Oncogene (2003) 22, 8031–8041

www.nature.com/onc

MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer

Ping Ji[1,5], Sven Diederichs[1,5], Wenbing Wang[1], Sebastian Böing[1], Ralf Metzger[2], Paul M Schneider[2], Nicola Tidow[3], Burkhard Brandt[3], Horst Buerger[4], Etmar Bulk[1], Michael Thomas[1], Wolfgang E Berdel[1], Hubert Serve*[,1] and Carsten Müller-Tidow*[,1]

[1]Department of Medicine, Hematology/Oncology, University of Münster, Germany; [2]Department of Visceral and Vascular Surgery, University of Cologne, Germany; [3]Institute for Laboratory Medicine and Clinical Chemistry, University of Münster, Germany; [4]Gerhard-Domagk-Institute of Pathology, University of Münster, Münster, Germany

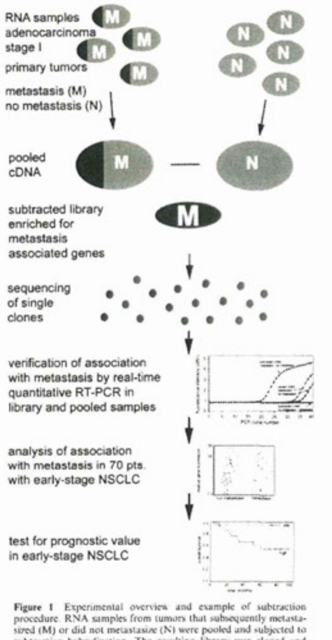


Figure 1 Experimental overview and example of subtraction procedure. RNA samples from tumors that subsequently metastasized (M) or did not metastasize (N) were pooled and subjected to subtractive hybridization. The resulting library was cloned and sequenced. The reliability of the subtractive hybridization was confirmed by real-time quantitative RT–PCR in the pooled samples and in the subtracted library. Then, the identified genes were analysed in 70 NSCLC patients

Accession number	*No. of clones*	*Name*	*Chromosomal localization*	*Complete name*	*Quantitative RT–PCR (fold increase)*
AF203815	22	MALAT-1	11q13	Metastasis associated in lung adenocarcinoma transcript 1	3.2
XM_007650	21	B2M	15q21-q22	Beta-2-microglobulin	
NM_021109	9	TMSB4X	Xq21.3-q22	Thymosin β4, X chromosome	1.02
XM_005047	9	RPL7	8q	Ribosomal protein L7	
NM_001829	6	CLCN3	4q33	Chloride channel 3	
NM_002933	5	RNASE1	Chr.14	Ribonuclease, RNase A family, 1	
XM_009451	5	NPCRP/PLUNC	20q11.2	(palate lung and nasal epithelium clone); tracheal epithelium-enriched protein	46.2
AK026534	4	FLJ22881		Ferritin L chain	
NM_014302	4	Sec61G		Sec61 γ	7.9
XM_009292	4	IFI30		Interferon, γ-inducible protein 30	
AC010196	3	RP11-202H2	12q seeder		
XM_004738	3	EGFR	7p12.3-p12.1	Epidermal growth factor receptor	
AF267861	3	EF1a-like protein	6q14	Eucaryotic translation elongation factor 1	
AF178581	3	NCG		Nasopharyngeal carcinoma gene sequence	
J03537	3	RPS6	9p21	Ribosomal protein S6	
HS644L1	3	RP4-644L1	20q12	Kreisler (mouse) maf-related leucine zipper homolog	
D82059	2	MRLC3	18	Myosin regulatory light chain 3	4.6
XM_002929	2	NDUFB4		NADH dehydrogenase (ubiquinone) 1, subcomlex, 4	
XM_004073	2	PGC	6p21.3-p21.1	Progastricsin (pepsinogen C)	
XM_008923	2	RPS11	19q	Ribosomal protein S11	
BC001392	2	RPS27a	Chr.1	Ribosomal protein S27a	
BC007273	2	RPL27		Ribosomal protein L27	
AF118092	2	PRO2061			
AK022248	2	FLJ12186		Weakly similar to ACTIN	
AF147331	2	YB24D08			
Y00052	2	CYPs	5q23	mRNA for T-cell cydophilin	
XM_007192	1	TPT1	13q12-q14	Tumor protein, translationally controlled 1	4.1
XM_005403	1	EHM2	9		3.4
XM_002072	1	SSR2	1q21-q23	Signal sequence receptor, β (translocation-associated protein β)	3.0
NM_001416	1	eIF4A1	17p13	Eucaryotic translation initiation factor 4A, isoform 1	2.3
NM_002719	1	PPP2R5C		Protein phosphatase 2, regulatory subunit B, γ isoform	1.7
AF144029	1	MDM2	12q14.3-q15	MDM2 gene, intron 9 and exon 10	1.4

Table 1 Metastasis-associated transcripts in NSCLC identified by subtractive hybridization

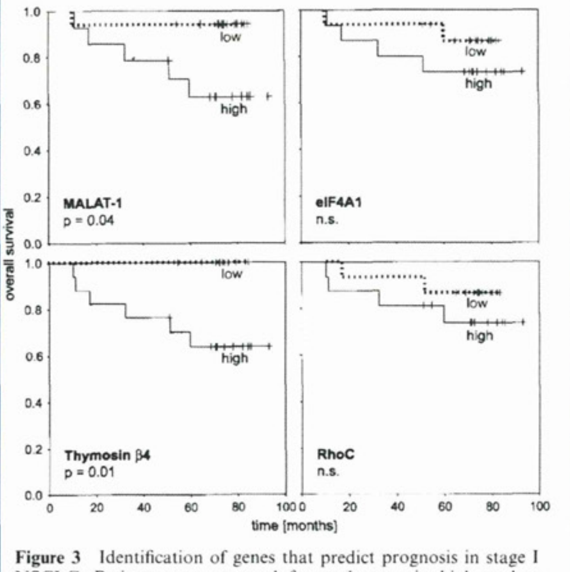
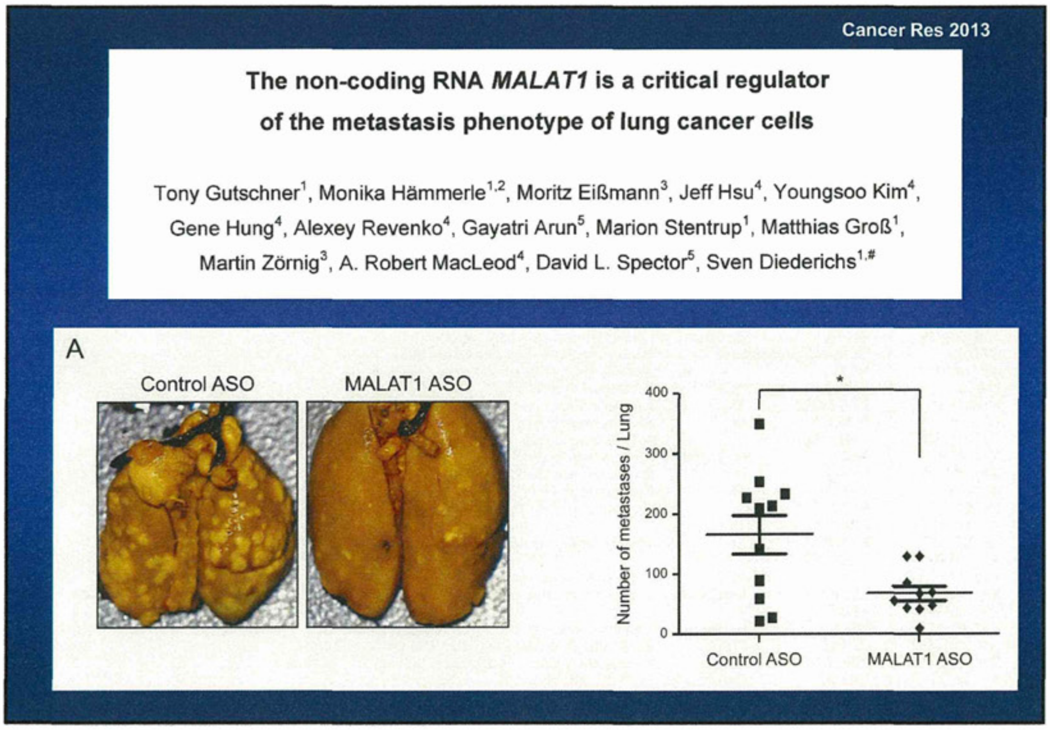
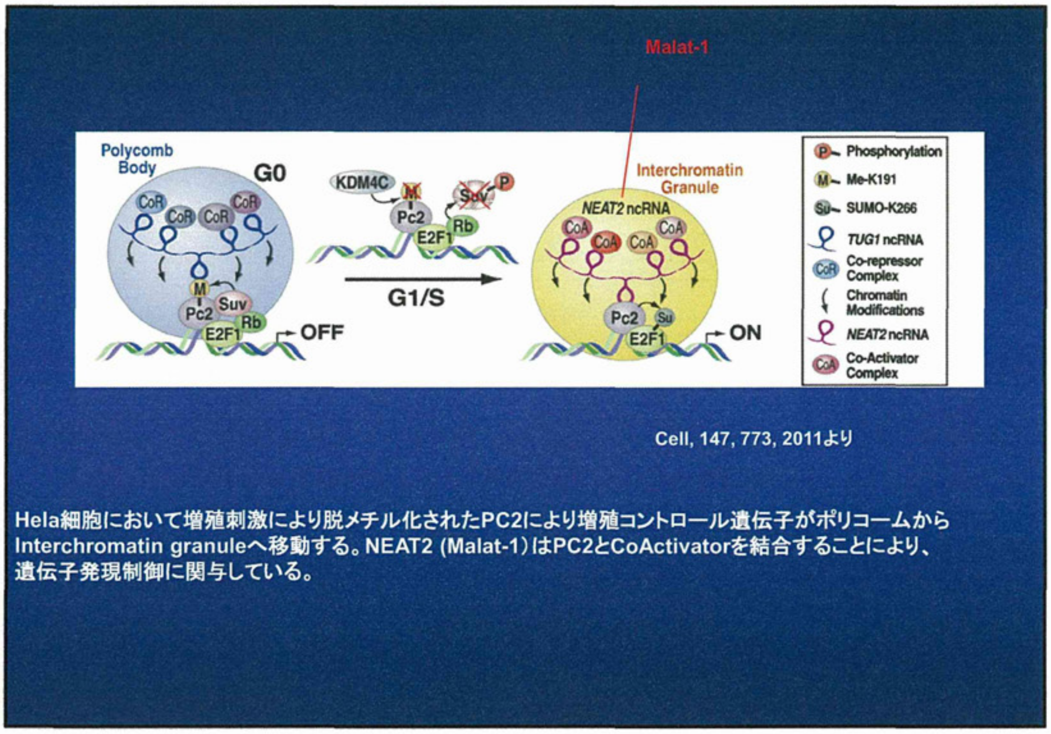


