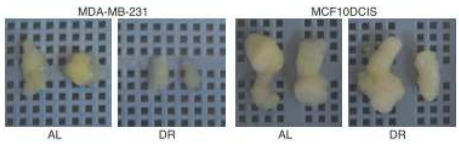

(Fig. 1a)

**DR-Resistant tumours**

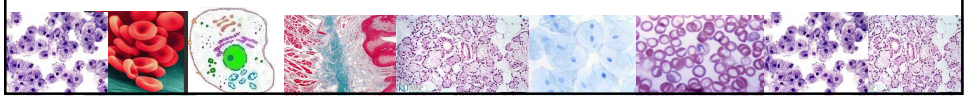
(PC3, U87-MG and MCF10DCIS)



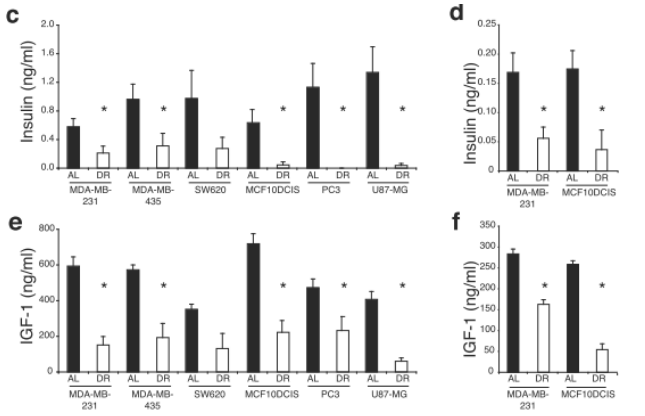

(Fig. 1b)



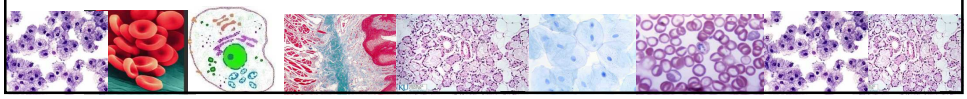

(Supp Fig. 1g)



Plasma levels of insulin and IGF1




Supp Fig 1c-f



**Constitutive PI3K signalling in DR-resistant tumours**

**Aim :2. whether the six cancer cell lines studied have differential requirements for Insulin and IGF-1, factors for their growth in tissue culture.**

Cell lines that form DR-sensitive tumours (MDA-MB-231, MDA-MB-435 and SW620) insulin or IGF1 caused a dose-dependent increase in cell numbers

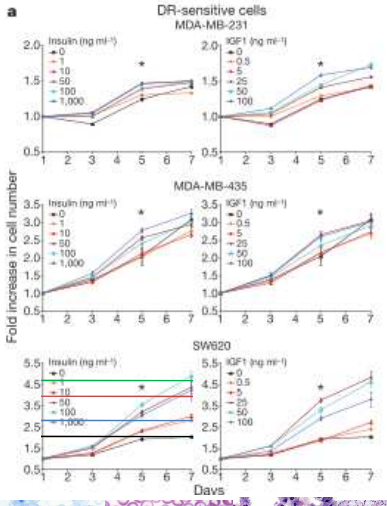
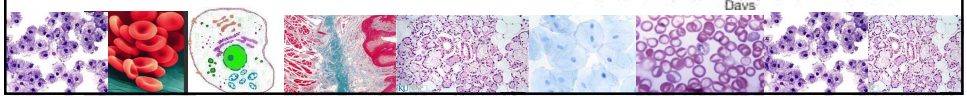



Fig 2a



Cell lines that generate DR-resistant tumours (MCF10DCIS, U87-MG and PC3) grew in culture in an insulin- and IGF1-independent fashion

