Transmembrane Prostatic Acid Phosphatase (TMPAP) Interacts with Snapin and Deficient Mice Develop Prostate Adenocarcinoma

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Abstract

The molecular mechanisms underlying prostate carcinogenesis are poorly understood. Prostatic acid phosphatase (PAP), a prostatic epithelial secretion marker, has been linked to prostate cancer since the 1930's. However, the contribution of PAP to the disease remains controversial. We have previously cloned and described two isoforms of this protein, a secretory (sPAP) and a transmembrane type-I (TMPAP). The goal in this work was to understand the physiological function of TMPAP in the prostate. We conducted histological, ultra-structural and genome-wide analyses of the prostate of our PAP-deficient mouse model (PAP⁻/⁻) with C57BL/6J background. The PAP⁻/⁻ mouse prostate showed the development of slow-growing non-metastatic prostate adenocarcinoma. In order to find out the mechanism behind, we identified PAP-interacting proteins by yeast two-hybrid assays and a clear result was obtained for the interaction of PAP with snapin, a SNARE-associated protein which binds Snap25 facilitating the vesicular membrane fusion process. We confirmed this interaction by co-localization studies in TMPAP-transfected LNCap cells (TMPAP/LNCap cells) and in vivo FRET analyses in transient transfected LNCap cells. The differential gene expression analyses revealed the dysregulation of the same genes known to be related to synaptic vesicular traffic. Both TMPAP and snapin were detected in isolated exosomes. Our results suggest that TMPAP is involved in endo-/exocytosis and disturbed vesicular traffic is a hallmark of prostate adenocarcinoma.

Introduction

The association between prostate cancer and serum prostatic acid phosphatase (PAP, ACPP; EC 3.1.3.2) has been known for more than 70 years [1]. Nevertheless, the molecular mechanisms underlying this association are still poorly understood. In spite of this, the connection between secreted PAP and prostate cancer contributed to the development of Sipuleucel-T, the first FDA-approved vaccine for cancer therapy targeting PAP-expressing non-metastatic prostate adenocarcinoma [2] even when in advanced/androgen-independent prostate cancer tissue, the expression of PAP is down-regulated [3]. Therefore, our goal is to elucidate the pathways where PAP and in particular its transmembrane isoform is involved.

PAP is a histidine acid phosphatase [4] from which two isoforms have been cloned, the secreted (sPAP) and the transmembrane type-I (TMPAP). Both are splice-variants of the same gene and widely expressed in different tissues, in both sexes [5]. The current evidence does not support the existence of a third, cytosolic cellular form of PAP, as it has been suggested in the literature but never cloned [6–8]. Topologically, TMPAP contains an N-terminal phosphatase activity domain which is extravascular when TMPAP is in the plasma membrane and intra-luminal when it is trafficking in vesicles, and a C-terminal domain with a cytosolic tyrosine-based endosomal-lysosomal (including MVE) targeting signal motif (YxxΦ) [5]. TMPAP also co-localizes with flotillin and LAMP2 [5], which are known markers for exosomes [9,10].

The prostate gland is fundamentally a secretory organ, and it is known that the secretion of specialized exosomes (prostasomes) is essential for the maintenance of the spermatozoan [11]. Exosomes are nanovesicles originated from multivesicular endosomes (MVE), which contain protein, lipid, DNA, RNA and/or microRNA molecules [12]. Also, it has been shown that exosomes are involved in the promotion of cancer cell proliferation and survival [13], and an increased level of prostasomes (exosomes) has been detected in plasma of prostate cancer patients [14].

PAP exerts its phosphatase activity in vitro against β-glycerophosphate [15], lysophosphatidic acid [16], and phosphoamino acids [17] and has 5'-nucleotidase activity [18]. In vivo, the ecto-5'-
nucleotidase activity of PAP is responsible of dephosphorylating adenosine monophosphate (AMP) to adenosine [18, 19] leading to the activation of A1-adenosine receptors in the dorsal root ganglia (DRG) [19]. PAP regulates the levels of adenosine and phosphatidylinositol 4,5-bisphosphate [PI (4,5) P2], an essential regulator of vesicular traffic [20], reducing sensitivity to painful stimuli [19, 21].

