

図3 5種の3Dポリマーによるヒト皮膚組織芽細胞とMT-4の増殖変化

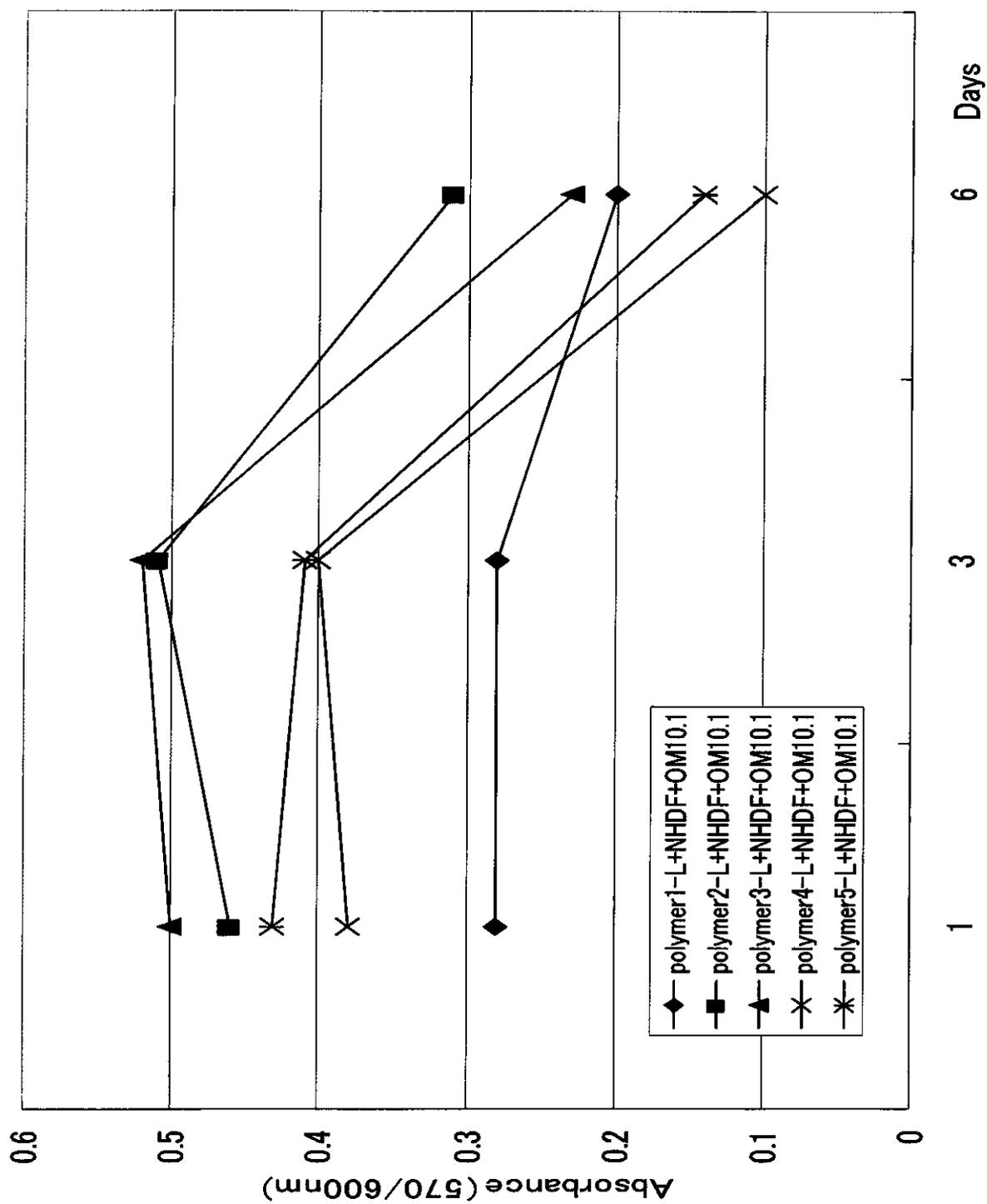


図4 5種の3Dポリマーによるヒト皮膚組織芽細胞とOM10.1の増殖変化

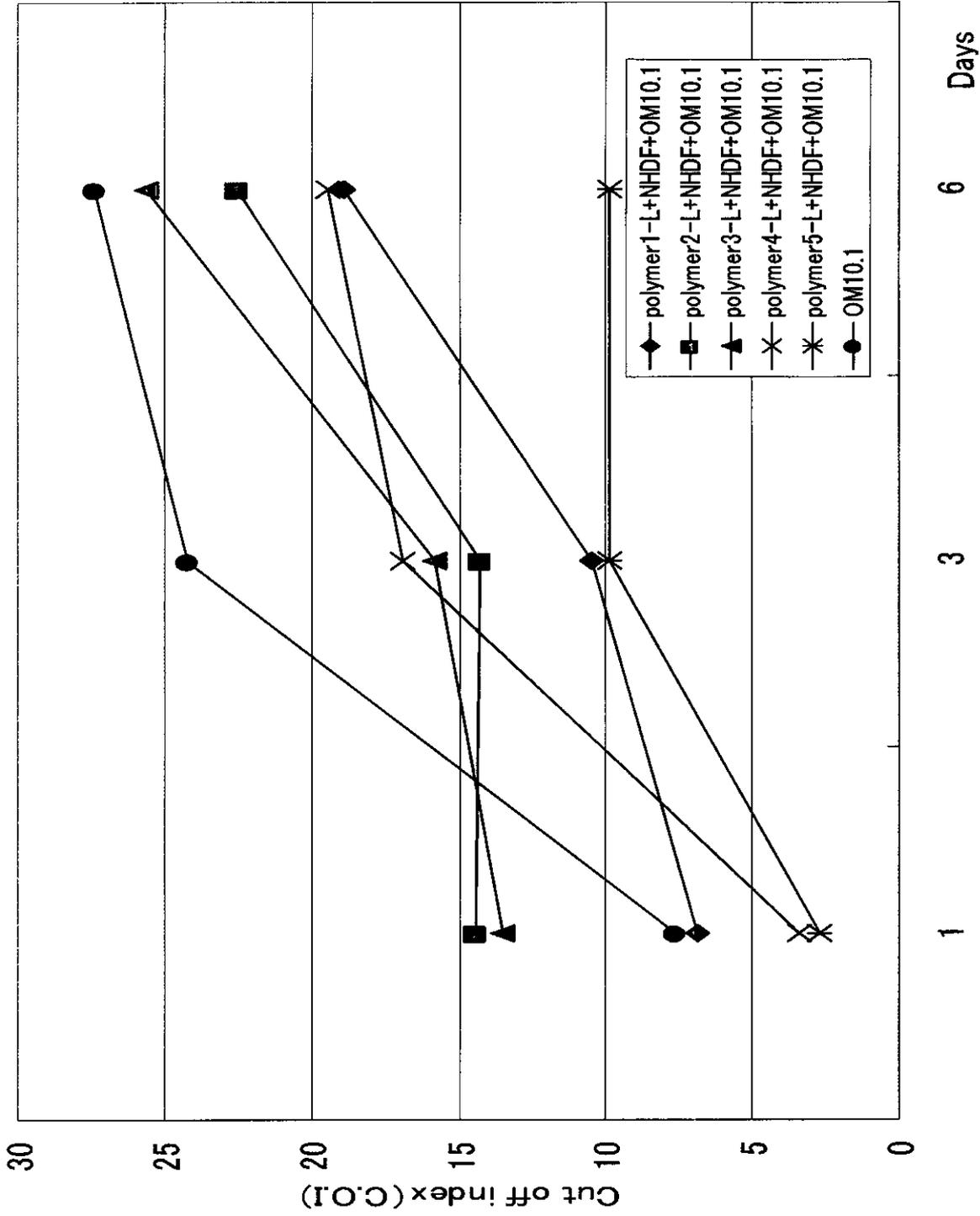
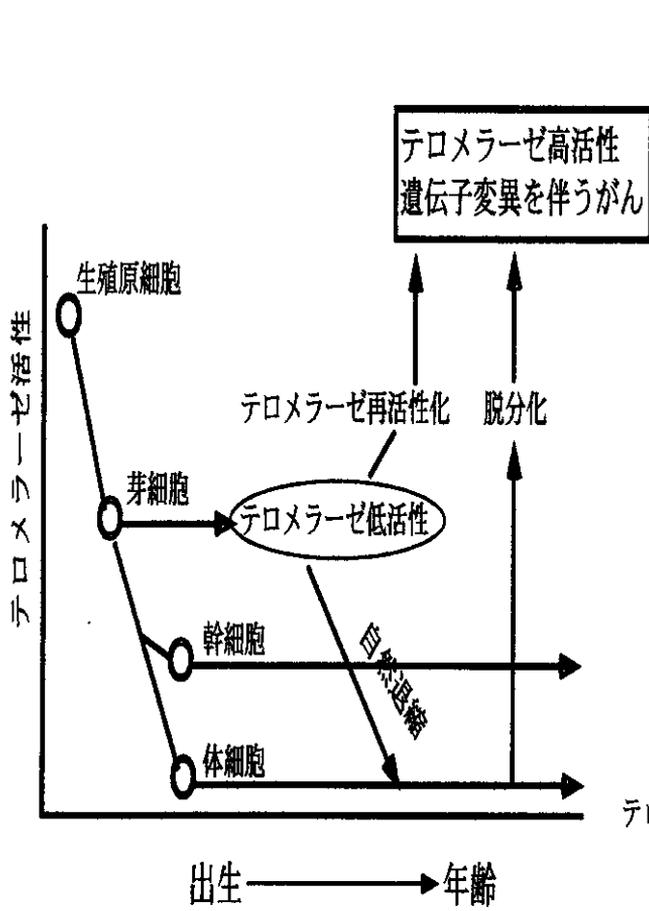
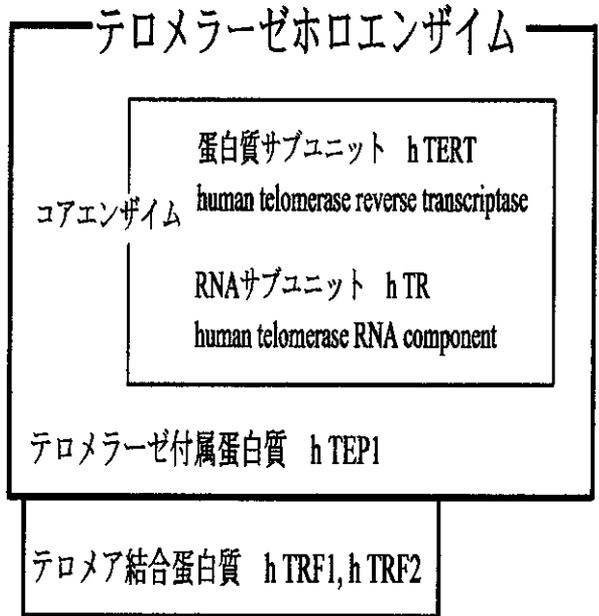


