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Lobe-specific proteome changes in the dorsal-lateral and ventral prostate of TRAMP mice vs. wild type mice

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Abstract

The transgenic adenocarcinoma of mouse prostate (TRAMP) is the most widely used transgenic model for prostate cancer chemoprevention studies. Although two lobe-specific lineages of carcinogenesis have been described, the molecular mechanisms are still poorly defined. Here, we concurrently profiled the proteome of dorsal-lateral (DLP) and ventral (VP) prostate lobes of both TRAMP and littermate wild type C57BL/6 mice of 18 weeks by 2D-LC-MALDI-TOF/TOF with iTRAQ labeling. A total of 483 and 748 proteins were identified at critical false discovery rates of 1% and 5%. In wild-type mice, 84 proteins were found to have different expression levels between DLP and VP. In TRAMP mice, 118 proteins significantly changed in DLP and/or VP during TRAMP carcinogenesis. Among them, 55 and 36 proteins were uniquely changed in DLP or VP lobe, respectively and 27 proteins in both DLP and LP lobe. Ingenuity Pathway Analysis (IPA) was able to segregate proteins changed in two lobes into different pathway networks. In addition to serving as reference for prostate proteomic profiles, our data suggest that different sets of proteins are involved in the carcinogenesis in DLP vs. in VP in the TRAMP model.

Keywords

iTRAQ; lobe; prostate cancer; proteomics; TRAMP mouse

The transgenic adenocarcinoma of mouse prostate (TRAMP) model was originally developed by Dr. Greenberg in 1995 [1]. This model, to some extent, mimics the natural history and progression of human prostate cancer (PCa) and the breeding scheme is straightforward. It is by far the most widely used model for PCa chemoprevention studies. Current understanding about this model is that TRAMP represents at least two models of prostate carcinogenesis: the AR/probasin promoter/T-Ag-mediated glandular prostate epithelial cancer formation in the dorsal-lateral prostate (DLP) and the development of neuroendocrine (NE)-carcinomas in the ventral prostate (VP) which is T-Ag driven but independent of AR [2]. The latter is thought to be important for human PCa in that the number of NE-cells correlates with the stage, Gleason grades and survival in castration-recurrent PCa in clinical cases [3]. Given the biological differences of these two lineages of

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carcinogenesis, their molecular pathways merit thorough studies. The information on the lobe-specific global protein expressions during TRAMP carcinogenesis can provide insights into mechanism studies of prostate carcinogenesis and development of targeted chemopreventive agents. We profiled the proteomes of the DLP and VP lobes of both TRAMP and their littermate wild-type mice. Here we present sets of lobe-specific proteins in wild-type mice and differentially expressed proteins during carcinogenesis.

The animal protocol was approved by the University of Minnesota IACUC committee. Eleven TRAMP and nine littermate wild-type mice at 18 weeks of age were sacrificed and the DLP and VP were further dissected on ice. Histopathology of the prostates has been described by our group previously [4]. Proteins were extracted from pooled prostate lobes and digested to peptides as reported [5]. The peptides were then labeled with iTRAQTM reagents, tags 114, 115, 116 and 117 were used to label the proteins extracted from wild type DLP, TRAMP DLP, wild type VP and TRAMP VP groups, respectively. Labeled samples were mixed, cleaned up by solid phase extraction with MCX cartridge and analyzed at the Mass Spectrometry and Proteomics facility at the University of Minnesota [6]. Labeled peptide mixture was first fractionated by strong-cation-exchange chromatography and all 14 fractions collected were subjected to capillary-LC with in-house packed columns with Magic C18AQ (Michrom BioResources, Auburn, CA). The LC elutes was spotted onto MALDI targets in a 1232-spot format. Spotting interval was 24 seconds and the plate voltage during spotting was 2.8 kV. The MS data on all LC spots were acquired on a 4800 MALDI-TOF/TOFTM analyzer (AB/MDS) with a 200 Hz repetition rate Nd:YAG laser. Full scan MS spectra were acquired from 800 – 4000 m/z. Data dependent tandem MS settings included acquisition of the top 30 most intense ion signals per spot.