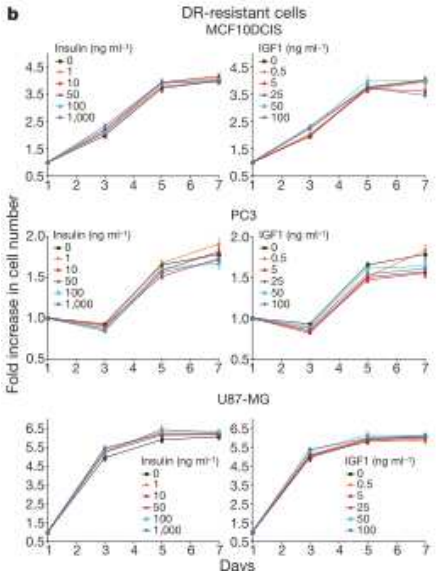
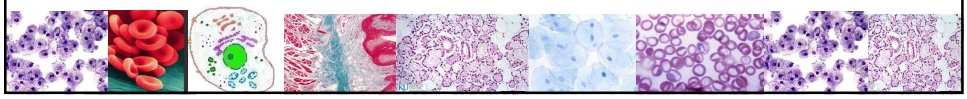



Fig 2b



These results indicated that the cancer cell lines forming DR-resistant tumours have a deregulation in an insulin/IGF1-activated signalling pathway, with the PI3K/Akt pathway.

**Aim:3. Whether DR causes diffrences in Akt phosphorylation or not .**

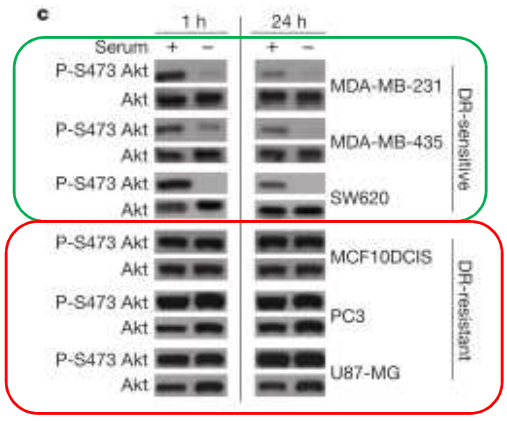
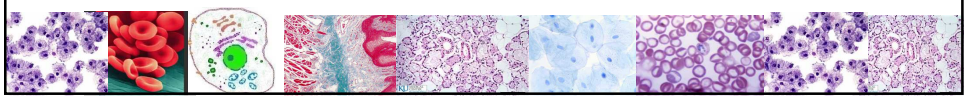



**Fig 2 c**



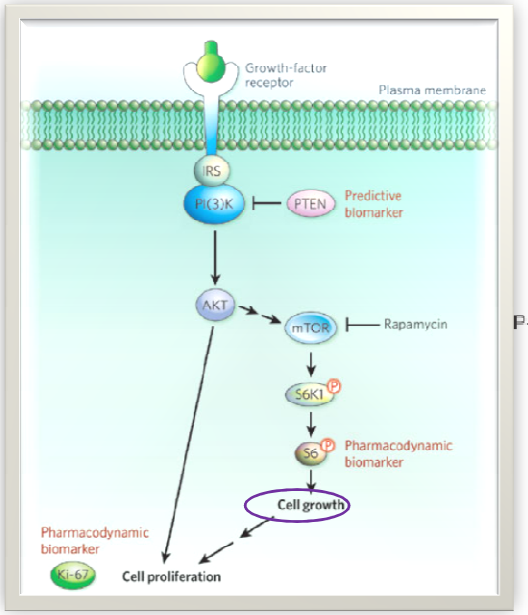
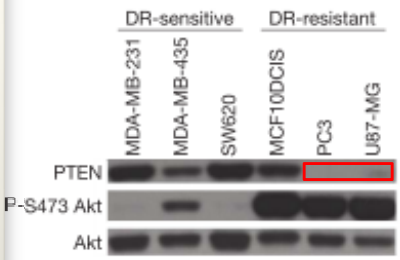
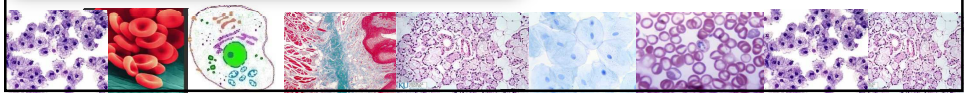



Fig 2d




Analysis of PTEN expression showed that PTEN loss could account for the serum-insensitive Akt activity 20 in PC3 and U87-MG, but not in MCF10DCIS