図5 5種の3Dポリマーによるヒト皮膚組織芽細胞へのOM10.1接触とHIV-1p24抗原の検出



楡山英三&楡山桂子
 がんとテロメア・テロメラーゼ 図 一部改変

図6 発生分化とがんの発生モデル



テロメラーゼ活性: h TERT mRNA発現量: h TERTプロモーター活性
 ほぼ相関性あり

h TERTプロモーター転写活性: がん細胞で転写レベルで
 活性化する機構あり

テロメラーゼ活性を指標としたリスク評価法を確立
 図7 がん特異的h TERTプロモーターを用いた
 がん化リスク評価系の確立

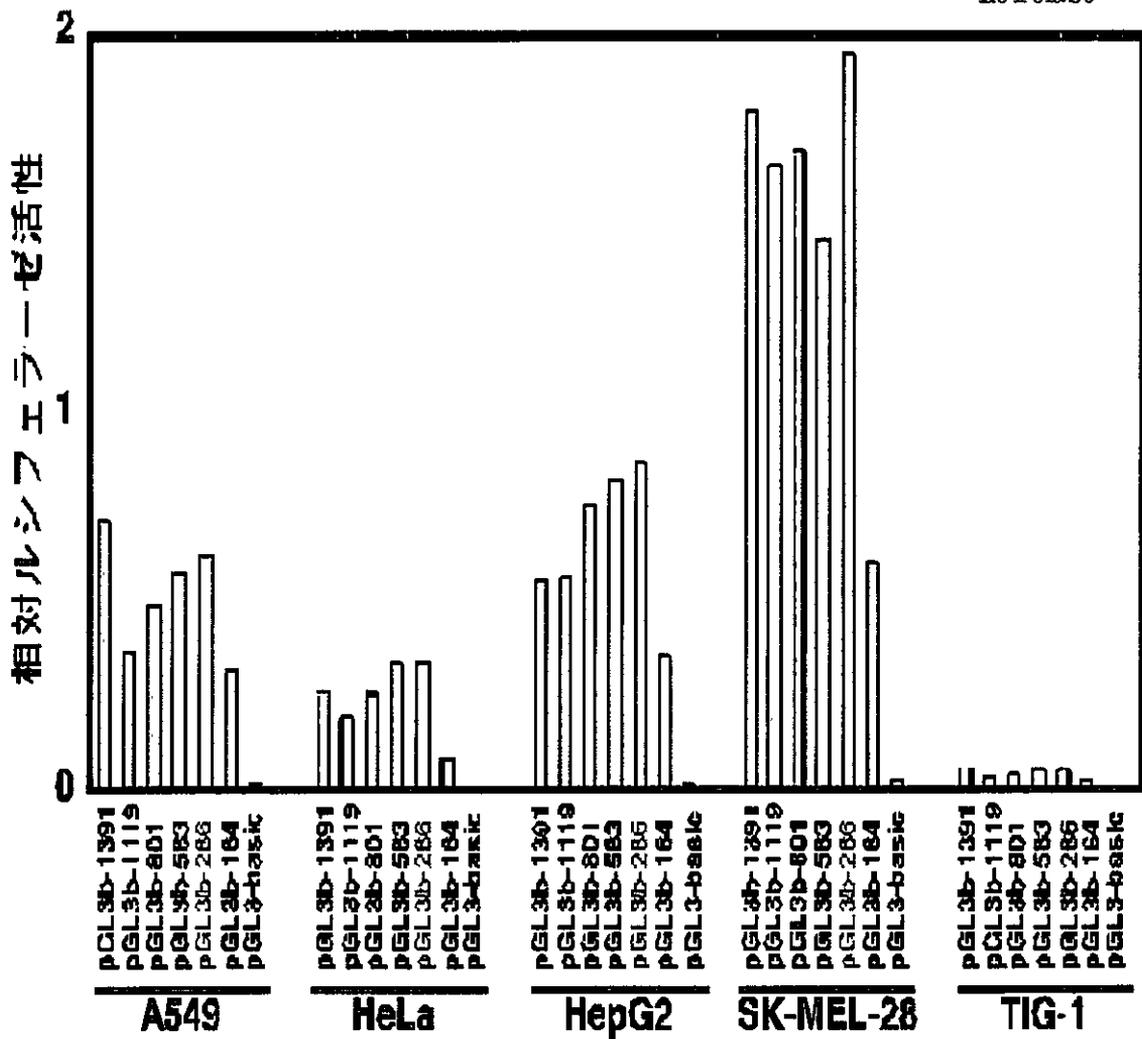
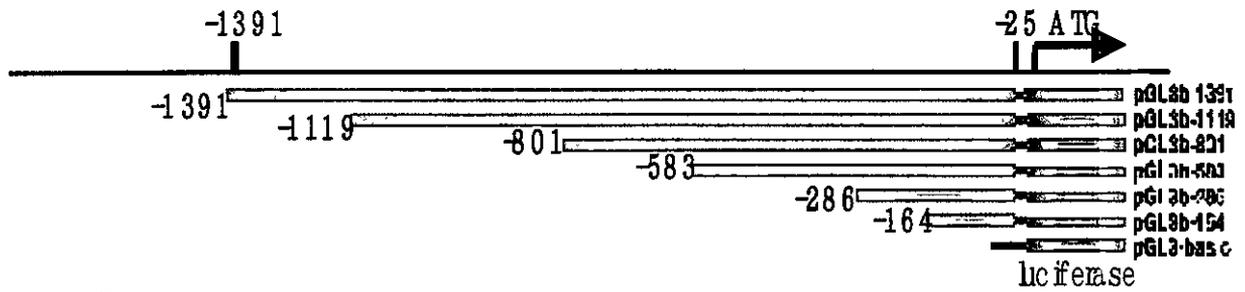


