Paper presentation JC Current Topics in Applied Immunology SS

ORIGINAL ARTICLE

STAT3- and GSK3β-mediated Mcl-1 regulation modulates TPF resistance in oral squamous cell carcinoma

Santanu Maji[1,2], Omprakash Shriwas[1,2], Sabindra K. Samal[1,2], Manashi Priyadarshini[1], Rachna Rath[3], Sanjay Panda[4,5], Saroj Kumar Das Majumdar[6], Dillip Kumar Muduly[7] and Rupesh Dash[1,*]

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Paper presentation JC Molecular Mechanisms of Cancer Therapeutics

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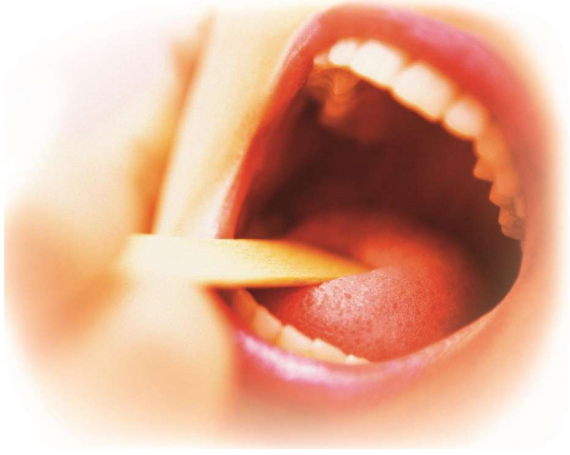
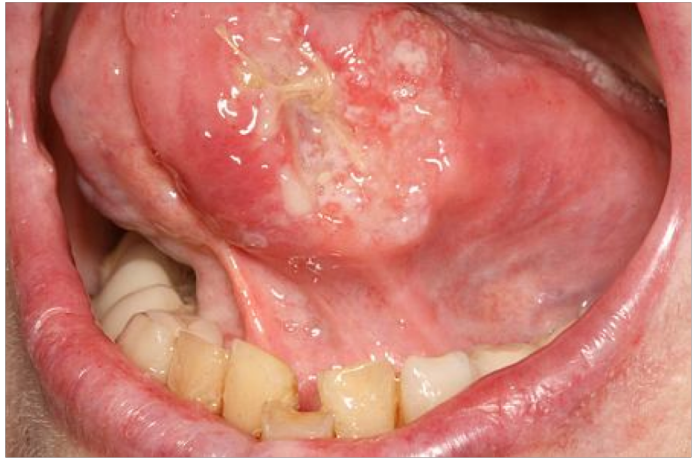
TPF = docetaxel (*Taxotere®*) + cisplatin + 5-FU

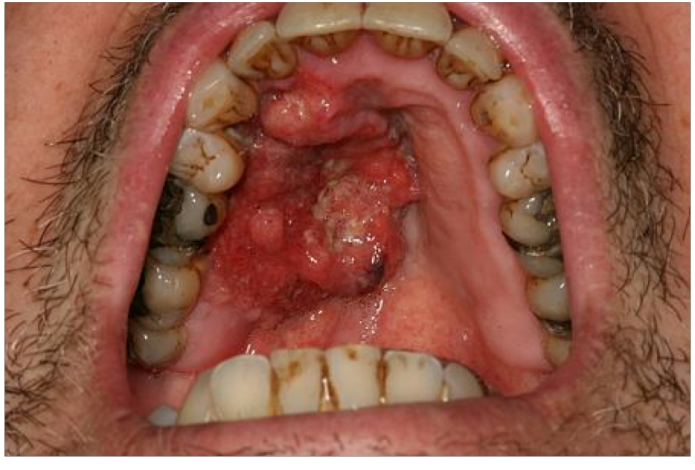
Facts about Oral Squamous Cell Carcinoma (OSCC)



- head and neck cancers ranked 6th most common cancer worldwide (♂>♀)
- 90% are SCC, which 60-70% in oral cavity (+ larynx)
- first diagnosed in advanced stages (III - IV)

Advanced stages:





How to treat OSCC:

- in general:
  surgery first
  if resectable !
- unresectable: RCHT (<70. Lj)

  ...combi shows benefit in 5-year SR compared to radiation therapy alone
- CHT increases radiation toxicity...

Leitlinienprogramm Onkologie

Mundhöhlenkarzinom

"Diagnostik und Therapie des Mundhöhlenkarzinoms"

AWMF-Register-Nummer (007-100OL)

Version 2.0 12.2012

Leitlinie (Langversion)

Limitations in OSCC treatment:

- overall 5-year SR:
  ~ 65%

- CHT-resistance
- relapse: tumor growth + metastatic disease
- therapeutic failure

Targets to restore cell death in CHT-R OSCC:

- inhibition of antiapoptotic factors
- re-activation of apoptotic factors

→ major antiapoptotic proteins: Bcl-2, Bcl-xL, Mcl-1

→ cancer cells modulate these to block CHT-induced apoptosis

OSCC CHT-R models / study set-up:

CHT-aR

1. OSCC cell lines of acquired Resistance
   H357, SCC4
   each sensitive & established resistant

- ▻ human tongue
- ▻ cultured 6-8 mon @ [IC_{50}]
- ▻ relapsed TU growth pattern

in vitro analysis

PDCs

2. OSCC patient samples

- ▻ n = 15
  neoadjuvant non-responders,
  transplanted to mice
- ▻ n = 15 CHT-naive patients
  („none“ CHT/RT/surgery)
- ▻ from indian cancer center hospital

clinical relevance

FACS-based Apoptosis assay / methods:

flow cytometry-based cell death assay

- apoptosis detection kit *by Annexin V/propidium iodide staining*
- „quantification (%) cell death“ in CHT-aR cell lines