ProteinPilotTM 3.0 software was used for data processing. The search was performed against NCBI database (version 2009-07)[5]. The quality of protein identification was further assessed by false-discovery-rate-analysis (FDR) and a 1% global FDR was set as a cut-off value for proteins subjected to quantization. Proteins identified as differentially expressed with $p < 0.05$ and $EF < 2$ [7], and expression ratio < 0.8 or > 1.2 against that of the corresponding control [5] were reported here. The final results were subjected to analysis with Ingenuity Pathway Analysis (IPA, version 8.5), a literature based software (Ingenuity Systems, Mountain View, CA).

In total, we identified 1068 proteins expressed in the prostate of 18-wk old mice. Among them, 813 and 619 proteins were identified with $> 95\%$ or $> 99\%$ confidence, whereas 483 and 748 proteins were identified at FDRs of 1% and 5% respectively. One example of the proteins identified was GRP78 (heat shock protein 5, a protein known for responding to androgen signaling) with a protein score of 61.07 and a local FDR of $7.34341E-09$. Quantization indicated that GRP78 was more abundant in DLP lobe (Table 1) and its expression was significantly decreased during TRAMP carcinogenesis in both DLP and VP lobes (Table 2C). These changes for GRP78 were further validated by immune-blot using GRP78 antibody from Santa Cruz (Supplemental figures).

Based on the criteria of protein identification, statistical significance and 20% cut-off threshold for abundance ratio [5], the expression levels of 84 proteins were different between DLP and VP in wild-type mice (Table 1). For example, transglutaminase 4 (TGM4), experimental autoimmune prostatitis antigen 2 (EAPA2), probasin, beta-tropomyosin, calponin-1, high mobility group box (HMGB) 1 & 2 and GRP78 were preferably expressed in DLP, whereas there were more prostatic spermine-binding protein (SBP), serine peptidase inhibitor Kazal type 3 (SPINK3), polymeric immunoglobulin receptor (PIGR), solute carrier family 12 member 2, epidermal growth factor (EGF) and

clusterin in VP lobe. The expression pattern of clusterin was also validated by Western blot (Supplemental figures).

Fujimoto *et al* identified 14 secreted proteins from mouse prostates and studied their mRNA abundance in different lobes [8]. They reported that the mRNA of EAPA2 was absent in mouse VP, and mRNA levels of probasin, GRP78, peroxiredoxin-6 (PRDX6), anterior gradient protein 2 homolog (AGR2), beta-microseminoprotein (PSP94) and calreticulin were higher in the DLP than VP in 11 weeks old C57BL mice. In addition, they found that there were no SBP and SPINK3 mRNA expression in VP [8]. Similar trends were found in rat by the same group recently [9]. Berquin *et al* profiled the transcriptomes of different prostate lobes of 6-week-old C57BL/N6Tac mice using RNAs extracted from laser-capture micro-dissected epithelium and stromal cells by microarray [10]. The lobe-specific expression profiles in these studies were in good agreement with our proteomic changes.

Some of the mouse prostatic proteins are known to be highly glycosylated, and exposure to e-glycosidation enzyme PNGase F led to changes of molecular weights of some proteins [8]. Cognizant of the possible effect of the heterogeneity of post-translational modification on protein identification and quantization, we excluded some proteins in the dataset. For example, AGR2 and PSP94 were identified with high confidence and quantitative data showed that their abundances were higher in DLP of wild-type mice (1.72 and 1.87 times higher compared to VP, respectively), which was consistent with previous report by Fujimoto *et al* [8]. However, they were not able to fulfill the threshold of $p < 0.05$ and $EF < 2$ described above. This was very likely due to heterogeneity of glycosylation.

