From Preclinical Drug Development to Clinical Application

Exploring Novel Therapeutic Strategies

Using the Plant Derived Polyphenol Curcumin

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Problems in Cancer Treatment

Innate & Acquired Resistance

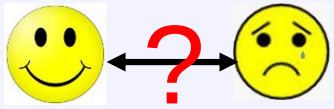
We need to understand variations in drug response

→ discover new substances for treatment

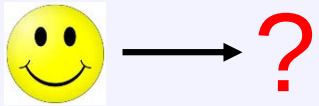
→ develop more efficient therapy strategies using (combinations of) existing drugs

Central questions to be solved

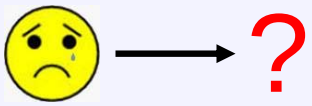
→ Does the substance work



→ How does it work



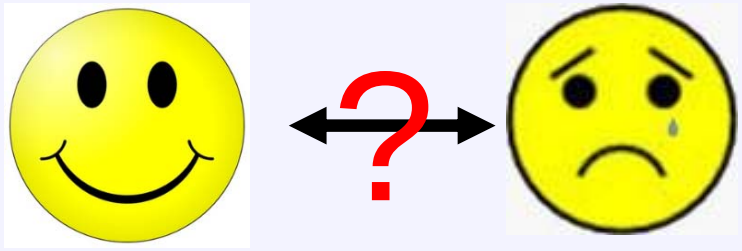
→ Why does it not work



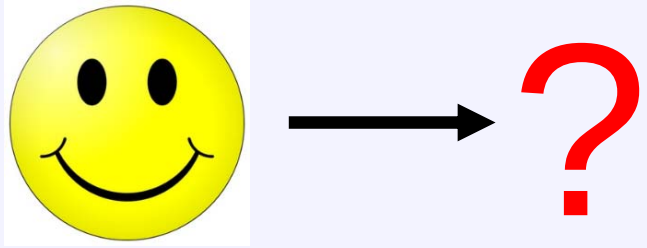
Solution: Test platform for bench to bedside approach

Central Questions / Problems to be solved

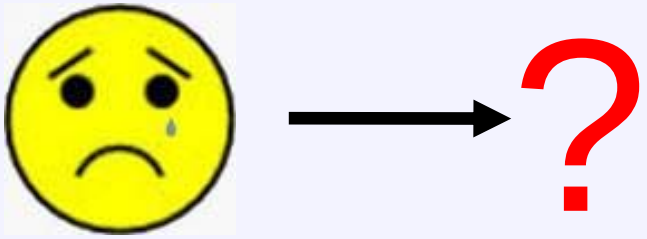
1. Does the substance work?



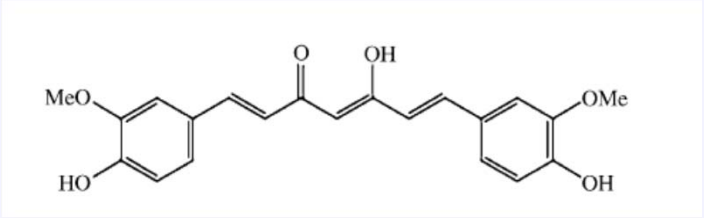
2. How does it work?



3. Why does it not work?

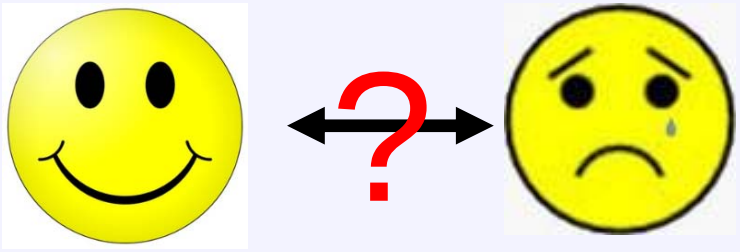


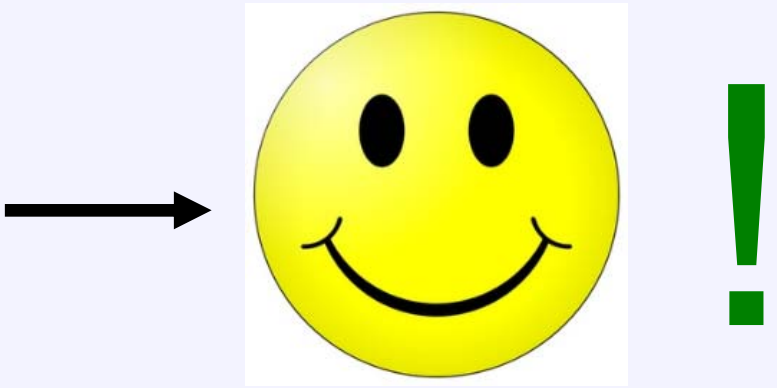
Model Compound: Curcumin



- natural product / belongs to polyphenolic phytochemicals
- harmless and well tolerated
- epidemiologic evidence for its chemopreventive effects in various tumors

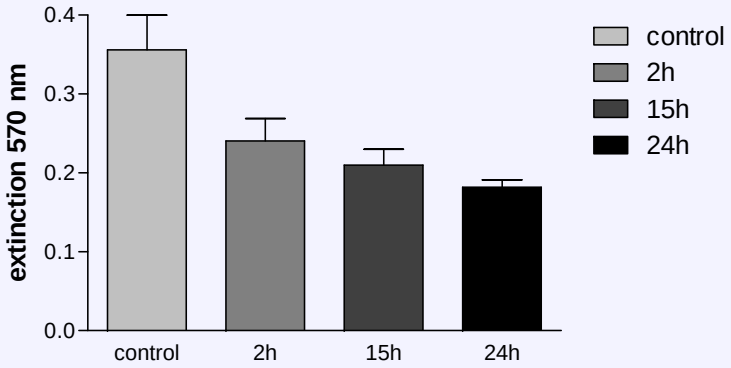
Responsiveness



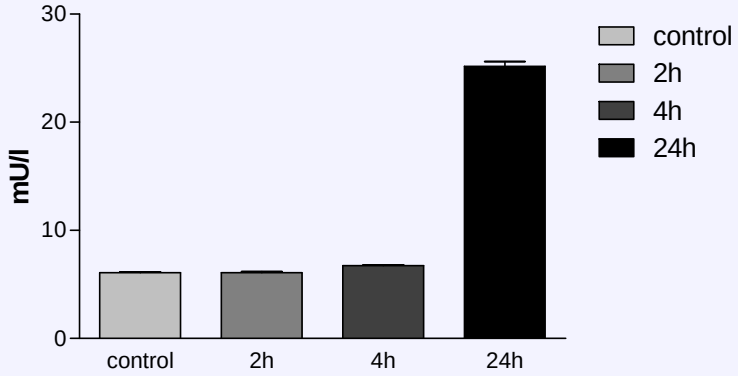


Curcumin induces Apoptosis

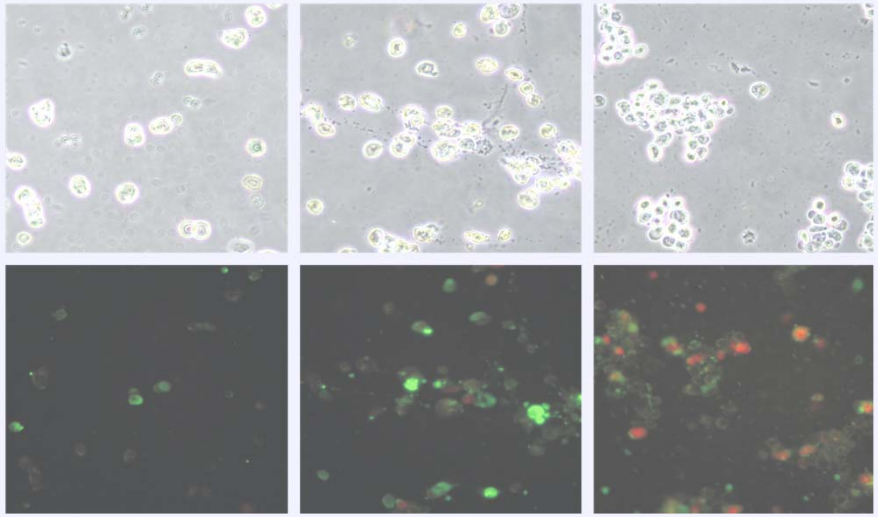
MTT-Test (cell vitality)



