Anti-cancer discovery and development: Targeting the sweet tooth of cancer.

Monde Ntwasa

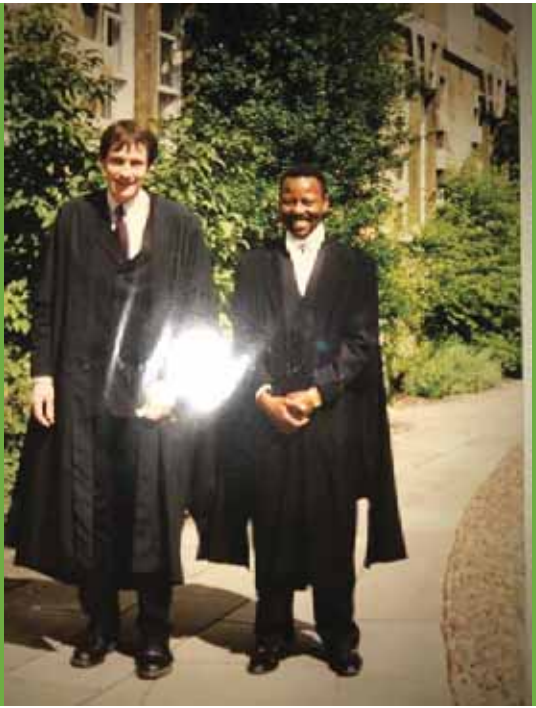
Department of Life & Consumer Sciences
University of South Africa
03 August 2017

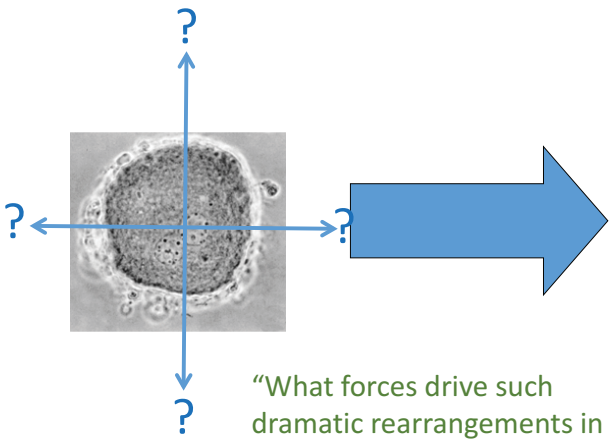
1. Historical background
2. Current work
3. Future
4. Reflections

Cambridge University


Jesus College
Jesus College


Sidney Sussex College
Christ's College Cambridge
Google

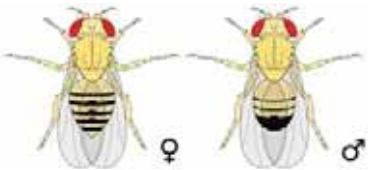


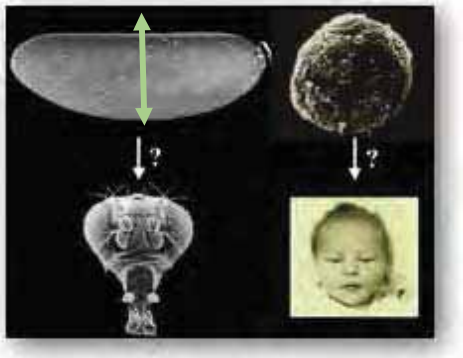


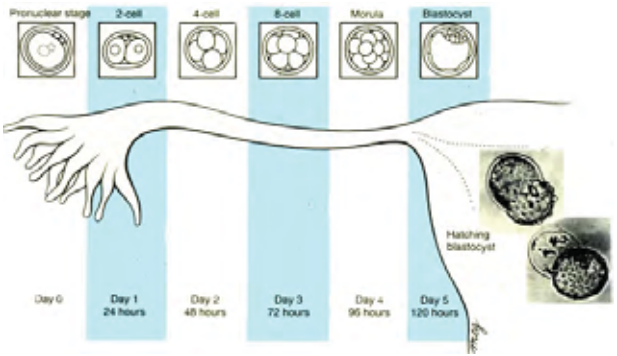
"What forces drive such dramatic rearrangements in cells?"

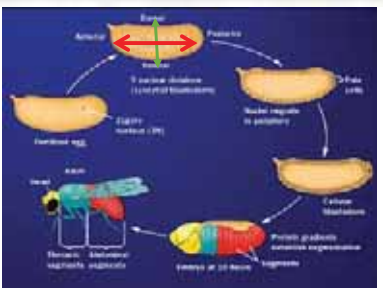
Question: Molecular mechanisms that control Dorso-ventral axis determination

Drosophila – The fruitfly

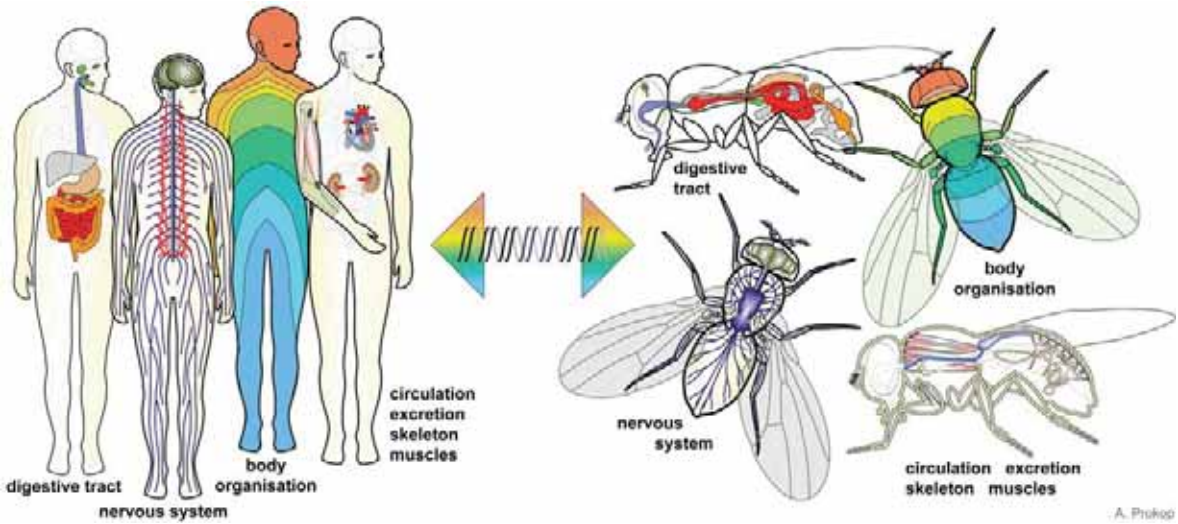








For almost every organ in humans there is a match in flies, and common genes

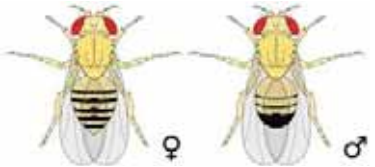


Nobel Prize in Physiology or Medicine 1995

for discoveries around the genetic control of early embryonic development

Eric F. Wieschaus

"I will never forget the thrill of seeing cleavage and gastrulation for the first time in living frog embryos."

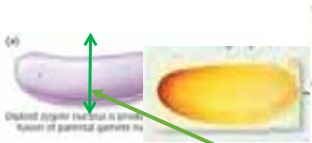


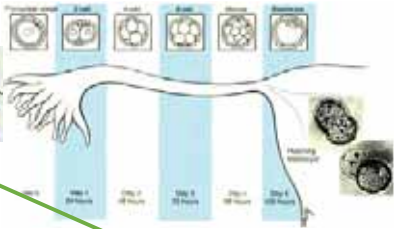
Edward B. Lewis

He generally avoided molecular explanations for his observations, in part due to a feeling of humility towards most things biochemical, and in part from a suspicion that the available molecular mechanisms couldn't explain the complexity he saw in the flies.