We found that the expression levels of 118 proteins were significantly altered in DLP and/or VP during TRAMP carcinogenesis. Among these proteins, 55 and 36 proteins were uniquely changed in DLP and VP lobe of TRAMP mice, respectively (Table 2A & 2B) and only 27 proteins were found to be significantly modulated in both lobes during TRAMP carcinogenesis (Table 2C). The majority (24 out of 27 proteins) shared the same trend, albeit the extent of change was not exactly the same. Shared changes in DLP and VP might indicate possible common molecular events associated with TRAMP carcinogenesis. For example, up-regulation of HMGB1&2 and nuclear proteins such as histone(s) might be related to increased proliferation potential [11]; down-regulation of myosin-11 [12] and calponin-1 [13] might indicate the disruption of stroma, whereas decreased antioxidant enzyme PRDX6 could suggest increased oxidative stress [14]. Interestingly, given lots of prostatic proteins were regulated by androgen signaling [8], we found that EAPA2 and calreticulin were down-regulated only in DLP whereas zinc-alpha-2-glycoprotein down-regulated only in VP during carcinogenesis. These observations suggested that aberrant AR signaling could play different roles in TRAMP carcinogenesis of different lobes. Proteins like clusterin, PIGR and aldose-reductase, whose expression changed in opposite directions in DLP *versus* VP between TRAMP and wild type mice, were more likely related to lobe-specific mechanisms of carcinogenesis. For example, clusterin is a gene expressing three protein isoforms with different sub-cellular locations. The role of clusterin in carcinogenesis is very complicated not only due to protein isoforms but also by the changes of cancer over time and the selective pressure from treatments [15]. It should be interesting to dynamically track the expression and location of clusterin isoforms during TRAMP carcinogenesis in each lobe and examine the impact of chemo-preventive treatments.

Our group has recently published the identification of TRAMP carcinogenesis related proteins in DLP in mice of 18 weeks old by 2D LC-ESI-Q-TOF[5], which could be considered as part of biological repeat of the current study. Relative protein abundance identified as being significantly changed during carcinogenesis in both experiments are highly consistent (Supplemental figures), which further strengthen the confidence of the

dataset presented here. In addition, more proteins related to carcinogenesis were identified in this study than our previous work probably due to twice as many proteins were identified in the current study. The functional significance of probasin, HMGB2, TGM4, PRDX6, GRP78, GRP170 and calponin-1 had been discussed [5]. Some other down-regulated proteins such as decorin [16] and beta-tropomyosin [13] might be tumor suppressors while up-regulated proteins such as Spliceosome RNA helicase Bat1 [17] and EGF [18] might function as onco-proteins as suggested by literature survey.

The lists of differentially expressed proteins associated with TRAMP carcinogenesis were further analyzed by IPA software for pathway connections based on gene ontology and functionality. Twenty-eight and twenty-five TRAMP related proteins in DLP were mapped to Immunological Disease/Cancer/Antigen Presentation and Post-Translational Modification/Protein Folding/Cancer networks (Figure 1A&B) with a score of 61 and 49, respectively. Distinct inferred network nodes 14-3-3 zeta/delta (YWHAZ, up-regulated only in DLP) and NF- κ B were identified in the first network, whereas epidermal growth factor (EGF, up-regulated only in DLP), ERK1/2, PKCs were identified in the second network. Accordingly, 14-3-3-mediated Signaling ($p=0.0018$) was among the top canonical pathways. With the same approach, we mapped sets of nineteen and fifteen proteins in VP to Connective Tissue Disorders/Developmental Disorder/Genetic Disorder and Cancer/Cell Cycle/Cellular Development pathways with a score of 48 and 36 (Figure 1C&D). ERK1/2 and FGF2 were identified as distinct network nodes in the two networks. In addition, we examined the expression patterns of network nodes NF- κ B p65 and FGF2, which were not identified by mass spectrometry but recognized by IPA software as important proteins in the TRAMP carcinogenesis of DLP and VP lobes, respectively. Western blot data showed that NF- κ B p65 was expressed at much higher level in VP lobe of wild type mice than in DLP, though it was up-regulated in both lobes during TRAMP carcinogenesis (Supplemental figures), in partial agreement with Shukla et al [19]. Different isoforms of FGF2 were expressed in each lobe and FGF2 was only up-regulated in VP lobe of TRAMP mice (Supplemental figures). These bioinformatics analyses and Western blot data further highlight the mechanistic difference between two lineages of carcinogenesis.