LDH-Test (cell damage)



Annexin V / Propidiumiodide



control 2h 24h

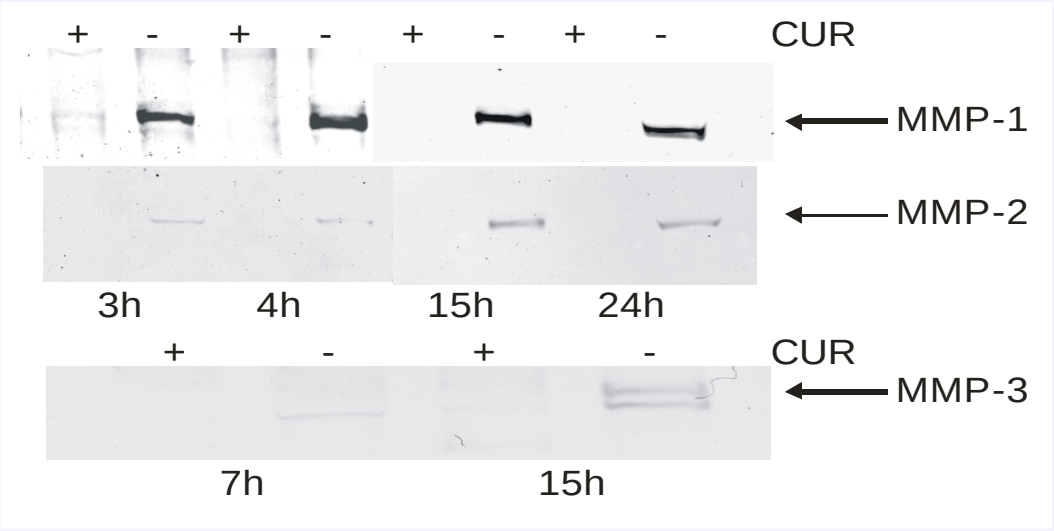
early Apoptosis

late Apoptosis

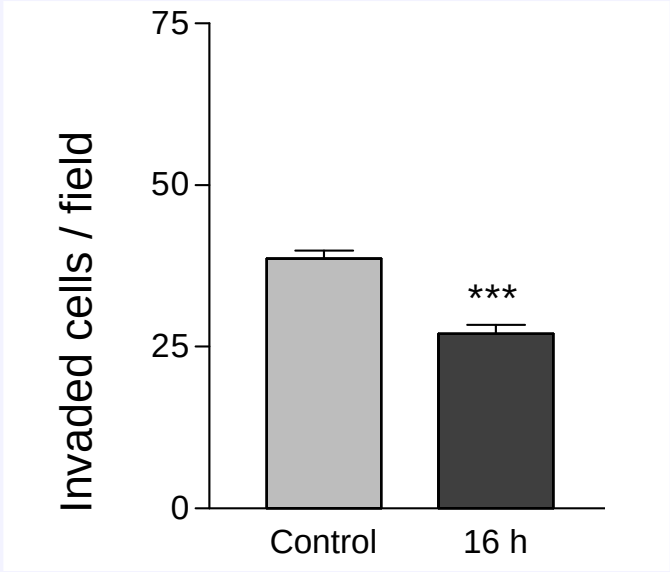
(Bachmeier *et al*., Cell. Physiol. Biol. 2007)

MMP-Expression/-Activity and Invasiveness

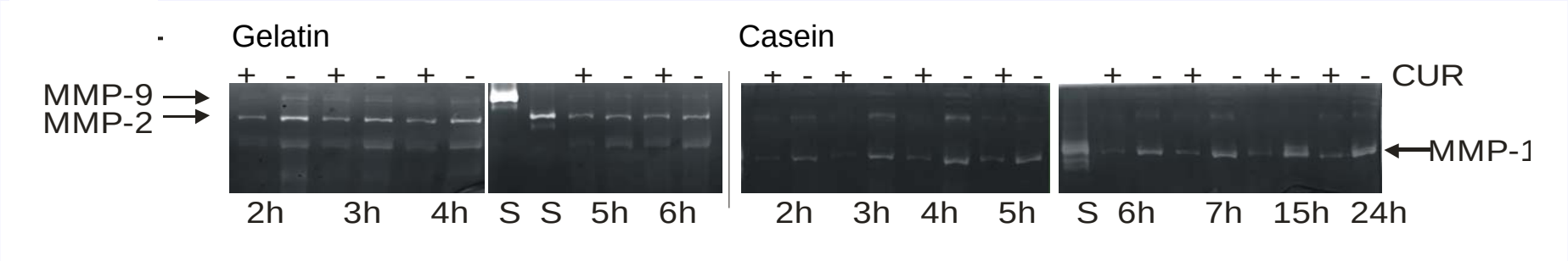
Western Blots



Matrigel-Assay (Boyden Chamber)