Activating mutations in the PIK3CA gene, which encodes the catalytic subunit of PI3K, also lead to constitutive Akt activity in cancer cells

**Aim:4. Is there any hot spot mutation in PIK3CA and PTEN gene sequence?**

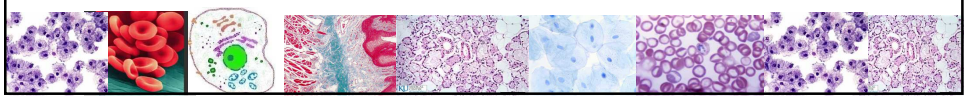
Fig 2e

e

| | | *PIK3CA* | *PTEN* | *TP53* | *KRAS or HRAS* | *BRAF* |
|---|---|---|---|---|---|---|
| DR-sensitive | MDA-MB-231 | WT | WT | Mut | Mut | Mut |
| | MDA-MB-435 | WT | WT | Mut | WT | Mut |
| | SW620 | WT | WT | Mut | Mut | WT |
| DR-resistant | MCF10DCIS | H1047R | WT | ND | Mut | ND |
| | PC3 | WT | – | – | WT | WT |
| | U87-MG | WT | – | WT | WT | WT |

**Mut, mutant; ND, not determined; WT, wild-type. '–' indicates absence of the gene.**

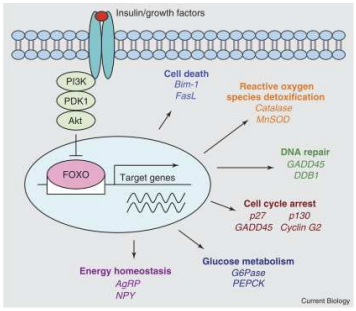
MCF10DCIS cells harbour a previously unknown **H1047R** mutation. None of the cell lines that form DR-sensitive tumours have lost PTEN function or carry PI3K activating mutations. These results indicate that tumour resistance to DR correlates with constitutive activation (for example, through PIK3CA mutations or PTEN loss), rather than with hyperactivation of the PI3K pathway.

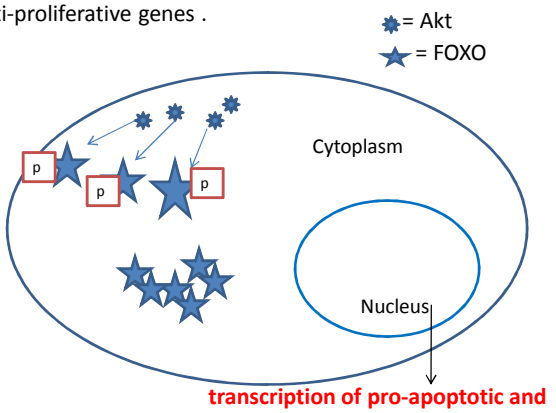


**Aim:5. To find whether the tumour size decrease due to increased enhanced cell death or Reduced proliferation .**

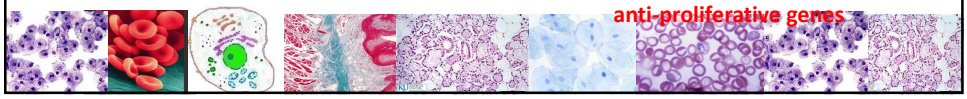
FOXO1 transcription factor a major downstream target of Akt that is selectively phosphorylated as a result of PI3K activation .

FOXO1 by Akt leads to its cytoplasmic sequestration and degradation A decrease in Akt signalling causes relocalization of FOXO1 to the nucleus, where it induces transcription of pro-apoptotic and anti-proliferative genes .

