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14. ABSTRACT
Protein palmitoylation, more accurately termed as protein S-acylation, is reversibly regulated by a group of Asp-His-His-Cys (DHHC) motif-containing palmitoyl acyltransferases (PATs) and several acylprotein thioesterases (APTs) (1). Its primary function is to regulate the spatial cycling of proteins between different compartments of cells, and thus to modulate protein activity and stability as well as multiprotein complex formation (1). By developing and applying unbiased palmitoyl-proteomics technologies, we and others showed that many palmitoylated proteins are enriched in cholesterol-rich membrane microdomains such as lipid rafts and tetraspanin-enriched microdomains (TEMs) (2).

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Introduction

Protein palmitoylation, more accurately termed as protein *S*-acylation, is reversibly regulated by a group of Asp-His-His-Cys (DHHC) motif-containing palmitoyl acyltransferases (PATs) and several acylprotein thioesterases (APTs) (*1*). Its primary function is to regulate the spatial cycling of proteins between different compartments of cells, and thus to modulate protein activity and stability as well as multiprotein complex formation (*1*). By developing and applying unbiased palmitoyl-proteomics technologies, we and others showed that many palmitoylated proteins are enriched in cholesterol-rich membrane microdomains such as lipid rafts and tetraspanin-enriched microdomains (TEMs) (*2*).

Our collaborator Dr. Martin Hemler is a founder in the TEMs field. His group, along with other research groups, has shown that TEMs are a critical signaling platform for tumor initiation, promotion and metastasis and that protein palmitoylation is essential for maintaining TEMs assembly and function (*3, 4*). In addition to tetraspanins, the TEMs are also rich in laminin-binding integrins (*e.g.*, α6β4, α6β1, and α3β1) (*5*). The Hemler lab showed that integrin α6 and β4 subunits undergo palmitoylation, and further determined that both integrin subunits were palmitoylated by the PAT DHHC3 protein. Moreover, ablation of DHHC3 disrupts TEMs and markedly changes cell morphology, invasion, and signaling through focal adhesion kinase (FAK) in breast cancer cell lines.

For the proposed project, the overall hypothesis is that **DHHC3 plays a major role during breast cancer cell growth, invasion, and metastasis, *in vitro* and *in vivo***. To test the hypothesis, the Hemler lab would examine the extent to which DHHC3 regulates TEMs assembly and explore mechanisms whereby FAK dephosphorylation is altered. Our role was to improve our palmitoyl-proteomics technology termed *Palm*itoyl *P*rotein *I*dentification and *S*ite *C*haracterization (PalmPISC) (*2*) and to identify DHHC3 substrates in an unbiased fashion, such that the signaling networks downstream of DHHC3 could be rapidly mapped. Briefly, in the PalmPISC method (Fig. 1), non-palmitoylated cysteine residues are irreversibly blocked by an alkylating group. Subsequently, via acyl-biotinyl exchange, the palmitoyl groups are replaced by biotin groups, so palmitoylated proteins can be enriched by streptavidin affinity purification and characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

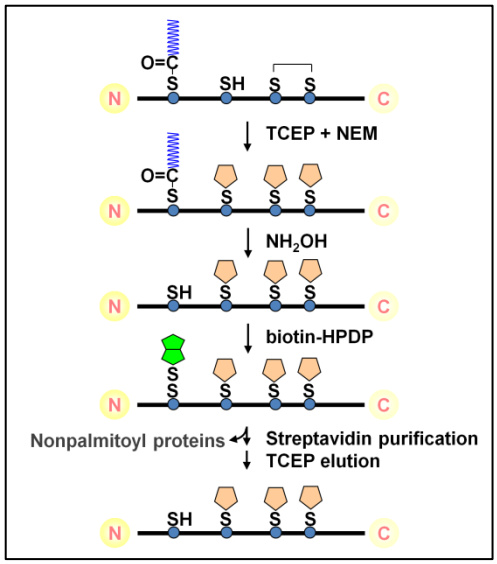


Figure 1. Schematic of the PalmPISC method. For details, see reference (*2*).

