Frauenheilkunde

Epigenetische Veränderungen beim Mammakarzinom

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Abteilung für Frauenheilkunde
Univ. Klinik für Gynäkologie und Geburtshilfe

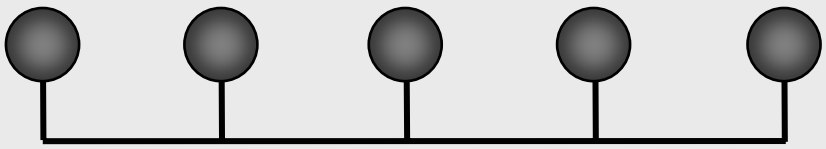
Überblick

Frauenheilkunde

- Epigenetik: DNA-Methylierung
- DNA-Methylierungsanalysen
- DNA–Methylierung beim Mammakarzinom

DNA-Methylierung

Der fünfte Baustein der DNA



C G T C G A C G G C G A C G G A A G G G A G A T C C T G G T

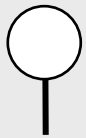
G C A G C T G C C G C T G C C T T C C C T C T A G G A C C A

Peter A. Jones

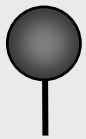
Adenin

Guanin

Thymin

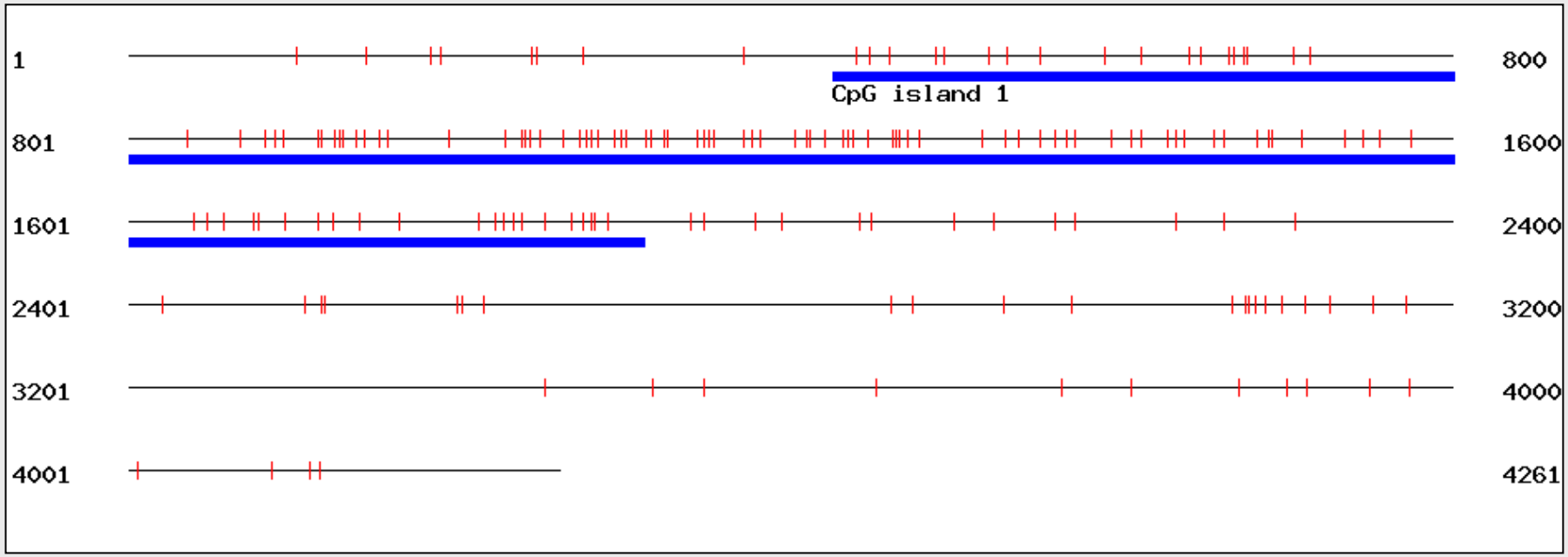


Cytosin

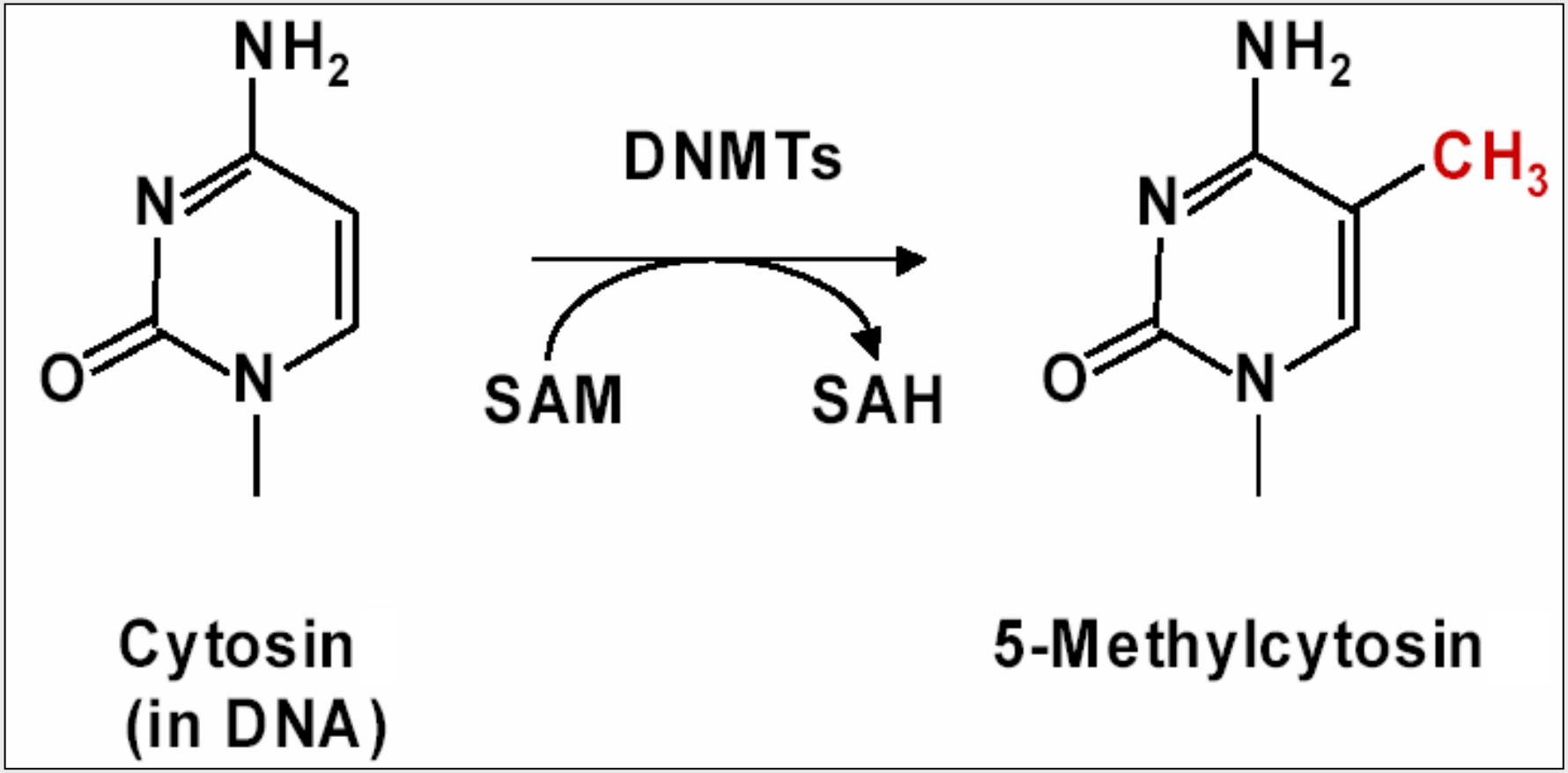


Methyl-Cytosin

GSTP1 CpG Island

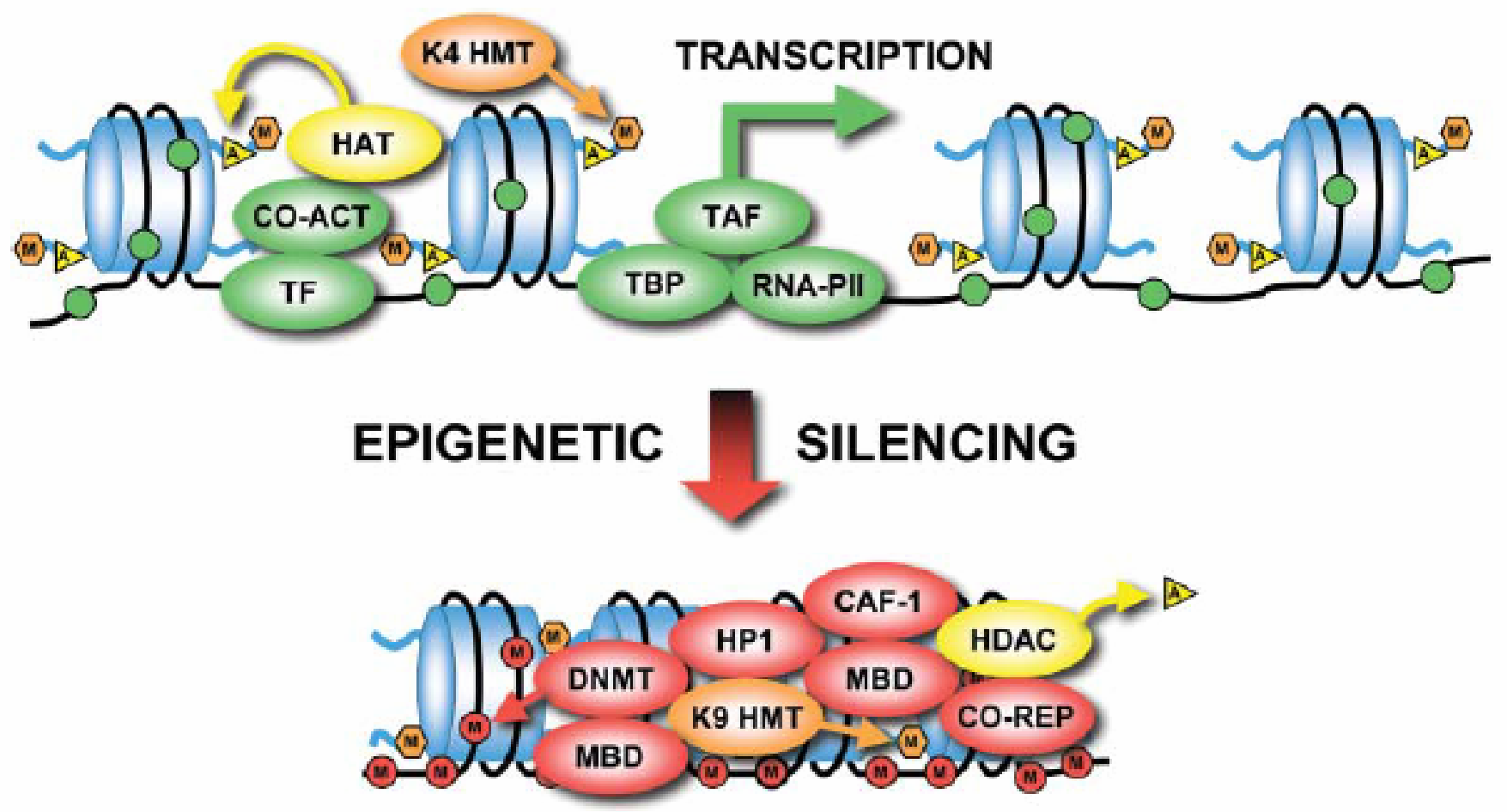


Cytosin Methylierung



De Novo Methylierung: DNMT 3A, DNMT 3B
Erhaltungs-Methylierung: DNMT 1

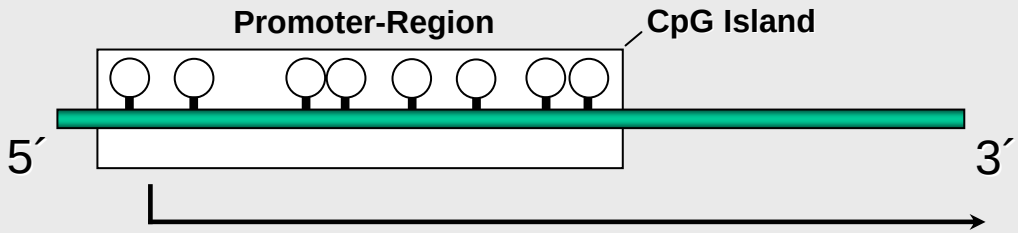
Epigenetik
Regulation der Gen Expression