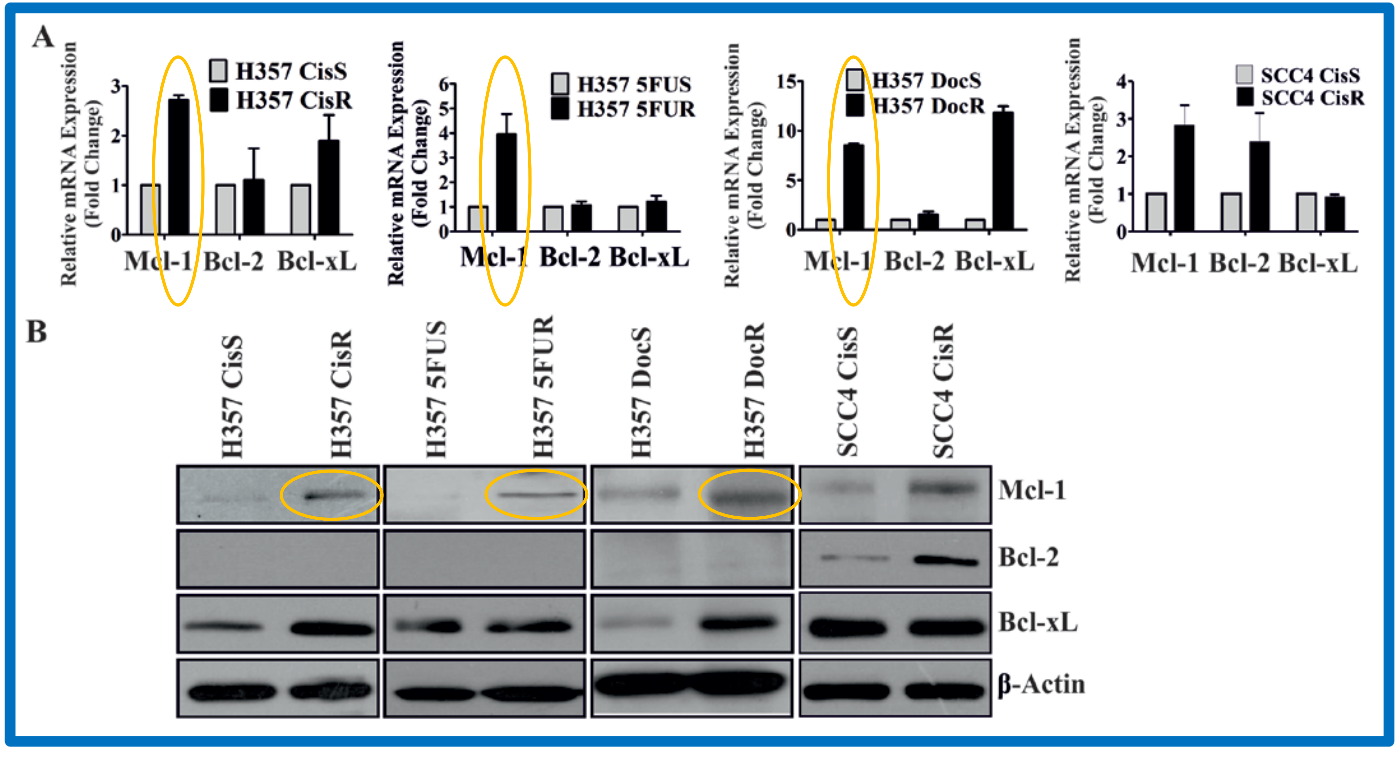
- sorting cells CHT resistant / sensitive

- resistant cells: further evaluation of expression of major Bcl2-antiapoptotic members & check for clinical relevance in PDCs

CHT-aR cell lines

PDCs pat. samples

results:

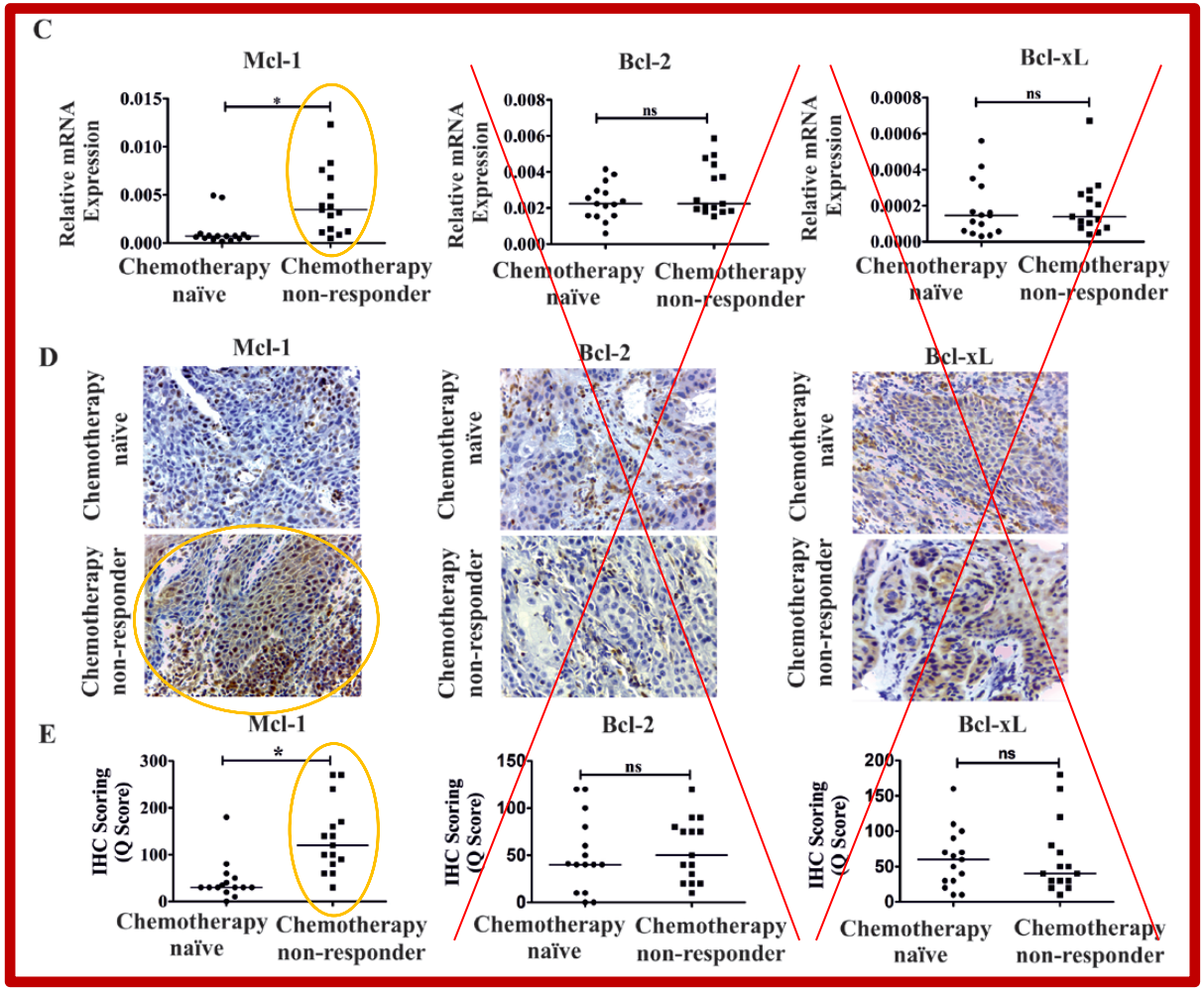


Mcl-1 mRNA expression ↑ (qRT-PCR)