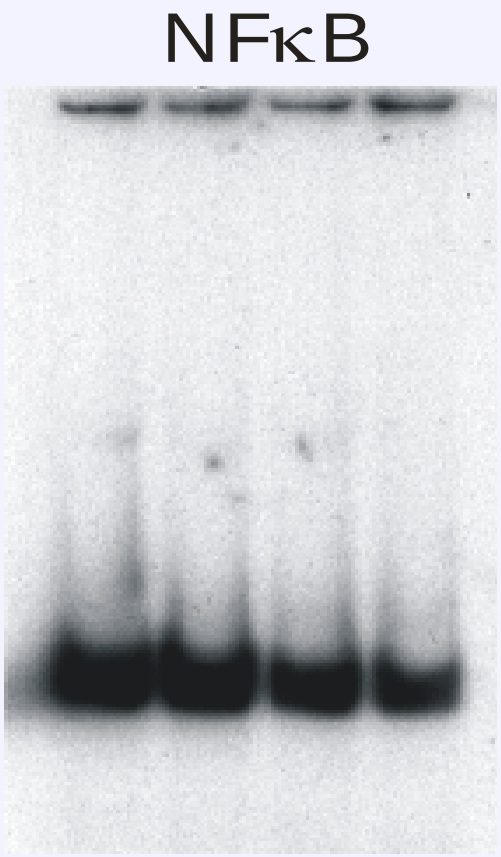
Zymography



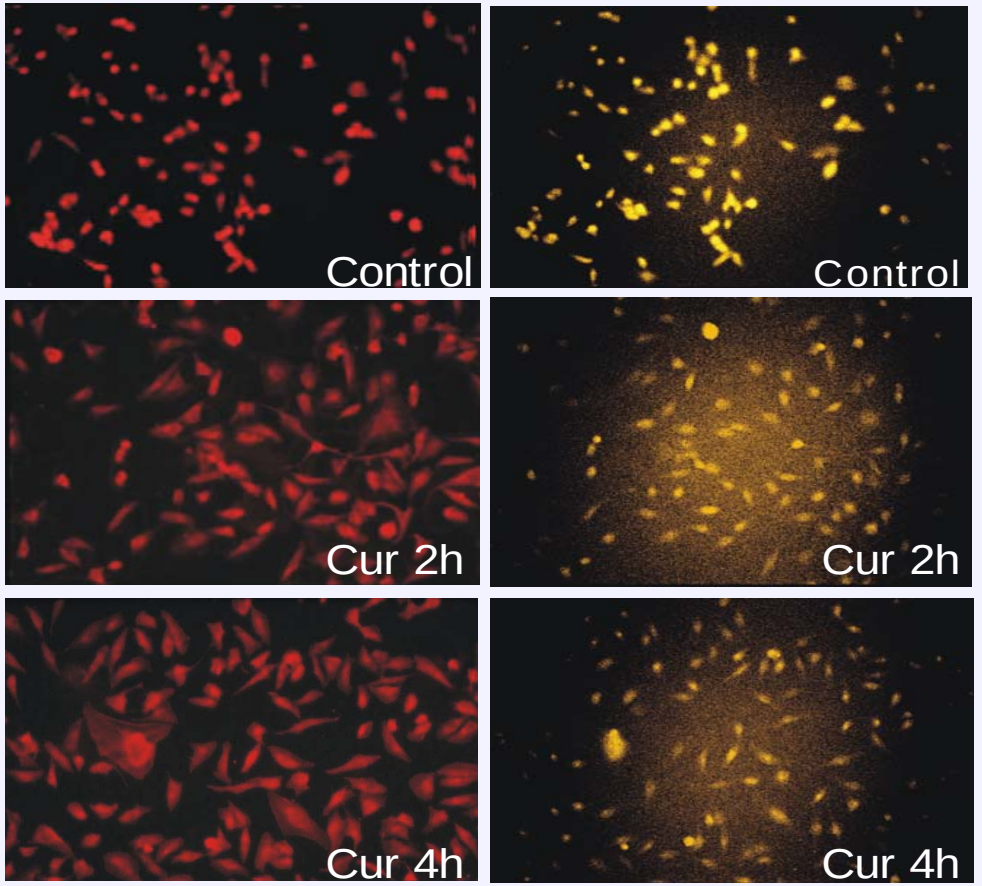
(Bachmeier *et al*., Cell. Physiol. Biol. 2007)

Transcription Factor Activity

EMSA



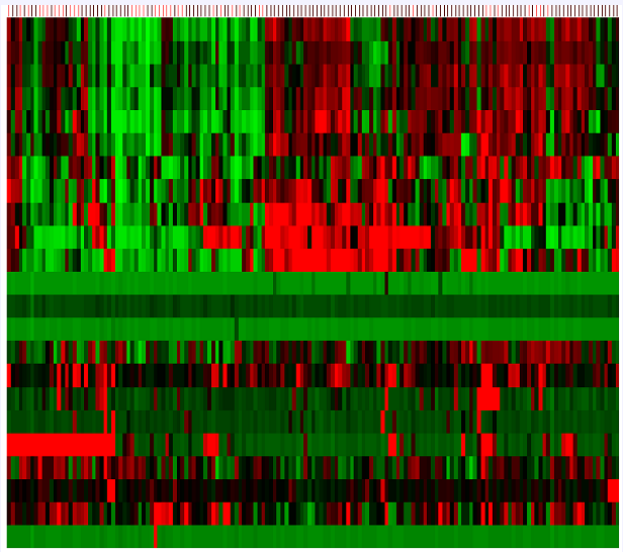
Translocation-Assay

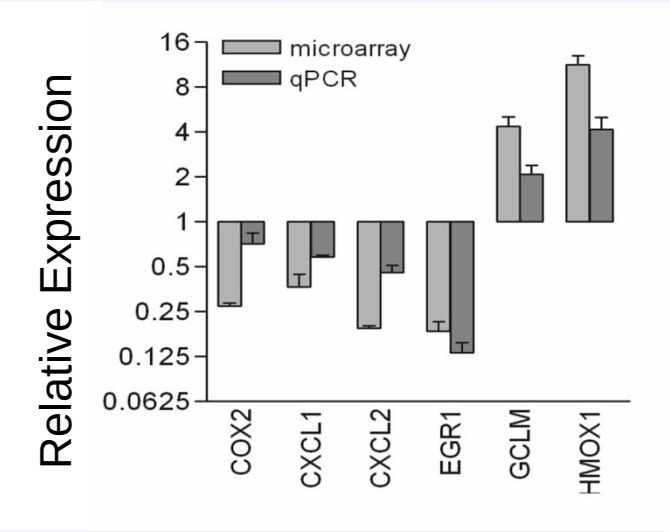


(Bachmeier *et al*., Cell. Physiol. Biol. 2007)

Differential Expression

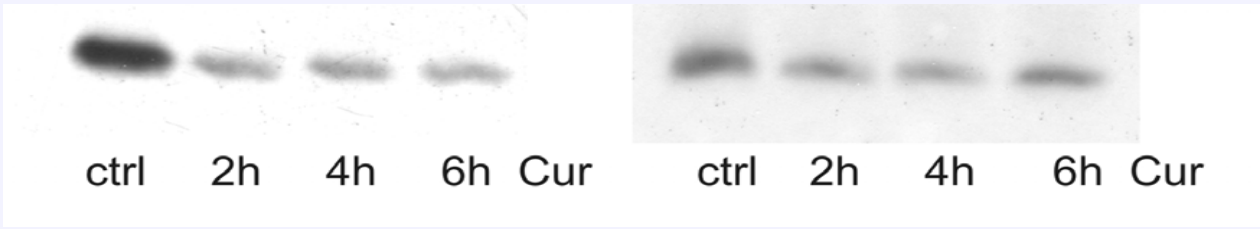
Genexpressions-Analysis with Micro-Arrays (Affymetrix) / Validation with quantitative RT-PCR and Western Blot





Western Blot

CXCL1 **CXCL2**

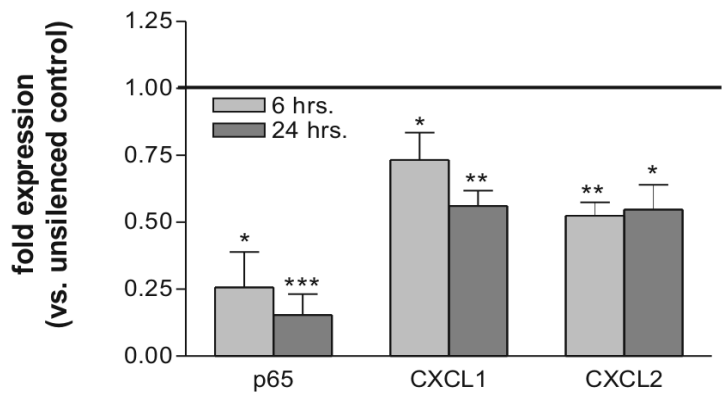


(Bachmeier *et al.*, Carcinogenesis 2007)