Keywords

DHHC3, Focal adhesion kinase, Integrin, Invasion, Metastasis, Palmitoylation, PalmPISC, SILAC, Tetraspanin, TEMs

Overall Project Summary

Improvement of the PalmPISC technology for unbiased palmitoyl-proteomics profiling

By evaluating our and other groups' palmitoyl-proteomics studies, we found that after the enrichment of palmitoyl proteins and LC-MS/MS analysis, most identified proteins are non-palmitoylated proteins (*1*). These proteins are typically highly abundant house-keeping proteins, whose co-enrichment masks the identification of very lowly abundant palmitoyl proteins. To substantially improve the sensitivity and specificity of detecting palmitoyl proteins, we have systematically optimized our PalmPISC method. By further blocking non-palmitoylated cysteine residues, only <0.1% (w/w) of total enriched proteins were derived from high-abundance contaminating proteins (Fig. 2). In contrast, using our original PalmPISC method, >20% (w/w) of enriched proteins are contaminating proteins (*2*). Taken together, our improved PalmPISC method drastically improves the selectivity for the purification of palmitoyl proteins.

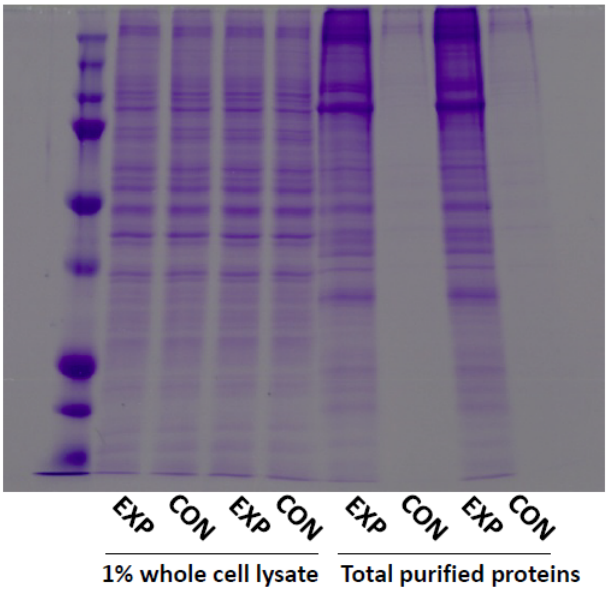
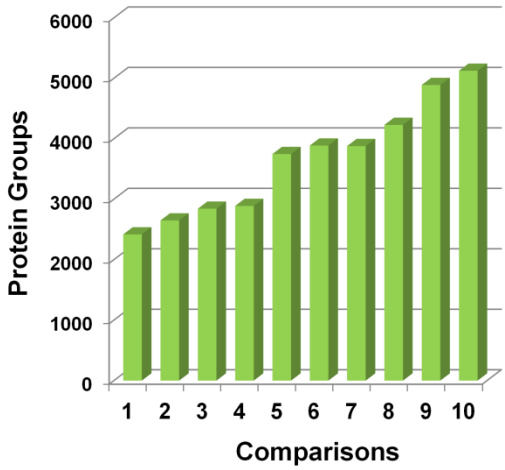


Figure 2. Over 99.9% of non-palmitoylated proteins can be depleted using our optimized PalmPISC protocol. Here, EXP indicates the experimental group, for which hydroxylamine was added to convert palmitoylated cysteines into free cysteines so that they can be specifically enriched by biotinylation of free cysteines followed by streptavidin affinity purification. CON indicates the control group, for which Tris buffer was used to replace hydroxylamine solution.

Palmitoyl proteins only account for a small subset (<2%) of cellular proteins, posing a challenge for proteome-scale analysis. To develop a highly sensitive LC-MS/MS method with low sample consumption, we systemically evaluated ten sets of LC-MS/MS settings and developed a single-shot proteomics workflow that enables the identification of over 5,000 proteins from 2 μg prostate cancer

LNCaP lysates in about 4 hours of LC-MS/MS analysis (Fig. 3). A detailed description of the method comparisons has been submitted to the *Journal of Proteome Research* and is currently under review.



