In Fröhlichkeit
den Menschen dienen

Allgemein öffentliches Krankenhaus
Elisabethinen Linz

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Epigenetics and Mutations

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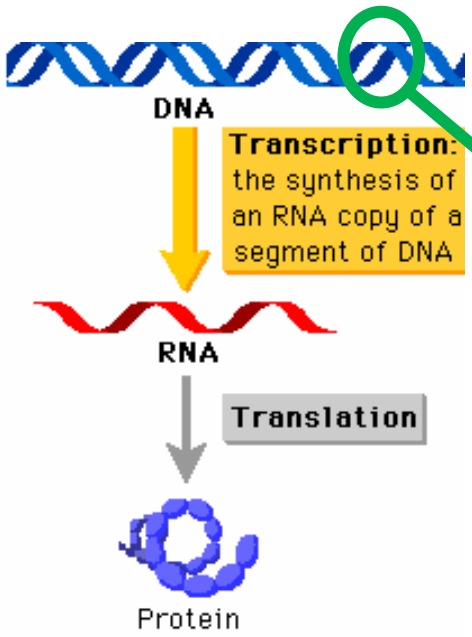
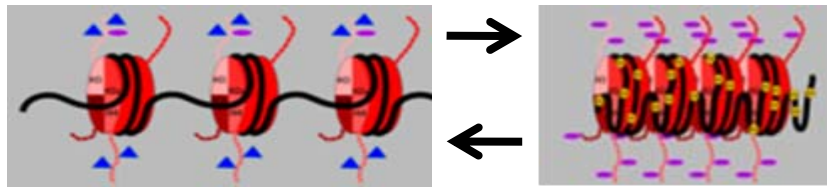
Genetics



Epigenetics

State of Chromatin (= DNA + Histones)

open, active	tight, inactive
transcription	no transcription



1. Epigenetics

Epigenetics and DNA Methylation

Epigenetics
Changes in gene function
without changes in DNA sequence.

found
on DNA (e.g. methylation),
on histones (e.g. acetylation),
and by microRNAs.

Heritable changes in mitosis and meiosis

Consequences
Epigenetic modifications have influence on gene expression:

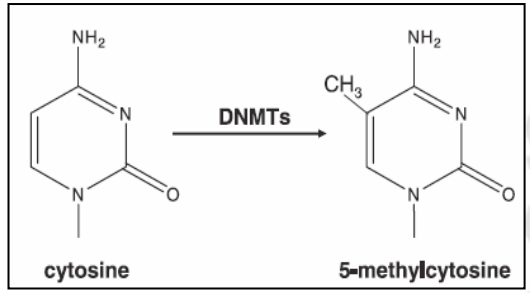
- WHEN
  (e.g. age specific)
- WHERE
  (e.g. cell type specific)

Epigenetics

DNA Methylation: 5´-methyl Cytosine

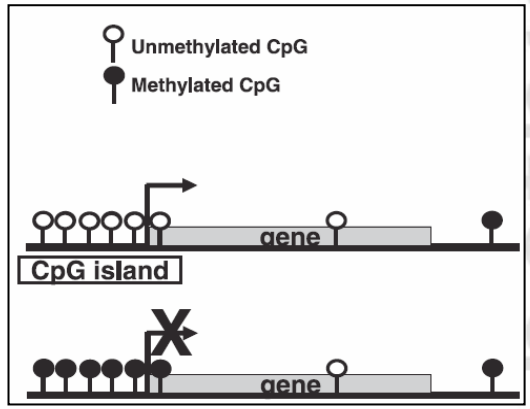
The „fifth“ base

Cytosine- and Guanine- rich (*CpG) islands*, found in promoter regions.



Methylation state in Tumorigenesis

genome wide HYPO-methylation and
local HYPER-methylation (e.g. tumor suppressor genes) leading to genetic instability.

