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PRINCIPAL INVESTIGATOR: Douglas S. Conklin, Ph.D.

CONTRACTING ORGANIZATION: State University of New York
Albany, NY 12201-0009

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Douglas S. Conklin, Ph.D.

E-Mail: dconklin@albany.edu

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14. ABSTRACT
The year 1 aims were primarily concerned with collecting shRNA constructs so that a small number that have inhibitory effects on breast cancer progression *in vivo* models could be identified. In the past year, we have a collated a set of encoded hairpins targeting genes overexpressed in ErbB-2 positive breast tumors. We have also spent considerable effort testing the compatibility of several assays for cellular correlates of tumorigenicty with high throughput gene transfer. To date, we have retrieved 65 shRNA constructs targeting 51 of the genes overexpressed ErbB2-positive breast cancer cells and tested them for effects on cell proliferation in a screen in BT474 cells. Somewhat surprisingly, almost 35% of the hairpins used in the screening cause similar or greater reduction of cell proliferation than ErbB-2 shRNA controls. Although several of the genes whose decreased expression compromises proliferation rate have been shown to play a role in tumor cell growth, it is clear that these constructs must now be tested for effects on normal cells, i.e. human mammary epithelial cells (HMECs), to assess whether these represent pathways that are required specifically for tumor cell proliferation.

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Introduction

The recent explosion of genome sequences and bioinformatic data has changed the nature of mammalian genetics. Systematic investigation of expression patterns of thousands of genes in breast tumors using cDNA microarrays and serial analysis of gene expression has yielded a long list of genes whose increased activity is correlated with the occurrence of breast cancer (1-4). In notable cases, associations between gene expression levels and clinical outcome have been determined. In most cases, however, the information is only correlative and serves as a rough guide to genes that might positively influence some aspect of breast cancer.

We are working towards identifying which of the nearly 100 genes typically overexpressed in ErbB-2 positive breast tumors are important for the survival and aggressiveness of breast cancer cells. Our approach makes use of DNA-encoded short hairpin RNAs that elicit an RNA interference silencing of the overexpressed genes. Using high throughput methodologies, we have constructed a library of sequence-verified hairpins that target most human genes (5). Constructs from the library are being used for the systematic validation of breast cancer molecular profiling data in ErbB-2 positive BT 474 cells. Cellular correlates of tumorigenicity such as proliferation rate, resistance to apoptosis and anchorage independent growth are assayed in cells containing shRNA constructs that target overexpressed genes. The overall objective of this proposal is to identify hairpins that have inhibitory effects on breast cancer progression in *in vivo* models.

This would add to our understanding of the genes involved in breast cancer and identify their products as potential small molecule therapeutic targets. It would also pave the way for testing of the hairpins that we identify as gene therapeutics.

The underlying hypothesis of this proposal is that functional genomic screening with DNA-encoded RNA interference can identify those genes that breast cancer cells actually rely on to cause cancer. We will systematically target the most commonly overexpressed genes in breast cancer cells especially those that are overexpressed in association with ErbB-2 amplification. Many of these genes are likely to be involved in granting some sort of survival advantage to these cells during cancer progression. Silencing key genes in this group should reverse the cancer phenotype. In this way, we expect to gain insights into which genes are actually required by breast cancer cells to cause cancer and would therefore make excellent therapeutic targets. Moreover, it may contribute directly to the development of gene-based therapeutics by identifying short hairpin RNAs that could someday be used clinically.

Body

There has been good progress on the proposed work for completion by the end of twelve months. The major goal of this time period as set forth in the Statement of Work was: **Creation of a set of encoded hairpins targeting genes overexpressed in ErbB-2 positive breast tumors.** This goal has been met.

An important first step was recruiting a postdoctoral fellow to work on the project, Dr. Antonis Kourtidis joined the lab in January. Antonis has recently received his PhD in molecular biology from the Aristotle University of Thessaloniki in Greece. He has been responsible for performing most of the work on the project to date.

