Prelecture to the 3rd LOEWE-Minisymposium

‚Tumor, Inflammation and Metabolism‘

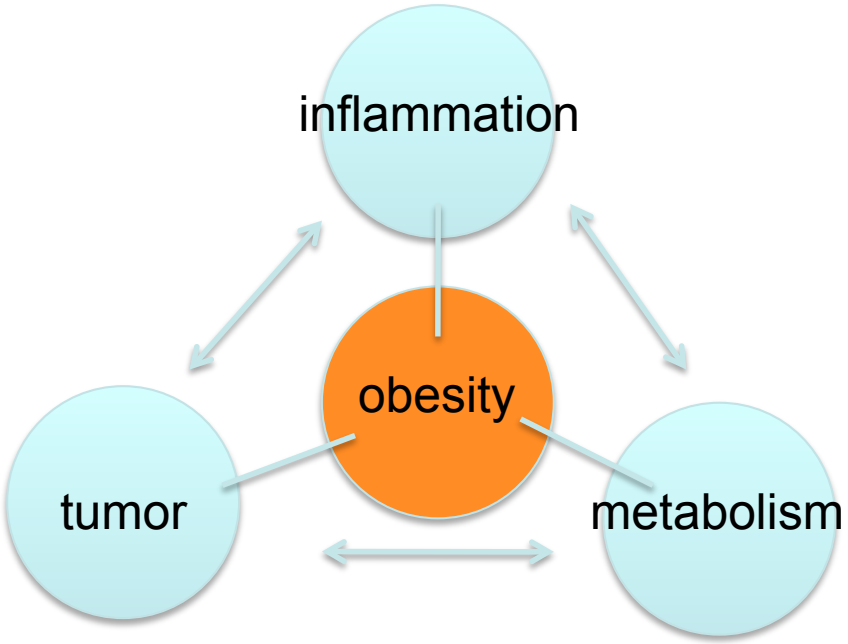
February 16, 2010

by Dr. Abdo Konur

TOPICS

- interdependence of tumor-inflammation-metabolic syndrome
- fundamentals of chronic (subclinical) inflammation
- impact of inflammation for tumor development
- inflammation and insulin resistance
- insulin resistance carcinogenesis axis
- treatment modalities insulin resistance/inflammation
- Good vs bad inflammation

Tumor, inflammation and metabolism



→ a subclincial inflammatory response in obesity

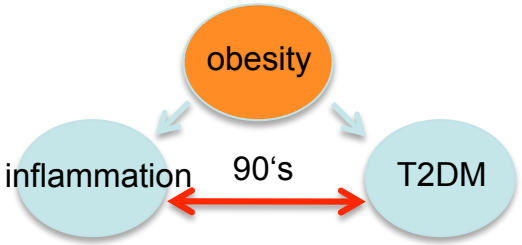
Historical perspectives
- linking inflammation to insulin resistance -

1857 W. Ebstein: treatment of diabetes patients with salicylates
→ decrease in blood glucose levels

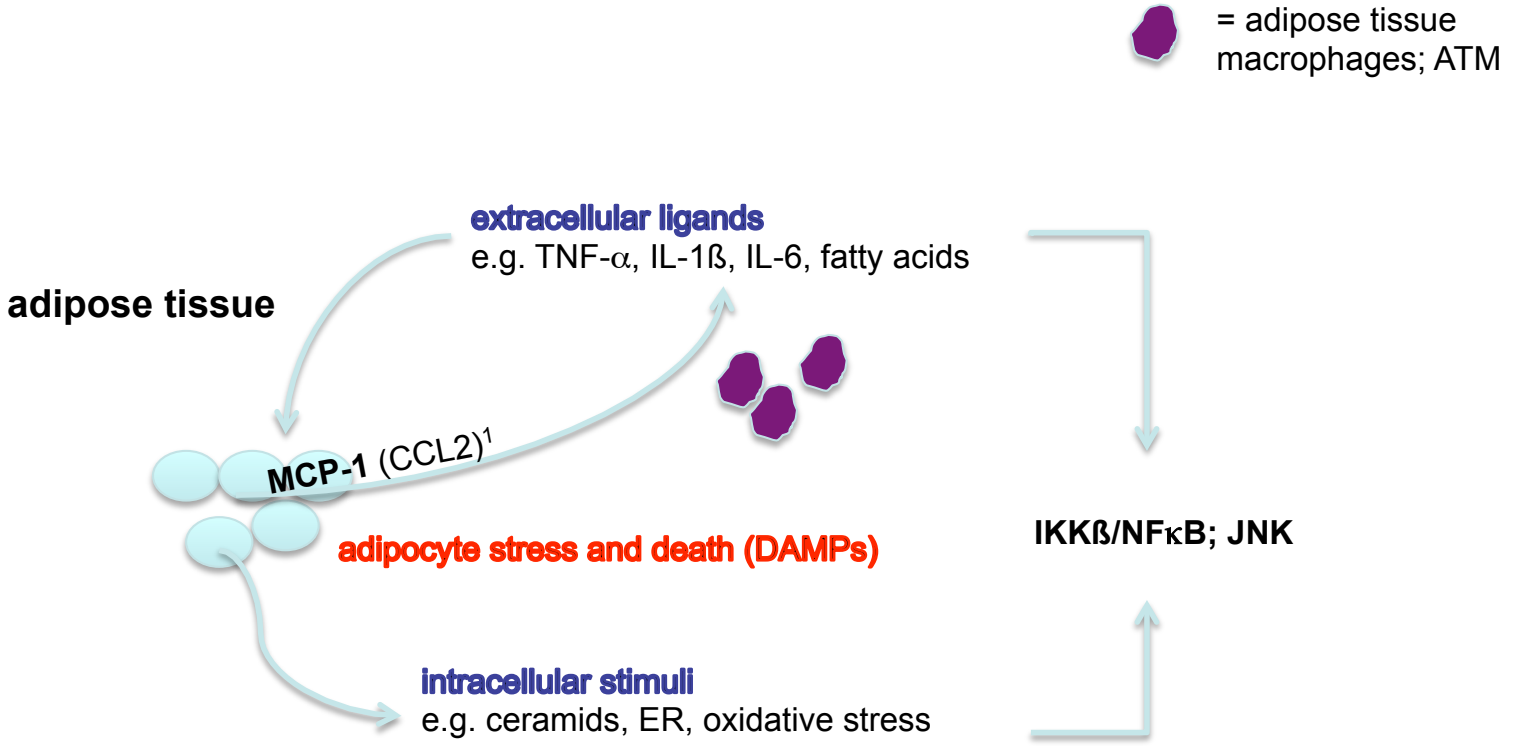
1902 Williamson: observed the salicylate-induced decrease in glucose production

1957: co-treatment of an diabetes patient with insulin injections and high-dose aspirin (to treat RA)
→ no longer requirement of daily insulin injections

in the 1950s: epidemiological evidence relating inflammation to T2DM and obesity, no link in terms of pathogenesis until the 1990s



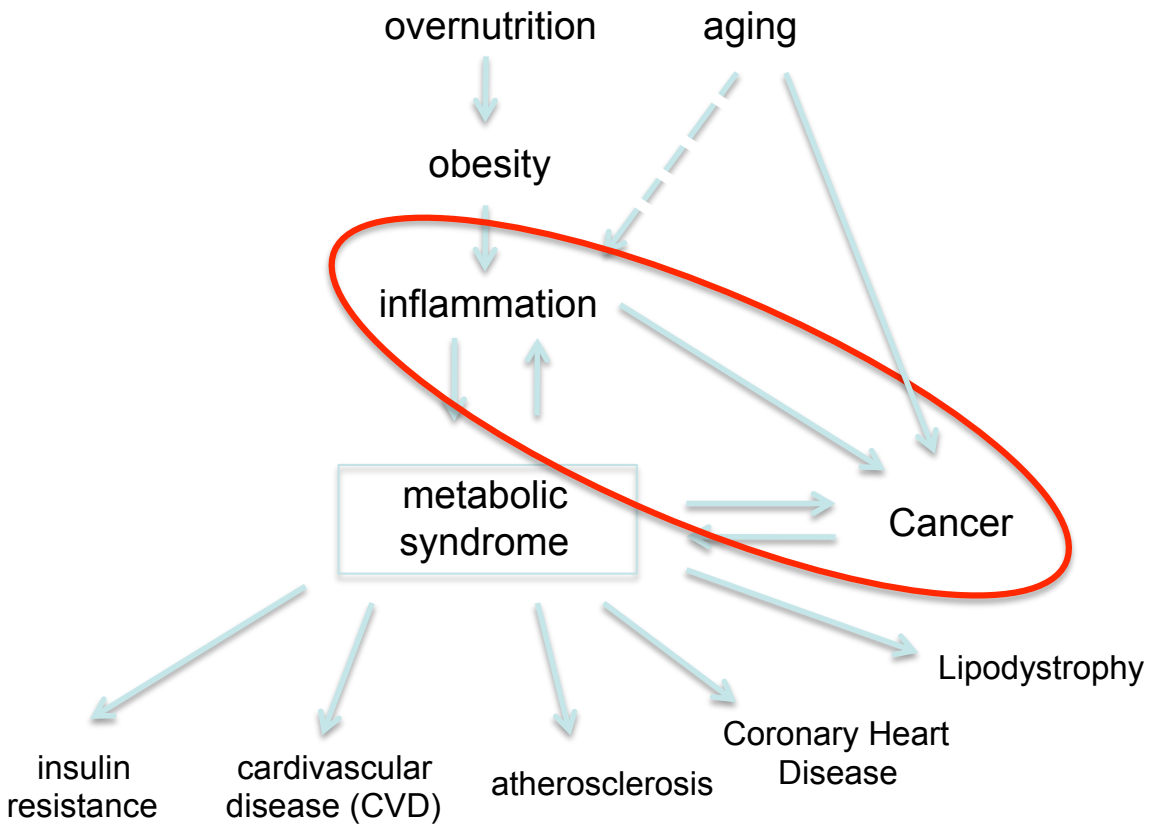
Adipose tissue as the starting site of inflammation



[1] Sartipy & Loskutoff, PNAS **100**:7265 (2003).

