pro-NPY, PCa, and Neuroendocrine Tumors

Terrence P McGarty
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There are many markers for PCa diagnosis and prognosis. This paper considers a recently remarked marker for prognosis of aggressive PCa, namely a small protein, pro-NPY. This is a neuroendocrine related protein. We examine this in some detail and also look at its relationship to the neuroendocrine tumors in PCa.

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1 INTRODUCTION

We continue to find new markers for the detection of and prognostication of prostate cancer. We herein examine one of the somewhat newer one the pro-NPY protein. There are always two questions that arise. The first is diagnostic; namely, does this patient have PCa? We have examined several of the recent methodologies in this area[1]. The second is prognostic tests, where we are looking for markers of aggressive PCa. Just because a patient is diagnosed with PCa it is well known that very few will die of the disease. Thus the issue of identifying that group and using aggressive treatments is an imperative.

As was noted almost three decades ago by Sakr et al:

The incidence of clinically detected prostate cancer is increasing with more frequent diagnosis in younger male patients. Whether this represents a genuine increase in incidence or earlier detection is not clear. To understand better the evolution and early changes of prostate cancer we evaluated 152 prostate glands from young male patients 10 to 49 years old. Of the prostates 98 were from African-Americans and 54 were from white patients. Prostatic intraepithelial neoplasia was identified in 0%, 9%, 20 and 44%, and small foci of histological cancer in 0%, 0%, 27% and 34% of the male patients in the second, third, fourth and fifth decades of age, respectively.

The majority of the cases of prostatic intraepithelial neoplasia were of low grade. High grade prostatic intraepithelial neoplasia, found in 5 prostates, was first identified in the fifth decade. All 5 cases occurred in prostates containing histological carcinoma. Incidental carcinoma was detected with a similar frequency in white and black patients. The cancerous foci were of similar size with a tendency for cancer in black patients to be multifocal, particularly in those in the fifth decade.

We conclude that prostatic intraepithelial neoplasia and histological cancers are surprisingly common in young male patients of both races. The evolution of prostatic intraepithelial neoplasia and focal histological cancers is not clear but it appears to present several decades earlier than clinically detected carcinoma. The natural history of prostate cancer must encompass many more years (decades) than has been previously realized. In addition, the initiating events leading to clinically relevant prostate cancers likely occur at a remarkably young age.

The young patients all too often have the most aggressive. However this is also not uncommon in the older male. Thus having the appropriate markers is essential. The USPTF recommendations have been critiqued by minions for a variety of reasons, not the least of which was the somewhat haphazard way in which the information was obtained. However, as has been recognized for decades now, the risk of PCa in an aggressive form, even in young men, is substantial.

This search for the markers has been going on for several decades. For example from Walker et al (1999) we have the following:

[1] See the list of White Papers attached for details. They are part of and an addition to the Draft book, Prostate Cancer

We wish to identify genes associated with disease. To do so, we look for novel genes whose expression patterns mimic those of known disease-associated genes, using a method we call Guilt-by-Association (GBA), on the basis of a combinatoric measure of association. Using GBA, we have examined the expression of 40,000 human genes in 522 cDNA libraries, and have discovered several hundred previously unidentified genes associated with cancer, inflammation, steroid-synthesis, insulin-synthesis, neurotransmitter processing, matrix remodeling, and other disease processes.

The majority of the genes, thus discovered, show no sequence similarity to known genes, and thus could not have been identified by homology searches. We present here an example of the discovery of eight genes associated with prostate cancer. Of the 40,000 most-abundant human genes, these 8 are the most closely linked to the known diagnostic genes, and thus are prime targets for pharmaceutical research.

They then present ten genes most associated with PSA:

p Value	***Gene***	***both***	***only PSA***	***only co-expressed***	***neither***
1.53E-31	glandular kallikrein[2]	26	12	3	481
1.65E-26	IPCA-3	22	16	2	482
7.48E-25	IPCA-4	26	12	14	470
8.12E-25	IPCA-9	23	15	6	478
3.38E-24	Prostate seminal protein	23	15	7	477
1.89E-23	PAP	24	14	11	473
6.87E-18	IPCA-10	19	19	9	475
9.01E-18	Prostate transglutaminase	14	24	0	484
4.61E-14	IPCA-11	27	11	66	418
1.58E-13	neuropeptide Y	16	22	11	473

As to the above they specifically note:

Table 1 shows that, for PSA, the most closely coexpressed genes are glandular kallikrein, three novel genes, prostate seminal protein, PAP, a fourth novel gene, prostate transglutaminase, a fifth novel gene, and neuropeptide Y. (IPCA-9, IPCA-10 and IPCA-11 are coexpressed with PSA but appear to be 38 untranslated sequences.) ....Neuropeptide Y is coexpressed with PSA. It has been reported to be associated with prostate cancer

The neuropeptide Y has thus been on the list as a putative marker for almost two decades. This specific one, namely pro-NPY, the predecessor of NPY, is the target of recent interest. A recent paper purports to establish a basis for pro-NPY. Namely from MedicalNews[3] we have the following:

[2] Note this is now used as part of the 4K test which we have discussed at length.

[3] http://www.medicalnewstoday.com/releases/305722.php

Researchers at the University of Copenhagen have identified a new prognostic biomarker: the neuropeptide pro-NPY, which may help determine the risk of dying from prostate cancer. This particular type of protein is very specific to prostate cancer cells and could help identify whether newly diagnosed patients require radical prostatectomy surgery or if it is safe to delay surgery. The research has been published in the journal, European Urology[4].

Using mass spectrometry, the researchers measured concentration changes in thousands of proteins in both normal and tumour tissue from prostate cancer. They discovered that in comparison to normal tissue, the prostate tumors exhibit numerous metabolic alterations including exacerbated activity of mitochondria.

Among the 9000 proteins identified, one protein, the neuropeptide, pro-NPY, was demonstrated to exhibit high levels in a subgroup of prostate cancer samples. Pro-NPY was analyzed in 750 patients with prostate cancer to show that pro-NPY levels correlate with increased risk of prostate cancer death.

"Our research shows that high pro-NPY levels are very specific to prostate cancer and can serve to predict prostate cancer related death among diagnosed patients who have not received surgical treatment," says Professor Amilcar Flores-Morales from the Department of Veterinary Disease Biology, University of Copenhagen.

"So identifying the biomarker pro-NPY could help us identify patients who would benefit from early active treatment, whereby we would also reduce unnecessary treatment of patients who undergo surgery when they have low-grade tumors that for the most part do not put their lives at risk. In the end, due to side effects, this could prove more harmful than beneficial to patients," adds Amilcar Flores-Morales.

Proteins are key effectors of cellular functions. Therefore, a better understanding of the protein signaling pathways deregulated in prostate cancer could lead to better preventive and therapeutic strategies for the treatment of this disease. Specifically, it is possible that metabolic alterations such as the increase in mitochondria activity could be targeted in the treatment of prostate cancer.

"We hope to contribute to the advance of translational cancer research and the implementation of precision medicine in the field of prostate cancer by providing a unique insight into the protein level alterations associated with tumor tissue in clinical samples," adds Flores-Morales.

This specific gene product pro-NPY has been studied by many, either directly or as its product NPY.

From Shay and Mangian we have a history of the identification and understanding of the function of NPY. Specifically they state:

[4] See References Inglesias-Gato et al.

In the 1970s, it was determined by Wurtman, Fernstrom and colleagues that dietary concentrations of tyrosine and tryptophan could affect the synthesis and concentrations of the neurotransmitters norepinephrine and serotonin. In turn, the diet-affected central concentrations of these neurotransmitters could affect the relative appetite/satiety state of an individual. These findings spurred nutrition researchers to connect zinc deficiency, dietary amino acid intake and anorexia. In 1980 it was reported that norepinephrine had a profound influence on feeding behavior within specific sites in the hypothalamus. Leibowitz and Brown (1980) reported that the predominantly inhibitory neurotransmitter norepinephrine had a strong stimulatory effect on food intake. When exogenous norepinephrine was delivered to the paraventricular nucleus (PVN) of the hypothalamus, short-term food intake increased. Later in the 1980s, neuropeptides were also discovered to have a profound impact on feeding behavior.

In 1982, neuropeptide Y (NPY) was first isolated from neural tissue within the porcine intestine. Soon after, NPY was found to have significant stimulatory effect on food intake (Clark et al. 1984). Although NPY may be synthesized by all neurons within the body, it is synthesized at very high levels within cell bodies derived in the arcuate nucleus of the hypothalamus. A high percentage of these neurons project to the PVN of the hypothalamus. Within the PVN, exogenously administered NPY has been demonstrated to stimulate appetite to a greater degree than any other agent yet tested, when considered on a molar basis. Interestingly, it was also found that the administration of NPY to the PVN specifically stimulated carbohydrate intake when rats were allowed to freely select from a three-choice macronutrient diet system.

Some investigators have also suggested that the results demonstrating an effect of NPY on macronutrient preference may be influenced by the past history or dietary preferences of rats chosen for study (Welch et al. 1994). Even specifics of the diet ingredients used in macronutrient choice studies may influence the results obtained (Glass et al. 1997). Because of its very potent effect on food intake, NPY has been investigated very vigorously at many laboratories. Targets of research have included the effects of NPY, the development of agonists and antagonists of NPY and the identification and study of NPY receptors. The development of an NPY antagonist with an appetite-modulating activity is of interest to pharmaceutical concerns.

Consistent with the complex nature of the appetite regulation system, NPY has proved to be a difficult target to study.

First, it has been found that there are a family of NPY receptors, and it is still unclear whether a single NPY receptor or a subset of a few receptors mediate the appetite-generating effect of NPY.

Second, the NPY knockout mouse regulates food intake in a relatively normal fashion.

This has led some to suggest that the large set of physiological studies investigating the effect of NPY on food intake may need to be reconsidered. A possible explanation for normal appetite in the NPY knockout mouse is that NPY action may be accommodated for by other neuropeptides during development. The paradox between physiological data and the NPY knockout results is of great interest and is likely to be further investigated.

Finally in the current Inglesias-Gato et al paper they conclude:

The evaluation of pro-NPY as a biomarker of disease progression in historic TURP-detected watchful-waiting cohorts has some limitations as PSA levels are not available and also as tumor tissue obtained through TURP could be different from small cancers located in the peripheral zone. However, it also has some strength. Watchful-waiting cohorts are superior for identifying patients with an excellent outcome also in the absence of treatment and for identifying tumors that will progress when left in situ.