To our knowledge, this is the first systematic report on the differences of global protein expression between DLP and VP lobes of mouse prostate. The information presented would benefit the understanding of prostatic biology of rodent animals. In addition, we also, for the first time, compared the different protein profile changes between DLP and VP lobes during TRAMP carcinogenesis. Our results provide further molecular support to the concept of TRAMP mouse representing two lineages of prostate carcinogenesis in DLP and VP lobes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

TRAMP	transgenic adenocarcinoma of mouse prostate
DLP	dorsal-lateral prostate
VP	ventral prostate
TGM4	transglutaminase 4
EAPA2	experimental autoimmune prostatitis antigen 2
SBP	prostatic spermine-binding protein
SPINK3	serine peptidase inhibitor Kazal type 3
GRP78	heat shock protein 5
MMTS	methyl methane thiosulfate
EF	error factors
IPA	Ingenuity Pathway Analysis

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B

D

Figure 1. Examples of IPA analysis results. (A) Twenty-eight TRAMP related proteins in DLP were mapped to Immunological Disease/Cancer/Antigen Presentation network with a score of 61; (B) Twenty-five TRAMP related proteins in DLP were mapped into Post-Translational Modification/Protein Folding/Cancer network with a score of 49. (C) Nineteen proteins changed in VP were mapped to Connective Tissue Disorders/Developmental Disorder/Genetic Disorder pathway with a score of 48. (D) Fifteen proteins changed in VP were mapped to Cancer/Cell Cycle/Cellular Development pathways with a score of 36.

Table 1

Proteins differentially expressed between DLP and VP of wild-type mice

Accession No.	Protein	Abundance Ratio
		VP/DLP
gi 89130373	Seminal vesicle secretory protein 5	0.02
gi 119850932	9530053A07Rik protein	0.05
gi 227500026	transglutaminase 4 (prostate);TGM4	0.11
gi 81892776	Experimental autoimmune prostatitis antigen 2;EAPA2	0.15
gi 56541062	Aldo-keto reductase family 1, member B7	0.18
gi 71051244	Major urinary protein 1	0.20
gi 76828138	Seminal vesicle secretory protein 2	0.25
gi 6225831	Probasin	0.39
gi 6680806	bisphosphoglycerate mutase	0.40
gi 417208	Creatine kinase B-type	0.40
gi 62510933	Protein disulfide-isomerase A6	0.40
gi 55391503	Aldo-keto reductase family 1, member B3 (aldose reductase)	0.41
gi 23396453	Protein ARMET; Mesencephalic astrocyte-derived neurotrophic factor	0.41
gi 45219865	Pdia4 protein	0.41
gi 119362	Endoplasmic; GRP94	0.44
gi 13431676	Myosin-11; SMMHC	0.46
gi 254540168	heat shock protein 5;GRP78	0.49
gi 548710	Reticulocalbin-1	0.51
gi 20522236	Tropomyosin beta chain; Beta-tropomyosin	0.54
gi 584951	Calponin-1	0.54
gi 94717658	Myosin light chain kinase, smooth muscle; MLCK	0.55
gi 62132964	Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	0.56
gi 6680836	calreticulin	0.57
gi 1351218	Testin	0.58
gi 1729962	Thiosulfate sulfurtransferase; Rhodanese	0.59
gi 1169687	FK506-binding protein 2; Peptidyl-prolyl cis-trans isomerase; PPIase	0.59
gi 146345480	Protein disulfide-isomerase A3	0.60
gi 54036890	Cysteine-rich protein 1	0.60
gi 6671507	actin, alpha 2, smooth muscle, aorta	0.62
gi 3219774	Peroxiredoxin-6	0.63
gi 124297829	Tropomyosin 1, alpha	0.64
gi 6680229	high mobility group box 2	0.64
gi 81906751	Hypoxia up-regulated protein 1; GRP-170	0.64
gi 58476928	Vimentin	0.67
gi 3915671	Desmin	0.69
gi 47605401	Cysteine and glycine-rich protein 1;CRP1	0.70
gi 33317937	heat shock protein 84b	0.74
gi 21542415	Lumican	0.76