Months 1-12 of the proposal were to be spent in the creation of a set of encoded hairpins that targeted those genes commonly overexpressed in ErbB-2 positive breast tumors. We had planned to make use of a library of over 20,000 sequence-verified hairpins that we had constructed. This library targeted approximately 10,000 human genes. Included were those genes which encoded all of the protein classes that are viewed to be accessible to medicinal chemistry. More importantly, the majority of sequences identified in ErbB-2 positive breast cancer molecular profiling experiments were also represented in the library. Although the original plan was to collect these from an existing short hairpin RNA library and synthesize any missing constructs for genes, advances in RNAi technology simplified this process. A second generation library was constructed in a vector that produces more infective viral particles and expresses short hairpin RNAs with better silencing properties. This library is available through a commercial vendor. As a developer of the original shRNA library detailed in the proposal, I have received the entire library targeting virtually every gene in the human library at cost from the supplier. Although this resource became available early this year, it has simplified assembling a sublibrary of shRNAs targeting overexpressed genes.

The year 1 aims were primarily concerned with collecting shRNA constructs so that a small number that have inhibitory effects on breast cancer progression *in vivo* models could be identified. This would add to our understanding of the genes involved in breast cancer and identify their products as potential small molecule therapeutic targets. It would also pave the way for testing of the hairpins that we identify as gene therapeutics. The accession numbers corresponding to the genes overexpressed in erb-B2 positive breast cancer are given in Table 1. A complete listing of the constructs is given in the appendix.

NM_000125	NM_001982	NM_003651	NM_004901	NM_005574	NM_014399	NM_001903
NM_000212	NM_002014	NM_003710	NM_004933	NM_005765	NM_016016	NM_052886
NM_000224	NM_002203	NM_003909	NM_005005	NM_005885	NM_017802	NM_002795
NM_000419	NM_002266	NM_004265	NM_005015	NM_005930	NM_018728	NM_080861
NM_000442	NM_002317	NM_004448	NM_005030	NM_006148	NM_018979	NM_004893
NM_000595	NM_002353	NM_004483	NM_005079	NM_006265	NM_020974	NM_178516
NM_001026	NM_002411	NM_004496	NM_005213	NM_006276	NM_021724	NM_005570
NM_001216	NM_002417	NM_004643	NM_005228	NM_006432	NM_021980	NM_203365
NM_001311	NM_002585	NM_004747	NM_005310	NM_006601	NM_024101	NM_012229
NM_001408	NM_002758	NM_004774	NM_005324	NM_006726	NM_032308	NM_033419
NM_001429	NM_002791	NM_004888	NM_005556	NM_007067	NM_033418	

Table 1. Accession numbers corresponding to the genes overexpressed in erb-B2 positive breast cancer

In the past year, we have spent considerable effort testing the compatibility of several assays for cellular correlates of tumorigenicty with high throughput gene transfer. Among these are standard soft agar assays, DAPI staining to quantify apoptotic nuclei, Cyquant assays, Rb and E2F luciferase-based reporter cell cycle progression assays, Alamar blue proliferation stains and live cell counts. Although the assays are straightforward, most were insufficiently robust for large scale analysis. Instead we have found that using Alamar blue (Biosource) reduction that is normalized to GFP transfection efficiency to measure shRNA containing cell proliferation is the most workable assay in high throughput. This assay is by far the most robust.

To date, we have retrieved 65 shRNA constructs targeting 51 of the genes overexpressed ErbB2-positive breast cancer cells and tested them for effects on cell proliferation in a screen in BT474 cells. A non-specific hairpin construct targeting the firefly luciferase gene was used as a negative control. Purified DNA for each construct was prepared and used to transiently transfect BT 474s in 96-well plates in triplicate. Transfection efficiency was monitored by co-transfecting with a plasmid construct expressing green fluorescent protein (EGFP). After three days, the abilty of the shRNA to decrease cell viability was determined by quantifying proliferation using Alamar blue (Biosource) reduction that had been normalized to GFP fluorescence. We have found that this is a relatively rapid and reliable method for quantification of proliferation changes.