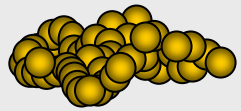


Epigenetik und Karzinogenese

Frauenheilkunde

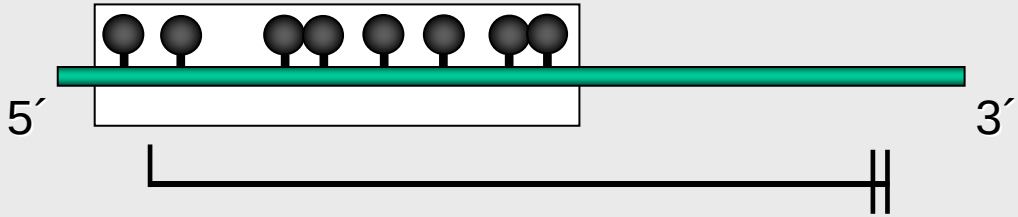
Aktives Tumorsuppressorgen



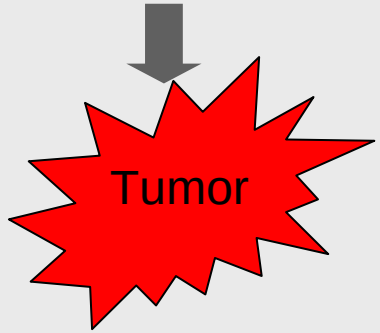


Tumorsuppressor Protein

Epigenetisch inaktiviertes Gen

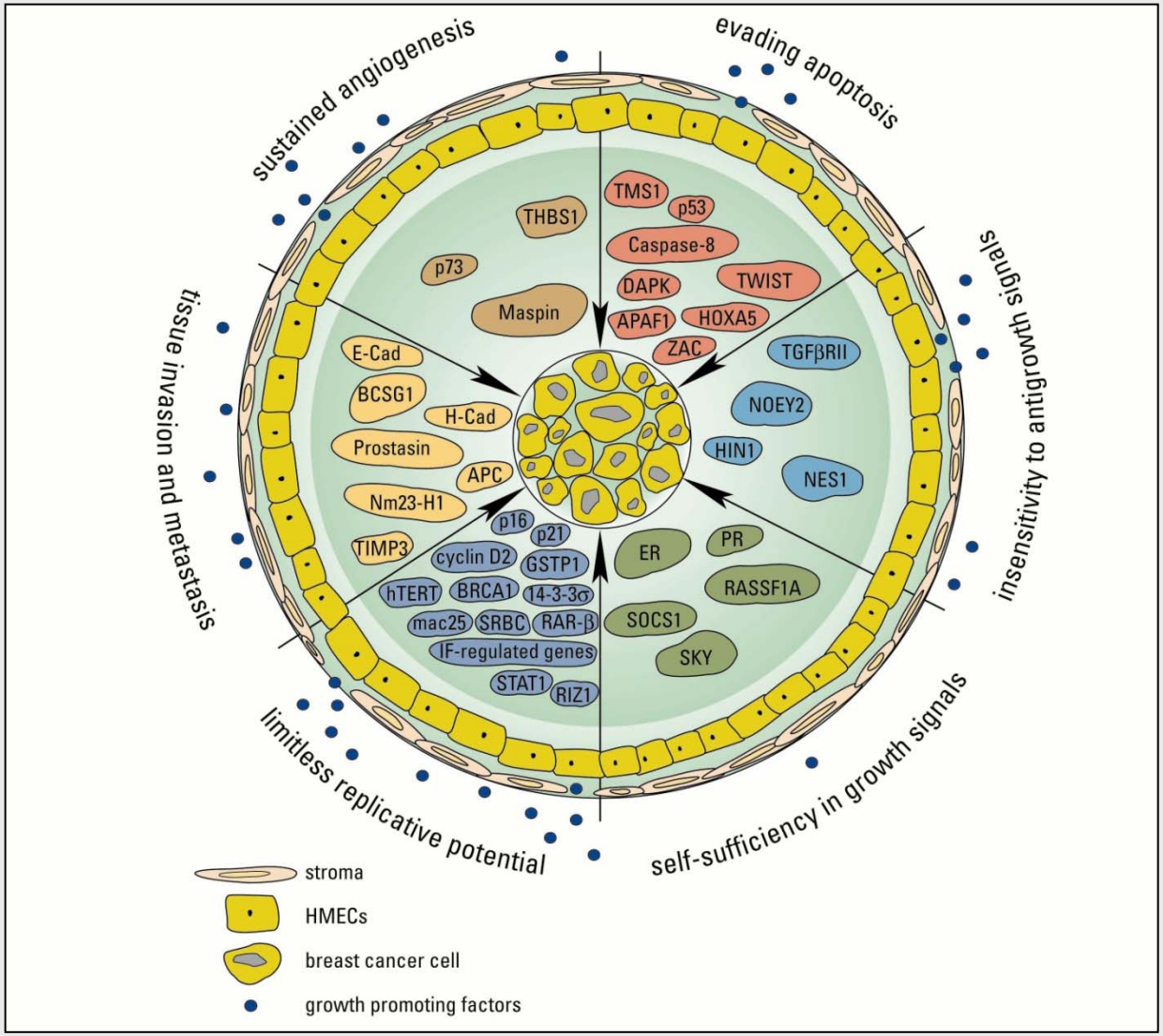


kein Tumorsuppressor Protein



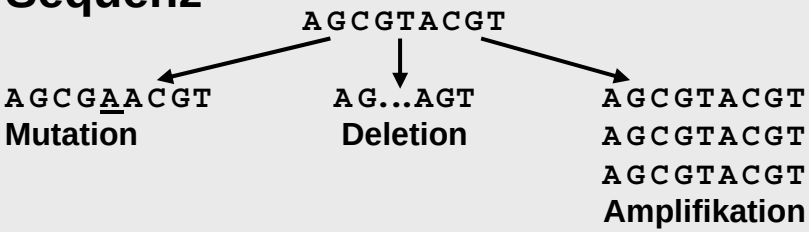
DNA Methylierung

Rolle im Zuge der Karzinogenese

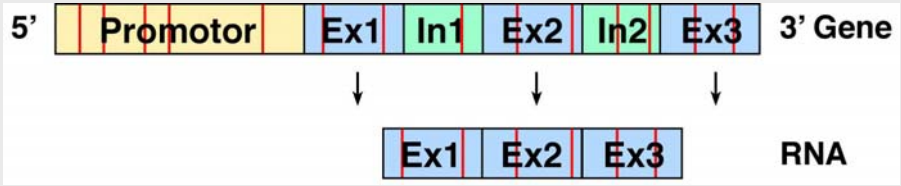


GENETIK

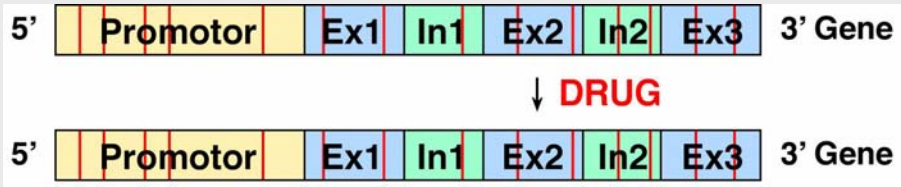
- **Veränderungen in der DNA Sequenz**



- **Zufällig verteilt**



- **Nicht reversibel durch Einsatz von Medikamenten**

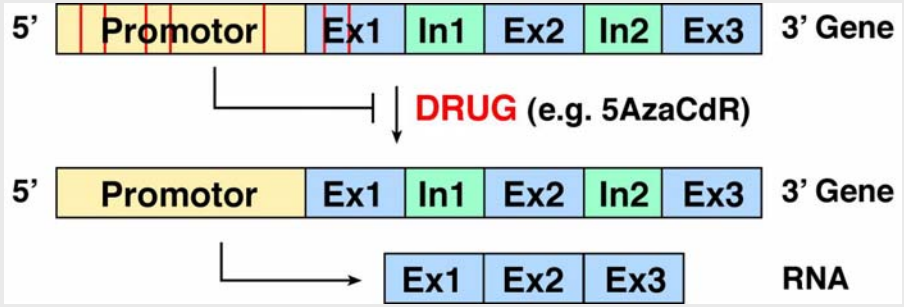


EPIGENETIK

- **Keine Veränderung in der DNA Sequenz**