1. Lengths of LC columns and gradients
2. Time windows for dynamic exclusion
3. LC flow rates
4. Mass windows
5. Monoisotopic precursor selection
6. Precursor ion intensity thresholds
7. Numbers of data-dependent MS/MS
8. Loading amounts
9. Protein digestion methods
10. LC gradient times

Figure 3. Development of a highly sensitive single-shot proteomics method. Systematic evaluations of 10 groups of settings led to markedly improved identification of proteins. After the optimization, we were able to identify over 5,000 proteins from merely 2 μg LNCaP lysates, more than doubling the number of proteins identified using a vendor-provided method.

Previous palmitoyl-proteomics studies identified at most 500 candidate palmitoyl proteins in a single mammalian cell line (*1*). To determine whether palmitoylation is a more pervasive modification (and thus play more versatile roles) than we think, we coupled our improved methods with Stable Isotope Labeling by Amino acids in Cell culture (SILAC) (*6*)—a widely used quantitative proteomics method—to perform an in-depth palmitoyl-proteome profiling of LNCaP cells. We identified a total of 1,137 candidate palmitoyl proteins (FDR <0.05 and EXP/CON > 1.5) from merely 400 μg LNCaP lysates. This represents the largest group of palmitoylated proteins identified in a single study. Notably, in this deep palmitoyl-proteomics profiling study, we identified 15 out of the 23 DHHC-PATs as well as the best characterized APT protein APT1 (also called LYPLA1) as palmitoylated.

Identification of DHHC3 substrates by quantitative palmitoyl-proteomics

By coupling our PalmPISC method with SILAC, we quantitatively compared the difference between the palmitoyl-proteomes of breast cancer MDA-MB-231 cells before and after stable DHHC3 knockdown. As shown in Figure 4, three populations of MDA-MB-231 were metabolically labeled with isotopically different SILAC amino acids in parallel. One group of control cells were cultured in "light" medium containing natural lysine (Lys0) and arginine (Arg0), DHHC3-knockdown cells were cultured in "heavy" medium containing $^{13}C_6$,$^{15}N_2$-lysine (Lys8) and $^{13}C_6$,$^{15}N_4$-arginine (Arg10), and the other group of control cells were cultured in "medium" medium containing 4,4,5,5-D_4-lysine (Lys4) and $^{13}C_6$-arginine (Arg6). After six doublings, when cellular proteins were at least 98% labeled with SILAC amino acids, control cells labeled with Lys0 and Arg0 and DHHC3-knockdown cells labeled with Lys8 and Arg10 were mixed at 1:1 ratio, and then palmitoyl proteins were isolated using our PalmPISC method. Moreover, to distinguish palmitoyl proteins from co-enriched contaminating proteins, we omitted hydroxylamine—a chemical provides selectivity for palmitoyl proteins—from our PalmPISC

condition and isolated the contaminating proteins from control cells labeled with Lys4 and Arg6. Finally, we mixed the purified proteins together and performed quantitative proteomics analyses and analyzed the SILAC dataset with MaxQuant (v1.0.13.13), a free software suite for SILAC data analysis (*7*).

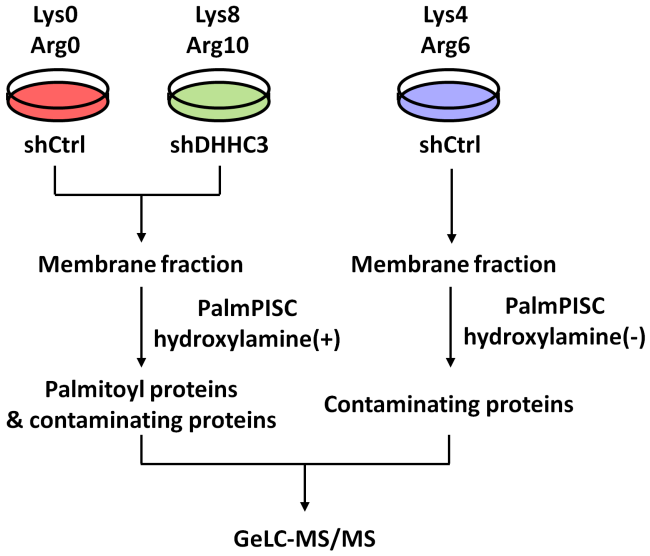


Figure 4. Workflow for the unbiased identification of DHHC3 substrates. See the main text for details.

