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13. ABSTRACT ***(Maximum 200 Words)***

The representational difference analysis (RDA) technique, and its variant the differential subtraction chain (DSC) technique were attempted on 10 paired samples of microdissected benign and malignant prostate epithelium. The resulting subtraction products were cloned and screened in Southern blots of the initial tester and driver amplicon populations. Truly subtracted products were sequenced and tested for markers of homozygous deletion in the original tissue samples by polymerase chain reaction. No homozygous deletions were found. The research strategy was changed to take advantage of the newly available technology of oligonucleotide microarrays, which have the potential to also achieve the aims of the proposed research. Results of validation studies are given that show that the bulk tissue collected at our institution can identify distinct gene expression clusters between samples of benign and malignant human prostate cancer. Data is given to show that microdissected tissue samples retain RNA of sufficient quality to perform gene expression profiling, and that RNA of the quantity present in microdissected samples can be amplified by an in vitro transcription protocol modified in our laboratory to levels sufficient for microarray analysis.

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Table of Contents

Introduction

The subject of this research is the genetic basis of tumor progression in the human prostate. The purpose is to understand the molecular basis for the etiology of human prostate carcinoma, in the hopes of affecting changes in risk factors that affect this disease, in refining diagnostic tests that can help guide therapy and providing information useful in the rational design of therapeutic agents. The scope of this work entails the procurement of high quality tissue samples of human prostate cancer, microdissecting pure sample of prostate cancer and normal cells from this tissue, the efficient extraction of nucleic acids from these microsamples, and the subsequent assay of these nucleic acids for differences between benign and malignant cells. Our initial evaluation of a technique known as representational difference analysis (RDA) has not proven successful in its application to such samples. We are currently developing techniques to use these microdissected samples on oligonucleotide microarrays, a strategy which should also provide global assays of genetic and transcriptional differences that underlie human prostate cancer.

Body of Summary

Tissue procurement and validation of microdissection/RNA isolation methods

We have continued to collect tissue samples from radical prostatectomy specimens with an enhanced protocol to ensure the best quality tissue in terms of RNA and DNA preservation. In our previous summary we had shown initial validation studies of the histologic and microdissection techniques we employ. We now present additional evidence that these procedures result in the preservation of a rare transcript in human prostate cancer.

Figure 1 shows an example of the precision with which targeted cell types can be collected with microdissection. This example utilizes a hydraulic micromanipulator, but we also have a Pixcell laser capture microdissection apparatus in our laboratory. Both apparatuses are used in cell collection.

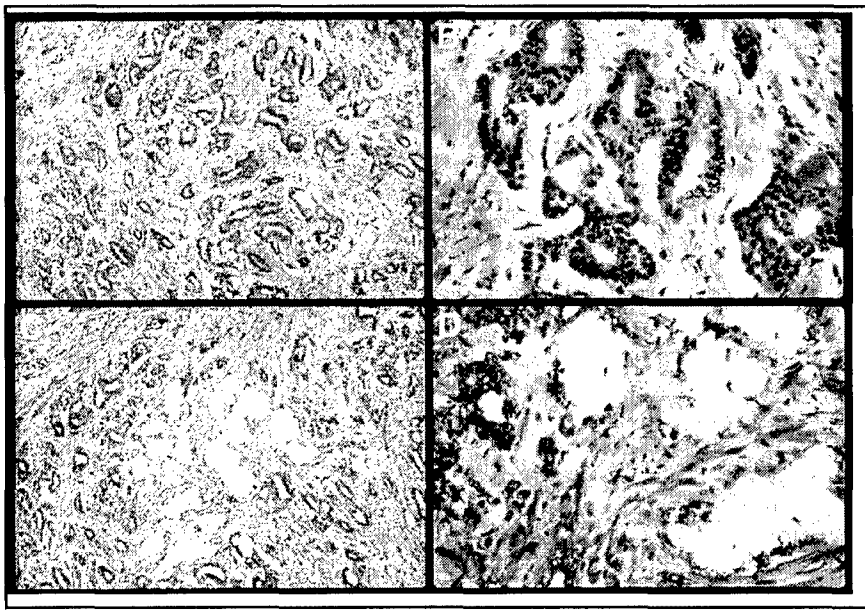

Figure 1. Example of tissue microdissection. Panel A shows a histologic section of Gleason pattern 3 human prostate cancer at 100 x original magnification. Panel B shows a higher magnification of the same section (400 x original magnification). Panel C shows a histologic section adjacent to the one shown in Panels A & B after microdissection had been performed to procure prostate carcinoma cells (100 x original magnification). Panel D shows a higher power of the microdissection (400 x original magnification). Only prostate carcinoma cells were selected by microdissection, surrounding stromal and inflammatory cells remain in the histologic section.