Mcl-1 protein levels ↑ (western blot)

results:

„in vivo check“ (Mcl-1 upregulated in CHT-non-responding tumors)



ONLY
Mcl-1 mRNA expression ↑ (qRT-PCR)

ONLY
Mcl-1 protein expression by IHC ↑

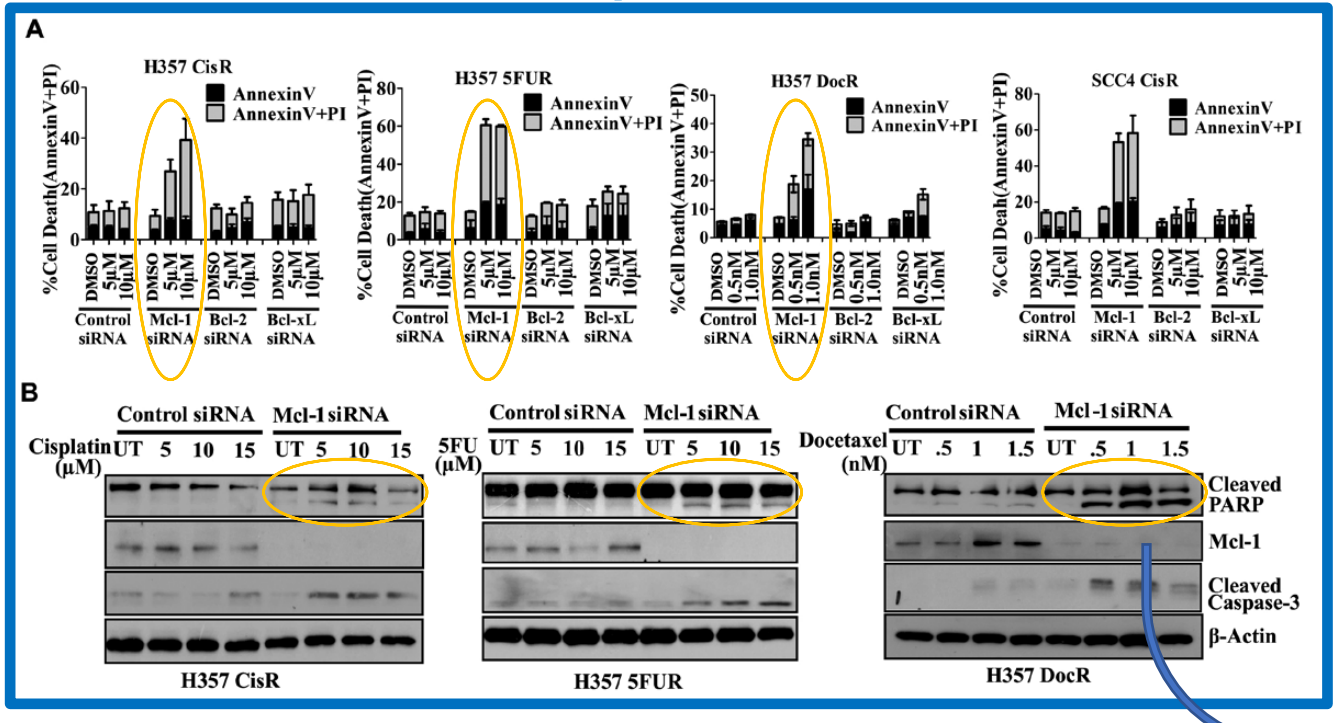
(Q score)

CHT-aR cell lines

PDCs pat. samples

results:

genetic inhibition / knock-down of Mcl-1 restores platin-derived cell death



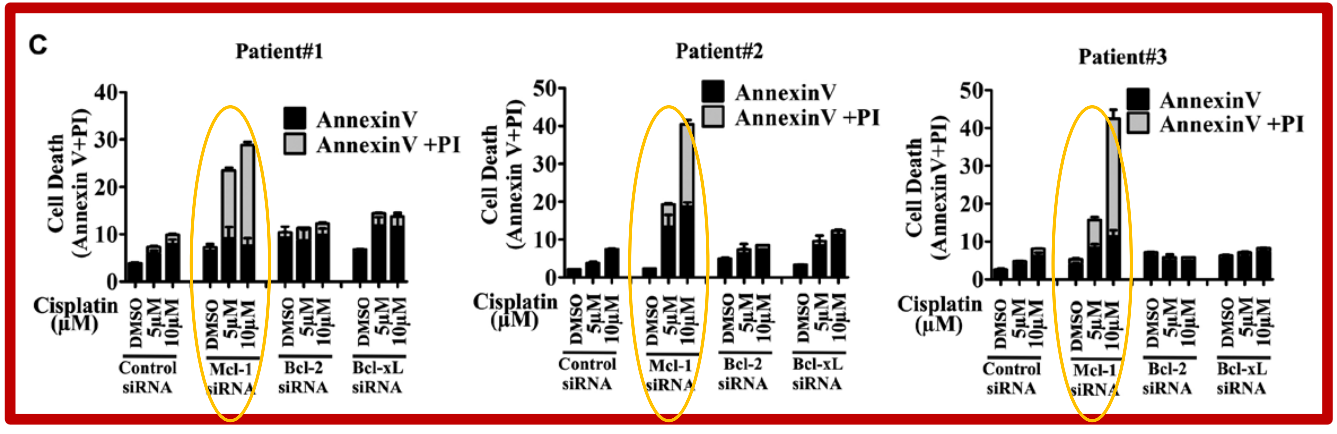
Mcl-1 knockdown restores drug-induced apoptosis in all 3 cell lines !