- **In 5`Region von Genen**

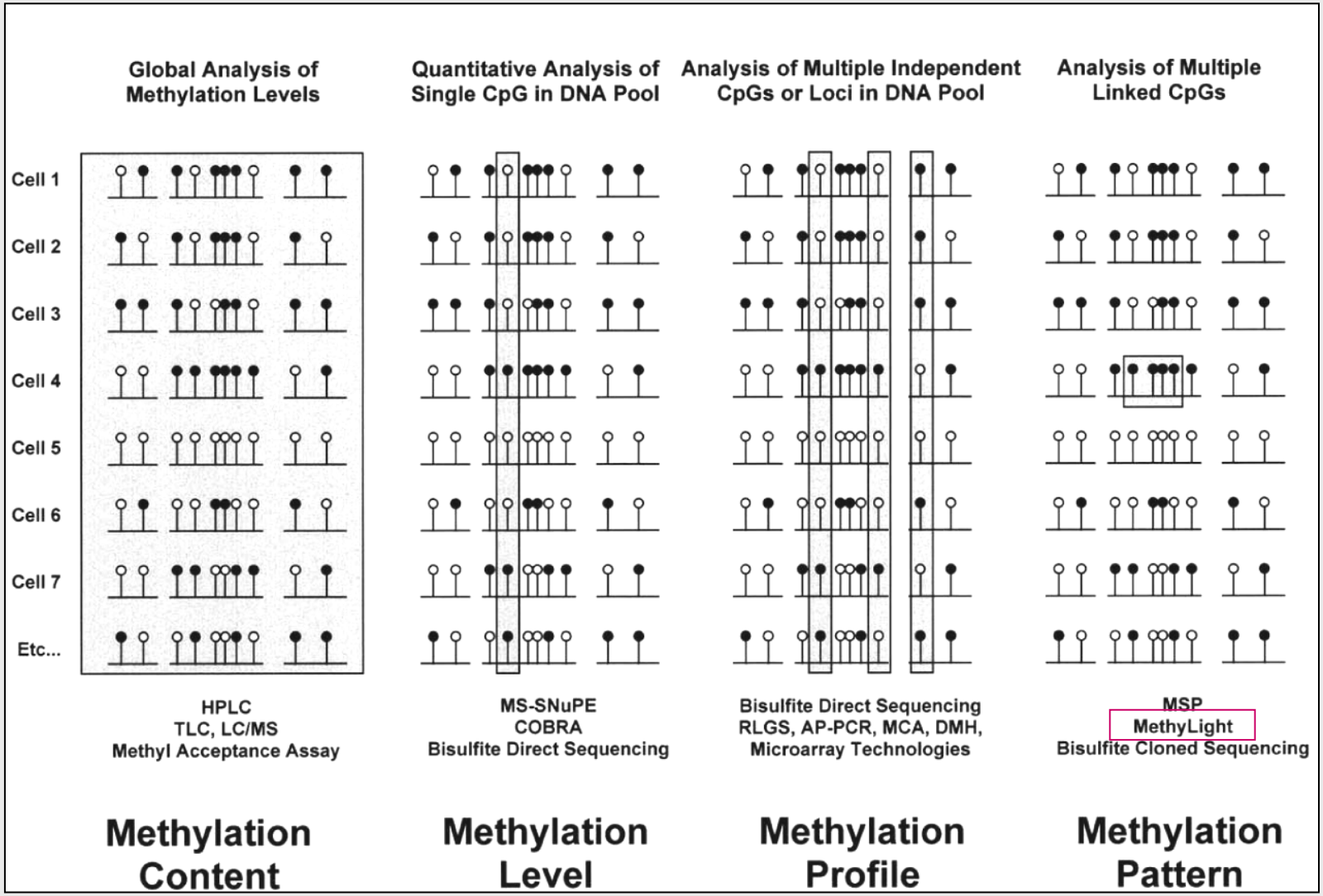
- **Reversibel durch Einsatz von Medikamenten**



DNA-Methylierungsanalysen

DNA Methylierungsanalysen

Frauenheilkunde



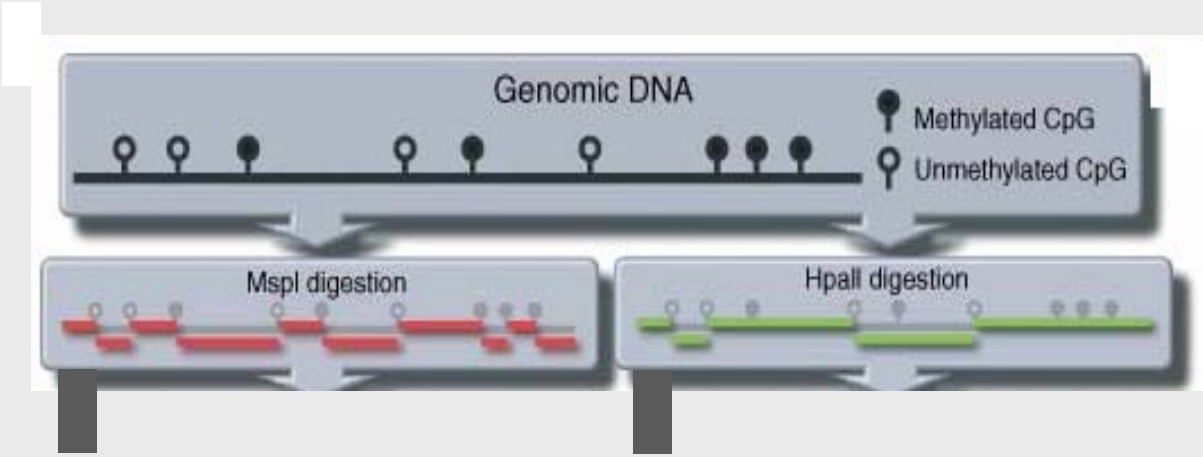
DNA Methylierungsanalysen
Erste Schritte

Frauenheilkunde

Methylierungs-sensitive Restriktionsenzyme
Bsp.: *Hpa*II und *Msp*I (CCGG Erkennungssequenz)

zunächst in Kombination mit anschl. Southern Blot Analyse
danach in Kombination mit PCR

→ Problem:
nur Methylierungsunterschiede an Enzymerkennungsstellen erkannt



DNA Methylierungsanalysen

Innovation in Down Under - Bisulfit-Sequenzierung

Frauenheilkunde

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1827–1831, March 1992
Genetics