図8 ERT プロモーターのガン細胞特異的活性化

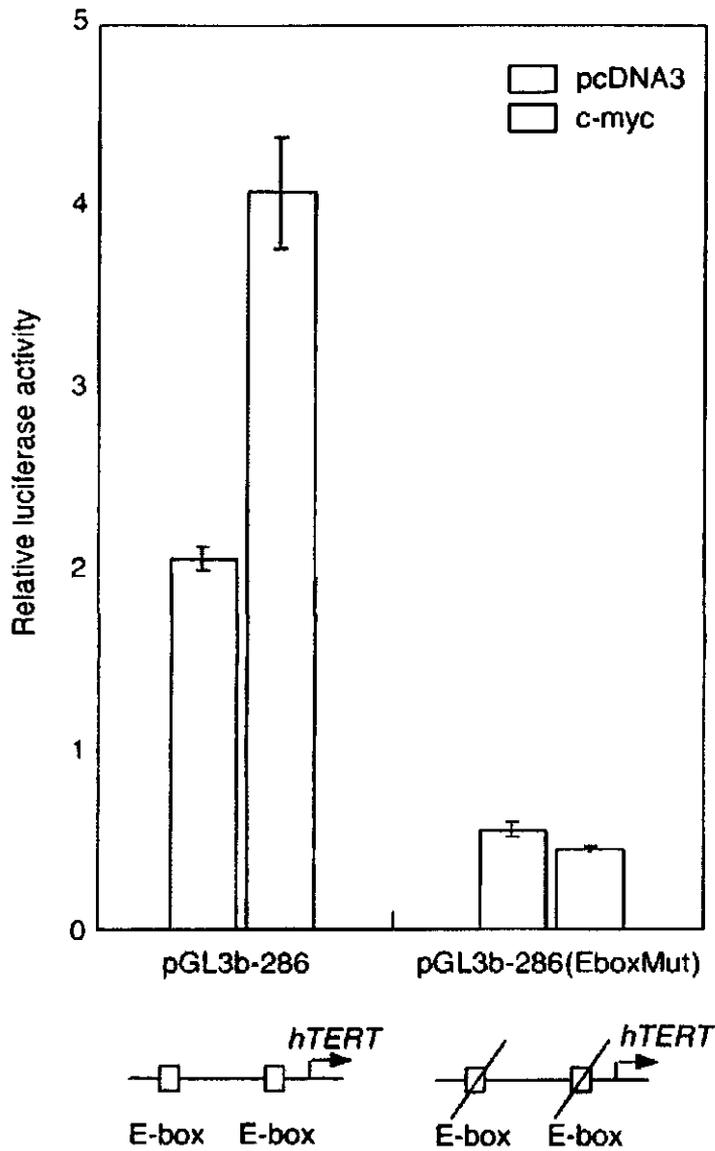


Figure Transcriptional activation of the core promoter region of *hTERT* by *c-myc*. A human *c-myc* cDNA expression plasmid (pcDNA3/HA-*c-myc*) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-286 or pGL3b-286 (EboxMut)) and the pRL-TK into the A549 cells. The means from the three independent experiments are shown. Bars indicate standard deviations.

図9 *c-myc*による *hTERT*のコアプロモーターの転写活性について

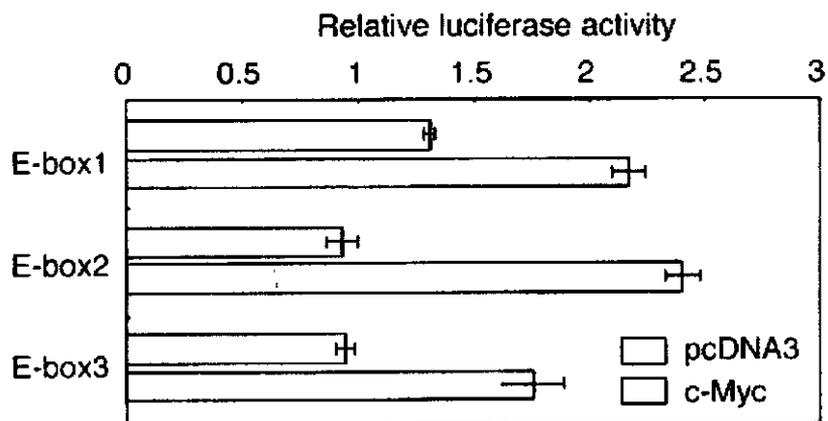
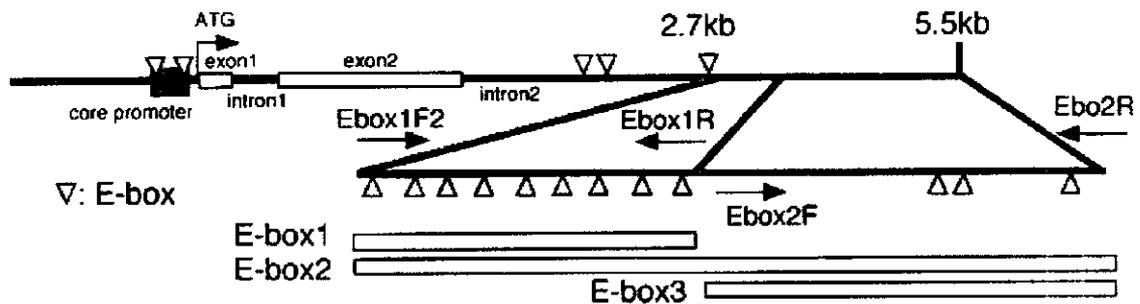


Figure Transcriptional activation of the *hTERT* promoter containing E-box cluster by *c-myc*. *Upper panel* shows the schematic representation of the *hTERT* promoter region. The genomic fragments of E-box cluster were amplified using the indicated primer sets (*arrows*) as described in "Materials and Methods". Then, the genomic fragments of E-box cluster (E-box1, 2, 3) were inserted into CMV promoter driven-luciferase construct (pGL3-Promoter), and used for analysis of transcriptional activation by *c-myc*. The c-Myc binding sites (E-box) are indicated by *triangles* (Wu et al., 1999), and *bent arrow* indicates translation start site (ATG). *Lower panel* shows the transcriptional activation of *hTERT* by *c-myc*. A *c-myc* expression vector (pcDNA3 /HA-*c-myc*) or control vector (pcDNA3) were co-transfected into A549 cells with different E-box cluster-reporter plasmids by LipofectAMINE PLUS reagent. For each transfection, the firefly luciferase activity of *hTERT* E-box cluster-reporter constructs shown in the *upper panel* was normalized with *Renilla reniformis* luciferase activity driven from the co-transfected pRL-SV40. The means from the three independent experiments are shown. *Bars* indicate standard deviations.