Regulation of CXCL1 und CXCL2 via NFκB / IκBα

p65 Gene Silencing → reduction

Quantitative RT-PCR

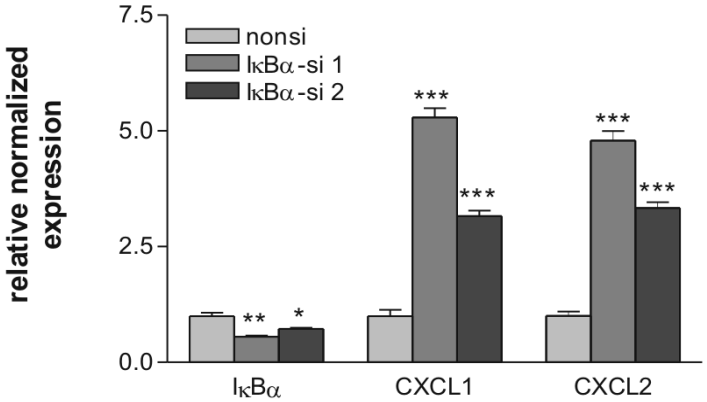


Western Blot

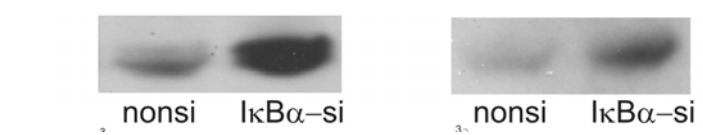
CXCL1 CXCL2

IκBα Gene Silencing → induction

Quantitative RT-PCR



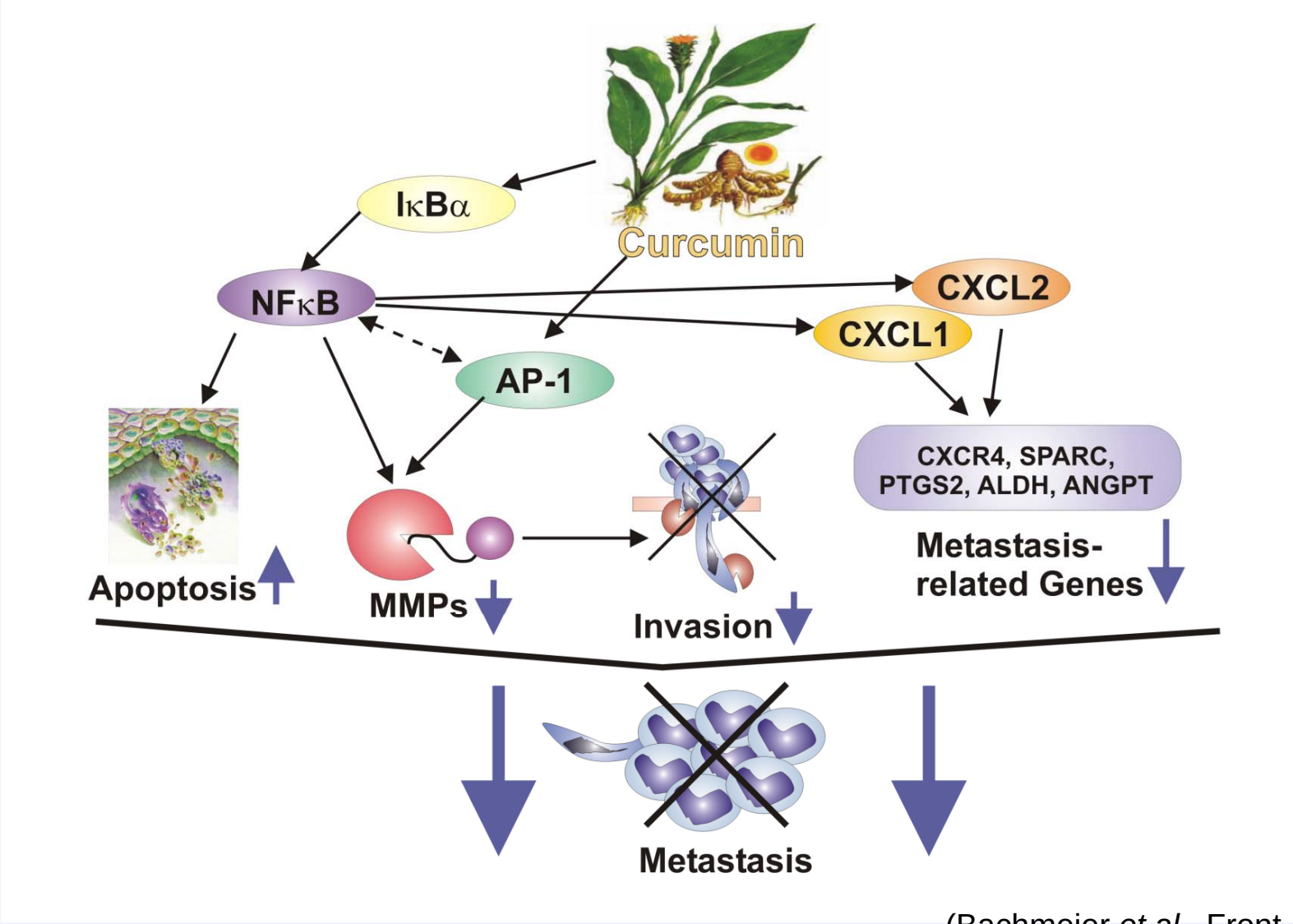
Western Blot



CXCL1 CXCL2

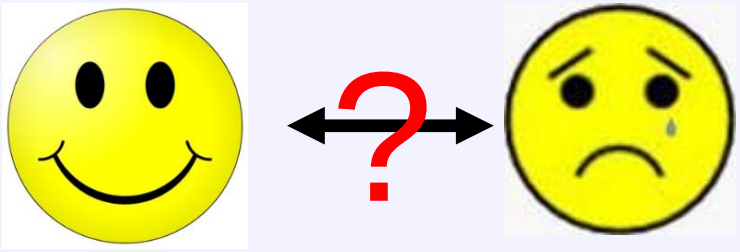
(Bachmeier *et al.*, Carcinogenesis 2007)

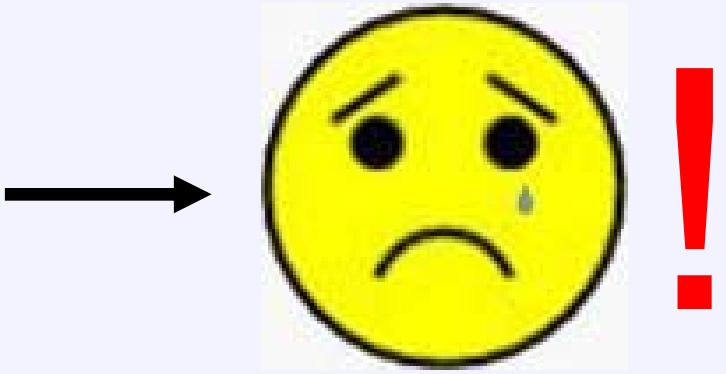
Mechanism of Action



(Bachmeier *et al.*, Front. Biosci., 2010)