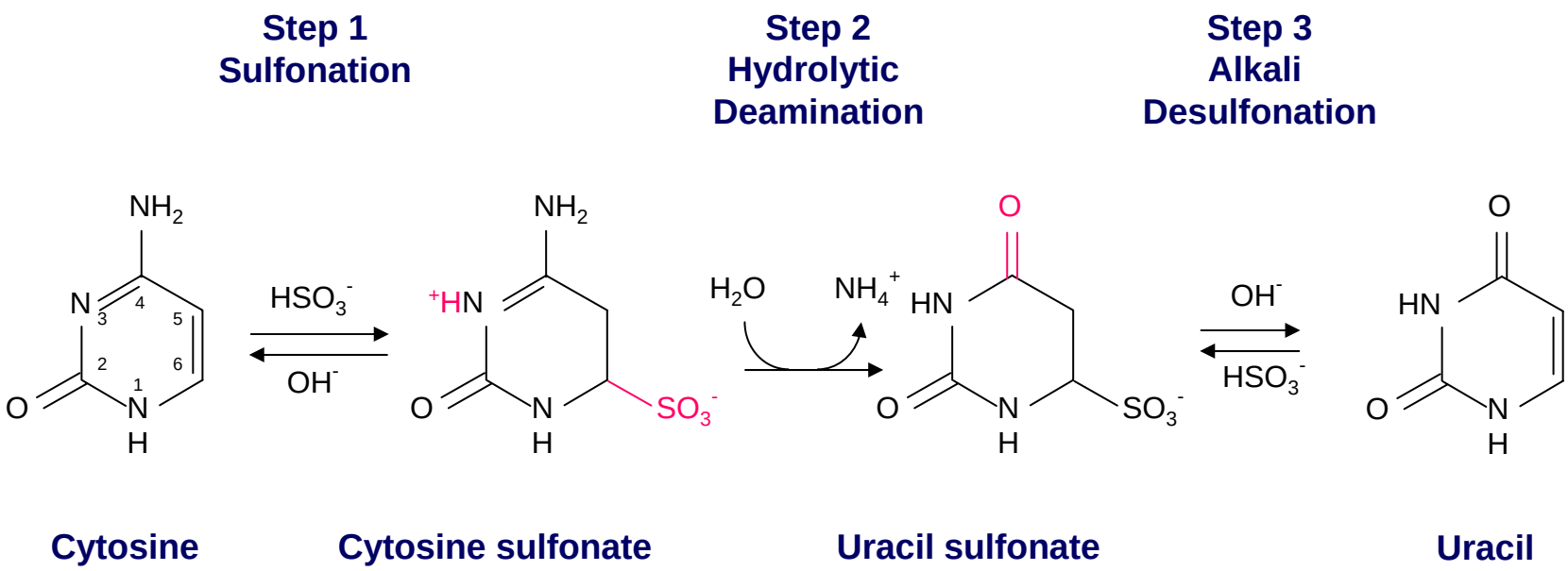
A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands

(genomic sequencing/DNA methylation/bisulfite modification/PCR/kininogen gene)

MARIANNE FROMMER*†, LOUISE E. MCDONALD*‡, DOUGLAS S. MILLAR*, CHRISTINA M. COLLIS†, FUJIKO WATT†, GEOFFREY W. GRIGG†, PETER L. MOLLOY†, AND CHERYL L. PAUL*

*The Kanematsu Laboratories, Royal Prince Alfred Hospital, Missenden Road, Camperdown, Sydney, NSW 2050, Australia; and †Division of Biomolecular Engineering, Laboratory for Molecular Biology, Commonwealth Scientific and Industrial Research Organization, 103 Delhi Road, North Ryde, Sydney, NSW 2113, Australia

Bisulfitmodifikation



DNA Methylierungsanalysen

Methylierungs-spezifische PCR MSP

Frauenheilkunde

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 9821–9826, September 1996
Medical Sciences

Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands

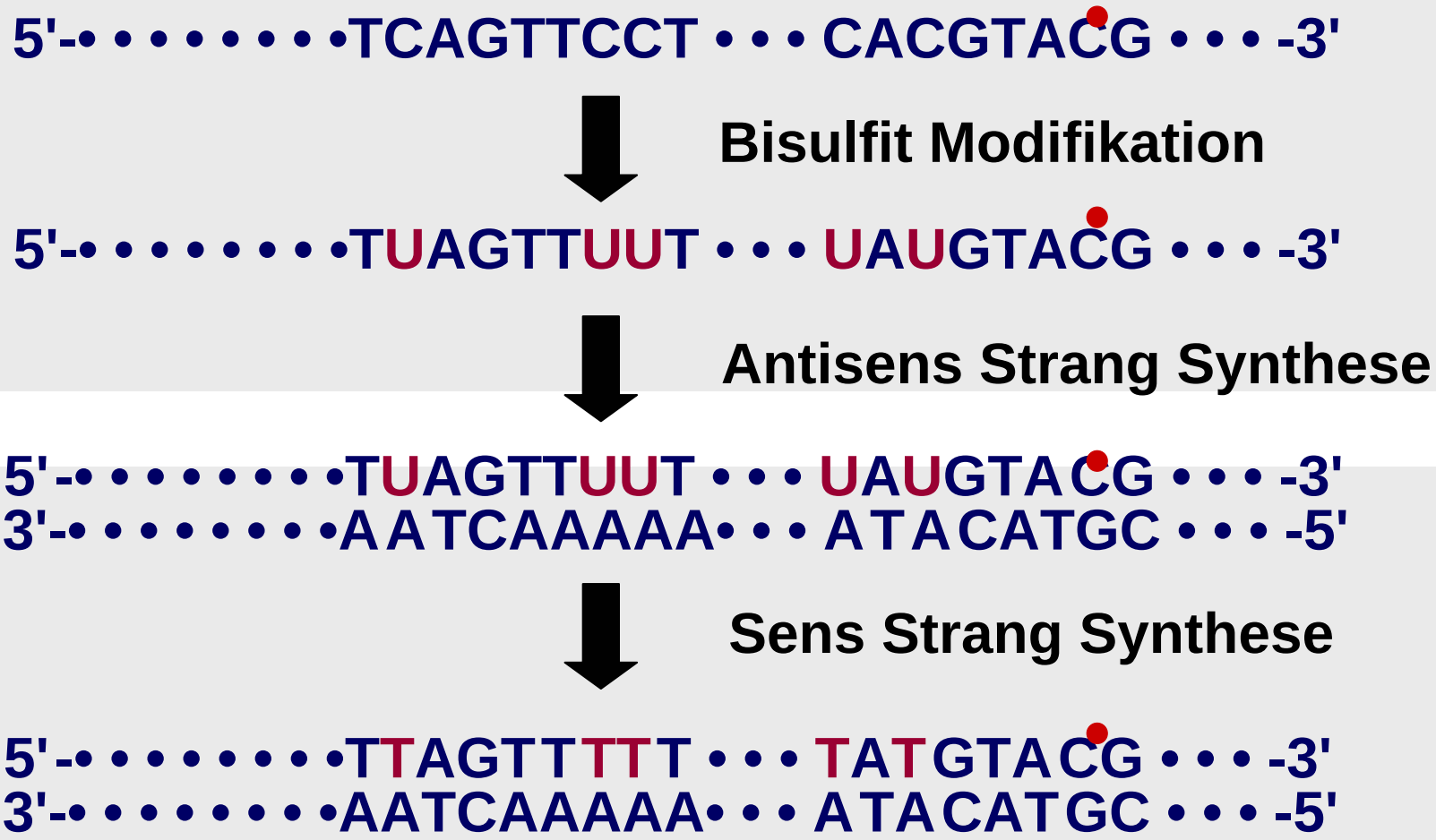
(DNA methylation/tumor suppressor genes/*p16*/*p15*)

JAMES G. HERMAN*†, JEREMY R. GRAFF*, SANNA MYÖHÄNEN*, BARRY D. NELKIN*, AND STEPHEN B. BAYLIN*‡

*Oncology Center and ‡Department of Medicine, The Johns Hopkins Medical Institutions, 424 North Bond Street, Baltimore, MD 21231

→ Weniger DNA benötigt
→ sensitiver
→ Lokus spezifisch

MSP


5'-• • • • • • • •TCAGTTCCT • • • CACGTACG • • • -3'
Bisulfit Modifikation
5'-• • • • • • • •TUAGTTUUT • • • UAUGTACG • • • -3'
Antisens Strang Synthese
5'-• • • • • • • •TUAGTTUUT • • • UAUGTACG • • • -3'
3'-• • • • • • • •AATCAAAAA• • • ATACATGC • • • -5'
Sens Strang Synthese
5'-• • • • • • • •TTAGTTTTT • • • TATGTACG • • • -3'
3'-• • • • • • • •AATCAAAAA• • • ATACATGC • • • -5'

DNA Methylierungsanalysen
MethyLight

Frauenheilkunde

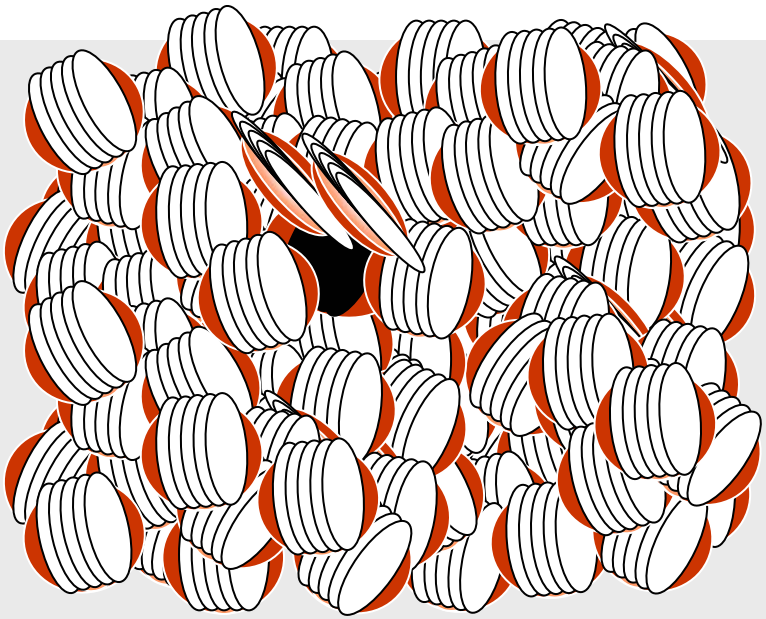
Nucleic Acids Research, 2000, Vol. 28, No. 8 e32