SNARE proteins comprise a large family found in yeast and mammalian cells, with the primary function to mediate docking and fusion of vesicles with the cell membranes [22] in regulated endo-/exocytosis [23]. Snapin is a SNARE-associated protein [24] interacting with Snap25, Snap23 or Snap29, and increasing the binding of the calcium sensor synaptotagmin to the SNARE complex [25]. Snapin also forms part of the BLOC1 protein complex, which is necessary for the biogenesis of vesicles in the endosomal-lysosomal pathway [26]. Increasing evidence shows that snapin is important in retrograde axonal transport, late endosomal-lysosomal trafficking and glucose-induced insulin exocytosis. In mediating retrograde axonal transport, snapin acts as a dynein adaptor protein for BDNF-TrkB (brain-derived neurotrophic factor – tyrosine kinase receptor B) activated signaling complexes. This interaction leads to the delivery of TrkB signaling endosomes from axonal terminals to cell bodies, which is an essential mechanism for dendritic growth of cortical neurons [27]. Moreover, snapin deficiency in neurons leads also to accumulation of immature lysosomes due to impaired delivery of cargo proteins from late endosomes to lysosomes [28]. In addition, snapin as a target of protein kinase A (PKA), was found to be a critical regulator of glucose-stimulated insulin exocytosis in pancreatic β-cells by promoting the interaction and assembly of insulin secretory vesicle-associated proteins Snap25, collectrin and Epac2 [29].

The mouse prostate consists of three different lobes: anterior (AP), dorsolateral (DLP) and ventral prostate (VP); and it does not show spontaneous development of neoplasia [30]. The mouse prostate lobes have characteristic histology which has been described previously [31, 32]. Briefly, all prostate lobes show a monolayer epithelium with eosinophilic columnar cells and cosinophilic secretion which is paler in VP than in AP and DLP. Each duct in the lobes is surrounded by a thin fibromuscular sheet composed mainly of smooth muscle cells and collagen fibers. In the AP epithelium, the cell nucleus is central and the epithelium has a high number of infoldings and papillary fibers. In the VP epithelium, the cell nucleus is central and the epithelium has a high number of infoldings and papillary fibers. The AP and VP of PAP−/− mice show no significant changes. Scale bars: 100 μm.

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Figure 1. DLP lobe exhibits the primary changes in the PAP−/− mouse prostate. The panels show an overview of the 12 month-old mice prostate dissected lobes. The DLP, AP and VP lobes were dissected from WT and PAP−/− mouse. The monolayer epithelium (white arrows) is seen in all the lobes of the WT mouse, whereas in the PAP−/− mouse an increased amount of cells is present in the lumen of the DLP lobe (black arrows). The AP and VP of PAP−/− mouse show no significant changes. Scale bars: 100 μm.

Materials and Methods

Ethics statement

The animal protocols were approved by the Animal Experimentation Committee of the University of Oulu and ELLA – The National Animal Experiment Board of Finland. The project license numbers are 044/11 and STH705A/ESLH-2009–08353/Ym-2.