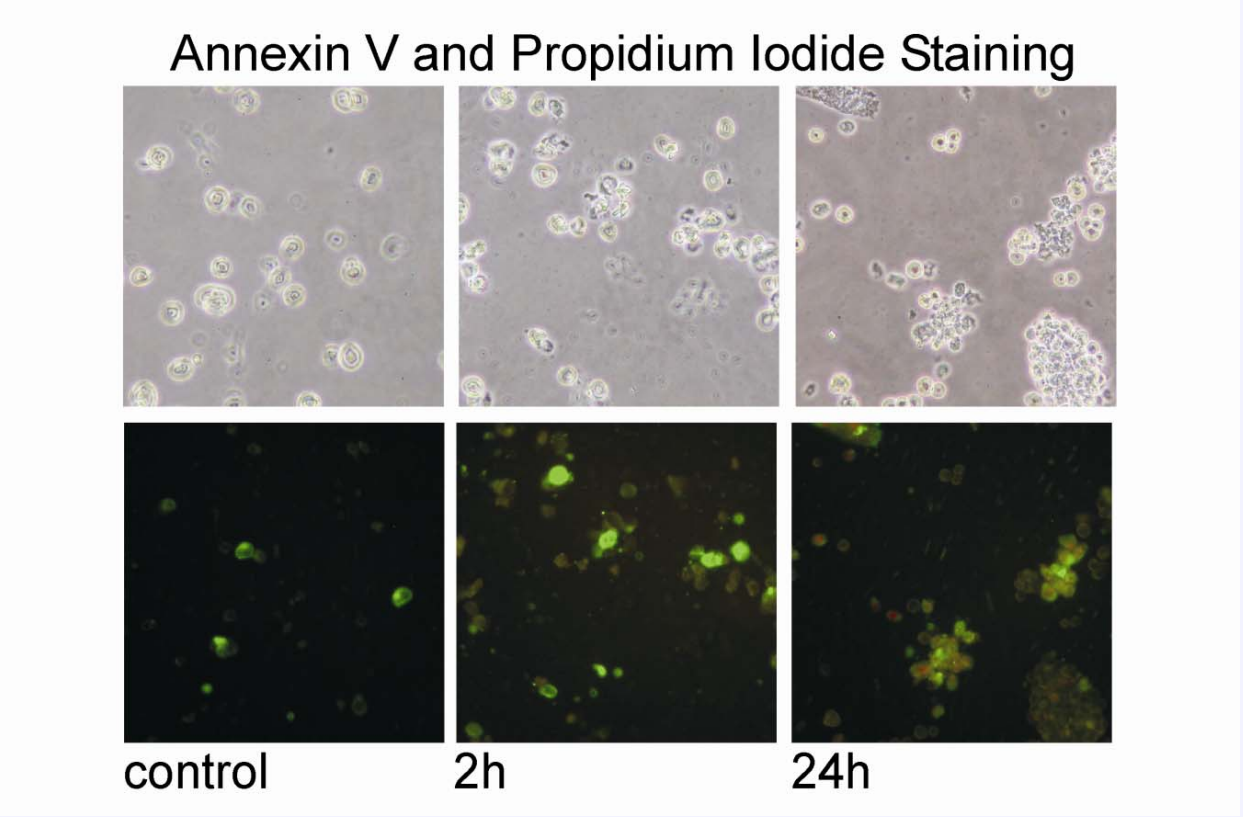
Resistance





Melanoma cells (partial resistance)

Curcumin does not induce late apoptosis/necrosis



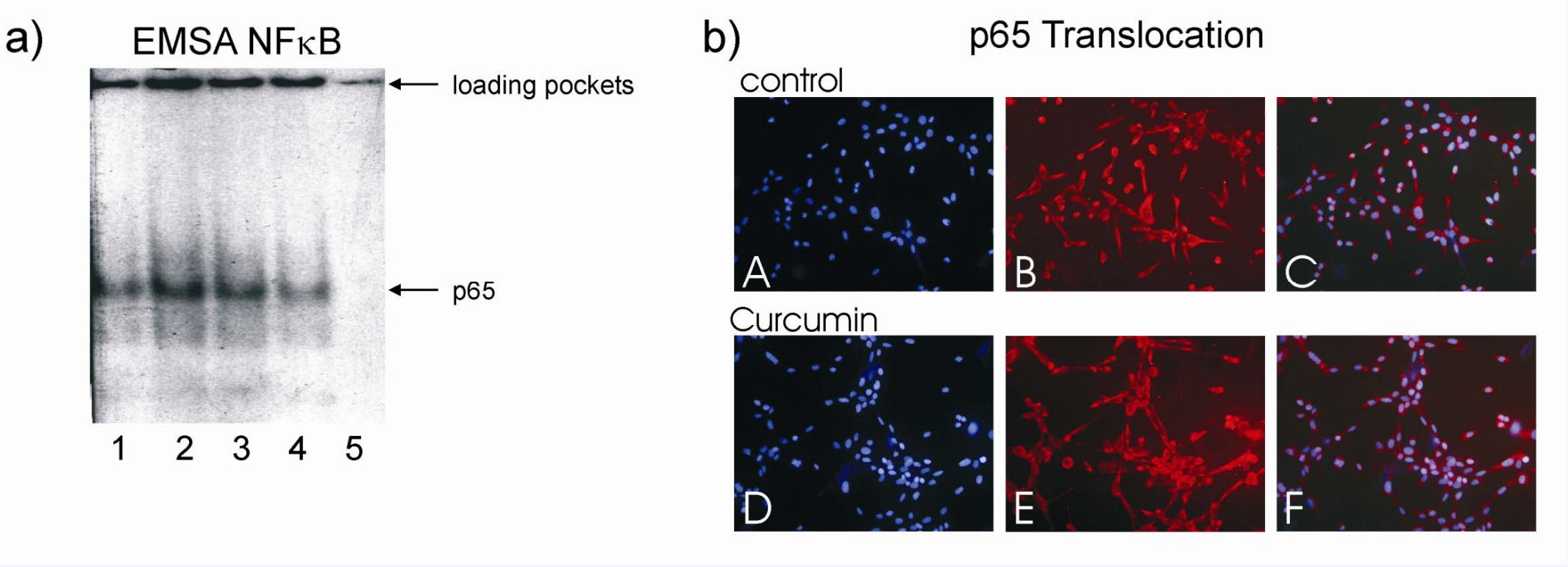
early Apoptosis

late Apoptosis is missing !!!!

(Bachmeier *et al*., Mol. Cancer, 2009)

Melanoma cells (partial resistance)

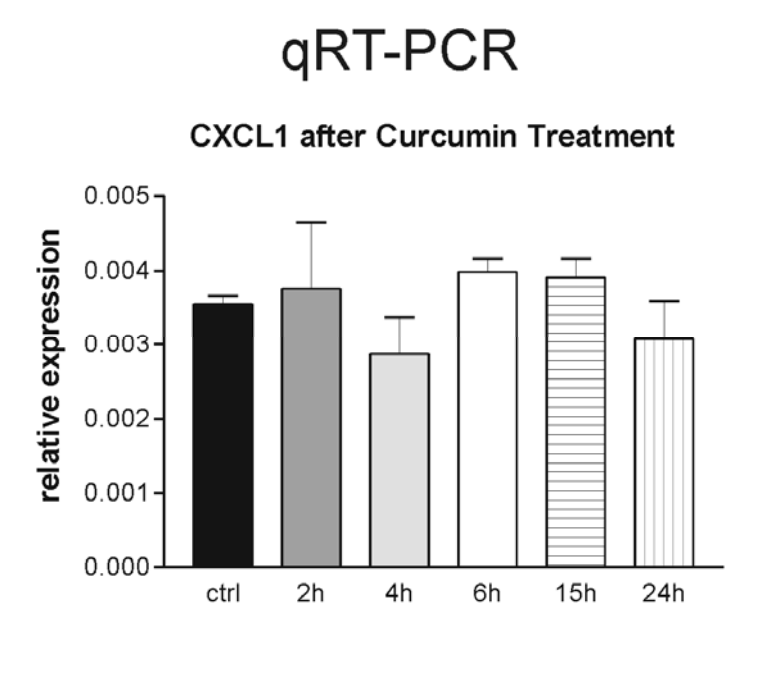
Curcumin does not inhibit NFκB transcription factor activity

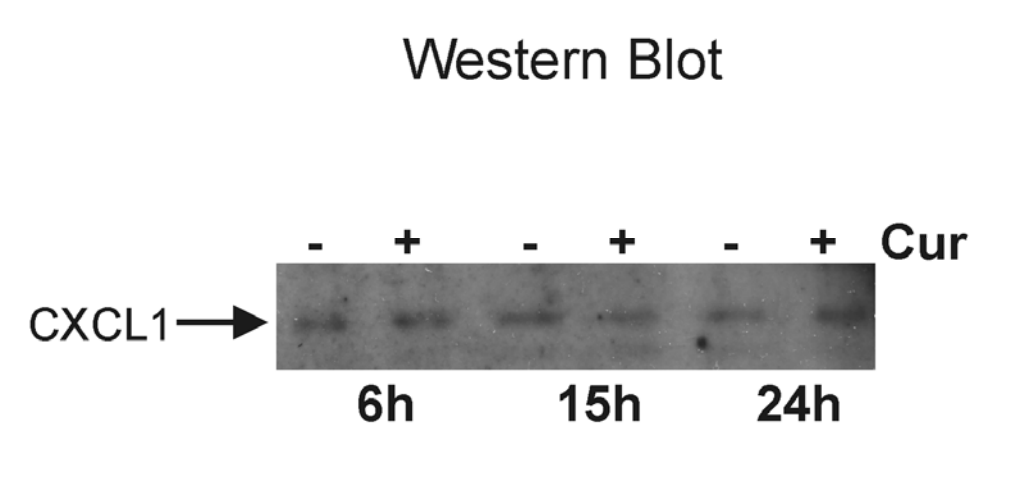


(Bachmeier *et al*., Mol. Cancer, 2009)