図10 E-boxクラスター領域のhTERT転写活性化へのc-mycの関与について

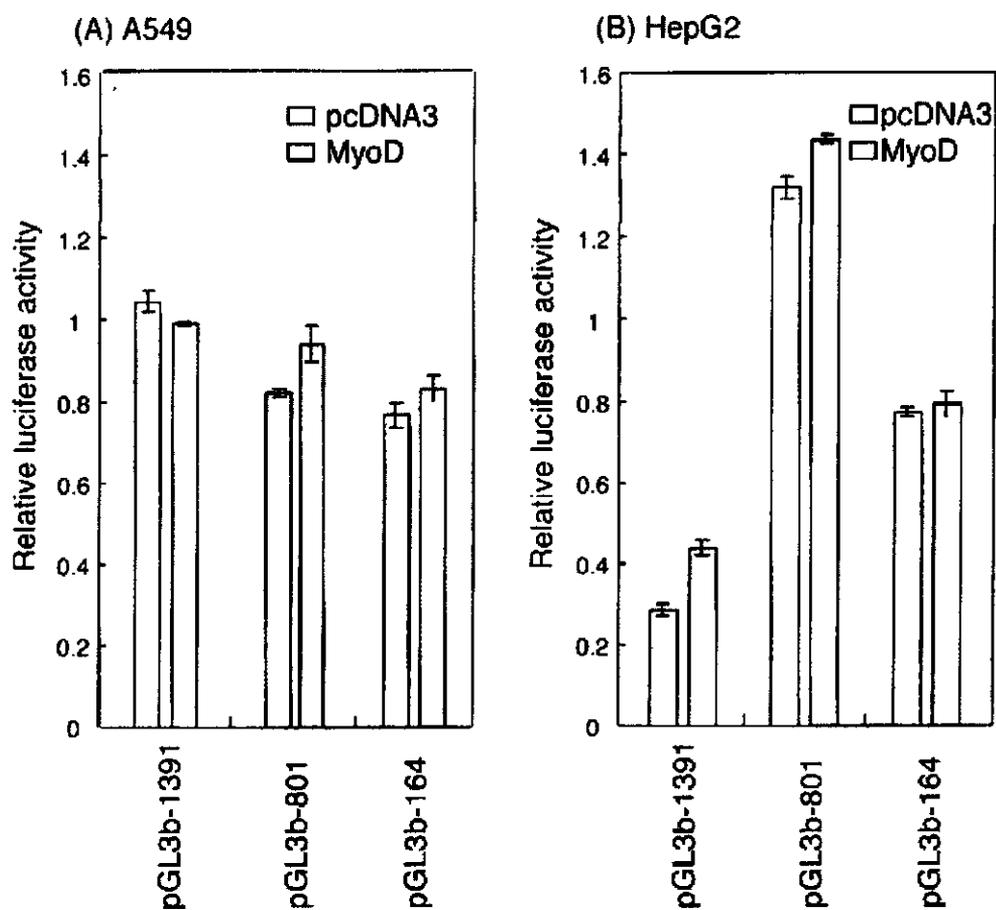


Figure Transcriptional repression of the *hTERT* promoter by MyoD. A human *MyoD* cDNA expression plasmid (pcDNA3-MyoD) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-1391, pGL3b-801 or pGL3b-286) and the pRL-TK into the A549 cells (A) or HepG2 (B). The means from the three independent experiments are shown. Bars indicate standard deviations.

図11 MyoDによるhTERTコアプロモーターの転写制御について

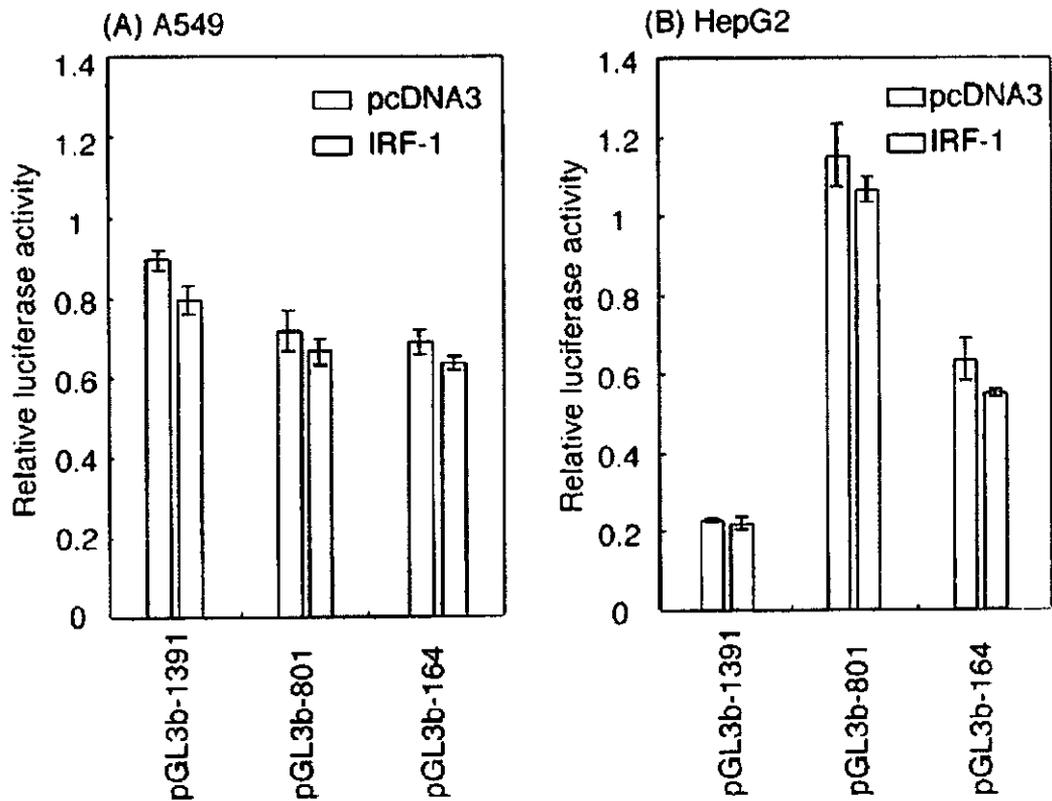


Figure Transcriptional repression of the *hTERT* promoter by IRF-1. A human *IRF-1* cDNA expression plasmid (pcDNA3-IRF-1) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-1391, pGL3b-801 or pGL3b-286) and the pRL-TK into the A549 cells (A) or HepG2 (B). The means from the three independent experiments are shown. Bars indicate standard deviations.

