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13. ABSTRACT (Maximum 200 Words)

In attempts to understand how the signaling by retinoic acid (the active vitamin A metabolite) is regulated we have been studying the retinoic acid binding protein called CRABP-II. These studies revealed that CRABP-II acts to enhance the transcriptional activities of RA and that it does so by directly delivering the hormone to its cognate transcription factor, RAR. Consequently CRABP-II dramatically sensitized cultured mammary carcinoma cells to RA-induced growth inhibition. Similarly, over-expression of CRABP-II inhibited mammary tumor growth in two different mouse models of cancer. CRABP-II may be a novel target for therapeutic and preventive strategies for retinoid-treatment of breast cancer. This project aims to delineate the mechanism by which CRABP-II modulates RA activity, especially as related to its ability to enhance the anti-proliferative action of the ligand. The first aim is to determine the extent to which CRABP-II acts in activating different isotypes of RAR. The second aim is to dissect the mechanism by which RA-induced growth inhibition is mediated. This will then allow for closer inspection of particular target genes that are under such control. The third aim is to understand the basis for RA-resistance of mammary carcinoma cells and how CRABP-II functions to overcome this resistance.

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Introduction

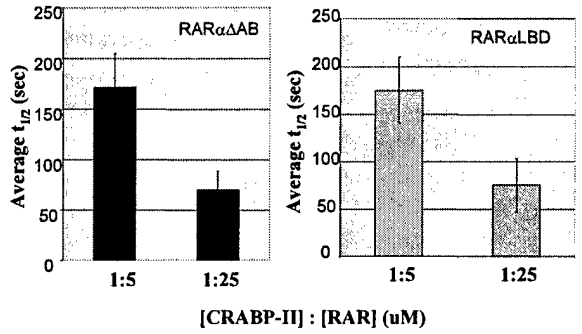
Retinoic acid (RA), the active metabolite of vitamin A, plays critical roles in embryonic development as well as growth and differentiation in adult mammals. Retinoic acid is currently used or is in clinical trials for treatment of a variety of different cancers, including breast cancer (*1*). Although retinoids are efficacious, pharmacological doses often result in toxicity (*2*). The ability of RA to induce growth arrest and apoptosis in carcinoma cells is mediated by the ligand-inducible transcription factors termed retinoic acid receptors (RARs). There are three isotypes of RAR, namely RARα, RARβ and RARγ (*3*). In cells, RA also associates with cellular retinoic acid binding proteins (CRABP-I and CRABP-II). We recently showed that CRABP-II carries RA from the cytoplasm to the nucleus where it channels the ligand directly to RARα via a transient protein-protein interaction (*4, 5*). This "ligand channeling" sensitizes cells to transcriptional activation by RAR (*4*). Indeed, over-expression of the binding protein dramatically lowered the effective RA concentration necessary to induce growth inhibition in mammary carcinoma cells (*5*). Similarly, over-expressing CRABP-II inhibited mammary tumor growth in two different mouse models of cancer. The goal of this project is to elucidate the mechanisms by which CRABP-II directs RA signaling to enhance anti-proliferative responses in mammary carcinoma cells.

Body

Task 1. To determine which RAR isotypes is/are under CRABP-II regulation. Subtasks will overlap during this time period:

Our group has shown that CRABP-II channels RA directly to RARα via a transient protein-protein interaction (*4*). Theoretically, transfer of RA from CRABP to RAR may occur by one of two possible mechanisms: (1) RA may dissociate from the binding protein to the bulk aqueous phase prior to its association with RAR. (2) RA may transfer from CRABP to RAR by 'channeling', i.e. by a process that involves direct protein-protein interactions and that bypasses the bulk aqueous phase. The two pathways may be distinguished by the dependence of the rate constants of transfer of RA from the donor (CRABP) to the acceptor (RAR) on the concentration of the acceptor. If transfer follows mechanism *(1)*, the rate-limiting step will be the dissociation of the ligand from CRABP, and the apparent rate constant will be <u>independent</u> of the concentration of RAR. On the other hand, if RA moves from CRABP to RAR by "channeling" (mechanism 2), then increasing the concentration of RAR (at a constant CRABP concentration), will result in a higher probability of productive donor-acceptor collisions, and thus, $t_{1/2}$ for the transfer reaction will become smaller as the acceptor concentration is raised. The observation that RA fluoresces much more when bound to CRABP-II versus when it is bound to RARα was used as a tool for measuring the transfer of ligand from binding protein to receptor (i.e. monitoring the loss of fluorescence of RA). The rate constants of transfer of RA from CRABP-I or CRABP-II to nearly full length RARα (RARαΔ AB) were thus measured at different receptor concentrations. The rate of transfer from CRABP-II, but not CRABP-I, was found to strongly depend on the concentration of acceptor protein (*4*). Hence, CRABP-II (but not CRABP-I) delivers RA to RARα via direct protein-protein interaction.

Figure 1 RAR LBD contains region necessary for ligand channeling with CRABP-II



To determine the region of RARα that mediates the interactions of the receptor with CRABP-II, we examined the ability of the ligand binding domain of RARα (RARαLBD) to engage in RA-channeling from the binding protein. Proteins were bacterially expressed and affinity purified. The dependence of the rate of ligand transfer from CRABP-II to the RAR LBD was then studied. As seen in figure 1, transfer was significantly facilitated upon increasing the acceptor/donor ratio from 1:5 to 1:25 with nearly identical kinetics of transfer as the nearly full length receptor. Hence, the LBD of the receptor contains the interaction domain for binding to CRABP-II and is

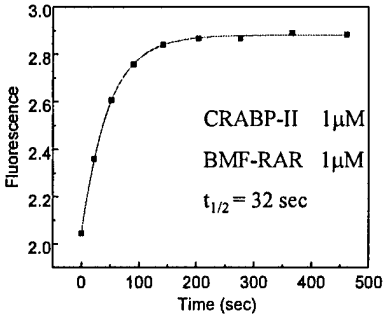
sufficient for ligand channeling.

In order to clarify which of the RAR isotypes is under direct control of CRABP-II, we planned to study the kinetic parameters of transfer of RA from CRABP-II to the three RAR isotypes. The ligand binding domains of RARβ and RARγ were cloned into bacterial expression vectors and purified to near homogeneity. The fluorescence properties of retinoic acid bound to both RARβ and RARγ were determined to ensure transfer experiments could be performed. Unfortunately, RA bound to these two isotypes fluoresces nearly as much as when bound to CRABP-II. Thus, transfer of RA from CRABP-II to RARβ and RARγ could not be monitored by RA fluorescence.