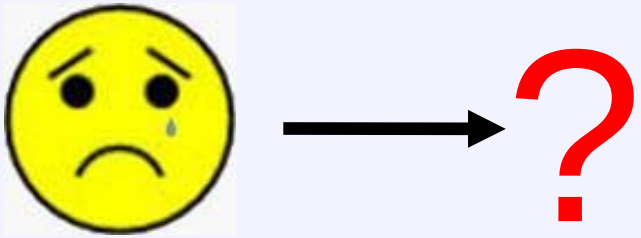
Melanoma cells (partial resistance)

Curcumin does not inhibit CXCL1 expression





(Bachmeier *et al*., Mol. Cancer, 2009)

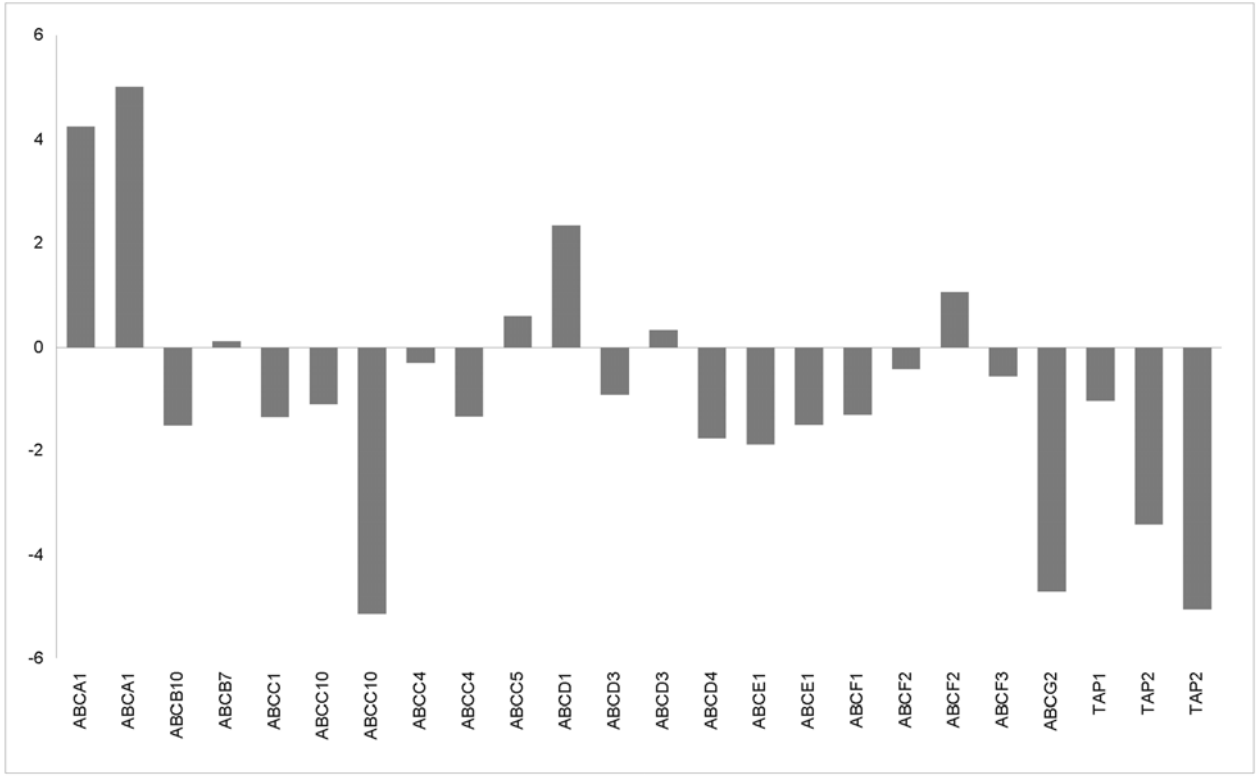


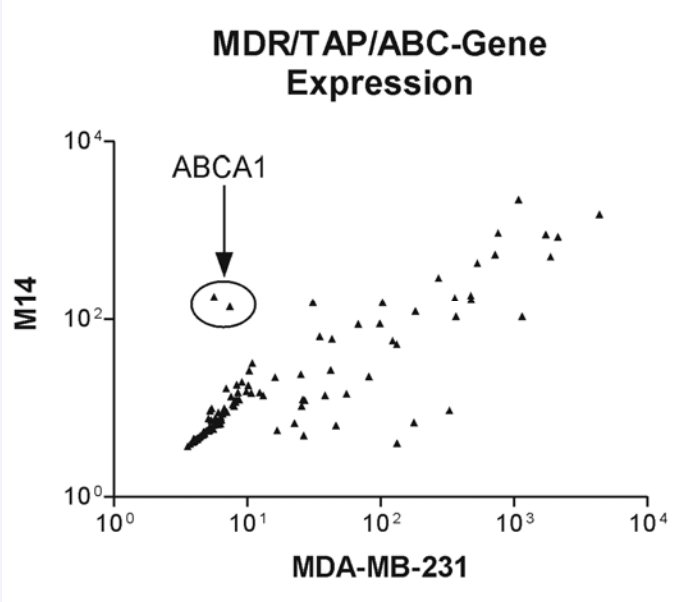
III. Cause of Resistance

Mechanism of Action
Gene Expression Studies

Molecular Mechanism of Resistance

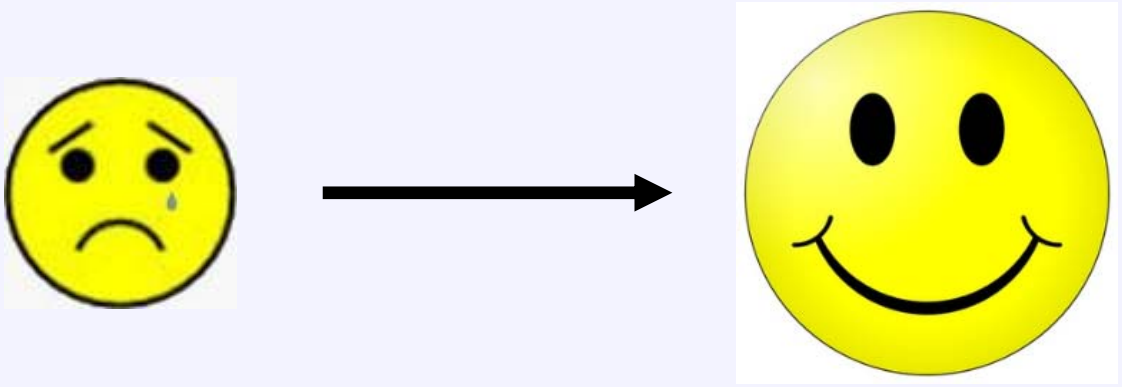
Gene Expression (Affymetix whole human array)





(Bachmeier *et al*., Mol. Cancer, 2009)