(cell death assay via flow cytometry)

restored apoptosis

cells transfected with siRNA

results:



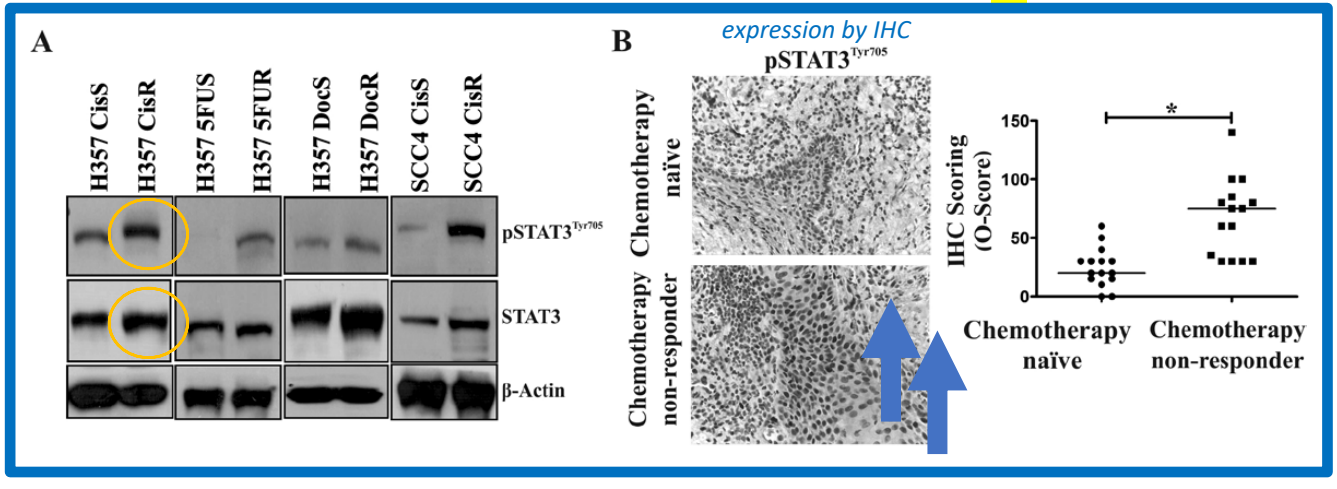
- PDCs from 3 different non-responders
- ONLY Mcl-1 knockdown restores platin-derived cell death ! (indicating major role of Mcl-1 above Bcl-2/Bcl-xL)

CHT-aR cell lines

PDCs pat. samples

results:

which mechanisms lead to upregulation of Mcl-1 in CHT-aR cells?



STAT3 & pSTAT3 upregulated in CHT-aR cells

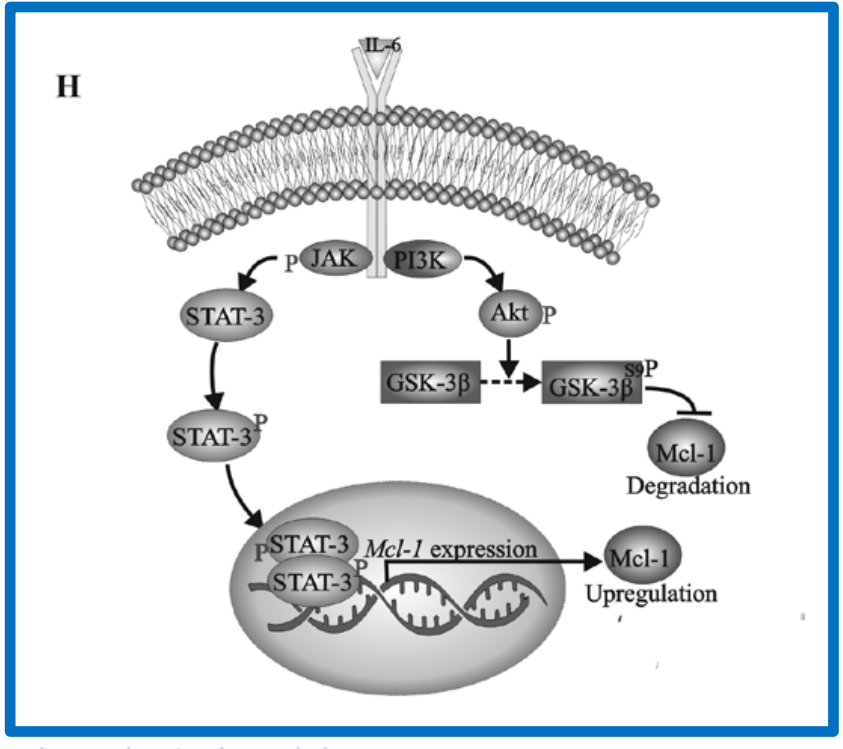
(Mcl-1 promoter contains a STAT3-binding site,

after STAT3-phosphorylation dimerized pSTAT3 translocates in the nucleus)

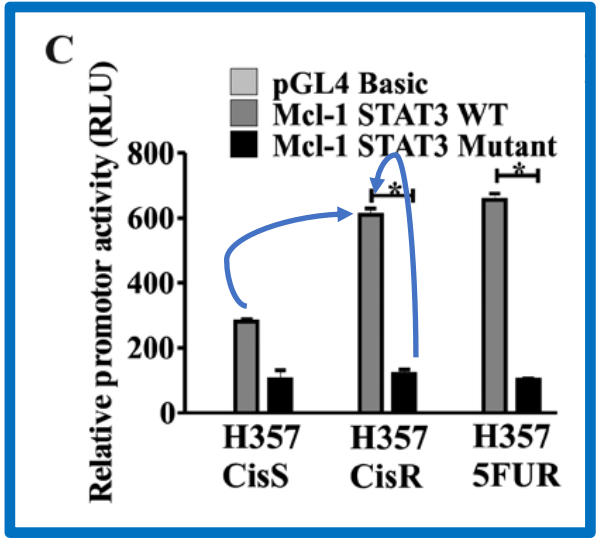
- CHT-R cells show elevated cancer stem cell population (→ stem cell PCR array)
- STAT3-mediated Mcl-1 mRNA upregulation

results:

which mechanisms lead to upregulation of Mcl-1 in CHT-aR cells?