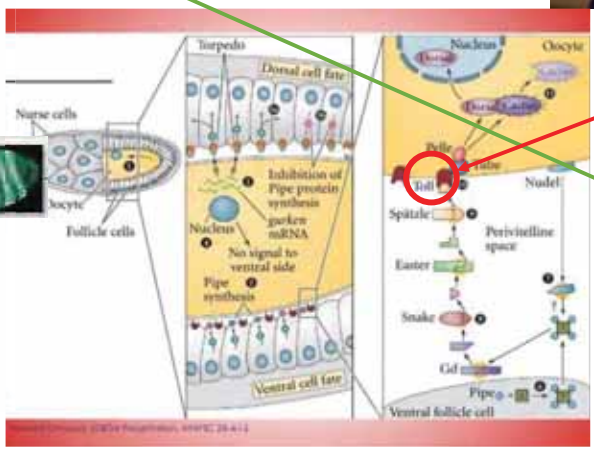
Christiane *Nüsslein-Volhard*

"I immediately loved working with flies. They fascinated me, and followed me around in my dreams."





Christiane *Nüsslein-Volhard*



Toll

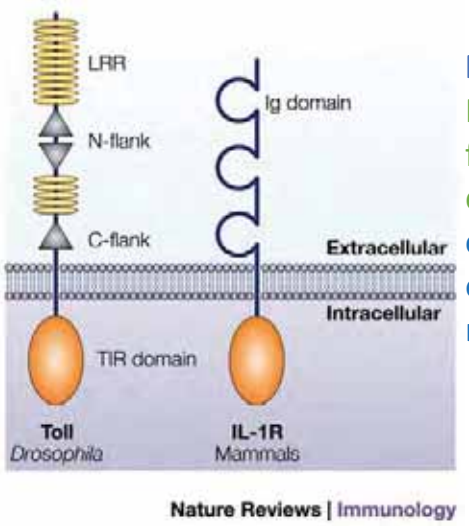
12 Maternal Effect Genes

Christiane *Nüsslein-Volhard*

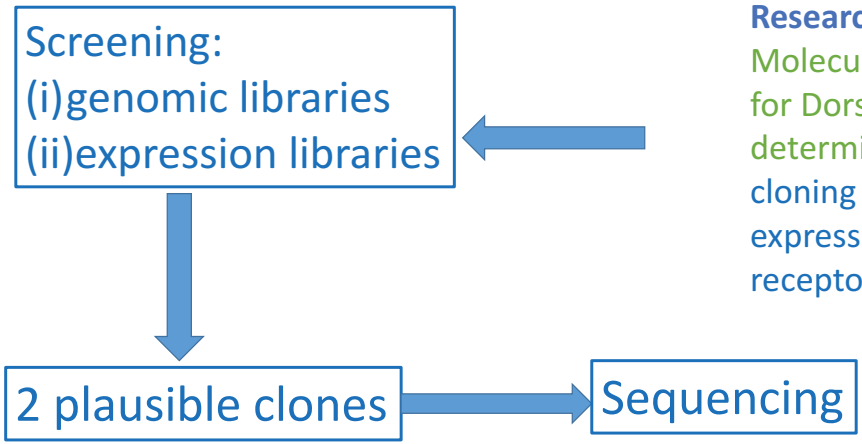
"The genetic ordering of the Toll pathway is probably the thing that has had the biggest impact because of the importance of Toll-like receptors in mammalian immunity," says Anderson.

Kathryn Anderson

Toll encodes a transmembrane protein and, together with its ligand, Spätzle, controls the entire dorsal-ventral pathway in *Drosophila* development. MAPS to position 97D on the left of chromosome 3.



Research project:
Molecular mechanisms for Dorso-ventral axis determination – The cloning and heterologous expression of the Toll receptor



Research project:
Molecular mechanisms for Dorso-ventral axis determination – The cloning and heterologous expression of the Toll receptor

LRR47

Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression

Recommended articles

Citing articles

Sequence and expression of LRR47, a novel embryonic leucine rich repeat protein of *Drosophila*

Abstract

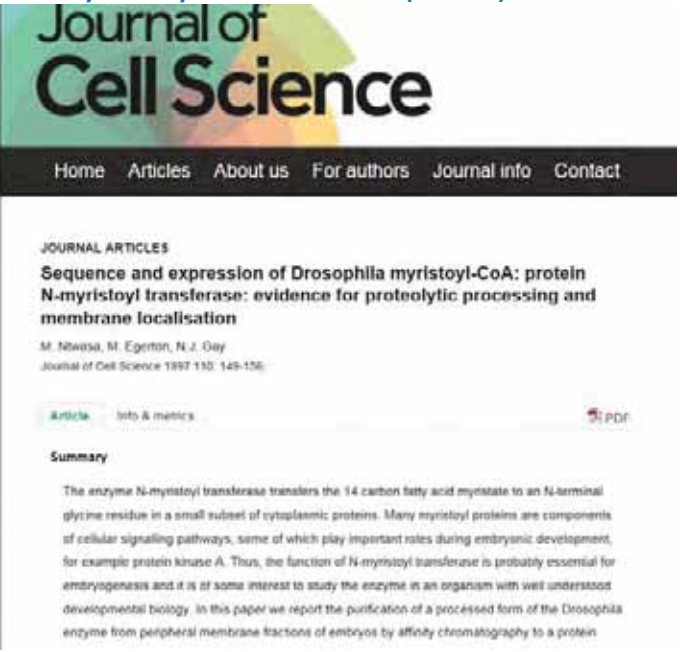
Leucine-rich repeats (LRRs) are 22–28 amino acid long sequence motifs found in a variety of extracellular, membrane and cytoplasmic proteins. They are believed to mediate specific protein-protein interactions and to function in cellular adhesion. In *Drosophila*, four LRR proteins are known and each plays an important role in embryogenesis. In this paper we report the cloning of a cDNA that encodes a fifth *Drosophila* embryonic LRR protein, LRR47. The sequence includes a hydrophobic N-terminal which may constitute an ER signal sequence, eight LRR copies and a unique C-terminal. The transcript of the LRR47 gene is detected in adult females and in early embryogenesis. It is not found in adult males and is only present at low levels in embryos after 6 h of development. In Western blot experiments, a protein of approx. 47 kDa, which is expressed in a similar developmental profile and purifies in peripheral membrane protein extracts, is detected by an antibody specific for LRR47. The LRR47 gene maps to position 32A on the left arm of chromosome 2, an interval in which three genes with semi-lethal maternal effects (*dal*, *hup* and *wdl*) are located.

Cloning of the Toll gene by screening gene libraries and isolated 2 clones

LRR47

- 47kDa protein with leucine-rich repeats
- Present in adult females
- Also during embryogenesis
- MAPS to position 32A on the left of chromosome 2

N-myristoyltransferase (NMT)



Journal of Cell Science

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JOURNAL ARTICLES

Sequence and expression of Drosophila myristoyl-CoA: protein N-myristoyl transferase: evidence for proteolytic processing and membrane localisation

M. Niwasa, M. Egerton, N.J. Gay

Journal of Cell Science 1997 110: 149-156

Article Info & metrics PDF

Summary

The enzyme N-myristoyl transferase transfers the 14 carbon fatty acid myristate to an N-terminal glycine residue in a small subset of cytoplasmic proteins. Many myristoyl proteins are components of cellular signalling pathways, some of which play important roles during embryonic development, for example protein kinase A. Thus, the function of N-myristoyl transferase is probably essential for embryogenesis and it is of some interest to study the enzyme in an organism with well understood developmental biology. In this paper we report the purification of a processed form of the Drosophila enzyme from peripheral membrane fractions of embryos by affinity chromatography to a protein

N-Myristoyltranferase (NMT)

- Essential for embryogenesis
- Catalyses lipid modification of proteins

Cape Town – Welcome Trust Fellowship

Cape Town – Welcome Trust Fellowship

Jasper Rees

Cape Town

The New Gene

- Identified an interesting gene in Chinese hamster ovary (CHO) cells using promoter trap mutagenesis to screen for genes involved in programmed cell death (apoptosis).