To ensure that microdissection techniques do not compromise the quality of the RNA in these tissue samples, tissue was microdissected using manual, hydraulic micromanipulator and laser capture microdissection methods. A panel of genes was successfully amplified from these samples. Figure 2 shows an example.

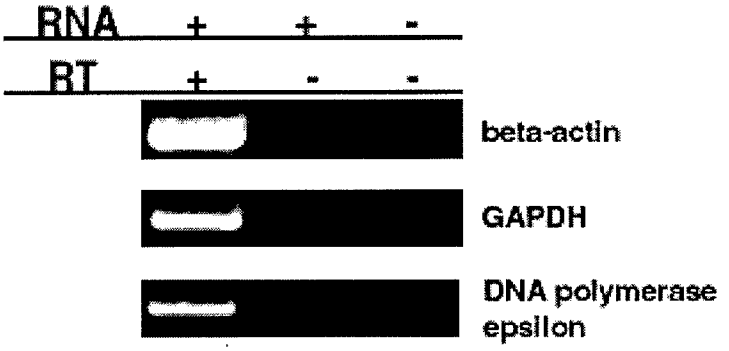

Figure 2. RT-PCR of a microdissected prostate cancer sample . 5000 cells were microdissected from a 14 uM histologic section and subjected to RNA isolation. One aliquot was treated with reverse transcriptase (RT) while the other was not. Samples corresponding to approximately 500 cell input were then subjected to PCR for target housekeeping genes. The left-hand lanes show that the aliquots contain RNA for an abundant transcript (beta-actin, 587 bp), a moderately expressed transcript (GAPDH, 456 bp) and a low abundance transcript (DNA polymerase epsilon, 606 bp). No genomic DNA contaminating the RNA preps is detected (middle lanes). Negative controls for PCR contamination are shown in the right-hand lanes.

Representational difference analysis of primary human prostate cancer

In our previous report we had reported the inadequacy of the originally published RDA protocol to achieve efficient isolation of differences from the small amounts of DNA present in microdissected tissue samples. Using an optimized protocol which we had validated on test samples (described in the previous annual report), we attempted RDA on seven separate samples of microdissected prostate samples. In addition, we tested a newly described variant of RDA known as differential subtraction chain (DSC) analysis on three paired samples (Luo et al., 1999). The resultant subtraction products from these procedures were cloned and used as probes in Southern blots of the initial driver and tester amplicon populations. Probes which were apparently selected from the driver population were sequenced. PCR reactions performed on microdissected samples of the original benign and malignant cell populations failed to show that any of the subtraction products identified homozygous deletions. This effort required the work of two people over a one year period.

New strategy utilizing oligonucleotide microarrays

We are currently uncertain whether the lack of detection of homozygous deletion by the RDA protocol is the result of technical limitations of the RDA procedure or if it reflects a relative lack of such lesions in the organ-confined prostate cancer which is what our tissue bank entirely consists of. Since the initial submission of this grant application, the technology of DNA microarrays has become available to us, and we intend to continue to pursue the aims of this grant combining aspects of our original protocol with the use of Affymetrix GeneChips® and single nucleotide polymorphism (SNP) chips. Microdissected samples of benign prostate epithelium and samples of prostate cancer will have both RNA and DNA isolated. Our plan is to perform both gene expression profiling with the GeneChips® and widespread allelotyping with the SNP chips. The SNP chips detect the presence of polymorphic markers in the test genome, hence can identify loss of a marker denoting deletion of one of the chromosomal pairs. This is akin to the microsatellite allelotype assays currently employed. However, while high density microsatellite allelotyping may require several person-years to perform on a sizeable number of tumor samples, the microarray strategy can yield a high density allelotype in a single overnight hybridization. By combining the results of both the GeneChip® and SNP analysis for an individual tumor, analysis software like GeneSpring can quickly correlate genes that are down-regulated with the physical location of chromosomal deletion. Such a combined analysis may more quickly pinpoint potential tumor suppressor gene candidates in a large area of genetic deletion.