DAMP: Danger associated molecular patterns
JNK: c-Jun N-terminal kinase

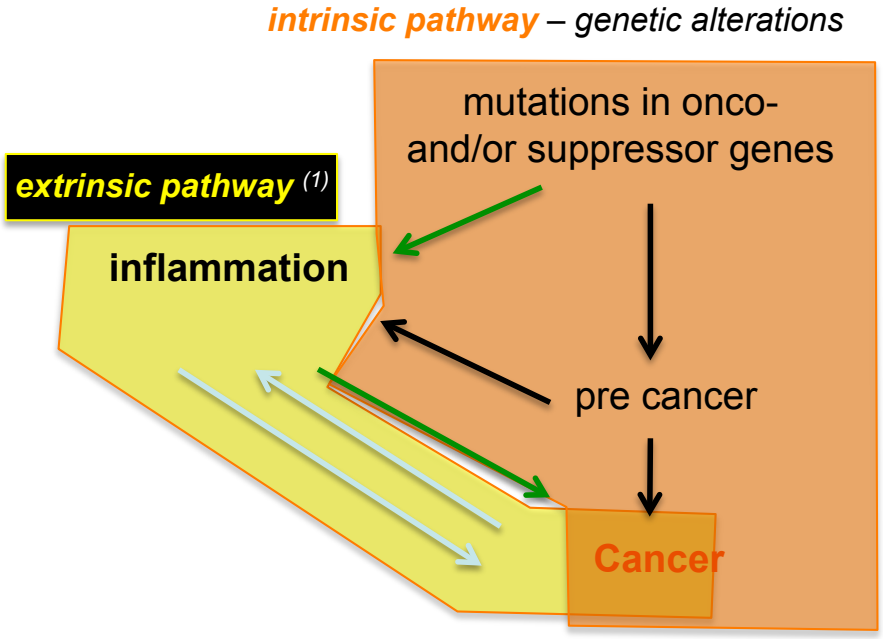
Tumor, inflammation and metabolism
- Inflammation and Cancer -



Inflammation associated cancers

Cancer entity	Details	Reference
colorectal Ca	correlation with higher levels of TNF-α and IL-6	Kim et al, Cancer Res; 68:323 (2008).
pancreatic Ca	TNF-α[1], IL1-α[2] (induction of metastasis by NFkB activation), inflammatory cytokines[3]	[1] Egberts et al, Cancer Res; 68:1443 (2009). [2] Melisi et al, Mol Cancer Res; 7:624 (2009). [3] Farrow et al, Ann Surg, 239:763 (2004).
(inflammatory) Breast Ca	approx 3% of all breast cancer; highly aggressive, association with high BMI[1,2]	[1] Levine & Veneroso, Semin Oncol; 35:11 (2008). [2] Chang et al, J Clin Oncol; 16:3731 (1998).
hepatocellular Ca (HCC)	90% of HCC cases have a natural history of unresolved inflammation[1]	[1]Elsharkawy & Mann, Hepatology; 46:590 (2007).

Intrinsic vs. extrinsic pathway



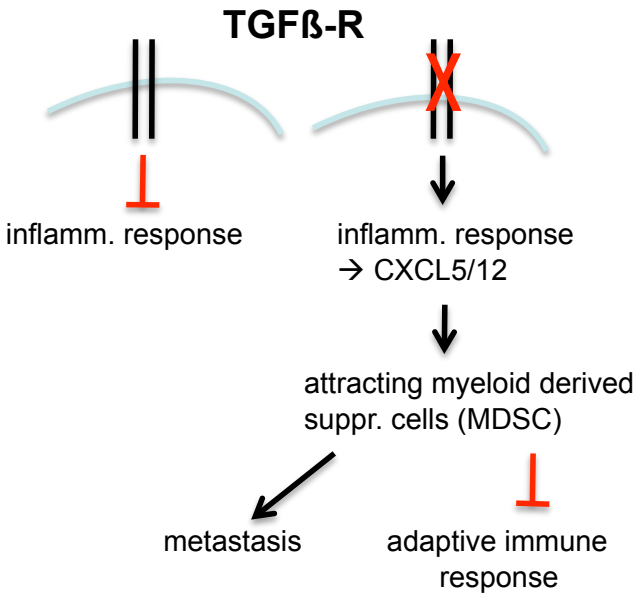
intrinsic pathway – genetic alterations:

- papillary thyroid Ca → activation of the protein tyrosine kinase RET by chromosome rearrangement
  → inflammatory transcriptional program

- *oncogenes*: Ras/Raf, RET, Myc
- *suppressor genes*: von Hippel-Lindau (VHL), TGFß, PTEN

→ **VHL** targets HIF1α for degradation; interaction of **HIF1α** with NFκB → production of inflammatory cytokines

→ **TGFß: a tumor suppressor protein**



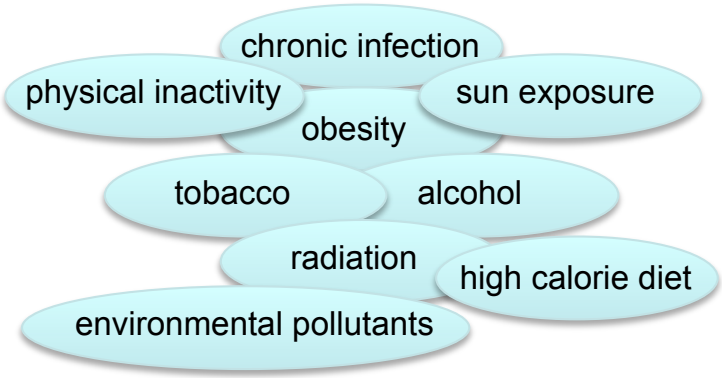
[1] Mantovani et al, Nat **454**:436 (2008).

RET: rearranged during transfection; a single pass transmembrane receptor, binds GDNF
PTEN: phosphatases and tensin homologue

Cancer risk factors
– linking cancer to inflammation -

→ approx. only 5–10% of all cancer cases can be attributed to genetic defects;
the remaining 90-95% have their roots in the environment and lifestyle[1,2];
approx. 20% of all malignancies are initiated or exacerbated by inflammation[3]

Cancer risk factors
- environmental and lifestyle -



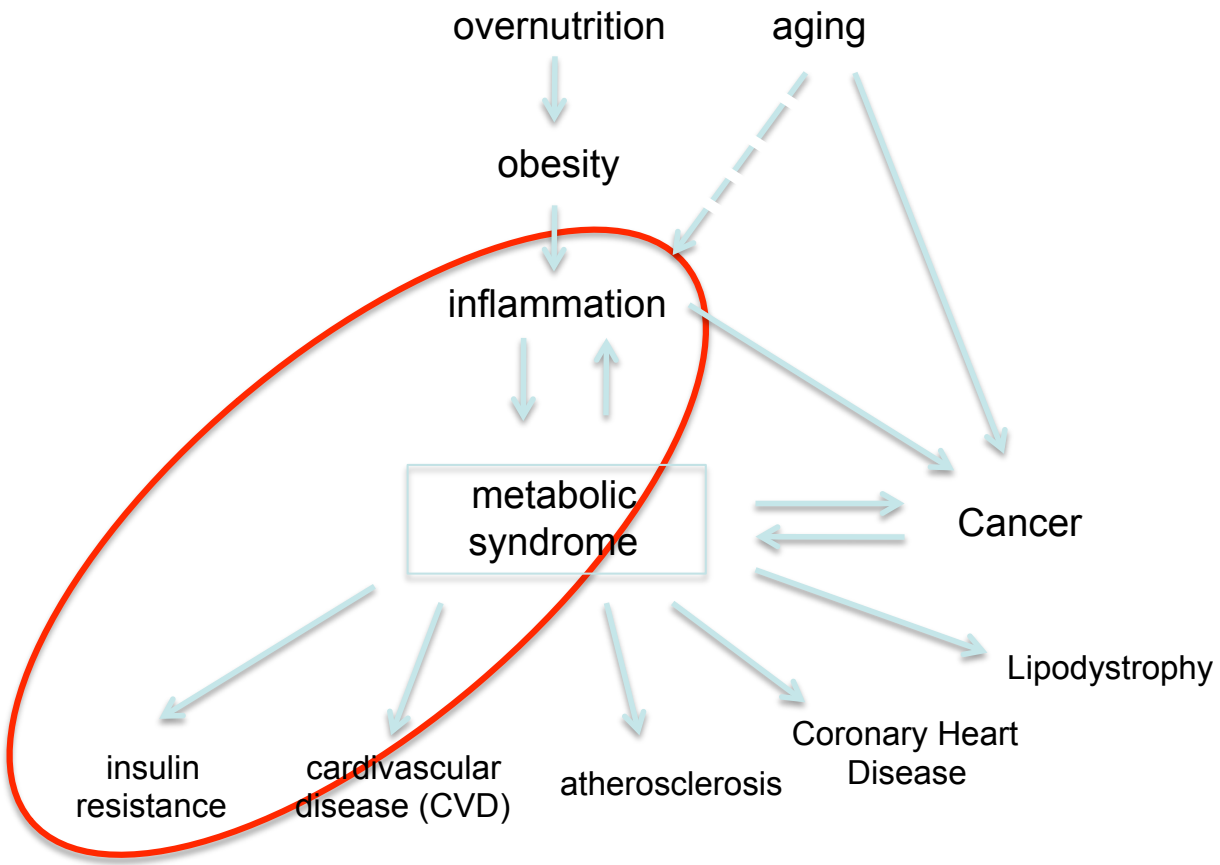
chronic Inflammation

activation of: NFκB, AP-1, STAT3

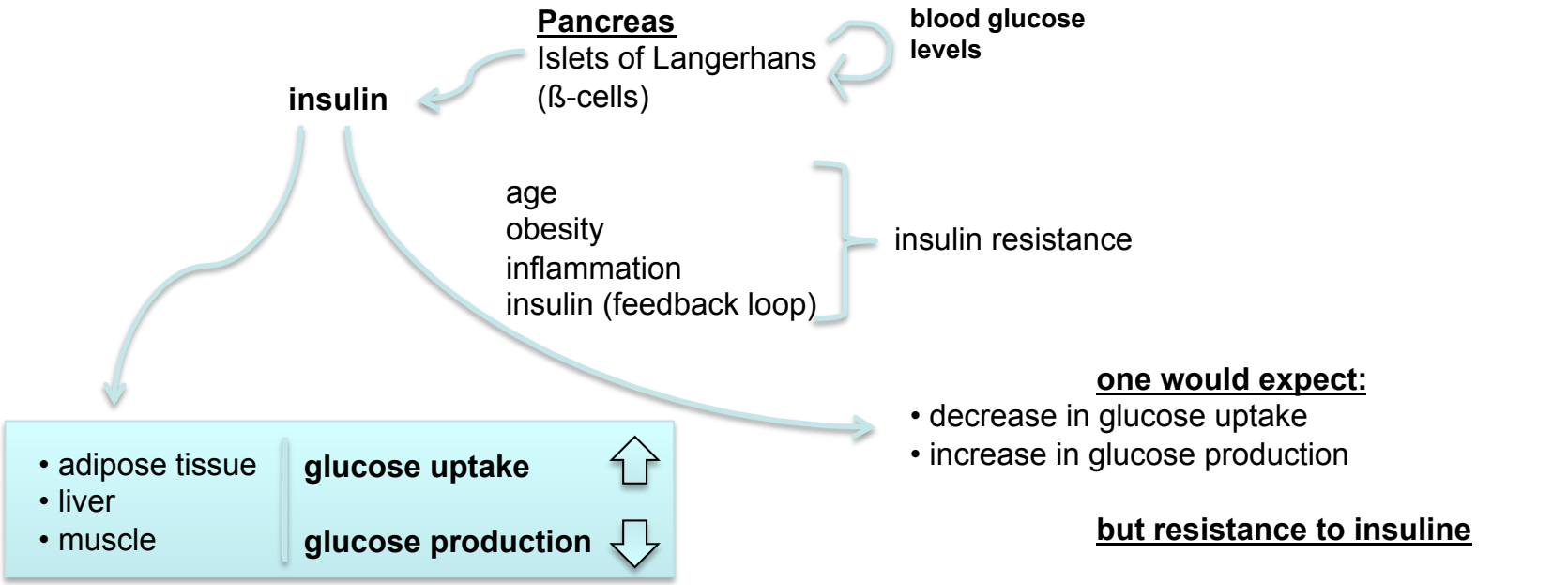
[1] Anand et al, Pharm Res; **25**:2097 (2008).
[2] Aggarwal & Gehlot, Curr Opin Pharmacol; **9**:351 (2009).
[3] Ono, Cancer Sci; **99**:1501 (2008).