To overcome this setback, fluorescent probes can be used to label either the donor protein (CRABP-II) or acceptor protein (RAR). If ligand binding changes the fluorescence of the probe, this can be used as a measure of ligand transfer. A variety of fluorescent probes were tested for labeling CRABP-II. Some did not label the protein, some labeled the protein but ligand binding did not affect the fluorescence of the label, while others both labeled and responded to RA but did not channel with RARα. Thus, no suitable probe was found that could be used as a read-out for ligand transfer between CRABP-II and RAR.

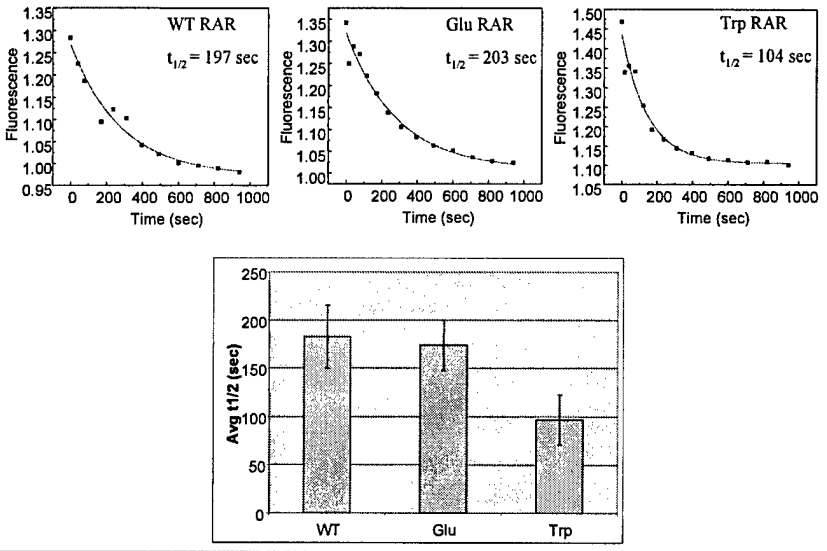
Next, RARs were labeled with fluorescent probes. Again, a variety of probes were tested for labeling RARα. Labeling with the cycteine-attacking probe (5-bromo-methyl-fluorescein (BMF)) resulted in a labeled RARα whose fluorescence properties responded to ligand binding. Specifically, this protein displayed fluorescence resonance energy transfer (FRET) between the RA and the protein-bound probe. Unfortunately, when RARβ and RARγ were labeled with the same probe, the fluorescence of neither protein responded to ligand binding. Therefore, labeling RARs with BMF could not be used as a measure of ligand transfer between CRABP-II and RARβ and RARγ.

Figure 2 Rate of transfer between CRABP-II and BMF-RAR is faster than with native RAR



Channeling experiments were performed between CRABP-II and BMF-labeled RARα. Surprisingly, RA channeled much faster to the labeled RARα as compared to the unlabeled RARα (Figure 2). RA transferred from CRABP-II to the labeled RAR with a $t_{1/2}$ of 36s, about 3-fold faster as compared with its rate of transfer to the native receptor. We hypothesize that RARα was labeled near the site of interaction with CRABP-II and that the label facilitated ligand transfer.

Figure 3 Rate of transfer between CRABP-II and RAR C336W is faster than with native RAR or C336E

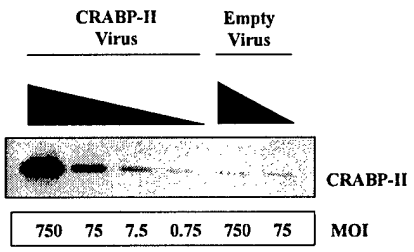


With the help of mass spectrometry at the Harvard Microchemistry facility the labeling site was determined to be cysteine 336. Mutational analysis was performed to mimic the structure of the BMF at the site of labeling and tested for ligand transfer with CRABP-II. In these Cys336 was substituted with either Trp or Glu. As seen in figure 3 introducing a Trp in this location resulted in a protein that accepted RA from CRABP-II at a much faster rate than either the wild type or the Cys336Glu receptor. This indicates that (1) this region in RAR may be the domain responsible for the interaction with CRABP-II, and (2) that adding a bulky hydrophobic group at this position facilitates

ligand-channeling between these two proteins. We are now working in collaboration with Dr. Richard Gillilan to computationally "dock" the binding protein and receptor to determine if the region around Cys336 in RARα could be the interaction domain with CRABP-II.

Task 2a. To determine the mechanism by which CRABP-II inhibits growth of mammary carcinoma cells.

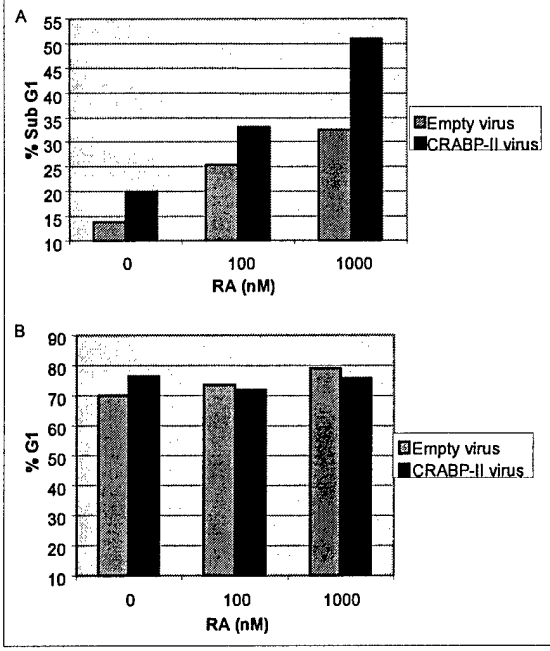
Figure 4 Over-expression of CRABP-II using Adenovirus Infection



Our group has previously shown that over-expression of CRABP-II enhances the ability of RA to inhibit the growth of the mammary carcinoma cell line MCF-7 (*5*). The goal of this project is to determine the mechanism by which these cells are growth inhibited. We tested two signaling pathways that may respond to RA through RAR-mediated transcriptional regulation to induce growth arrest: (1) Apoptosis- RA may induce programmed cell death, characterized by cleavage of cytosolic cysteine proteases, caspases, and DNA fragmentation. (2) Cell cycle arrest- RA could induce growth arrest by blocking the cell cycle at a particular stage.