Our results showed that hairpins targeting genes with known impact on breast cancer, such as ErbB-2, ErbB-3 and EGFR, resulted in reduction of proliferation up to 23% compared to the non-specific hairpin construct (Fig. 1). Somewhat surprisingly, almost 35% of the hairpins used in the screening resulted in similar or greater reduction of cell proliferation when compared to the ErbB-2 shRNA controls (Fig. 1). Although several of the genes whose decreased expression compromises proliferation rate have been shown to play a role in tumor cell growth, it is clear that these constructs must now be tested for effects on normal cells, i.e. human mammary epithelial cells (HMECs), to

assess whether these represent pathways that are required specifically for tumor cell proliferation.

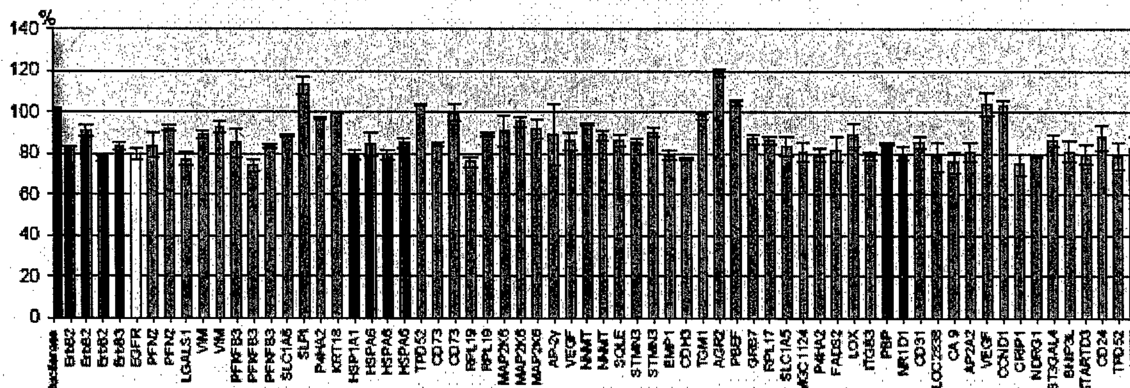

Figure 1. Relative quantification of BT474 cells proliferation based on Alamar blue reduction, on a pilot screening using shRNA constructs targeting overexpressed genes. Numbers on the Y axis represent the mean value of the % of Alamar blue fluorescent units, comparing to the firefly luciferase control hairpin (black bar). Error bars indicate standard deviation as resulted from a triplicate experiment. All results have been normalized for transfection efficiency. Red bars indicate ErbB2 hairpins, the orange and yellow indicate ErbB3 and EGFR hairpins respectively, the deep blue indicate the HSP70 hairpins and the green the PBP and NR1D1 hairpins. Proliferation rates are likely an underestimate of shRNA effect, since cyclohexamide treatment of these cells, a control not available with this dataset, typically results in only a 40% decrease in Alamar blue signal.

In addition to well known targets, it was expected that this sort of approach would reveal novel pathways. Although the results are still at an early stage, we are optimistic. For example, hairpins targeting several HSP70s, the peroxisome proliferator activated receptor (PPAR) binding protein (PPARBP, PBP) and the nuclear receptor 1s, D1 (NR1D1, RevErbα) all resulted in reduced proliferation (Fig. 1). Cell proliferation was reduced both by a hairpin targeting the major heat-inducible HSPA1A gene and by three different hairpins targeting the also inducible HSPA6 (Fig. 1). Recently, RNAi against another member of the HSP70 family, the moderately-expressed, testis associated, HSP70-2 gene, also resulted in attenuation proliferation of the cells (6). The role of the HSP70 genes in breast cancer cell proliferation, metastasis, poor differentiation, and drug resistance is well established (7), but the downstream targets of the HSP70 genes during cancer development have not been extensively studied yet. It has been suggested by a limited number of studies that these two genes may be functionally related to HSP70. PBP is a co-activator of the PPAR receptors, which some of their ligands have been shown to induce HSP70 expression (8,9). PPARα overexpression in rat hydrocephalus cells was concurrent to HSP70 overexpression (10). An indirect link could be also that of the HSP90 stress protein, which binds to the HSP70 during stress response, but it also acts as a repressor of the PPARα, without the biological consequences being clarified yet (11). The NR1D1/RevErbα receptor has been found to be induced by the activated PPARγ during adipocyte differentiation of 3T3-L1 cells (12). It would be very interesting to examine all the possible functional links between the above genes during breast cancer progression. Under this point of view, it is also interesting that PBP was found to interact with the estrogen receptor alpha (ERα),

serving as a co-activator in the ER signaling and therefore suspected to be involved in breast cell tumorigenicity (13). In addition, there has also been shown that there is a regulatory link between NR1D1 and the NFκB pathway (14).