The SNP chip strategy may also be capable of detecting homozygous deletions, and has the advantage of speed over the RDA procedure. The RDA procedure that we have employed requires approximately two months of work to obtain subtraction products which must then be screened as markers of homozygous deletion. The detection of a lack of hybridization from the tile of probes for a SNP marker in a tumor sample compared to the tumor, particular if this occurs in a series of physically linked SNPs, will denote the presence of a homozygous deletion.

Affymetrix gene chip analysis of prostate cancer specimens

In a collaboration with Dr. Henry Frierson of the Dept. of Pathology at the University of Virginia and

with members of the Novartis Research Foundation, gene expression profiling of 24 primary samples of prostate cancer from our tissue bank was performed on Affymetrix GeneChips®. The tissues selected for analysis had been examined by cryostat histologic sectioning. Areas containing cancer were trimmed to contain no less than 70% carcinoma cells relative to surrounding benign cellular constituents. The results of the hybridization pattern after hierarchical clustering are shown in the figure in the Appendix. There were clusters of gene expression that clearly separated samples of prostate cancer from those of benign prostate tissue. However, patterns of gene expression were present in the tumor samples that would have been expected in stromal and inflammatory cells. The confounding effect that this degree of tissue heterogeneity has on any specific gene probe is unknown at this point. These results show the utility of this technique in identifying gene expression that differs between neoplastic and non-neoplastic prostate epithelial cells, but indicates the potential that microdissection has for obtaining more uniform cell populations for study.

Amplification of RNA isolated from microdissected samples

In order to use microdissected tissue samples in microarray analysis, the RNA present in the original samples will have to be linearly amplified to microgram quantities. We have optimized a modified procedure based on the "aRNA" procedure developed by Eberwine and colleagues (Van Gelder et al., 1990). The protocol is present in the Appendix. Using this protocol we have been able to amplify the RNA present in aliquots of 10,000 microdissected cells to 20 ug, and aliquots of 100 ng of purified RNA to 8-10 ug after 2 rounds of amplification. We have just submitted some of these amplified RNA pools for microarray hybridization, and will compare the results with those of non-amplified RNA present in the original pool. The hybridization intensity for each microarray probe will be plotted among samples to observe the linearity of results among samples (Wang et al., 2000). This type of analysis will be performed to determine the optimal number of cells to microdissect and the maximum number of amplification rounds allowed in order to obtain no detectable shifts in transcriptional representation due to amplification.

Example of screening and validation methodologies

Validation of differentially expressed genes will be carried out initially by RT-PCR assays of microdissected specimens. To verify that RT-PCR of microdissected tissue samples as a validation method for the candidate differentially-expressed genes identified in this proposal, 500 cells each of benign prostate epithelium and prostate cancer were microdissected from another of the archived frozen tissue samples. After RNA isolation and cDNA synthesis, half of each aliquot (250 cell equivalents) was subjected to duplex PCR for GAPDH (a control housekeeping gene) and GSTπ, an enzyme that has previously shown to be down regulated in prostate cancer relative to normal prostate epithelium (Lee et al., 1994; Moskaluk et al., 1997). Figure 3 shows that this strategy successfully showed the GSTπ transcript is greatly diminished in the cancer specimen relative to normal epithelium.

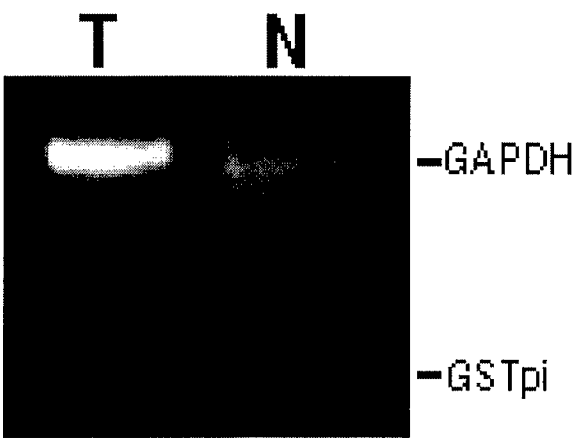

Figure 3 . Differential gene expression of GSTπ identified in semiquantitative RT-PCR amplifications of microdissected prostate tissue. The figure shows a gel electrophoresis of duplex RT-PCR products. The upper product corresponds to GAPDH (456 bp) which serves as a relative control for the amount of input RNA. the lower band corresponds to GSTπ (150 bp), an enzyme present in benign prostate epithelium, but which is known to be down-regulated in most prostatic carcinomas. The T lane corresponds to 250 prostate cancer cell equivalents assayed. the N lane corresponds to 250 cell equivalents of benign prostate epithelium obtained from the same patient. Although in this example the normal sample did not amplify as well as the tumor sample, the GSTπ product is clearly absent from the tumor sample.