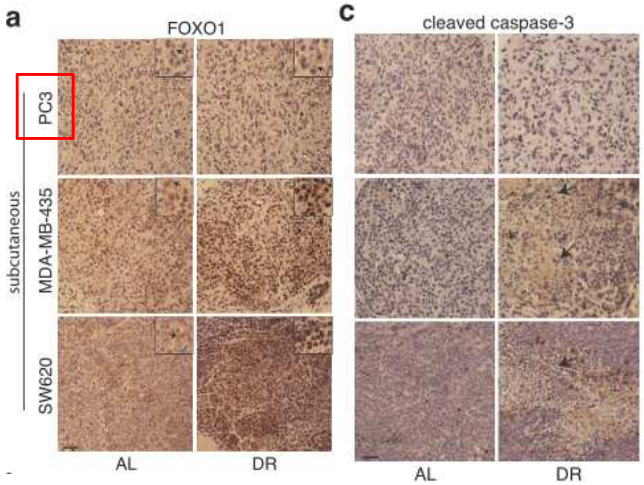


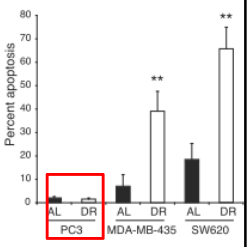




**transcription of pro-apoptotic and anti-proliferative genes**

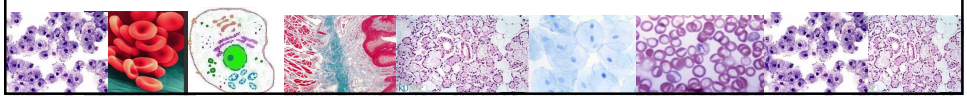


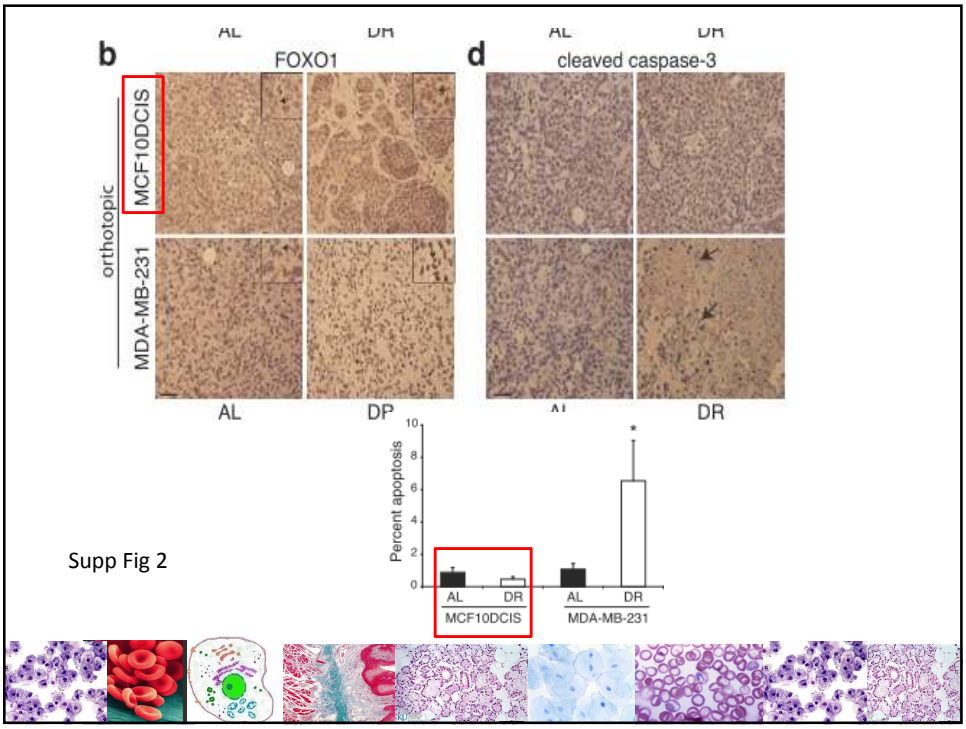
In the DR-resistant tumours (PC3 and MCF10DCIS), FOXO1 was predominantly localized to the cytoplasm of tumour cells in both ad libitum and DR mice




Supp Fig 2






Supp Fig 2

The correlation between tumour sensitivity to DR and the activation status of the PI3K pathway led us to consider that constitutive PI3K signalling in tumour cells is sufficient to decrease the sensitivity of tumours to DR.

To begin testing this idea, they used two cell lines derived from the DLD-1 colorectal cancer cell line 21 . These cells are isogenic except that one carries a wild-type allele (DLD-WT) and the other has a constitutively active mutant allele (E545K; DLD-mut) of PIK3CA.