Cape Town – Welcome Trust Fellowship

Jasper Rees

Sonti Aapies

Cape Town – Welcome Trust Fellowship

Departure from Cape Town

Sonti Aapies

Johannesburg

Johannesburg

(i) NMT work continues

Sonti Aapies

Arshad Mather

Characterization of the new gene

N-myristoyltransferase

Experimental Cell Research
Volume 262, Issue 2, 10 January 2001, Pages 134-144

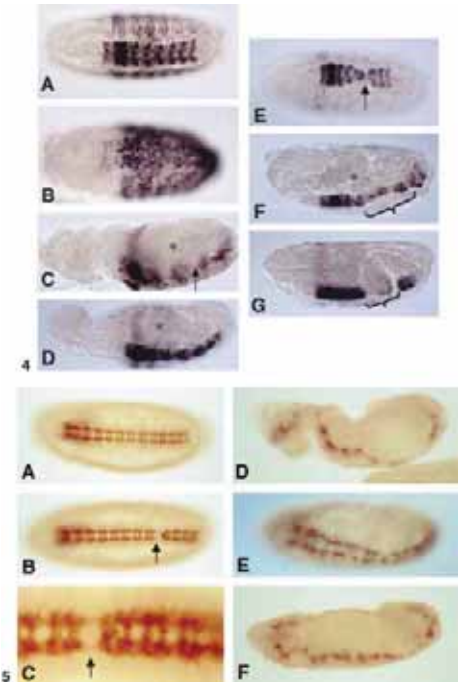
Drosophila Embryos Lacking *N*-Myristoyltransferase Have Multiple Developmental Defects

Mondo Nirasawa, Scott Aspers, David A. Schaffmann, Nicholas J. Gay

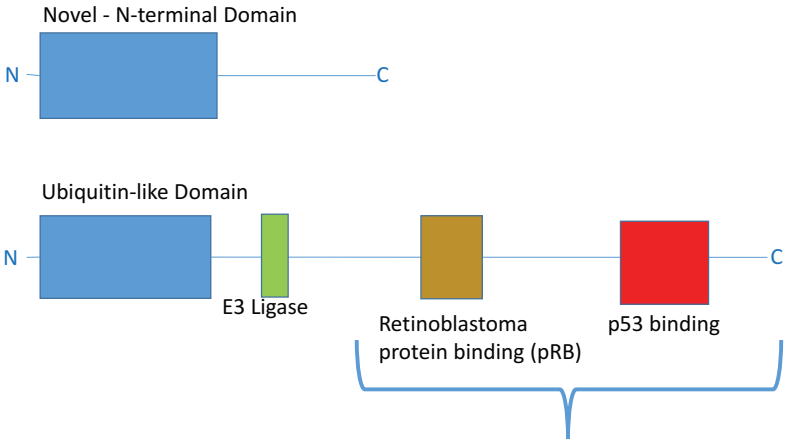
https://doi.org/10.1006/excr.2000.5096

Abstract

Lipid modification of proteins by the addition of myristic acid to the N-terminal is important in a number of critical cellular processes, for example, signal transduction and the modulation of membrane association by myristoyl switches. Myristic acid is added to proteins by the enzyme *N*-myristoyltransferase (NMT) and in this paper we detail the effects on embryonic development of a null mutation in the *Drosophila NMT* gene. Mutant embryos display a range of phenotypes, including failures of head involution, dorsal closure, and germ-band retraction, morphogenetic processes that require cellular movements. Embryos with milder phenotypes have more specific defects in the central nervous system, including thinning of the ventral nerve chord and, in some embryos, specific scission at parasegment 10. Staining of mutant embryos with phalloidin shows that the mutant embryos have a disrupted actin cytoskeleton and abnormal cell morphology. These phenotypes are strikingly similar to those caused by genes involved in dynamic rearrangement of the actin cytoskeleton. For example the myristoylated nonreceptor tyrosine kinases Dsrc42A and Dsrc64B were shown recently to be key regulators of dorsal closure. In addition, analysis of cell death reveals widespread ectopic apoptosis. Our findings are consistent with the hypothesis that the myristoyl switches



The New Gene



- Tumour suppressors
- Play critical roles in the cell cycle

SNAMA probably plays an anti-apoptotic function

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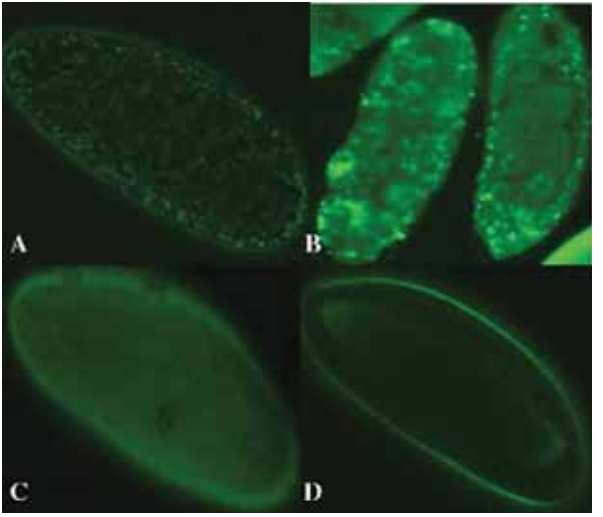
SNAMA, a novel protein with a DWNN domain and a RING finger-like motif: A possible role in apoptosis

Arshad Mather, Mpho Rakgotho, Monde Ntwasa*

We have characterized SNAMA a hitherto uncharacterized *Drosophila* protein that appears to play a role in apoptosis. SNAMA (something that sticks like glue) is a 1231 amino acid protein with a conserved 76 residue N-terminal domain called Domain With No Name (DWNN). The DWNN domain was first identified in cytotoxic T Cell-resistant CHO cells using promoter trap mutagenesis to screen for genes involved in apoptosis. Subsequently, this domain was identified in other eukaryotic organisms including animals and plants. The SNAMA transcript is abundant early in embryogenesis but reduced in older embryos and in adult males and females. Human and mouse homologues of SNAMA are known to bind to p53 and to the retinoblastoma protein (Rb) suggesting a role in transcriptional regulation and cell cycle control. We took advantage of a *P*-element insertion line in which the *P*-element is inserted in the first intron, to investigate the biological function of the gene. These mutants are lethal when homozygous. Apoptosis appears early during embryogenesis and is observed virtually throughout the gastrula. The DWNN domain has a ubiquitin-like fold and may interact with a subset of cellular proteins. There is also a conserved RING finger-like motif along the sequence of SNAMA following a C2HC zinc finger.