Larger scale screening assays to determine the clinical implication of differential gene expression will be carried out by immunohistochemical staining of a large number of clinical specimens. In collaboration with Dr. Henry Frierson in the Department of Pathology at UVA, technicians have been trained to create tissue microarrays on a manual tissue puncher/arrayer (Beecher Instruments, Silver Spring, MD) that belongs to the UVA Department of Pathology (see letter in Appendix). Figure 4 shows an example of a microarray made from archival pathology paraffin blocks of prostate tissue at UVA. It contains 100 separate tissue samples of benign prostate glands, prostatic intraepithelial neoplasia, organ-confined prostate cancer (well, moderately and poorly differentiated) and metastatic prostate cancer. Although this particular array was created to support other research projects, Dr. Frierson has agreed to provide sections sufficient to perform pilot studies, and will provide the original paraffin blocks to create more tissue microarrays.

A search of records at the UVA Tumor registry (see Research Methods) yielded over 4,000 patients with prostate cancer seen at the University of Virginia Health System during the last 75 years. For the years 1990-1998, 470 patients with prostate cancer have undergone radical prostatectomy procedures performed at UVA. All of these cases have paraffin blocks on file in the archives of the Pathology Dept., and are available for the construction of tissue microarrays.

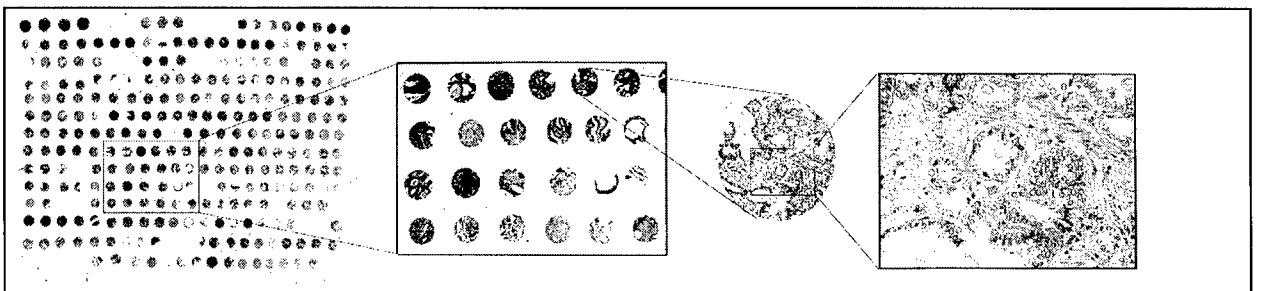

Figure 4. Existing prostate cancer tissue microarray at UVA. The figure to the left shows the entire microarray, with increasing magnification of selected areas going from left to right.

Key research accomplishments

-Optimized tissue procurement procedures resulting in samples of human prostate cancer with high quality DNA and RNA populations

-Optimized RNA isolation and RT-PCR protocols that allow the identification of rare mRNA transcripts from small microdissected samples of human prostate cells

-Optimization of RNA amplification procedures to allow microdissected prostate cancer samples to be used on oligonucleotide microarrays

Reportable outcomes

A grant application to continue and extend this work has been submitted to the National Institutes of Health in response to the National Cancer Institute PAR-99-102 (Applications of innovative technologies for the molecular analysis of cancer). The grant application is entitled

"Differential Gene Expression in Primary Prostate Cancer".

Conclusions

Currently, the discovery of gene expression correlated with the tumor progression in human prostate is an active area of research, but is hampered by the lack of samples of prostate tissue which are of sufficient size and quality to perform such analyses, and by the confounding effects of non-neoplastic cells which "contaminate" such samples of prostate cancer. The microdissection/amplification methodology that we are developing require a much lower number of neoplastic cells to profile, and will allow a greater number of samples to be analyzed in such a manner. In addition, the use of pure samples of prostate cancer will remove the artifacts associated with cell culture, epithelial extraction procedures, and contaminating non-cancer tissue from such analyses. Our use of the representational difference analysis procedure on such samples showed that this is not an efficient technology in screening large numbers of cases for relatively rare events. However, the adaptation of the microdissection and nucleic acid amplification techniques to the emerging microarray technologies shows great promise as an efficient screening procedure for studies utilizing larger numbers of samples.