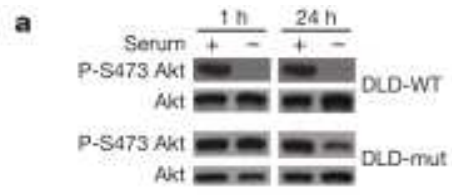



Fig 3a

Cell proliferation in response to Insulin and IGF-1

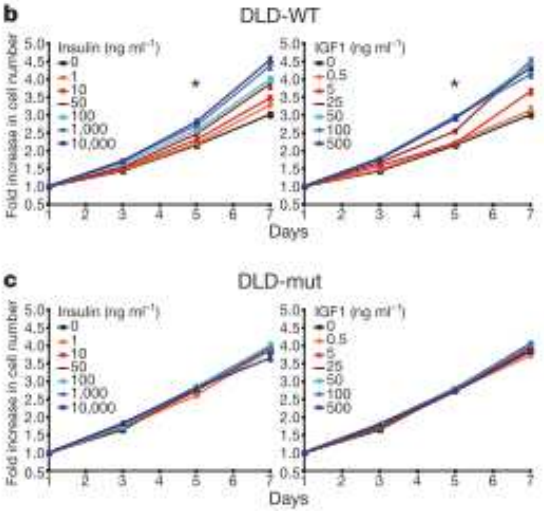
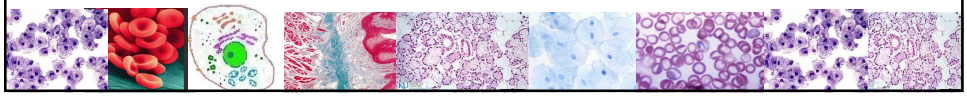



Fig 3b and 3c



Tumor Volume

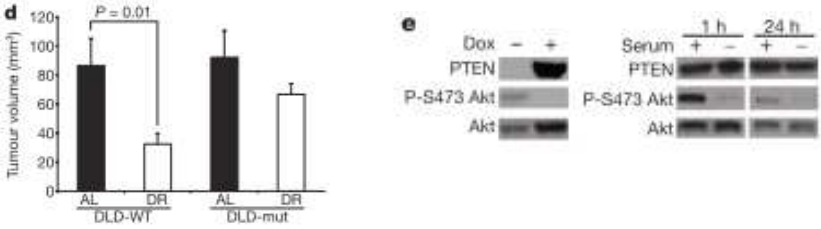
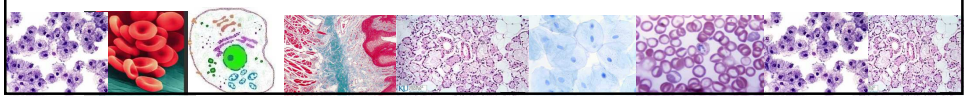



**Fig 3 d and e**

Doxycyline increases PTEN express invitro



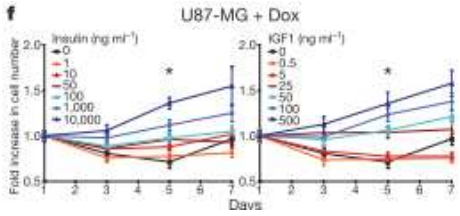
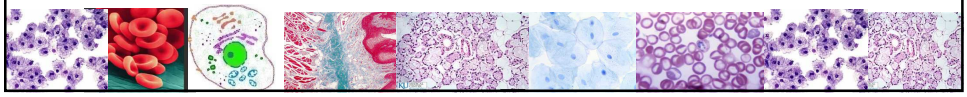