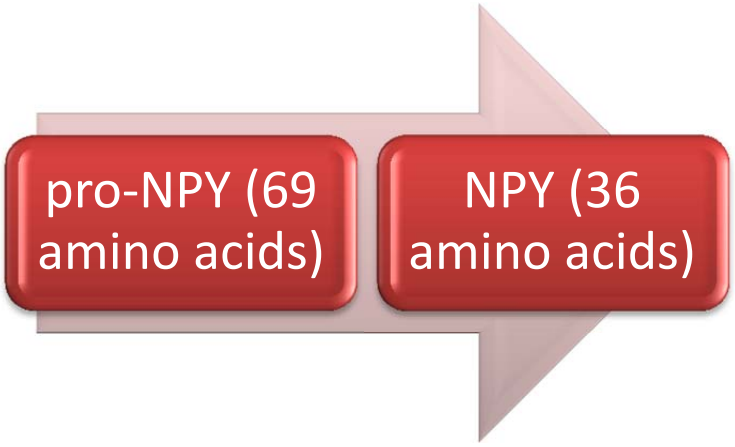
Markers for long-term indolent tumors, or tumors that will eventually progress when left in situ are difficult to identify in cohorts where patients are treated at an early stage. However, in order to implement pro-NPY measurements into current practice, the prognostic potential of pro-NPY should be addresses in modern, PSA tested cohorts, to evaluate its performance relative to current standard of care.

Furthermore, pro-NPY's nature as a secreted peptide and its high specificity for PCa would support additional studies to validate pro-NPY as a prognostic blood biomarker for disease progression in PSA screened patients and patients on active surveillance.

2 NEUROPEPTIDES

Neuropeptides are a class of molecules that have the capability of establishing communications between neurons. They are most common therefore amongst the nerve cells. Thus their presence in the prostate and especially their over-expression is of some interest. One of the key questions one may ask is; why do we see such an overexpression of neuropeptides in aggressive PCa? We will examine also the presence of neuroendocrine cells in the prostate as well. Neuroendocrine cells are a special class of cells that receive signals from nerve cells in the form of neurotransmitters, such as the neuropeptides, and then release various hormones[5]. Neuroendocrine PCa is a highly aggressive and androgen receptive negative form of PCa with associated fatal results[6]. We shall focus on this collection of relationships to interpret some of the results of the paper in discussion.

Specifically we focus on pro-NPY and not its successor NPY. We note the difference below:



As Wulff et al noted the following about NPY, a successor to pro-NPY:

Peptide hormones, neuropeptides, and most other biologically active peptides are generated from larger precursors through proteolytic processing at dibasic or monobasic sites . In recent years a series of enzymes have been characterized that appear to be involved in this maturation process.

The two so-called precursor convertases, PC2, cloned from a human insulinoma and from mouse pituitary, and PC3, cloned from mouse pituitary and from AtT-20 cells , both have the expected specificity for certain pairs of basic residues. These enzymes are also expressed exclusively in peptide producing neuronal and endocrine cells . Coexpression of proopiomelanocortin and the two precursor convertases indicate that the balance between the expression of PC2 and PC3 probably can explain certain cases of tissue specific processing of precursors .

[5] See Mydlo & Godec, pp 149-155.

[6] See Staibano, pp 87-109.

Furthermore, the processing of proopiomelanocortin in AtT-20 cells has been suppressed by antisense constructs of PC3 . Thus PC2 and PC3 appear to constitute key enzymes of the precursor processing machinery in the neuroendocrine system.

PP and NPY belong to the so-called PP-fold family of peptides, which have relatively simple precursors with a single dibasic processing site, plus, in pro-PP, an additional mono basic processing site.

The overall homology of the secreted products, PP and NPY, is 45%, and this homology is mainly restricted to residues that are important in the stabilization of the PP-fold structure and to the C-terminal part of the molecules, which is involved in receptor recognition.

From NCBI they discuss the NPY gene and its product:[7]

This gene encodes a neuropeptide that is widely expressed in the central nervous system and influences many physiological processes, including cortical excitability, stress response, food intake, circadian rhythms, and cardiovascular function. The neuropeptide functions through G protein-coupled receptors to inhibit adenylyl cyclase, activate mitogen-activated protein kinase (MAPK), regulate intracellular calcium levels, and activate potassium channels. A polymorphism in this gene resulting in a change of leucine 7 to proline in the signal peptide is associated with elevated cholesterol levels, higher alcohol consumption, and may be a risk factor for various metabolic and cardiovascular diseases. The protein also exhibits antimicrobial activity against bacteria and fungi.

First we examine NPY, a 36 amino acid molecule derived from pro-NPY which as we will discuss is 69 amino acids in size. From Silva et al we have the following discussion on NPY:

NPY (neuropeptide Y) is a 36-amino-acid peptide involved in the regulation of the cardiovascular system. It has vasopressor effects and potentiates the effect of other vasoconstrictor molecules such as noradrenaline or histamine. When used at low, non-vasoconstrictive doses on cultured vascular SMCs (smooth muscle cells), NPY stimulates SMC proliferation, an effect potentiated by noradrenaline.

NPY also acts on vascular ECs (endothelial cells). The potentiating effect of NPY on noradrenaline-induced vasoconstriction has been shown to be endothelium dependent on human saphenous veins.

NPY is capable of promoting EC proliferation, migration and adhesion on the extracellular matrix. It also stimulates capillary tube formation in vitro and angiogenesis in vivo. Similar to other secreted peptides, NPY is produced as a pre-pro-peptide. After removal of the signal peptide in the endoplasmic reticulum, pro-NPY is further cleaved by successive enzymes to generate the biologically active amidated NPY.

[7] http://www.ncbi.nlm.nih.gov/gene/4852

In neuroendocrine cells, mature NPY is localized in secretory granules, e.g. in neurons, chromaffin cells or in the pituitary. Immunoreactive NPY has been detected in HUVEC (human umbilical-vein endothelial cells), but NPY synthesis, storage and secretion have not been studied.

The potential for NPY to promote endothelial cell proliferation may be one of the factors in its excess and the growth of PCa beyond AR inhibition. It may facilitate angiogenesis via its endothelial action. Again this is speculative. The identification in neuroendocrine cells may also be significant in view of the aggressiveness of neuroendocrine based PCa. Again we are speculating here as well.

But pro-NPY is a more complex molecule as described by Eggelkraut-Gottanka:

Similar to many other hormones and neurotransmitters, neuropeptide Y (NPY) is derived from a larger precursor molecule, the 69 amino acid pro-neuropeptide Y (pro-NPY). Precursor proteins undergo a highly specific conversion process to yield their biologically active products. As part of a finely tuned regulation network, the biosynthesis of hormones and neuropeptides plays a major role in many physiological and pathophysiological processes.

Diseases such as diabetes, obesity and diverse sorts of cancer could be associated with dysfunctions in the biosynthetic pathways.

From Silva et al who connect pro-NPY and NPY as follows:

The present study shows the expression of NPY and its precursor pro-NPY in HUVEC at the mRNA and protein levels as demonstrated by RT–PCR and ELISA. NPY expression has previously been determined in HUVEC only in a small number of HUVEC cultures. In the present study, NPY expression was evidenced in all cell preparations tested, independent of the medium used for cell culture (results not shown).

The difference between the results of these two studies may be due to a different initial amount of RNA used for RT–PCR and the number of passages, since we used cells at passage 1, whereas …The presence of NPY and pro-NPY in HUVEC was also assessed by immunofluorescence. NPY immunoreactivity appeared as small punctate granular structures disseminated in the cytoplasm.

The antibody NPY02 used in our study is directed against an epitope borne by both NPY and pro-NPY, thus leading to the labelling of both NPY and its precursor. The small punctate granular appearance of endocytic vesicles …. However, it is unlikely that intracellular NPY derives from cell-culture medium since NPY staining is not lost or altered in HUVEC incubated in the absence of serum. Furthermore, labelling of EEA1, an early endosome marker, showed a staining completely different from the immunolabelling of NPY.

From the work of Magni and Motta (2001) we know that:

By showing that NPY receptors are expressed in the androgen-independent cell line PC-3 and that their activation results in cell proliferation, the present date suggest that NPY-related

mechanisms might be relevant in certain stages of CaP, such as the progression of the disease during the androgen-independent stage.

They continue to state:

Prostate cancer (CaP) is initially often androgen dependent, and it may progress to androgen independence in later stages. In this condition, hormonal therapy is no longer useful and the prognosis becomes worse. It is believed that the molecular basis underlying this transition includes a host of factors, some of which are now being identified as peptidic molecules, such as growth factors and neurohormones. Several studies suggest that neuroendocrine mechanisms play an important role in the control of the development and the function of the normal prostate, as well as of the progression of CaP to androgen independence.

Few data, however, are presently available about one of these neuroendocrine modulators, neuropeptide Y (NPY), and on the related receptors in the normal as well as in the tumoral prostate. NPY, a peptide of 36 aminoacids, is abundantly distributed through the nervous system, and activates specific membrane receptors that exist in at least five different isoforms .

NPY participates to the regulation of a variety of physiological functions, including regulation of neuroendocrine mechanisms, cognitive functions, eating behavior and cardiovascular activity, and has also been shown to stimulate cell proliferation . In the context of the normal human prostate, NPY is mainly localized in the nerve fibers, and in the neuroendocrine (NE) cells .

In conclusion, the present study, together with other data present in the literature , suggests that the prostatic NPY neuroendocrine system might participate in the modulation of the proliferation of CaP cells. Moreover, the presence and the activation of NPY receptors might represent a marker of CaP progression toward a stage sensitive to non-androgenic trophic and proliferative agents. Further studies in this field might also give indications about possible novel future lines for the treatment of CaP, especially when this disease has progressed to the androgen-independent stage.

The regulation of neuroendocrine regulation may be a significant factor in the presence of pro-NPY in PCa and its aggressive forms. Again we have examined also the neuroendocrine types and these are driven by neuropeptides and are AR independent.

From the recent paper (2015) which we are examining by Inglesias et al[8]:

Clinical management of the prostate needs improved prognostic tests and treatment strategies. Because proteins are the ultimate effectors of most cellular reactions, are targets for drug actions and constitute potential biomarkers; a quantitative systemic overview of the proteome changes occurring during prostate cancer (PCa) initiation and progression can result in clinically relevant discoveries. To study cellular processes altered in PCa using system-wide

[8] http://www.europeanurology.com/article/S0302-2838%2815%2901087-8/abstract/the-proteome-of-primary-prostate-cancer

quantitative analysis of changes in protein expression in clinical samples and to identify prognostic biomarkers for disease aggressiveness.