Eventually even the material present in small needle biopsies of the prostate or metastatic tumors may be amenable to such global assays of gene expression and genetic deletion. As the diagnostic, prognostic and therapeutic implications of gene expression and genetic deletions in prostate cancer become better understood, the ability to assay such minimal tissue samples will be crucial to the translation of this research to clinical care.

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Appendices

1. Protocol for *in vitro* RNA amplification procedure
2. Figure showing gene clusters and dendogram analysis of prostate cancer gene expression profiling study

RNA amplification protocol

cDNA Synthesis Round 1 from total RNA

3. Mix total RNA sample with T7-polydT primer as follows:

100 ng total RNA in DEPC-treated dH_2O	9.0 µL
1 µg/µL glycogen carrier	1.0 µL
100 µM T7-polydT primer	1.0 µL
total volume	11.0 µL

4. Incubate at 70 °C for 10 minutes and then at 42 °C for 5 minutes.
5. Add the following to the above mixture in the order specified:

RNasin	1.0 µL
5X First Strand Buffer	4.0 µL
10mM dNTPs	1.0 µL
2 µL 0.1M DTT	2.0 µL
Superscript II	1.0 µL
total volume	20.0 µL

   Mix and incubate at 42 °C for 1 hour.
6. Add the following to the first strand reaction for second strand cDNA synthesis:

5X Second Strand Buffer	30.0 µL
10mM dNTPs	3.0 µL
l DNA polymerase I	4.0 µL
l DNA ligase	1.0 µL
RNase H	1.0 µL
DEPC-treated dH_2O	92.0 µL
total volume	151.0 µL

   Mix and incubate at 16 °C for 2 hours.
7. Add 2.0 µL of T4 DNA polymerase and incubate at 16 °C for 10 minutes.
8. Use Qiagen MiniElute PCR Purification Kit for cleanup. If using the IEC Micromax refrigerated centrifuge for this procedure, do not exceed 12,000 RPM, since this equipment produces very high RCF.
   a) Add 4 volumes of Buffer PB(600 µL) to 1 volume of reaction (~150 µL).
   b) Mix and transfer to a Qiagen MiniElute column.
   c) Place the column in the provided 2 mL catchtube. Spin at miximum speed for 1 minute.
   d) Discard the flow through and place the column back in the same catch tube.
   e) Add 750 µL of Buffer PE to the column and spin at maximum speed for 1 minute.
   f) Again, discard the flow through and place the column back in the same catch tube.
   g) Centrifuge for 1 minute additional at maximum speed to complete wash buffer removal.
   h) Transfer the column to a clean 1.5 mL tube and elute using 10 µL Buffer EB. Pipette the buffer directly onto the center of the filter membrane. Allow the column to stand at RT for 1 min and then spin for 1 minute at maximum speed.
7. Samples may be held at –20°C after this step is completed.

T7-In Vitro Transciption (Epicentre Ampliscribe Kit)

1. Adjust the volume of the double stranded cDNA prepared above to 8.0 µL (correct using DEPC-treated dH_2O), then add the following in the order specified:

10X T7 Ampliscribe Reaction Buffer	2.0 µL
100 mM ATP	1.5 µL
100 mM CTP	1.5 µL
100 mM GTP	1.5 µL
100 mM UTP	1.5 µL
100 mM DTT	2.0 µL
Ampliscribe T7 Enzyme Mix	2.0 µL
total volume	20.0 µL

   Mix and incubate at 37 °C for 3 hours.
2. Purify using Qiagen RNeasy columns. If using the IEC Micromax refrigerated centrifuge for this procedure, do not exceed 12,000 RPM, since this equipment produces very high RCF.
   a) Add 80 µL water to bring reaction volume to 100 µL.
   b) Add 350 µL lysis buffer (prepare an aliquot by adding 10 µL β-mercaptoethanol per 1 mL of lysis buffer; stable at 4°C for 30 days).
   c) Add 250 µL absolute ethanol. Mix and transfer to a RNeasy column.
   d) Spin at max speed for 15 seconds.
   e) Wash once with 500 µL of Wash Buffer RPE and spin at max speed for 2 minutes.
   f) Transfer column to a new 1.5 mL microcentrifuge tube and elute with 50 µL of water. Add the water directly to the center of the membrane and spin at max speed for 1 min.
3. Speed-vac until 5-10 µL volume remain (approx. 20-25 minutes). Samples may be held at –70°C prior to this step or after it is completed.