図12 IRF-1によるhTERTコアプロモーターの転写制御について

表3 . 分離された形質転換巢の形質転換能確認のための
トランスフォーメーション試験

parental or transformant cells	cloning efficiency (% of control)	No. of foci/plate*
A 3111	100	0.3 ± 0.48
A 5	106.3	2.3 ± 1.23
A 6	110.8	47.0 ± 6.28
L11	144.8	114.1 ± 13.32
L21	126.2	84.5 ± 5.10
A 3111+3-M C**	2.2	30.0 ± 5.15

*average with SD of 15 plates

**A 3111 cells were treated with 0.5 µg/ml of 3-M C (positive control)

表4 . 発現が親株の5倍以上亢進した遺伝子

Code	Gene
A08e	integrin beta 7
A14l	insulin-like growth factor binding protein 10
B02a	c-fos proto-oncogene
B02b	FBJ osteosarcoma oncogene B
B02l	Jun oncogene
B02m	junB proto-oncogene
B06g	HSP27, HSP25, HSPB1, growth-related 25-kDa protein
B07e	N-oxide forming dimethylaniline monooxygenase 1 hepatic flavin-containing monooxygenase 1 dimethylaniline oxidase 1
C01k	retinoic acid receptor beta, nuclear receptor subfamily 1 group B member 2
C03m	caspase-activated DNase, DNase inhibited by DNA fragmentation factor
D05b	insulin-like growth factor II precursor, multiplication-stimulating polypeptide
D05e	leukemia inhibitory factor, cholinergic differentiation factor
D06d	proliferin
E07k	non-receptor type 16 protein tyrosine phosphatase

表 5 発現が親株の5倍以上抑制された遺伝子

Code	Gene
A06f	Cdk 6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C
A08c	fat tumor suppressor homolog (Drosophila)
A10n	thrombospondin 1
A11c	vascular cell adhesion molecule 1 precursor
A12a	cysteine rich intestinal protein
A12e	delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG
B01l	EB1 APC-binding protein
B14d	HSP60, HSP65, HSPD1, mitochondrial matrix protein P1 precursor, 60-kDa chaperonin, GroEL, protein
C01c	apoptosis inhibitor 1
C03j	clusterin precursor, clusterin, apolipoprotein J, sulfated glycoprotein 2
C07m	platelet-derived growth factor receptor alpha precursor
C12n	Hek2 murine homolog, Mdk5 mouse developmental kinase, Eph-related tyrosine-protein kinase receptor
D06b	pleiotrophin
D06l	small inducible cytokine A9
D14g	avian sarcoma virus CT10 (v-crk) oncogene homolog
E02n	non-receptor type 11 protein tyrosine phosphatase, phosphotyrosine phosphatase
E04f	Cdk7, CDC2-related kinase 4, Cdk-activating kinase, 39-kDa protein kinase, MO15, MPK7
E08f	serum-inducible kinase
E13k	histidine triad nucleotide-binding protein, protein kinase Ciota
E13n	menage a trois 1
F01i	a disintegrin-like and metalloprotease with thrombospondin type 1 motif protein 1
F02g	matrix metalloproteinase 9
F03a	large multifunctional protease 7
F09h	developmentally d neural precursor cell expressed
F10j	tubulin cofactor a

表6 . 発現が親株の5倍以上変化した遺伝子

trans-formant	Genes upregulated	Genes downregulated
A 5	c-fos proto-oncogene (33.0) FBJ osteosarcoma oncogene B Jun oncogene junB proto-oncogene HSP27, HSP25, HSPB1, growth-related 25-kDa protein N-oxide forming dimethylaniline monooxygenase 1 hepatic flavin-containing monooxygenase 1 dimethylaniline oxidase 1 insulin-like growth factor II precursor, multiplication-stimulating polypeptide	large multifunctional protease 7
A 6	integrin beta 7 retinoic acid receptor beta, nuclear receptor subfamily 1 group B member 2 proliferin (10.7)	fat tumor suppressor homolog (Drosophila) thrombospondin 1 (15.8) delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG (12.7) clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 pleiotrophin matrix metalloproteinase 9 large multifunctional protease 7 (20.7)
L11	insulin-like growth factor binding protein 10 c-fos proto-oncogene (79.8) FBJ osteosarcoma oncogene B (10.9) Jun oncogene (26.3) caspase-activated DNase, DNase inhibited by DNA fragmentation factor leukemia inhibitory factor, cholinergic differentiation factor	Cdk6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C (11.5) fat tumor suppressor homolog (Drosophila) thrombospondin 1 vascular cell adhesion molecule 1 precursor (17.0) delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG (11.2) EB1 APC-binding protein HSP60, HSP65, HSPD1, mitochondrial matrix protein P1 precursor, 60-kDa chaperonin, GroEL protein apoptosis inhibitor 1 clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 (10.0) platelet-derived growth factor receptor alpha precursor Hek2 murine homolog, Mdk5 mouse developmental kinase, Eph-related tyrosine-protein kinase receptor pleiotrophin (25.2) small inducible cytokine A9 avian sarcoma virus CT10 (v-crk) oncogene homolog (11.0) non-receptor type 11 protein tyrosine phosphatase, phosphotyrosine phosphatase Cdk7, CDC2-related kinase 4, Cdk-activating kinase, 39-kDa protein kinase, MO15, MPK7 serum-inducible kinase histidine triad nucleotide-binding protein, protein kinase C iota (34.4) menage a trois 1 a disintegrin-like and metalloprotease with thrombospondin type 1 motif protein 1 (11.8) matrix metalloproteinase 9 large multifunctional protease 7 (10.4) developmentally neuronal precursor cell expressed (15.0) tubulin cofactor a (11.8)
L21	c-fos proto-oncogene (30.3) Jun oncogene (11.1) proliferin non-receptor type 16 protein tyrosine phosphatase	Cdk6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C fat tumor suppressor homolog (Drosophila) cysteine rich intestinal protein delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 (10.0) pleiotrophin (25.2) histidine triad nucleotide-binding protein, protein kinase C iota (17.2) large multifunctional protease 7 (16.6)

Genes in black and red were up- or down-regulated more than 5-fold or less than 10-fold and more than 10-fold, respectively. Figures in parentheses indicate fold-increase or -decrease in gene expression compared to parental cells.