Tumor, inflammation and metabolism

- Inflammation, metabolic syndrome and insulin resistance -

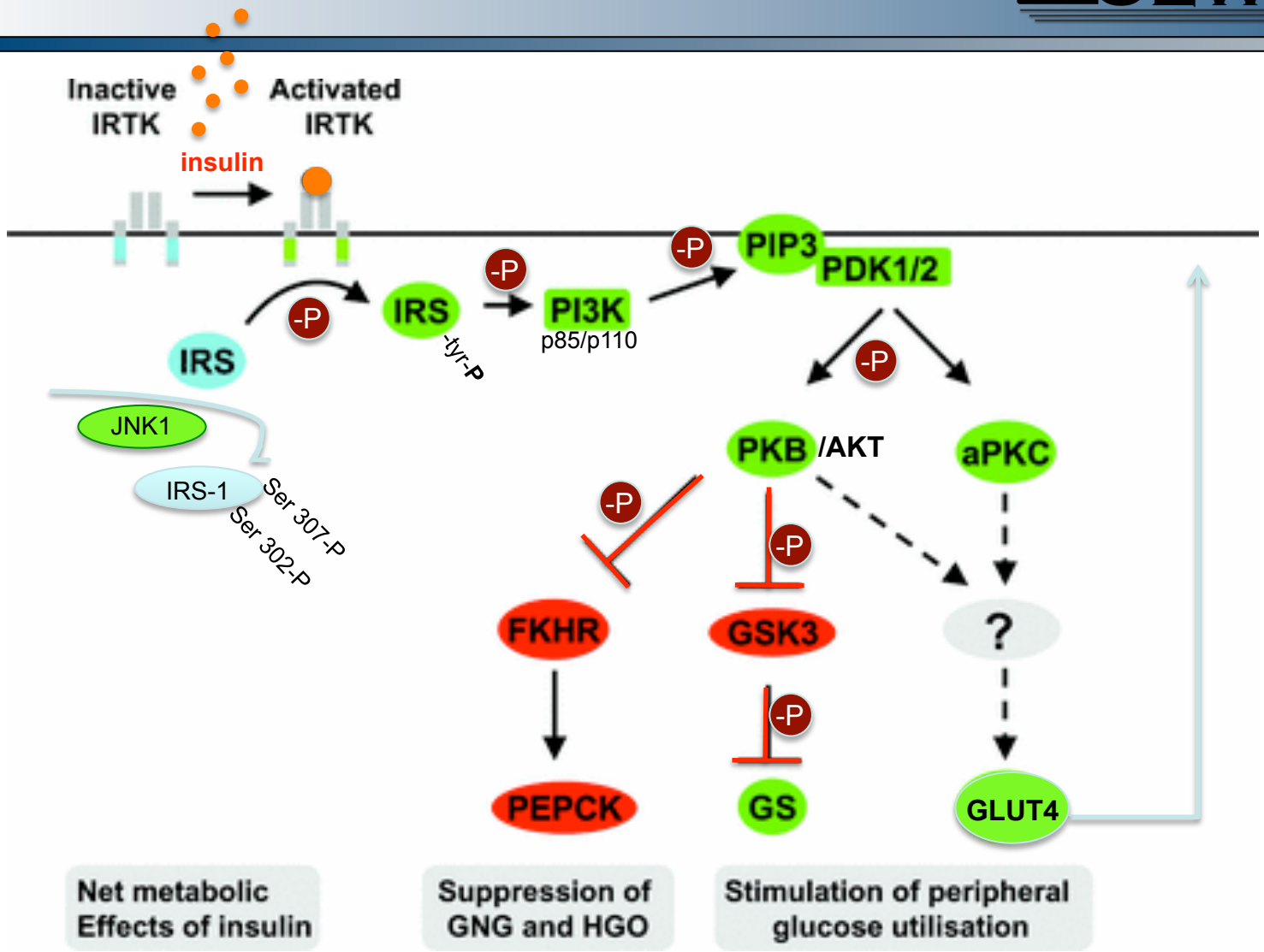


How insulin works:
physiological and pathological conditions



Hyperinsulinaemia: insulin concentrations above physiological levels
insulin resistance: diminished response of insulin-responsive tissues to insulin

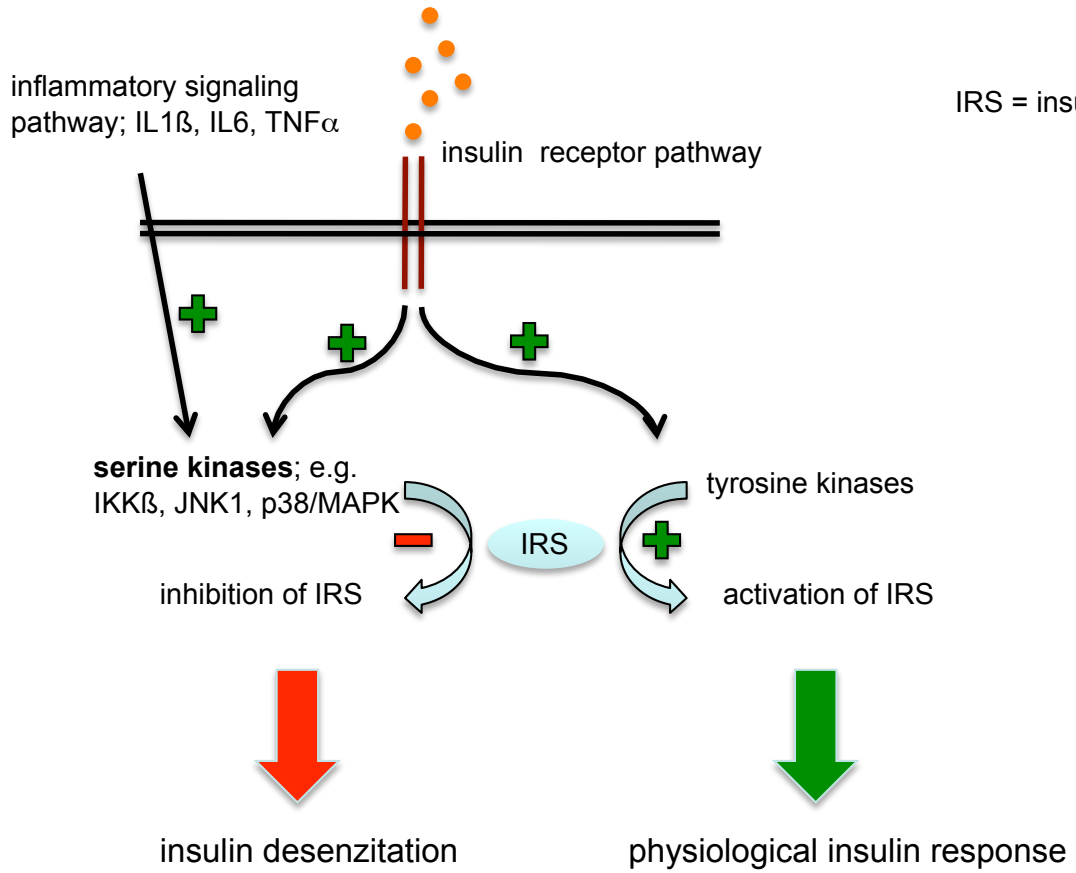
Intracellular insulin signaling



IRS: insulin receptor substrate
GSK3: glycogen synthase kinase 3
GS: glycogen synthase
GLUT4: glucose transporter

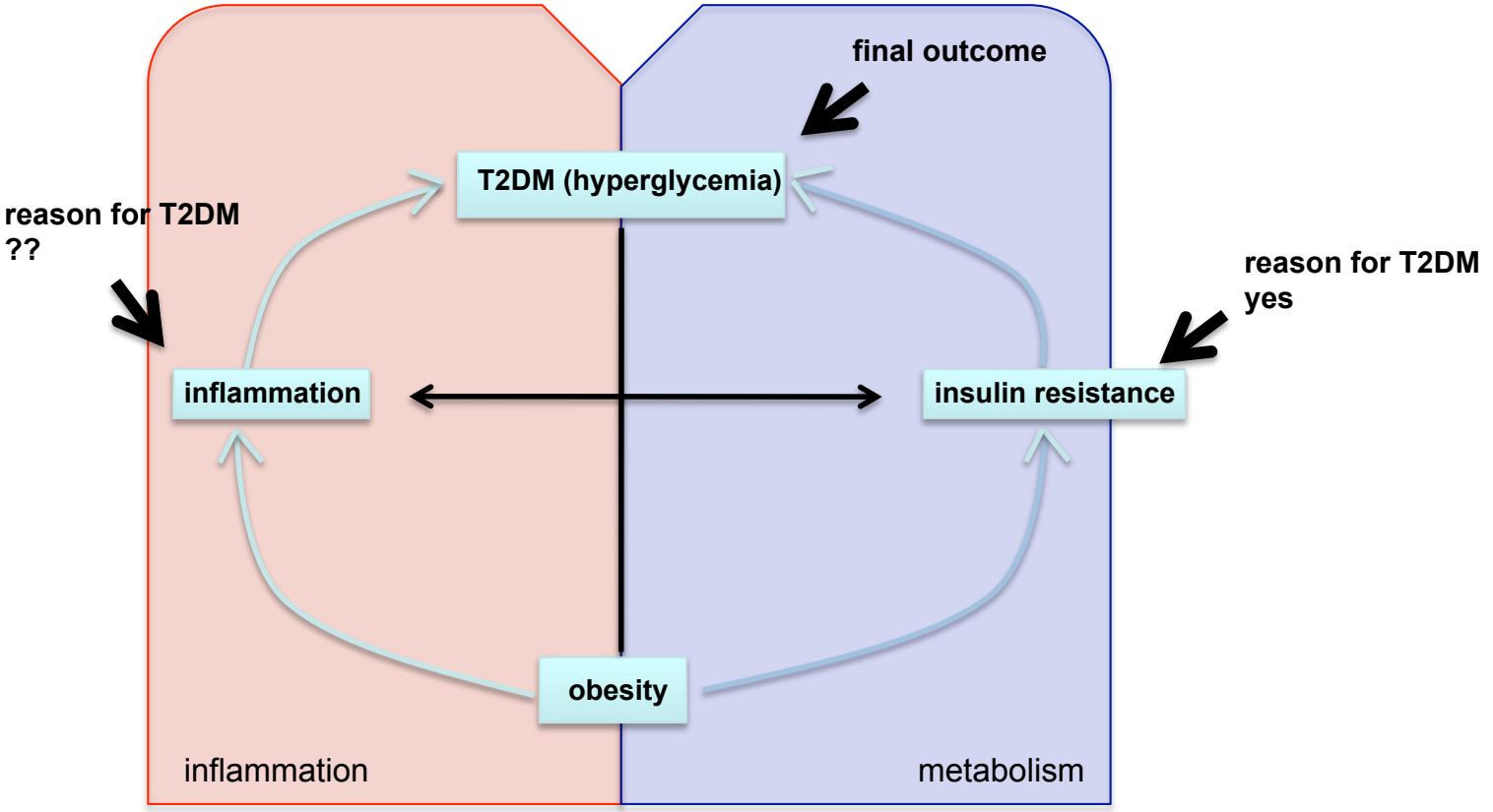
PI3K: phosphatidylinositol-3 kinase
PDK1/2: protein kinase 3-phosphoinositide-dependent protein kinase-1
FKHR: forkhead transcription factor (Foxo1) :
PEPCK: phosphoenolpyruvate carboxykinase