Theoretically, proteins that are palmitoylated by DHHC3 (*i.e.*, DHHC3 substrates) will have a pattern of SILAC spectra shown in Fig. 5A, because the knockdown of DHHC3 reduces the palmitoylation level of its substrates while the omission of hydroxylamine prevents the purification of the substrates. In contrast, DHHC3 knockdown will not affect other palmitoylated proteins, thereby non-DHHC3-substrates will have a pattern shown in Fig. 5B. In addition, contaminating proteins will have a ratio of 1:1:1 (Fig. 5C), because DHHC3 knockdown or the presence/absence of hydroxylamine will not affect their purification.

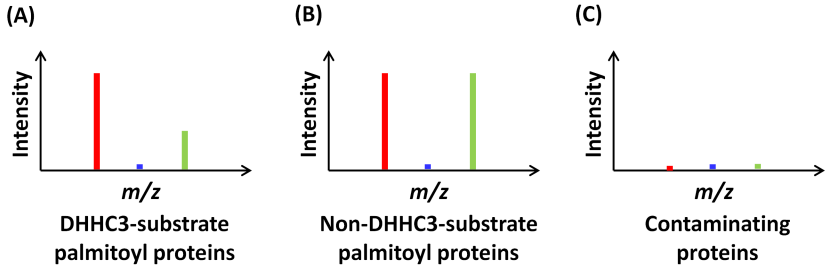


Figure 5. Theoretic patterns of SILAC spectra for (A) palmitoylated proteins that are DHHC3 substrates, (B) palmitoylated proteins that are not DHHC3 substrates, and (C) contaminating proteins. See the main text for details.

Our quantitative palmitoyl-proteomics analysis led to the identification of 1097 proteins with a false-discovery rate of 1%; among these proteins about 930 were quantitated. Using a cutoff value of 0.606 (p<0.05) for the "medium"/ "light" (M/L) SILAC ratio, we identified 687 candidate palmitoyl proteins,

representing the largest breast cancer palmitoyl-proteome assembled so far. Furthermore, using a cutoff value of 0.606 ($p<0.05$) for the "heavy"/ "light" (H/L) SILAC ratio, we identified 70 candidate palmitoyl proteins as candidate DHHC3 substrates (Table 1). Notably, the known DHHC3 substrate integrin α6 subunit was identified as a top DHHC3 substrate protein, validating the effectiveness of our quantitative palmitoyl-proteomics approach.