Cells lacking SNAMA commit suicide

SNAMA

International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

The *Drosophila* Retinoblastoma Binding Protein 6 Family Member Has Two Isoforms and Is Potentially Involved in Embryonic Patterning

Rodney Hull [1], Brent Oosthuysen [1], Umar Farug Cajee [1], Lehlogonolo Mokgolkloa [1], Ekene Nweke [1], Ricardo Jorge Antunes [1], Theresa H. T. Coetzer [2] and Monde Ntwasa [1,*]

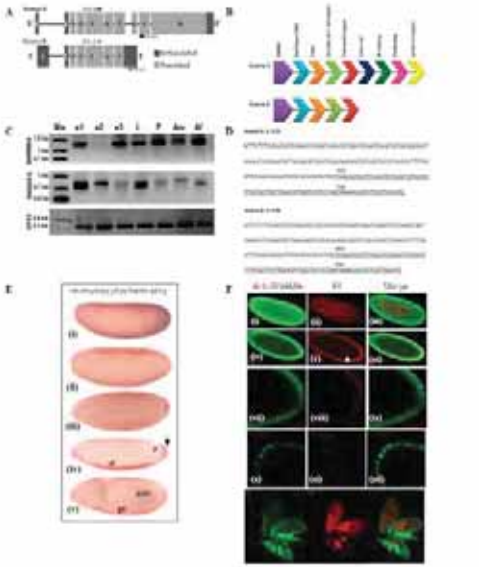
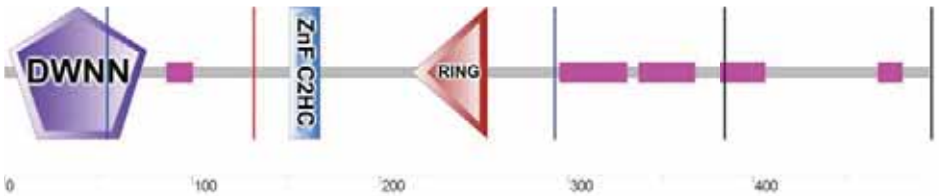


Figure 2. Differential expression of SNAMA during oogenesis and embryogenesis. (A) Schematic representation of the human genomic organization showing the exon structure of Isoform A and B transcripts; (B) domain structure of SNAMA A and B

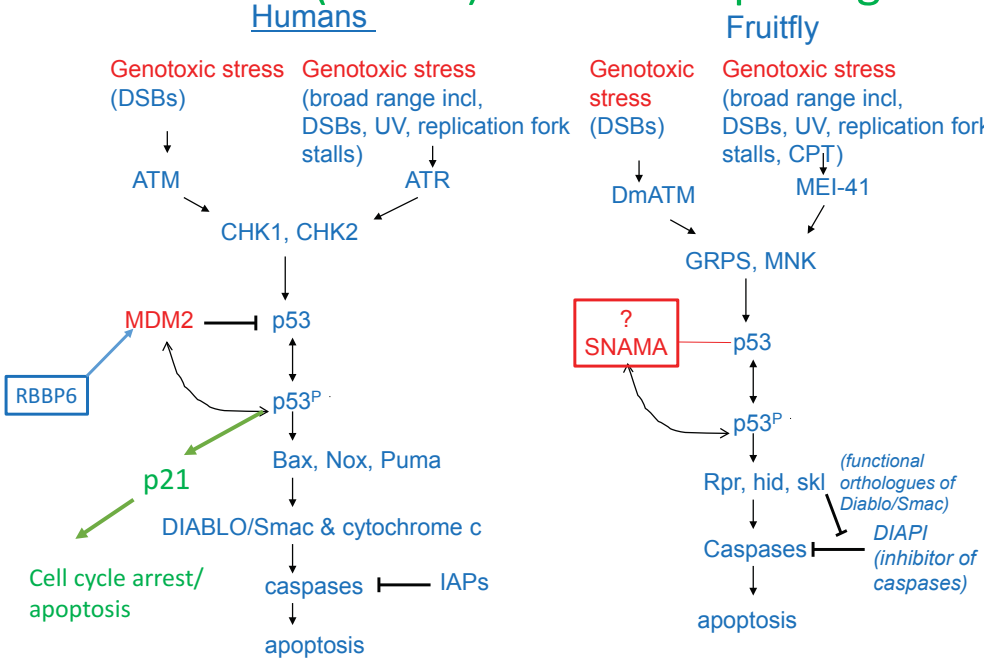
- Two isoforms – SNAMA A & B
- Differential expression
- Spatial
- Temporal
- Expressed in pole cells

Human RBBP6 – and *Drosophila* SNAMA



- Ubiquitin-like domain called DWNN – Domain With No Name
- Zinc finger – CCHC
- RING finger like motif – probably with E3 ligase activity
- *Proline-rich region*
- Lysine-rich region
- RS-region
- coiled-coil
- p53 – binding domain (downstream the RING finger)
- pRB – binding domain (also downstream the RING finger)

Is SNAMA (RBBP6) involved in p53 signaling?



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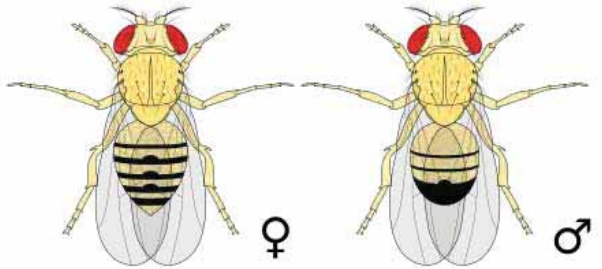

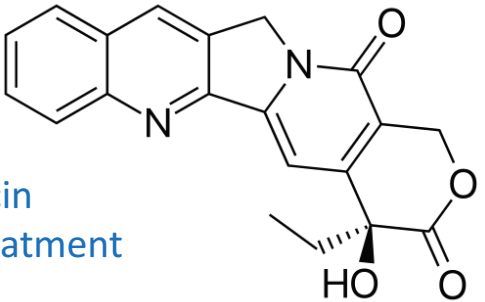

- Flies were challenged with camptothecin
- Proteomic analysis during and after treatment

Is SNAMA (RBBP6) involved in p53 signaling?

ANTI-CANCER DRUGS

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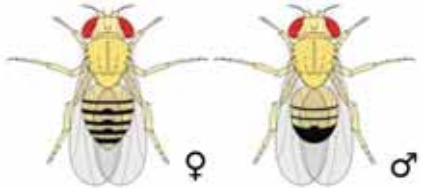
Glycolytic flux occurs in Drosophila melanogaster recovering from camptothecin treatment

Hull, Rodney; Nkwasa, Monde McMillan

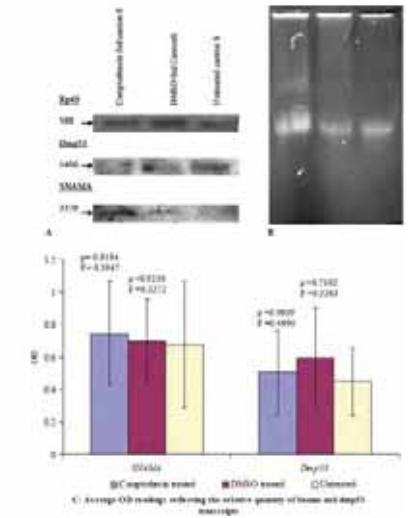
Anti-Cancer Drugs. November 2010 - Volume 21 - Issue 10 - pp 943-957
doi: 10.1097/CAD.0b013e32833e2f60
Preclinical Reports

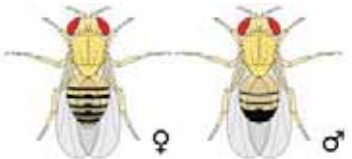
Abstract | Author Information

Camptothecin (CPT) and CPT-derived drugs are widely used against gynaecological and colorectal cancers. On account of their mechanism of action these drugs target rapidly dividing cells and may have an adverse effect on normal tissues. We sought to investigate their impact on normal cells by using *Drosophila* as a model. We investigated the possible involvement of *Drosophila* homologue of p53 (Dmp53) and a member of the retinoblastoma binding protein II family, known as Snama. On account of its molecular features and experimental evidence gleaned from mammalian studies we propose Snama as a candidate in Dmp53 regulation. We have



Is SNAMA (RBBP6) involved in p53 signaling?