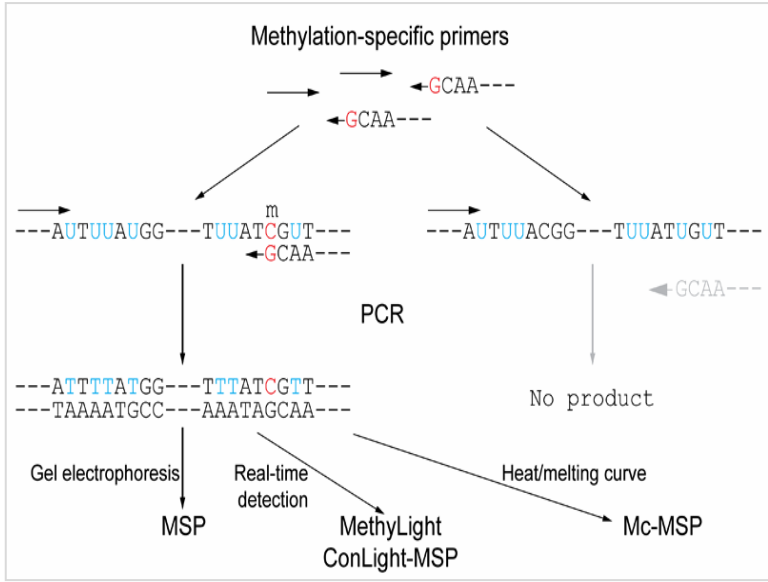


Chuang et.al. 2007

Epigenetics

Detection of methylated DNA with Bisulfite Modification

Unmethlyated Cytosines → Uracile
Methylated Cytosines → Cytosines



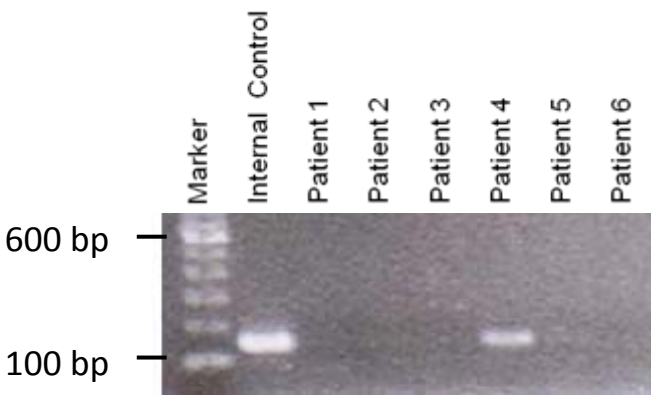
Basics for Assays

- Sequencing
- Methylation Specific PCR = MSP

Epigenetics

Detection of methylation status in **SOCS1**

in patients with essential thrombocythemia

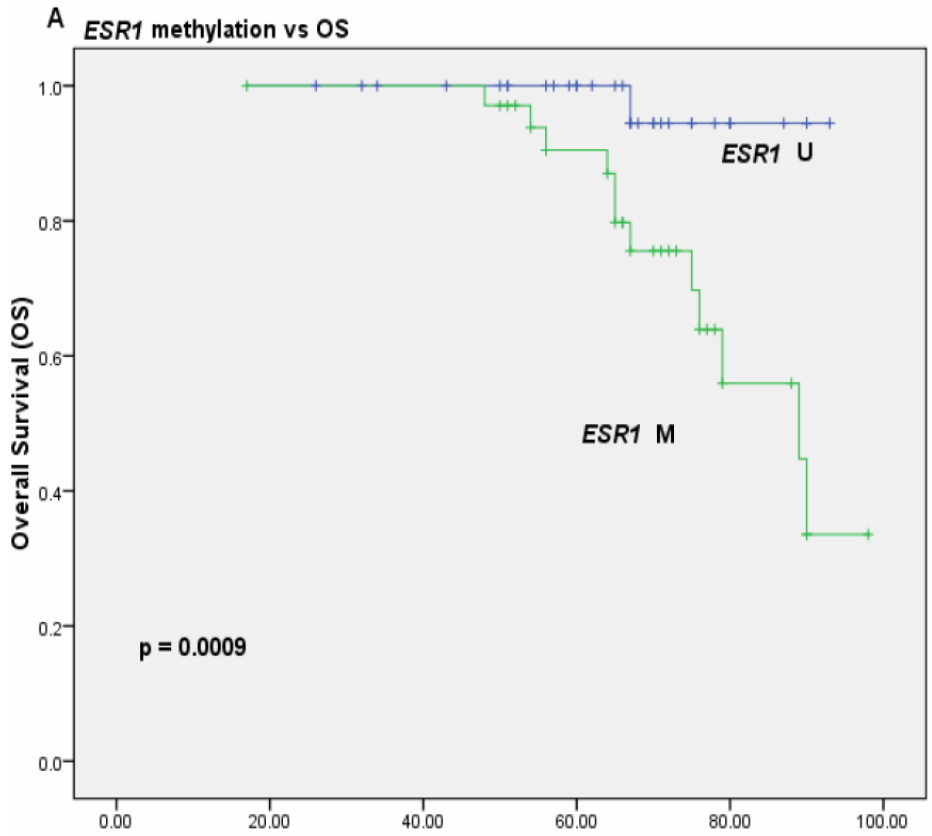


DNA Methylation	SOCS1 n [%]
Healthy controls (n=22)	10 [46]
ET patients (n=93)	20 [22]
P value	**< 0.05**

DNA Methylation in Breast Cancer

Hypermethylation of
ESR1 and **CXCL12 island 4**

prognostic factor for
OS, MFS (n = 69)