Mass spectrometry was used for genome-scale quantitative proteomic profiling of 28 prostate tumors (Gleason score 6–9) and neighboring nonmalignant tissue in eight cases, obtained from formalin-fixed paraffin-embedded prostatectomy samples. Two independent cohorts of PCa patients (summing 752 cases) managed by expectancy were used for immunohistochemical evaluation of proneuropeptide-Y (pro-NPY) as a prognostic biomarker.

Over 9000 proteins were identified as expressed in the human prostate. Tumor tissue exhibited elevated expression of proteins involved in multiple anabolic processes including fatty acid and protein synthesis, ribosomal biogenesis and protein secretion but no overt evidence of increased proliferation was observed. Tumors also showed increased levels of mitochondrial proteins, which was associated with elevated oxidative phosphorylation capacity measured in situ.

Molecular analysis indicated that some of the proteins overexpressed in tumors, such as carnitine palmitoyltransferase 2 (CPT2, fatty acid transporter), coatomer protein complex, subunit alpha (COPA, vesicle secretion), and mitogen- and stress-activated protein kinase 1 and 2 (MSK1/2, protein kinase) regulate the proliferation of PCa cells. Additionally, pro-NPY was found overexpressed in PCa (5-fold, $p < 0.05$), but largely absent in other solid tumor types. Pro-NPY expression, alone or in combination with the ERG status of the tumor, was associated with an increased risk of PCa specific mortality, especially in patients with Gleason score ≤ 7 tumors.

This study represents the first system-wide quantitative analysis of proteome changes associated to localized prostate cancer and as such constitutes a valuable resource for understanding the complex metabolic changes occurring in this disease. We also demonstrated that pro-NPY, a protein that showed differential expression between high and low risk tumors in our proteomic analysis, is also a PCa specific prognostic biomarker associated with increased risk for disease specific death in patients carrying low risk tumors.

The identification of proteins whose expression change in prostate cancer provides novel mechanistic information related to the disease etiology. We hope that future studies will prove the value of this proteome dataset for development of novel therapies and biomarkers. Deep and quantitative proteomic profiling was obtained from formalin-fixed paraffin-embedded prostate cancer specimens and revealed that: prostate cancer cells preferably use oxidative phosphorylation for energy production; and proneuropeptide-Y expression defines a subgroup of prostate cancer patients with worsened prognosis, who might benefit from active intervention.

As with many such markers this results is not a causative result but a fortuitous result from observation. It appears to provide a prognostic marker. From Science Daily[9]:

A new prognostic biomarker has been identified by researchers: the neuropeptide pro-NPY, which may help determine the risk of dying from prostate cancer. This particular type of protein

[9] http://www.sciencedaily.com/releases/2016/01/160127115510.htm

is very specific to prostate cancer cells and could help identify whether newly diagnosed patients require radical prostatectomy surgery or if it is safe to delay surgery.

Researchers at the University of Copenhagen have identified a new prognostic biomarker: the neuropeptide pro-NPY, which may help determine the risk of dying from prostate cancer. This particular type of protein is very specific to prostate cancer cells and could help identify whether newly diagnosed patients require radical prostatectomy surgery or if it is safe to delay surgery.

The research has been published in the journal, European Urology.

Using mass spectrometry, the researchers measured concentration changes in thousands of proteins in both normal and tumour tissue from prostate cancer. They discovered that in comparison to normal tissue, the prostate tumors exhibit numerous metabolic alterations including exacerbated activity of mitochondria. Among the 9000 proteins identified, one protein, the neuropeptide, pro-NPY, was demonstrated to exhibit high levels in a subgroup of prostate cancer samples. Pro-NPY was analyzed in 750 patients with prostate cancer to show that pro-NPY levels correlate with increased risk of prostate cancer death.

"Our research shows that high pro-NPY levels are very specific to prostate cancer and can serve to predict prostate cancer related death among diagnosed patients who have not received surgical treatment," says Professor Amilcar Flores-Morales from the Department of Veterinary Disease Biology, University of Copenhagen.

"So identifying the biomarker pro-NPY could help us identify patients who would benefit from early active treatment, whereby we would also reduce unnecessary treatment of patients who undergo surgery when they have low-grade tumors that for the most part do not put their lives at risk. In the end, due to side effects, this could prove more harmful than beneficial to patients," adds Amilcar Flores-Morales.

Proteins are key effectors of cellular functions. Therefore, a better understanding of the protein signaling pathways deregulated in prostate cancer could lead to better preventive and therapeutic strategies for the treatment of this disease. Specifically, it is possible that metabolic alterations such as the increase in mitochondria activity could be targeted in the treatment of prostate cancer.

"We hope to contribute to the advance of translational cancer research and the implementation of precision medicine in the field of prostate cancer by providing a unique insight into the protein level alterations associated with tumor tissue in clinical samples," adds Flores-Morales.

This work is the result of collaborations between the research groups of Professor Flores-Morales at IVS, Professor Matthias Mann at Novo Nordisk Foundation Center for Protein Research both from the Faculty of Health and Medical Sciences together with the Danish Cancer Society Research Center and Associate Professor Pernilla Wikström from the Umeå University, Sweden. The validation of pro-NPY as a biomarker was possible due to the contribution of patients and clinical researchers from several institutions in Sweden.

The creation of NPY from pro-NPY is discussed in Brakch et al as noted below:

Proneuropeptide Y (ProNPY) undergoes cleavage at a single dibasic site Lys38-Arg39 resulting in the formation of 1-39 amino acid NPY which is further processed successively by carboxypeptidase-like and peptidylglycine alpha-amidating monooxygenase enzymes.

To investigate whether prohormone convertases are involved in ProNPY processing, a vaccinia virus derived expression system was used to coexpress recombinant ProNPY with each of the prohormone convertases PC1/3, PC2, furin, and PACE4 in Neuro2A and NIH 3T3 cell lines as regulated neuroendocrine and constitutive prototype cell lines, respectively. The analysis of processed products shows that only PC1/3 generates NPY in NIH 3T3 cells while both PC1/3 and PC2 are able to generate NPY in Neuro2A cells.

The convertases furin and PACE4 are unable to process Pro-NPY in either cell line.

Moreover, comparative in vitro cleavage of recombinant NPY precursor by the enzymes PC1/3, PC2 and furin shows that only PC1/3 and PC2 are involved in specific cleavage of the dibasic site.

Kinetic studies demonstrate that PC1/3 cleaves ProNPY more efficiently than PC2. The main difference between the cleavage efficiency is observed in the Vmax values whereas no major difference is observed in Km values.

In addition the cleavage by PC1/3 and PC2 of two peptides reproducing the dibasic cleavage site with different amino acid sequence lengths namely (20-49)-Pro-NPY and (28-43)-Pro-NPY was studied. These shortened Pro-NPY substrates, when recognized by the enzymes, are more efficiently cleaved than Pro-NPY itself.

The shortest peptide is not cleaved by PC2 while it is by PC1/3.

On the basis of these observations it is proposed,

first, that the constitutive secreted NPY does not result from the cleavage carried out by ubiquitously expressed enzymes furin and PACE4;

second, that PC1/3 and PC2 are not equipotent in the cleavage of Pro-NPY; and

third, substrate peptide length might discriminate PC1/3 and PC2 processing activity.

3 MARKERS

Various markers have been proposed for PCa status over the years. We can divide them into the categories as shown in the Table below:

Gene Expression (Proteins or RNA)	*Benign*	*Malignant Confined*	*Malignant Metastatic*
Under-Expressed			
Over-Expressed			pro-NPY

Filling this Table in is of significant importance. The following reference by Khan does help in this area. From Khan et al:

A total of 80 proteins was found to be elevated in PCA compared with Benign. Included among these were previously known alterations for prostate cancer, namely GOLM1 , transcription elongation factor B (SIII), polypeptide 1 (15 kDa; elongin C or TCEB1) , neuropeptide Y , Parkinson disease (autosomal recessive, early onset) 7 (PARK7 or DJ-1) , anterior gradient homolog-2 (AGR2) , growth differentiation factor 15 (GDF15, MIC-1, or NAG-1) , ferritin heavy chain (FTH1) , tumor necrosis factor, -induced protein 9 (STAMP2 or STEAP4) , fatty acid-binding protein (FABP5) , and VIM .

A similar analysis for down-regulated proteins revealed 81 proteins whose expression was decreased in PCA compared with Benign. Prominent among these were lactotransferrin , 2-glycoprotein (AZGP1) , microseminoprotein (prostatic secretory protein of 94 amino acids, PSP94, or MSMB) , isoforms of glutathione transferase (GSTP1 and GSTM3) (58–60), lactate dehydrogenase B , and N-myc downstream regulated gene (NDRG1) , all of which have been reported earlier to be down-regulated in organ-confined disease.

In the Appendix we reiterate the lists of genes in detail as noted by Khan et al. There are four such tables; up-expressed, down-expressed and non-metastasized and metastasized. This is an exceptionally useful set of genes and should be referred to from time to time.

From Inglesias-Gato et al:

Over 9000 proteins were identified as expressed in the human prostate. Tumor tissue exhibited elevated expression of proteins involved in multiple anabolic processes including fatty acid and protein synthesis, ribosomal biogenesis and protein secretion but no overt evidence of increased proliferation was observed. Tumors also showed increased levels of mitochondrial proteins, which was associated with elevated oxidative phosphorylation capacity measured in situ. Molecular analysis indicated that some of the proteins overexpressed in tumors, such as carnitine palmitoyltransferase 2 (CPT2, fatty acid transporter), coatomer protein complex,

subunit alpha (COPA, vesicle secretion), and mitogen- and stress-activated protein kinase 1 and 2 (MSK1/2, protein kinase) regulate the proliferation of PCa cells. Additionally, pro-NPY was found overexpressed in PCa (5-fold, $p < 0.05$), but largely absent in other solid tumor types. Pro-NPY expression, alone or in combination with the ERG status of the tumor, was associated with an increased risk of PCa specific mortality, especially in patients with Gleason score 7 tumors.

They continue at length:

Pro-NPY as a novel biomarker of disease progression Patients with primary Gleason grade at diagnosis have a more aggressive disease course ..... Therefore, we next compared the proteome of tumor areas scored as GS 7 (4+3), GS 8 (4+4), and GS 9 (4+5), n = 16, with the proteome from GS 6 (3+3) and GS 7 (3+4) areas, n = 12, in an attempt to identify candidate biomarkers of disease progression.