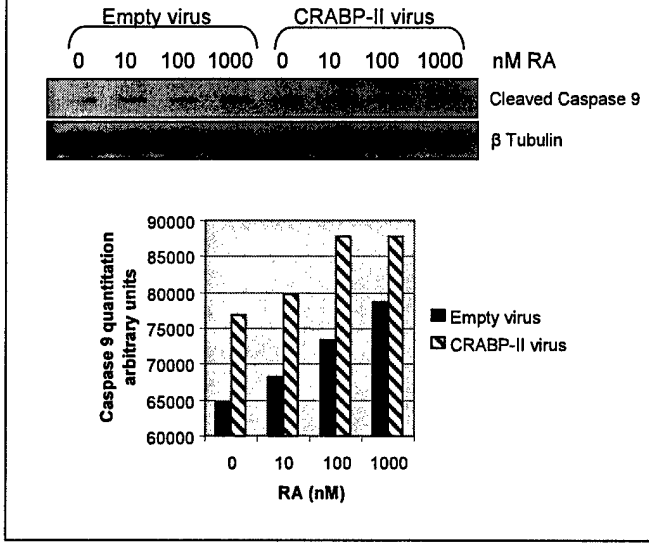
Apoptosis and cell cycle distribution were monitored following RA treatment in MCF-7 cells that over-express CRABP-II compared to wild type cells. Over-expression of the binding protein in these cells was accomplished by viral infection with an adenovirus encoding the cDNA for CRABP-II (Figure 4). A multiplicity of infection (MOI) of 600 was used in subsequent experiments. Nuclei from MCF-7 cells treated with an empty virus (Ad0) or with the adenovirus harboring CRABP-II (Ad-CRABP-II) and treated with RA for 5 days were analyzed for DNA content by flow cytometry. In these experiments, a shift in percentage of G1 nuclei would indicate a cell cycle arrest while an increase in sub G1 population (fragmented DNA) would indicate the occurrence of programmed cell death. As seen in figure 5, retinoic acid treatment of MCF-7 cells caused an increase in the amount of fragmented DNA but had little effect on the cell cycle distribution. Over-expression of CRABP-II enhanced the accumulation of fragmented DNA in response to RA in MCF-7 cells, again, with little effect on the portion of cells in G1. Therefore, we can conclude that RA causes growth inhibition in MCF-7 cells by induction of apoptosis and over-expression of CRABP-II enhances this response.

Figure 5 CRABP-II Enhances DNA Fragmentation in Response to RA Treatment



Apoptotic responses are coordinated by a tightly regulated group of cysteine proteases named caspases. In a normal healthy cell, these proteins are found in an inactive state. Upon stimulation of apoptosis, procaspases are cleaved

Figure 6 CRABP-II Enhances Caspase 9 Cleavage in Response to RA



to produce active proteases which then cleave cellular targets to propagate the apoptotic signal and induce death. Therefore, apoptotic responses can be detected by monitoring the appearance of the cleaved caspases. MCF-7 cells were treated with RA for 5 days and cell extracts were probed by western blotting for cleaved caspase 9 (Figure 6). Treatment of MCF-7 cells with RA caused a dose dependent activation of caspase 9. Cells that over-express CRABP-II show enhanced caspase 9 cleavage. Therefore, we conclude that RA-induced apoptosis in MCF-7 cells is a caspase 9 mediated event and over-expression of CRABP-II enhances the caspase response.

Task 2b. Effect of RA and CRABP-II on gene expression profiles in MCF-7 cells.

RA treatment causes growth inhibition in mammary carcinoma cells including MCF-7 cells. MCF-7 cells undergo apoptosis in response to RA treatment and this response is enhanced upon over-expression of CRABP-II. We would like to identify the genes involved in these responses. To this end, we carried out Affymetrix expression array analysis. In these, RNA is extracted from cells under particular assay conditions and corresponding cDNA is made with a fluorescent probe incorporated. This cDNA is then hybridized to a U133A chip containing single stranded DNA from most genes in the human genome, one gene at every spot on the chip. The labeled cDNA hybridizes to the spot containing its complimentary sequence. The more a given gene is expressed, the more signal will be measured from its spot on the chip. Thus, comparisons of gene expression can be made on an entire genome by comparing spot signal intensities from different conditions. We performed expression array studies using Affymetrix cDNA hybridization chips to identify genes that are up-regulated in response to RA and also to over-expression of CRABP-II. Four conditions were analyzed by expression analysis, each one in triplicate; (1) empty virus, no RA treatment, (2) empty virus, 50 nM RA, 4 hours, (3) CRABP-II virus, no RA treatment, (4) CRABP-II virus, 50 nM RA, 4 hours. When MCF-7 cells were treated with RA, the expression of numerous genes was induced. Appendix 1 contains the entire list of genes induced by at least 1.32-fold in response to RA treatment. Contained in this list are many known RA target genes such as the RA degrading enzymes cytochrome P450s and homeobox genes. Importantly, the expression of many genes involved in apoptotic responses was also induced, as shown by the partial list of genes in Table 1. Specifically, caspase 7 and caspase 9 genes were both induced in response to RA. These observations support the experimental findings that RA triggers an apoptotic response in MCF-7 cells and suggest a mechanism by which the response is accomplished.