hypothetical model



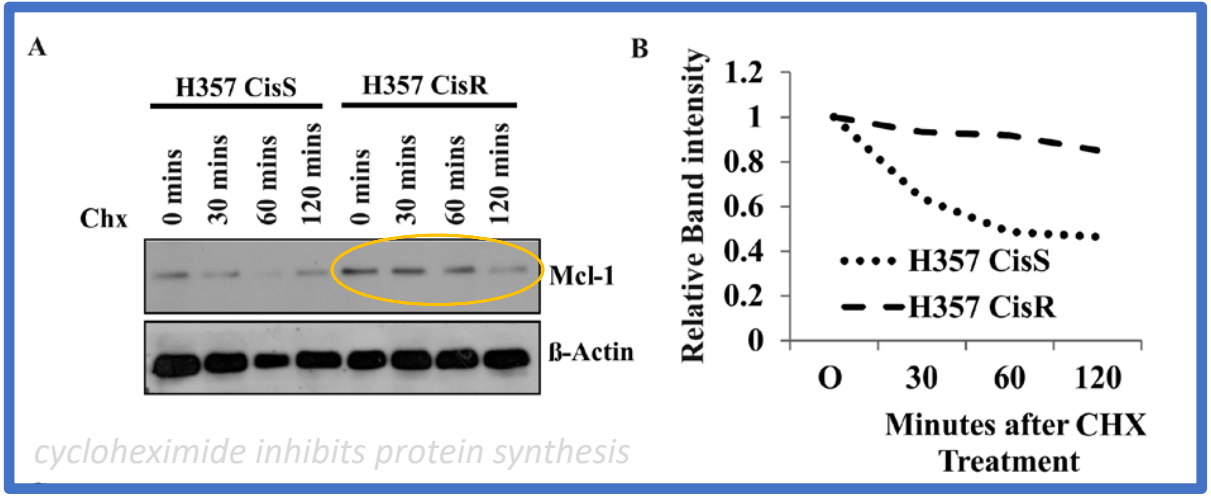
Mcl-1 promoter activity ↑ in CHT-aR compared to CHT-S cell lines *(by luciferase assay)*

Cell lines cotransfected with either WT or Mutant STAT3 binding site (of Mcl-1 promoter)

→ activated STAT3 upregulates Mcl-1 in CHT-R cells !

results:

which mechanisms lead to upregulation of Mcl-1 in CHT-aR cells?



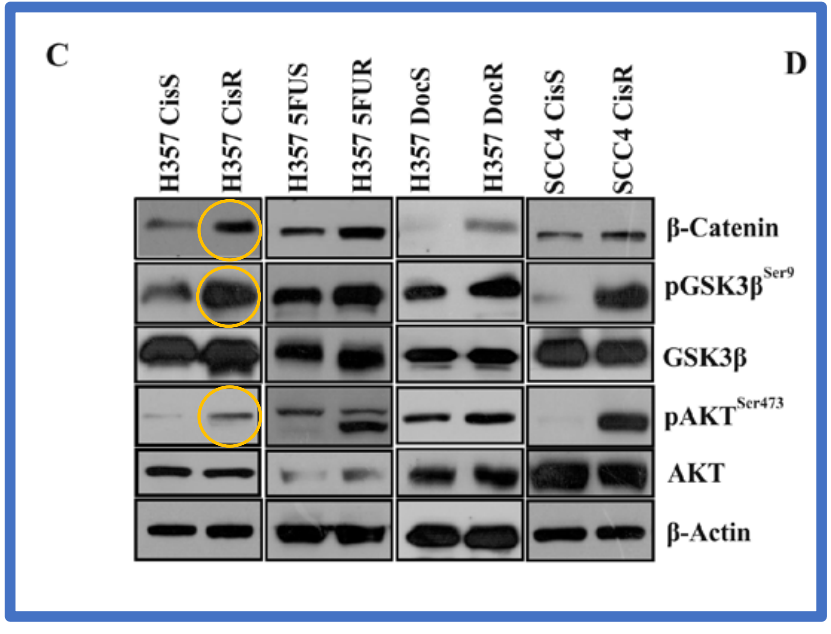
cycloheximide inhibits protein synthesis

Mcl-1 half-life: < 1h

- Mcl-1 stabilization in CHT-aR Vs. CHT-S !
- (Mcl-1 is more stable in cisplatin-resistant cells, less proteasomal degradation)

results:

which mechanisms lead to upregulation of Mcl-1 in CHT-aR cells?



immunoblotting

CHT-aR cells show enhanced:

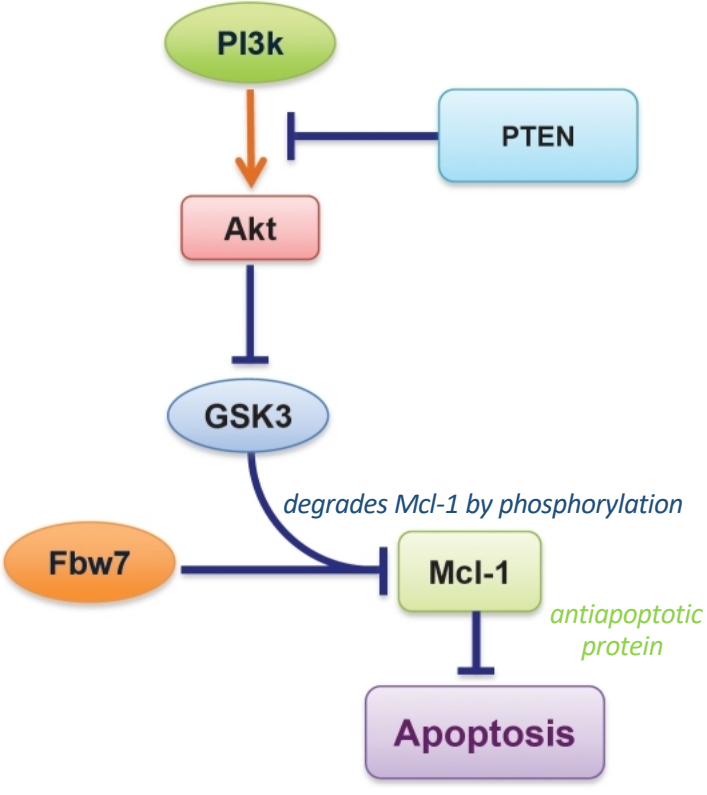
→ ß-catenin expression

→ inactivation of GSK3ß (by phosphorylation Ser9)

→ activation of AKT (by phosphorylation Ser473)

results:

which mechanisms lead to upregulation of Mcl-1 in CHT-aR cells?