cDNA synthesis Round 2 from Amplified RNA

1. Adjust volume of cRNA from above to 10.0 µL with DEPC-treated dH_2O as necessary, then add 1.0 µL of 1 µg/µL random hexamer primers (total volume 11.0 µL).
2. Incubate at 70 °C for 10 minutes. Chill on ice, then incubate at room temperature for 10 minutes. Spin down briefly.
3. Add the following to the mixture above in the order specified:

RNasin	1.0 µL
5X First Strand Buffer	4.0 µL
10 mM dNTPs	1.0 µL
100 mM DTT	2.0 µL
Superscript II	1.0 µL
total volume	20.0 µL

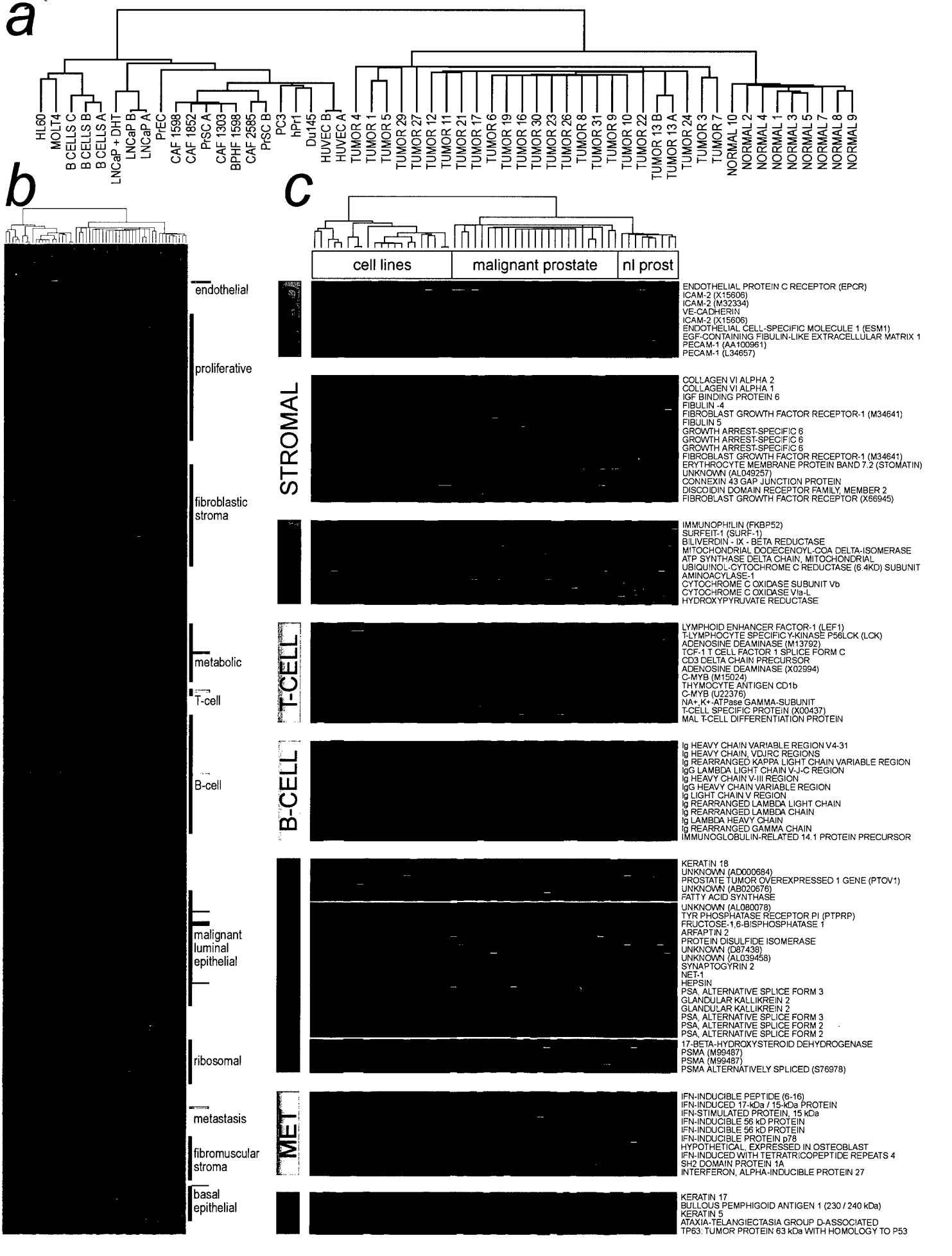
   Mix and incubate at 37 °C for 1 hour.
4. Add 1 µL of RNase H and incubate for 20 minutes at 37 °C.
5. Heat at 94 °C for 2 minutes and chill on ice. Spin down briefly and correct the volume to 21.0 µL with DEPC-treated dH_2O as necessary.