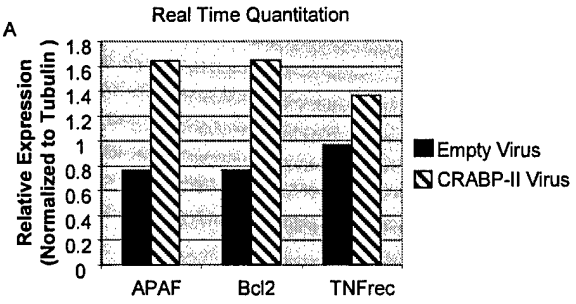
Table 1: Pro-Apoptotic Genes Induced by RA

Gene Name	Fold Change	P-Value
interferon regulatory factor 1	1.66	0.012061
tumor necrosis factor receptor superfamily, member 1A	1.65	3.99E-06
caspase 7, apoptosis-related cysteine protease	1.39	0.023309
caspase 9, apoptosis-related cysteine protease	1.37	0.012061
TIA1 cytotoxic granule-associated RNA binding protein	1.33	0.027382
small inducible cytokine subfamily E, member 1	1.32	0.067476
interleukin 6 signal transducer (gp130, oncostatin M receptor)	1.32	0.067476

The known function of CRABP-II is to bind RA and deliver it to RAR in the nucleus thereby enhancing the transcriptional activity of the receptor. This binding protein has no known function in the absence of RA. Surprisingly, the expression array screens indicated that a number of pro-apoptotic genes are induced by simply over-expressing CRABP-II in the absence of RA treatment (Figure 7B). Among these are 3 genes that we chose to use in order to validate the Affymetrix data, namely: Apoptosis protease activating factor, BCL2/adenovirus E1b 19kDa interacting protein 3, and tumor necrosis factor receptor superfamily, member 11b. For validation studies, quantitative real-time PCR (Q-PCR) was carried out (Figure 7A). cDNA from the array was subjected to Q-PCR using Taqman probes. Primer and probe sets for all genes tested were obtained from Applied Biosystems as Assays on Demand. The relative standard curve method was used to quantitate the relative expression for each gene. Fold induction was calculated by dividing the expression of each gene in the CRABP-

II over-expressing cDNA by the expression of that gene in the empty virus treated cDNA. As seen in figure 7, all three genes were found to be induced in samples from CRABP-II over-expressing cells. The Q-PCR thus verified the validity of the observations derived from the Affymetrix array screen. This up-regulation of pro-apoptotic genes may explain how, in the previous assays, over-expression of CRABP-II in the absence of RA caused an increase in all the apoptotic markers tested. Importantly, this may point to a novel, RA-independent function of CRABP-II. Our current working hypothesis is that CRABP-II may heighten cellular responses not only to RA signaling via RAR but also to a variety of other apoptotic signals. We are currently testing the effect of over-expression of CRABP-II on the effectiveness of other known apoptosis inducers such as etoposide or TNFα.

Figure 7 CRABP-II Over-expression induces expression of pro-apoptosis genes



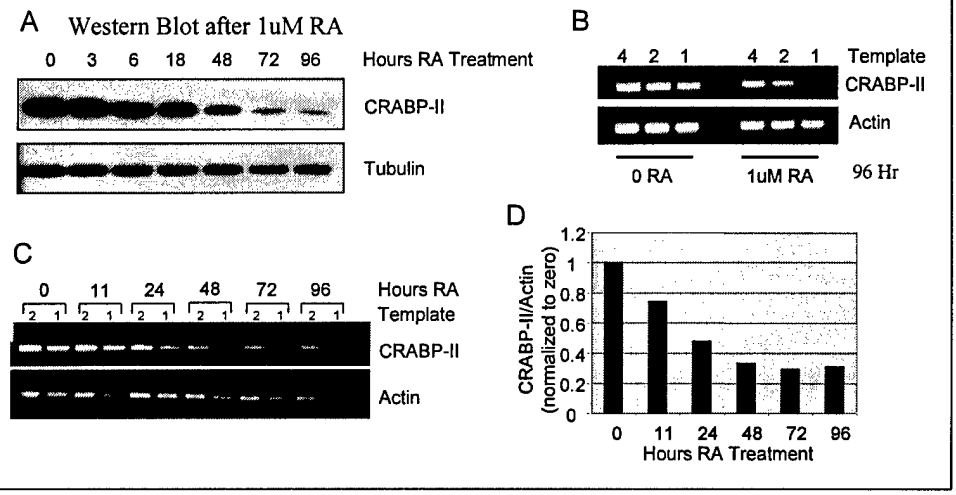
B

Gene Name	Fold Induction Affymetrix
Apoptotic protease activating factor	1.25
BCL2/adenovirus E1B 19kDa interacting protein 3	1.30
Tumor necrosis factor receptor superfamily, member 11b	1.24

Task 3. Understand the underlying basis for RA resistance in mammary carcinoma cells

A frequent complication in utilizing RA for cancer therapy is the development of RA-resistance in tumors. Understanding the mechanisms that underlie RA-resistance in cancer cells are thus of significant clinical importance. We have observed that mammary carcinoma cells that over-express CRABP-II become more sensitive to RA-induce growth inhibition. Conversely, we find that cells that do not express CRABP-II are resistant to this anti-proliferative effect. Therefore, we examined the effect of RA on the expression of CRABP-II. The data reveal that RA treatment resulted in a decrease in CRABP-II protein in MCF-7 cells (Figure 8A). This effect may originate from two possible responses: (1) RA treatment may lead to a degradation of CRABP-II protein, or (2) RA treatment may result in a decrease in mRNA levels. To determine whether this loss of protein is due to a decrease in CRABP-II mRNA level, semi-quantitative PCR was performed on extracts from cells treated with RA. Figure 8B-D shows that the CRABP-II RNA level in MCF-7 cells is down regulated in response to RA treatment. RA-induced down-regulation of CRABP-II expression may comprise an important feature through which carcinoma cells are rendered RA-resistant. On going studies aim to understand the molecular mechanisms underlying down regulation of CRABP-II expression.

Figure 8 RA treatment causes reduced expression of CRABP-II



Key Research Accomplishments

- The ligand binding domain of RARα is sufficient for interaction and ligand channeling with CRABP-II
- The region around Cys336 is RARα is the potential interaction region with CRABP-II necessary for channeling
- Retinoic acid causes apoptosis in MCF-7 cells with little effect on cell cycle distribution.
- Over-expression of CRABP-II in MCF-7 cells enhances the apoptotic response to RA.
- Expression array data indicate that expression of certain pro-apoptotic genes is induced upon treatment with RA
- Over-expression of CRABP-II in the absence of RA causes an increase in pro-apoptotic genes indicating a novel ligand-independent function for this binding protein.
- RA-resistance of carcinoma cells may stem from RA-induced down-regulation of CRABP-II.

Reportable Outcomes:
There are no reportable outcomes to this date.