Only a small number of proteins were differentially expressed ($p < 0.05$, fold change > 1.6) between these groups. Among proteins downregulated in more aggressive tumors, we found several enzymes involved in the catabolism of amino acids, as well as proteins involved in cell adhesion and cell–cell contact as well as smooth muscle cell markers. Among the up-regulated proteins we found the nuclear receptor coactivator NCOA7 and the known biomarker of disease aggressiveness, FOLH1 (PSMA) , but the neuropeptide pro-NPY had the highest measurable concentration increase. These findings support the notion that in addition to metabolic changes, stroma-epithelium interactions and tissue remodeling contribute to disease progression.

In order to further select candidate biomarkers for clinical validation, we also employed a supervised learning approach, support vector machines, and combined it with feature selection.

A minimal classification error rate of 28.6% was observed when the 56 top ranked features were used in the validation procedure (Supplementary Tables 5 and 6). Importantly, pro-NPY was part of the top performing features that gave lower error rates. We also observed a significant up-regulation of pro-NPY in localized tumors in comparison to benign tissue (fold change = 5.16, $p < 0.05$), in line with previous reports .

The pro-NPY derived tryptic peptide most commonly identified by MS corresponded to a portion of the protein C terminal end, which is normally proteolytically removed to generate mature NPY (Fig. 4B). Therefore, we used an antibody generated against the C-terminal domain of pro-NPY to assess its expression levels by IHC. Pro-NPY immunoreactivity (IR) was stratified as negative, weak, moderate, and strong (Fig. 4C). In a panel of 400 surgical samples from 10 tumor types (prostate, breast, colorectal, pancreatic, lung, liver, uterus, oral, stomach, and ovarian), staining for pro-NPY was mainly observed in PCa (Fig. 4D).

This specificity for PCa was further confirmed in a panel of tumors available from the human protein atlas database.

We evaluated pro-NPY as a prognostic biomarker for PCa specific mortality in a cohort of PCa patients– Elevated mitochondrial content and activity in prostate tumors. (A)

Immunohistochemical staining of mitochondrial proteins ACAD9 and NDUFAF1 in radical prostatectomy specimens containing both tumor and benign prostate tissue. Immunoreactivity was estimated in a 0–3 scale. Student t test p values are indicated. (B) Mitochondrial complex IV activity was measured with histochemistry in frozen radical prostatectomy specimens containing both tumor (arrows) and benign prostate tissue.

Analysis of cell proliferation of 22rv1 prostate cancer cells treated with increasing amounts of the carnitine palmitoyltransferase II (CPT2) inhibitor L-aminocarnitine, or the fatty acid oxidation inhibitor trimetazidine…not screened for PSA that were diagnosed after TURP and followed up for a median of 15 y.

TMAs included PCa specimens from 289 patients from whom 196 had associated benign prostate tissue available. IHC analysis revealed that 40% of the tumors exhibited moderate or strong staining for pro-NPY with significant differences ($p < 0.001$) in expression levels of pro-NPY between normal and tumor tissues. According to the clinical routines at that time, patients were managed by watchful-waiting after diagnosis ($n = 196$) until metastasis or symptoms developed.

Patients on watchful-waiting with high levels of pro-NPY exhibited a significantly increased risk of PCa specific death compared with cases with low (negative to weak) pro- NPY levels. When samples were dichotomized based on their histological score, pro-NPY levels were no longer predictive of death for patients with high GS tumors (>7).

Instead, increased mortality was observed for patients with low GS tumors (<7) and high levels of pro-NPY. Next, we performed multivariate Cox proportional hazard analysis using age and a combined Gleason/ pro-NPY score as covariates. Patients with GS 7 or less and low pro-NPY expression have the lowest risk of disease specific death while high GS or high pro-NPY tumors have a significant increased risk of PCa death. We also analyzed a cohort of 122 prostatectomy samples and found that high pro-NPY expression did not correlate with increased biochemical recurrence after radical prostatectomy

4 NEUROENDOCRINE PCA

PCa has several modes or presentation, the most common is the adenocarcinoma type of the gland, basal and luminal, and also a neuroendocrine variety, less well known but highly aggressive. Both Staibano and Mydlo and Godec provide an extensive discussion of this form. We briefly examine it here since it relates to neuropeptide presence. Neuroendocrine Differentiation, NED, is considered a normal and ultimately lethal step in PCa progression. The neuroendocrine, NE, are a small part of the prostate cell mass. They tend to infiltrate the basal layers and at times may even penetrate into the lumen. They get signals from nerve cells and in turn emit stimulants to the surrounding cells. The set of surrounding cells impacted by this signalling also includes the blood network and their endothelial structures[10].

The NE have some specific characteristics of note here:

1. They are AR negative. Namely they androgen receptors are non-functional
2. They are PSA negative
3. They emit NPY to the surrounding cells. This is the nexus we have been examining. Perhaps it is the NE PCa via the NED that is the reason we have the nexus between lethal PCa and the presence of pro-NPY

From PCF we have a brief description of neuroendocrine PCa[11]:

Over 90 % of malignant prostate cancers occur in the form of adenocarcinoma, which is characterized by uncontrolled growth of the prostate cells that secrete the prostate specific antigen (PSA). This is why many men with malignant adenocarcinoma of the prostate have elevated PSA levels. Generally adenocarcinoma is highly treatable with excellent cure rates, even though every prostate cancer of this type has the subtype neuroendocrine cancer cells scattered throughout the tumor. (Even benign, normal prostate glands have a tiny population, roughly about 0.1 %, of neuroendocrine cells, nested throughout the gland. See Figure One. It is thought these neuroendocrine cells normally play a role in early prostate development or perhaps function.)

Cells staining brown are neuroendocrine cells in normal prostate (left); adenocarcinoma prostate cancer tissue not treated with hormone therapy (middle); and advanced metastatic treatment-resistant prostate cancer tissue treated with hormone therapy (right). As hormone therapy treatment commences the number of neuroendocrine cells significantly increases.

The neuroendocrine cells scattered throughout adenocarcinoma of the prostate generally make up about 1% or less of the total tumor. Neuroendocrine prostate cancer (NEPC) is diagnosed when vast numbers of neuroendocrine cells are found in a tumor. "Neuroendocrine prostate

[10] See Mydlo and Godec p 150.

[11] http://www.pcf.org/site/c.leJRIROrEpH/b.8747629/k.F201/Subtype_of_Highly_Aggressive_Prostate_Cancer_Increasing_Tied_to_Drug_Resistance_to_Hormone_Therapy.htm

cancer cells look small under the microscope," …"And they tend to metastasize not just to bone, as is common in adenocarcinoma, but to liver or other abdominal visceral organs." There are also a number of biochemical markers for NEPC that can be detected by tissue-staining lab tests, which aids in diagnosis of this disease.

Very rarely are men newly diagnosed with prostate cancer that is the neuroendocrine subtype. When this does occur it is called de novo NEPC, referring to the thought that this subtype of cancer has been there de novo, or from the beginning. Far more commonly, NEPC is a result of treatment with hormone therapy, and is known as treatment-related NEPC, or t-NEPC.

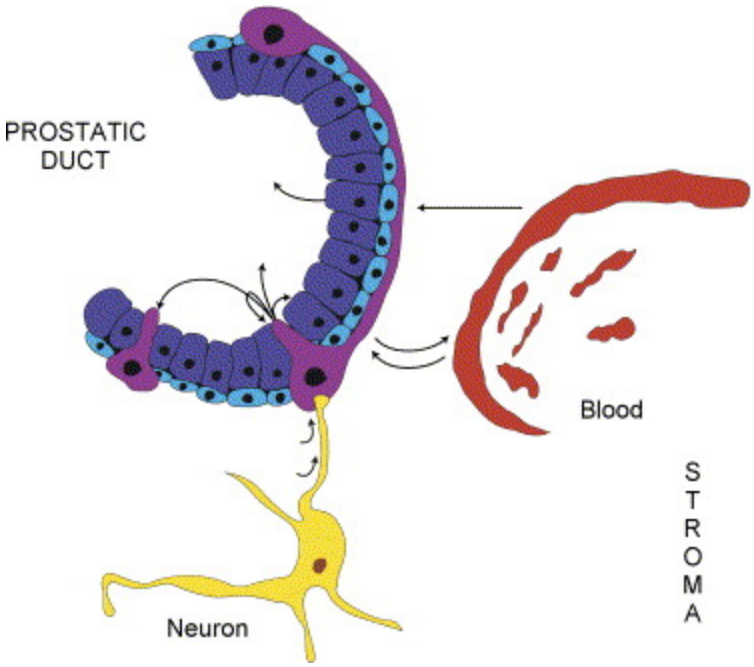
From Aggarwal et al:

Neuroendocrine prostate cancer (NEPC) encompasses various clinical contexts, ranging from the de novo presentation of small cell prostatic carcinoma to a treatment-emergent transformed phenotype that arises from typical adenocarcinoma of the prostate. The development of resistance to potent androgen receptor signaling inhibition may be associated with the emergence of aggressive phenotype, advanced castration-resistant NEPC.

Clinically, small cell prostate cancer and NEPC are often manifested by the presence of visceral or large soft tissue metastatic disease, a disproportionately low serum prostate-specific antigen level relative to the overall burden of disease, and a limited response to targeting of the androgen signaling axis. These tumors are often characterized by loss of androgen receptor expression, loss of retinoblastoma tumor suppressor copy number or expression, amplification of Aurora kinase A and N-Myc, and activation of the PI3K pathway.

However, a consensus phenotype-genotype definition of NEPC has yet to emerge, and molecularly based biomarkers are needed to expand on traditional morphologic and immunohistochemical markers of NEPC to fully define the spectrum of this aggressive, androgen receptor-independent disease. Emerging studies implicate a shared clonal origin with prostatic adenocarcinoma in many cases, with the adaptive emergence of unique cellular programming and gene expression profiles.

Ongoing clinical studies are focused on developing novel targeted therapeutic approaches for this high-risk, lethal subset of disease, to improve on the limited durations of response often observed with traditional platinum-based chemotherapy.



Schematic drawing illustrating possible regulatory pathways of the prostatic neuroendocrine cell. Regulation may be endocrine, paracrine (from adjacent neuroendocrine cells), autocrine (self-regulation), neurocrine or, in the open cell type, from the luminal contents. In addition, possible secretory pathways of these cells include endocrine, paracrine, neurocrine, and exocrine (lumencrine) secretion.

As Parimi et al state:

Neuroendocrine cells are one of the epithelial populations in the prostate. Neuroendocrine differentiation (NED) has been observed in prostate cancer. In addition to small cell neuroendocrine carcinomas and carcinoid tumors of the prostate, prostatic adenocarcinomas may have NED. The incidence and clinical relevance of NED in prostatic adenocarcinoma is not clearly understood because of conflicting results in the reported studies, and evaluation of NED is not routinely performed in clinical practice. …we are stratifying these lesions into separate subtypes based on histologic parameters such as tumor morphology, neuroendocrine cell density and distribution and clinical parameters.