Table 1. Candidate DHHC3 substrates in breast cancer MDA-MB-231 cells

Protein IDs	Protein Names	Gene Names	Ratio M/L[a)]	Ratio H/L[b)]
IPI00027462	Protein S100-A9	S100A9	0.160	0.008
IPI00306383	Secretory carrier-associated membrane protein 3	SCAMP3	0.009	0.034
IPI00910862	cDNA FLJ54105, highly similar to Sulfatase-modifying factor 2	SUMF2	0.075	0.075
IPI00220739	Membrane-associated progesterone receptor component 1	PGRMC1	0.470	0.280
IPI00011229	Cathepsin D	CTSD	0.297	0.294
IPI00152890	Nucleolar protein 6	NOL6	0.382	0.305
IPI00014172	Lysosomal-associated transmembrane protein 4A	LAPTM4A	0.099	0.340
IPI00043490	CKLF-like MARVEL transmembrane domain-containing protein 3	CMTM3	0.136	0.345
IPI00025512	Heat shock protein beta-1	HSPB1	0.520	0.369
IPI00216225	Integrin alpha-6	ITGA6	0.163	0.372
IPI00855873	Transmembrane protein 192	TMEM192	0.090	0.380
IPI00017515	Melanoregulin	MREG	0.008	0.380
IPI00940822	Abhydrolase domain-containing protein FAM108A1	FAM108A1	0.021	0.396
IPI00020004	Transmembrane protein 97	TMEM97	0.155	0.407
IPI00005107	Niemann-Pick C1 protein	NPC1	0.122	0.409
IPI00217465	Histone H1.2	HIST1H1C	0.553	0.416
IPI00015801	CKLF-like MARVEL transmembrane domain-containing protein 6	CMTM6	0.222	0.475
IPI00334453	Transmembrane protein 179B	TMEM179B	0.014	0.480
IPI00183603	Oligosaccharyltransferase complex subunit OSTC	OSTC	0.293	0.492
IPI00103599	BRI3-binding protein	BRI3BP	0.353	0.494
IPI00175029	Transmembrane protein 55A	TMEM55A	0.100	0.501
IPI00642584	Uncharacterized protein KIAA0090	KIAA0090	0.449	0.505
IPI00940046	N-acylsphingosine amidohydrolase 1	ASAH1	0.055	0.507
IPI00103867	E3 ubiquitin-protein ligase rififylin	RFFL	0.014	0.509
IPI00442274	NF-X1-type zinc finger protein NFXL1	NFXL1	0.370	0.514
IPI00009976	Transmembrane emp24 domain-containing protein 1	TMED1	0.138	0.517
IPI00012434	MLN64 N-terminal domain homolog	STARD3NL	0.010	0.519
IPI00019488	U3 small nucleolar ribonucleoprotein protein IMP3	IMP3	0.451	0.519
IPI00291695	Probable glutathione peroxidase 8	GPX8	0.161	0.522
IPI00025049	Cation-dependent mannose-6-phosphate receptor	M6PR	0.007	0.525
IPI00005202	Membrane-associated progesterone receptor component 2	PGRMC2	0.501	0.529
IPI00019141	1-acyl-sn-glycerol-3-phosphate acyltransferase alpha	AGPAT1	0.030	0.529
IPI00006211	Vesicle-associated membrane protein-associated protein B/C	VAPB	0.522	0.532
IPI00090807	Methyltransferase-like protein 7B	METTL7B	0.045	0.535
IPI00016046	Reactive oxygen species modulator 1	ROMO1	0.059	0.541
IPI00027230	Endoplasmin	HSP90B1	0.446	0.545
IPI00926820	Solute carrier family 4 sodium bicarbonate cotransporter member 7	SLC4A7	0.223	0.548
IPI00942906	Major facilitator superfamily domain-containing protein 1	MFSD1	0.129	0.550
IPI00924549	BET1 homolog	BET1	0.117	0.552
IPI00031000	Vitamin K epoxide reductase complex subunit 1	VKORC1	0.389	0.556
IPI00028338	Ethanolaminephosphotransferase 1	SELI	0.595	0.559
IPI00304264	Derlin-2	DERL2	0.352	0.561
IPI00737871	Uncharacterized protein KIAA0754	KIAA0754	0.493	0.562
IPI00555597	cDNA FLJ32471 fis, clone SKNMC2000322, highly similar to Peptidyl-tRNA hydrolase 2, mitochondrial (EC 3.1.1.29)	PTRH2	0.144	0.563
IPI00103057	Protein FAM36A	FAM36A	0.416	0.564
IPI00219755	Signal peptidase complex subunit 1	SPCS1	0.221	0.566
IPI00026087	Barrier-to-autointegration factor	BANF1	0.259	0.566

IPI00015713	CDK5 regulatory subunit-associated protein 1-like 1	CDKAL1	0.284	0.566
IPI00334579	Mitochondrial ribosomal protein L43	MRPL43	0.456	0.566
IPI00012913	Protein sprouty homolog 2	SPRY2	0.169	0.568
IPI00001891	Ancient ubiquitous protein 1	AUP1	0.046	0.568
IPI00141318	Cytoskeleton-associated protein 4	CKAP4	0.025	0.571
IPI00008207	Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase	MAN1B1	0.021	0.572
IPI00015954	GTP-binding protein SAR1a	SAR1A	0.546	0.573
IPI00011937	Peroxiredoxin-4	PRDX4	0.572	0.575
IPI00745125	Sterol O-acyltransferase 1	SOAT1	0.301	0.582
IPI00177968	Scavenger receptor class B member 1	SCARB1	0.079	0.582
IPI00011662	Kunitz-type protease inhibitor 2	SPINT2	0.509	0.583
IPI00012535	DnaJ homolog subfamily A member 1	DNAJA1	0.448	0.589
IPI00385495	Lipase maturation factor 2	LMF2	0.125	0.589
IPI00465059	Mitochondrial Rho GTPase 2	RHOT2	0.453	0.592
IPI00796337	Poly(RC)-binding protein 2 isoform b variant	PCBP2	0.496	0.592
IPI00216298	Thioredoxin	TXN	0.178	0.596
IPI00006280	Sterol regulatory element-binding protein cleavage-activating protein	SCAP	0.138	0.597
IPI00008350	Probable palmitoyltransferase ZDHHC6	ZDHHC6	0.015	0.597
IPI00289876	Syntaxin-7	STX7	0.109	0.599
IPI00465028	Triosephosphate isomerase	TPI1	0.549	0.601
IPI00010491	Ras-related protein Rab-27B	RAB27B	0.539	0.602
IPI00465290	DnaJ homolog subfamily C member 11	DNAJC11	0.332	0.602
IPI00446875	Endoplasmic reticulum-Golgi intermediate compartment protein 3	ERGIC3	0.050	0.603