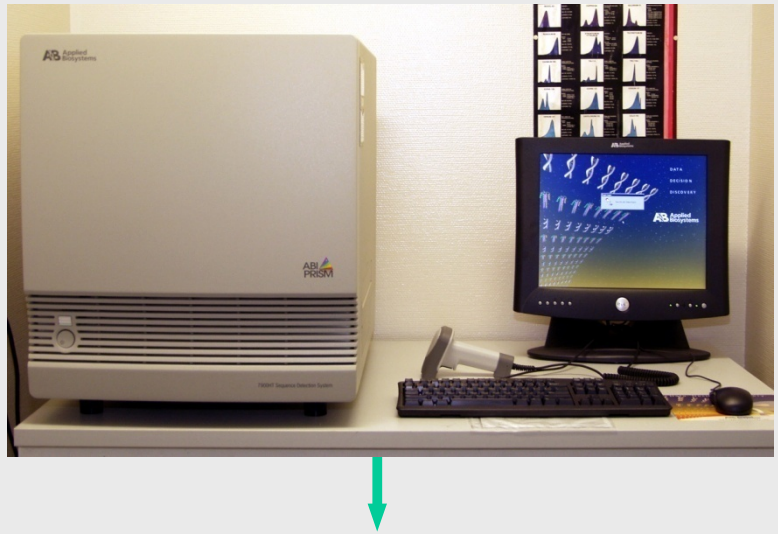
MethyLight: a high-throughput assay to measure DNA methylation

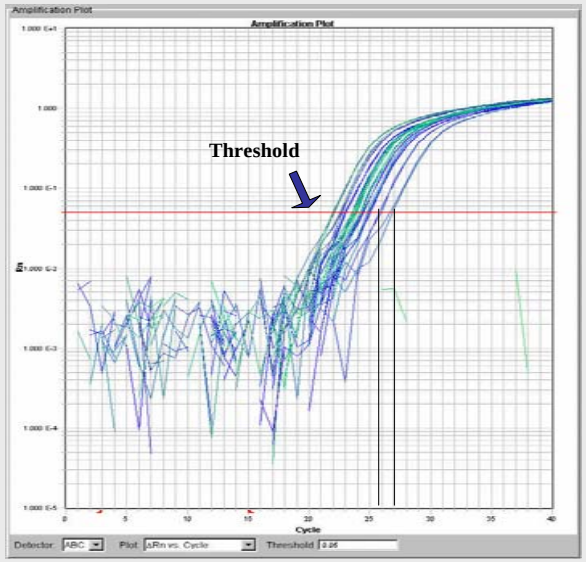
Cindy A. Eads[1,2], Kathleen D. Danenberg[2], Kazuyuki Kawakami[2], Leonard B. Saltz[4], Corey Blake[3], Darryl Shibata[3], Peter V. Danenberg[2] and Peter W. Laird[1,2,*]

[1]Department of Surgery, [2]Department of Biochemistry and Molecular Biology and [3]Department of Pathology, University of Southern California School of Medicine, Norris Comprehensive Cancer Center, Mail Stop # 73, Room 6418, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA and [4]Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10021-6094, USA

MethyLight

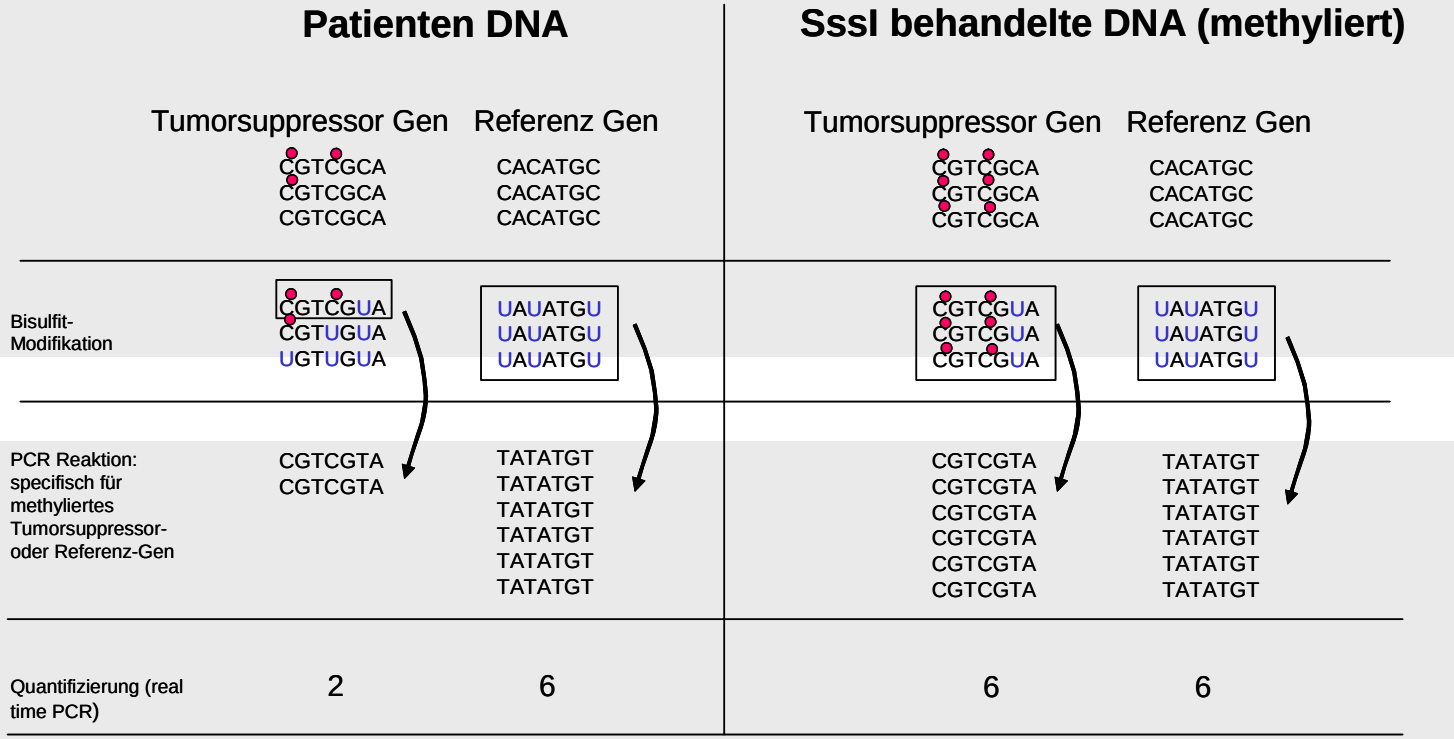






PMR Berechnung

MethyLight



$$\text{Percentage of Methylated Reference (PMR)} = \frac{\frac{2}{6}}{\frac{6}{6}} \times 100 = 33$$

DNA Methylierungsanalysen

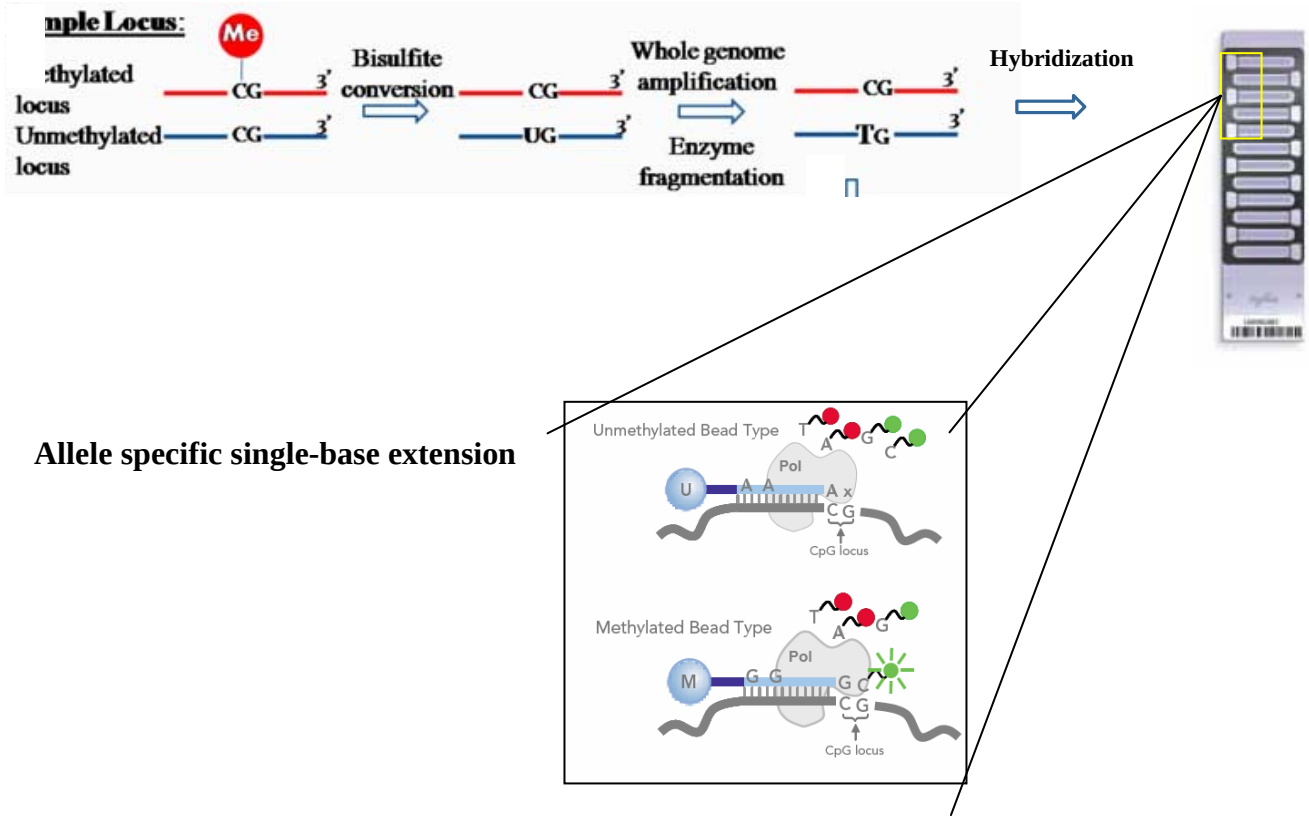
Illumina Infinium Human Methylation27 BeadChip