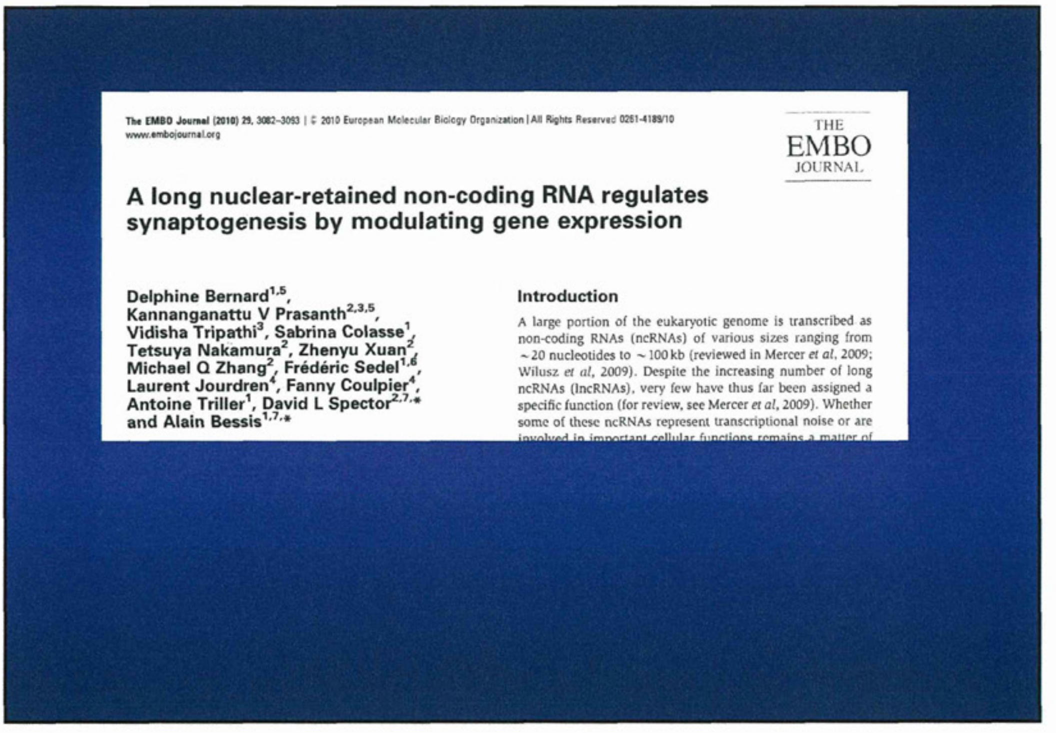
Figure 3 Identification of genes that predict prognosis in stage I NSCLC. Patients were grouped for each gene in high vs low expressing tumors based on its gene expression in comparison to the median expression of all tumors. The Kaplan–Meier survival plots are shown for patients with adenocarcinoma or squamous cell carcinoma and stage I disease ($n = 31$). The log-rank test was used to calculate statistical significance

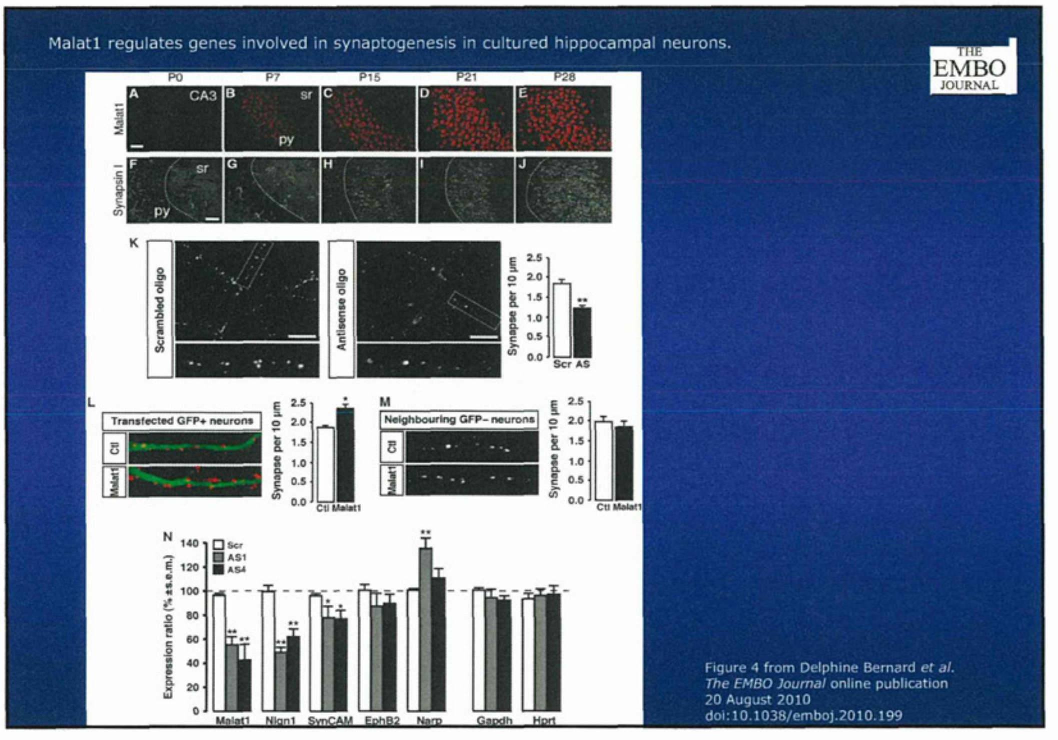


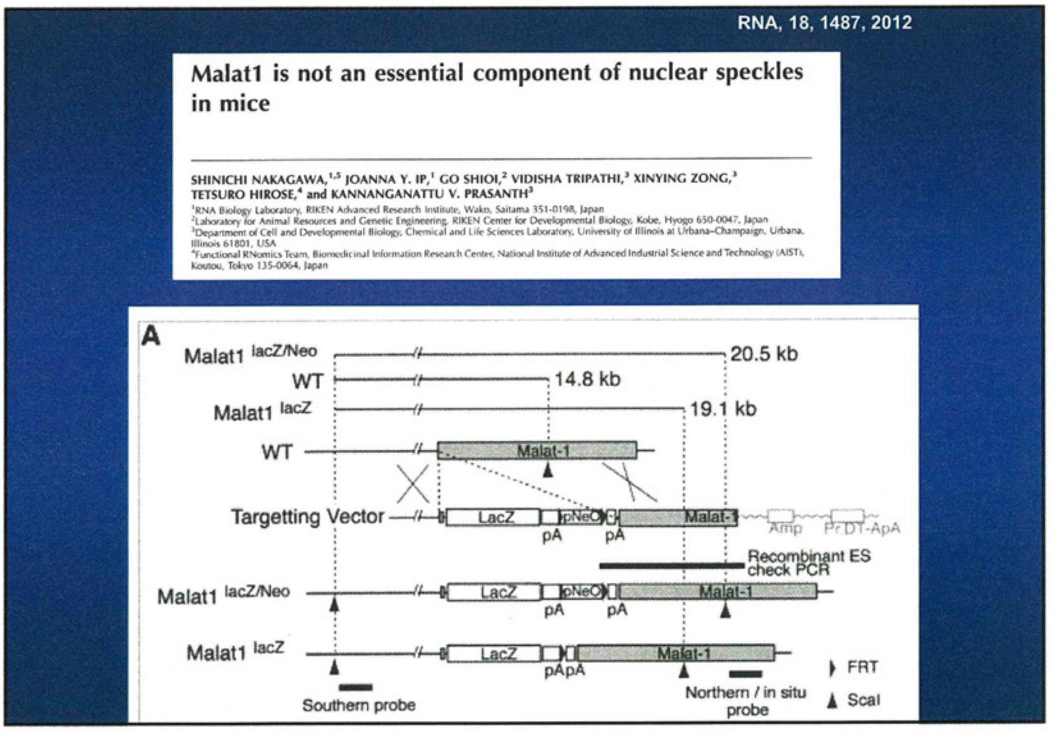
Cancer Res 2013
The non-coding RNA MALAT1 is a critical regulator
of the metastasis phenotype of lung cancer cells
Tony Gutschner[1], Monika Hämmerle[1,2], Moritz Eißmann[3], Jeff Hsu[4], Youngsoo Kim[4],
Gene Hung[4], Alexey Revenko[4], Gayatri Arun[5], Marion Stentrup[1], Matthias Groß[1],
Martin Zörnig[3], A. Robert MacLeod[4], David L. Spector[5], Sven Diederichs[1,#]
A
Control ASO
MALAT1 ASO
Number of metastases / Lung
400
300
200
100
0
*
Control ASO
MALAT1 ASO