Fig 3f and 3g



**Aim:6.Effects of modulation of PI3K signalling on the apoptotic response of tumours to DR.**

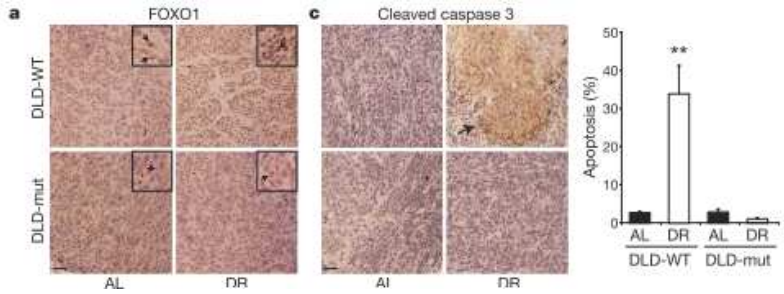
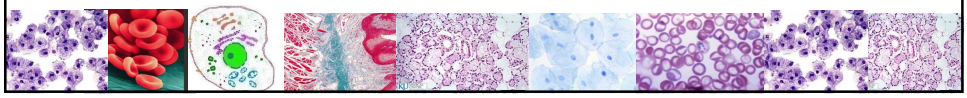



Fig 4a and c



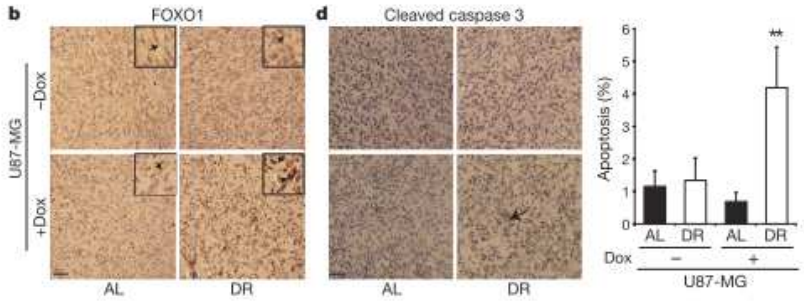
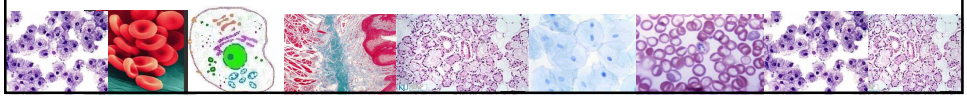
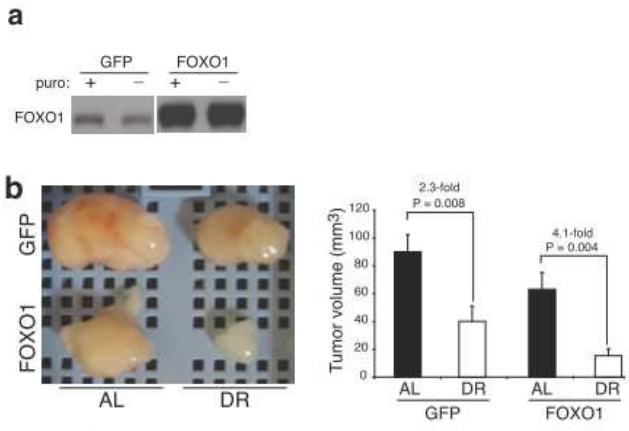