a) Ratio M/L (median/light) shows to what extent the purified protein was derived from the non-palmitoylated form (*i.e.*, hydroxylamine(-)/hydroxylamine(+)).

b) Ratio H/L (heavy/light) shows to what extent the palmitoylated form was still present after DHHC3 knockdown (*i.e.*, shDHHC3/shCtrl).

Figure 6A shows a representative SILAC spectrum of a peptide derived from cytoskeleton-associated protein 4 (CKAP4), a known palmitoyl protein (*8*). DHHC3 knockdown led to the decrease of the palmitoylation level of CKAP4, suggesting that CKAP4 is a candidate substrate of DHHC3. In contrast, as shown in Figure 6B, the palmitoylation level of flotillin-1 (FLOT1), also a known palmitoyl protein (*9*), was not affected by DHHC3 knockdown, indicating that flotillin-1 is unlikely a DHHC3 substrate.

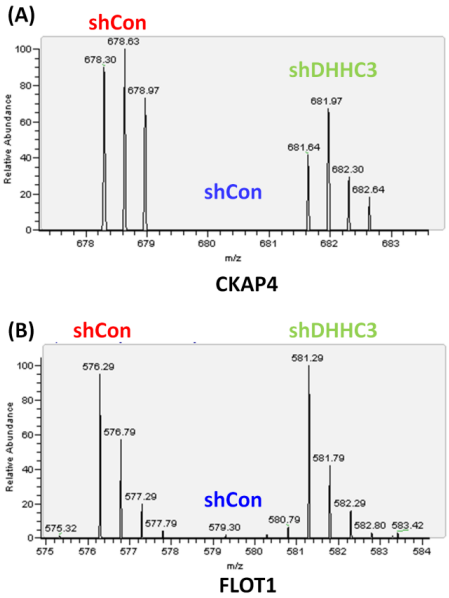


Figure 6: Representative SILAC spectrum of a peptide derived from (A) a candidate DHHC3 substrate cytoskeleton-associated protein 4 (CKAP4) and (B) an unlikely DHHC3 substrate flotillin-1 (FLOT1).

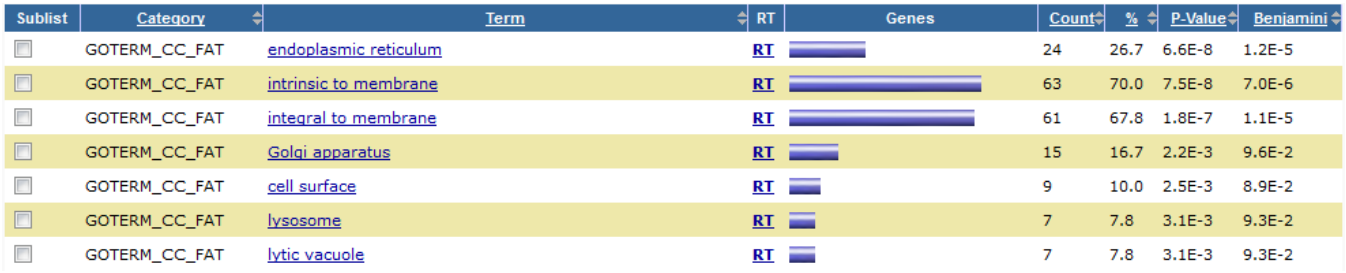
As a validation and extension of our findings in breast cancer cells, we applied the same approach to discover DHHC3 substrates in prostate cancer PC3 cells. We identified 529 candidate palmitoyl proteins, among which 30 were accepted as candidate DHHC3 substrates, with a H/L ratio cutoff of 0.667 (Table 2). These candidate substrates include integrin subunits α6 and β4 and tetraspanin-9. Among the 30 candidates, eight were identified as candidate DHHC3 substrates in both breast cancer MDA-MB-231 and prostate cancer PC3 cells. This suggests that, in addition to shared functions, DHHC3 may play different roles in different cancer types via regulating different sets of substrates. Alternatively, DHHC3 substrates are expressed or palmitoylated at different levels in different cancer cell lines. A more comprehensive analysis may identify more DHHC3 substrates common in different cancer types and cell lines.