Frauenheilkunde

Humanmethylation27 Beadchip Highlights

- **High Throughput:**
  > 27,000 CpG assays per sample, 12 samples per BeadChip
- **Specific:**
  Single CpG resolution
- **Streamlined Workflow:**
  PCR-free protocol
- **Low Sample Input:**
  500 ng manual or 1 µg automated processing
- **Product Integration:**
  Content overlap with Illumina gene expression BeadChips, integrated analysis supported by BeadStudio Software

Illumina Infinium Human Methylation27 BeadChip
Prinzip



DNA Methylierungsanalysen

Goldengate Methylation Assay

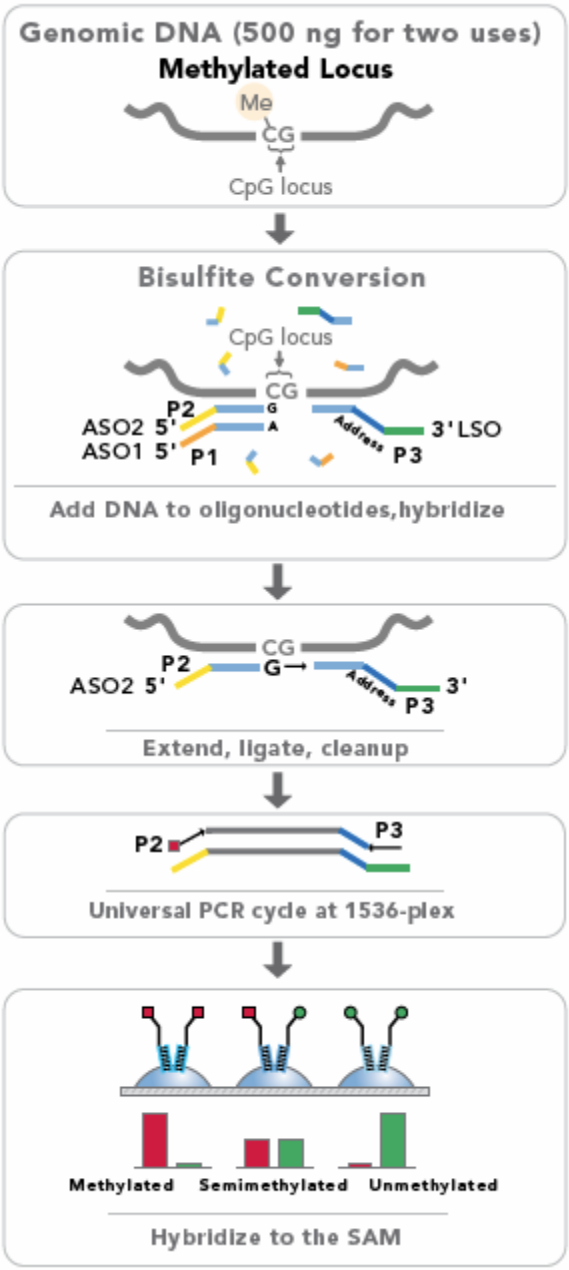
Frauenheilkunde

Goldengate Methylation on Beadarray Highlights

- **High Throughput:**
  Up to 1,536 CpG sites assayed simultaneously
- **Flexible Content:**
  Standard and custom panels
- **Streamlined Workflow:**
  96 samples per array, with a convenient 3-day workflow
- **Robust Performance:**
  High sensitivity and reproducibility

- **High Multiplex Array Platform:** Simultaneously analyze up to 1,536 CpG sites

Goldengate Methylation Assay
Prinzip



ASO = Allele-Specific Oligo
LSO = Locus-Specific Oligo
P1, P2, P3 = Universal PCR primers
Address = Sequence unique targeting a particular bead type

96-sample Universal Array Matrix (SAM) contains universal sequences or addresses that hybridize to complementary sequences in the prepared sample.

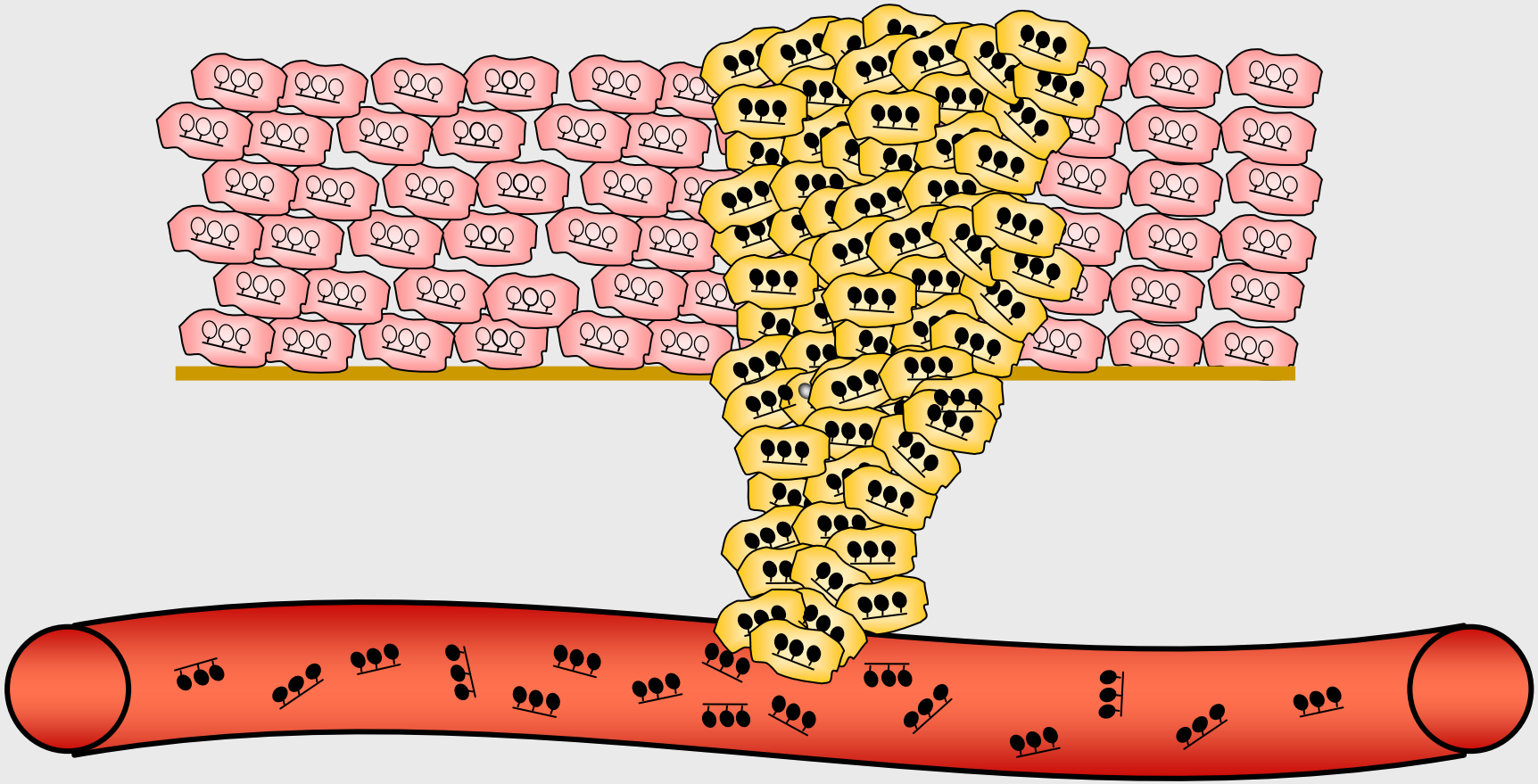
Frauenheilkunde

DNA-Methylierung:
prognostische und prädiktive Möglichkeiten beim Mammakarzinom

DNA Methylierung

Marker um minimale Tumorerkrankung im Serum festzustellen?