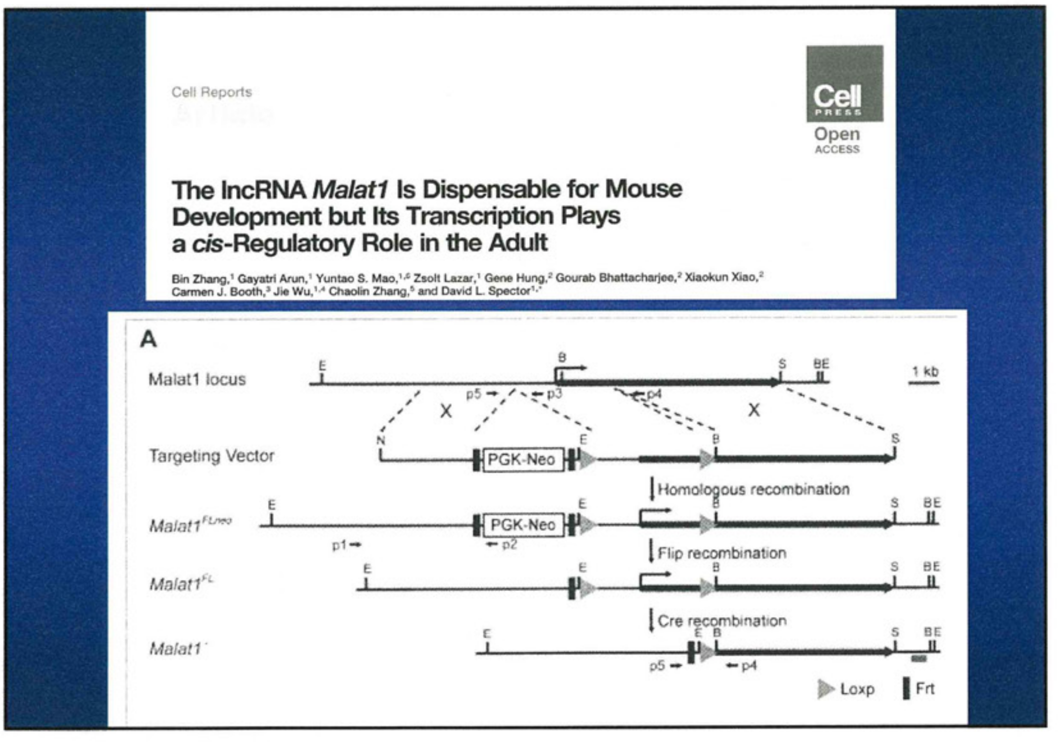


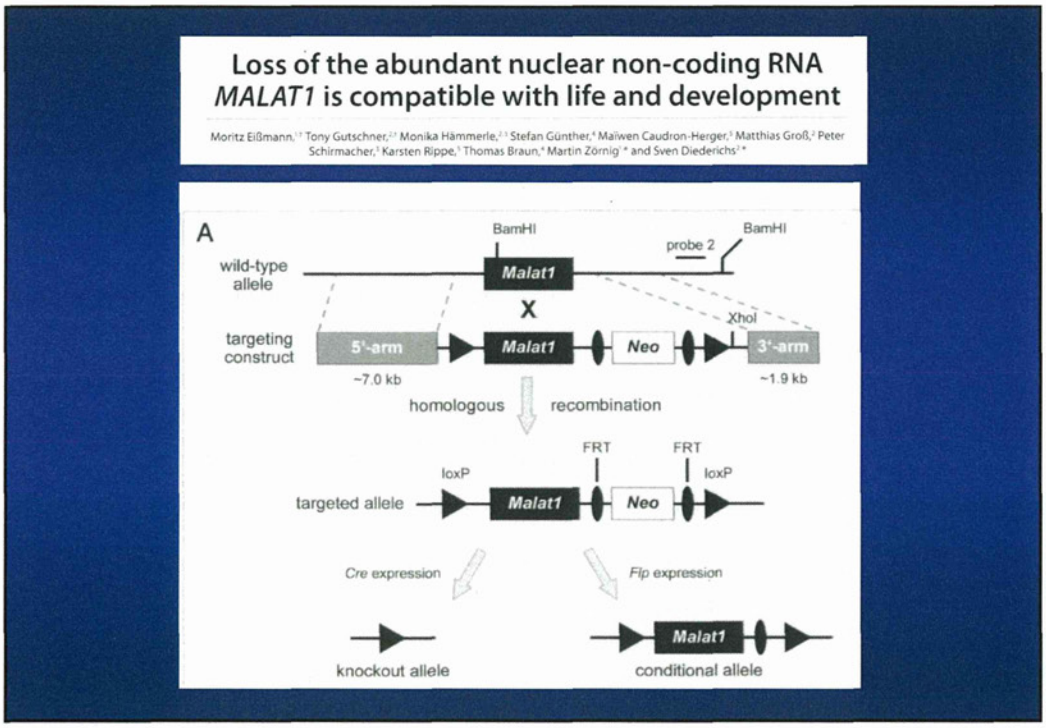
Malat-1
Polycomb Body
G0
CoR
M
Pc2
Suv
Rb
E2F1
OFF
KDM4C
G1/S
Interchromatin Granule
NEAT2 ncRNA
CoA
Su
ON
Phosphorylation
Me-K191
SUMO-K266
TUG1 ncRNA
Co-repressor Complex
Chromatin Modifications
NEAT2 ncRNA
Co-Activator Complex
Cell, 147, 773, 2011より
Hela細胞において増殖刺激により脱メチル化されたPC2により増殖コントロール遺伝子がポリコームから
Interchromatin granuleへ移動する。NEAT2 (Malat-1)はPC2とCoActivatorを結合することにより、
遺伝子発現制御に関与している。

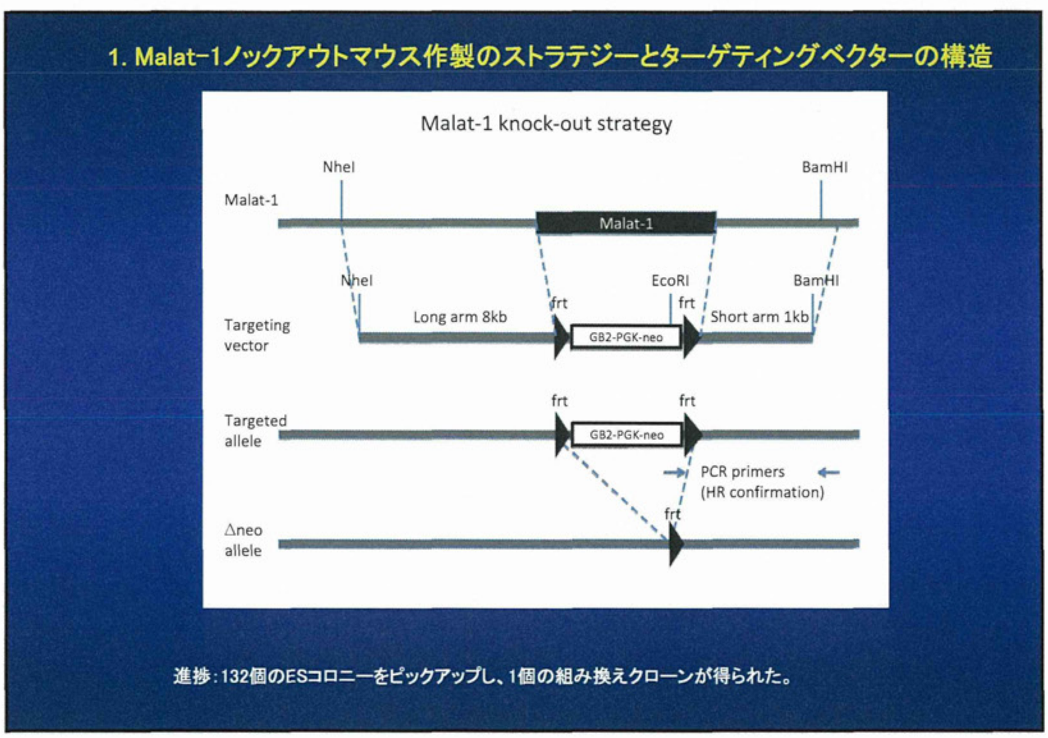

The EMBO Journal (2010) 29, 3082–3093 | © 2010 European Molecular Biology Organization | All Rights Reserved 0261-4189/10
www.embojournal.org
THE EMBO JOURNAL
A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression
Delphine Bernard1,5, Kannanganattu V Prasanth2,3,5, Vidisha Tripathi3, Sabrina Colasse1, Tetsuya Nakamura2, Zhenyu Xuan2, Michael Q Zhang2, Frédéric Sedel1,6, Laurent Jourdren4, Fanny Coulpier4, Antoine Triller1, David L Spector2,7,* and Alain Bessis1,7,*
Introduction
A large portion of the eukaryotic genome is transcribed as non-coding RNAs (ncRNAs) of various sizes ranging from ~20 nucleotides to ~100 kb (reviewed in Mercer et al, 2009; Wilusz et al, 2009). Despite the increasing number of long ncRNAs (lncRNAs), very few have thus far been assigned a specific function (for review, see Mercer et al, 2009). Whether some of these ncRNAs represent transcriptional noise or are


Malat1 regulates genes involved in synaptogenesis in cultured hippocampal neurons.
THE EMBO JOURNAL
P0
P7
P15
P21
P28
Malat1
Synapsin I
CA3
sr
py
Scrambled oligo
Antisense oligo
Synapse per 10 µm
Scr AS
Transfected GFP+ neurons
Neighbouring GFP− neurons
Ctl
Malat1
Ctl Malat1
Expression ratio (% ±s.e.m.)
Scr
AS1
AS4
Malat1
Nlgn1
SynCAM
EphB2
Narp
Gapdh
Hprt
Figure 4 from Delphine Bernard et al.
The EMBO Journal online publication
20 August 2010
doi:10.1038/emboj.2010.199

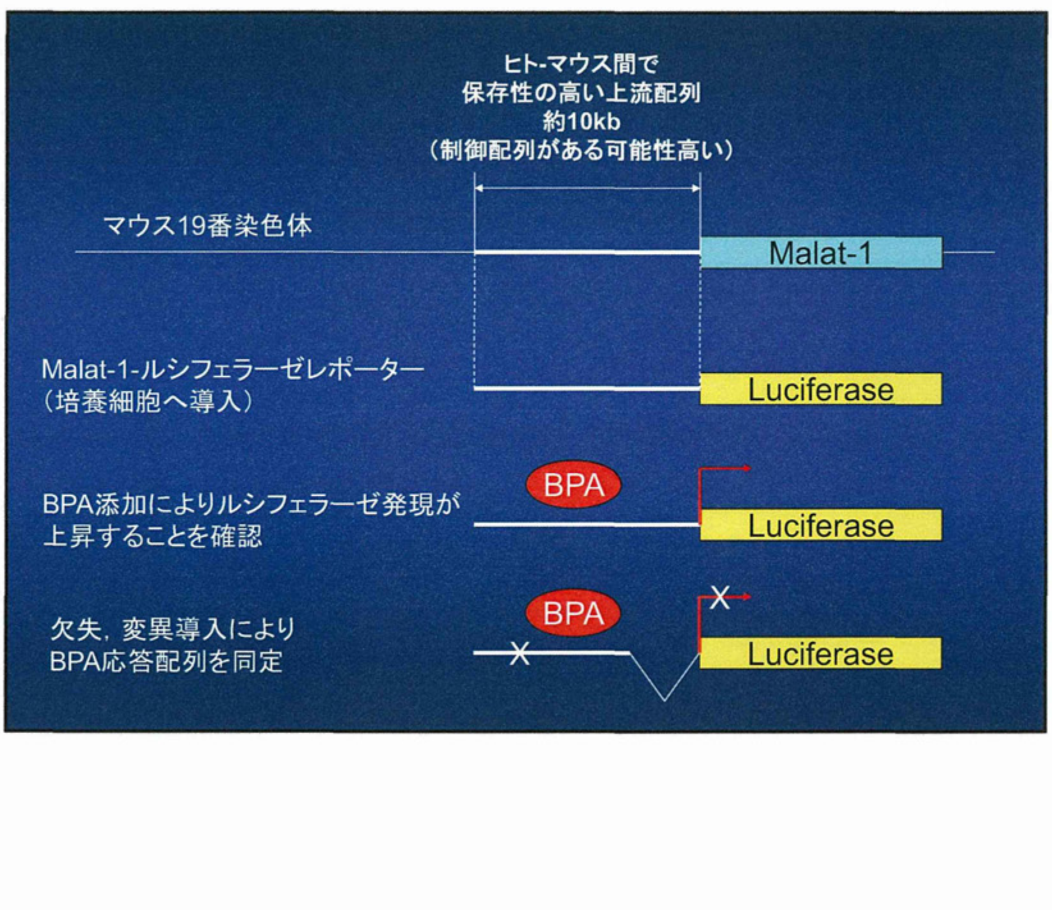

RNA, 18, 1487, 2012
Malat1 is not an essential component of nuclear speckles in mice
SHINICHI NAKAGAWA,[1,5] JOANNA Y. IP,[1] GO SHIOI,[2] VIDISHA TRIPATHI,[3] XINYING ZONG,[3] TETSURO HIROSE,[4] and KANNANGANATTU V. PRASANTH[3]
[1]RNA Biology Laboratory, RIKEN Advanced Research Institute, Wako, Saitama 351-0198, Japan
[2]Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan
[3]Department of Cell and Developmental Biology, Chemical and Life Sciences Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, USA
[4]Functional RNomics Team, Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Koutou, Tokyo 135-0064, Japan
A
Malat1 lacZ/Neo
20.5 kb
WT
14.8 kb
Malat1 lacZ
19.1 kb
WT
Malat-1
Targetting Vector
LacZ
pNeO
Malat-1
Amp
pA
pA
Recombinant ES check PCR
Malat1 lacZ/Neo
LacZ
pNeO
Malat-1
pA
pA
Malat1 lacZ
LacZ
Malat-1
pApA
FRT
Southern probe
Northern / in situ probe
ScaI