On-going and future work:

Little is changed from the original proposal. Those constructs that target overexpressed genes not yet tested for effects on proliferation will be tested in the proliferation assay. Constructs that are without effect on human mammary epithelial cells (HMECs) but that inhibit the growth of tumor cells will be tested in mouse models as proposed. In addition, the available hairpin constructs will be packaged in amphotropic retroviral packaging cells. Retroviral supernatants will be used to infect BT474 cells. Infected populations will be monitored for phenotypic changes related to tumorigenicity that are not suited for high throughput analysis. These include resistance to apoptosis and anchorage independent.

Although as proposed, it was not our intent to carry out a detailed investigation of the phenotypes that result from expression of a particular hairpin. It should be clear that the information gained in this investigation can hopefully be used in further studies. It is possible, for example, to carry out a functional genomic analysis using RNAi in breast cancer cell lines of potential relationships between the HSP70, PPARBP-PBP and NR1D1-RevErbα genes. In this way we can study in depth the pathways that are implicated as well as their relationship to the breast tumor progression.

Key Research Accomplishments

- Adopted a new vector and shRNA production methodology
- Created a set of encoded hairpins targeted against genes overexpressed in ErbB-2 positive breast tumors
- Developed a relatively rapid and reliable method for quantification of proliferation changes due to shRNA expression in cells
- **Identified approximately 30 genes that negatively impact the growth of Erb-B2 positive breast cancer cells**

Reportable Outcomes

Manuscripts:

Baroni, T.E., Lastro, M.T., Ranganathan, A.C., Tenenbaum, S.A., Conklin, D.S. and Aguirre-Ghiso, J.A., 2005 Combinatorial ribonomics and shRNA gene targeting to investigate functional gene programs ***Methods in Molecular Biology*** *in press*

Abstracts:

Functional Genomic Analysis of Breast Cancer Cell Tumorigenicity

Antonios Kourtidis, Matthew Curley and Douglas S. Conklin
Gen*NY*Sis Center for Excellence in Cancer Genomics
University at Albany, State University of New York
dconklin@albany.edu

The recent explosion of genome sequences and bioinformatic data has changed the nature of mammalian genetics. Systematic investigation of expression patterns of thousands of genes in breast tumors using cDNA microarrays and serial analysis of gene expression has yielded a long list of genes whose increased activity is correlated with the occurrence of breast cancer. In notable cases, associations between gene expression levels and clinical outcome have been determined. In most cases, however, the information is only correlative and serves as a rough guide to genes that might positively influence some aspect of breast cancer.

We are working towards identifying which of the nearly 100 genes typically overexpressed in ErbB-2 positive breast tumors are important for the survival and aggressiveness of breast cancer cells. Our approach makes use of DNA-encoded short hairpin RNAs that elicit an RNA interference silencing of the overexpressed genes. Using high throughput methodologies, we have constructed a library of over 20,000 sequence-verified hairpins. These library constructs target approximately 10,000 human genes which encode all of the protein classes that are viewed to be accessible to medicinal chemistry. More importantly, the majority of sequences identified in ErbB-2 positive breast cancer molecular profiling experiments are also represented in the library. Constructs from the library are being used for the systematic validation of breast cancer molecular profiling data in ErbB-2 positive BT 474 cells. Cellular correlates of tumorigenicity such as proliferation rate, resistance to apoptosis and anchorage independent growth are assayed in cells containing shRNA constructs that target overexpressed genes.

The overall objective of this proposal is to identify hairpins that have inhibitory effects on breast cancer progression in *in vivo* models. This would add to our understanding of the genes involved in breast cancer and identify their products as potential small molecule therapeutic targets. It would also pave the way for testing of the hairpins that we identify as gene therapeutics.