Molecular pathways of insulin resistance

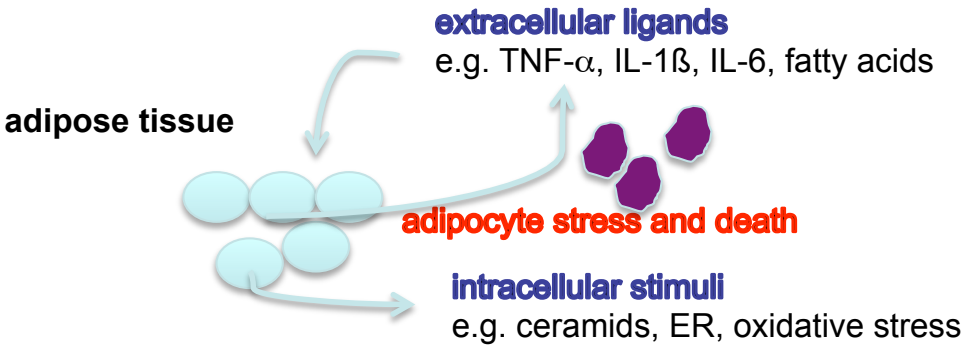


[1] Werner et al, 2004; JBC **34**:35298

Linking Obesity to insulin resistance



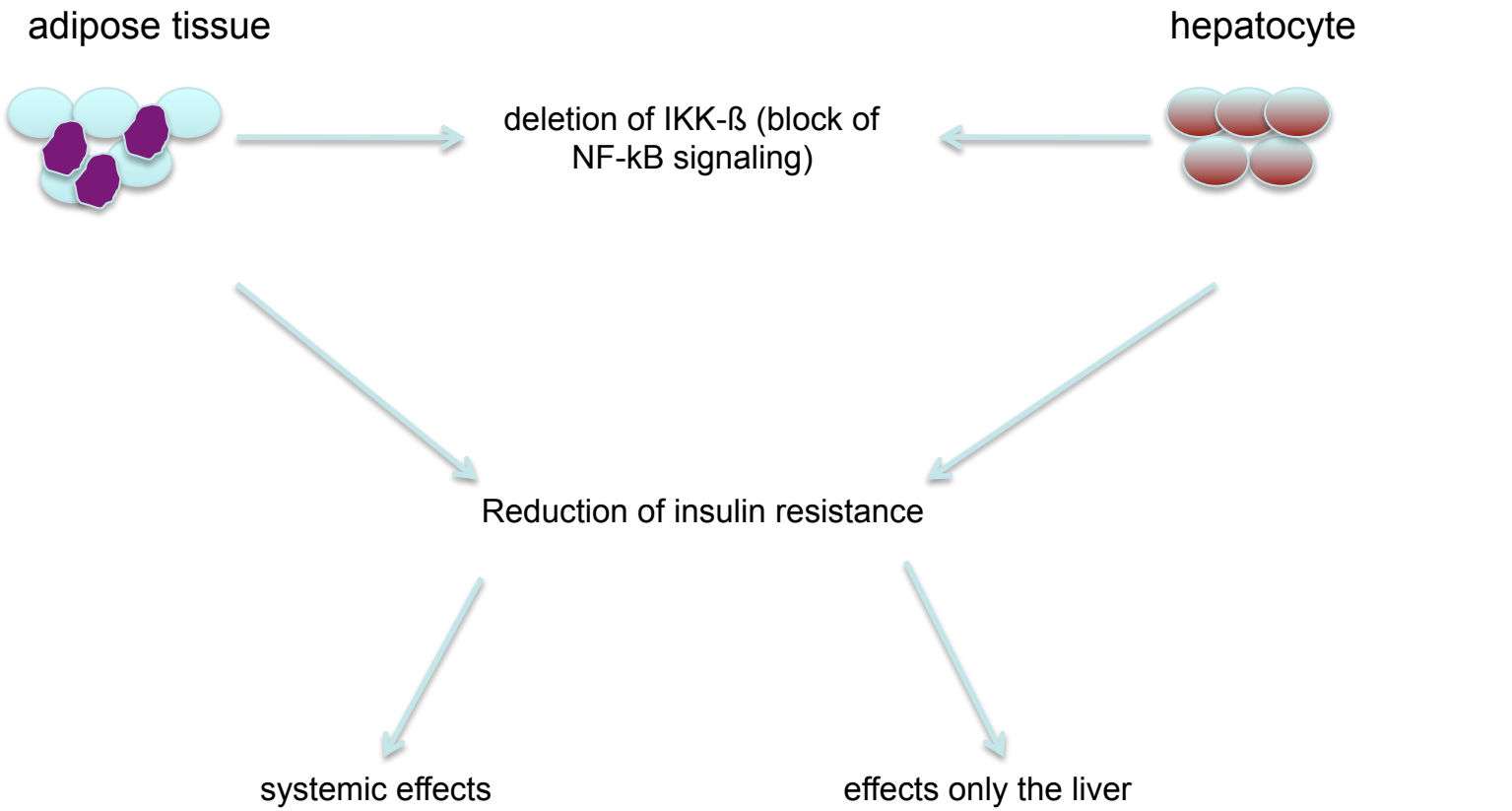
Insulin resistance and cytokines



cytokines

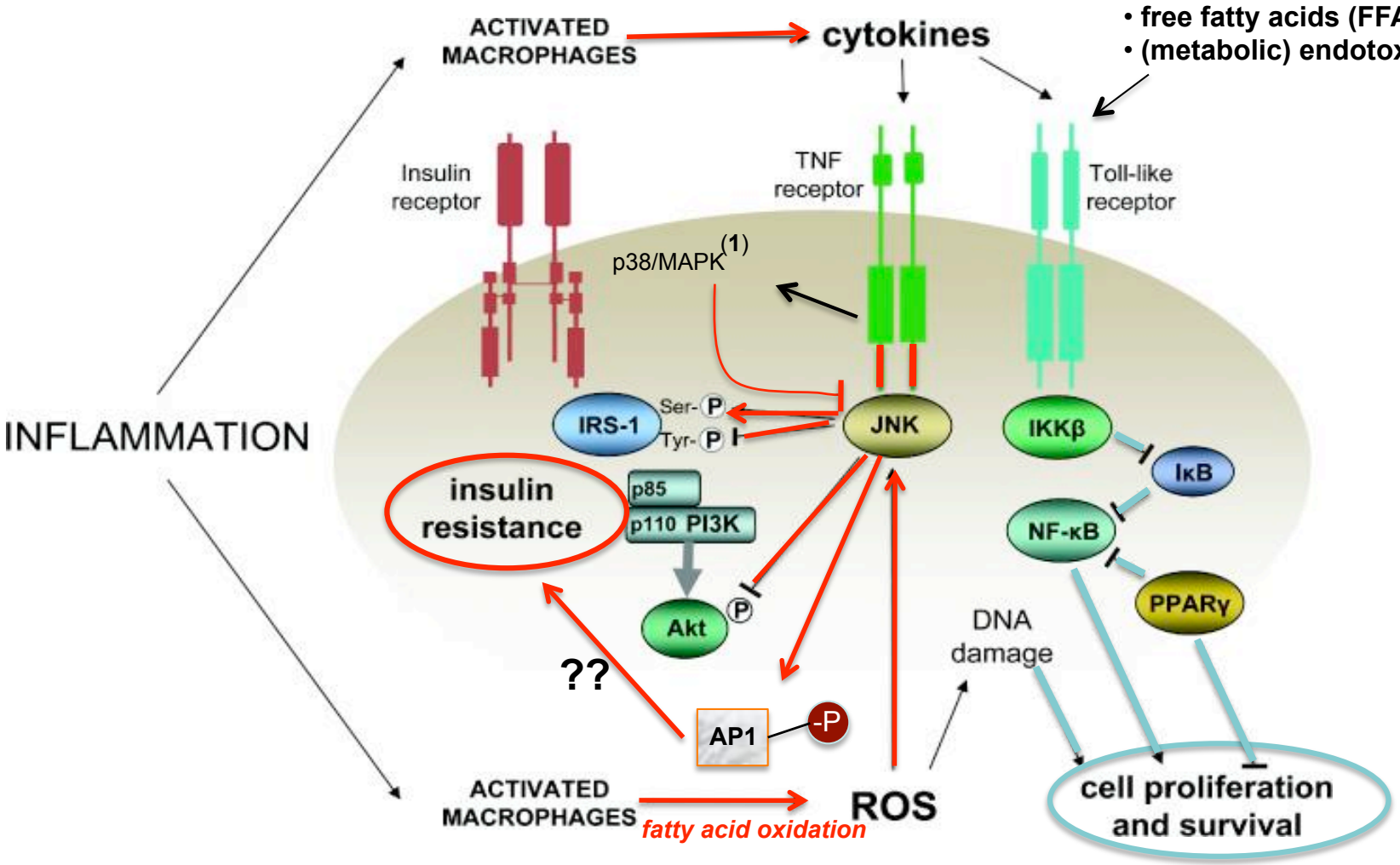
inflammatory	anti-inflammatory
e.g. TNF-α, IL-1ß, IL-6, MCP-1	e.g. IL-10
↓	↓
insulin desensitizing	insulin-sensitizing

Role of macrophages in systemic insulin resistance



[1] Arkan et al, Nat Med 2005; **11**:191.