Cell Reports
Cell PRESS
Open ACCESS
The lncRNA Malat1 Is Dispensable for Mouse Development but Its Transcription Plays a cis-Regulatory Role in the Adult
Bin Zhang,[1] Gayatri Arun,[1] Yuntao S. Mao,[1,6] Zsolt Lazar,[1] Gene Hung,[2] Gourab Bhattacharjee,[2] Xiaokun Xiao,[2] Carmen J. Booth,[3] Jie Wu,[1,4] Chaolin Zhang,[5] and David L. Spector[1,*]
A
Malat1 locus
1 kb
p5
p3
p4
Targeting Vector
PGK-Neo
Homologous recombination
Malat1 FL/neo
PGK-Neo
p1
p2
Flip recombination
Malat1 FL
Cre recombination
Malat1
p5
p4
Loxp
Frt

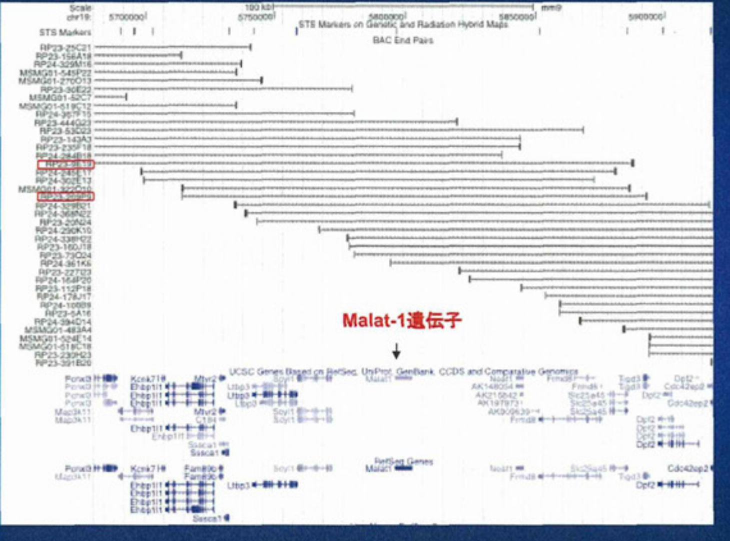

Loss of the abundant nuclear non-coding RNA
MALAT1 is compatible with life and development
Moritz Eißmann, Tony Gutschner, Monika Hämmerle, Stefan Günther, Maïwen Caudron-Herger, Matthias Groß, Peter Schirmacher, Karsten Rippe, Thomas Braun, Martin Zörnig and Sven Diederichs
A
BamHI
BamHI
probe 2
wild-type allele
Malat1
X
XhoI
targeting construct
5'-arm
Malat1
Neo
3'-arm
~7.0 kb
~1.9 kb
homologous
recombination
FRT
FRT
loxP
loxP
targeted allele
Malat1
Neo
Cre expression
Flp expression
knockout allele
Malat1
conditional allele


1. Malat-1ノックアウトマウス作製のストラテジーとターゲティングベクターの構造
Malat-1 knock-out strategy
NheI
BamHI
Malat-1
Malat-1
NheI
EcoRI
BamHI
frt
frt
Targeting vector
Long arm 8kb
GB2-PGK-neo
Short arm 1kb
frt
frt
Targeted allele
GB2-PGK-neo
PCR primers
(HR confirmation)
frt
Δneo allele
進捗：132個のESコロニーをピックアップし、1個の組み換えクローンが得られた。

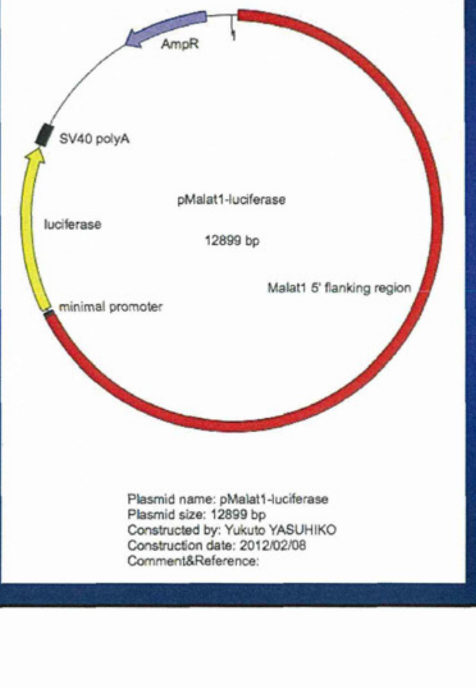
2.Malat-1遺伝子のプロモーター領域をルシフェラーゼ遺伝子に結合させたコンストラクトと同定した受容体遺伝子をCos7あるいはES細胞にco-transfectさせ、ルシフェラーゼアッセイを行い、BPAによるMalat-1誘導に必要な領域を限定する。



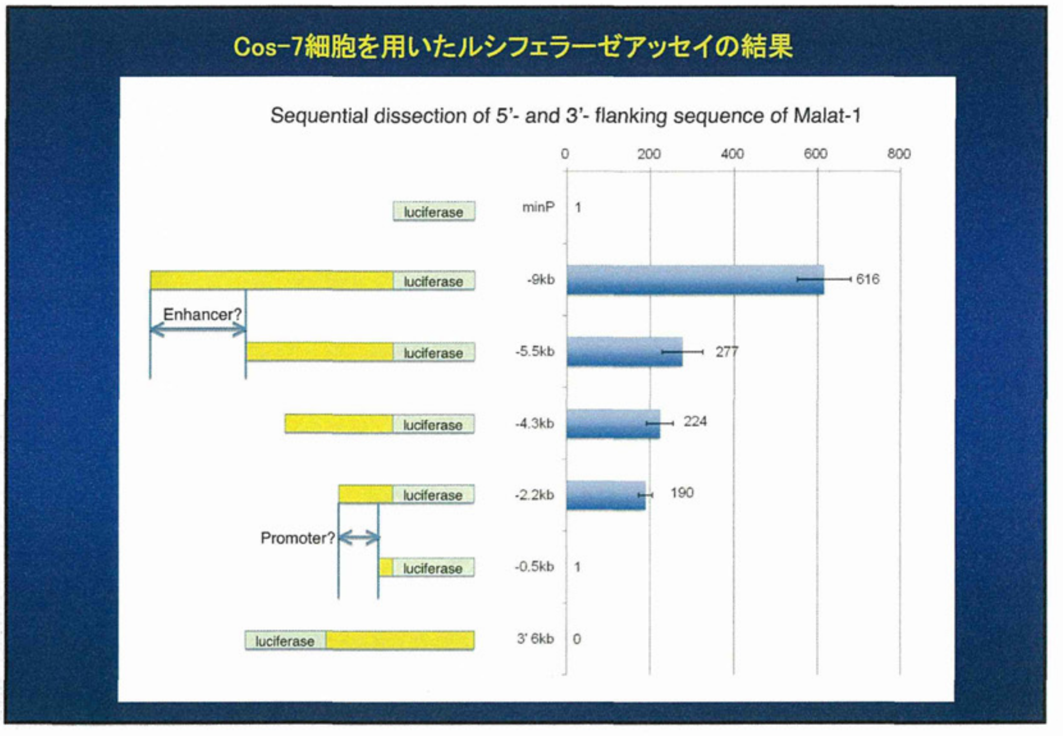
UCSC Genome Browser からBAC cloneの選択

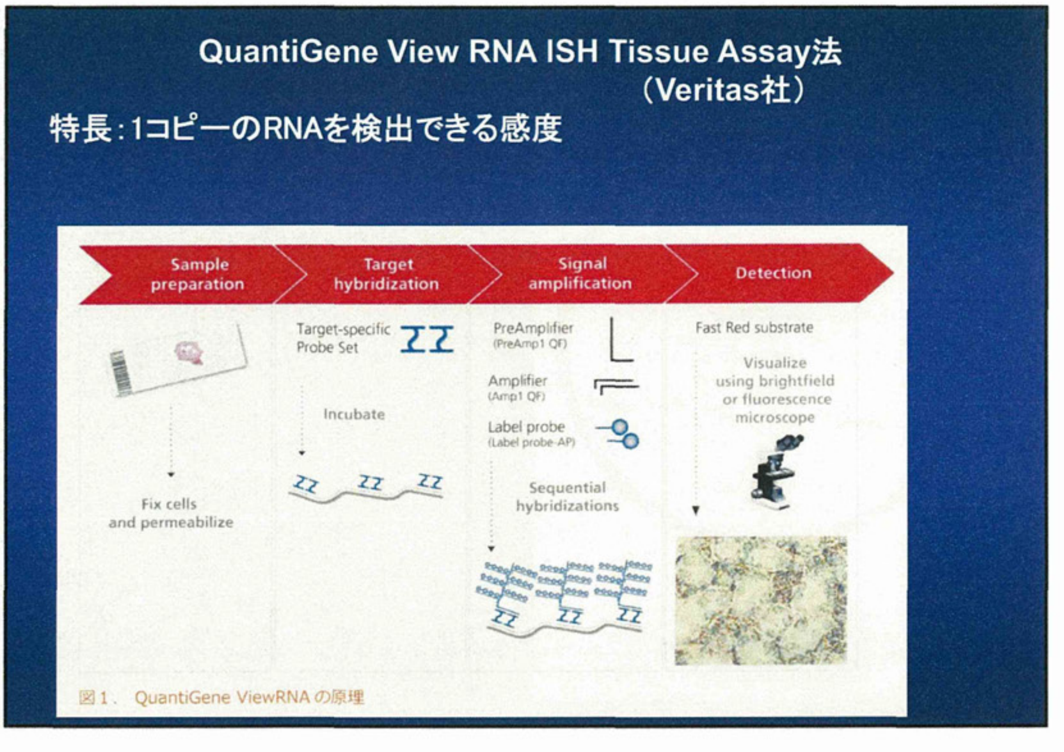


完成したconstruct

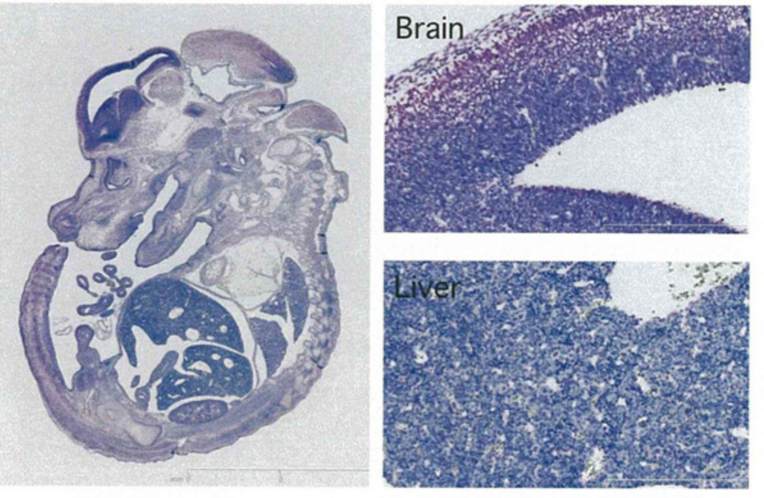


C57BL/6マウスのMalat-1遺伝子およびその上流・下流領域を含むBAC(細菌人工染色体)クローン(RP23-9E19)をIn vitrogen社から入手し、Malat-1上流9kbを制限酵素EcoRIおよびNheIで切り出してレポーターベクターpGL4-23(プロメガ社)に挿入し、ベクターを構築した。