Frauenheilkunde



Cancer Res 2005; 65: (4). February 15, 2005

Priority Reports

Circulating Tumor-Specific DNA: A Marker for Monitoring Efficacy of Adjuvant Therapy in Cancer Patients

Heidi Fiegl,[1] Simone Millinger,[1] Elisabeth Mueller-Holzner,[1] Christian Marth,[1] Christian Ensinger,[2] Andreas Berger,[3] Helmut Klocker,[3] Georg Goebel,[4] and Martin Widschwendter[1]

Departments of [1]Obstetrics and Gynecology, [2]Pathology, [3]Urology, and [4]Biostatistics and Documentation, Innsbruck Medical University, Innsbruck, Austria

Studiendesign

Frauenheilkunde

148 Patienten

Prä- und posttherapeutische Seren

Bei Diagnose keine Metastasen

Tamoxifenbehandlung für 5 Jahre

Kein Rezidiv nach 1 Jahr

RASSF1A DNA Methylierung

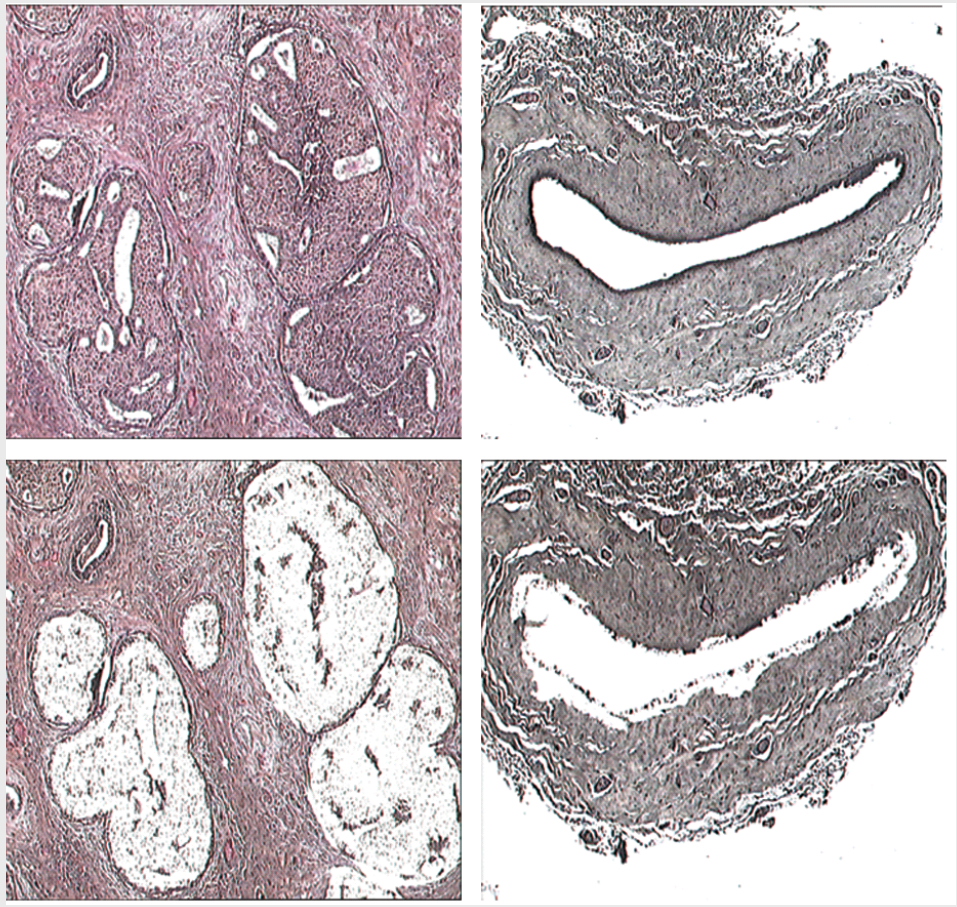
Stammt sie vom Tumorepithel?

Frauenheilkunde

TUMOR NORMAL

vorher

danach

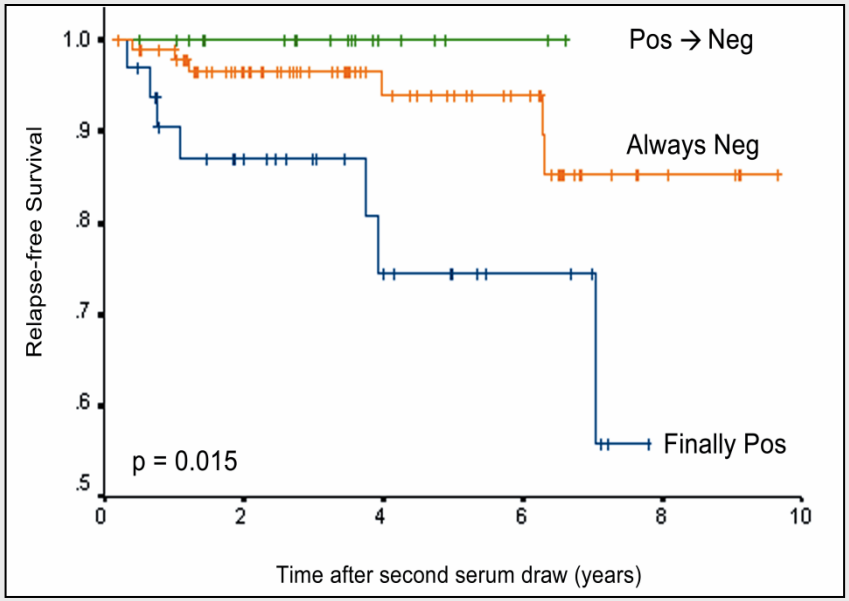


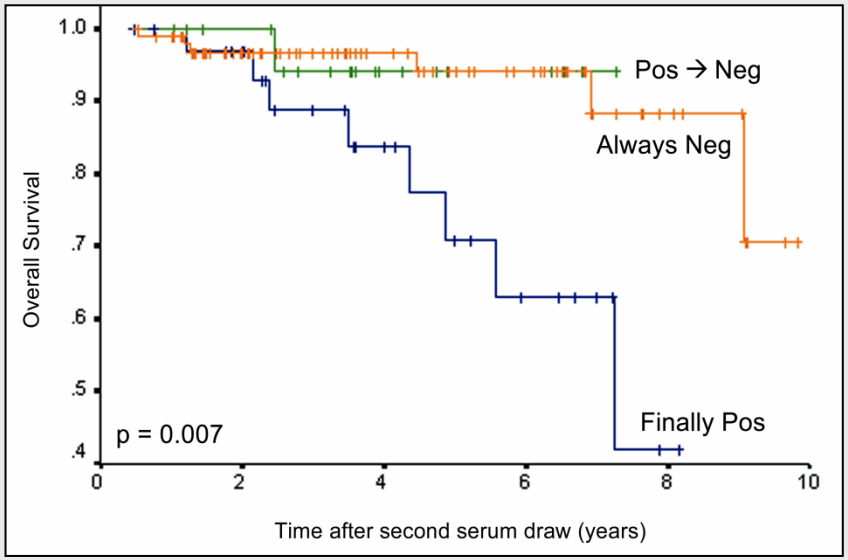
Pat.ID	RASSF1A methylation			
	Tumor Epithelium	Tumor Stroma	Non-neoplastic Epithelium	Non-neoplastic Stroma
3167	+	–	–	–
2914	+	n.d.	–	–
217	+	–	n.d.	n.d.
2668	+	–	–	n.d.
1724	+	–	n.d.	n.d.
3161	+	–	+	–
673	+	–	–	–
3248	+	–	n.d.	–
3527	+	n.d.	–	–
3591	+	–	+	–
202	+	n.d.	–	n.d.
3610	+	–	–	–
1649	+	+	–	–

DNA Methylierung

Überwachung adjuvanter endokriner Therapie

Frauenheilkunde



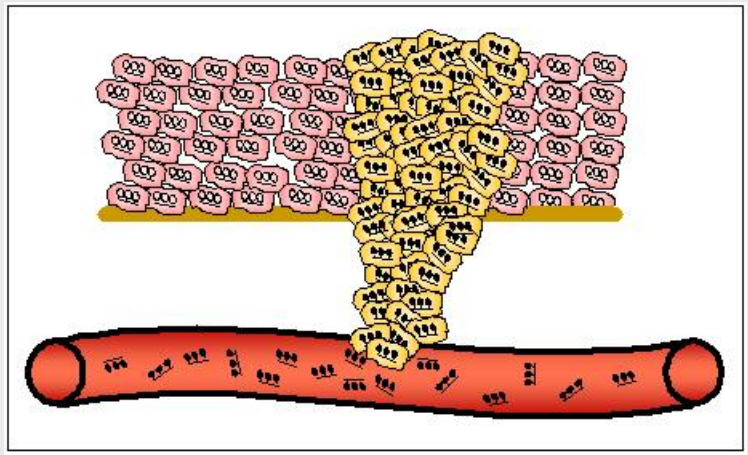


Characteristic	Pos → Neg	Finally Pos	Fishers´ Exact Test
	no. of patients		
Size of tumor			0.78
T1	11	19	
T2/3/4	10	14	
Tumor grade			0.07
I	3	13	
II/III	17	20	
Lymph node metastases			1.00
Negative	10	18	
Positive	8	13	
Menopausal status			1.00
Premenopausal	1	3	
Postmenopausal	20	30	
Hormone-receptor status			0.39
Positive	20	33	
Negative	1	0	
Adjuvant radiation therapy			0.76
No	7	13	
Yes	14	20	
Additional chemotherapy			0.26
No	15	18	
Yes	6	15	
Type of surgery			0.59
BE	9	17	
ME	12	16	