Molecular pathways of insulin resistance
- linking inflammation to the insulin pathway -



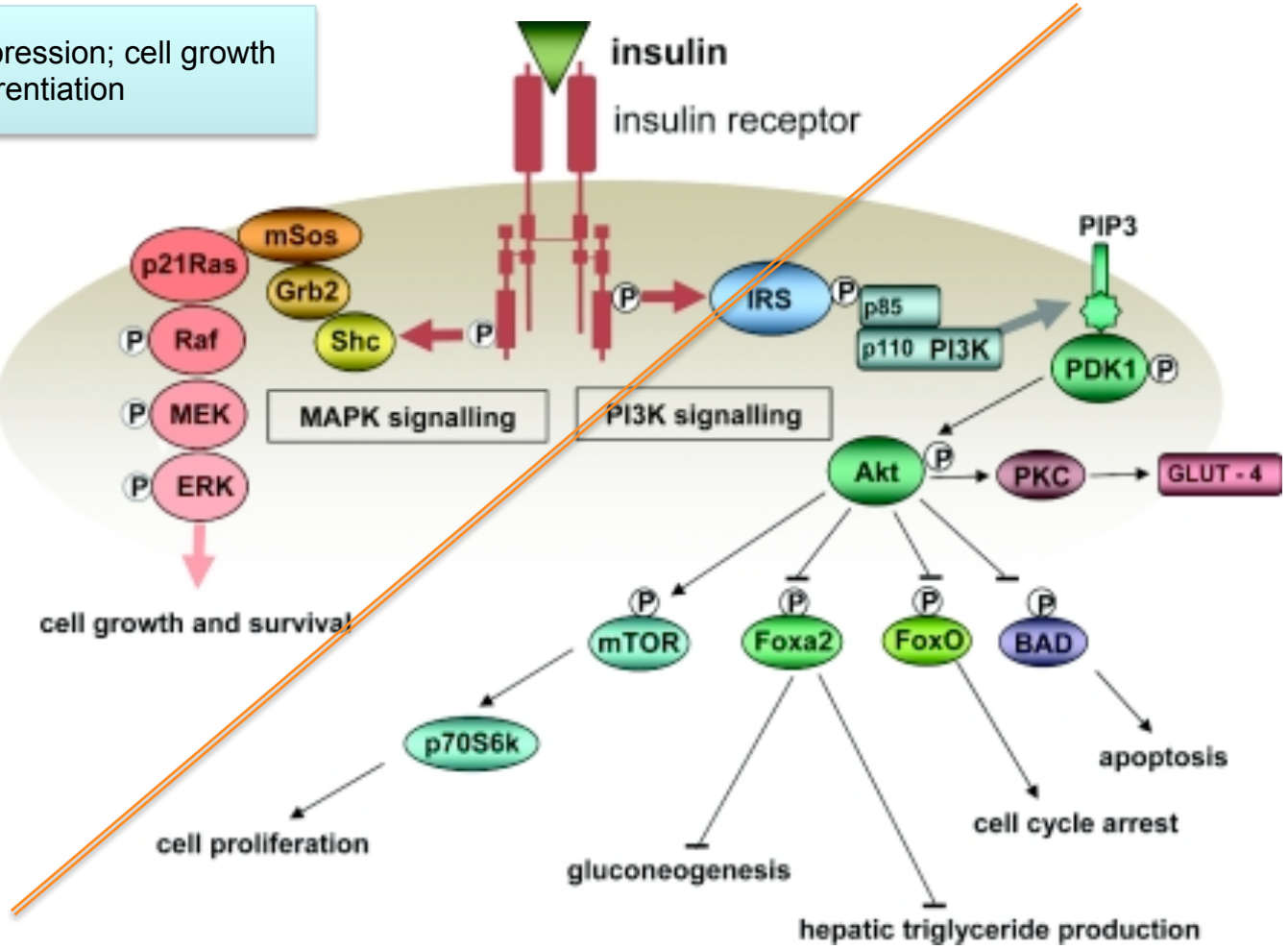
[1] Gaestel et al, Nat Rev Drug discov **8**:480 (2009).
[2] Cani et al, Diabetes **56**:1761 (2007).

JNK: c-Jun N-terminal kinase
PPAR: peroxisome-proliferator activated receptor

Molecular pathways of insulin resistance
- linking resistance to Cancer biology -

LOEWE Tumor & Inflammation

gene expression; cell growth and differentiation

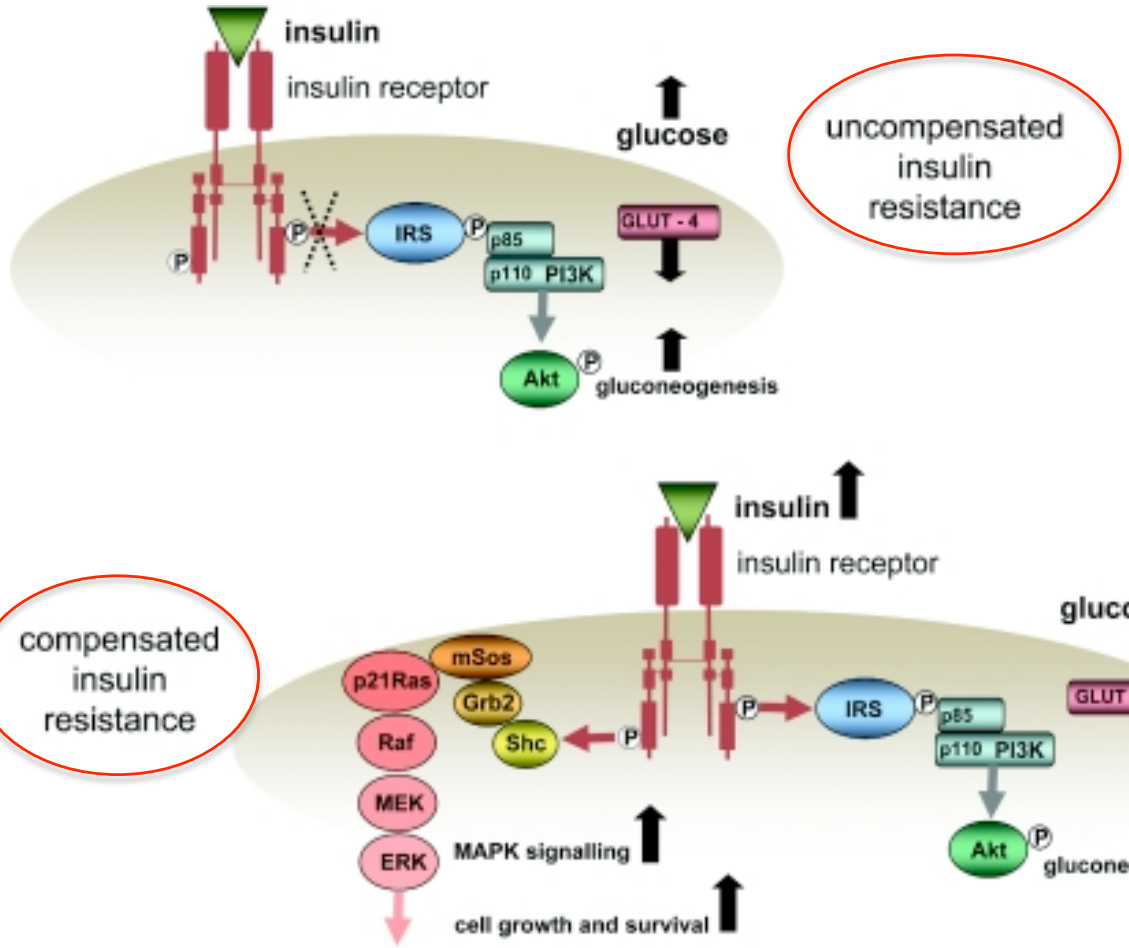


glucose uptake and gluconeogenese

Godsland IF. Insulin resistance and hyperinsulinaemia in the development and progression of cancer. Clin Sci; **118**:315 (2009).

Prelecture to 3rd LOEWE Minisymposium A. Konur

Molecular pathways of insulin resistance
- linking resistance to Cancer biology -



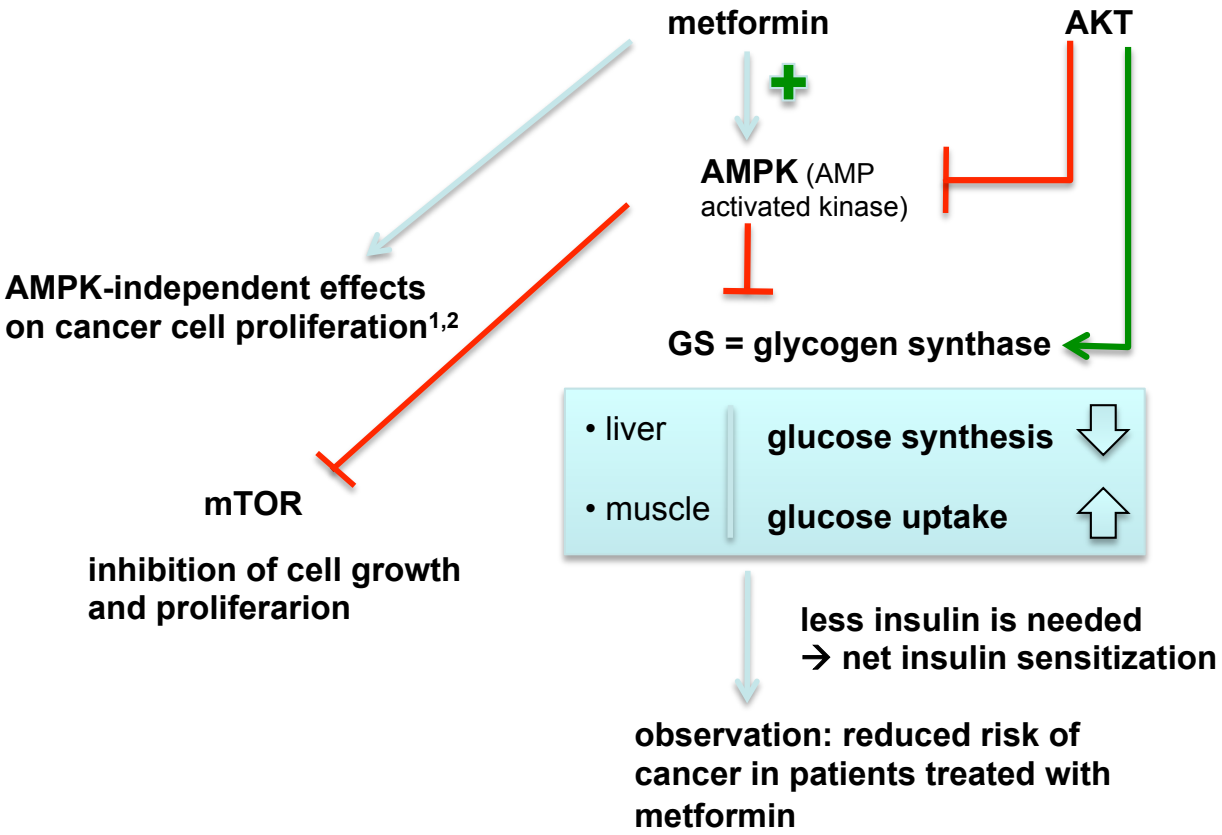
injection of insulin in T2DM

↓

increased colorectal cancer risk[1]