Accession No.	Protein	Abundance Ratio
		VP/DLP
gi 71774133	peptidylprolyl isomerase B	0.77
gi 73620809	DnaJ homolog subfamily C member 3;Protein kinase inhibitor p58	0.77
gi 84105496	High mobility group box 1	0.80
gi 6756041	14-3-3 protein zeta/delta	1.21
gi 51338604	Ras-related protein Rab-1A; YPT1-related protein	1.23
gi 60391192	14-3-3 protein epsilon	1.23
gi 2495110	Glutathione S-transferase theta-2	1.24
gi 2851390	Triosephosphate isomerase	1.28
gi 29840803	Plastin-2; L-plastin; Lymphocyte cytosolic protein 1	1.31
gi 55976751	Sodium/potassium-transporting ATPase subunit alpha-1	1.32
gi 68534059	Heat shock protein 1 (chaperonin 10)	1.34
gi 90110028	Cytokeratin-8; CK-8; Keratin-8	1.41
gi 62948081	Transaldolase 1	1.41
gi 231557	Apolipoprotein A-I	1.42
gi 2497916	Zinc-alpha-2-glycoprotein	1.44
gi 84794552	phosphatidylethanolamine binding protein 1	1.45
gi 59808087	Histone cluster 1, H1c	1.46
gi 21759182	Hemopexin	1.49
gi 1352102	Complement C3	1.50
gi 7304875	alpha-2-HS-glycoprotein	1.50
gi 254540068	keratin 18	1.53
gi 55153937	Keratin 19	1.53
gi 116961	Serine protease inhibitor A3K; Contrapsin; SPI-2	1.61
gi 2492905	Annexin A4	1.68
gi 81881843	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	1.70
gi 63101527	Phosphomannomutase 2	1.70
gi 62027488	Transferrin	1.71
gi 2494022	Cystatin-B; Stefin-B	1.72
gi 1169205	3,2-trans-enoyl-CoA isomerase, mitochondrial	1.75
gi 42560536	Aldehyde dehydrogenase family 1 member A1; ALDH-E1	1.79
gi 73919875	3-ketoacyl-CoA thiolase, mitochondrial; Acetyl-CoA acyltransferase	1.80
gi 23821758	UMP-CMP kinase	1.85
gi 17366650	L-gulonate 3-dehydrogenase	1.92
gi 21263402	Argininosuccinate lyase;ASAL	1.92
gi 46396900	Glycogen phosphorylase, brain form	1.96
gi 267133	Tissue inhibitor of metalloproteinases 2; TIMP-2	2.11
gi 62185775	Epidermal growth factor; EGF	2.25
gi 113986	NADPH-dependent carbonyl reductase 2; Lung carbonyl reductase	2.48
gi 729152	Clusterin;SGP-2	2.62
gi 124517716	solute carrier family 12, member 2	2.98
gi 23503057	Protein FAM3D; Oncoprotein-induced protein 1; Protein EF-7	3.49

Accession No.	Protein	Abundance Ratio
		VP/DLP
gi 81898174	Putative DMBT1-like protein	5.13
gi 109730807	Serine peptidase inhibitor, Kazal type 5; SPINK5	5.52
gi 6225856	Polymeric immunoglobulin receptor; PIGR	7.31
gi 56541044	Serine peptidase inhibitor, Kazal type 3; SPINK3	14.70
gi 134788	Prostatic spermine-binding protein; SBP	18.60

Table 2

TRAMP related proteins. (A) Proteins uniquely changed in DLP. (B) Proteins uniquely changed in VP. (C) Proteins modulated in both DLP and VP