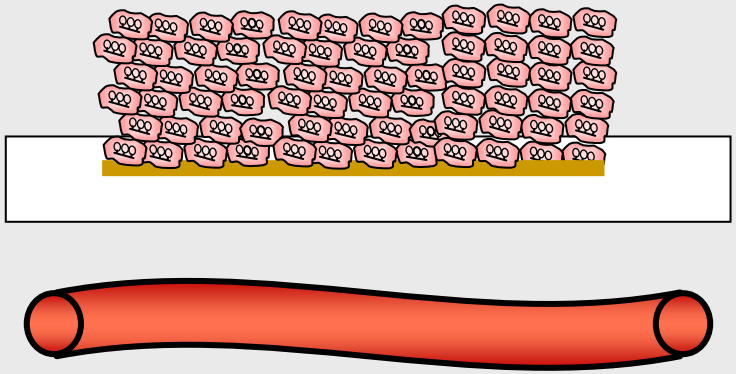
DNA Methylierung

Überwachung adjuvanter endokriner Therapie

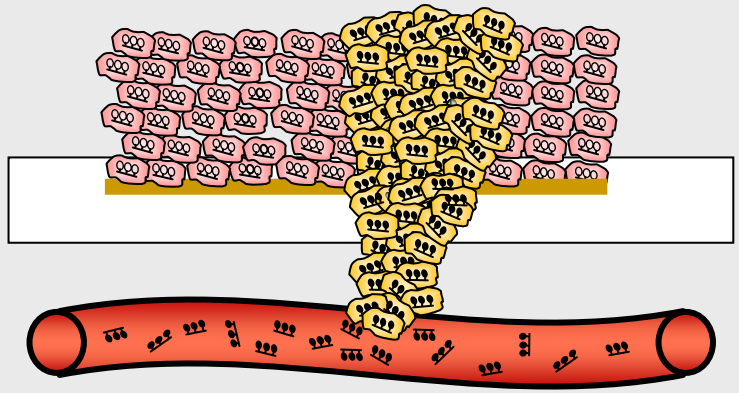
Frauenheilkunde



+ Tamoxifen > 6 Monate



gute Prognose



schlechte Prognose

Breast Cancer Res Treat
DOI 10.1007/s10549-010-1160-0

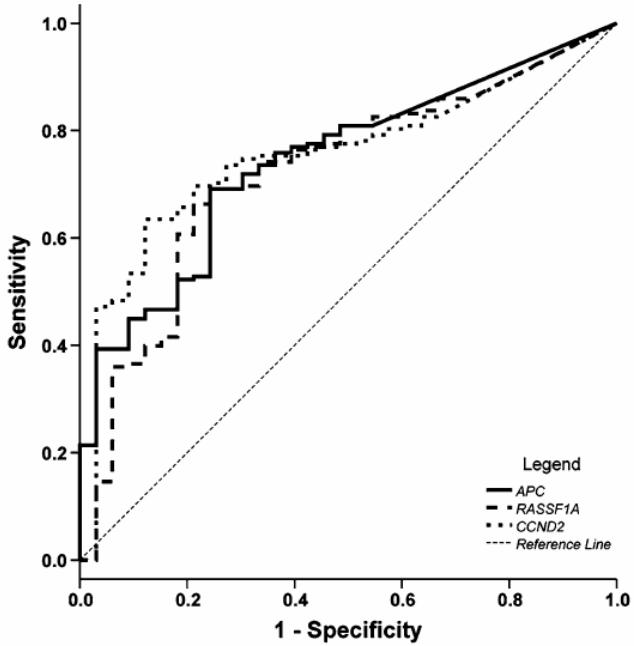
PRECLINICAL STUDY

High *RASSF1A* promoter methylation levels are predictive of poor prognosis in fine-needle aspirate washings of breast cancer lesions

Ana Teresa Martins · Paula Monteiro · João Ramalho-Carvalho · Vera L. Costa · Mário Dinis-Ribeiro · Conceição Leal · Rui Henrique · Carmen Jerónimo

FNA & *RASSF1A, APC* u. *CCND2* DNA Methylierungsanalysen Diagnose

Frauenheilkunde



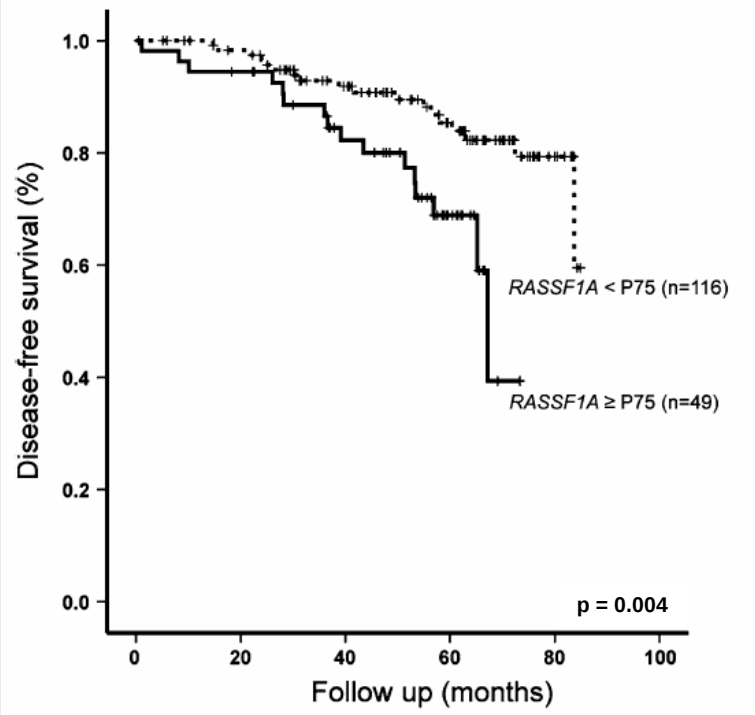
211 Patientinnen mit Veränderungen der Brust

178	maligne Veränderungen
33	benigne Veränderungen

Validity estimates	Number of markers with positive result in each case		
	1	2	3
Se (95% CI)	0.88 (0.82–0.92)	0.78 (0.71–0.83)	0.37 (0.30–0.44)
Sp (95% CI)	0.42 (0.24–0.62)	0.79 (0.62–0.89)	0.91 (0.76–0.97)
AUC (95% CI)	0.75 (0.70–0.81)	0.85 (0.81–0.90)	0.77 (0.71–0.83)

FNA und *RASSF1A* DNA Methylierungsanalysen Prognose

Frauenheilkunde



Model tested	Variables	Odds ratio (OR)	95% CI for OR	*P*
Overall survival	pTNM	1.37	1.12–1.67	0.002
Disease-specific survival	pTNM	1.51	1.19–1.92	<0.001
	Grade	3.71	1.45–9.51	0.006
Disease-free survival	pTNM	1.46	1.16–1.80	0.001
	Grade	3.26	1.42–7.50	0.005
	RASSF1A methylation ≥p75	2.53	1.09–5.87	0.031

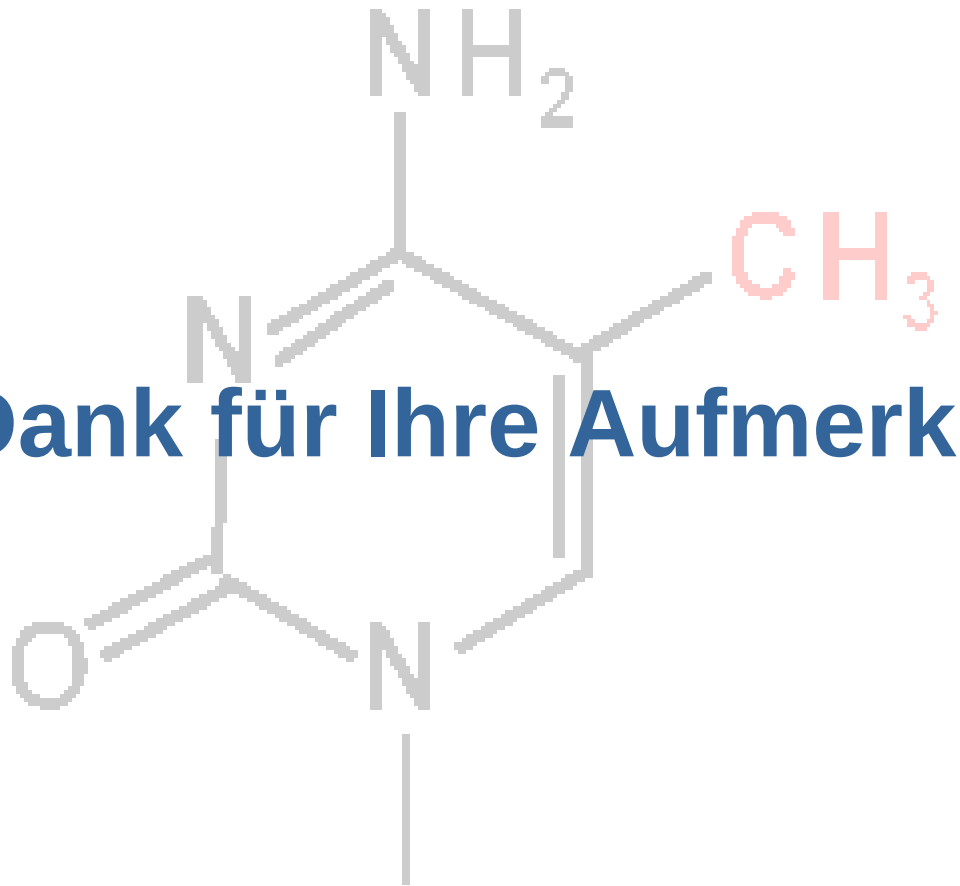
Multivariate Analyse:
unabhängige Assoziation mit schlechtem Disease-free survival

Vorteile von DNA Methylierungsanalysen

Frauenheilkunde

- hohe Sensitivität (Amplifikation mittels PCR)
- biologische und chemische Stabilität
- spezifische Regionen im Genbereich
- tumorspezifisch

Frauenheilkunde



Vielen Dank für Ihre Aufmerksamkeit!