[1] Hemkens et al, Diabetologia 52:1732 (2009)

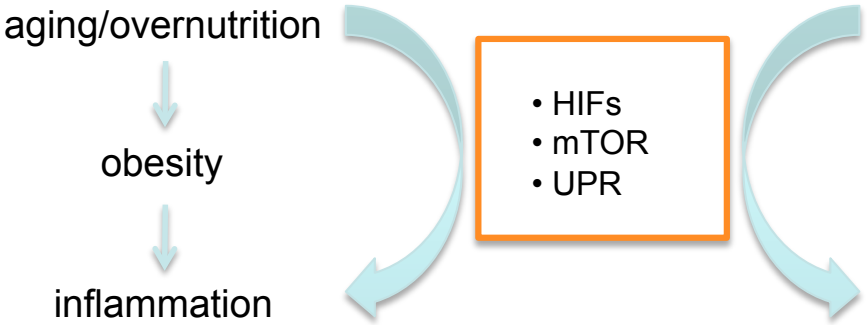
Metformin for treatment of hyperinsulinaemia:
an anticancer drug?



→ **however:** **this doesn‘t proof that insulin is directly responsible for cancer, but metformin may act via other intracellular targets...**

[1] Ben Sahra et al, Oncogene **27**:3576 (2008).
[2] Vazquez-Martin et al, Cell Cycle **8**:88 (2009).

The mTOR pathway
- merging overnutriation, inflammation and cellular stress networks -



Majority of tumors[1]

O(2)-sensitive signaling pathways (hypoxia)

angiogenesis
metastasis
resistance to therapy
tumorigenesis/tumor growth
metabolism

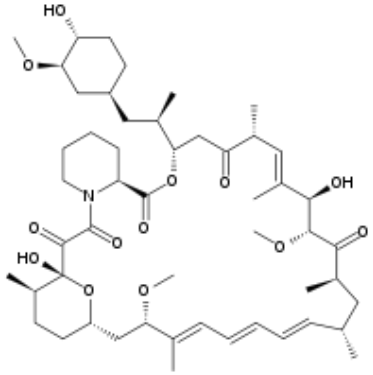
• UPR → lipid accumulation in fat and liver → activation of JNK

[1] Wouters & Koritzinsky, Nat. Rev. Cancer 2008; **11**:851.
[2] Ron & Walter, Nat Rev Mol Cell Biol 2007; **7**: 519.

• HIFs (hypoxia-inducible factor family of transcription factors)
• mTOR (mammalian target of rapamycin (mTOR) kinase)
• UPR (unfolded protein response; misfolded protein response of the ER)

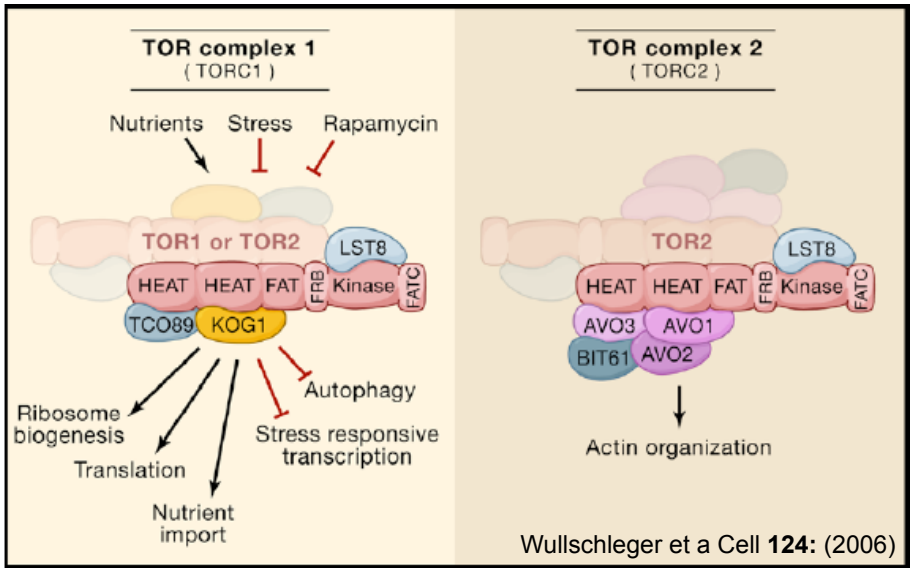
The mTOR pathway

rapamycin:



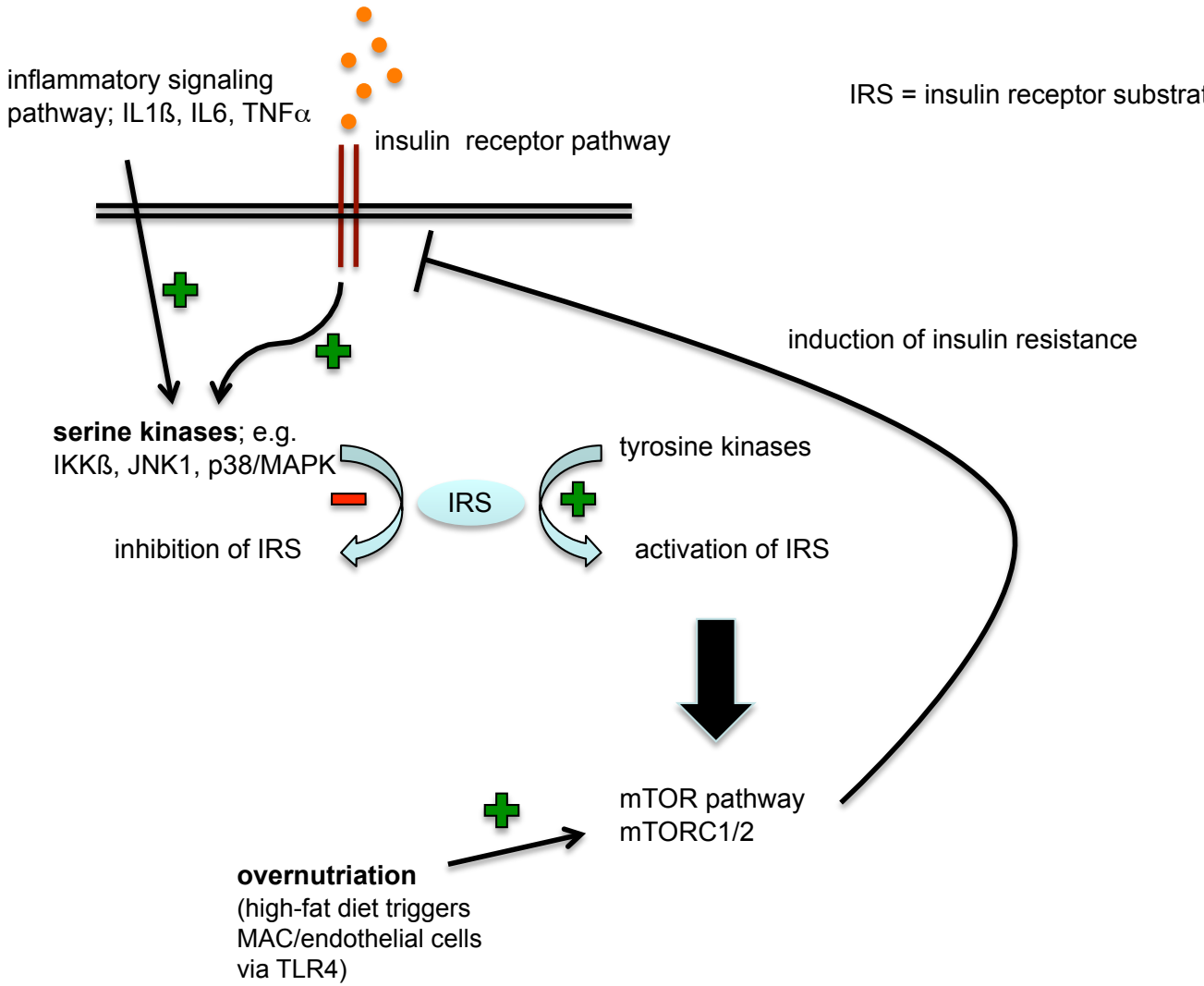
→ a macrocyclic lactone
→ isolated in the 1970s from a bacterial strain (Streptomyces hygroscopicus) from a soil sample from Easter Island (in the local language Rapa Nui)
→ antifungal, but also inhibits proliferation of mammalian cells
→ immunosuppressive properties; used in renal transplantations in combination with cyclosporine

→ rapamycin binds via the intracellular cofactor FKBP12 to TOR (target of rapamycin)
→ 2 TOR complexes exist (TORC1 and TORC2) but only TORC1 is rapamycin-sensitiv
→ TOR is a **Ser/Thr kinase**