DMSO
Untreated
Camptothecin

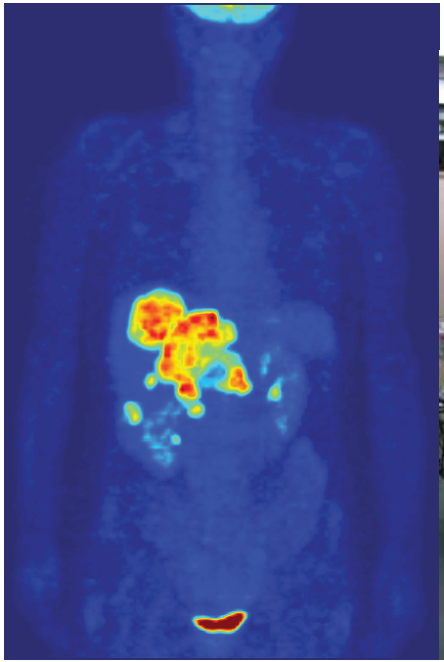
- *Dmp53* increases following camptothecin treatment
- *SNAMA* decreases following camptothecin treatment

Otto Warburg

In the 1920s Otto Warburg found that, under aerobic conditions, tumour tissues metabolize approximately tenfold more glucose to lactate in a given time than normal tissues, a phenomenon known as the Warburg effect.

Sidney Weinhouse (1976). “I feel that as our perspectives have broadened over the years, the burning issues of glycolysis and respiration in cancer now flicker only dimly; they have receded in importance, and are no longer in the mainstream of cancer research”.

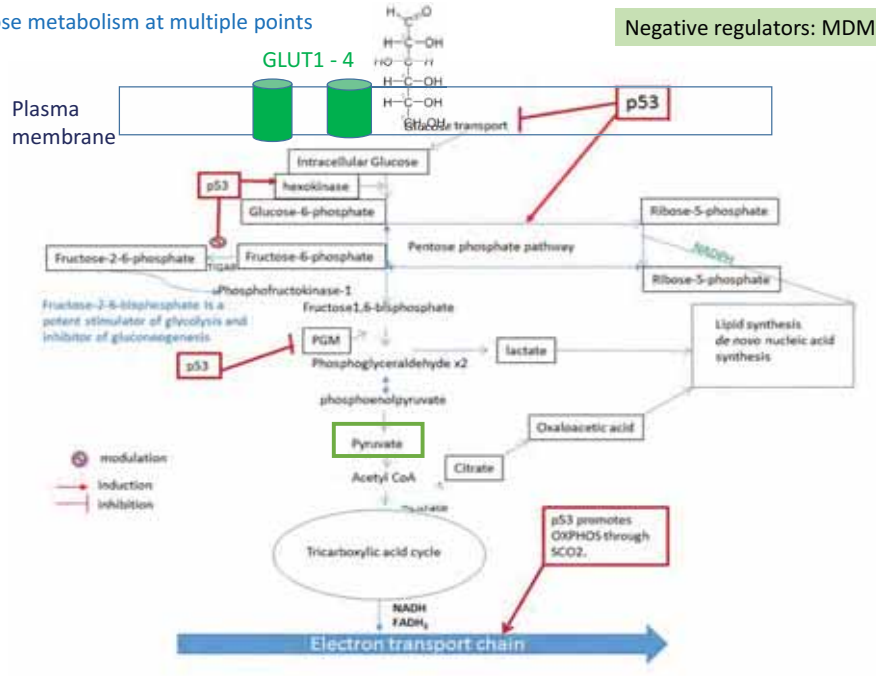
The Warburg Effect is a widespread cancer-associated trait. It is exploited as a diagnostic e.g. positron emission tomography (PET) whereby 18-fluorodeoxyglucose (FDG), preferentially accumulates in tumour cells as a result of their rapid uptake of glucose. Because of the prevalence of this phenotype, PET is an effective clinical imaging technique to detect most cancers and monitor therapeutic responses.



Positron Emission Tomography (PET)

p53 controls glucose metabolism at multiple points

Negative regulators: MDM2 and RBBP6



p53 controls glucose metabolism at multiple points

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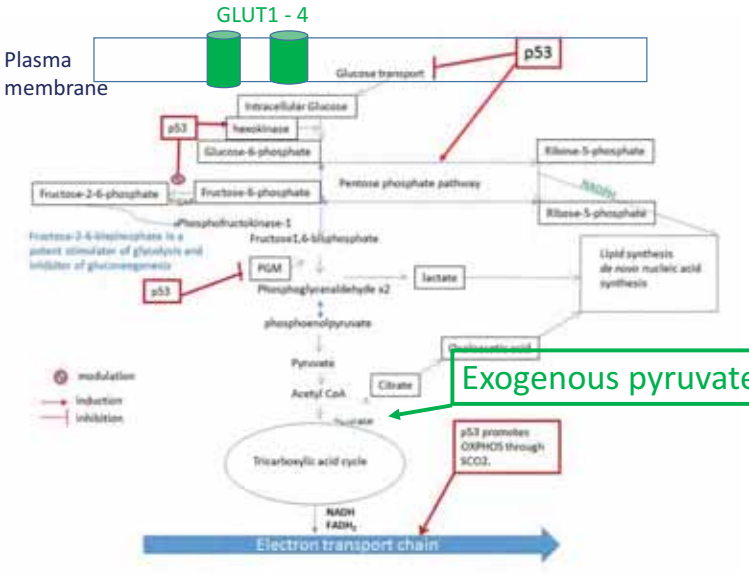
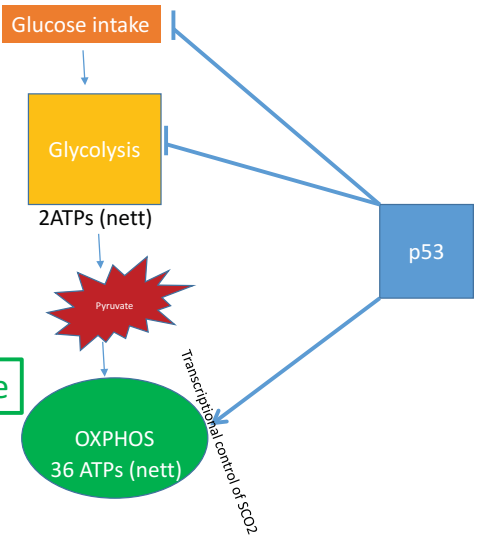
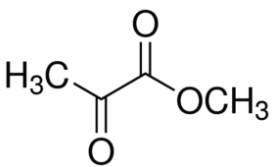


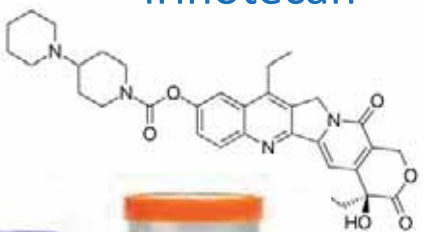


Would by-pass of the glycolytic pathway selectively protect normal cells during chemotherapy?

Methyl pyruvate



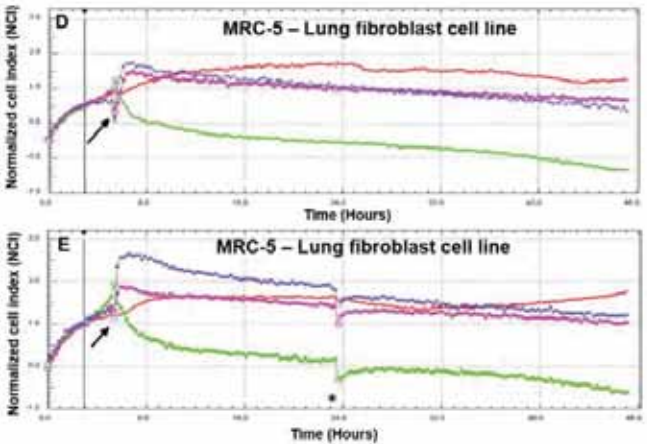
Irinotecan

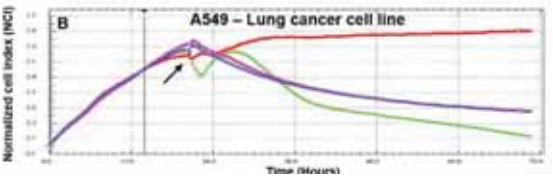


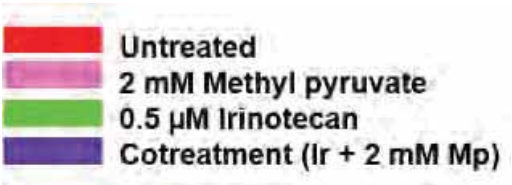
xCELLigence RTCA (real-time cell analysis) C