We also want to identify current controversies and confusing issues not totally resolved in this topic for further investigations. Eventually a clearer understanding of this phenomenon and appropriate handling NED in prostate cancer will benefit clinical practice.

They continue to describe NE as follows and then details some of the specifics in type:

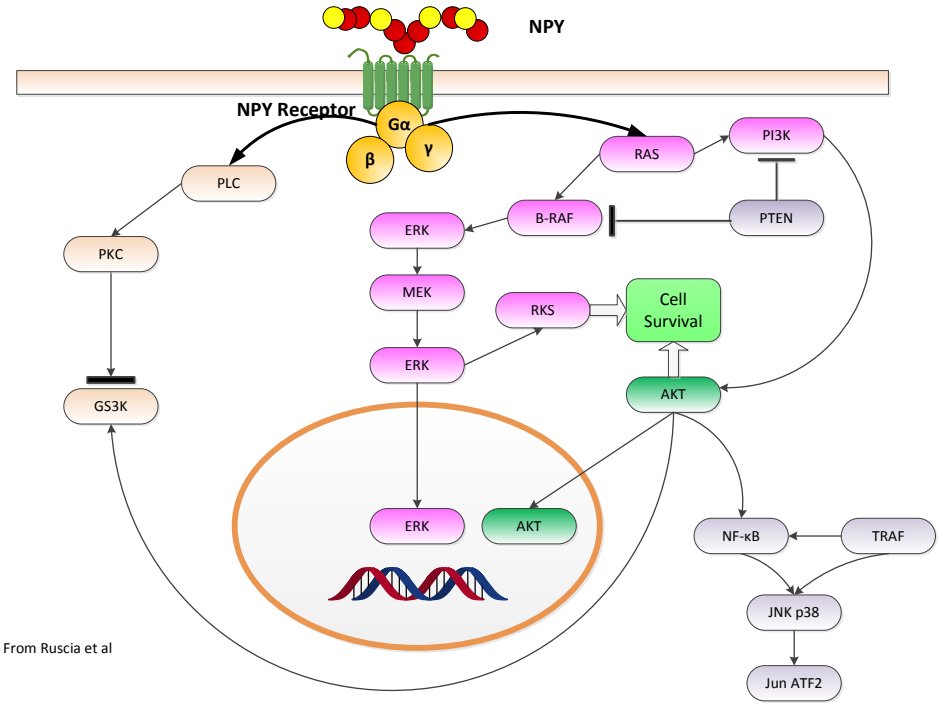
Even though the definition of neuroendocrine prostatic carcinomas is still emerging, in this review from a morphologic standpoint, neuroendocrine prostatic carcinomas is considered as a special type of neuroendocrine differentiation of prostatic epithelial neoplasms (Table 2). Neuroendocrine carcinoma of the prostate may represent a subset of prostate cancer phenotypes which may be linked to resistance to androgen receptor (AR) signaling inhibition with

aggressive tumor characteristics and a largely dismal prognosis. Neuroendocrine prostatic carcinomas (NEPC) are often diagnosed on primary prostate needle biopsy or on biopsies of metastatic lesions with negative or low PSA levels.

Based on morphological characteristics, proliferative index and grade, this spectrum tumor phenotypes can be subdivided into: small cell carcinomas, large cell neuroendocrine carcinomas and carcinoid tumors. Neuroendocrine differentiation within conventional PCa will be discussed below separately. In general, NEPC differ from conventional PCa histologically by presence of neuroendocrine cells which do not express generic PCa markers like AR, P501S, PSMA, PSAP and PSA but characteristically expresses neuroendocrine markers such as chromogranin A, synaptophysin, CD56, and NSE

Thus the existence of NED and the relationship to NPY and pro-NPY is worthy of further examination.

Also, the work by Ruscica et al provides a substantial basis for examining the putative pathway influence of NPY on PCa. The following Figure depicts the putative pathways they propose and have examined in multiple cell lines.



The authors state as to the above:

Proposed mechanisms of ERK1/2 activation by NPY in human prostate cancer cells. In PC3 cells, NPY activates ERK1/2 via PKC and, possibly, via RAS/RAF, whereas in DU145 cells, PKC activation is not required for NPY-induced ERK1/2 phosphorylation.

This may then provide a reasonable method of activation under PCa.

5 OBSERVATIONS

As noted we continue to see the reporting of many new markers for both detection of and prognosis of PCa. However in many cases these markers are not always causative but adventitious. They just happen to be there and may or may not reflect a process, one which may allow for a therapeutic approach.

As we have noted in Inglesias-Gato et al where they conclude:

The combined assessment of pro-NPY levels and ERG status improves prediction of PCa related death High NPY protein expression was recently demonstrated in prostate tumors harboring the TMPRSS2-ERG fusion gene .

Previous evaluation of ERG expression in the … cohort showed it to be related to increased risk of disease- related mortality. Therefore we analyzed whether pro- NPY expression correlated with ERG expression in our sample cohort. Forty-four percent of the tumors included in the analysis were positive for ERG expression and of these, 52% showed high pro-NPY levels.

Patients with tumors expressing high levels of pro-NPY with positive ERG protein expression have a significant increase in the relative risk of PCa related death, especially within the low GS group. Accordingly, multivariate regression analysis shows that high expression of both ERG and NPY increases the risk for PCa mortality independently of GS

We use this to examine a few issues.

5.1 CAUSATIVE RESULTS

The above indicate the ERG expression which we know has causative effect, especially when we see the ERG: TMPRSS merge. However the relationship and systemic details on the pro-NPY effect are missing. One would like to see this in some detail.

5.2 THERAPEUTIC TARGETS

It is not clear if NPY or pro-NPY can be therapeutic targets or even more so the underlying gene. Since the causative effect does not seem apparent one wonders if having a prognostic marker is of substantial value. Metastasis is most likely an existing fact, albeit on a micro scale. Thus the question can be stated: of what clinical value do we have measuring pro-NPY?

5.3 PROCESS CONTROL

What are the pathways, receptors, ligand, and the like that are involved in pro-NPY and NPY generation, communication, and activation? The details appear to be missing in this overall analysis.

5.4 NEUROENDOCRINE DIFFERENTIATION

When we examined the NED type of progression we saw the presence of NPY as a secretion from the NE cells to the remainder of the prostate. We also understand the evolution of NED in most PCas and the question posed would be; what is the driver for NED? Also does the NE communications somehow relate to stress activation via the nerve cells communicating with the NE calls. Also is it the endothelial enhancement of the NE cells that facilitate the metastatic growth.

6 APPENDIX (KHAN ET AL TABLES)

Supplementary Table 3. List of proteins up-regulated in localized prostate cancer compared to benign. A total of 80 proteins were up-regulated in at least 2 of the localized prostate cancer samples analyzed compared to benign specimens.

Gene Symbol	IPI Number	Description
AGR2	IPI00007427	AGR2
ANP32B	IPI00007423	Isoform 1 of Acidic leucine-rich nuclear phosphoprotein 32 family member B
ANXA7	IPI00002460	Isoform 1 of Annexin A7
ASPN	IPI00418431	ASPN protein
ATP5C1	IPI00395769	Isoform Heart of ATP synthase gamma chain, mitochondrial precursor
ATP5D	IPI00024920	ATP synthase delta chain, mitochondrial precursor
BCAM	IPI00002406	Lutheran blood group glycoprotein precursor
BCAM	IPI00793616	28 kDa protein
C14orf166	IPI00006980	Protein C14orf166
C1orf116	IPI00028392	specifically androgen-regulated protein
C21orf33	IPI00024913	Isoform Long of ES1 protein homolog, mitochondrial precursor
C21orf33	IPI00218482	Isoform Short of ES1 protein homolog, mitochondrial precursor
CA2	IPI00218414	Carbonic anhydrase 2
CA3	IPI00216983	Carbonic anhydrase 3
CBX3; LOC653972	IPI00297579	Chromobox protein homolog 3
CKMT1B; CKMT1A	IPI00658109	Creatine kinase, ubiquitous mitochondrial precursor
CNN3	IPI00216682	Calponin-3
CRYAB	IPI00021369	Alpha crystallin B chain
CYB5R3	IPI00328415	Isoform 1 of NADH-cytochrome b5 reductase
DLAT	IPI00021338	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial precursor
EIF4B	IPI00012079	Eukaryotic translation initiation factor 4B
FABP5; FABP5L7	IPI00007797	Fatty acid-binding protein, epidermal
FBN1	IPI00328113	Fibrillin-1 precursor
FKBP1A	IPI00413778	FKBP1A protein
FTH1	IPI00554521	Ferritin heavy chain
FUBP3	IPI00377261	Isoform 1 of Far upstream element-binding protein 3
GDF15	IPI00306543	Growth/differentiation factor 15 precursor
GOLM1	IPI00171411	Golgi phosphoprotein 2
GPX3	IPI00026199	Glutathione peroxidase 3 precursor
HDGF	IPI00020956	Hepatoma-derived growth factor
HEBP2	IPI00644697	HEBP2 protein (Fragment)