Cos-7細胞を用いたルシフェラーゼアッセイの結果
Sequential dissection of 5'- and 3'- flanking sequence of Malat-1
0
200
400
600
800
luciferase
minP
1
luciferase
-9kb
616
Enhancer?
luciferase
-5.5kb
277
luciferase
-4.3kb
224
luciferase
-2.2kb
190
Promoter?
luciferase
-0.5kb
1
luciferase
3' 6kb
0


QuantiGene View RNA ISH Tissue Assay法
（Veritas社）
特長：1コピーのRNAを検出できる感度
Sample preparation
Target hybridization
Signal amplification
Detection
Target-specific Probe Set
PreAmplifier
(PreAmp1 QF)
Amplifier
(Amp1 QF)
Label probe
(Label probe-AP)
Fast Red substrate
Visualize using brightfield or fluorescence microscope
Incubate
Sequential hybridizations
Fix cells and permeabilize
図1. QuantiGene ViewRNA の原理

3. 胎生14.5日のマウス胎児におけるmalat-1遺伝子のin situ hybridization結果



RNA, 18, 1487, 2012

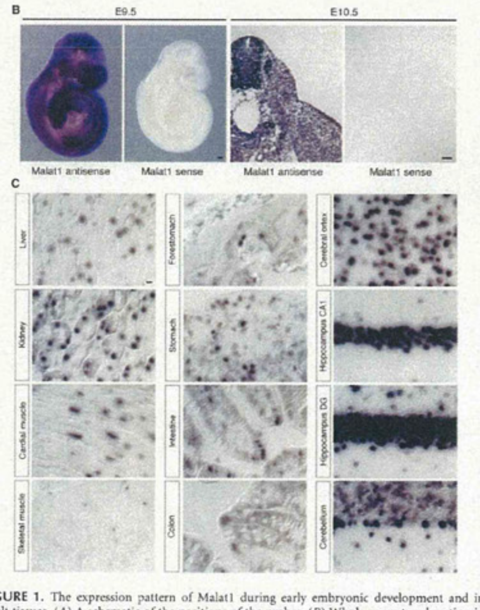
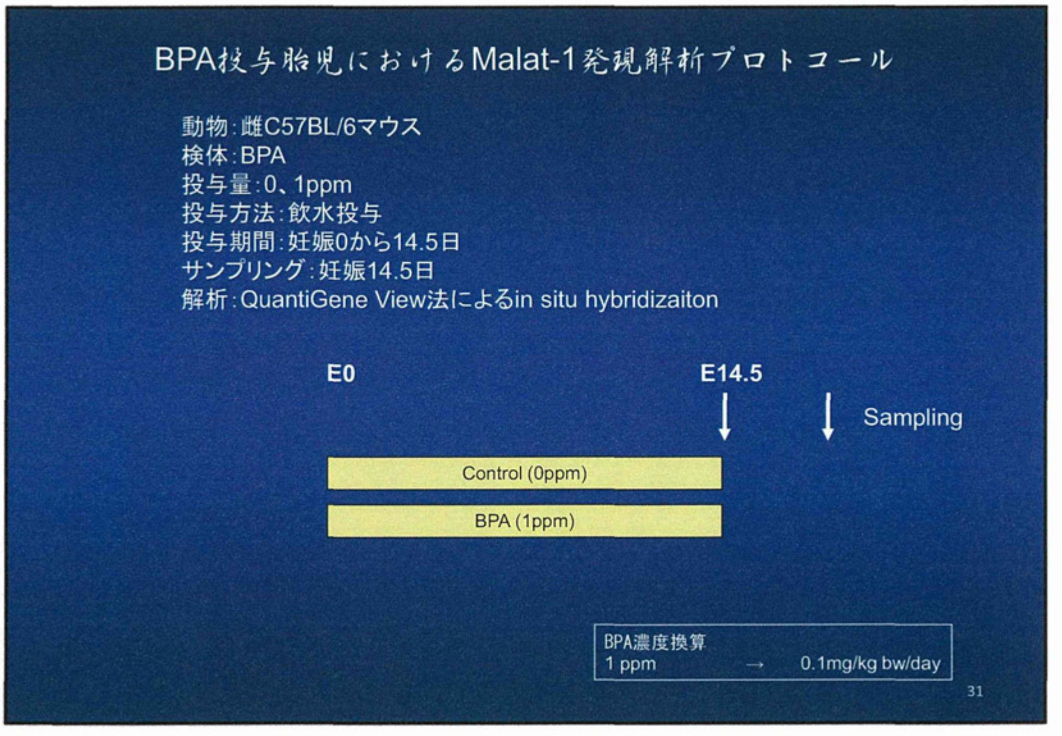
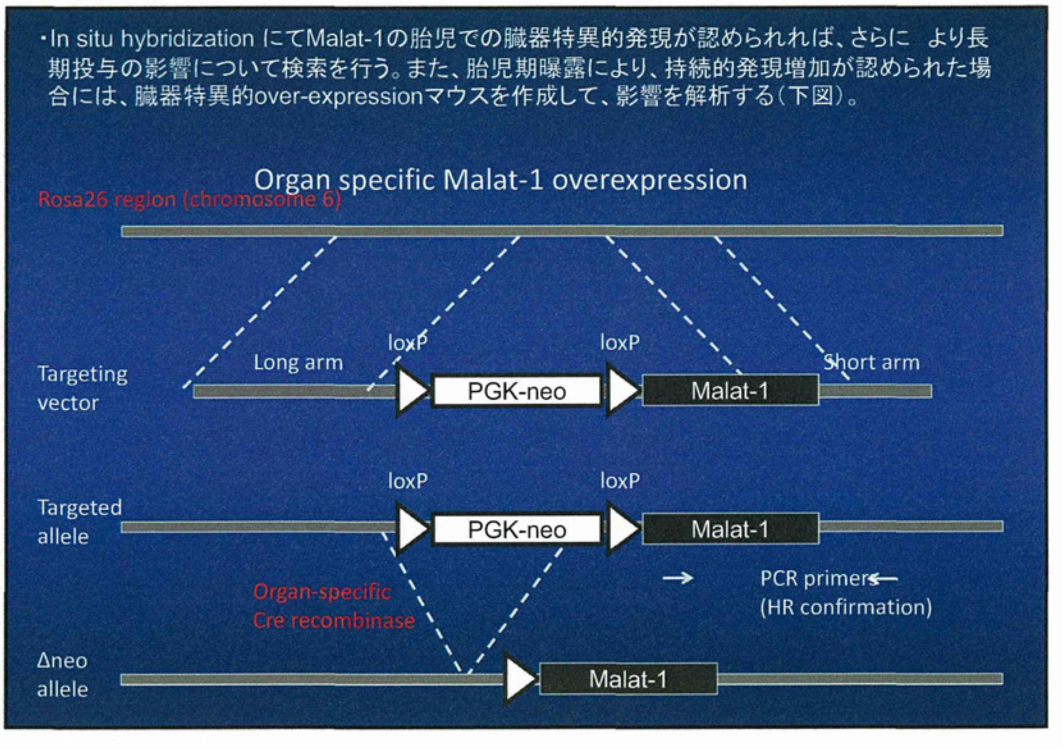


FIGURE 1. The expression pattern of Malat1 during early embryonic development and in adult tissues. (*A*) A schematic of the positions of the probes. (*B*) Whole-mount and section in situ hybridization of E9.5 and E10.5 mouse embryos with Malat1 antisense and sense probes. Note that strong and uniform expression was detected using the antisense probes, whereas no expression was detected using the sense probes. (*C*) In situ hybridization of adult organs using Malat1 antisense probes. (CA1) Hippocampus CA1; (DG) dentate gyrus. Scale bars, (*B*) 100 µm; (*C*) 10 µm.


BPA投与胎児におけるMalat-1発現解析プロトコール
動物：雌C57BL/6マウス
検体：BPA
投与量：0、1ppm
投与方法：飲水投与
投与期間：妊娠0から14.5日
サンプリング：妊娠14.5日
解析：QuantiGene View法によるin situ hybridizaiton
E0
E14.5
Sampling
Control (0ppm)
BPA (1ppm)
BPA濃度換算
1 ppm → 0.1mg/kg bw/day
31


・In situ hybridization にてMalat-1の胎児での臓器特異的発現が認められれば、さらに より長期投与の影響について検索を行う。また、胎児期曝露により、持続的発現増加が認められた場合には、臓器特異的over-expressionマウスを作成して、影響を解析する（下図）。
Organ specific Malat-1 overexpression
Rosa26 region (chromosome 6)
Targeting vector
Long arm
loxP
loxP
Short arm
PGK-neo
Malat-1
Targeted allele
loxP
loxP
PGK-neo
Malat-1
Organ-specific Cre recombinase
PCR primers
(HR confirmation)
Δneo allele
Malat-1

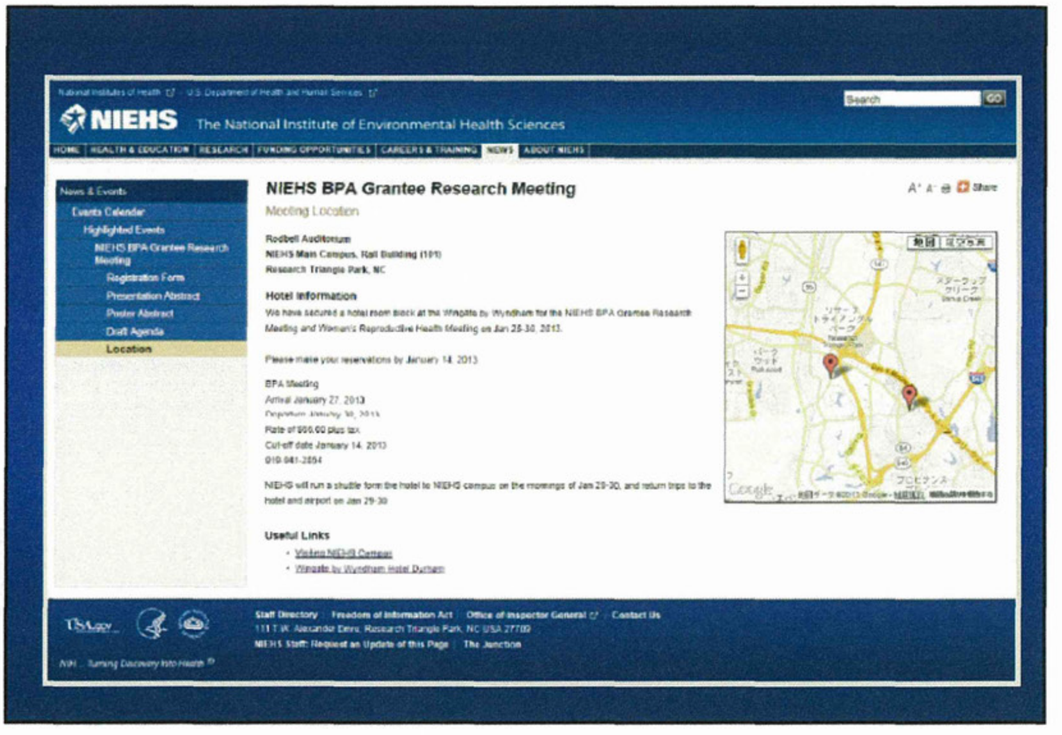
まとめ

Malat1遺伝子上流9kbを含むレポーターベクター、Malat-1遺伝子ノックアウトマウス作製のためのターゲティングベクター、Malat-1の*in situ* hybridizationの系の確立を本年度末から来年度初頭までに完了し予定どおりのBPAの影響解析を目指す。