Normal Lung fibroblast cell line

Lung cancer cell line







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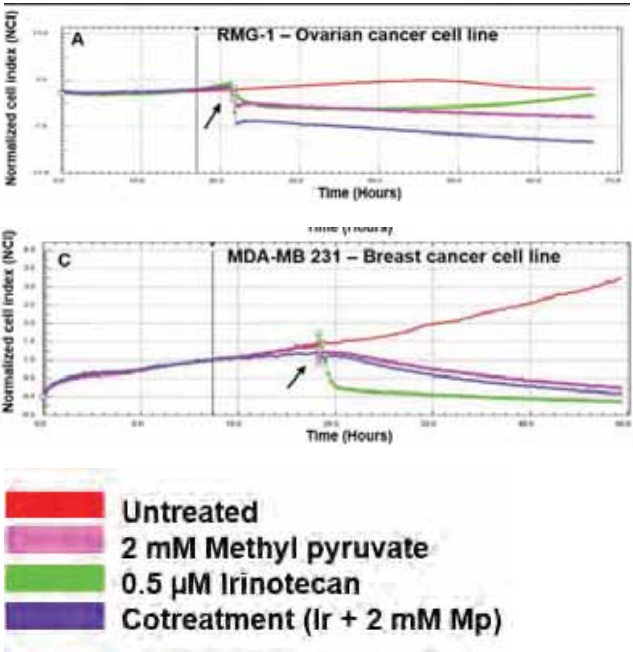
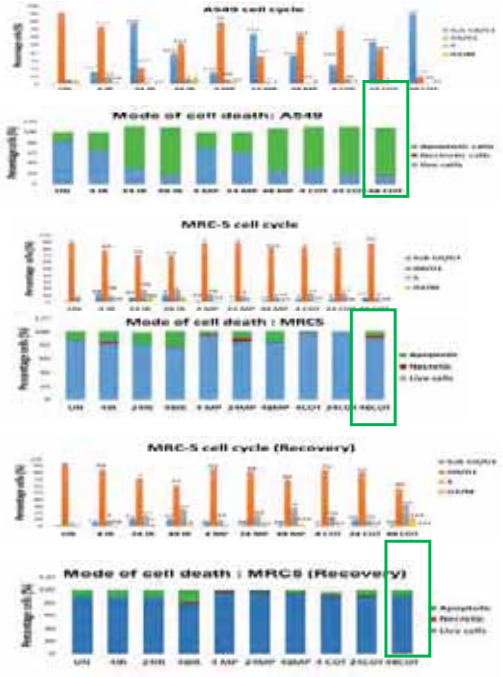
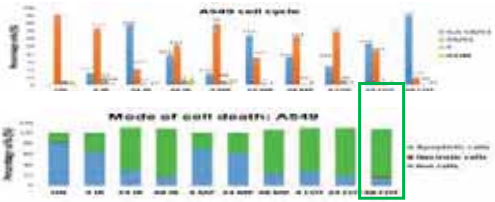
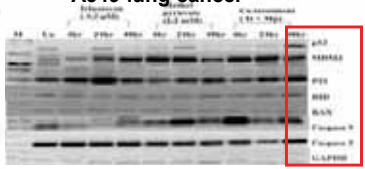


- Methyl pyruvate protects a MRC-5 lung fibroblasts from irinotecan-induced cell death
- Methyl pyruvate accelerates the death of cancer lung fibroblasts


A549 cell cycle
Mode of cell death: A549
MRC-5 cell cycle
Mode of cell death : MRC5
MRC-5 cell cycle (Recovery)
Mode of cell death : MRC5 (Recovery)

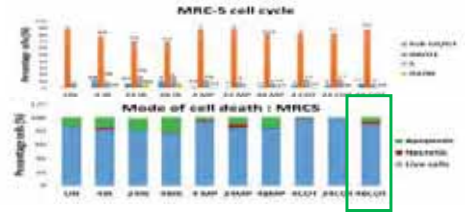


A549 lung cancer

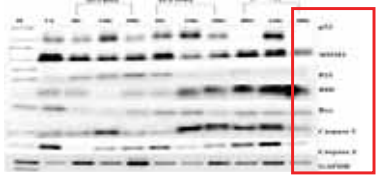


A549 lung cancer

Cytochrome c

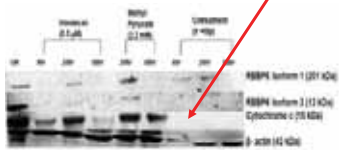


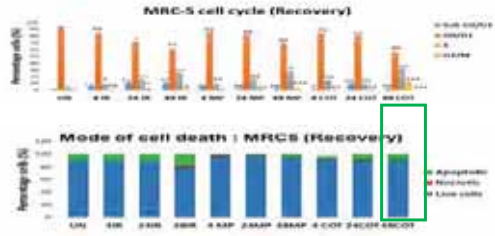
MRC-5 lung fibroblast



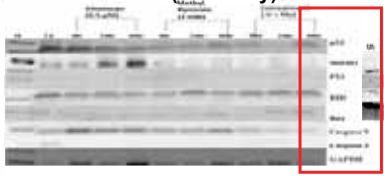
MRC-5 lung fibroblast

Cytochrome c





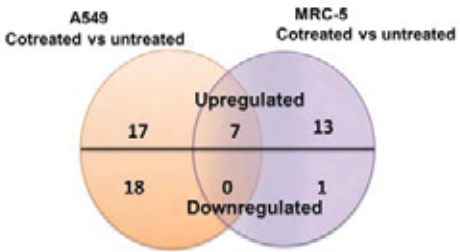
MRC-5 (Recovery)



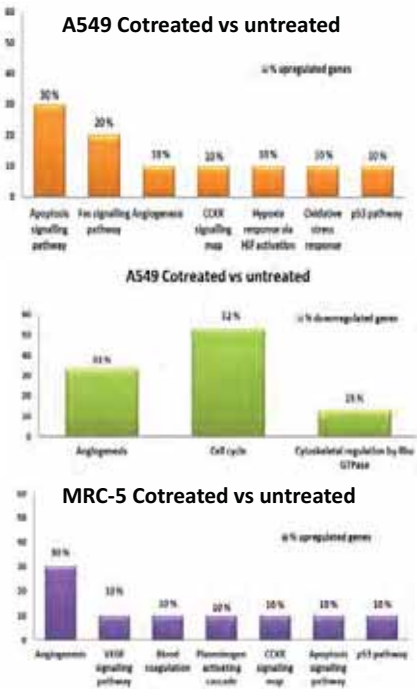
MRC-5 (Recovery)

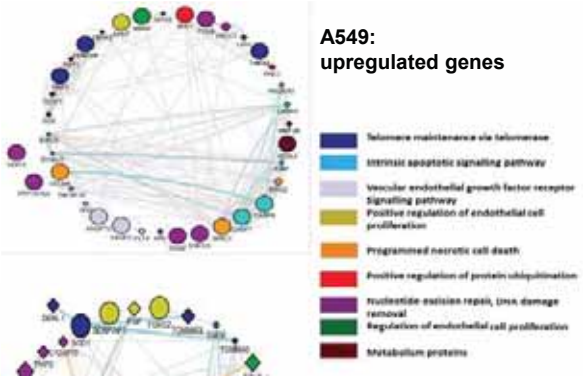
Cytochrome c

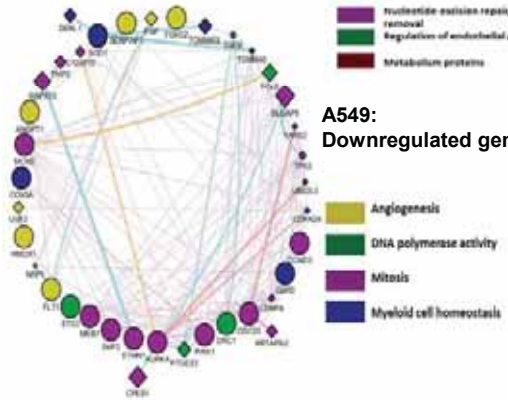
Affected Biological processes - GENMANIA