Accession No.	Protein	Abundance Ratio TRAMP/Wild Type
(A) Proteins significantly changed only in DLP during carcinogenesis		
gi 6225831	Probasin	0.20
gi 227500026	transglutaminase 4 (prostate); TGM4	0.22
gi 81892776	Experimental autoimmune prostatitis antigen 2; EAPA2	0.24
gi 119362	Endoplasmic; GRP94; Tumor rejection antigen gp96	0.36
gi 6680836	calreticulin	0.39
gi 45219865	Pdia4 protein	0.39
gi 71051244	Major urinary protein 1	0.39
gi 1169687	Peptidyl-prolyl cis-trans isomerase; Rotamase	0.49
gi 417753	Cysteine-rich secretory protein 1; CRISP-1	0.50
gi 1729962	Thiosulfate sulfurtransferase; Rhodanese	0.51
gi 62533148	Prolyl 4-hydroxylase, beta polypeptide (PDI)	0.51
gi 71774133	peptidylprolyl isomerase B	0.55
gi 6681143	decorin	0.57
gi 20140233	Signal peptidase complex subunit 2	0.61
gi 215274228	Prostate and testis expressed protein 4; Caltrin	0.64
gi 76364095	Complement C1r-B subcomponent	0.65
gi 31077036	Endoplasmic reticulum resident protein ERp44	0.67
gi 6671507	actin, alpha 2, smooth muscle, aorta	0.67
gi 6680806	bisphosphoglycerate mutase	0.74
gi 23822106	Ribosome-binding protein 1	0.74
gi 48474741	Vesicle-trafficking protein SEC22b	0.75
gi 47605401	Cysteine and glycine-rich protein 1; CRP1	0.76
gi 20522236	Tropomyosin beta chain; Tropomyosin-2; Beta-tropomyosin	0.77
gi 5915871	Calumenin; Crocalbin	0.79
gi 58476928	Vimentin	0.79
gi 49035535	ER-Golgi intermediate compartment 53 kDa protein	0.79
gi 3915671	Desmin	0.80
gi 205371802	Myosin-9; NMMHC II-a	1.22
gi 82571480	Glyceraldehyde-3-phosphate dehydrogenase	1.22
gi 46396900	Glycogen phosphorylase, brain form	1.24
gi 71051228	Peptidylprolyl isomerase A	1.25
gi 6756041	14-3-3 protein zeta/delta	1.25
gi 52783213	Plasminogen activator inhibitor 1 RNA-binding protein	1.26
gi 62185775	Epidermal growth factor; EGF	1.30
gi 730956	Transketolase	1.30
gi 85681898	Seminal vesicle secretory protein 4; Seminal vesicle protein 2	1.31

Accession No.	Protein	Abundance Ratio
		TRAMP/Wild Type
gi 62900337	S-formylglutathione hydrolase	1.33
gi 71119320	Hnrpl protein	1.35
gi 89130373	Seminal vesicle secretory protein 5	1.36
gi 55153937	Keratin 19	1.37
gi 81901906	Acyl-CoA synthetase family member 2, mitochondrial	1.41
gi 17366650	L-gulonate 3-dehydrogenase	1.43
gi 61217662	Spliceosome RNA helicase Bat1; DEAD box protein UAP56; DDX39	1.43
gi 1351942	Annexin A5	1.46
gi 82568930	S-adenosylhomocysteine hydrolase	1.47
gi 46577330	Heterogeneous nuclear ribonucleoprotein H2	1.49
gi 56754803	Tubulin beta-5 chain	1.50
gi 124028629	Heterogeneous nuclear ribonucleoproteins A2/B1	1.52
gi 2498955	Src substrate cortactin	1.53
gi 1345935	Cystatin-C; Cystatin-3	1.65
gi 124517716	solute carrier family 12, member 2	1.76
gi 81911775	Histone H2B type 1-C/E/G	1.79
gi 2492905	Annexin A4	1.83
gi 8928084	RNA-binding protein FUS; Protein pigpen	1.90
gi 267133	Tissue inhibitor of metalloproteinases 2; TIMP-2	2.51
(B) Proteins significantly changed only in VP during carcinogenesis		
gi 23503057	Protein FAM3D; Oncoprotein-induced protein 1; Protein EF-7	0.32
gi 62027488	Transferrin	0.50
gi 119850932	9530053A07Rik protein	0.51
gi 134035924	Alpha-2-macroglobulin	0.53
gi 81898174	Putative DMBT1-like protein	0.56
gi 7304875	alpha-2-HS-glycoprotein	0.58
gi 42560536	Aldehyde dehydrogenase family 1 member A1;ALDH-E1	0.61
gi 231557	Apolipoprotein A-I	0.61
gi 21263374	ATP-citrate synthase	0.73
gi 6755392	S100 calcium binding protein A6 (calcyclin)	0.73
gi 2497916	Zinc-alpha-2-glycoprotein	0.73
gi 46395721	Alpha-actinin-1	0.75
gi 1703331	Apolipoprotein A-IV	0.76
gi 28381362	Gelsolin; Actin-depolymerizing factor	0.76
gi 1352250	Aldehyde dehydrogenase, mitochondrial; ALDH1	0.76
gi 462608	Moesin; Membrane-organizing extension spike protein	0.76
gi 68534059	Heat shock protein 1 (chaperonin 10)	0.76
gi 38257560	Filamin-A	0.77
gi 63101587	Aconitase 2, mitochondrial	0.78
gi 81882043	Coatmer subunit gamma	0.79
gi 68534451	Ribosomal protein S12	1.21