(from „Mcl-1 Ubiquitination and Destruction“ DOI: 10.18632/oncotarget.242)

CHT-aR cells show:

→ ß-catenin expression

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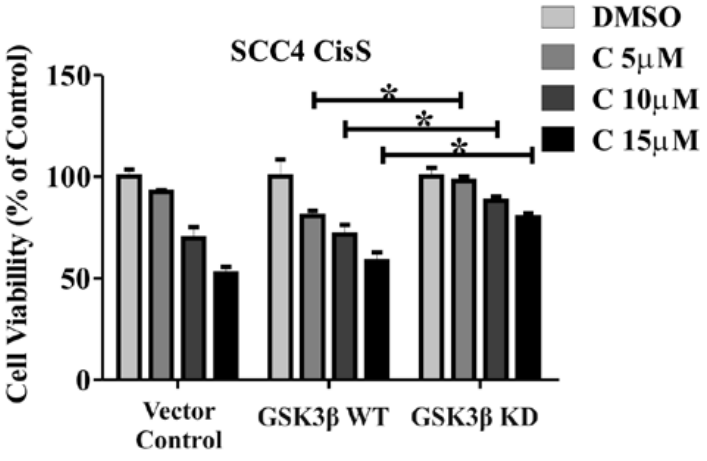
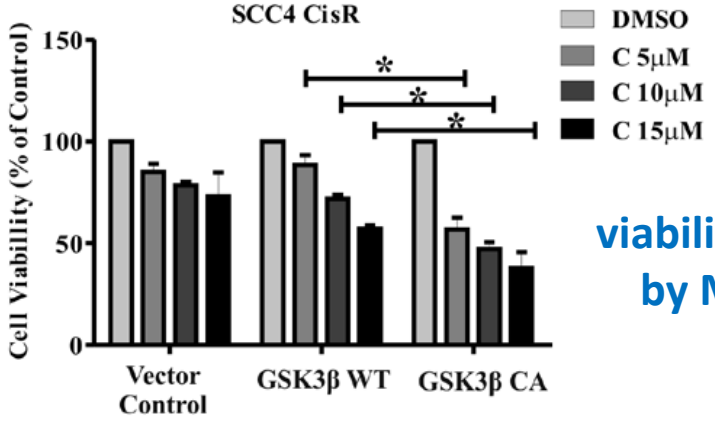
Stabilization of Mcl-1 !

results: role of GSK3ß in de-stabilization of Mcl-1





viability measured by MTT assay

- ectopically overexpression of GSK3ß WT / KD (kinase dead)

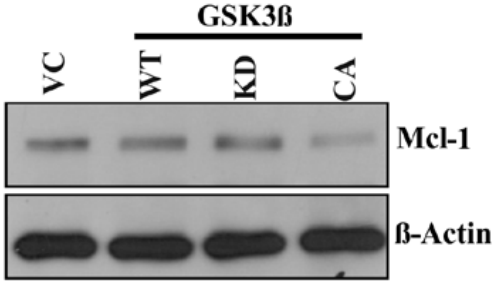
→ ect. expr. of GSK3ß KD rescued Cis-S cells from cisplatin toxicity

→ ect. expr. of GSK3ß CA in Cis-R cells restores cisplatin-induced cell death

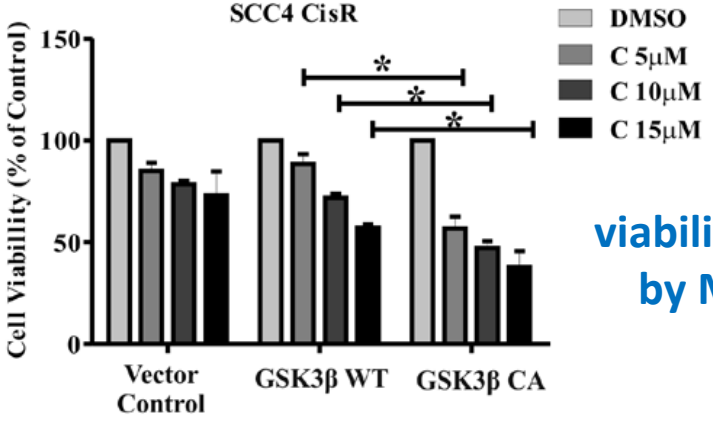
CA = constitutively active

results:

role of GSK3ß in de-stabilization of Mcl-1



as consequence of reduced Mcl-1 expression



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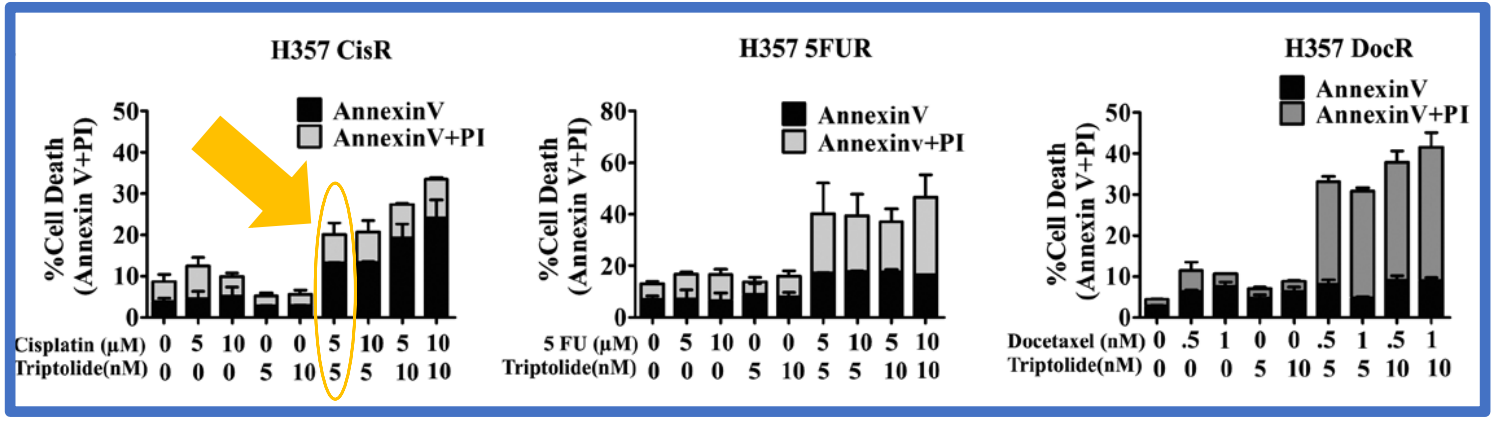
results: inhibition of Mcl-1 by Triptolide (pharmacological)