発生、発育後の遅発性影響との関連を、発現欠失モデル、選択的過剰発現モデル等の所見から明らかにする方向へ進展させる基盤を構築する。

OECD/WHO関連

厚労省「試験スキーム」の拡充に関連し、米国NIEHSにおける内分泌かく乱化学物質研究費研究班成果発表会にコメンテータとして参加し、情報収集と情報交換を行う。また、OECDの拡張型第一世代繁殖試験をサポートするためのTG443に付随するガイダンスドキュメント151(GD151)の内容に関する意見交換を本年度も実施した。以上の活動を基盤に、厚労省「試験スキーム」の拡充に関わる厚労省検討会の活動について化学物質安全対策室との調整を開始した。



<u>January 28</u>

8:30 AM	Welcome, Linda Birnbaum, *Director NIEHS/NTP*
8:45	Introduction and Overview, Jerry Heindel, *NIEHS*

Session I: BPA Biomonitoring and Pharmacokinetics

9:00	Tracey Woodruff, *University of California San Francisco;* Laura Vandenberg, *Tufts University*
9:40	Discussion
10:00	NIEHS/NTP BPA Pharmacokinetic and Dermal Exposure Study, Linda Birnbaum, *Director NIEHS/NTP*
10:30	Break
10:50	BPA pharmacokinetics across species, Louis Toutain, *Ecole Nationale Veterinaire de Toulouse, France*
11:20	Human fetal tissue BPA metabolism, Dana Dolinoy, *University of Michigan*
11:40	Discussion
12:00 PM	Lunch, NIEHS Cafeteria
12:50	Discussion

Session II: NIEHS BPA Grantees: Cancer (30 minute presentations and 15 minute discussion for all grantee presentations)

1:00
Cheryl, Walker, *Texas A &M*
1:30
Gail Prins, *University of Illinois, Chicago*
2:00
Ana Soto, *Tufts University*
2:30
Nameer Kirma, *University of Texas Health Science Center*
2:45
Open Discussion
3:00
Break

3:15 Session III: Posters (all grantees invited to present a poster)

5:30
Adjourn, Bus back to Hotel
6:30
Reception and Dinner, TBD

January 29
Session IV: NIEHS BPA Grantees: Metabolism, Reproduction and Sexual Behavior
8:00 AM
Shana Swan, *Mt Sinai School of Medicine*
8:30
David Waxman, *Boston University*
9:00
Cheryl Rosenfeld, *University of Missouri-Columbia*
9:30
Fred vom Saal, *University of Missouri-Columbia*
10:00
Open Discussion
10:30
Break
10:45
Beverly Rubin/Andrew Greenberg, *Tufts University*
11:15
Kim Harley, *University of California, Berkeley*
10:45
Open Discussion
12:00 PM
Lunch, NIEHS Cafeteria
1:00
Pat Hunt, *Washington State University*

Session V: Immunity

1:30
Katie Donohue, *Columbia University*

2:00
Steve Georas, *University of Rochester School of Medicine & Dentistry*

Session VI: Cardiology

2:30
Scott Belcher, *University of Cincinnati*

Session VII: BPA Consortium

3:00
Low Dose studies on BPA, Laura Vandenberg, *Tufts University*

3:30
CLARITY- BPA U01 Consortium, Luisa Camacho, *FDA/NCTR*

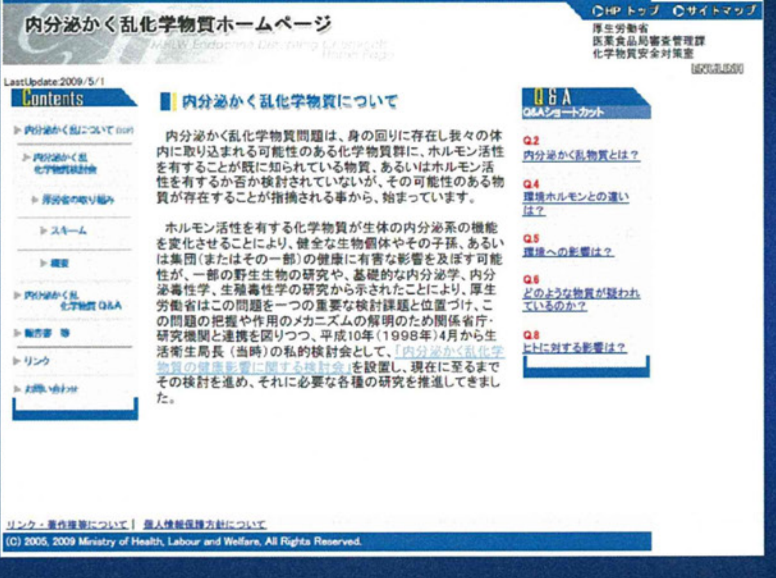
4:00
Open Discussion

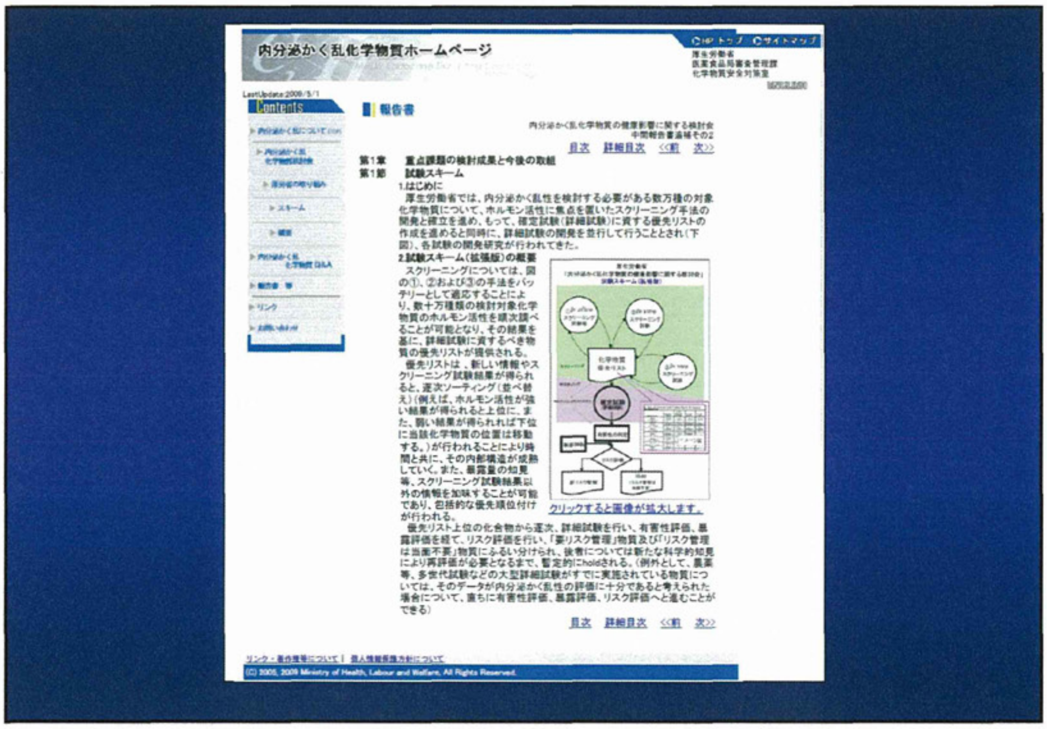
4:20
Virtual consortium assessment: Program Analysis Branch, *DERT*

4:40
Meeting Wrap-up (Discussion of future directions including EHP reviews), Jerry Heindel, *NIEHS*

5:00
Adjourn

6:30
Dinner, TBD




内分泌かく乱化学物質ホームページ
HPトップ
サイトマップ
厚生労働省
医薬食品局審査管理課
化学物質安全対策室
LastUpdate:2009/5/1
Contents
リンク
報告書
内分泌かく乱化学物質の健康影響に関する検討会
中間報告書追補その2
目次 詳細目次 <<前 次>>
第1章 重点課題の検討成果と今後の取組
第1節 試験スキーム
1.はじめに
厚生労働省では、内分泌かく乱性を検討する必要がある数万種の対象化学物質について、ホルモン活性に焦点を置いたスクリーニング手法の開発と確立を進め、もって、確定試験（詳細試験）に資する優先リストの作成を進めると同時に、詳細試験の開発を並行して行うこととされ（下図）、各試験の開発研究が行われてきた。
2.試験スキーム（拡張版）の概要
スクリーニングについては、図の①、②および③の手法をバッテリーとして適応することにより、数十万種類の検討対象化学物質のホルモン活性を順次調べることが可能となり、その結果を基に、詳細試験に資するべき物質の優先リストが提供される。
優先リストは、新しい情報やスクリーニング試験結果が得られると、逐次ソーティング（並べ替え）（例えば、ホルモン活性が強い結果が得られると上位に、また、弱い結果が得られれば下位に当該化学物質の位置は移動する。）が行われることにより時間と共に、その内部構造が成熟していく。また、暴露量の知見等、スクリーニング試験結果以外の情報を加味することが可能であり、包括的な優先順位付けが行われる。
化学物質優先リスト
クリックすると画像が拡大します。
優先リスト上位の化合物から逐次、詳細試験を行い、有害性評価、暴露評価を経て、リスク評価を行い、「要リスク管理」物質及び「リスク管理は当面不要」物質にふるい分けられ、後者については新たな科学的知見により再評価が必要となるまで、暫定的にholdされる。（例外として、農薬等、多世代試験などの大型詳細試験がすでに実施されている物質については、そのデータが内分泌かく乱性の評価に十分であると考えられた場合について、直ちに有害性評価、暴露評価、リスク評価へと進むことができる）
目次 詳細目次 <<前 次>>
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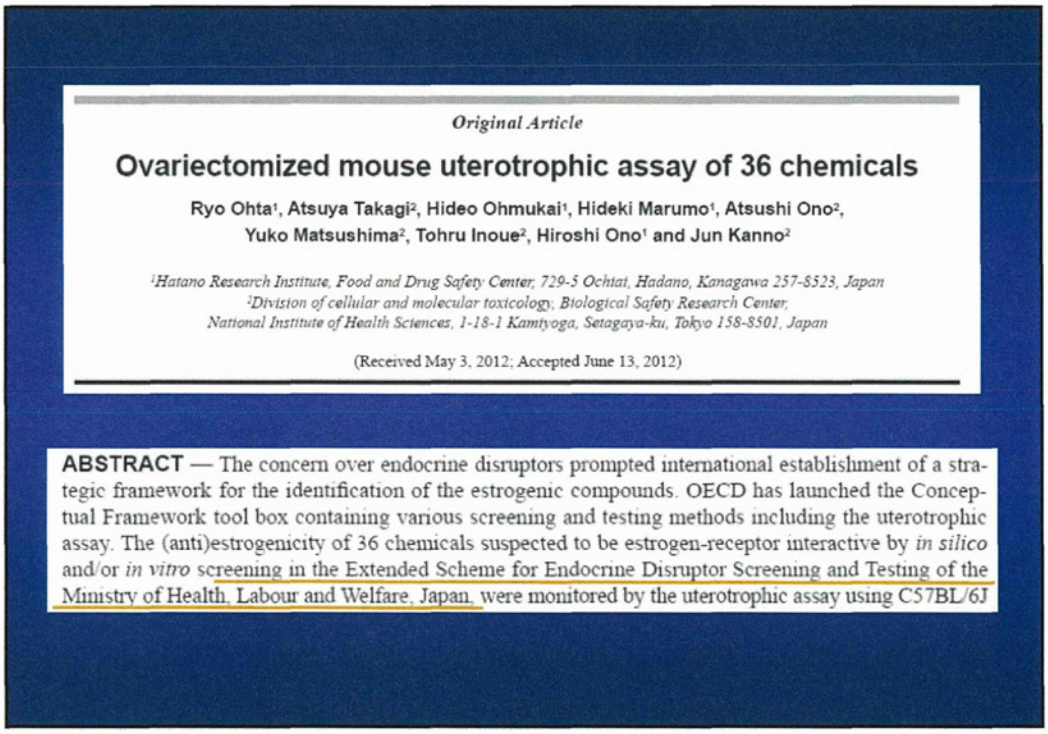
Original Article
Ovariectomized mouse uterotrophic assay of 36 chemicals
Ryo Ohta[1], Atsuya Takagi[2], Hideo Ohmukai[1], Hideki Marumo[1], Atsushi Ono[2], Yuko Matsushima[2], Tohru Inoue[2], Hiroshi Ono[1] and Jun Kanno[2]
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ABSTRACT — The concern over endocrine disruptors prompted international establishment of a strategic framework for the identification of the estrogenic compounds. OECD has launched the Conceptual Framework tool box containing various screening and testing methods including the uterotrophic assay. The (anti)estrogenicity of 36 chemicals suspected to be estrogen-receptor interactive by in silico and/or in vitro screening in the Extended Scheme for Endocrine Disruptor Screening and Testing of the Ministry of Health, Labour and Welfare, Japan, were monitored by the uterotrophic assay using C57BL/6J