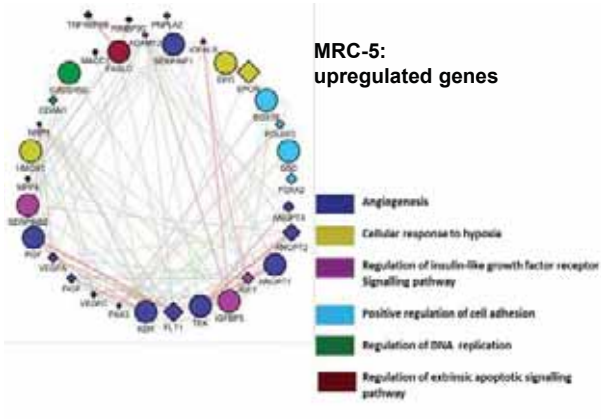


- upregulated in the MRC-5 lung fibroblasts promote angiogenesis, cell cycle regulation, cell survival and control glucose metabolism.
- upregulated genes in A549 fibroblasts mediate apoptosis









- methyl pyruvate directly triggers opposite outcomes in these cells: pro-angiogenic and pro-survival factors in the MRC-5 fibroblasts and anti-angiogenic and apoptotic factors in fibroblasts.

Key observations

- **methyl pyruvate protects irinotecan-treated normal lung fibroblast cell line (MRC-5) probably by turning off the p53/p21 axis of the apoptotic pathways.**
- **When the MRC-5 fibroblasts recover in drug-free medium, the intrinsic apoptotic pathway is also turned off**
- **In contrast, the mere introduction of exogenous pyruvate kills the cancer lung fibroblasts (A549).**
- **When combined with irinotecan cell death occurs in cancer cells but not in the normal cell line.**

Methyl pyruvate protects a normal lung fibroblast cell line from irinotecan-induced cell death: Potential use as adjunctive to chemotherapy

Bernice Monchusi[1] and Monde Ntwasa[2*]

Food for thought

- **Sugar feeds cancer**
- **Carbohydrate diet may no longer be overlooked during the management of cancer**
- **Problem – carbohydrates are required by all cells**
- **Bypassing glycolysis and boosting respiration may be a good strategy to protect normal cells during chemotherapy and in the management of cancer in general.**

"Poor carbohydrate diet supports therapy with amatin."

Cell
Science & Therapy

Review Article Open Access

Tumor Therapy with *Amanita phalloides*: Remission of a Tumor Disease and Dietary Effect of Sugar

Isolde Riede*

Independent Cancer Research, Im Anlass 7, D-88662 Überlingen, Germany

Abstract

Molecular events that cause tumor formation upregulate a number of HOX genes, called switch genes, encoding for RNApolymeraseII transcription factors. Thus, RNApolymeraseII is used to full extent in tumor cells but not in somatic cells. *Amanita phalloides* contains amanitin, inhibiting RNApolymeraseII. Application of *Amanita phalloides* influences tumor cell (but not normal cell) activity. Dilutions of *Amanita phalloides* are applied to a patient with both, colon carcinoma and thyroid carcinoma. Monitoring tumormarkers, different doses of Amanita are applied. After two years of stabilization, somatic investigations and imaging methods reveal complete remission. Change of dietary practice with the addition of daily 70 gram sugar lead to increase of tumormarker values. Following dietary without sugar and reduced carbohydrates decrease tumormarker values. With sugar, tumor activity increase despite Amanita tumor therapy, therefore, poor carbohydrate diet supports the therapy.

PLOS | ONE

RESEARCH ARTICLE

Ethyl Pyruvate Combats Human Leukemia Cells but Spares Normal Blood Cells

Gerd Birkenmeier[1]*, Nasr Y. A. Hemdan[1], Susanne Kurz[1], Marina Bigl[1], Philipp Pieroh[2,3], Tewodros Debebe[3,4], Martin Buchold[1], Rene Thieme[5], Gunnar Wichmann[6], Faramarz Dehghani[7]

2016

RADIATION RESEARCH 168, 552–559 (2007)
0033-7587/07 $15.00

Ethyl Pyruvate, a Potentially Effective Mitigator of Damage after Total-Body Irradiation

Michael Epperly,[a] ShunQian Jin,[a] Suhua Nie,[a] Shaonan Cao,[a] Xichen Zhang,[a] Darcy Franicola,[a] Hong Wang,[a] Mitchell P. Fink[b,c,d] and Joel S. Greenberger[a,1]

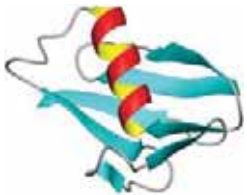
Departments of [a] Radiation Oncology, [b] Critical Care Medicine, [c] Surgery and [d] Pharmacology, University of Pittsburgh Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213

The Future

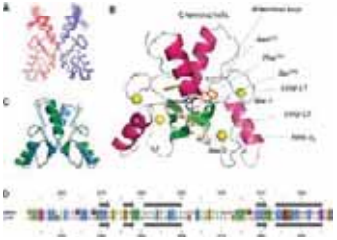
Eureka

The Future

Is RBBP6 drugable?



Pugh et.al; BMC: 2006



Kappo et,al: JBC VOL. 287, NO. 10, pp. 7146–7158, March 2, 2012

The Future

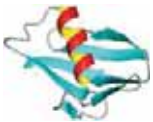
Is RBBP6 drugable?