Table 1. Candidate DHHC3 substrates in prostate cancer PC3 cells

Protein description	Gene Name	Ratio H/L	Also in 231 cells?
Palmitoyl acyltransferase DHHC3	ZDHHC3	0.244	
Linker for activation of T-cells family member 2	LAT2	0.324	
Chromobox protein homolog 5	CBX5	0.337	
Hephaestin-like protein 1	HEPHL1	0.356	
Anoctamin-1	ANO1	0.395	
Integrin alpha-6	ITGA6	0.407	Yes
Transmembrane protein 192	TMEM192	0.446	Yes
Transmembrane emp24 domain-containing protein 1	TMED1	0.459	Yes
CD58	CD58	0.485	
Cytoskeleton-associated protein 4	CKAP4	0.490	Yes
CKLF-like MARVEL transmembrane domain-containing protein 6	CMTM6	0.510	Yes
Beta-1,4-galactosyltransferase 1	B4GALT1	0.515	
Tetraspanin-9	TSPAN9	0.515	
Transmembrane protein 97	TMEM97	0.546	Yes
24-dehydrocholesterol reductase	DHCR24	0.549	
B-cell receptor-associated protein 29	BCAP29	0.559	
Cytochrome b	MT-CYB	0.559	
Protein disulfide-isomerase TMX3	TMX3	0.571	
Carboxypeptidase D	CPD	0.585	
Integrin beta-4	ITGB4	0.585	
Surfeit locus protein 4	SURF4	0.588	
CD44 antigen	CD44	0.599	
Tumor necrosis factor receptor superfamily, member 6 isoform 1 variant	FAS	0.617	
Guanine nucleotide-binding protein subunit beta-2-like 1	GNB2L1	0.637	
Endoplasmic reticulum-Golgi intermediate compartment protein 2	ERGIC2	0.645	
Cation-dependent mannose-6-phosphate receptor	M6PR	0.645	Yes
N-acylsphingosine amidohydrolase 1	ASAH1	0.658	Yes
Disintegrin and metalloproteinase domain-containing protein 17	ADAM17	0.658	
Protein jagged-1	JAG1	0.667	

40S ribosomal protein S27a	RPS27A	0.667	

In addition, gene ontology analysis of the total 92 identified DHHC3 substrate candidates by DAVID (the Database for Annotation, Visualization and Integrated Discovery, v6.7) showed that about two-thirds (61 out of 92) of candidate DHHC3 substrates are integral membrane proteins (Fig. 7), consistent with the previous report that DHHC3 itself is an integral membrane protein (*10*). In addition, the most significant biological processes regulated by DHHC3 are cell motion and vesicle-mediated transport (Fig. 8), two processes important for cancer cell invasion and metastasis.



Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_CC_FAT	endoplasmic reticulum	RT		24	26.7	6.6E-8	1.2E-5
	GOTERM_CC_FAT	intrinsic to membrane	RT		63	70.0	7.5E-8	7.0E-6
	GOTERM_CC_FAT	integral to membrane	RT		61	67.8	1.8E-7	1.1E-5
	GOTERM_CC_FAT	Golgi apparatus	RT		15	16.7	2.2E-3	9.6E-2
	GOTERM_CC_FAT	cell surface	RT		9	10.0	2.5E-3	8.9E-2
	GOTERM_CC_FAT	lysosome	RT		7	7.8	3.1E-3	9.3E-2
	GOTERM_CC_FAT	lytic vacuole	RT		7	7.8	3.1E-3	9.3E-2

Figure 7. Gene ontology analysis of the candidate DHHC3 substrates showed that most DHHC3 substrates are integral membrane proteins.