Ramos et al. 2010

DNA Methylation and Prognosis

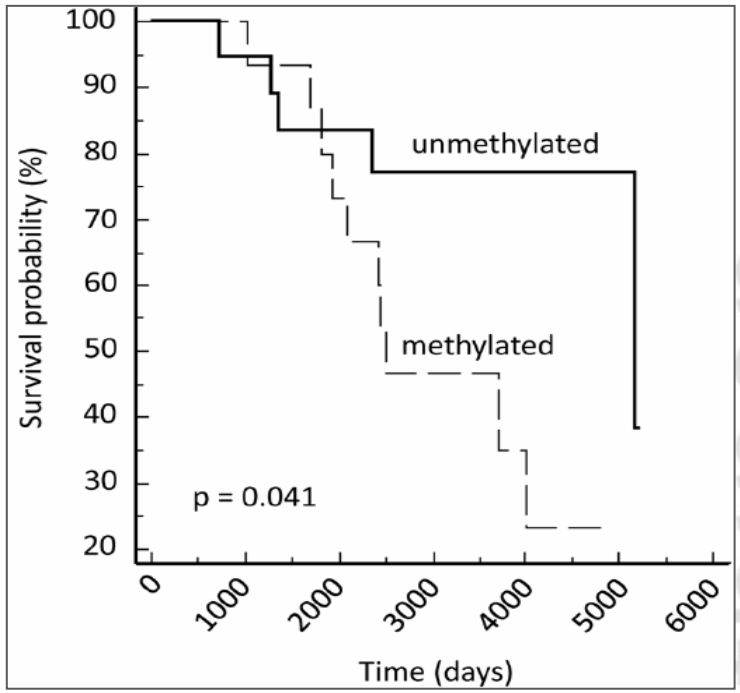
High Throughput Assays

Hypermethylation of **EMILIN2**

Association with

- poor clinical outcome (n = 60)
- ER/PR positivity

Hill et al. Molecular Cancer, 2010



DNA Methylation and Prognosis