The U.S. Army Medical and Materiel Command under W81XWH- 04-1-0474 supported this work.

Presentations:

Genetic screening in mammalian cells, (invited presentation), Roswell Park Cancer Institute, April 2005

shRNA libraries in cancer target discovery, (invited presentation) RNAi in vivo, Marcus Evans, London, April 2005

Genetic screening in mammalian cells, (invited presentation), In vitro screens in drug metabolism, Cambridge Health Inc., Orlando, December 2004

RNAi-based mammalian functional genomics, (invited presentation), Taconic Laboratories Inc, October 2004

RNAi-based mammalian functional genomics, (invited presentation), University at Albany, Dept of Biological Sciences, September 2004

Development of cell lines, tissue or serum repositories:

As was proposed, the collection of shRNA constructs targeting overexpressed genes that contribute to breast cancer tumorigenicity will undoubtedly be useful to many investigators and will be made available as soon as testing is complete.

Funding applied for based on work supported by this award:

None. With further progress it is hoped that sufficient data will be available for submission of an RO1.

Employment or research opportunities applied for and/or received based on experience/training supported by this award:

This award enabled the PI and a post doctoral fellow to attend the Era of Hope Meeting in Philadelphia. Several potential collaborative projects were initiated at this meeting.

Conclusions

Year 1 of funding has resulted in the collation of a large set of constructs targeting genes overexpressed in ErbB-2 positive breast tumors. We have also developed a relatively rapid and reliable method for quantification of proliferation changes due to shRNA expression in cells. Using this we have identified approximately 30 genes that negatively impact the growth of Erb-B2 positive breast cancer cells.

Little has changed with respect to Year 2 Proposed Work. Those constructs not yet tested for effects on proliferation will be tested in the proliferation assay. All shRNA constructs that have tested positively will also be tested for similar effects in human mammary epithelial cells. Constructs that are without effect on HMECs but that inhibit the growth of tumor cells will be tested in mouse models as proposed. In addition, the available hairpin constructs will be packaged in amphotropic retroviral packaging cells. Retroviral supernatants will be used to infect BT474 cells. Infected populations will be monitored for phenotypic changes related to tumorigenic potential including apoptosis and growth in soft agar.

"So what?" one might ask.

Although we are still determining the effect that suppressing the expression of these genes has on the tumorigenicity of breast cancer cells, that we have identified a large number of RNAi constructs that inhibit breast tumor cell proliferation is encouraging. With further study it is hoped that this ultimately will add to our understanding of the genes encoding potential small molecule therapeutic targets in breast and other cancers. It should also pave the way for testing of the hairpins that we identify as gene therapeutics.

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Appendix

List of all shRNA constructs targeting genes overexpressed in erb-B2 breast cancer:

Current Accession	Name
BC043350	Protein kinase C, delta
NM_000125	Estrogen receptor 1
NM_000125	Estrogen receptor 1
NM_000212	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
NM_000212	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
NM_000224	Keratin 18
NM_000419	Integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)
NM_000442	Platelet/endothelial cell adhesion molecule (CD31 antigen)
NM_000442	Platelet/endothelial cell adhesion molecule (CD31 antigen)
NM_000595	Calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha
NM_001026	Ribosomal protein S24
NM_001026	Ribosomal protein S24
NM_001216	Carbonic anhydrase IX
NM_001311	Cysteine-rich protein 1 (intestinal)
NM_001408	
NM_001408	Actin related protein 2/3 complex, subunit 5-like
NM_001408	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)
NM_001429	
NM_001903	Catenin (cadherin-associated protein), alpha 1, 102kDa
NM_001982	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
NM_001982	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
NM_002014	
NM_002014	FK506 binding protein 4, 59kDa
NM_002014	FK506 binding protein 4, 59kDa
NM_002203	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
NM_002203	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
NM_002203	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
NM_002266	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
NM_002266	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
NM_002317	Lysyl oxidase
NM_002353	Tumor-associated calcium signal transducer 2
NM_002353	Tumor-associated calcium signal transducer 2
NM_002411	Secretoglobin, family 2A, member 2
NM_002417	Antigen identified by monoclonal antibody Ki-67
NM_002417	Antigen identified by monoclonal antibody Ki-67
NM_002417	Antigen identified by monoclonal antibody Ki-67
NM_002417	Antigen identified by monoclonal antibody Ki-67
NM_002421	Matrix metalloproteinase 1 (interstitial collagenase)
NM_002421	Matrix metalloproteinase 1 (interstitial collagenase)