6. Add 1.0 µL of 100 µM T7-polydT primer. Incubate at 70° C for 5 minutes and then 42°C for 10 minutes.
7. Add the following for second strand synthesis:

5X Second Strand Buffer	30.0 µL
10 mM dNTPs	3.0 µL
DNA polymerase I	4.0 µL
RNase H	1.0 µL
DEPC-treated dH_2O	92.0 µL
total volume	150.0 µL

   Mix and incubate at 16 °C for 2 hours.
8. Add 2.0 µL of T4 DNA polymerase and incubate at 16 °C for 10 minutes.
9. Repeat the MiniElute PCR Purification protocol for cleanup as above
10. For additional rounds of amplification, repeat the T7 RNA synthesis.

Figure legend: Expression of 3,530 genes in 55 experimental samples. Rows represent individual genes; columns represent individual samples. The samples included prostate cancer cell lines PC3, LNCaP, and DU145, fibroblastic cell strains CAF 1598, CAF 1303, CAF 1852, CAF 2585 which were expanded from fragments of prostates removed for adenocarcinoma, fibroblastic cell line BPHF 1598 propagated from fragments of prostate tissue that contained benign hypertrophic glands, and normal prostate stromal cells (PrSC) A and B [Clonetics (San Diego, California)]. Primary tissue included 24 primary cancer specimens, one lymph node metastasis and 9 samples of benign prostate. Messenger RNAs from T-lymphoblastic MOLT4 and myeloleukemic HL60 cells were purchased from Clontech (Palo Alto, California) and RNA from endothelial cells and activated B-cells were gifts from Drs. Akira Kawamura (The Scripps Research Institute, San Diego, California) and Michael Cooke (Novartis GNF), respectively. Labeled complementary RNA (cRNA) was prepared and hybridized to high-density oligonucleotide arrays containing 12,626 probe sets representing > 12000 human genes ("U95a," Affymetrix, Santa Clara, California) as previously described [Wodicka, 1997 #821]. Each cell in the matrix represents the expression level of a single transcript in a single sample, with red and green indicating transcript levels above and below the median for that gene across all samples, respectively. Color saturation is proportional to magnitude of the difference from the mean. ***a***, dendrogram of samples showing overall similarity in gene expression profiles across the samples. ***b***, demonstration of overall groupings of genes and samples. Colored bars to the right indicate gene clusters of special interest. ***c***, enlarged view of selected clusters of genes .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HL60
MOLT4
B CELLS C
B CELLS B
B CELLS A
LNCaP + DHT
LNCaP B
LNCaP A
PrEC
CAF 1598
CAF 1852
PrSC A
CAF 1303
BPHF 1598
CAF 2585
PrSC B
PC3
hPr1
Du145
HUVEC B
HUVEC A
TUMOR 4
TUMOR 1
TUMOR 5
TUMOR 29
TUMOR 27
TUMOR 12
TUMOR 11
TUMOR 21
TUMOR 17
TUMOR 6
TUMOR 19
TUMOR 16
TUMOR 30
TUMOR 23
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TUMOR 8
TUMOR 31
TUMOR 9
TUMOR 10
TUMOR 22
TUMOR 13 B
TUMOR 13 A
TUMOR 24
TUMOR 3
TUMOR 7
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NORMAL 2
NORMAL 4
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NORMAL 3