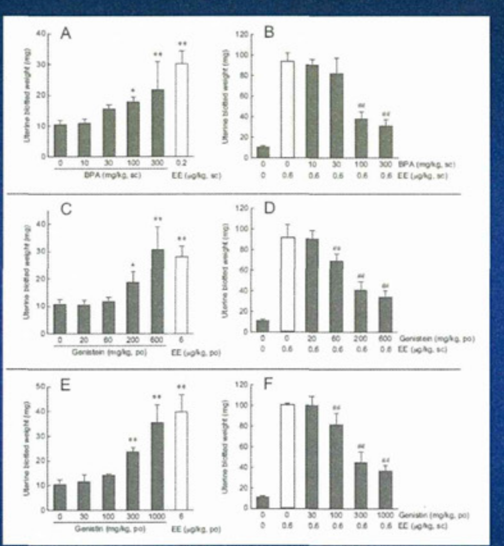
Table 1. Test chemicals

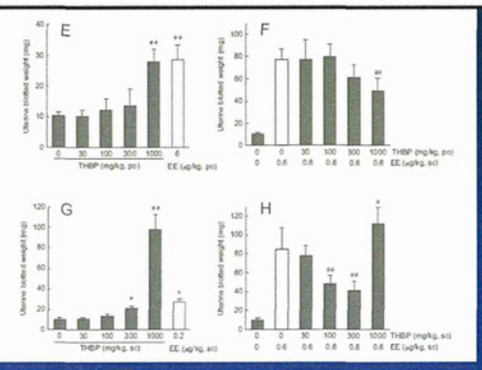
No.	Chemical	Abbreviation	CAS No.	Source	Purity (%)	Vehicle
ref	Ethynyl estradiol	EE	57-63-6	Wako Pure Chemical	99.0	Corn oil
a	Bisphenol A	BPA	80-05-7	Wako Pure Chemical	99.0	Corn oil
b	Genistein	Genistein	446-72-0	Wako Pure Chemical	>98	Corn oil
c	Genistin	Genistin	529-59-9	Wako Pure Chemical	>98	Corn oil
d	Daidzein	Daidzein	486-66-8	Wako Pure Chemical	>98	Corn oil
1	2-[Bis(4-hydroxyphenyl)methyl]benzylalcohol	BHPMBA	81-92-5	Tokyo Kasei Kogyo Co	98.3	Corn oil
2	2,2',4,4'-Tetrahydroxybenzophenone	THBP	131-55-5	Wako Pure Chemical	>95	Corn oil
3	2,4-Dihydroxybenzophenone	DHBP	131-56-6	Wako Pure Chemical	98	Corn oil
4	3,3',5-Triiodothyroacetic acid	Tiratricol	51-24-1	Sigma-Aldrich Japan	99.4	Corn oil
5	New fuchsin	NF	3248-91-7	ICN Biomedicals, Inc	Unknown	Distilled Water
6	alpha-Naphtholbenzein	ANB	6948-88-5	ICN Biomedicals Inc.	Unknown	Corn oil
7	N,N-Diphenyl-p-phenylenediamine	PDD	2350-01-8	Wako Pure Chemical	>90	Corn oil
8	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone	DDB	131-54-4	Wako Pure Chemical	>95	Corn oil
9	n-Butyl 4-hydroxybenzoate	BHB	94-26-8	Wako Pure Chemical	>98	Corn oil
10	Reserpine	Res	50-55-5	Wako Pure Chemical	>98	Distilled Water
11	Benzo [a] pyrene	B[a]P	50-32-8	Wako Pure Chemical	>98	Corn oil
12	Benz [a] anthracene	B[a]A	56-55-3	Wako Pure Chemical	>99	Corn oil
13	Dibenz [a,h] anthracene	DB[a,h]A	53-70-3	Wako Pure Chemical	>90	Corn oil
14	2-(2H-Benzotriazol-2-yl)-4,6-di(t-pentyl)phenol	BTPP	25973-55-1	Wako Pure Chemical	>97	Corn oil
15	Rosmarinic acid	RMA	20283-92-5	Sigma-Aldrich Co.	99.9	Corn oil
16	meta-Thymol	MT	3228-03-3	Sigma-Aldrich Co.	99.4	Corn oil
17	6-Gingerol	6-gin	23513-14-6	Wako Pure Chemical	>98	Corn oil
18	Colchicine	Col	64-86-8	Wako Pure Chemical Ind	>95	Distilled Water
19	Malachite green base	MGB	510-13-4	Sigma-Aldrich Co.	90	Distilled Water
20	Fenbuconazole	FBZ	114369-43-6	GL Sciences Inc.	99	Corn oil
21	Lead acetate	LA	6080-56-4	Wako Pure Chemical	>99	Distilled Water
22	n-Heptyl 4-hydroxybenzoate	HHB	1085-12-7	Tokyo Kasei Kogyo Co	99	Corn oil
23	Tetrazolium violet	TZV	1719-71-7	Sigma-Aldrich Co.	>99	Corn oil
24	Pravastatin sodium salt	Pra	81131-70-6	Wako Pure Chemical	99.4	Distilled Water
25	Physostigmine, salicylate (1:1)	PHS	57-64-7	Sigma-Aldrich Co.	99.5	Distilled Water
26	Nordihydroguaiaretic acid	NDGA	500-38-9	ICN Biomedicals Inc.	100	Corn oil
27	o-Cresolphthalein	CP	596-27-0	Wako Pure Chemical	[illegible]	[illegible]
28	1,3-Dinitrobenzene	DNB	99-65-0	Wako Pure Chemical	[illegible]	[illegible]
29	C.I.Pigment orange	PO	12236-62-3	NIHS	[illegible]	[illegible]
30	Tetrabromobisphenol-A	TBBPA	79-94-7	Wako Pure Chemical	[illegible]	[illegible]
31	2-Hydroxy-4-methoxybenzophenone	HMB	131-57-7	Wako Pure Chemical	[illegible]	[illegible]
32	Ethylparaben	EP	120-47-8	Wako Pure Chemical	[illegible]	[illegible]
33	Propyl p-hydroxybenzoate	PHB	94-13-3	Wako Pure Chemical	[illegible]	[illegible]
34	Kaempferol	K	520-18-3	Tokyo Kasei Kogyo Co	[illegible]	[illegible]
35	2-(2-Benzotriazolyl)-p-cresol	BTC	2440-22-4	Wako Pure Chemical	[illegible]	[illegible]
36	Phenolphthalein	PP	77-09-8	Sigma-Aldrich Co.	[illegible]	[illegible]

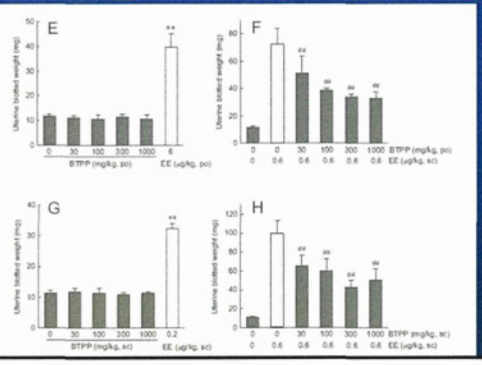
Table 2. Study design

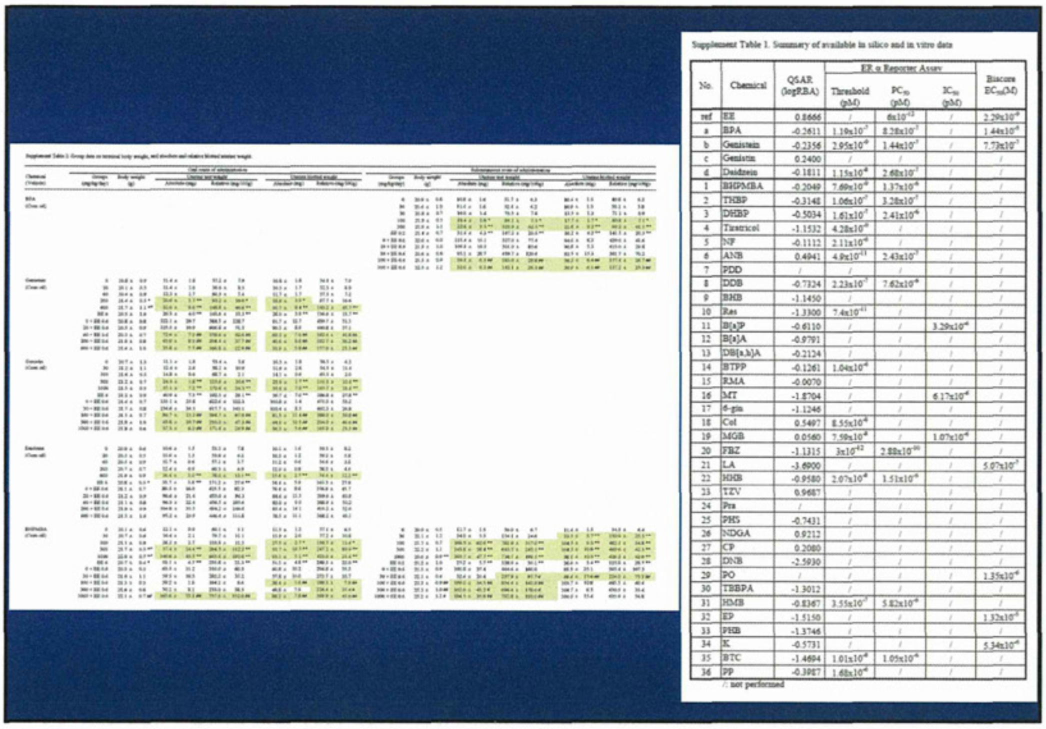
Group	Gr. No.	Agonist detection	Gr. No.	Antagonist detection
Negative control	1	Vehicle	7	Vehicle plus EE [b]
Low dose	2	Test substance	8	Test substance plus EE [b]
Medium low dose	3	Test substance	9	Test substance plus EE [b]
Medium high dose	4	Test substance	10	Test substance plus EE [b]
High dose	5	Test substance	11	Test substance plus EE [b]
Positive control	6	EE [a]	/ [c]	/ [c]