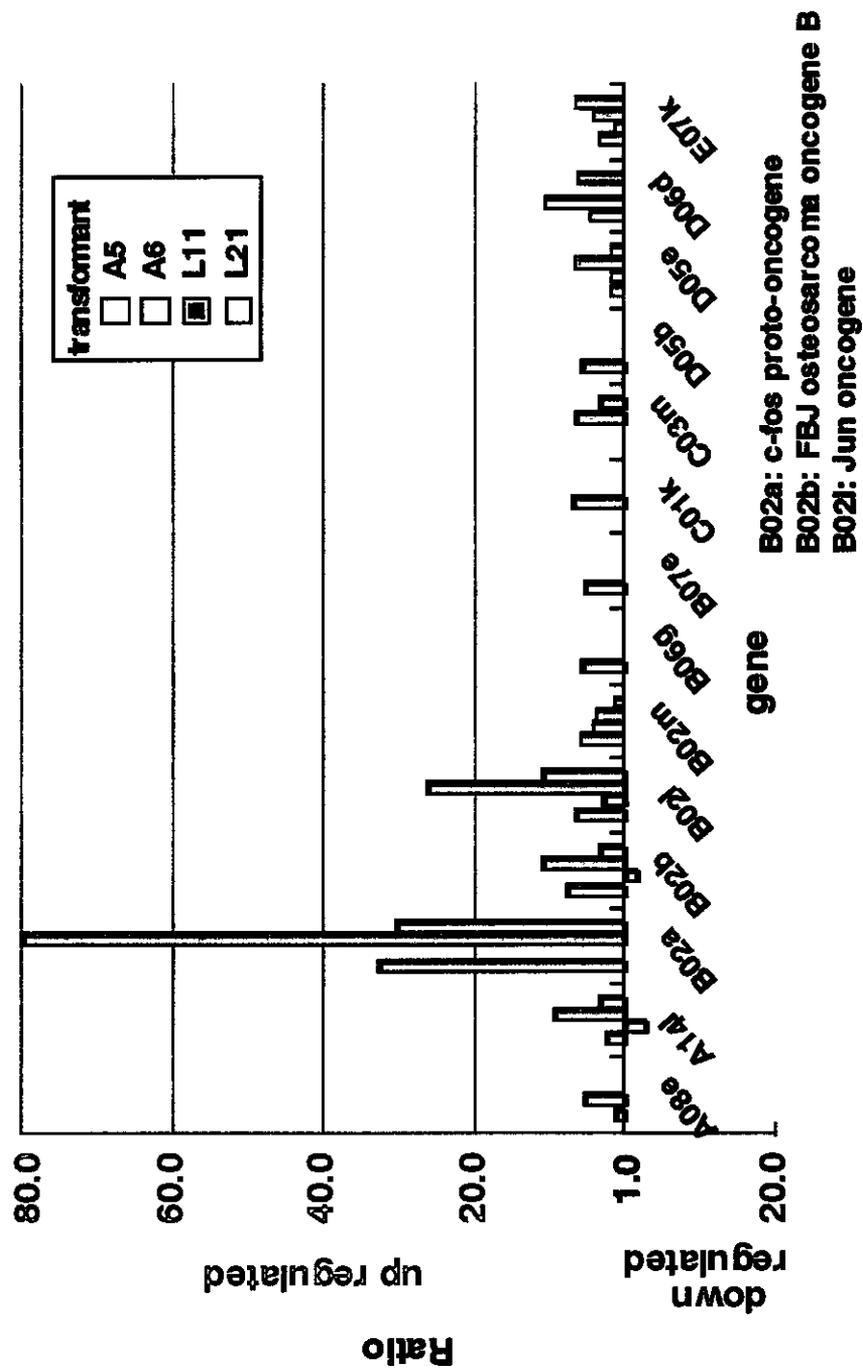


図13 いずれかの形質転換株で、その発現が親株の5倍以上亢進した遺伝子

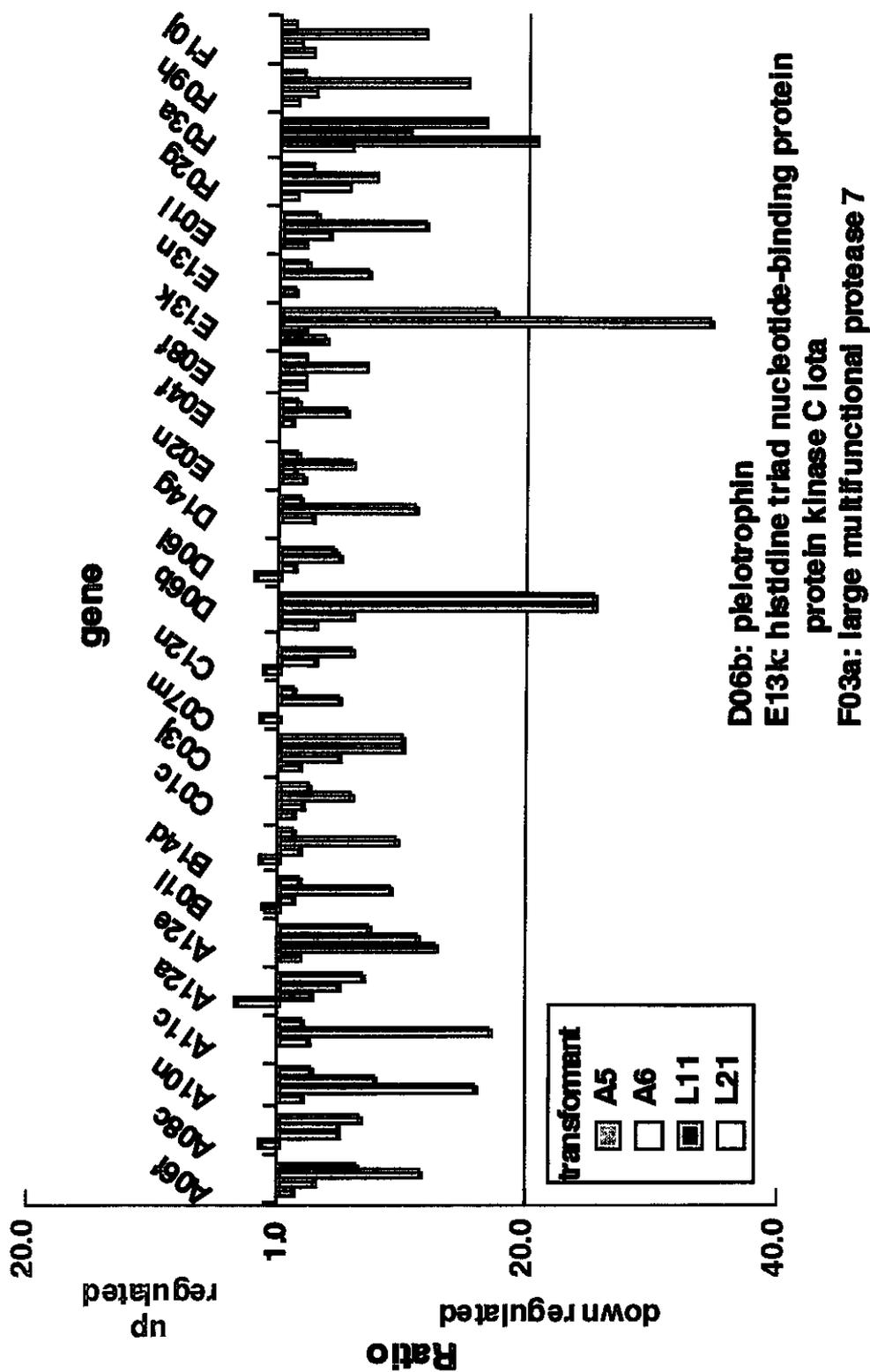


図14 いずれかの形質転換株で、その発現が親株の5倍以上抑制された遺伝子

Oncogenes and tumor suppressor genes

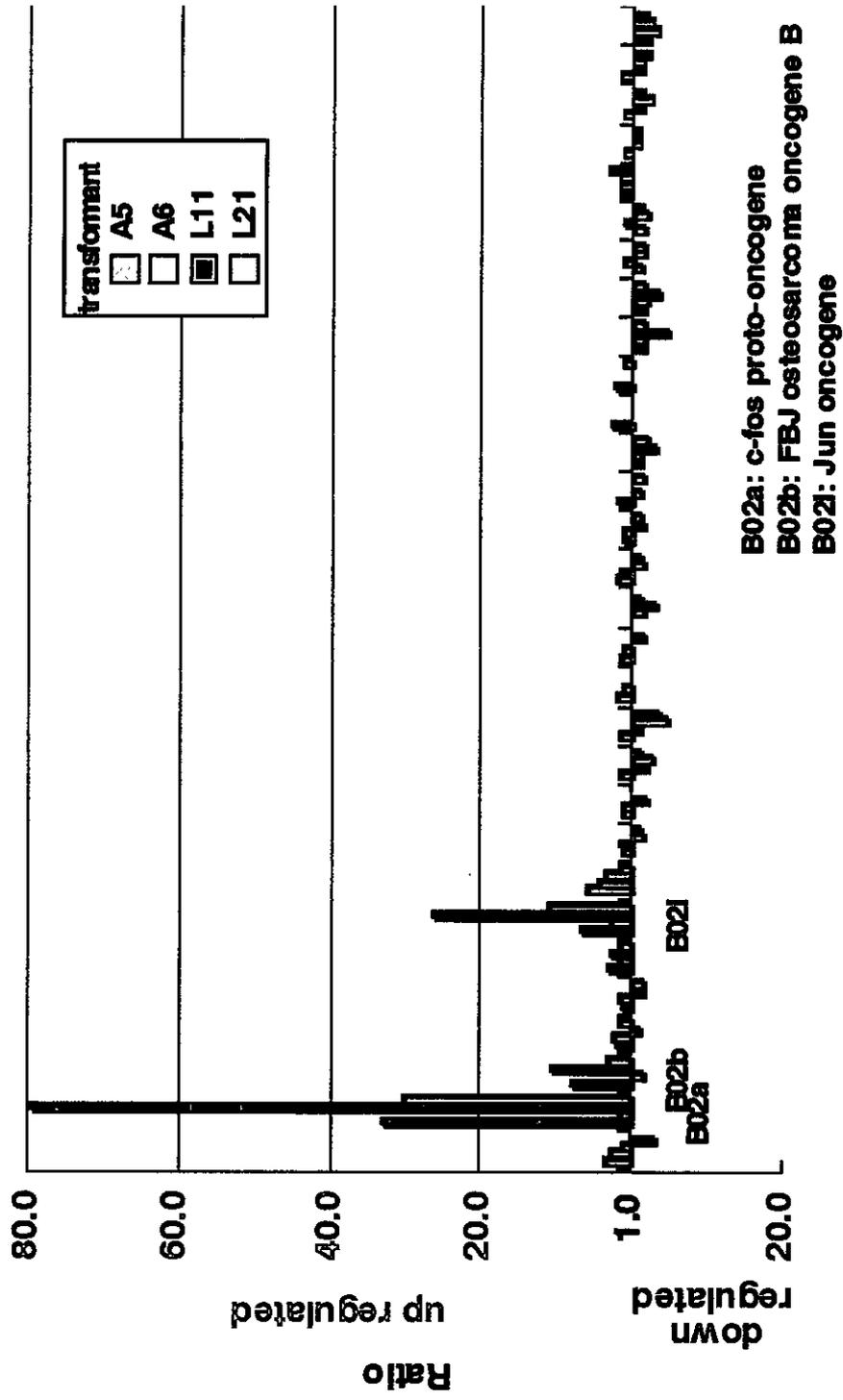