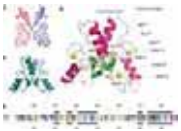
DWNN

RING domain

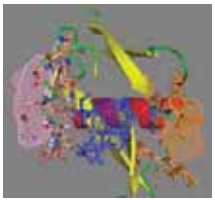
p53BD

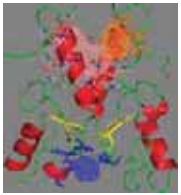
3-D structure

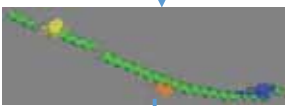




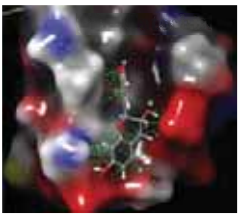
Putative
Binding sites

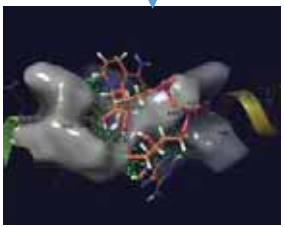






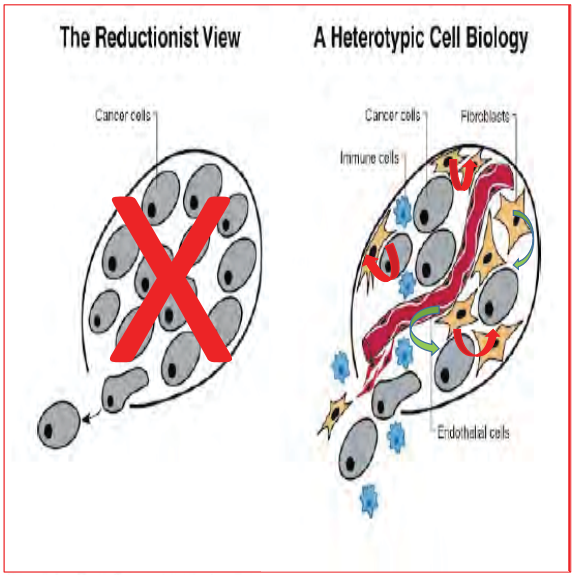
Molecular
docking & drug
discovery





The Future

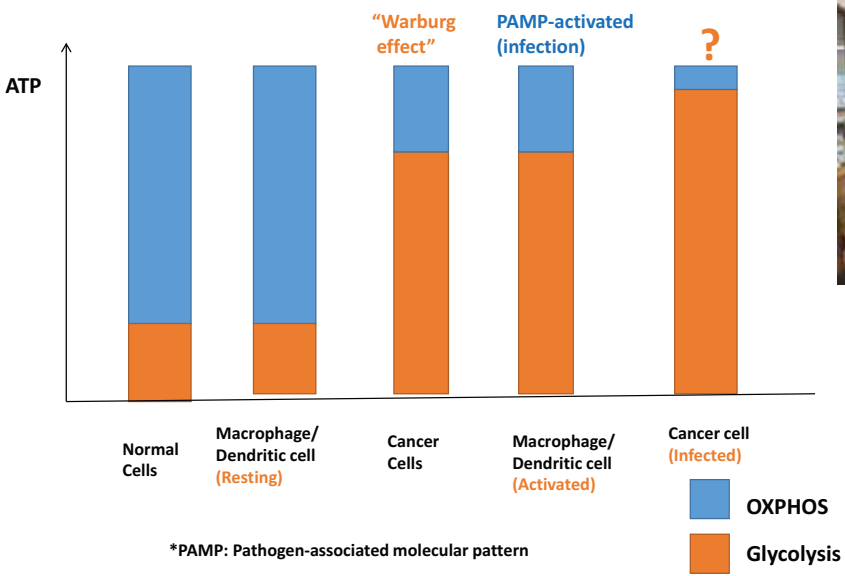
The field of cancer has largely been guided by a reductionist focus on cancer cells



Design, construction and testing of a micro-system to simulate the tumor microenvironment.

The Future

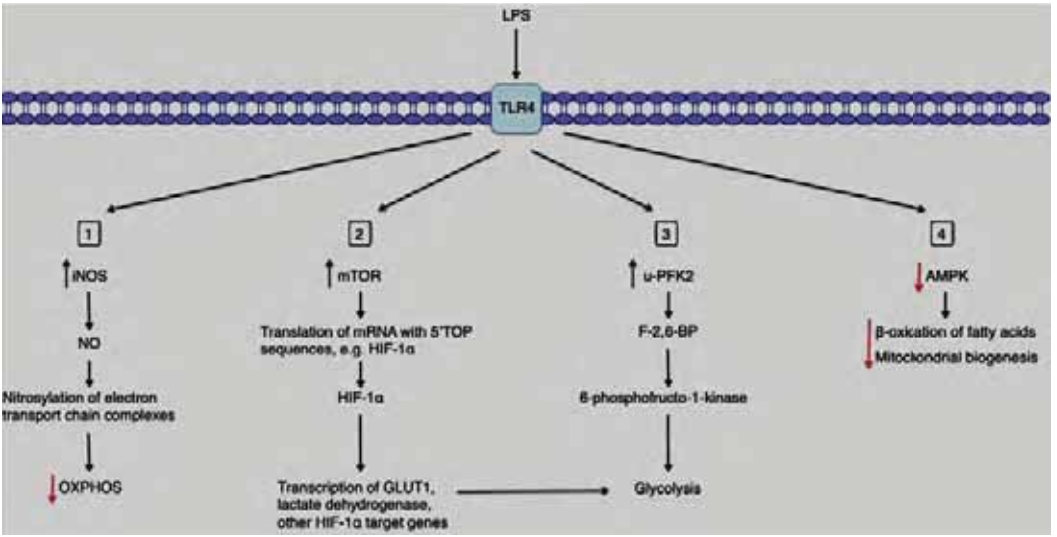
Metabolic reprogramming and the immune system



*PAMP: Pathogen-associated molecular pattern

Ntombi and Phil

How LPS promotes Warburg metabolism in macrophages and Dendritic cells

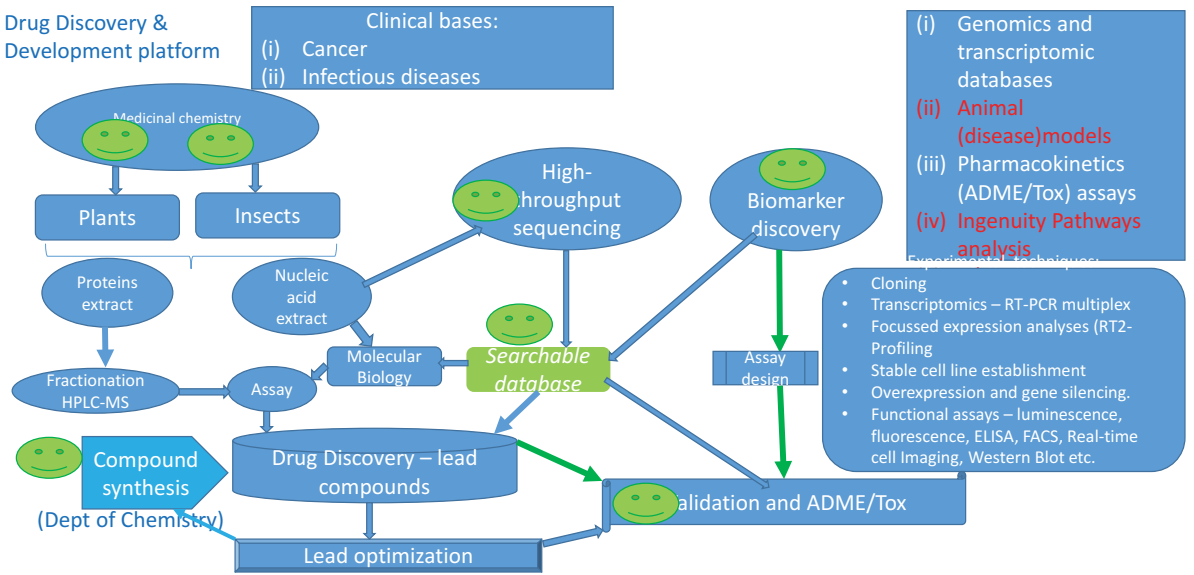


(Kelly et al. 2005).

Future

Biomarkers for early Diagnosis of Pancreatic cancer.

- Validation of pyruvate esters as adjunctive to chemotherapy
- Development of drug leads


Drug Discovery & Development platform
Clinical bases:
(i) Cancer
(ii) Infectious diseases
(i) Genomics and transcriptomic databases
(ii) Animal (disease)models
(iii) Pharmacokinetics (ADME/Tox) assays
(iv) Ingenuity Pathways analysis
Medicinal chemistry
Plants
Insects
High-throughput sequencing
Biomarker discovery
Proteins extract
Nucleic acid extract
Molecular Biology
Searchable database
Assay design
Fractionation HPLC-MS
Assay
Compound synthesis
(Dept of Chemistry)
Drug Discovery – lead compounds
Lead optimization
alidation and ADME/Tox
Cloning
Transcriptomics – RT-PCR multiplex
Focussed expression analyses (RT2-Profiling
Stable cell line establishment
Overexpression and gene silencing.
Functional assays – luminescence, fluorescence, ELISA, FACS, Real-time cell Imaging, Western Blot etc.

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(Cape Town)


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Jean-Marc
(Strasbourg, France)


Shoichiro
(Tohoku, Sendai)


Hazel (MIT, USA)

Professor
Emeritus
Barry
Fabian


Jean Marc
and Friends

Best Friends

Flylab people

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