NM_002585	Pre-B-cell leukemia transcription factor 1
NM_002585	Pre-B-cell leukemia transcription factor 1
NM_002758	Mitogen-activated protein kinase kinase 6
NM_002758	Mitogen-activated protein kinase kinase 6
NM_002758	Mitogen-activated protein kinase kinase 6
NM_002758	Mitogen-activated protein kinase kinase 6
NM_002758	Mitogen-activated protein kinase kinase 6
NM_002791	Proteasome (prosome, macropain) subunit, alpha type, 6
NM_002795	Proteasome (prosome, macropain) subunit, beta type, 3
NM_002795	Proteasome (prosome, macropain) subunit, beta type, 3
NM_003651	Cold shock domain protein A
NM_003651	Cold shock domain protein A
NM_003651	Cold shock domain protein A
NM_003710	Serine protease inhibitor, Kunitz type 1
NM_003909	Copine III
NM_003909	Copine III
NM_003909	Copine III
NM_003909	Copine III
NM_004265	Fatty acid desaturase 2
NM_004265	Fatty acid desaturase 2
NM_004448	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2,
NM_004483	Glycine cleavage system protein H (aminomethyl carrier)
NM_004483	Glycine cleavage system protein H (aminomethyl carrier)
NM_004483	Glycine cleavage system protein H (aminomethyl carrier)
NM_004496	Forkhead box A1
NM_004496	Forkhead box A1
NM_004496	Forkhead box A1
NM_004643	Poly(A) binding protein, nuclear 1
NM_004643	Poly(A) binding protein, nuclear 1
NM_004747	Discs, large homolog 5 (Drosophila)
NM_004774	PPAR binding protein
NM_004774	PPAR binding protein
NM_004774	PPAR binding protein
NM_004888	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
NM_004888	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
NM_004888	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
NM_004888	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
NM_004888	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
NM_004893	H2A histone family, member Y
NM_004893	H2A histone family, member Y
NM_004901	Ectonucleoside triphosphate diphosphohydrolase 4
NM_004901	Ectonucleoside triphosphate diphosphohydrolase 4
NM_004933	Cadherin 15, M-cadherin (myotubule)
NM_004933	Cadherin 15, M-cadherin (myotubule)
NM_005005	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa
NM_005015	Oxidase (cytochrome c) assembly 1-like
NM_005015	Oxidase (cytochrome c) assembly 1-like
NM_005030	Polo-like kinase 1 (Drosophila)

NM_005030	Polo-like kinase 1 (Drosophila)
NM_005030	Polo-like kinase 1 (Drosophila)
NM_005079	Tumor protein D52
NM_005079	Tumor protein D52
NM_005213	Cystatin A (stefin A)
NM_005213	Cystatin A (stefin A)
NM_005213	Cystatin A (stefin A)
NM_005213	Cystatin A (stefin A)
NM_005213	Cystatin A (stefin A)
NM_005213	Cystatin A (stefin A)
NM_005228	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog,
NM_005228	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog,
NM_005310	Growth factor receptor-bound protein 7
NM_005324	H3 histone, family 3B (H3.3B)
NM_005324	H3 histone, family 3B (H3.3B)
NM_005324	H3 histone, family 3B (H3.3B)
NM_005324	H3 histone, family 3B (H3.3B)
NM_005348	Heat shock 90kDa protein 1, alpha
NM_005556	Keratin 7
NM_005556	Keratin 7
NM_005570	Lectin, mannose-binding, 1
NM_005570	Lectin, mannose-binding, 1
NM_005570	Lectin, mannose-binding, 1
NM_005570	Lectin, mannose-binding, 1
NM_005574	LIM domain only 2 (rhombotin-like 1)
NM_005574	LIM domain only 2 (rhombotin-like 1)
NM_005574	LIM domain only 2 (rhombotin-like 1)
NM_005652	Telomeric repeat binding factor 2
NM_005652	Telomeric repeat binding factor 2
NM_005652	Telomeric repeat binding factor 2
NM_005765	ATPase, H+ transporting, lysosomal accessory protein 2
NM_005765	ATPase, H+ transporting, lysosomal accessory protein 2
NM_005765	ATPase, H+ transporting, lysosomal accessory protein 2
NM_005885	Membrane-associated RING-CH protein VI
NM_005885	Membrane-associated RING-CH protein VI
NM_005885	Membrane-associated RING-CH protein VI
NM_005930	CTAGE family, member 5
NM_006148	LIM and SH3 protein 1
NM_006148	LIM and SH3 protein 1
NM_006148	LIM and SH3 protein 1
NM_006148	LIM and SH3 protein 1
NM_006148	LIM and SH3 protein 1
NM_006148	LIM and SH3 protein 1
NM_006254	Protein kinase C, delta
NM_006265	RAD21 homolog (S. pombe)
NM_006265	RAD21 homolog (S. pombe)
NM_006265	RAD21 homolog (S. pombe)
NM_006265	RAD21 homolog (S. pombe)