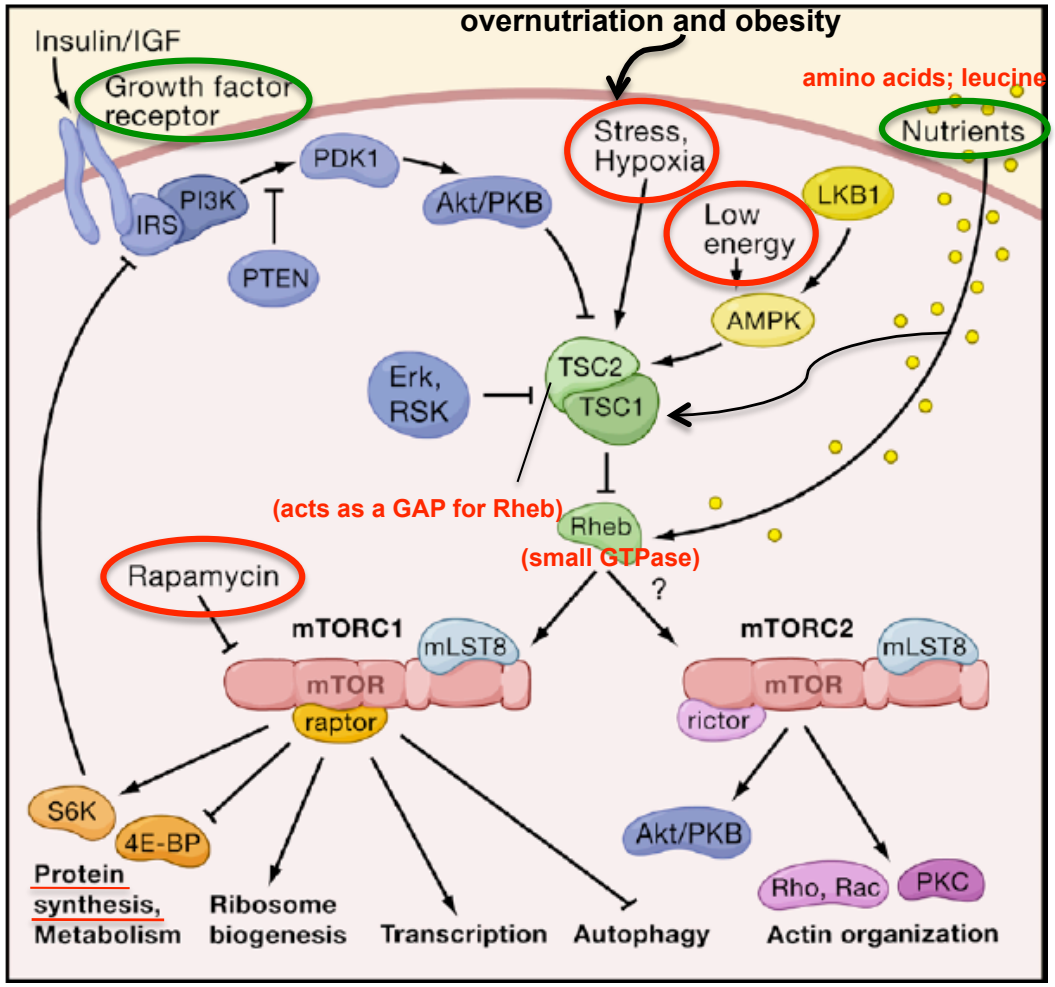


Wullschleger et a Cell **124:** (2006)

The mTOR pathway
- merging overnutriation, inflammation and cellular stress networks -



The mTOR pathway
- merging overnutriation, inflammation and cellular stress networks -



Wullschleger et al, Cell 2005; **124**:471.

pathways increasing mTORC1 activity also increase:
→ cell size and mass
→ protein synthesis
→ transcription
but also is involved in
→ induction of insulin resistance

pathways decreasing mTORC1
→ induction of katabolic processes

TSC: tuberous sclerosis complex; TSC1=hamartin; TSC2=tuberin)
PTEN: phosphatases and tensin homologue; lipid phosphatase
GAP: GTPase activating protein
Rheb: Ras-related small G protein Ras homologue enriched in brain)

Rapamycin – a life prolonging drug

Anti-Aging-Wirkstoff
Osterinsel-Medizin lässt Mäuse älter werden

Ein Naturstoff von der Osterinsel verspricht ein längeres Leben - zumindest gilt das für Mäuse. Ältere Nager lebten mit Hilfe von Rapamycin rund zehn Prozent länger. Der Wirkstoff dämpft das Immunsystem und verhindert nach Organtransplantationen Abstoßungsreaktionen.

Spiegel 9.7.2009

nature Vol 460|16 July 2009|doi:10.1038/nature08221

LETTERS

Rapamycin fed late in life extends lifespan in genetically heterogeneous mice

David E. Harrison[1]*, Randy Strong[2]*, Zelton Dave Sharp[3], James F. Nelson[4], Clinton M. Astle[1], Kevin Flurkey[1], Nancy L. Nadon[5], J. Erby Wilkinson[6], Krystyna Frenkel[7], Christy S. Carter[8]†, Marco Pahor[8]†, Martin A. Javors[9], Elizabeth Fernandez[2] & Richard A. Miller[10]*

Rapamycin – a life prolonging drug

life span extension (median and maximum) by:

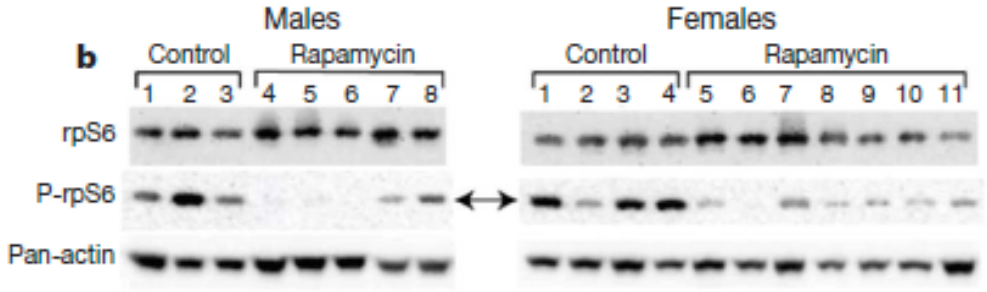
diet

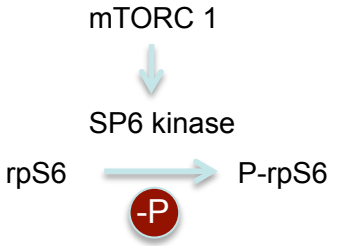
- reduction in body weight
- no benefit if started in elder mice

Rapamycin
(→ inactivation of mTORC1)

- no changes in body weight
- even effectiv in elder mice

(> 600 d→ equivalent to 60-year-old humans)



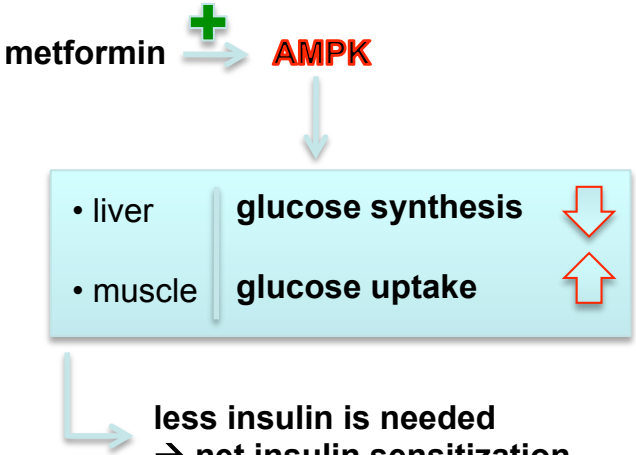


Drugs used for treatment of insulin resistance

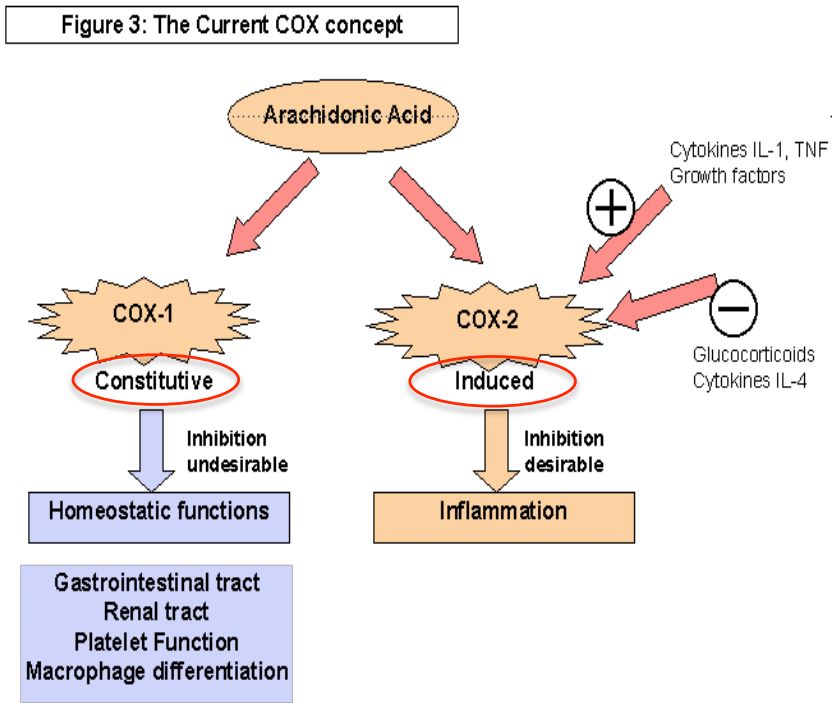
Thiazolidinedione (TZD; e.g. rosiglitazone):
- fatty acid sequestration in the adipose tissue → reduction of stress response in adipocytes, liver and muscle
- TZD-dependent SUMOylation of **PPARγ** which targets it to nuclear receptor corepressor histone deacetylase-3 complexes on inflammatory gene promoters (such as NFkB and AP1). Binding of PPARγ stabilizes the corepressor complex

Salicylate (used since more than a century)
- directly target inflammation by inhibition of the **NFkB/IKKß** signaling pathway

Metformin: suppressing hepatic glucose production



COX-inhibitors (NSAID):
- Aspirine → transacetylation of COX-1 and -2; other NSAIDs bind directly to the enzyme



Cyclooxygenase-inhibitors

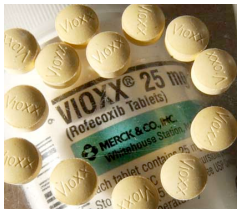
COX1: ‚constitutively expressed' in most cells; involved in many physiological processes;
e.g. PGE_2 (protective role in the gastrointestinal tract); platelet aggregation
COX2: inducible, mainly in inflammatory immune cells, but also in tumor cells
{COX3}: splice variant of COX1; function unknown

COX inhibitors: NSAIDs (non steroidal anti inflammatory drugs)

→ *non selective* (‚classical') NSAIDs inhibit both, COX1 and COX2:
e.g. acetylsalicylic acid (Aspirin/ASA), Diclofenac, Ibuprofen
specificity with regard to COX1 is approx. 10 – 100 times higher

→ *selective* COX2 inhibitors; e.g. Rofecoxib (VIOXX)

Specificity of NSAIDs



COX-2

DFP
L-7445,337
Rofecoxib *VIOXX*
NS-398

>50fach COX-2-selektiv

Etodolac
Meloxicam
Celecoxib
Nimesulid

5-50fach COX-2-selektiv

Diclofenac
Sulindac Sulfid
Meclofenamat
Tomoxiprol
Piroxicam
Diflusinal
Natriumsalizylat
Niflumic Acid
Zomepirac