Conclusions:
RA is currently used or is in clinical trials for therapy of a variety of cancers, however, at pharmacological doses it is often toxic. Our lab is investigating approaches that will allow for sensitization of cancer cells to RA chemotherapy in order to increase the therapeutic efficacy of this compound. We have found that a RA binding protein (CRABP-II) functions to inhibit mammary carcinoma cell proliferation in culture as well as tumor progression *in vivo*. Therefore, CRABP-II may be a novel target for therapeutic and preventive strategies for treatment of breast cancer. The mechanisms by which this binding protein acts to modulate RA signaling and enhance the anti-proliferative activities of this compound in breast cancer are being studied. CRABP-II enhances RA-induced apoptosis in MCF-7 cell. Interestingly, CRABP-II appears to function by two separate mechanisms. The protein enhances RA-induced apoptosis and it also increases expression of pro-apoptotic genes even in the absence of RA. These observations suggest that CRABP-II possesses a novel ligand-independent function which contributes to its anti-carcinogenic activity. Studies carried out during this funding period also revealed that RA down-regulates the expression of CRABP-II in carcinoma cells. This activity may be an important factor in the development of RA-resistance in carcinomas.

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5. A. S. Budhu, N. Noy, *Mol Cell Biol* **22**, 2632-41 (Apr, 2002).

Appendix 1 RA-induced genes

Gene ID	Mean Log2 Ratio	UniGene Name
206424_at	4.19	cytochrome P450, family 26, subfamily A, polypeptide 1
219825_at	3.04	cytochrome P450 retinoid metabolizing protein
213844_at	2.99	homeo box A5
202481_at	2.11	short-chain dehydrogenase/reductase 1
214639_s_at	2.08	homeo box A1
202936_s_at	1.78	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
201510_at	1.62	E74-like factor 3 (ets domain transcription factor, epithelial-specific)
210827_s_at	1.56	E74-like factor 3 (ets domain transcription factor, epithelial-specific)
209324_s_at	1.41	regulator of G-protein signalling 16
211458_s_at	1.35	GABA(A) receptors associated protein like 3
206289_at	1.32	homeo box A4
203140_at	1.25	B-cell CLL/lymphoma 6 (zinc finger protein 51)
204677_at	1.25	cadherin 5, type 2, VE-cadherin (vascular epithelium)
221042_s_at	1.24	calmin (calponin-like, transmembrane)
201236_s_at	1.2	BTG family, member 2
201506_at	1.17	transforming growth factor, beta-induced, 68kDa
209277_at	1.17	tissue factor pathway inhibitor 2
208763_s_at	1.16	delta sleep inducing peptide, immunoreactor
212240_s_at	1.16	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
202464_s_at	1.15	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
209278_s_at	1.12	tissue factor pathway inhibitor 2
205870_at	1.11	bradykinin receptor B2
212719_at	1.11	pleckstrin homology domain containing, family E (with leucine rich repeats) member 1
209325_s_at	1.08	regulator of G-protein signalling 16
206504_at	1.07	cytochrome P450, family 24, subfamily A, polypeptide 1
212444_at	1.06	retinoic acid induced 3
221880_s_at	1.05	Homo sapiens, clone IMAGE:4816940, mRNA
200878_at	1.04	endothelial PAS domain protein 1
203453_at	1.02	sodium channel, nonvoltage-gated 1 alpha
201889_at	1	family with sequence similarity 3, member C
202342_s_at	1	tripartite motif-containing 2
202510_s_at	0.99	tumor necrosis factor, alpha-induced protein 2
205074_at	0.99	solute carrier family 22 (organic cation transporter), member 5
206632_s_at	0.99	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
202388_at	0.98	regulator of G-protein signalling 2, 24kDa

Appendix 1 RA-induced genes

217999_s_at	0.98	pleckstrin homology-like domain, family A, member 1
209682_at	0.97	Cas-Br-M (murine) ecotropic retroviral transforming sequence b
220840_s_at	0.97	hypothetical protein FLJ10706
51158_at	0.97	Homo sapiens, clone IMAGE:4816940, mRNA
204908_s_at	0.96	B-cell CLL/lymphoma 3
213397_x_at	0.96	ribonuclease, RNase A family, 4
217906_at	0.96	kelch domain containing 2
202934_at	0.94	hexokinase 2
213839_at	0.94	calmin (calponin-like, transmembrane)
221541_at	0.94	hypothetical protein DKFZp434B044
218676_s_at	0.93	phosphatidylcholine transfer protein
206478_at	0.92	KIAA0125 gene product
210517_s_at	0.92	A kinase (PRKA) anchor protein (gravin) 12
211962_s_at	0.92	zinc finger protein 36, C3H type-like 1
202431_s_at	0.91	v-myc myelocytomatosis viral oncogene homolog (avian)
202599_s_at	0.91	nuclear receptor interacting protein 1
219694_at	0.9	hypothetical protein FLJ11127
212462_at	0.89	MYST histone acetyltransferase (monocytic leukemia) 4
217997_at	0.89	pleckstrin homology-like domain, family A, member 1
203148_s_at	0.88	tripartite motif-containing 14
212977_at	0.87	G protein-coupled receptor
203911_at	0.85	RAP1, GTPase activating protein 1
209211_at	0.85	Kruppel-like factor 5 (intestinal)
213528_at	0.85	hypothetical protein MGC9084
201150_s_at	0.84	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
202969_at	0.84	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
218330_s_at	0.82	neuron navigator 2
206277_at	0.81	purinergic receptor P2Y, G-protein coupled, 2
218507_at	0.81	hypoxia-inducible protein 2
221773_at	0.81	ELK3, ETS-domain protein (SRF accessory protein 2)
201042_at	0.79	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
219369_s_at	0.79	chromosome 14 open reading frame 137
208937_s_at	0.78	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
213572_s_at	0.78	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
202935_s_at	0.76	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)
209723_at	0.76	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 9