Gene Symbol	IPI Number	Description
HEXA	IPI00027851	Beta-hexosaminidase alpha chain precursor
HEXB	IPI00012585	Beta-hexosaminidase beta chain precursor
HNRPF	IPI00003881	Heterogeneous nuclear ribonucleoprotein F
HSP90AB1	IPI00414676	Heat shock protein HSP 90-beta
ILF3	IPI00219330	Isoform 5 of Interleukin enhancer-binding factor 3
LUC7L2	IPI00006932	Isoform 1 of Putative RNA-binding protein Luc7-like 2
LUM	IPI00020986	Lumican precursor
MARCKS	IPI00219301	Myristoylated alanine-rich C-kinase substrate
MARCKSL1	IPI00641181	MARCKS-related protein
MB	IPI00217493	Myoglobin
MIA3	IPI00374065	similar to melanoma inhibitory activity 3 isoform 1
NPY	IPI00001506	Neuropeptide Y precursor
OGN	IPI00025465	Mimecan precursor
PARK7	IPI00298547	Protein DJ-1
PHB	IPI00017334	Prohibitin
PPIB	IPI00646304	peptidylprolyl isomerase B precursor
RAB10	IPI00016513	Ras-related protein Rab-10
RANBP1	IPI00414127	Ran-specific GTPase-activating protein
RAP1B	IPI00015148	Ras-related protein Rap-1b precursor
RPS7	IPI00013415	40S ribosomal protein S7
RRBP1	IPI00215743	Isoform 3 of Ribosome-binding protein 1
SERBP1	IPI00410693	Isoform 1 of Plasminogen activator inhibitor 1 RNA-binding protein
SFRS7	IPI00003377	Isoform 1 of Splicing factor, arginine/serine-rich 7
SLC25A3	IPI00022202	Isoform A of Phosphate carrier protein, mitochondrial precursor
SNRPA	IPI00012382	U1 small nuclear ribonucleoprotein A
SOD3	IPI00027827	Extracellular superoxide dismutase [Cu-Zn] precursor
SPTBN1	IPI00005614	Isoform Long of Spectrin beta chain, brain 1
SRP14	IPI00293434	Signal recognition particle 14 kDa protein
SSB	IPI00009032	Lupus La protein
STEAP4	IPI00002856	CDNA: FLJ23153 fis, clone LNG09441
TCEB1	IPI00300341	Transcription elongation factor B polypeptide 1
TCEB1	IPI00791185	11 kDa protein
TMSB10	IPI00220827	Thymosin beta-10
TPP1	IPI00298237	Isoform 1 of Tripeptidyl-peptidase 1 precursor
TPP1	IPI00554617	Isoform 2 of Tripeptidyl-peptidase 1 precursor
TROVE2	IPI00019450	Isoform Long of 60 kDa SS-A/Ro ribonucleoprotein
U2AF2	IPI00031556	Splicing factor U2AF 65 kDa subunit
UGDH	IPI00031420	UDP-glucose 6-dehydrogenase
UQCRB	IPI00220416	Ubiquinol-cytochrome c reductase complex 14 kDa protein
UQCRC1	IPI00013847	Ubiquinol-cytochrome-c reductase complex core protein 1, mitochondrial precursor
VIM	IPI00418471	Vimentin
YBX1	IPI00031812	Nuclease sensitive element-binding protein 1

Gene Symbol	IPI Number	Description
-	IPI00022970	Nucleoprotein TPR
-	IPI00329745	CDNA FLJ43793 fis, clone TESTI4000014, highly similar to 130 kDa leucine-rich protein
-	IPI00397828	20 kDa protein
-	IPI00472102	61 kDa protein
-	IPI00555692	ANXA4 protein
-	IPI00783839	Spectrin, beta, non-erythrocytic 1 isoform 1 variant
-	IPI00788958	Heat shock 70kDa protein 9B variant (Fragment)

Supplementary Table 4. List of proteins down-regulated in localized prostate cancer compared to benign. A total of 81 proteins were down-regulated in at least 2 of the localized prostate cancer samples analyzed compared to benign specimens.

Gene Symbol	IPI Number	Description
ACPP	IPI00289983	ACPP protein
ACPP	IPI00396434	Prostatic acid phosphatase precursor
ACTN1	IPI00013508	Alpha-actinin-1
ALDH9A1	IPI00479877	aldehyde dehydrogenase 9A1
ANXA3	IPI00024095	Annexin A3
APOA1BP	IPI00168479	apolipoprotein A-I binding protein precursor
ASAH1	IPI00059685	ASAH1 protein
ATP5F1	IPI00029133	ATP synthase B chain, mitochondrial precursor
ATP6V1A	IPI00007682	Vacuolar ATP synthase catalytic subunit A, ubiquitous isoform
AZGP1	IPI00166729	alpha-2-glycoprotein 1, zinc
C10orf116	IPI00020017	Adipose most abundant gene transcript 2
C4A;C4B	IPI00032258	Complement C4-A precursor
CALD1	IPI00014516	Isoform 1 of Caldesmon
CAPG	IPI00027341	Macrophage capping protein
CBR1	IPI00295386	Carbonyl reductase [NADPH] 1
CBR1	IPI00795334	24 kDa protein
CPE	IPI00031121	Carboxypeptidase E precursor
CSRP1	IPI00442073	Cysteine and glycine-rich protein 1
DES	IPI00465084	Desmin
DLD	IPI00015911	Dihydrolipoyl dehydrogenase, mitochondrial precursor
DMN	IPI00299301	Isoform 1 of Desmuslin
DMN	IPI00299302	Isoform 2 of Desmuslin
DSP	IPI00013933	Isoform DPI of Desmoplakin
FBLN1	IPI00218803	Isoform B of Fibulin-1 precursor
FLNA	IPI00302592	filamin A, alpha
GANAB	IPI00011454	Isoform 2 of Neutral alpha-glucosidase AB precursor
GLUD1	IPI00016801	Glutamate dehydrogenase 1, mitochondrial precursor
GSTP1	IPI00219757	Glutathione S-transferase P
HSPB1	IPI00025512	Heat-shock protein beta-1
IDH2	IPI00011107	Isocitrate dehydrogenase [NADP], mitochondrial precursor
IGHA1; IGHV3OR16-13	IPI00061977	IGHA1 protein
IGL@	IPI00154742	IGL@ protein
IGLL1	IPI00013438	Immunoglobulin lambda-like polypeptide 1 precursor
KRT1	IPI00220327	Keratin, type II cytoskeletal 1
LAMC1	IPI00298281	Laminin subunit gamma-1 precursor
LDHB	IPI00219217	L-lactate dehydrogenase B chain
LPP	IPI00023704	Lipoma-preferred partner
LTF	IPI00298860	Growth-inhibiting protein 12

Gene Symbol	IPI Number	Description
M6PRBP1	IPI00303882	Isoform B of Mannose-6-phosphate receptor-binding protein 1
MSMB	IPI00414609	Isoform PSP94 of Beta-microseminoprotein precursor
MSN	IPI00219365	Moesin
MYH11	IPI00020501	Myosin-11
MYH11	IPI00024870	smooth muscle myosin heavy chain 11 isoform SM2A
MYL9	IPI00030929	myosin regulatory light chain 9 isoform b
MYL9	IPI00220278	Myosin regulatory light chain 2, smooth muscle isoform
MYLK	IPI00221255	Isoform 2 of Myosin light chain kinase, smooth muscle
MYLK	IPI00221259	Isoform Del-1790 of Myosin light chain kinase, smooth muscle
NANS	IPI00147874	Sialic acid synthase
NDRG1	IPI00022078	Protein NDRG1
NID2	IPI00028908	Nidogen-2 precursor
NONO	IPI00304596	Non-POU domain-containing octamer-binding protein
PALLD	IPI00292009	palladin
PBEF1	IPI00018873	Isoform 1 of Nicotinamide phosphoribosyltransferase
PCBP2	IPI00012066	poly(rC)-binding protein 2 isoform b
PDLIM7	IPI00023122	Isoform 1 of PDZ and LIM domain protein 7
PEA15	IPI00014850	Astrocytic phosphoprotein PEA-15
PGK1	IPI00169383	Phosphoglycerate kinase 1
PGRMC1	IPI00220739	Membrane-associated progesterone receptor component 1
PHGDH	IPI00011200	D-3-phosphoglycerate dehydrogenase
PPP2R1A	IPI00168184	cDNA FLJ34068 fis, clone FCBBF3001918, highly similar to serine/threonine protein phosphatase 2A, 65 kDa regulatory subunit A, alpha isoform
PRDX5	IPI00024915	Isoform Mitochondrial of Peroxiredoxin-5, mitochondrial precursor
S100A6	IPI00027463	Protein S100-A6
SELENBP1	IPI00745729	selenium binding protein 1
SH3BGRL	IPI00025318	SH3 domain-binding glutamic acid-rich-like protein
SMTN	IPI00024007	Isoform B of Smoothelin
SOD2	IPI00022314	Superoxide dismutase [Mn], mitochondrial precursor
SORD	IPI00216057	Sorbitol dehydrogenase
SYNPO2	IPI00173549	synaptopodin 2
SYNPO2	IPI00735855	Synaptopodin-2
TAGLN	IPI00216138	Transgelin
TNC	IPI00220211	Isoform 2 of Tenascin precursor
TNC	IPI00220216	Isoform 6 of Tenascin precursor
TPM1	IPI00000230	tropomyosin 1 alpha chain isoform 2
TPM1	IPI00455050	Sarcomeric tropomyosin kappa
TPSAB1	IPI00010274	Isoform 1 of Tryptase alpha-1 precursor
TPSB2	IPI00382751	Mast cell tryptase beta III
UBE2L3	IPI00021347	Ubiquitin-conjugating enzyme E2 L3

Gene Symbol	IPI Number	Description
UQCRC2	IPI00305383	Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial precursor
YWHAB	IPI00216318	Isoform Long of 14-3-3 protein beta/alpha
-	IPI00219910	23 kDa protein
-	IPI00419307	alpha isoform of regulatory subunit A, protein phosphatase 2

Supplementary Table 5. List of proteins up-regulated in metastatic prostate cancer compared to localized prostate cancer. A total of 141 proteins were elevated in at least 60 % of the metastatic samples analyzed compared to localized cancer.