NORMAL 5
NORMAL 7
NORMAL 8
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malignant luminal epithelial
ribosomal
metastasis
fibromuscular stroma
basal epithelial
c
cell lines
malignant prostate
nl prost
STROMAL
T-CELL
B-CELL
MET
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ICAM-2 (X15606)
ICAM-2 (M32334)
VE-CADHERIN
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COLLAGEN VI ALPHA 2
COLLAGEN VI ALPHA 1
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FIBULIN 5
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UNKNOWN (AL049257)
CONNEXIN 43 GAP JUNCTION PROTEIN
DISCOIDIN DOMAIN RECEPTOR FAMILY, MEMBER 2
FIBROBLAST GROWTH FACTOR RECEPTOR (X66945)
IMMUNOPHILIN (FKBP52)
SURFEIT-1 (SURF-1)
BILIVERDIN - IX - BETA REDUCTASE
MITOCHONDRIAL DODECENOYL-COA DELTA-ISOMERASE
ATP SYNTHASE DELTA CHAIN, MITOCHONDRIAL
UBIQUINOL-CYTOCHROME C REDUCTASE (6.4KD) SUBUNIT
AMINOACYLASE-1
CYTOCHROME C OXIDASE SUBUNIT Vb
CYTOCHROME C OXIDASE VIa-L
HYDROXYPYRUVATE REDUCTASE
LYMPHOID ENHANCER FACTOR-1 (LEF1)
T-LYMPHOCYTE SPECIFIC Y-KINASE P56LCK (LCK)
ADENOSINE DEAMINASE (M13792)
TCF-1 T CELL FACTOR 1 SPLICE FORM C
CD3 DELTA CHAIN PRECURSOR
ADENOSINE DEAMINASE (X02994)
C-MYB (M15024)
THYMOCYTE ANTIGEN CD1b
C-MYB (U22376)
NA+,K+-ATPase GAMMA-SUBUNIT
T-CELL SPECIFIC PROTEIN (X00437)
MAL T-CELL DIFFERENTIATION PROTEIN
Ig HEAVY CHAIN VARIABLE REGION V4-31
Ig HEAVY CHAIN, VDJRC REGIONS
Ig REARRANGED KAPPA LIGHT CHAIN VARIABLE REGION
IgG LAMBDA LIGHT CHAIN V-J-C REGION
Ig HEAVY CHAIN V-III REGION
IgG HEAVY CHAIN VARIABLE REGION
Ig LIGHT CHAIN V REGION
Ig REARRANGED LAMBDA LIGHT CHAIN
Ig REARRANGED LAMBDA CHAIN
Ig LAMBDA HEAVY CHAIN
Ig REARRANGED GAMMA CHAIN
IMMUNOGLOBULIN-RELATED 14.1 PROTEIN PRECURSOR
KERATIN 18
UNKNOWN (AD000684)
PROSTATE TUMOR OVEREXPRESSED 1 GENE (PTOV1)
UNKNOWN (AB020676)
FATTY ACID SYNTHASE
UNKNOWN (AL080078)
TYR PHOSPHATASE RECEPTOR PI (PTPRP)
FRUCTOSE-1,6-BISPHOSPHATASE 1
ARFAPTIN 2
PROTEIN DISULFIDE ISOMERASE
UNKNOWN (D87438)
UNKNOWN (AL039458)
SYNAPTOGYRIN 2
NET-1
HEPSIN
PSA, ALTERNATIVE SPLICE FORM 3
GLANDULAR KALLIKREIN 2
GLANDULAR KALLIKREIN 2
PSA, ALTERNATIVE SPLICE FORM 3
PSA, ALTERNATIVE SPLICE FORM 2
PSA, ALTERNATIVE SPLICE FORM 2
17-BETA-HYDROXYSTEROID DEHYDROGENASE
PSMA (M99487)
PSMA (M99487)
PSMA ALTERNATIVELY SPLICED (S76978)
IFN-INDUCIBLE PEPTIDE (6-16)
IFN-INDUCED 17-kDa / 15-kDa PROTEIN
IFN-STIMULATED PROTEIN, 15 kDa
IFN-INDUCIBLE 56 kD PROTEIN
IFN-INDUCIBLE 56 kD PROTEIN
IFN-INDUCIBLE PROTEIN p78
HYPOTHETICAL, EXPRESSED IN OSTEOBLAST
IFN-INDUCED WITH TETRATRICOPEPTIDE REPEATS 4
SH2 DOMAIN PROTEIN 1A
INTERFERON, ALPHA-INDUCIBLE PROTEIN 27
KERATIN 17
BULLOUS PEMPHIGOID ANTIGEN 1 (230 / 240 kDa)
KERATIN 5
ATAXIA-TELANGIECTASIA GROUP D-ASSOCIATED
TP63: TUMOR PROTEIN 63 kDa WITH HOMOLOGY TO P53

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

MCMR-RMI-S (70-1y) 15 May 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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