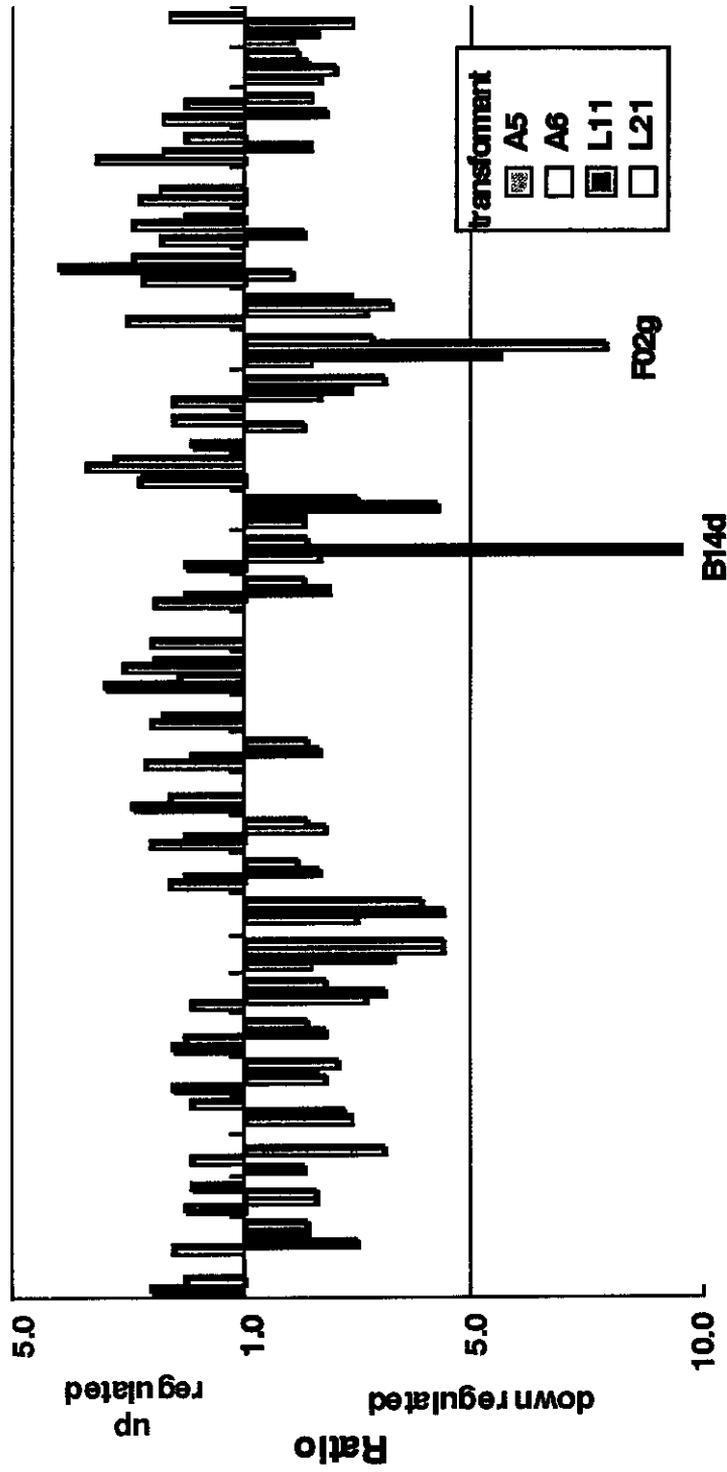


図15 癌遺伝子および癌抑制遺伝子の発現変化

Extracellular matrix related genes



B14d: HSP60, HSP65, HSPD1, mitochondrial matrix protein
 P1 precursor, 60-kDa chaperonin, GroEL protein
 F02g: MMP9