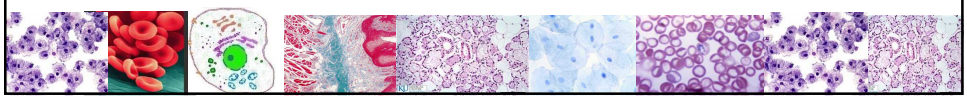

Fig 4b and d

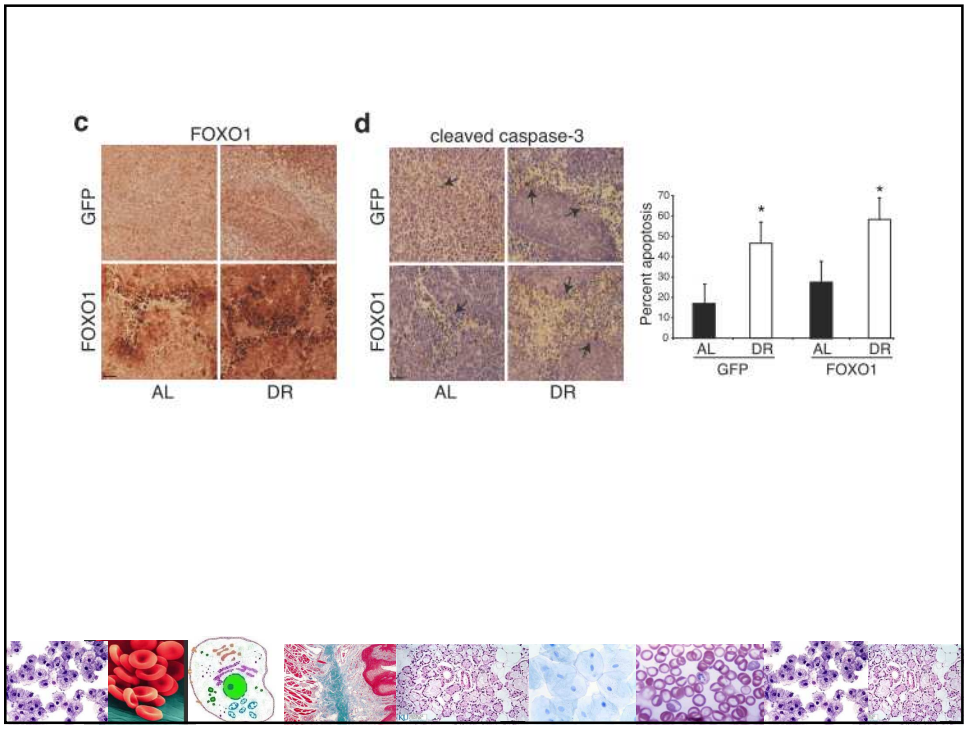


**FOXO1 overexpression sensitizes tumours to DR**




Supp Fig 4 a and b

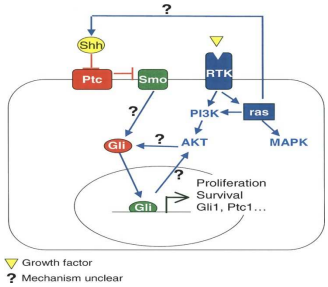





**A murine PTEN-null prostate cancer is resistant to DR**

They examined the effects of DR in two engineered mouse models of cancer. The first model (probasin-Cre; PTEN lox/lox ,hereafter Pb-Cre; PTEN L/L )) is driven by PTEN loss in the prostate and recapitulates human prostate cancer progression

The second (KRAS LA2 ; p53-lox-stop-lox/WT—hereafter KRAS LA2 ; p53 LSL/WT) is driven by KRAS activation as well as p53 heterozygosity and leads to the development of lung adenocarcinoma.



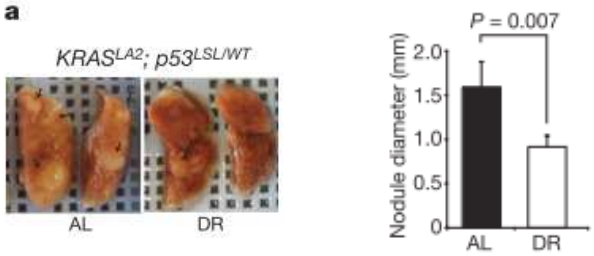
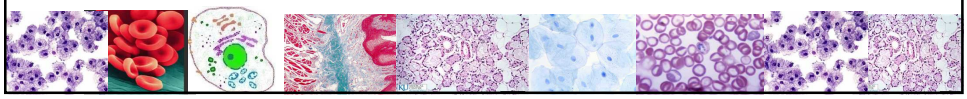