EE: Ethynyl estradiol
[a] 6 μg/kg/day for oral route and 0.2 μg/kg/day for subcutaneous route, respectively
[b] subcutaneous injection of 0.6 μg/kg/day for both oral and subcutaneous route, 15 min after test substance
[c] not performed





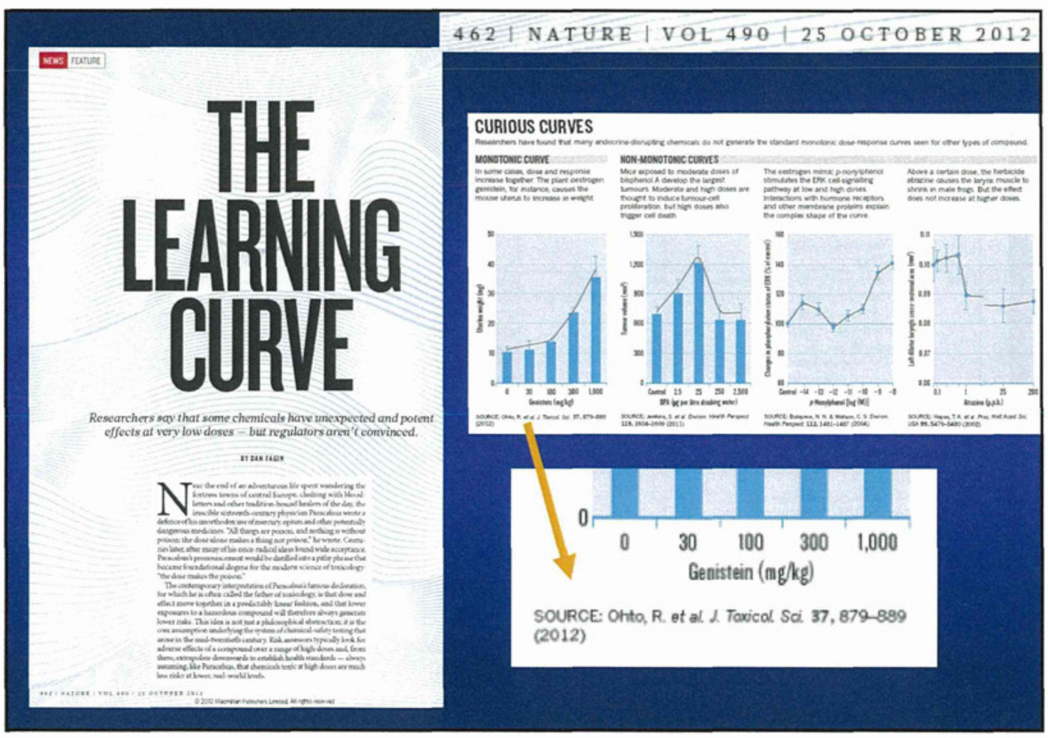




Supplement Table 1. Summary of available in silico and in vitro data

No.	Chemical	QSAR (logRBA)	ER α Reporter Assay: Threshold (pM)	ER α Reporter Assay: PC_{50} (pM)	ER α Reporter Assay: IC_{50} (pM)	Biacore EC_{50}(M)
ref	EE	0.8666	/	$6x10^{-12}$	/	$2.29x10^{-9}$
a	BPA	-0.2611	$1.19x10^{-7}$	$8.28x10^{-7}$	/	$1.44x10^{-6}$
b	Genistein	-0.2356	$2.95x10^{-9}$	$1.44x10^{-7}$	/	$7.73x10^{-7}$
c	Genistin	0.2400	/	/	/	/
d	Daidzein	-0.1811	$1.15x10^{-8}$	$2.68x10^{-7}$	/	/
1	BHPMBA	-0.2049	$7.69x10^{-9}$	$1.37x10^{-6}$	/	/
2	THBP	-0.3148	$1.06x10^{-7}$	$3.28x10^{-7}$	/	/
3	DHBP	-0.5034	$1.61x10^{-7}$	$2.41x10^{-6}$	/	/
4	Triatricol	-1.1532	$4.28x10^{-8}$	/	/	/
5	NF	-0.1112	$2.11x10^{-6}$	/	/	/
6	ANB	0.4941	$4.9x10^{-11}$	$2.43x10^{-7}$	/	/
7	PDD	/	/	/	/	/
8	DDB	-0.7324	$2.23x10^{-7}$	$7.62x10^{-8}$	/	/
9	BHB	-1.1450	/	/	/	/
10	Res	-1.3300	$7.4x10^{-11}$	/	/	/
11	B[a]P	-0.6110	/	/	$3.29x10^{-6}$	/
12	B[a]A	-0.9791	/	/	/	/
13	DB[a,h]A	-0.2124	/	/	/	/
14	BTPP	-0.1261	$1.04x10^{-6}$	/	/	/
15	RMA	-0.0070	/	/	/	/
16	MT	-1.8704	/	/	$6.17x10^{-6}$	/
17	6-gin	-1.1246	/	/	/	/
18	Col	0.5497	$8.55x10^{-8}$	/	/	/
19	MGB	0.0560	$7.59x10^{-8}$	/	$1.07x10^{-6}$	/
20	FBZ	-1.1315	$3x10^{-12}$	$2.88x10^{-10}$	/	/
21	LA	-3.6900	/	/	/	$5.07x10^{-7}$
22	HHB	-0.9580	$2.07x10^{-8}$	$1.51x10^{-6}$	/	/
23	TZV	0.9687	/	/	/	/
24	Pra	/	/	/	/	/
25	PHS	-0.7431	/	/	/	/
26	NDGA	0.9212	/	/	/	/
27	CP	0.2080	/	/	/	/
28	DNB	-2.5930	/	/	/	/
29	PO	/	/	/	/	$1.35x10^{-6}$
30	TBBPA	-1.3012	/	/	/	/
31	HMB	-0.8367	$3.55x10^{-7}$	$5.82x10^{-8}$	/	/
32	EP	-1.5150	/	/	/	$1.32x10^{-5}$
33	PHB	-1.3746	/	/	/	/
34	K	-0.5731	/	/	/	$5.34x10^{-6}$
35	BTC	-1.4694	$1.01x10^{-8}$	$1.05x10^{-6}$	/	/
36	PP	-0.3987	$1.68x10^{-8}$	/	/	/

/: not performed



Bisphenol A 低用量影響文献リスト by Dr. M. Yamamoto

- 1日の体重当たりの投与量がマイクログラムオーダ以下のものを低用量とした。
- 119文献を抽出。
- 抽出手順
  1. PubMedにおいて『Bisphenol A』により抽出される2000年から2012年12月09日までの約5400文献（総説を含む）
  2. 要旨（必要に応じて本文）から投与量がマイクログラム/kg体重のオーダー以下の実験を抽出
  3. フランス食品衛生・環境・労働安全機関（ANSES）のbisphenol Aに関するヒト健康影響のレポート『Effets sanitaires et usages du bisphénol A』（仏語）のリストを確認し、漏れているものを抽出
  4. 総説『Golub, M.S., Wu, et al. (2010). Bisphenol A: developmental toxicity from early prenatal exposure. Birth Defects Res B Dev Reprod Toxicol 89, 441-466.』のリストより漏れを抽出
  5. 出版年、動物種、投与経路（orally, subcutaneously, Pumps s.c.）、投与量（microg/kg/day）、投与期間、エンドポイント（【behavior】、【immune】など大項目と、具体的な記述を要旨や本文からの引用）、影響（要旨や本文から引用）、TOXJC No.（すでに発表したもののみ）をリスト化
  6. リストにある全て*の文献はPDF化し電子ファイルとして保存（紙媒体文献はスキャナーで取り込みOCRで文字化してPDF化）

  * 『Zhang, X.F., Zhang, L.J., Feng, Y.N., Chen, B., Feng, Y.M., Liang, G.J., Li, L., and Shen, W. (2012). Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Mol Biol Rep.』のみ現在取り寄せ中。

117	2001	Markey, C.M., Luque, E.H., Munoz De Toro, M., Sonnenschein, C., and Soto, A.M. (2001). In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol Reprod 65, 1215–1223.	CD-1 mice	Pumps s.c.	25 and 250 microg/kg/day	from Day 9 of pregnancy to 10 days, 1 mo, and 6 mo of age	【mammary gland】 development of the mammary gland	Mammary glands of BPA-exposed mice showed differences in the rate of ductal migration into the stroma at 1 mo of age and a significant increase in the percentage of ducts, terminal ducts, terminal end buds, and alveolar buds at 6 mo of age. The percentage of cells that incorporated BrdU was significantly decreased within the epithelium at 10 days of age and increased within the stroma at 6 mo of age.
118	1999	Farabollini, F., Porrini, S., and Dessi-Fulgherit, F. (1999). *Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats.* Pharmacol Biochem Behav 64, 687–694.	Sprague-Dawley rats	orally	40, 400 microg/kg/day	from 10 days before mating *until the* weaning of the pups (40 mug), GD 14 PND 6 (400 mug),	【behavior, brain】 *exploration,* (hole-board) anxiety (elevated pluz maze)	in treated males both the motivation to explore and anxiety are reduced, while in *females, motor activity and motivation to* explore are depressed
119	1997	Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M., and Welshons, W.V. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect 105, 70–76.	CF1 mice	orally	2 and 20 microg/kg/day	from GD11 to GD17	【prostate】	increased their adult prostate weight relative to control males

まとめ

BPAとその標的の一つであることが強く示唆されるMalat-1を端緒に所謂低用量暴露による遅発性影響の分子基盤を明らかにすると共に、OECD/WHO対応を含む厚労省試験法スキームの拡充と完成に向けての、特に遅れている確定試験法の確立を含む諸手続きに必要な作業を進める。