Appendix 1 RA-induced genes

204733_at	0.75	kallikrein 6 (neurosin, zyme)
214155_s_at	0.75	c-Mpl binding protein
218245_at	0.75	hypothetical protein, estradiol-induced
202531_at	0.73	interferon regulatory factor 1
203455_s_at	0.72	spermidine/spermine N1-acetyltransferase
207643_s_at	0.72	tumor necrosis factor receptor superfamily, member 1A
210002_at	0.72	GATA binding protein 6
36711_at	0.72	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
204667_at	0.71	forkhead box A1
221215_s_at	0.7	ankyrin repeat domain 3
35254_at	0.7	FLN29 gene product
202786_at	0.69	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)
209250_at	0.69	degenerative spermatocyte homolog, lipid desaturase (Drosophila)
202638_s_at	0.68	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
204563_at	0.68	selectin L (lymphocyte adhesion molecule 1)
206613_s_at	0.68	TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa
212637_s_at	0.68	WW domain-containing protein 1
221840_at	0.68	protein tyrosine phosphatase, receptor type, E
222309_at	0.68	chromosome 6 open reading frame 62
204204_at	0.67	solute carrier family 31 (copper transporters), member 2
204709_s_at	0.67	kinesin family member 23
211967_at	0.67	pro-oncosis receptor inducing membrane injury gene
212148_at	0.67	pre-B-cell leukemia transcription factor 1
214775_at	0.67	Nedd4 binding protein 3
218960_at	0.67	transmembrane protease, serine 4
203386_at	0.66	TBC1 domain family, member 4
217851_s_at	0.66	chromosome 20 open reading frame 45
218539_at	0.66	F-box only protein 34
202149_at	0.65	neural precursor cell expressed, developmentally down-regulated 9
203706_s_at	0.65	frizzled homolog 7 (Drosophila)
212531_at	0.65	lipocalin 2 (oncogene 24p3)
201170_s_at	0.64	basic helix-loop-helix domain containing, class B, 2
202545_at	0.64	protein kinase C, delta
202968_s_at	0.64	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
210367_s_at	0.64	prostaglandin E synthase
212449_s_at	0.64	lysophospholipase I

Appendix 1 RA-induced genes

212586_at	0.64	calpastatin
218590_at	0.64	chromosome 10 open reading frame 2
208056_s_at	0.63	core-binding factor, runt domain, alpha subunit 2; translocated to, 3
218574_s_at	0.63	LIM and cysteine-rich domains 1
221843_s_at	0.62	KIAA1609 protein
215001_s_at	0.61	glutamate-ammonia ligase (glutamine synthase)
204971_at	0.6	cystatin A (stefin A)
205896_at	0.6	solute carrier family 22 (organic cation transporter), member 4
209512_at	0.6	hypothetical protein MGC10940
219681_s_at	0.6	Rab coupling protein
221577_x_at	0.6	prostate differentiation factor
201218_at	0.59	C-terminal binding protein 2
202904_s_at	0.59	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae)
203910_at	0.59	PTPL1-associated RhoGAP 1
203102_s_at	0.58	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
204526_s_at	0.58	TBC1 domain family, member 8 (with GRAM domain)
212124_at	0.58	retinoic acid induced 17
213260_at	0.58	forkhead box C1
65438_at	0.58	KIAA1609 protein
201829_at	0.57	neuroepithelial cell transforming gene 1
203232_s_at	0.57	spinocerebellar ataxia 1 (olivopontocerebellar ataxia 1, autosomal dominant, ataxin 1)
211953_s_at	0.57	karyopherin (importin) beta 3
213005_s_at	0.57	kidney ankyrin repeat-containing protein
217853_at	0.57	tumor endothelial marker 6
221727_at	0.57	activated RNA polymerase II transcription cofactor 4
219370_at	0.56	candidate mediator of the p53-dependent G2 arrest
219581_at	0.56	hypothetical protein MGC2776
201424_s_at	0.55	cullin 4A
201975_at	0.55	restin (Reed-Steinberg cell-expressed intermediate filament-associated protein)
203394_s_at	0.55	hairy and enhancer of split 1, (Drosophila)
204256_at	0.55	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)
204807_at	0.55	transmembrane protein 5
209679_s_at	0.55	hypothetical protein from clone 643
210970_s_at	0.55	inhibitor of Bruton's tyrsoine kinase
212709_at	0.55	nucleoporin 160kDa
213262_at	0.55	spastic ataxia of Charlevoix-Saguenay (sacsin)

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218605_at	0.55	transcription factor B2, mitochondrial
219540_at	0.55	zinc finger protein 267
201830_s_at	0.54	neuroepithelial cell transforming gene 1
203705_s_at	0.54	frizzled homolog 7 (Drosophila)
203780_at	0.54	epithelial V-like antigen 1
204019_s_at	0.54	likely ortholog of mouse Sh3 domain YSC-like 1
208078_s_at	0.54	transcription factor 8 (represses interleukin 2 expression)
209187_at	0.54	down-regulator of transcription 1, TBP-binding (negative cofactor 2)
210512_s_at	0.54	vascular endothelial growth factor
212180_at	0.54	v-crk sarcoma virus CT10 oncogene homolog (avian)-like
212646_at	0.54	raft-linking protein
212684_at	0.54	zinc finger protein 3 (A8-51)
213984_at	0.54	KIAA0648 protein
217996_at	0.54	pleckstrin homology-like domain, family A, member 1
218446_s_at	0.54	family with sequence similarity 18, member B
218696_at	0.54	eukaryotic translation initiation factor 2-alpha kinase 3
219178_at	0.54	hypothetical protein FLJ12960
221193_s_at	0.54	hypothetical protein FLJ20094
201219_at	0.53	zinc finger, RAN-binding domain containing 1
203100_s_at	0.53	chromodomain protein, Y chromosome-like
205083_at	0.53	aldehyde oxidase 1
212522_at	0.53	phosphodiesterase 8A
213139_at	0.53	snail homolog 2 (Drosophila)
214771_x_at	0.53	Rho interacting protein 3
216268_s_at	0.53	jagged 1 (Alagille syndrome)
218251_at	0.53	hypothetical protein STRAIT11499
221020_s_at	0.53	mitochondrial folate transporter/carrier
201675_at	0.52	A kinase (PRKA) anchor protein 1
202039_at	0.52	TGFB1-induced anti-apoptotic factor 1
202376_at	0.52	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
202727_s_at	0.52	interferon gamma receptor 1
202837_at	0.52	FLN29 gene product
207935_s_at	0.52	keratin 13
212060_at	0.52	U2-associated SR140 protein
212249_at	0.52	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
213304_at	0.52	KIAA0423 protein