NM_006276	Splicing factor, arginine/serine-rich 7, 35kDa
NM_006276	Splicing factor, arginine/serine-rich 7, 35kDa
NM_006276	Splicing factor, arginine/serine-rich 7, 35kDa
NM_006432	Niemann-Pick disease, type C2
NM_006432	Niemann-Pick disease, type C2
NM_006601	Unactive progesterone receptor, 23 kD
NM_006601	Unactive progesterone receptor, 23 kD
NM_006601	Unactive progesterone receptor, 23 kD
NM_006726	LPS-responsive vesicle trafficking, beach and anchor containing
NM_007067	MYST histone acetyltransferase 2
NM_007067	MYST histone acetyltransferase 2
NM_007067	MYST histone acetyltransferase 2
NM_007246	Kelch-like 2, Mayven (Drosophila)
NM_007246	Kelch-like 2, Mayven (Drosophila)
NM_007246	Kelch-like 2, Mayven (Drosophila)
NM_007355	Heat shock 90kDa protein 1, beta
NM_007355	Heat shock 90kDa protein 1, beta
NM_007355	Heat shock 90kDa protein 1, beta
NM_007355	Heat shock 90kDa protein 1, beta
NM_012229	5'-nucleotidase, cytosolic II
NM_012229	5'-nucleotidase, cytosolic II
NM_012229	5'-nucleotidase, cytosolic II
NM_014399	Transmembrane 4 superfamily member 13
NM_014399	Transmembrane 4 superfamily member 13
NM_016016	CGI-69 protein
NM_017802	Hypothetical protein FLJ20397
NM_018695	Erbb2 interacting protein
NM_018695	Erbb2 interacting protein
NM_018728	Myosin VC
NM_018979	Protein kinase, lysine deficient 1
NM_018979	Protein kinase, lysine deficient 1
NM_018979	Protein kinase, lysine deficient 1
NM_020974	Signal peptide, CUB domain, EGF-like 2
NM_020974	Signal peptide, CUB domain, EGF-like 2
NM_020974	Signal peptide, CUB domain, EGF-like 2
NM_021724	Nuclear receptor subfamily 1, group D, member 1
NM_021980	Optineurin
NM_021980	Optineurin
NM_021980	Optineurin
NM_021980	Optineurin
NM_024101	Melanophilin
NM_024101	Inter-alpha (globulin) inhibitor H2
NM_032308	RPA interacting protein
NM_032308	RPA interacting protein
NM_033418	Hypothetical protein MGC9084
NM_033419	Per1-like domain containing 1
NM_033419	Per1-like domain containing 1
NM_052886	Mal, T-cell differentiation protein 2

NM_080861	SPRY domain-containing SOCS box protein SSB-3
NM_080861	SPRY domain-containing SOCS box protein SSB-3
NM_178516	Hypothetical protein LOC283849
NM_203365	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1
NM_203365	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1