Mice

Mice deficient in PAP were generated by replacing exon 3 (ACPPD3/D3) of the prostatic acid phosphatase gene (ACPP, PAP) with the neo gene as described earlier [33] thereby abolishing the expression of both PAP isoforms. The fertility status in the PAP−/− mice was not affected by the gene modification. PAP−/− mice were backcrossed to the C57BL/6J strain (Harlan Laboratories Inc.) for 16 generations to obtain homogenous background. Age-matched C57BL/6J male mice were used as controls in all the experiments.
Figure 2. PAP−/− mice develop prostate adenocarcinoma. A, age related histological changes in PAP−/− mouse DLP. Epithelial hyperplasia was present in DLP of 3 month-old PAP−/− mice. The lumen was filled with dysplastic epithelial cells, and mPIN structures were observed in 6 month-old animals. Bulging of epithelial cells into the stroma (black arrows) through loosen fibromuscular sheath and prostate adenocarcinoma were present in 12 month-old mice. Scale bars: 100 μm. (n = 8). B, morphological abnormalities present in 12 month-old PAP−/− mouse prostates. Glands...
were filled with epithelial cells (black arrowhead). Dyscohesive cells with double nuclei were present (white arrows), as well as sites of microinvasions of hyperchromatic epithelial cells with prominent nucleoli (black arrow). Cribriform structures (white arrowhead) and blood vessels among neoplastic epithelial cells (*) were also observed. Scale bars: 100 μm. (n = 8). C, 24 month-old PAP /−/− mouse DLPs. Cells invaded the surrounding stroma and inflammation is present. Microinvasion of cells into the stroma and bulging is clearly observed (black arrows). Scale bars: 100 μm. (n = 8). FS: fibromuscular sheath, L: lumen, mL: monolayer epithelium.

Figure 3. The prostate adenocarcinoma in PAP−/− mice is also detected by immunohistochemistry and electronmicroscopy. A, smooth muscle actin (SMA) immunohistochemistry in 12 month-old mice. Monolayer epithelium (mL) and open lumen in PAP+/− DLP. White arrows show the broken fibromuscular sheath (SM, smooth muscle) and bulging of epithelial cells to the stroma. Prostate adenocarcinoma (black arrows) is present in AP and DLP, showing a multilayer epithelium (ML) and inflammatory cells (black arrowhead) spreading in neighboring areas. Scale bars: 100 μm. (n = 4, per group). B, ultrastructural changes in 3 month-old and 12 month-old PAP−/− mouse DLPs. Monolayer epithelium, regular basement membrane (BM) and apical secretion are clearly seen in PAP+/− mouse DLPs. 3 month-old PAP−/− DLPs show irregular BM and numerous apical vacuoles (red arrow head), as well the presence of basal lysosomes (Ly). In 12 month-old PAP−/− mouse DLPs, the epithelium has transformed to a multilayer epithelium containing hyperchromatic nuclei with multiple nucleoli. Pseudolumens (pL) have formed as a result of the growing and fusion of the epithelium. Invaginations of BM (red arrows) into the epithelium and numerous vesicles in the basal side of the cells (blue arrow heads) were additional signs of the transformation in the cells. Scale bars: 2000 nm (n = 4, per group).

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Transmission electron microscopy

DLP samples from age-matched PAP<sup>−/−</sup> and PAP<sup>+/+</sup> mice were fixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer for TEM. The samples were post-fixed in 1% osmium tetroxide, dehydrated in acetone, embedded in Epon Embed 812 (Electron Microscopy Sciences) and analyzed at the Biocenter Oulu EM core facility using Philips 100 CM Transmission Electron Microscope with CCD camera.

Yeast two-hybrid analysis

To screen for interacting partners of human TMPAP, yeast two-hybrid screening was performed using the Matchmaker Gal4 two-hybrid System 3 (Clontech) in accordance with the manufacturer's instructions. The bait construct consisted of the coding region of human TMPAP (GeneBank accession BC007460, nucleotides 51–1304, except the starting methionine was changed to valine) cloned in frame into NcoI/SmaI sites of pGBK7 using PCR generated linkers. A human thymus cDNA library cloned in pACT2 (Clontech) was used as the prey. The bait and prey

Figure 4. PAP<sup>−/−</sup> mouse DLPs release exosomal-like microvesicles. A, electronmicroscopy images show the presence of electron-dense (white arrow) and electron-lucent (black arrow) microvesicles (~30 to 80 nm) in the lumen of the acini, and MVE containing microvesicles in the apical part of the cell. Scale bar: 1,000 nm. B, numerous microvesicles are present in the apical region of PAP<sup>−/−</sup> DLP and secreted into the lumen, decreased amount of microvill is observed (black arrowheads) (scale bar: 1,000 nm). C, microvesicles are secreted into basolateral intercellular space of PAP<sup>−/−</sup> DLP (scale bar: 2000 nm). D, lamellar body-like structures (*) are inside the epithelial cell (scale bar: 500 nm) and E, released into the lumen (*). Scale bar: 200 nm. F, TMPAP and snapin are also present in exosomes. Immunoblots of exosomes isolated from TMPAP/LNCaP cell culture medium. Flotillin and CD13 were used as exosomal and prostasomal marker respectively.