Intervention



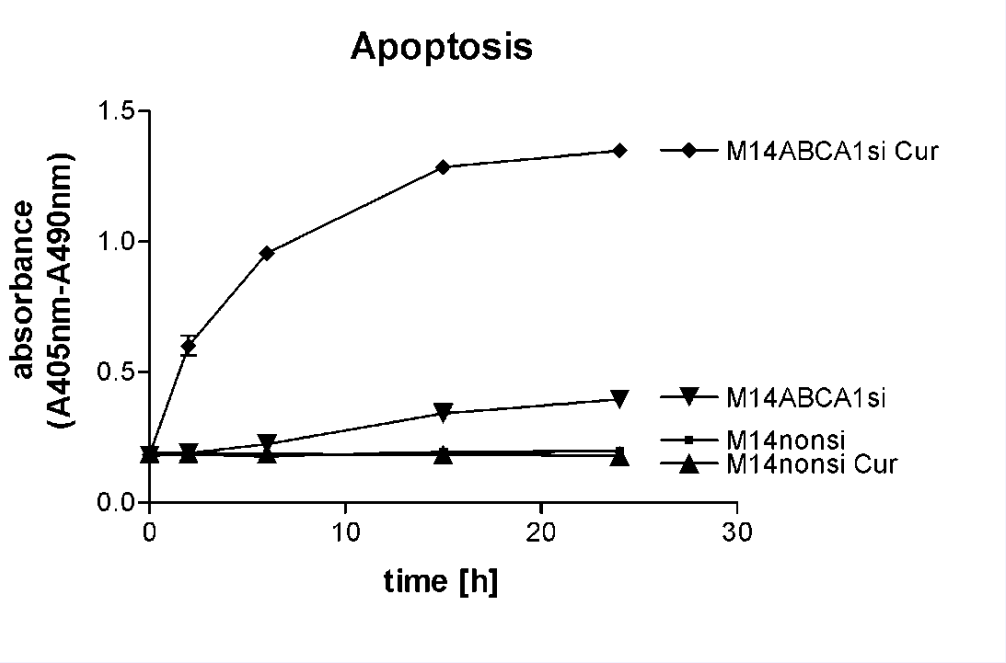
Resistance → **Responder**

Modulation on Molecular Level

ABCA1-silenced Melanoma cells (Curcumin responder)

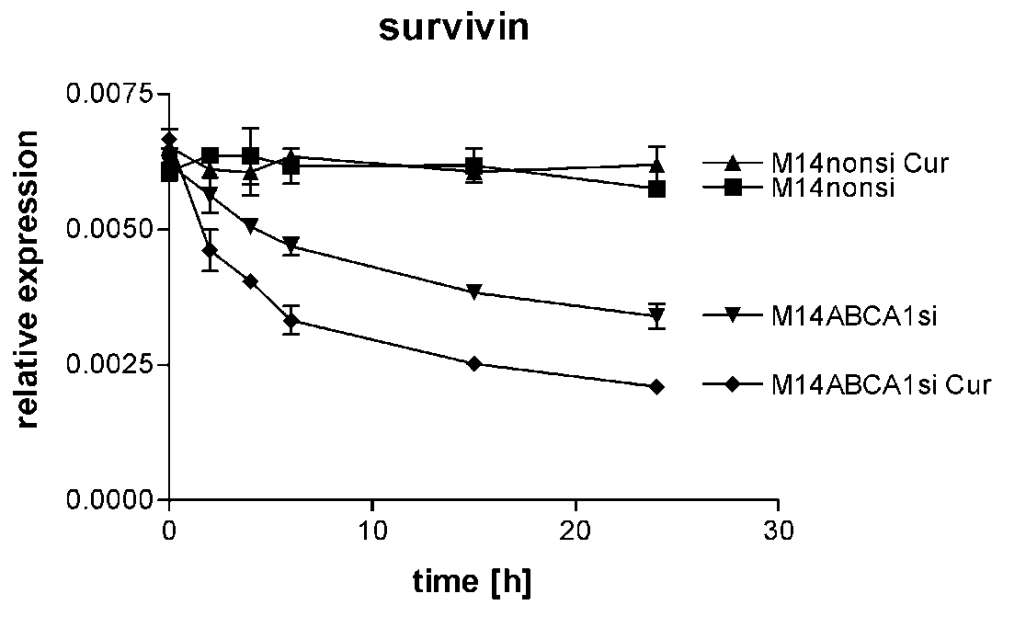
Curcumin induces apoptosis

Cell Death ELISA (Roche)



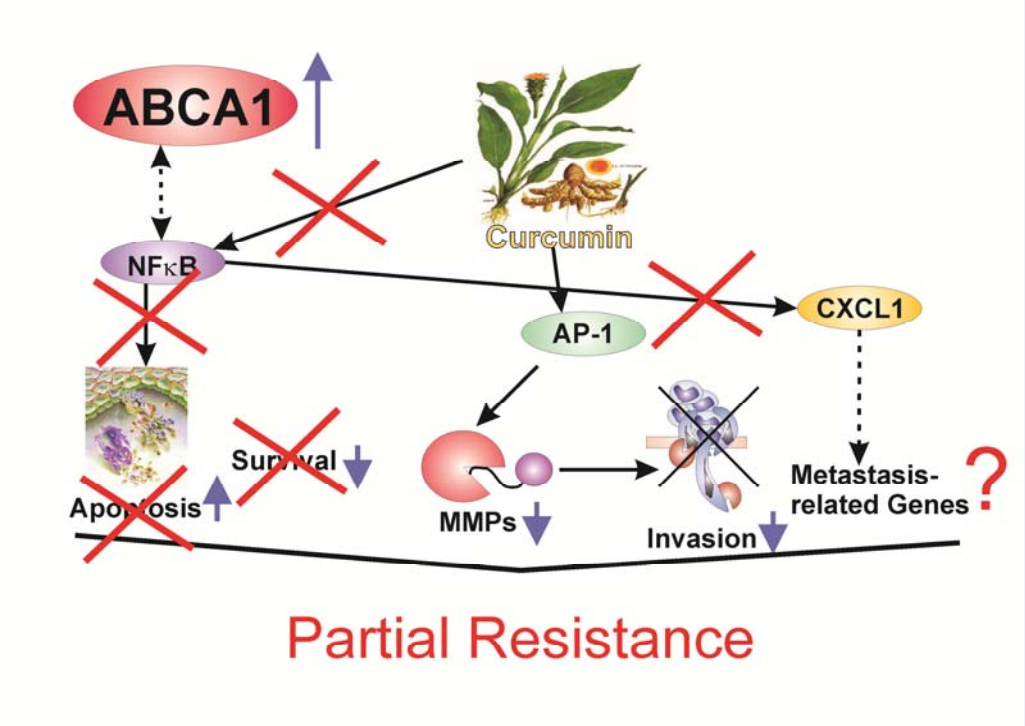
Curcumin inhibits survival

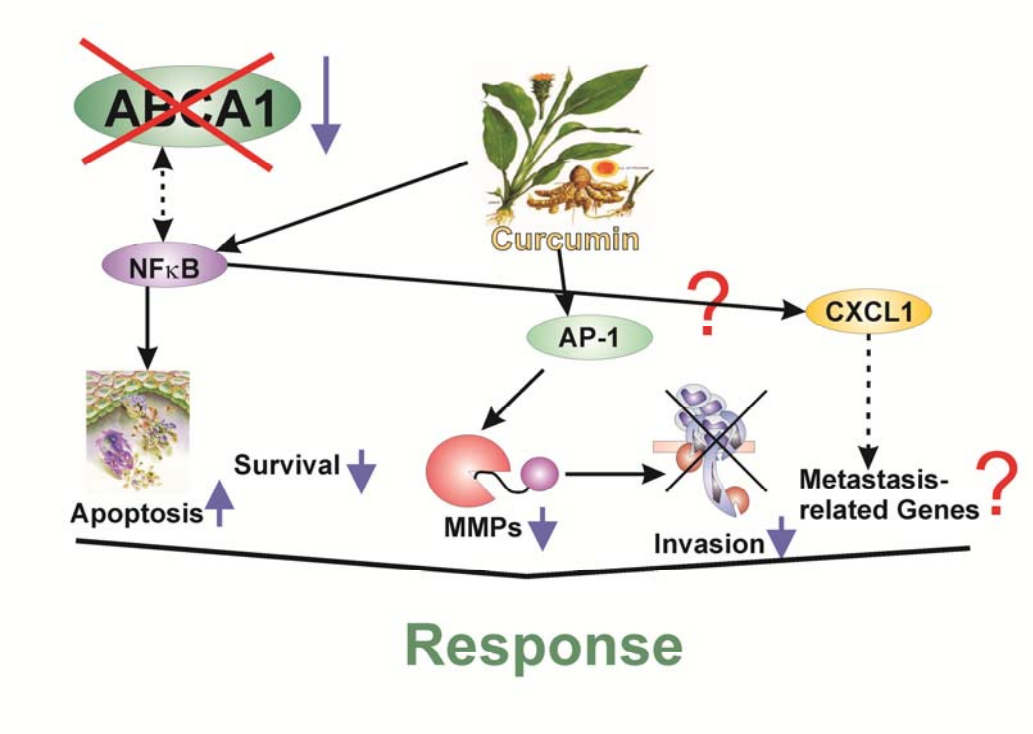
qRT-PCR



(Bachmeier *et al*., Mol. Cancer, 2009)