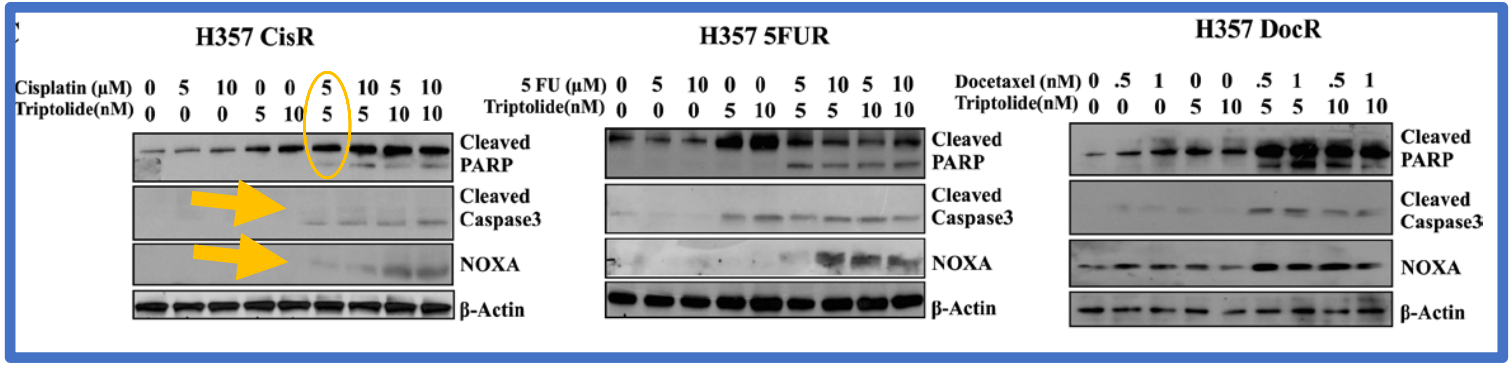
- inhibits mRNA expression of Mcl-1 specifically

→ synergistic effect to CHT induces apoptosis in Cis-R cell lines

→ apoptosis markers significantly increased (cleaved PARP + caspase-3 / NOXA level ↑)

→ similarly apoptosis induction in CHT non-responding PDCs !

results: inhibition of Mcl-1 by Triptolide (pharmacological)



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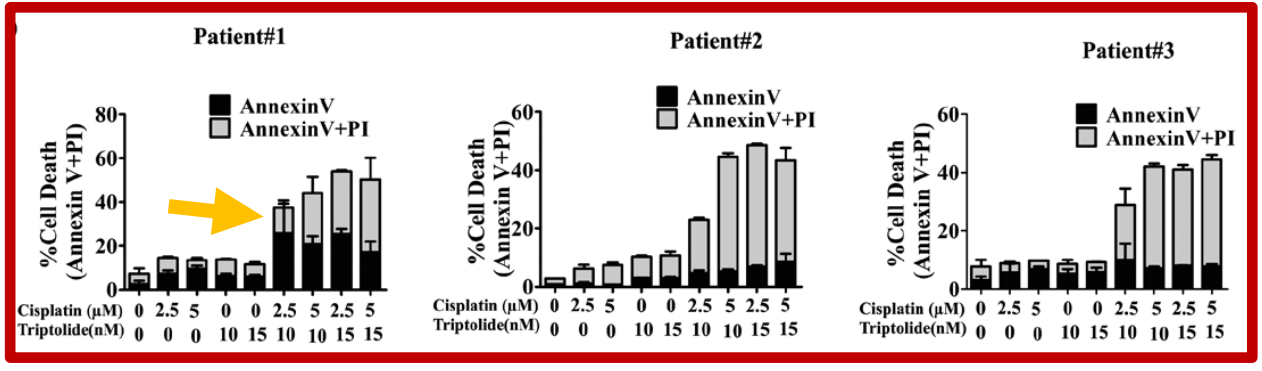

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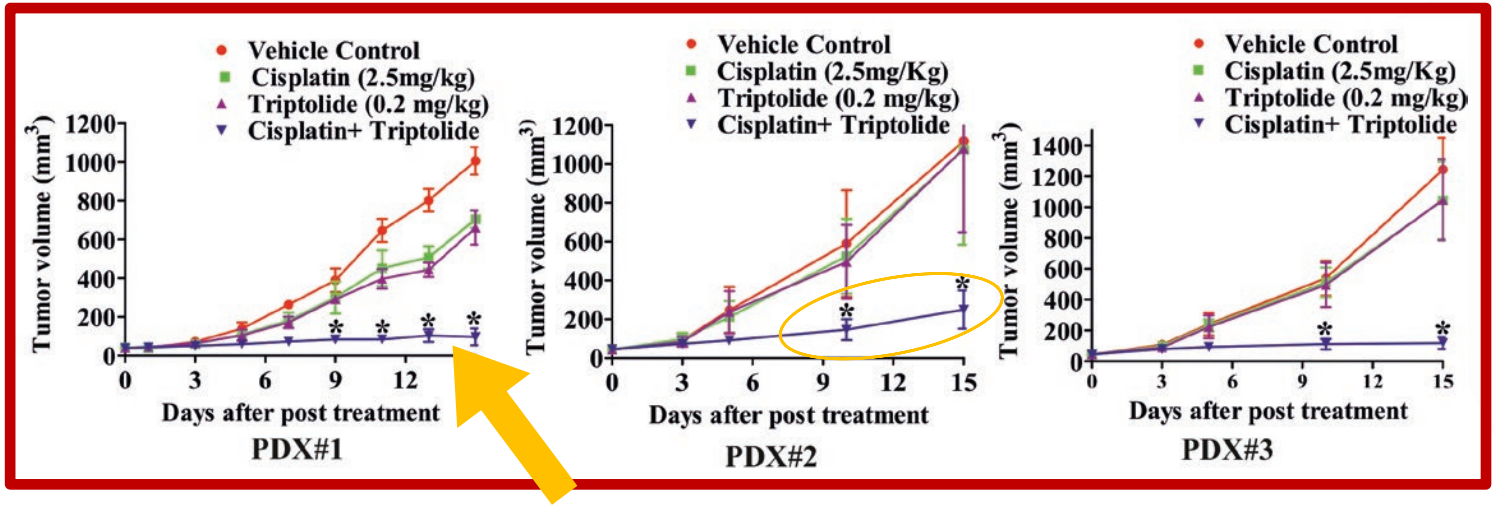
results: inhibition of Mcl-1 by Triptolide (in vivo)