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Figure 5. Proliferation of DLP cells is increased in PAP<sup>−/−</sup> mice compared to WT mice. A, bar plot showing the ratio (as percentage) between proliferative cell counts and total amount of cells. (**, P value <0.01; ***, P value <0.001). Error bars indicate S.E.M. values. B, bar plot showing the ratio (as percentage) between apoptotic cell counts and total amount of cells. Error bars indicate S.E.M. values.

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plasmids were co-transformed into *Saccharomyces cerevisiae* Mav 203 strain according to the Clontech’s two-hybrid protocols. Inserts of positive clones were amplified by PCR, and the DNA was automatically sequenced.

The Förster resonance energy transfer (FRET) analysis

The FRET variant acceptor photobleaching was used. In this technique, the efficiency of energy transfer between two molecules (and consequently the interaction between them) is measured by comparing the fluorescence of the donor molecule before and after the selective photobleaching of the acceptor molecule [34]. Human TMPAP-GFP and the control GFP plasmid constructs have been previously described [5]. Human snapin (NM_012437, nt 76–486) was cloned into pDsRed-Monomer-C1 vector, between SalI/BamHI restriction sites. LNCaP cells were obtained from the American Tissue Culture Collection (ATCC) and

| GO ID | \(P\)-value | Term | Genes |
|-------|-------------|------|-------|
| 5576  | <0.0001     | extracellular region | Nrxn1, Timp4, Syn2, Pnoc, Htr3b, Edn1, Timp4, Syn2, Gria1, Tac1, Ncam1, Kif5a, Pnoc, Snap25, Htr3a, Syn2, Syp, Bcan, Rab3c, Syt10, Cplx1, Gad2, Syt4, Syt10, Abpa, Grm7, Rasgrf1, Cart, Syt1 |
| 7268  | <0.0001     | synaptic transmission | Gria1, Gria3, Tac1, Ncam1, Mapt, Kif5a, Uchl1, Esr2, Cnntn2, Prph1, Nef3, Thby1, Sncg, Syt4, Gad2, Nef3, Gap43, Syt1, Kif5c |
| 43005 | <0.0001     | neuron projection | Gria1, Gria3, Tac1, Ncam1, Mapt, Kif5a, Uchl1, Esr2, Cnntn2, Prph1, Nef3, Thby1, Sncg, Syt4, Gad2, Nef3, Gap43, Syt1, Kif5c |
| 45202 | <0.0001     | synapse | Nrxn1, Htr3b, Syn2, Thbs4, Gria1, Gria3, Snap25, Htr3a, Nef3, Syn2, Bcan, Syngrr1, Syn2, Syt10, Syt4, Gad2, Gabra4, Mgl1, Grm7, Syt1 |
| 30424 | <0.0001     | axon | Tyh1, Sncg, Tac1, Gad2, Ncam1, Mapt, Nef3, Gap43, Uchl1, Cnntn2, Prph1, Nef3 |
| 8021  | <0.0001     | synaptic vesicle | Syngrr1, Timp4, Syn2, Syt10, Syt4, Syt4, Syp, Rab3c, Syt1 |
| 4293  | <0.0001     | tissue kallikrein activity |
| 42044 | <0.0001     | fluid transport |
| 32501 | 0.0001      | multicellular organismal process |
| 7267  | 0.0001      | cell-cell signaling |
| 19226 | 0.0001      | transmission of nerve impulse | Nrxn1, Timp4, Syn2, Pnoc, Htr3a, Cldn11, Rab3c, Cplx1, Gad2, Syt4, Grm7, Rasgrf1, Edn1, Htr3b, Gria1, Ncam1, Tac1, Kif5a, Snap25, Syt4, Bcan, Syt10, Abpa, Cart, Syt1 |
| 3001  | 0.0001      | generation of a signal involved in cell-cell signaling |
| 45055 | 0.0001      | regulated secretory pathway | Nrxn1, Timp4, Lat, Syn2, Cplx1, Syt4, Abpa, Snap25, Cart, Rab3c, Syt1 |
| 30672 | 0.0001      | synaptic vesicle membrane | Syngr1, Syn2, Bcan, Syt1 |