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	GOTERM_BP_FAT	cell motion	RT		11	12.2	9.4E-5	7.7E-2
	GOTERM_BP_FAT	vesicle-mediated transport	RT		11	12.2	4.4E-4	1.7E-1

Figure 8. Gene ontology analysis of the candidate DHHC3 substrates showed that DHHC3 substrates are mainly involved in cell motion and vesicle-mediated transport.

After the identification of these DHHC3 substrate candidates, our collaborator Dr. Hemler's lab would validate certain proteins-of-interest and perform functional assays to delineate the mechanisms of how DHHC3 regulates breast cancer cell growth, invasion, and metastasis (see the Hemler report).

Key research Accomplishments

- Dramatically improved the selectivity of purifying palmitoylated proteins by optimizing our original PalmPISC method.
- Developed a highly sensitive single-shot proteomics method by systematically evaluating 10 sets of LC-MS/MS settings.
- Conducted an in-depth palmitoyl-proteomics profiling study of cancer cells.
- Identified candidate DHHC3 substrates in breast cancer MDA-MB-231 cells by quantitative palmitoyl-proteomics.
- Identified candidate DHHC3 substrates in prostate cancer PC3 cells by quantitative palmitoyl-proteomics.

Conclusion

By substantially improving the selectivity of our PalmPISC method as well as the speed and sensitivity of LC-MS/MS analysis, we developed a very powerful palmitoyl-proteomics profiling method. By

coupling the method with duplex SILAC, we conducted so far the most comprehensive palmitoyl-proteome profiling study. Moreover, by integrating RNA interference (RNAi), triplex SILAC, and PalmPISC, we identified 70 and 30 DHHC3 substrate candidates in breast cancer MDA-MB-231 cells and prostate cancer PC3 cells, respectively. Gene ontology enrichment analyses showed that most DHHC3 substrates are integral membrane proteins and that DHHC3 significantly regulates cell motion and vesicle-mediated transport.

Publications, Abstracts, and Presentations

1. Zhou, B., An, M., Freeman, M. R., and Yang, W. Technologies and challenges in proteomic analysis of protein S-acylation. J. Proteomics Bioinform. 2014, 7: 256-263.
2. Zhou, B., Freeman, M. R., and Yang, W. Single-shot proteomics profiling and quantification of human prostate cancer cells to a depth of 6,500 proteins. Submitted to J. Proteome Res.
3. Zhou, B., Freeman, M. R., and Yang, W. Single-shot proteomics profiling and quantification of human prostate cancer cells. Abstract for the 14th Human Proteome Organization (HUPO) World Congress (2015, Vancouver, Canada)

Inventions, Patents and Licenses

N/A

Reportable Outcomes

- A markedly improved method for purification of palmitoyl proteins with high (>99.9%) selectivity.
- A powerful LC-MS/MS analysis method for highly sensitive identification of proteins from micrograms of proteins.
- The largest group of palmitoyl proteins identified in a single study so far.
- DHHC3 substrates identified from breast cancer cells.
- DHHC3 substrates identified from prostate cancer cells.
- Cell motion is the most significantly regulated biological process by DHHC3 in cancer.

Other Achievements

DoD W81XWH-15-1-0167 Yang (PI) 06/15/15-06/14/16
DHHC2, a Palmitoylating Enzyme, is a Key Suppressor of Prostate Cancer Metastasis and Castration Resistance
The objective of this project is to test the overall hypothesis that DHHC2 suppresses prostate cancer metastasis and castration resistance via the palmitoylation of certain substrates critical for prostate cancer progression to metastatic castration-resistant prostate cancer.

References

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(10) Ohno, Y., Kihara, A., Sano, T., and Igarashi, Y. Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. *Biochim Biophys Acta* 2006, *1761*, 474-483.

Appendices

N/A