図16 細胞外マトリックス関連遺伝子の発現変化

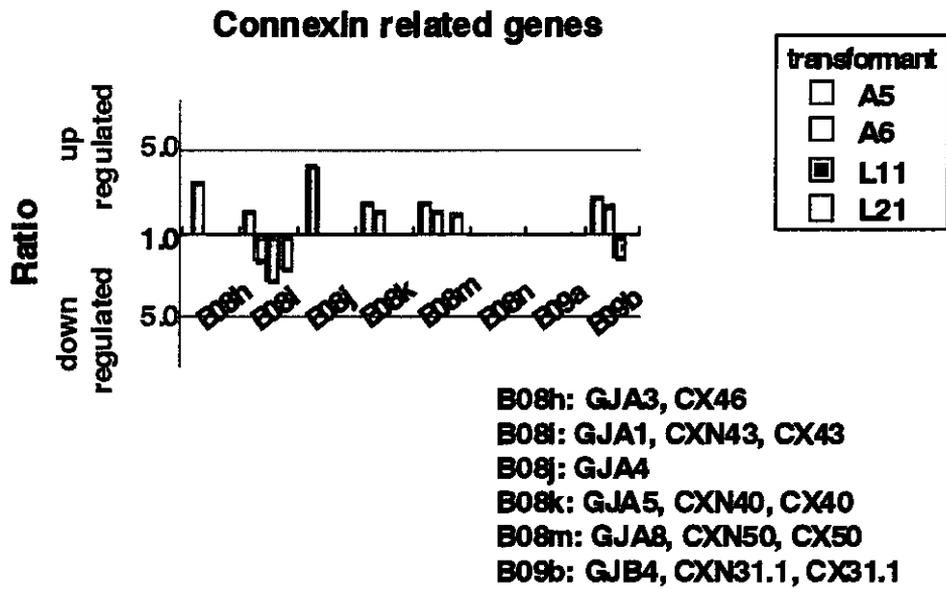


図 17 . コネキシン関連遺伝子の発現変化

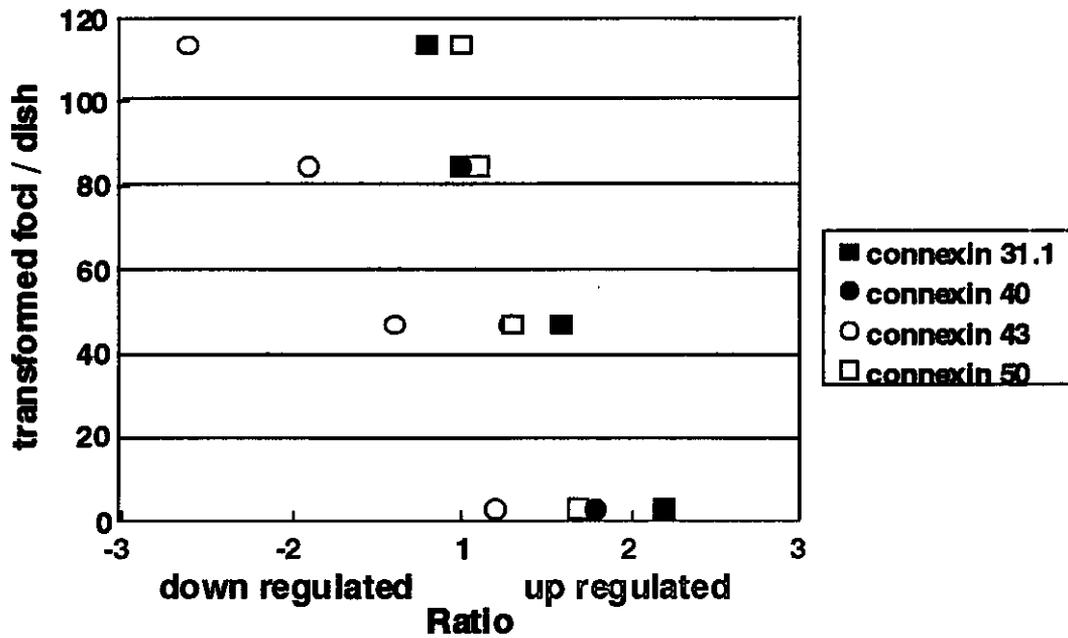
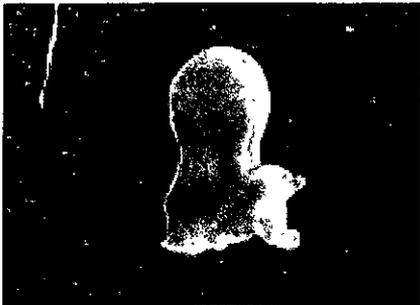
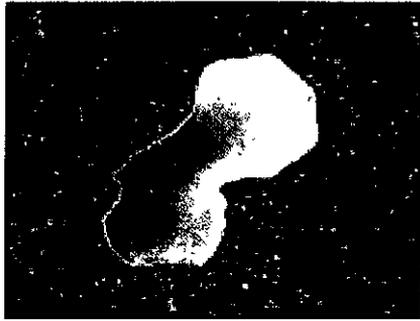


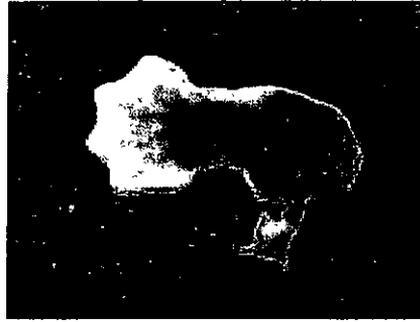
図 18 . 悪性度とコネキシン遺伝子発現との相関



13th



14th



15th



16th



17th

図19 ラット胎児の肢器官のアルシアンブルー染色標本

Control



14th

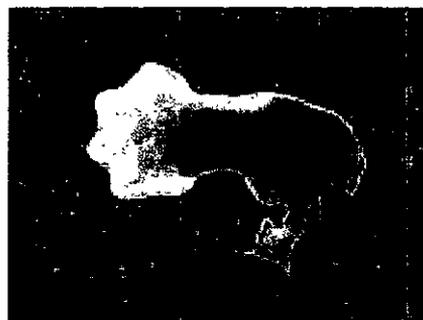
14th 1 week



14th in Bag 1 week

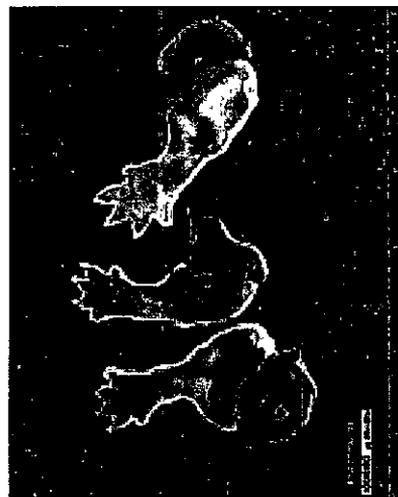


Control



15th

15th 1 week



15th in Bag 1 week

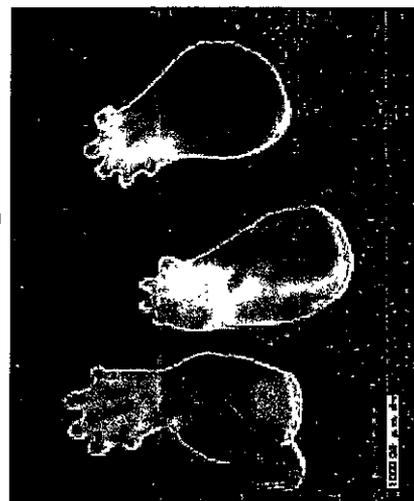
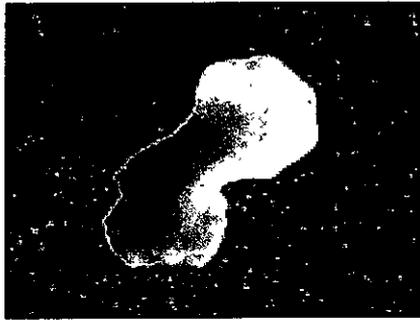


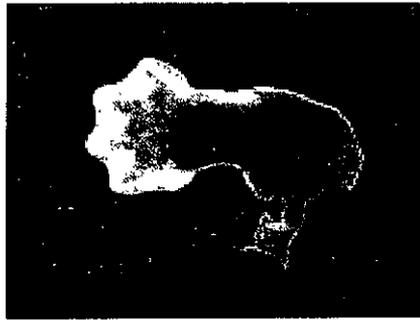
図20 肢芽に動的ストレスを与えて培養した際の形態変化と軟骨分化能



14th



14th Limb buds in bags for 1 week



15th

図21 マイクロメンブレン・バッグに入れ1週間腹腔内培養した肢芽と、胎齡14日及び15日目の肢芽との比較