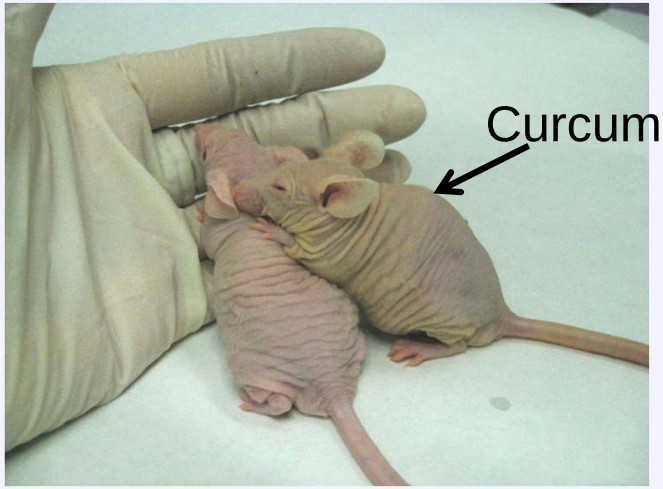
Mechanism of Resistance (Melanoma)

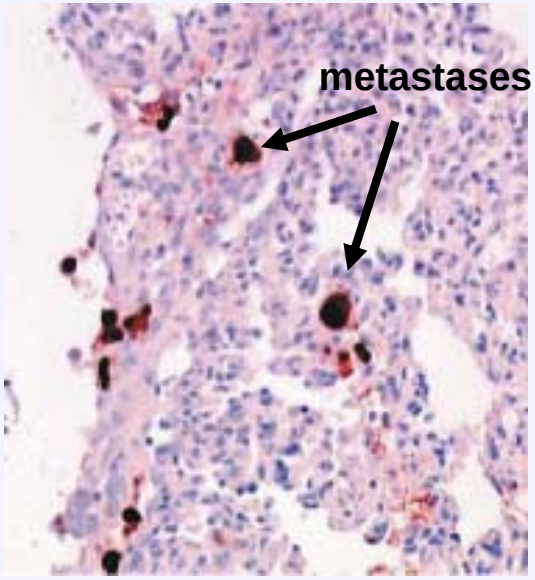




According to:
Bachmeier *et al*., Mol. Cancer 2009
Bachmeier *et al*., unpublished data

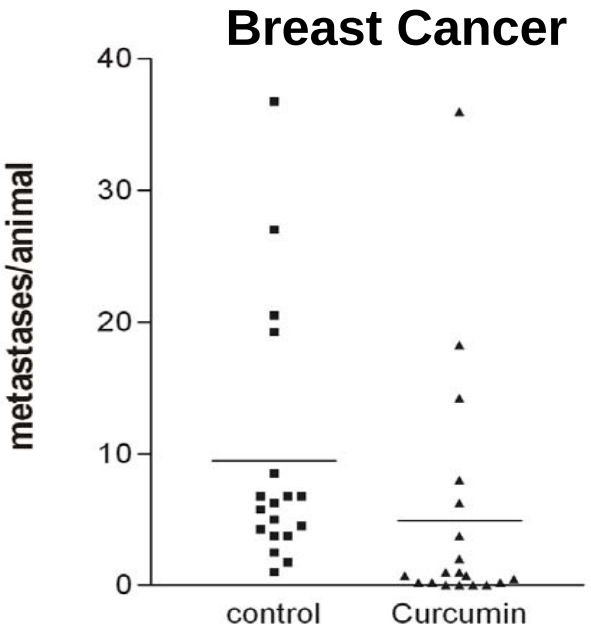
Metastases *in vivo*





human ki67

Lung Metastases



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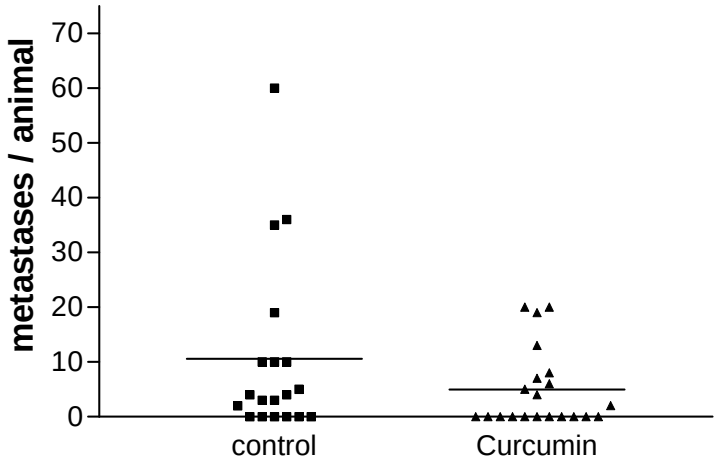

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(Bachmeier *et al*., Cell. Physiol. Biol. 2007; Killian et al, Carcinogenesis, 2012)

Summary of Preclinical Studies

1. **<u>Curcumin has anti-tumor effects in prostate and breast cancer</u>**
   - in vitro
   - in vivo
   - underlying molecular mechanisms partially unraveled (CXCL1/2)

2. **<u>Occurance of resistance in advanced metastatic melanoma</u>**
   - mechanism of resistance unraveled
   - strategy developed to circumvent resistance

→ Curcumin has high potential for chemoprevention and therapy of cancer

Prostate Cancer – an Ideal Target for Chemoprevention

- high incidence
- long latency
- specific tumor marker (PSA)
- identifiable neoplastic leasions

Conclusion

Optimization for Future Studies

- diligent selection of patients
- better monitoring of compliance
- longer (>6 weeks) study period
- Biomarker studies (responsiveness) on blood samples
  - mRNA
  - miRNA
  - mutation sequencing

Perspectives

- Pilot Study with patients with advanced therapy resistant prostate cancer
  - few patients already included (preliminary data)
  - more patients to be recruited
- Further identification of molecular mechanisms and biomarkers
  - miRNAs
  - transcriptomics

→ Recruitment of Research funding

Acknoledgements

- Prof. Dr. M. Jochum
- Prof. Dr. C. Sommerhoff
- Dr. C. Iancu
- I. Mohrenz
- C. Höhnecke
- D. Hutu
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- E. Kronski

- … and all collegues at my „home institute“

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- Prof. Dr. D. Brenner, Ann Arbor, USA
- Dr. A. Albini, Milan, Italy
- Prof. Dr. D. Noonan, Varese, Italy
- Prof. Dr. E. Schleicher, Tübingen
- PD Dr. C. Weigert, Tübingen
- Prof. T. Efferth, Mainz
- Prof. Dr. A. Nerlich, Munich

Thank you for your attention !!!