| 15026 | 0.0001      | coreceptor activity |
| 5372  | 0.0001      | water transporter activity |
| 15250 | 0.0001      | water channel activity |
| 15722 | 0.0001      | canalicular bile acid transport |
| 5179  | 0.0002      | hormone activity |
| 7269  | 0.0002      | neurotransmitter secretion | Nrxn1, Timp4, Syn2, Cplx1, Syt4, Abpa, Snap25, Cart, Rab3c, Syt1 |
| 1772  | 0.0002      | immunological synapse |
| 6833  | 0.0003      | water transport |
| 31226 | 0.0005      | intrinsic to plasma membrane | Nrxn1, Treh, Aqp7, Kcnj5, Aqp8, Aqp5, Cldn11, Cntntn2, Htr3a, Cd28, Rhbg, Kcnc1, Lat, Vlrc12, Itga9, Acrv1b, Aqp8, Tcrb-V13, Kcnv2, Gabra4, Tpbq, Tyro3, Gps5, Slc12a2, Htr3b, Gria1, Gria3, P2rx1, Mme, Slc1a6, Abcb11, Snap25, Xtrp3s1, Cntr1, Acdy3, Raet1a, Syngr1, Cd5, V1rg9, Mup1, Ppko, Slc15a2, Omg, Adora1, Olfh3, Slc1a1 |
| 45104 | 0.0005      | intermediate filament cytoskeleton organization and biogenesis | Nef, Krt6b, Prph1, Nef3 |
| 5883  | 0.0005      | neurofilament | Nef, Prph1, Nef3 |
| 30136 | 0.0006      | clathrin-coated vesicle | Syngr1, Timp4, Syn2, Syt10, Syt4, Syp, Ncald, Bcan, Syt10, Rab3c, Syt1 |
| 5232  | 0.0006      | serotonin-activated cation-selective channel activity |
| 5615  | 0.0007      | extracellular space |
| 48812 | 0.0007      | neuropeptide morphogenesis |
| 48667 | 0.0007      | neuron morphogenesis during differentiation |
| 7409  | 0.0008      | axonorganization |

**GO ID**: gene ontology ID accession number. **\(P\)-value**: \(P\)-value for the number of changed genes in the input list, significant \(P\)-value < 0.05. **Term**: associated ontological term. **Rows in bold**: relevant ontological groups for vesicular transport. Mice in microarray experiment per group, \(n = 3\).

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cultured in 10% FBS (Promocell), L-Glutamine (2 mM), Penicillin (100 U/ml), Streptomycin (100 µg/ml), RPMI-1640 (Sigma) on polylysine-coated petridishes (Mattek). Cells were transfected using Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. In our experiments we used GFP and DsRed fluorophores as donor and acceptor, respectively, and co-expressed TMPAP-GFP and snapin-DsRed plasmids in LNCaP cells. GFP/DsRed FRET pair provides excellent wavelength separation of donor and acceptor spectra. For FRET positive control we used a DNA construct produced by the connection of EGFP and DsRed sequences with a 23-amino acid linker. Co-transfected pEGFP-C1 and pDsRed-Monomer-C1 vectors have been used as negative controls.

Cultured cells were mounted 24 hours after transfection and epifluorescent images were acquired using an Olympus CellR imaging system with 60 x oil immersion NA 1.45 objective. Images were collected with a CCD camera (Orca, Hamamatsu). The system was equipped with automated filter wheels for excitation filters and emission beam-splitter/emission-filter cubes for epifluorescence imaging. GFP fluorescence was excited at 450 nm and collected at 510/40 nm. DsRed fluorescence was excited at 575 nm and collected at 640/50 nm. Acceptor fluorescence was bleached for 5 minutes with maximal burner power.