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213704_at	0.52	Rab geranylgeranyltransferase, beta subunit
218538_s_at	0.52	MRS2-like, magnesium homeostasis factor (S. cerevisiae)
218889_at	0.52	AD24 protein
222361_at	0.52	similar to beta-tubulin 4Q
39891_at	0.52	similar to zinc finger protein 366
202345_s_at	0.51	fatty acid binding protein 5 (psoriasis-associated)
203217_s_at	0.51	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)
204700_x_at	0.51	hypothetical protein MGC29875
209422_at	0.51	chromosome 20 open reading frame 104
218313_s_at	0.51	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7)
221701_s_at	0.51	stimulated by retinoic acid gene 6
207467_x_at	0.5	calpastatin
208673_s_at	0.5	splicing factor, arginine/serine-rich 3
209706_at	0.5	NK3 transcription factor related, locus 1 (Drosophila)
212197_x_at	0.5	Rho interacting protein 3
214079_at	0.5	dehydrogenase/reductase (SDR family) member 2
214193_s_at	0.5	hypothetical protein MGC29875
217892_s_at	0.5	epithelial protein lost in neoplasm beta
218764_at	0.5	protein kinase C, eta
221514_at	0.5	serologically defined colon cancer antigen 16
35148_at	0.5	tight junction protein 3 (zona occludens 3)
201948_at	0.49	nucleolar GTPase
203726_s_at	0.49	laminin, alpha 3
205366_s_at	0.49	homeo box B6
205453_at	0.49	homeo box B2
208912_s_at	0.49	2',3'-cyclic nucleotide 3' phosphodiesterase
213702_x_at	0.49	N-acylsphingosine amidohydrolase (acid ceramidase) 1
218395_at	0.49	actin-related protein 6
218940_at	0.49	chromosome 14 open reading frame 138
218947_s_at	0.49	hypothetical protein FLJ10486
220890_s_at	0.49	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47
203563_at	0.48	actin filament associated protein
203759_at	0.48	sialyltransferase 4C (beta-galactoside alpha-2,3-sialyltransferase)
207181_s_at	0.48	caspase 7, apoptosis-related cysteine protease
208754_s_at	0.48	nucleosome assembly protein 1-like 1
214658_at	0.48	CGI-109 protein

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215444_s_at	0.48	tripartite motif-containing 31
216159_s_at	0.48	Homo sapiens cDNA FLJ13695 fis, clone PLACE2000124.
218284_at	0.48	DKFZP586N0721 protein
218815_s_at	0.48	hypothetical protein FLJ10199
221497_x_at	0.48	egl nine homolog 1 (C. elegans)
221931_s_at	0.48	sec13-like protein
202006_at	0.47	protein tyrosine phosphatase, non-receptor type 12
202396_at	0.47	transcription elongation regulator 1 (CA150)
203049_s_at	0.47	KIAA0372 gene product
203073_at	0.47	component of oligomeric golgi complex 2
204362_at	0.47	src family associated phosphoprotein 2
205890_s_at	0.47	ubiquitin D
207760_s_at	0.47	nuclear receptor co-repressor 2
208264_s_at	0.47	eukaryotic translation initiation factor 3, subunit 1 alpha, 35kDa
208786_s_at	0.47	microtubule-associated protein 1 light chain 3 beta
214683_s_at	0.47	CDC-like kinase 1
218772_x_at	0.47	hypothetical protein FLJ10493
218882_s_at	0.47	WD repeat domain 3
220161_s_at	0.47	erythrocyte membrane protein band 4.1 like 4B
221648_s_at	0.47	CGI-146 protein
202307_s_at	0.46	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
204341_at	0.46	tripartite motif-containing 16
206935_at	0.46	protocadherin 8
208802_at	0.46	signal recognition particle 72kDa
209498_at	0.46	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
211764_s_at	0.46	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)
213188_s_at	0.46	myc-induced nuclear antigen, 53 kDa
213280_at	0.46	KIAA1039 protein
213934_s_at	0.46	zinc finger protein 23 (KOX 16)
218963_s_at	0.46	keratin 23 (histone deacetylase inducible)
219316_s_at	0.46	chromosome 14 open reading frame 58
220643_s_at	0.46	Fas apoptotic inhibitory molecule
202130_at	0.45	RIO kinase 3 (yeast)
202133_at	0.45	transcriptional co-activator with PDZ-binding motif (TAZ)
202392_s_at	0.45	phosphatidylserine decarboxylase
203632_s_at	0.45	G protein-coupled receptor, family C, group 5, member B

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203984_s_at	0.45	caspase 9, apoptosis-related cysteine protease
204175_at	0.45	zinc finger protein
204244_s_at	0.45	activator of S phase kinase
204495_s_at	0.45	DKFZP434H132 protein
205253_at	0.45	pre-B-cell leukemia transcription factor 1
205618_at	0.45	proline-rich Gla (G-carboxyglutamic acid) polypeptide 1
208407_s_at	0.45	catenin (cadherin-associated protein), delta 1
209451_at	0.45	TRAF family member-associated NFKB activator
210980_s_at	0.45	N-acylsphingosine amidohydrolase (acid ceramidase) 1
218319_at	0.45	pellino homolog 1 (Drosophila)
218331_s_at	0.45	hypothetical protein FLJ20360
218701_at	0.45	lactamase, beta 2
200776_s_at	0.44	basic leucine zipper and W2 domains 1
201193_at	0.44	isocitrate dehydrogenase 1 (NADP+), soluble
201963_at	0.44	fatty-acid-Coenzyme A ligase, long-chain 2
202704_at	0.44	transducer of ERBB2, 1
203395_s_at	0.44	hairy and enhancer of split 1, (Drosophila)
204521_at	0.44	protein predicted by clone 23733
205398_s_at	0.44	MAD, mothers against decapentaplegic homolog 3 (Drosophila)
205479_s_at	0.44	plasminogen activator, urokinase
206875_s_at	0.44	Ste20-related serine/threonine kinase
212268_at	0.44	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
212364_at	0.44	myosin IB
212499_s_at	0.44	chromosome 14 open reading frame 32
213150_at	0.44	homeo box A10
214240_at	0.44	galanin
214830_at	0.44	solute carrier family 38, member 6
218326_s_at	0.44	G protein-coupled receptor 48
218640_s_at	0.44	pleckstrin homology domain containing, family F (with FYVE domain) member 2
219443_at	0.44	chromosome 20 open reading frame 13
219832_s_at	0.44	homeo box C13
202820_at	0.43	aryl hydrocarbon receptor
203023_at	0.43	hypothetical protein HSPC111
203401_at	0.43	phosphoribosyl pyrophosphate synthetase 2
204249_s_at	0.43	LIM domain only 2 (rhombotin-like 1)
207222_at	0.43	phospholipase A2, group X