- transplantation of CHT non-responders PDCs to mice
- combi treatment of Triptolide + Cisplatin

→ far less tumor growth compared to single agent-treated groups !!

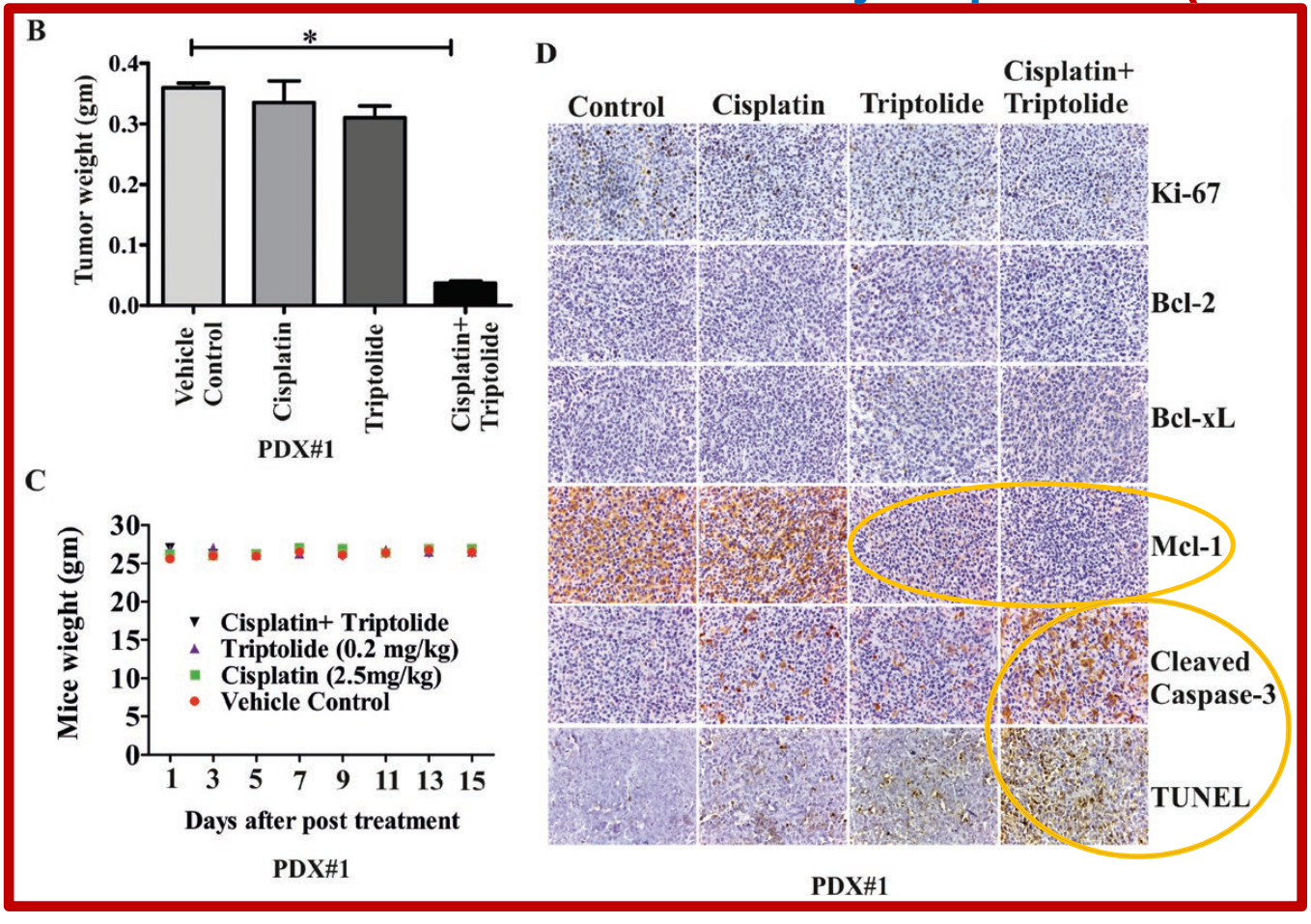
(+ less TU-weight at the end of experiment)

CHT-aR cell lines

PDCs pat. samples

results:

inhibition of Mcl-1 by Triptolide (in vivo)



IHC:

Triptolide inhibits only Mcl-1 !

Cleaved caspase-3 & TUNEL only in combi treatment !

discussion:

- reduced apoptotic response as a hallmark of CHT-R (by overexpressing Bcl-2 antiapoptotic proteins)
- but multiple mechanisms of CHT-R (i.e. drug distribution)
- progress in development of inhibitors against Bcl-2, but less data about specific Mcl-1 inhibitor
- Triptolide (Tripterygium wilfordii) currently in Phase-2-trials on CHT-R pancreatic CA

evaluation:

Pros:

- high-standard Paper (impact factor 5,2)
- multiple methods and equal supplementary figures
- high quality figures
- In vitro & in vivo assay (PDX-bearing mice)
- rediscovery of Triptolide for OSCC patients

Cons:

- requires experience in the methods
- confusing hypothetical model (fig. 4H)