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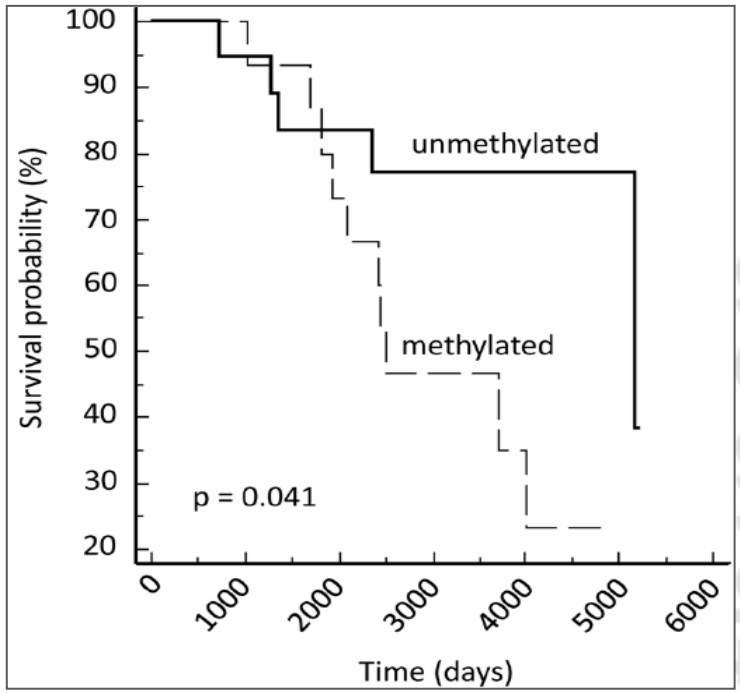


Hypermethylation of **p73, RAR β**

(n = 49)

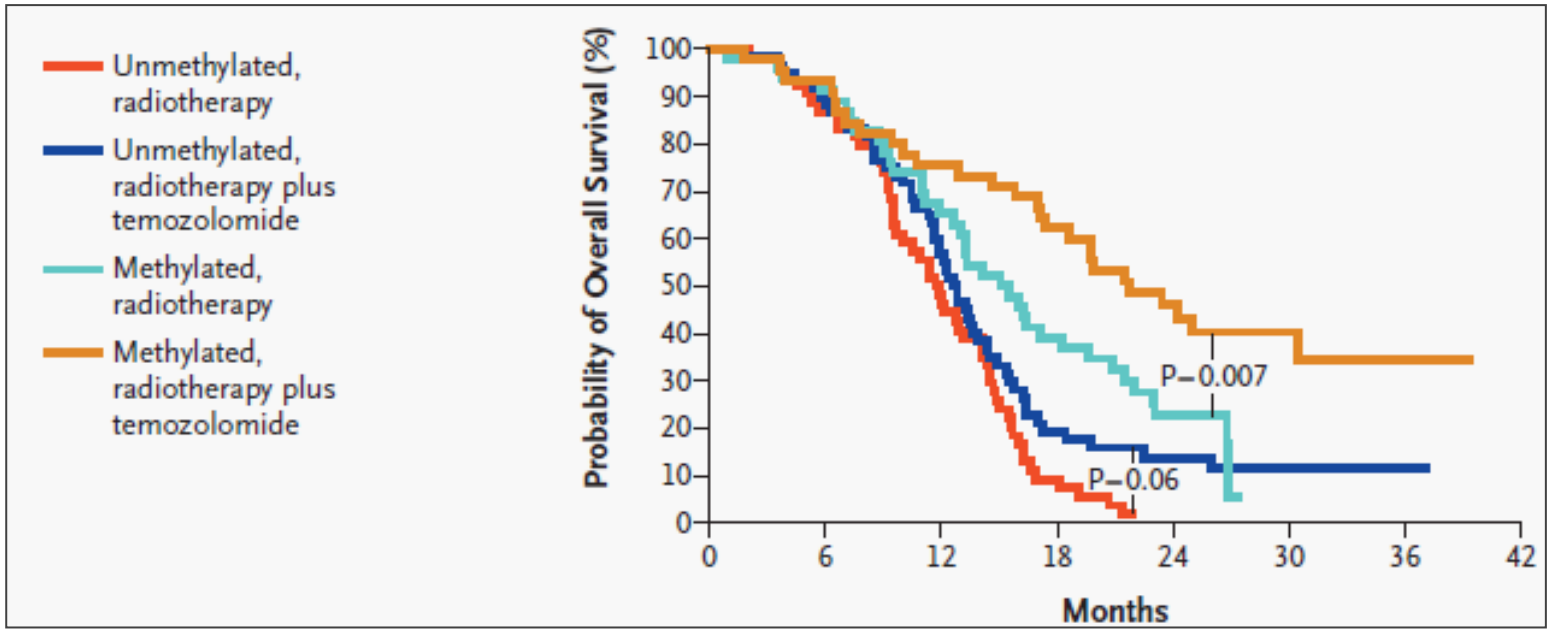
Association with

Aberrant Gene Methylation	p73	RAR β
High Tumor Grade	X	
Lymph Node Metastasis		X
Triple Negative Tumors	X	X