Gene Symbol	IPI Number	Description
ABHD11	IPI00171152	abhydrolase domain containing 11 isoform 2
ACAT1	IPI00062003	ACAT1 protein
ACLY	IPI00021290	ATP-citrate synthase
ACLY	IPI00394838	ATP citrate lyase isoform 2
ALCAM	IPI00015102	Isoform 1 of CD166 antigen precursor
ANP32B	IPI00007423	Isoform 1 of Acidic leucine-rich nuclear phosphoprotein 32 family member B
APEX1	IPI00215911	DNA-(apurinic or apyrimidinic site) lyase
ARF1	IPI00215914	ADP-ribosylation factor 1
ATP5A1	IPI00440493	ATP synthase subunit alpha, mitochondrial precursor
C20orf3	IPI00031131	Adipocyte plasma membrane-associated protein
CANX	IPI00020984	Calnexin precursor
CBX1	IPI00010320	Chromobox protein homolog 1
CBX3; LOC653972	IPI00297579	Chromobox protein homolog 3
CCT2	IPI00297779	T-complex protein 1 subunit beta
CCT3	IPI00290770	chaperonin containing TCP1, subunit 3 isoform b
CCT3	IPI00553185	T-complex protein 1 subunit gamma
CCT5	IPI00010720	T-complex protein 1 subunit epsilon
CCT8	IPI00302925	Chaperonin containing TCP1, subunit 8 (Theta) variant
CCT8	IPI00784090	T-complex protein 1 subunit theta
COX5A	IPI00025086	Cytochrome c oxidase subunit 5A, mitochondrial precursor
COX7A2	IPI00026570	Cytochrome c oxidase polypeptide VIIa-liver/heart, mitochondrial precursor
CS	IPI00025366	Citrate synthase, mitochondrial precursor
CTSD	IPI00011229	Cathepsin D precursor
CYCS	IPI00465315	Cytochrome c
DDX17	IPI00023785	Isoform 1 of Probable ATP-dependent RNA helicase DDX17
DDX17	IPI00651653	Isoform 3 of Probable ATP-dependent RNA helicase DDX17
DECR1	IPI00003482	2,4-dienoyl-CoA reductase, mitochondrial precursor
ECH1	IPI00011416	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial precursor
ECH1	IPI00445114	cDNA FLJ44603 fis, clone BRACE1000475, highly similar to Delta3,5- delta2,4-dienoyl-CoA isomerase, mitochondrial
EIF4G1	IPI00220365	EIF4G1 variant protein (Fragment)
ENO1	IPI00465248	Isoform alpha-enolase of Alpha-enolase
EZR	IPI00746388	Ezrin
FASN	IPI00026781	Fatty acid synthase
FBP1	IPI00073772	Fructose-1,6-bisphosphatase 1
FUBP1	IPI00375441	Isoform 1 of Far upstream element-binding protein 1
GANAB	IPI00011454	Isoform 2 of Neutral alpha-glucosidase AB precursor

Gene Symbol	IPI Number	Description
GNB2L1	IPI00641950	Lung cancer oncogene 7
GPD2	IPI00017895	Isoform 1 of Glycerol-3-phosphate dehydrogenase, mitochondrial precursor
HADH	IPI00294398	Isoform 1 of Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial precursor
HADHB	IPI00022793	Trifunctional enzyme beta subunit, mitochondrial precursor
HDGF2	IPI00013290	hepatoma-derived growth factor-related protein 2 isoform 1
HIST2H2BE	IPI00003935	Histone H2B type 2-E
HMG1L10	IPI00018755	High mobility group protein 1-like 10
HMGB2	IPI00219097	High mobility group protein B2
HNRNPA2B1	IPI00396378	Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1
HNRNPC	IPI00216592	Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2
HNRPAB	IPI00106509	Isoform 4 of Heterogeneous nuclear ribonucleoprotein A/B
HNRPAB	IPI00334587	Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B
HNRPD	IPI00028888	Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0
HNRPD	IPI00220683	Isoform 2 of Heterogeneous nuclear ribonucleoprotein D0
HNRPD	IPI00220684	Isoform 3 of Heterogeneous nuclear ribonucleoprotein D0
HNRPF	IPI00003881	Heterogeneous nuclear ribonucleoprotein F
HNRPH1	IPI00013881	Heterogeneous nuclear ribonucleoprotein H
HSD17B10	IPI00017726	Isoform 1 of 3-hydroxyacyl-CoA dehydrogenase type-2
HSD17B10	IPI00336094	Isoform 2 of 3-hydroxyacyl-CoA dehydrogenase type-2
HSP90AA1	IPI00382470	Heat shock protein HSP 90-alpha 2
HSP90AB1	IPI00414676	Heat shock protein HSP 90-beta
HSP90B1	IPI00027230	Endoplasmin precursor
HSPA8	IPI00003865	Isoform 1 of Heat shock cognate 71 kDa protein
HSPA9	IPI00007765	Stress-70 protein, mitochondrial precursor
HSPE1	IPI00220362	10 kDa heat shock protein, mitochondrial
ILF3	IPI00219330	Isoform 5 of Interleukin enhancer-binding factor 3
IMMT	IPI00009960	Isoform 1 of Mitochondrial inner membrane protein
IVD	IPI00645805	Isovaleryl-CoA dehydrogenase, mitochondrial precursor
KPNB1	IPI00001639	Importin beta-1 subunit
LDHA	IPI00217966	Isoform 1 of L-lactate dehydrogenase A chain
LDHA	IPI00607708	Isoform 2 of L-lactate dehydrogenase A chain
LMNB1	IPI00217975	Lamin-B1
LOC387867	IPI00398958	similar to 40S ribosomal protein SA
LOC646195; RPS28; LOC645899	IPI00719622	40S ribosomal protein S28
LONP1	IPI00005158	Lon protease homolog, mitochondrial precursor
LONP1	IPI00642982	Hypothetical protein
MDH2	IPI00291006	Malate dehydrogenase, mitochondrial precursor
NAP1L1	IPI00023860	Nucleosome assembly protein 1-like 1
NAP1L1	IPI00789029	43 kDa protein
NDUFS3	IPI00025796	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial precursor

Gene Symbol	IPI Number	Description
NHP2L1	IPI00026167	NHP2-like protein 1
NOLC1	IPI00216654	Isoform Beta of Nucleolar phosphoprotein p130
NONO	IPI00304596	Non-POU domain-containing octamer-binding protein
NPM1	IPI00220740	Isoform 2 of Nucleophosmin
NUCKS1	IPI00022145	Isoform 1 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate
NUCKS1	IPI00514586	Isoform 2 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate
P4HB	IPI00010796	Protein disulfide-isomerase precursor
PCBP1	IPI00016610	Poly(rC)-binding protein 1
PCBP2	IPI00012066	poly(rC)-binding protein 2 isoform b
PDIA4	IPI00009904	Protein disulfide-isomerase A4 precursor
PDIA6	IPI00299571	Isoform 2 of Protein disulfide-isomerase A6 precursor
PFN2	IPI00107555	Isoform IIb of Profilin-2
PGK1	IPI00169383	Phosphoglycerate kinase 1
PHB	IPI00017334	Prohibitin
PHB2	IPI00027252	Prohibitin-2
PPA1	IPI00015018	Inorganic pyrophosphatase
PRDX1	IPI00000874	Peroxiredoxin-1
PRDX3	IPI00024919	Thioredoxin-dependent peroxide reductase, mitochondrial precursor
PTBP1	IPI00179964	Isoform 1 of Polypyrimidine tract-binding protein 1
RAB7A	IPI00016342	Ras-related protein Rab-7
RAN	IPI00643041	GTP-binding nuclear protein Ran
RBBP4	IPI00328319	Histone-binding protein RBBP4
RBBP7	IPI00395865	Histone-binding protein RBBP7
RPLP2	IPI00008529	60S acidic ribosomal protein P2
RPN1	IPI00025874	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor
SET	IPI00072377	Isoform 1 of Protein SET
SFPQ	IPI00010740	Isoform Long of Splicing factor, proline- and glutamine-rich
SFRS2	IPI00005978	Splicing factor, arginine/serine-rich 2
SFRS6	IPI00012345	Isoform SRP55-1 of Splicing factor, arginine/serine-rich 6
SFRS7	IPI00003377	Isoform 1 of Splicing factor, arginine/serine-rich 7
SLC25A3	IPI00022202	Isoform A of Phosphate carrier protein, mitochondrial precursor
SLC25A5	IPI00007188	ADP/ATP translocase 2
SNRPA	IPI00012382	U1 small nuclear ribonucleoprotein A
SNRPE	IPI00029266	Small nuclear ribonucleoprotein E
SSBP1	IPI00029744	Single-stranded DNA-binding protein, mitochondrial precursor
STIP1	IPI00013894	Stress-induced-phosphoprotein 1
STMN1	IPI00479997	Stathmin
STMN1	IPI00642012	Stathmin 1/oncoprotein 18
TAF15	IPI00020194	Isoform Short of TATA-binding protein-associated factor 2N
TBCA	IPI00217236	Tubulin-specific chaperone A
TCEB1	IPI00300341	Transcription elongation factor B polypeptide 1

Gene Symbol	IPI Number	Description
TCEB1	IPI00791185	11 kDa protein
TIA1	IPI00291398	Isoform Long of Nucleolysin TIA-1 isoform p40
TRIM28	IPI00438229	Isoform 1 of Transcription intermediary factor 1-beta
TRIM28	IPI00438230	Isoform 2 of Transcription intermediary factor 1-beta
TUBB2C	IPI00007752	Tubulin beta-2C chain
TUFM	IPI00027107	Tu translation elongation factor, mitochondrial
UBA1	IPI00645078	Ubiquitin-activating enzyme E1
UQCRC1	IPI00013847	Ubiquinol-cytochrome-c reductase complex core protein 1, mitochondrial precursor
UQCRC2	IPI00305383	Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial precursor
VCP	IPI00022774	Transitional endoplasmic reticulum ATPase
VDAC1	IPI00216308	Voltage-dependent anion-selective channel protein 1
VDAC2	IPI00024145	Isoform 1 of Voltage-dependent anion-selective channel protein 2
VDAC3	IPI00031804	Isoform 1 of Voltage-dependent anion-selective channel protein 3
YWHAG	IPI00220642	14-3-3 protein gamma
YWHAZ	IPI00021263	14-3-3 protein zeta/delta
-	IPI00030154	Proteasome activator complex subunit 1
-	IPI00216027	Isoform 4 of Voltage-dependent anion-selective channel protein 2
-	IPI00218323	Tumor protein D52
-	IPI00334775	85 kDa protein
-	IPI00382617	P37 AUF1
-	IPI00419307	alpha isoform of regulatory subunit A, protein phosphatase 2
-	IPI00472102	61 kDa protein
-	IPI00645907	fatty acid synthase
-	IPI00742905	ATP-dependent RNA helicase A

Supplementary Table 6. List of proteins down-regulated in metastatic prostate cancer compared to localized prostate cancer. A total of 165 proteins were down-regulated in at least 60 % of the metastatic samples analyzed compared to localized cancer.