Figure 6. TMPAP co-localize and interact with snapin in the cell lamellipodia. A, co-localization (yellow) of TMPAP (green) with snapin (red) was observed in the vesicles and lamellipodia of the TMPAP/LNCaP cells. Arrows mark the co-localization points in the upper panel (scale bar: 20 µm). Lower panel (scale bar: 3 µm) showing the lamellipodia region, amplification of the area marked with a box in the upper panel (left). B, intensification of donor (TMPAP-GFP) fluorescence in LNCaP cells was observed after acceptor (snapin-DsRed) photobleaching which confirms FRET between two molecules (Scale bar: 2 µm).

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Images were quantified and processed using Olympus Biosystems AnalySIS software, ImageJ (freely available at http://rsb.info.nih.gov/ij/) and ImagePro 5.1 (Media Cybernetics). Background fluorescence was subtracted prior to calculations. The FRET efficacy defined as the percentage of donor fluorescence increase was calculated with the following equation: $E = 1 - (I_b/I_a)$, where $I_b$ is the fluorescence intensity of the donor before photobleaching and $I_a$ is the post-bleach fluorescence intensity.

Histology, immunohistochemistry, proliferation and apoptosis analyses, microarray analyses, generation of stable transfected LNCaP cells, immunofluorescence and co-localization studies, comparative genomic hybridization (CGH), isolation of exosomes and Western blot analyses

The detailed methodology can be found in the Supplementary Methodology in File S1.

**Accession codes**

Gene expression files containing microarray raw-data can be accessed from ArrayExpress repository database (accession number E-MTAB-1191).

**Results**

PAP-deficiency in mouse prostates leads to development of prostate adenocarcinoma

PAP-deficiency led to slow development of prostate neoplasia in DLP and AP. The progressive changes in mouse DLP were observed in all the PAP$^{+/+}$ mice examined ($n=8$), detecting a hyperplastic growth already at the age of 3 months, followed by mouse prostatic intraepithelial neoplasia (mPIN) at 6 months and prostate adenocarcinoma at 12 months (Fig. 1 and 2A). The follow-up of the disease in mice spanned until 26 month-old, where the presence of other pathologies arose as strain background or due to mouse aging.
All the PAP−/− mice analyzed at the age of 12 months had developed prostate adenocarcinoma (n = 6, Fig. 2B). Pathological acini were filled with non-cohesive pleomorphic epithelial cells, with enlarged hyperchromatic nuclei and prominent nucleoli. The presence of neoplastic acinar cells in the lumen was confirmed with pan cytokeratin staining (Fig. S1 in File S1). The fibrotic presence of neoplastic acinar cells in the lumen was confirmed at 6-, 15- and 25 month-old showed no differences in the copy number between the genotypes (wild type C57BL/6J and 2 mice lost the regular structure of the fibromuscular sheath and invasion of the acini, we determined the status of proliferation and apoptosis in the tumor. As a result, the proliferation was significantly increased in the AP lumen leading to the development of prostate adenocarcinoma at the age of 12 months. Despite the presence of inflammatory cells was detected in sites of microinvasive adenocarcinoma, we did not detect metastatic lesions in other studied organs such as brain, liver, lungs and lymph nodes at any age analyzed.