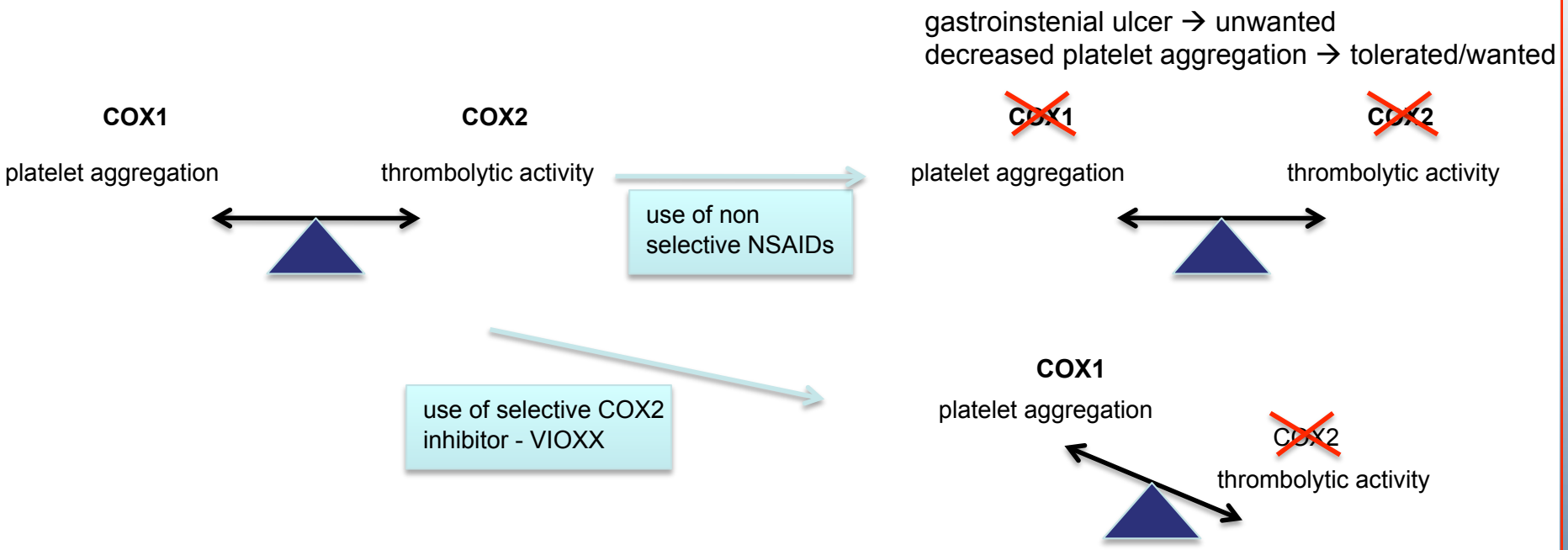
zunehmend COX-2-selektiv

zunehmend COX-1-selektiv

COX-1

Fenoprofen
Ampyron
Ibuprofen
Tolmetin
Naproxen
Aspirin
Indomethacin
Ketoprofen
Suprofen
Flurbiprofen
Ketorolac

COX2 specific NSAIDs – *VIOXX*



→ no gastrointestinal ulcer but increased risk for cardiovascular events and stroke; market withdrawal of Vioxx in 2004

NSAIDs in cancer therapy??

NSAIDs used in Clinical studies for pancreatic cancer treatment

A Prospective Study of Aspirin Use and the Risk of Pancreatic Cancer in Women. Schernhammer et al, JNCI, 2004; 96:22.

→ *...regular aspirin use appear to be associated with a statistically significantly increased risk of pancreatic cancer among women*

Association Between Nonsteroidal Anti-Inflammatory Drug Use and the Incidence of Pancreatic Cancer. Anderson et al, JNCI, 2002; 94:1168.

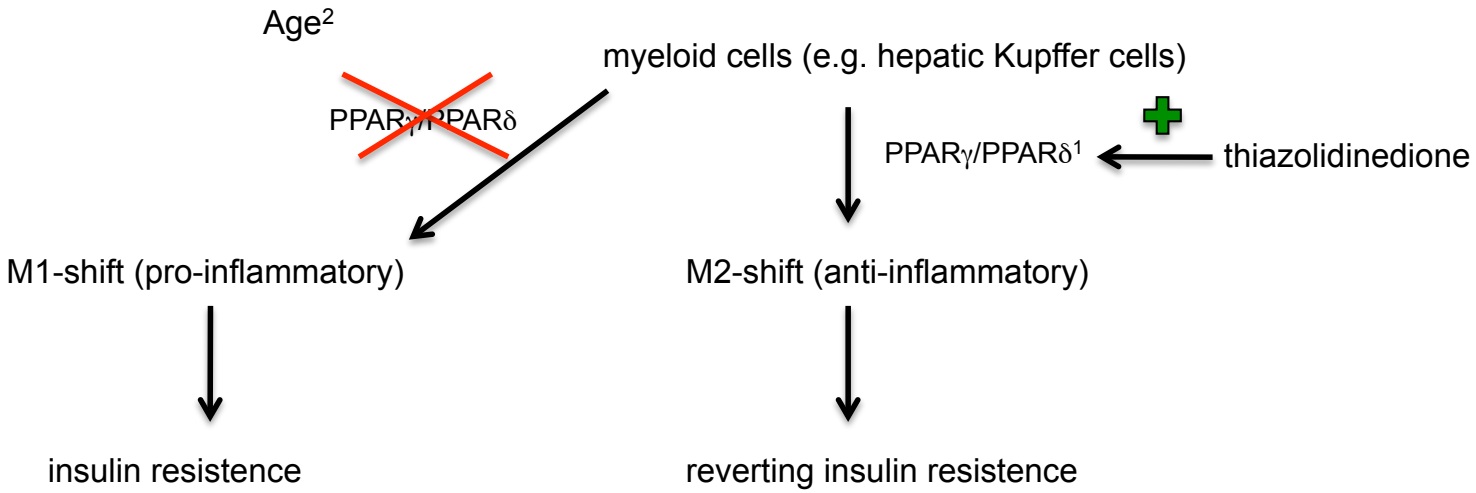
→ *...indicate that aspirin might be chemopreventive for pancreatic cancer.*

Aspirin and Nonsteroidal Anti-inflammatory Drug Use and Risk of Pancreatic Cancer: A Meta-analysis. Larsson et al, Cancer Epidemiol, Biomarkers & Prev 2006; 15:2561.

→ *...does not indicate that use of aspirin or NSAIDs lowers the risk of pancreatic cancer.*

COX-2 inhibitors: a new class of anticancer agents?
Gasparini et al, The Lancet Oncology; 2003 **4:** 605.

PPARs as targets for insulin sensitizer



[1] Odegaard et al, Cell Metab 2008; 7:496.
[2] Ye et al, Gerontology 2006; 52:69.

PPAR = peroxisome proliferator-activated receptors; TF located in the nucleus; their ligands are fatty acids/eicosanoids

Adipose tissue is not the same at all

Dicker Po ist gesund[1]

...dicker Bauch nicht

...the gluteofemoral fat depot is more passive than the abdominal depot...[2]
→ long term fatty acid storage

...leptin and adiponectin levels are positively associated with gluteofemoral fat while the level of inflammatory cytokines is negatively associated.[2]

→ gluteofemoral fat is not only less harmful compared to visceral fat depots, but even protective against cardio-vascular diseases ←

[1] Spiegel online 14.1.2010
[2] Manolopoulos et al, 2010, Int J Obes; Jan 12. {Epub ahead of print}

→ leptin is a protein hormon, produced mainly by adipocytes, controls appetite (low leptin levels, big appetite)
→ leptin concentrations proportional to body fat

Inflammation is not the same at all times

- lipodystrophy vs obesity-

common metabolic consequneces
- insuline resistance
- hepatic steatosis

	Lipodystrophy[1]	Obesity
systemic inflammation	yes	yes
ATMs	yes (only in adipose tissue)	yes
salicylates effective in improving insulin resistance	no	yes
adipocyte apoptosis	yes	no
resp. of ATMs to LPS	no	yes
inh. of inflamm. cytokines by salicylates	no	yes
deletion of IKKß for improving insulin resistance	no	yes

[1] Herrero et al, PNAS, 2010; **107**:220.

→ lipodystrophic mice as a model for e.g. human HIV-associated lipodystrophy and tumor cachexie

mechanisms leading to insuline resistance in lipodystrophic mice are not the same compared to ob/ob mice, despite the fact that in both mice systemic inflammation is observed

Psoriasis

→ marked dermal inflammatory response; not associated with an increased risk for developing skin cancer

→ tumors with a better prognosis in the presence of inflammatory cells; e.g. eosinophils in colon tumors, TAMs in a subset of breast tumors and pancreatic tumors

type of inflammation

‚bad' infl. ‚good' infl.

cancer promoting
MΦ - **M2** phenotyp

cancer inhibitory
MΦ - **M1** phenotyp

M2-MΦ (upon IL4, IL10, IL13, TGFß stimulation)
→ high IL10- no/low IL12 and IL-23 production, arginase-pos[1,2]

M1-MΦ (upon IFNγ/LPS stimulation)
→ high IL12- low IL10 production, inflamm. cytokines IL-1ß, IL-6, TNF-α, tumoridical activity

[1] Martinez et al. JI 177:7303 (2006)
[2] Murdoch et al Nat Rev 8:618 (2008)

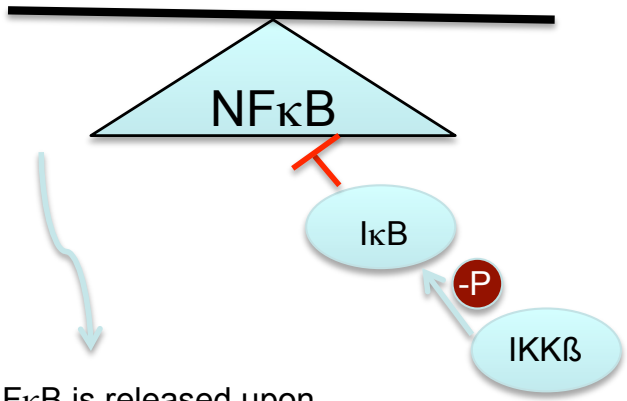
Bad vs good inflammation

type of inflammation

‚bad' infl. **‚good' infl.**

cancer promoting
MΦ - **M2** phenotyp

cancer inhibitory
MΦ - **M1** phenotyp



NFκB is released upon phosphorylation of IκB by IKKß

→ NFκB maintains the immunosuppressive (**M2**)-phenotyp in MΦ
→ Inactivation of IKKß in MΦ (thereby inhibiting NFκB-activity) reverts from a **M2**- to an **M1**-phenotype[3]
→ Re-education of TAM seems to be possible

inflammation
induced by dying tumor cells

‚bad' infl.

TLR-independent
(via sterile inflammation; non bacterial); recruitment of neutrophils by the IL-1R-MyD88 pathway[2]

polarization to **M2**-phenotyp also via the IL-1R-MyD88 pathway[3]

‚good' infl.

TLR-dependent (e.g. TLR-4-dependent cross-presentation of tumor antigens on DCs during chemo-and radiotherapy)[1]

promotes **M1**-MΦ differentiation and the development of a sustained and effective adaptive immune response

[1] Apetoh et al. Nat Med **13**:1050 (2007).
[2] Chen et al. Nat Med **13**:851 (2007).
[3] Hagemann et al. JEM **205**:1261 (2008).

Thank you for your attention