Accession No.	Protein	Abundance Ratio
		TRAMP/Wild Type
gi 7305443	ribosomal protein L7A	1.21
gi 66267516	Pyruvate kinase, muscle	1.23
gi 62825991	Ribosomal protein L4	1.23
gi 73919875	3-ketoacyl-CoA thiolase, mitochondrial; Acetyl-CoA acyltransferase	1.24
gi 33317937	heat shock protein 84b	1.25
gi 6677809	ribosomal protein S6	1.27
gi 52783339	40S ribosomal protein S9	1.29
gi 148840438	Selenium-binding protein 2	1.30
gi 46395611	Acidic leucine-rich nuclear phosphoprotein 32 family member A	1.48
gi 81918016	Heterogeneous nuclear ribonucleoprotein F; hnRNP F	1.50
gi 9910833	Proteasome subunit alpha type-1	1.59
gi 6647713	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthetase 2	1.67
gi 729000	Heterogeneous nuclear ribonucleoprotein A/B;hnRNP A/B	1.93
gi 78099787	PDZ and LIM domain protein 1	2.01
gi 123794006	FUSE-binding protein 2	2.11

Accession No.	Protein	Aundance Ratio (TRAMP/Wild Type)	
		DLP	VP
(C) Proteins significantly changed in both DLP and VP during carcinogenesis			
gi 56541044	Serine peptidase inhibitor, Kazal type 3; SPINK3	0.21	0.05
gi 134788	Prostatic spermine-binding protein; SBP	0.31	0.15
gi 254540168	heat shock protein 5, GRP78	0.29	0.65
gi 62510933	Protein disulfide-isomerase A6	0.29	0.76
gi 23396453	Protein ARMET	0.32	0.73
gi 3219774	Peroxiredoxin-6	0.39	0.66
gi 81906751	Hypoxia up-regulated protein 1; GRP-170	0.47	0.73
gi 146345480	Protein disulfide-isomerase A3	0.53	0.75
gi 584951	Calponin-1	0.74	0.79
gi 13431676	Myosin-11; SMMHC	0.77	0.69
gi 109730807	Serine peptidase inhibitor, Kazal type 5;SPINK5	0.73	0.27
gi 6680229	high mobility group box 2	2.75	2.45
gi 84105496	High mobility group box 1	1.51	1.74
gi 58475965	Hnrpk protein	1.55	1.84
gi 128843	Nucleolin; Protein C23	1.45	1.74
gi 76827483	Erh protein	1.47	1.64
gi 82571753	H1 histone family, member 0	1.60	1.92
gi 85057093	Hist4h4 protein	1.96	2.31
gi 6679108	nucleophosmin 1	1.62	1.97
gi 80474659	Chromobox homolog 3 (Drosophila HP1 gamma)	1.98	2.21
gi 33516945	Heterogeneous nuclear ribonucleoprotein D0	1.58	1.52
gi 2851390	Triosephosphate isomerase	1.31	1.28

Accession No.	Protein	<u>Aundance Ratio (TRAMP/Wild Type)</u>	
		DLP	VP
gi 62948081	Transaldolase 1	1.31	1.44
gi 59808087	Histone cluster 1, H1c	2.09	1.66
gi 6225856	Polymeric immunoglobulin receptor; PIGR	1.48	0.61
gi 55391503	Aldo-keto reductase family 1, member B3 (aldose reductase)	0.63	1.32
gi 729152	Clusterin; SGP-2	3.53	0.67