Gene Symbol	IPI Number	Description
A1BG	IPI00022895	Alpha-1B-glycoprotein precursor
ACPP	IPI00289983	ACPP protein
ACPP	IPI00396434	Prostatic acid phosphatase precursor
ACTN1	IPI00013508	Alpha-actinin-1
ACTN1	IPI00759776	Actinin alpha 1 isoform b
ADH5	IPI00746777	Class III alCohol dehydrogenase 5 Chi subunit
AHSG	IPI00022431	Alpha-2-HS-glycoprotein precursor
ALDH1B1	IPI00103467	Aldehyde dehydrogenase X, mitochondrial precursor
AMBP	IPI00022426	AMBP protein precursor
ANXA1	IPI00218918	Annexin A1
ANXA3	IPI00024095	Annexin A3
ANXA6	IPI00002459	annexin VI isoform 2
AOC3	IPI00004457	Membrane copper amine oxidase
APEH	IPI00337741	Acylamino-acid-releasing enzyme
APOA1	IPI00021841	Apolipoprotein A-I precursor
APOA2	IPI00021854	Apolipoprotein A-II precursor
APOA4	IPI00304273	Apolipoprotein A-IV precursor
APOH	IPI00298828	Beta-2-glycoprotein 1 precursor
ARL6IP5	IPI00007426	PRA1 family protein 3
AZGP1	IPI00166729	alpha-2-glycoprotein 1, zinc
B2M	IPI00004656	Beta-2-microglobulin precursor
BAG2	IPI00000643	BAG family molecular chaperone regulator 2
BCAM	IPI00002406	Lutheran blood group glycoprotein precursor
BCAM	IPI00793616	28 kDa protein
BCOR	IPI00100291	Isoform 2 of BCL-6 corepressor
BGN	IPI00010790	Biglycan precursor
BGN	IPI00385748	CDNA FLJ35635 fis, clone SPLEN2011805, highly similar to bone/cartilage proteoglycan I
C4A; C4B	IPI00032258	Complement C4-A precursor
CALD1	IPI00014516	Isoform 1 of Caldesmon
CAST	IPI00760715	calpastatin isoform e
CAV1	IPI00009236	Isoform Alpha of Caveolin-1
CAV1	IPI00759683	Isoform Beta of Caveolin-1
CKB	IPI00022977	Creatine kinase B-type
CLIC4	IPI00001960	Chloride intracellular channel protein 4
CNN1	IPI00021264	Calponin-1
CNN3	IPI00216682	Calponin-3
COL14A1	IPI00176193	Isoform 1 of Collagen alpha-1(XIV) chain precursor
COL18A1	IPI00022822	Isoform 2 of Collagen alpha-1(XVIII) chain precursor
COL18A1	IPI00479309	71 kDa protein

Gene Symbol	IPI Number	Description
COL1A2	IPI00304962	Collagen alpha-2(I) chain precursor
COL6A1	IPI00291136	Collagen alpha-1(VI) chain precursor
COL6A2	IPI00304840	Isoform 2C2 of Collagen alpha-2(VI) chain precursor
COL6A3	IPI00072917	alpha 3 type VI collagen isoform 3 precursor
CPE	IPI00031121	Carboxypeptidase E precursor
CRYAB	IPI00021369	Alpha crystallin B chain
CSRP1	IPI00442073	Cysteine and glycine-rich protein 1
DBI	IPI00010182	Isoform a 1 of Acyl-CoA-binding protein
DES	IPI00465084	Desmin
DHRS7	IPI00006957	Isoform 1 of Dehydrogenase/reductase SDR family member 7 precursor
DHRS7	IPI00470418	Hypothetical protein DKFZp564H1664
DMN	IPI00299301	Isoform 1 of Desmuslin
DMN	IPI00299302	Isoform 2 of Desmuslin
DSTN	IPI00473014	Destrin
EHD2	IPI00100980	EH domain-containing protein 2
EMILIN1	IPI00013079	EMILIN-1 precursor
FABP3	IPI00219684	Fatty acid-binding protein, heart
FAM129A	IPI00328350	Niban protein
FHL1	IPI00014398	Four and a half LIM domains 1 variant
FKBP1A	IPI00413778	FKBP1A protein
FLNA	IPI00302592	filamin A, alpha
FLNC	IPI00178352	Isoform 1 of Filamin-C
FSTL1	IPI00029723	Follistatin-related protein 1 precursor
GPX3	IPI00026199	Glutathione peroxidase 3 precursor
GSN	IPI00026314	Isoform 1 of Gelsolin precursor
GSTM3	IPI00246975	Glutathione S-transferase Mu 3
GSTO1	IPI00019755	Glutathione transferase omega-1
GSTP1	IPI00219757	Glutathione S-transferase P
HIST1H1C	IPI00217465	Histone H1.2
HIST1H1E	IPI00217467	Histone H1.4
HLA-A29.1; HLA-C; HLA-A; MICA; LOC730410; HLA-B	IPI00718924	HLA class I histocompatibility antigen, B-51 alpha chain precursor
HSPB6	IPI00022433	Alpha crystallin family protein
HSPG2	IPI00024284	Basement membrane-specific heparan sulfate proteoglycan core protein precursor
IGL@	IPI00154742	IGL@ protein
ILK	IPI00013219	Integrin-linked protein kinase
ITGA5	IPI00306604	Integrin alpha-5 precursor
KLK3	IPI00010858	Prostate-specific antigen precursor
KLK3	IPI00640523	prostate specific antigen isoform 4 preproprotein

Gene Symbol	IPI Number	Description
KRT18	IPI00554788	49 kDa protein
KRT5	IPI00009867	Keratin, type II cytoskeletal 5
KRT77	IPI00376379	Keratin 77
LAMA4	IPI00329482	Isoform 1 of Laminin subunit alpha-4 precursor
LAMA5	IPI00783665	Laminin subunit alpha-5 precursor
LAMB2	IPI00296922	Laminin subunit beta-2 precursor
LAMC1	IPI00298281	Laminin subunit gamma-1 precursor
LGALS1	IPI00219219	Galectin-1
LGALS3	IPI00465431	Galectin-3
LPP	IPI00023704	Lipoma-preferred partner
LTF	IPI00298860	Growth-inhibiting protein 12
LUM	IPI00020986	Lumican precursor
MFAP4	IPI00022792	Microfibril-associated glycoprotein 4 precursor
MSMB	IPI00414609	Isoform PSP94 of Beta-microseminoprotein precursor
MYH11	IPI00020501	Myosin-11
MYH11	IPI00024870	smooth muscle myosin heavy chain 11 isoform SM2A
MYL2	IPI00216798	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
MYL6B; MYL6	IPI00027255	Myosin light polypeptide 6B
MYL9	IPI00220278	Myosin regulatory light chain 2, smooth muscle isoform
MYLK	IPI00221255	Isoform 2 of Myosin light chain kinase, smooth muscle
MYLK	IPI00221259	Isoform Del-1790 of Myosin light chain kinase, smooth muscle
NID1	IPI00026944	Isoform 1 of Nidogen-1 precursor
NID2	IPI00028908	Nidogen-2 precursor
NID2	IPI00293033	NID2 protein
OGN	IPI00025465	Mimecan precursor
PALLD	IPI00292009	palladin
PARVA	IPI00018963	Isoform 1 of Alpha-parvin
PCP4	IPI00010148	Brain-specific polypeptide PEP-19
PDLIM4	IPI00032206	Isoform 1 of PDZ and LIM domain protein 4
PDLIM7	IPI00023122	Isoform 1 of PDZ and LIM domain protein 7
PFN1	IPI00216691	Profilin-1
PGM5	IPI00014852	Phosphoglucomutase-like protein 5
PKP2	IPI00005264	Isoform 2 of Plakophilin-2
POSTN	IPI00007960	Isoform 1 of Periostin precursor
PRELP	IPI00020987	Prolargin precursor
PRKAR2A	IPI00063234	PRKAR2A protein
PTRF	IPI00176903	Isoform 1 of Polymerase I and transcript release factor
PYGB	IPI00004358	Glycogen phosphorylase, brain form
RCADH5	IPI00176678	similar to Alcohol dehydrogenase class 3 chi chain (Alcohol dehydrogenase class III chi chain) (S-(hydroxymethyl)glutathione dehydrogenase) (Glutathione-dependent formaldehyde dehydrogenase) (FDH) isoform 1

Gene Symbol	IPI Number	Description
RRAS	IPI00020418	Ras-related protein R-Ras
RSU1	IPI00017256	Ras suppressor protein 1
S100A4	IPI00032313	Protein S100-A4
S100A6	IPI00027463	Protein S100-A6
SCRN1	IPI00289862	Secernin-1
SERPINB6	IPI00513699	Serpin peptidase inhibitor, clade B (Ovalbumin), member 6
SERPINC1	IPI00032179	Antithrombin III variant
SH3BGRL	IPI00025318	SH3 domain-binding glutamic acid-rich-like protein
SMTN	IPI00024007	Isoform B of Smoothelin
SNCG	IPI00297714	Gamma-synuclein
SOD3	IPI00027827	Extracellular superoxide dismutase [Cu-Zn] precursor
SORBS1	IPI00002491	Isoform 9 of Sorbin and SH3 domain-containing protein 1
SORBS2	IPI00061793	SORBS2 protein
SORD	IPI00787158	similar to sorbitol dehydrogenase
SYNPO2	IPI00173549	synaptopodin 2
SYNPO2	IPI00735855	Synaptopodin-2
TAGLN	IPI00216138	Transgelin
TAGLN3	IPI00005981	Neuronal protein NP25
TES	IPI00024097	Isoform 1 of Testin
TF	IPI00022463	Serotransferrin precursor
TGFB1I1	IPI00396399	Transforming growth factor beta 1 induced transcript 1
TGFBI	IPI00018219	Transforming growth factor-beta-induced protein ig-h3 precursor
TGM2	IPI00294578	Isoform 1 of Protein-glutamine gamma-glutamyltransferase 2
TLN1	IPI00298994	271 kDa protein
TNC	IPI00031008	Isoform 1 of Tenascin precursor
TNC	IPI00220211	Isoform 2 of Tenascin precursor
TNC	IPI00220216	Isoform 6 of Tenascin precursor
TNS1	IPI00307545	Tensin-1
TPM1	IPI00000230	tropomyosin 1 alpha chain isoform 2
TPM1	IPI00455050	Sarcomeric tropomyosin kappa
TPM2	IPI00013991	Isoform 1 of Tropomyosin beta chain
TPM4	IPI00010779	Isoform 1 of Tropomyosin alpha-4 chain
TPM4	IPI00216975	Isoform 2 of Tropomyosin alpha-4 chain
TPSAB1	IPI00010274	Isoform 1 of Tryptase alpha-1 precursor
TPSB2	IPI00382751	Mast cell tryptase beta III
TTR	IPI00022432	Transthyretin precursor
TTR	IPI00646384	13 kDa protein
VCL	IPI00291175	Isoform 1 of Vinculin
VCL	IPI00307162	Isoform 2 of Vinculin
-	IPI00296291	HP1-BP74
-	IPI00384438	Sarcoma antigen NY-SAR-77 (Fragment)
-	IPI00555692	ANXA4 protein

Gene Symbol	IPI Number	Description
-	IPI00641693	400 kDa protein
-	IPI00643346	RSU1 protein (Fragment)
-	IPI00783128	Gamma filamin variant
-	IPI00784273	Talin-1
-	IPI00784459	24 kDa protein
-	IPI00784669	Hypothetical protein
-	IPI00794372	17 kDa protein

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