Marzese et al. ASCO 2010

DNA Methylation and Prediction

Hypermethylation of **MGMT**
in 206 Glioma patients correlated with temozolomide response



Hegi et al. 2005

DNA Methylation and Prediction

Gene	Function	Evidence for Role in Drug Sensitivity	Tumor Type	Reference
Apaf I	proapoptotic	Unmethylated: increased sensitivity to doxorubicin	melanoma cells	Soengas et al. 2001
Caspase 8	proapoptotic	Unmethylated: increased sensitivity to doxorubicin, etoposide, cisplatin	Ewings sarcoma, neuroblastoma, medulloplastoma	Fulda et al. 2001
hMLH I	DNA mismatch repair	Methylated: resistance to cisplatin	Ovarian cancer	Gifford et al. 2004
MGMT	Removes mutagenic alkyl-groups from Guanine	Methylated: response to temozolomide, BCNU, increased OS, PFS when treated with cyclophosphamide-containing regimens	Primary glioma Patents with DLBCL	Paz et al. 2004
p73	DNA damage checkpoint	Methylated: Sensitivity to alcylating agents as e.g. Cisplatin	Renal cancer	Shen et al. 2007
…	…	…	…	…

2. Mutations

Genetic Aberrations

Mutations
(< 1% in population)

point mutations
translocations
inversions
deletions
insertions

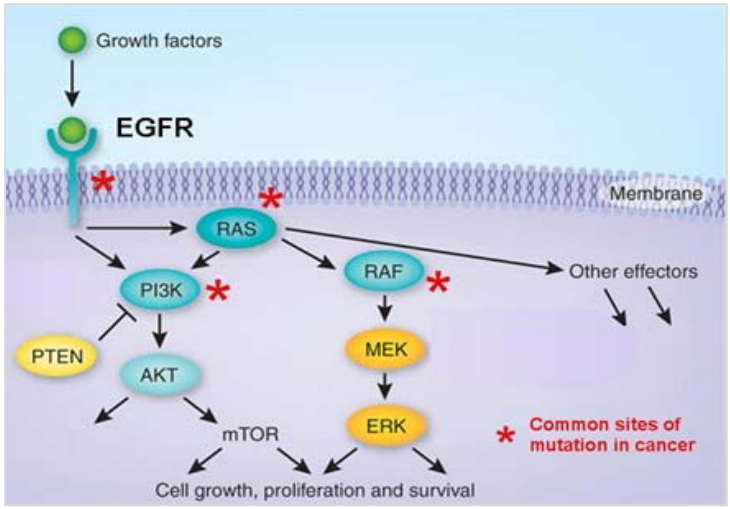
Polymorphisms
(> 1% in population)

point mutations

Detection: Sequencing, PCR, Cytogenetics, FISH

Mutations: Prognosis and Prediction, Colorectal Cancer

Prognosis



	WT vs Mutation in	Significant Difference in PFS, OS, RR
K-RAS	exon 12/13	yes
B-RAF		yes
PIK3CA	exon 20	yes

Prediction

EGFR Antagonists

Patient Population	RR
No Selection	24,4%
Selection: K-Ras	36,3%
Selection: K-Ras, B-Raf, N-Ras, PIK3CA	41,2%