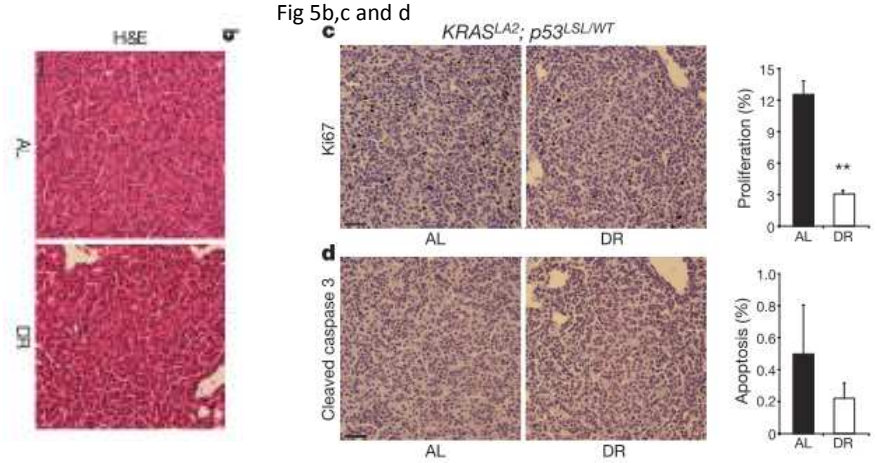

Fig 5a

lungs (graph) of 7-week-old KRAS $^{LA2}$; p53 $^{LSL/WT}$ mice under AL or DR conditions



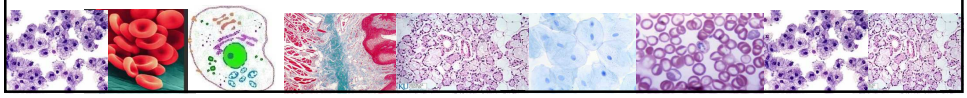
**Proliferation vs Apoptosis : KRAS mutant (lung)**

Fig 5b,c and d

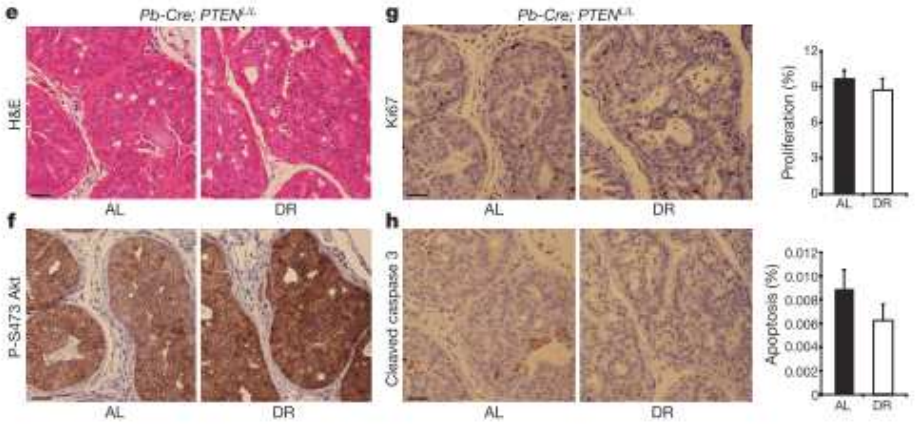


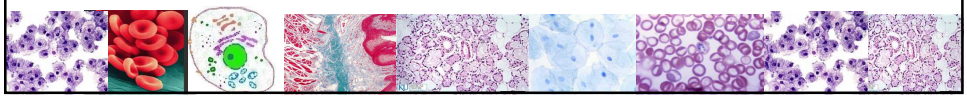

**KI-67** is a nuclear protein that is associated with and may be necessary for cellular proliferation.

**cleaved caspase-3** detects high level of apoptosis



**Proliferation vs Apoptosis : PTEN mutant (prostrat)**



**Discussion :**

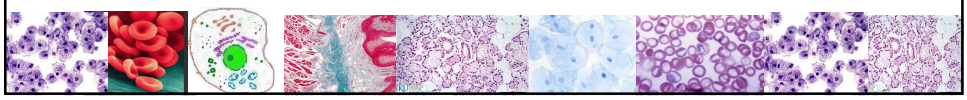
Genetic alterations in PIK3CA or PTEN can predict the response of tumours to DR, classifying them into DR-sensitive and DR-resistant tumours.

Differential levels of PI3K activation in tumours contribute to their differential sensitivities to DR.

Reminiscent of the cell-type- and tissue-microenvironment-dependent effects of the FOXO factors on proliferation and apoptosis .

DR causes strong suppression of proliferation in the engineered mouse lung cancer model, while having a prominent pro-apoptotic effect in the human tumour xenograft models.

After DR, FOXO moves from the cytoplasm to the nucleus only in the cells of DR-sensitive tumours and overexpression of FOXO sensitizes tumours to the anti-growth effects of DR.



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