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207431_s_at	0.43	degenerative spermatocyte homolog, lipid desaturase (Drosophila)
208760_at	0.43	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)
209536_s_at	0.43	EH-domain containing 4
209744_x_at	0.43	itchy homolog E3 ubiquitin protein ligase (mouse)
210868_s_at	0.43	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)
211137_s_at	0.43	ATPase, Ca++ transporting, type 2C, member 1
213320_at	0.43	protein arginine N-methyltransferase 3(hnRNP methyltransferase S. cerevisiae)-like 3
214058_at	0.43	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)
214835_s_at	0.43	succinate-CoA ligase, GDP-forming, beta subunit
219147_s_at	0.43	hypothetical protein FLJ20559
222056_s_at	0.43	CGI-105 protein
46323_at	0.43	Ca2+-dependent endoplasmic reticulum nucleoside diphosphatase
201407_s_at	0.42	protein phosphatase 1, catalytic subunit, beta isoform
201816_s_at	0.42	glioblastoma amplified sequence
202034_x_at	0.42	RB1-inducible coiled-coil 1
202079_s_at	0.42	OGT(O-Glc-NAc transferase)-interacting protein 106 KDa
202930_s_at	0.42	succinate-CoA ligase, ADP-forming, beta subunit
203177_x_at	0.42	transcription factor A, mitochondrial
205135_s_at	0.42	nuclear fragile X mental retardation protein interacting protein 1
205266_at	0.42	leukemia inhibitory factor (cholinergic differentiation factor)
205965_at	0.42	basic leucine zipper transcription factor, ATF-like
206463_s_at	0.42	dehydrogenase/reductase (SDR family) member 2
210005_at	0.42	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase
211421_s_at	0.42	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)
211686_s_at	0.42	RNA binding protein
212037_at	0.42	pinin, desmosome associated protein
212434_at	0.42	GrpE-like 1, mitochondrial (E. coli)
217094_s_at	0.42	itchy homolog E3 ubiquitin protein ligase (mouse)
217873_at	0.42	MO25 protein
218092_s_at	0.42	HIV-1 Rev binding protein
218102_at	0.42	CGI-26 protein
218989_x_at	0.42	solute carrier family 30 (zinc transporter), member 5
219036_at	0.42	p10-binding protein
220926_s_at	0.42	chromosome 1 open reading frame 22
221513_s_at	0.42	serologically defined colon cancer antigen 16
200626_s_at	0.41	matrin 3

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201038_s_at	0.41	hypothetical gene supported by U60823; U73477; X75090; AF025684; AY007110; BC007200; NM_006305
201448_at	0.41	TIA1 cytotoxic granule-associated RNA binding protein
202350_s_at	0.41	matrilin 2
202363_at	0.41	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)
203556_at	0.41	transcription factor ZHX2
204241_at	0.41	acyl-Coenzyme A oxidase 3, pristanoyl
204656_at	0.41	SHB (Src homology 2 domain containing) adaptor protein B
204689_at	0.41	hematopoietically expressed homeobox
204699_s_at	0.41	hypothetical protein MGC29875
206302_s_at	0.41	nudix (nucleoside diphosphate linked moiety X)-type motif 4
209037_s_at	0.41	EH-domain containing 1
211733_x_at	0.41	sterol carrier protein 2
211998_at	0.41	H3 histone, family 3B (H3.3B)
212096_s_at	0.41	mitochondrial tumor suppressor gene 1
212183_at	0.41	nudix (nucleoside diphosphate linked moiety X)-type motif 4
212675_s_at	0.41	KIAA0582 protein
218352_at	0.41	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1
218712_at	0.41	hypothetical protein FLJ20508
218795_at	0.41	lysophosphatidic acid phosphatase
219032_x_at	0.41	opsin 3 (encephalopsin, panopsin)
219231_at	0.41	nuclear receptor coactivator 6 interacting protein
220183_s_at	0.41	nudix (nucleoside diphosphate linked moiety X)-type motif 6
37793_r_at	0.41	RAD51-like 3 (S. cerevisiae)
49111_at	0.41	Homo sapiens mRNA; cDNA DKFZp762M127 (from clone DKFZp762M127)
200608_s_at	0.4	RAD21 homolog (S. pombe)
201129_at	0.4	splicing factor, arginine/serine-rich 7, 35kDa
201410_at	0.4	pleckstrin homology domain containing, family B (evectins) member 2
201599_at	0.4	ornithine aminotransferase (gyrate atrophy)
202541_at	0.4	small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)
203098_at	0.4	chromodomain protein, Y chromosome-like
203124_s_at	0.4	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2
203693_s_at	0.4	E2F transcription factor 3
203745_at	0.4	holocytochrome c synthase (cytochrome c heme-lyase)
204137_at	0.4	transmembrane 7 superfamily member 1 (upregulated in kidney)
204342_at	0.4	calcium-binding transporter
204354_at	0.4	protection of telomeres 1

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205158_at	0.4	ribonuclease, RNase A family, 4
205434_s_at	0.4	AP2 associated kinase 1
205681_at	0.4	BCL2-related protein A1
206770_s_at	0.4	solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member A3
208322_s_at	0.4	sialyltransferase 4A (beta-galactoside alpha-2,3-sialyltransferase)
209101_at	0.4	connective tissue growth factor
212195_at	0.4	interleukin 6 signal transducer (gp130, oncostatin M receptor)
212511_at	0.4	phosphatidylinositol binding clathrin assembly protein
213225_at	0.4	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform
213251_at	0.4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
213626_at	0.4	hypothetical protein FLJ14431
213792_s_at	0.4	insulin receptor
217833_at	0.4	NS1-associated protein 1
218035_s_at	0.4	RNA-binding protein
218158_s_at	0.4	adaptor protein containing pH domain, PTB domain and leucine zipper motif
218984_at	0.4	hypothetical protein FLJ20485
221478_at	0.4	BCL2/adenovirus E1B 19kDa interacting protein 3-like
43511_s_at	0.4	Homo sapiens mRNA; cDNA DKFZp762M127 (from clone DKFZp762M127)