De Rook et al. 2010

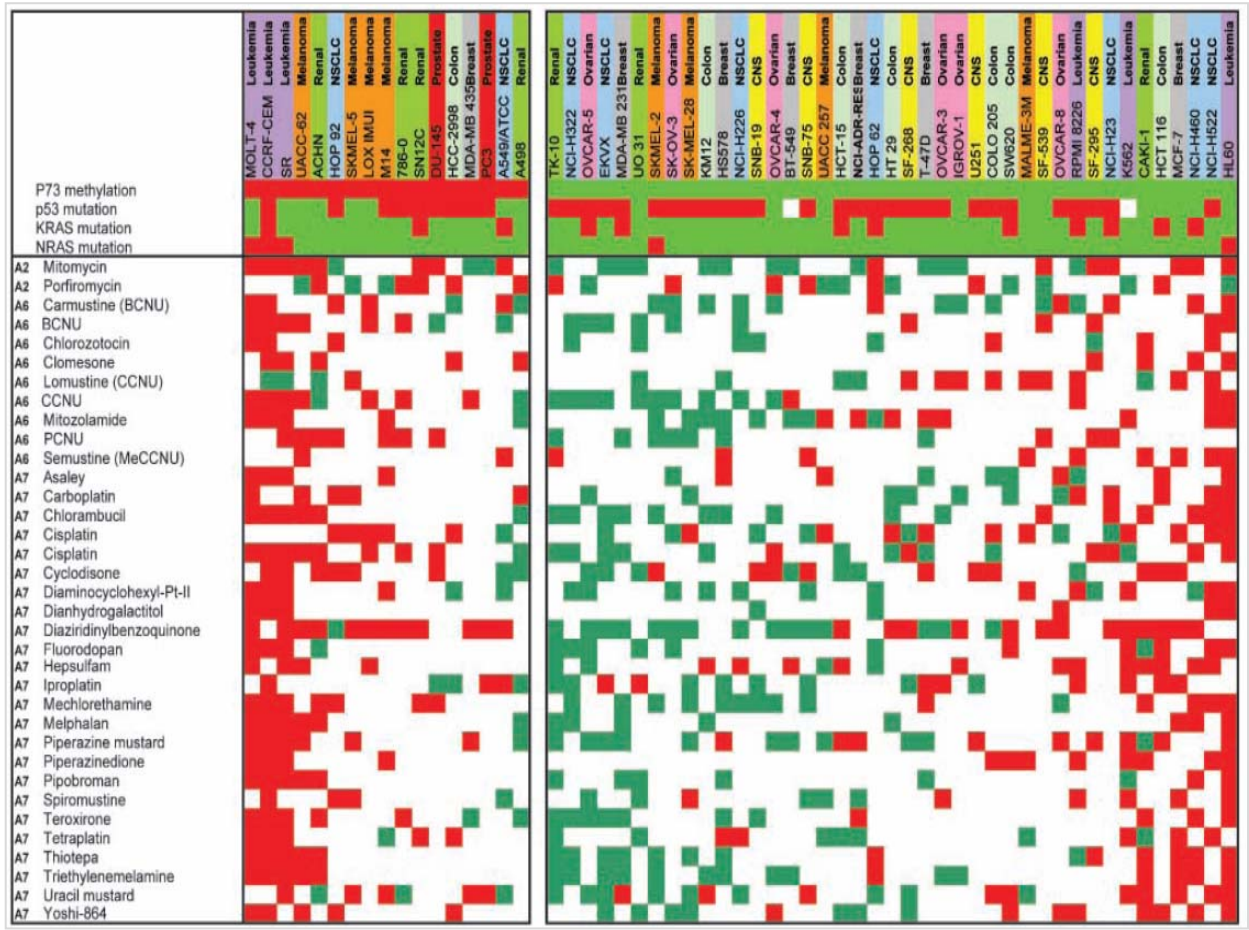
3. Epigenetics and Mutations

Shen et al. 2007

Prediction

p73 *methylation* is associated with sensitivity to alkylating agents.

Additional information for drug sensitivity by **p53, K-RAS, N-RAS *mutation*** status.



no methylation = transcription / WT = no mutation / chemotherapy sensibility

methylaton = **no** transcription / **mutation** / **no** chemotherapy sensibility

4. Summary

DNA Methylation plays an important role in **development** and **tumor progression**.

The **combination** of
gene expression
mutation detection
and epigenetic analysis
achieve higher importance:

in **prognosis**

in **predicting** chemotherapy response

in improvement of tumor profile establishment

Thank You

for Your Attention