Due to the gradually increased number of cells in the prostate acini, we determined the status of proliferation and apoptosis in the tissue. As a result, the proliferation was significantly increased in the three- (P-value = 4.3 × 10−7, n = 4) and 12 month-old (P-value = 3.9 × 10−5, n = 4) PAP−/− mice DLP cells and their contents secreted into the lumen (Fig. 4D and E). To validate the yeast two-hybrid result, double-immunofluorescence staining of PAP and snapin in TMAAP/LNCaP cells showed co-localization of these two proteins in vesicular structures and cell membrane (Fig. 6A). The quantification studies displayed relatively low Pearson’s correlation coefficient when the whole cell was analyzed (0.405 ± 0.012). However, when the co-localization was quantified exclusively in the cell lamellipodia the Pearson’s correlation coefficient reached a value of 0.680 ± 0.013. This coefficient value not only shows that TMAAP co-localized with snapin but it could imply an interaction between TMAAP and snapin in these cell regions. Therefore, to confirm this hypothesis of interaction, the FRET variant acceptor photobleaching, which gives an in vivo proof of the physical protein-protein interaction, was used to determine the interaction between TMAAP and snapin. Data analysis revealed significant FRET between TMAAP and snapin (FRET efficacy 9.3 ± 1%, n = 12 cells) compared to experiments with negative control (−0.4 ± 0.7%, n = 9 cells, P ≤ 0.0001) while in experiments with positive control FRET efficacy reached a level of 37.2 ± 6.5% (Fig. 6B).

Hence our previous results have showed the co-localization of PAP and flotillin, which is a protein also used as exosomal marker [9], wccorrorporate by Western blot the presence of PAP isolated exosomes produced by stable transfected TMAAP/LNCaP cells. The results showed the presence of TMAAP as well as snapin in the exosomal fraction in addition to flotillin and CD13, a prostatic marker [35] (Fig. 4F).

**Discussion**

Prostate cancer is a disease of complex etiology, in which genetic and epigenetic mechanisms are involved. PAP was the first prostate cancer marker, and its usefulness was based in the assessment of its serum activity levels. We have previously shown that in addition to the secretory PAP, a transmembrane isoform is widely expressed in different mouse organs such as prostate, salivary glands, thymus, lung, kidney and brain, amongst others. TMAAP is also present in androgen sensitive prostate cancer cells (LNCaP), but absent in androgen insensitive prostate cancer cells (PC3) [5]. In all the PAP−/− mice, progressive changes were observed in the prostatic tissue leading to the development of prostate adenocarcinoma at the age of 12 months. Despite the presence of prostate cancer, we have not detected any metastatic lesions. Histologically, the mouse DLP has been considered to be the analogous area to the peripheral zone of the human prostate, where the majorities of adenocarcinomas reside [36]. In this regard, Roy-Burman et al. suggested that the mouse models carrying genetic modifications that affect the tumor development in the DLP are more significant for the studies on those pathologies associated with the peripheral zone of the human prostate [37]. In humans, it has been observed that PTEN (phosphatase and tensin homolog deleted on chromosome 10) is downregulated in prostate cancer tissue specimens [38]. PTEN antagonize PI3K (phosphoinositoid 3-kinase) activity by dephosphorylating phosphoinositol (3,4,5)-trisphosphate which is an activator of the AKT pathway leading to cell survival. The PTEN prostate cancer mouse model showed a development of prostate cancer which resembles the stages of the disease in humans, starting with hyperplasia at 4 weeks of age, mPIN, prostate microinvasive adenocarcinoma in all prostatic lobes and finally metastasizing in
TMPAP interacts with Snapin

**Supporting Information**

**File S1 Supplementary file includes:** Figure S1: Pan cytokeratin immunohistochemistry of DLP from 12 month-old animals. Tables S1: Proliferative cell count statistics. Tables S2: Proliferative and non-proliferative cell counts. Tables S3: Apoptotic cell count statistics. Tables S4: Apoptotic and non-apoptotic cell counts. Tables S5: Significant ontological groups in the biological process category obtained with Genomatix Bibliosphere software from animals. Tables S6: Significant ontological groups in the biological process category obtained with Genomatix Bibliosphere software from two-color microarrays experiments. Supplementary methodology. (DOCX)

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**Author Contributions**

Conceived and designed the experiments: IQ AH AP SH LK PV. Performed the experiments: IQ AH AP EP MR. Analyzed the data: IQ AH CA AP SH KO EP EK YS RS LK PV. Contributed reagents/materials/analysis tools: EK. Wrote the paper: IQ AH CA AP SH KO EP EK LK PV.
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