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The roles of zDHHCs in cancer and as potential targets

Abstract: zDHHCs are proteins that are involved in multiple pathways in the cell. Palmitoylation of proteins happens when forming a thioester bond of palmitoyl-CoA and cysteine residue in DHHC-CR domain. There are multiple zDHHCs that are involved in multiple cancers, both as oncogenic and tumor suppressor. However, for targeting zDHHCs as potential targets in cancer there is more research needed.

Introduction: Discovery and mechanism of zDHHCs

The process of palmitoylation was first described in 1951[10], however the enzymes that catalyze palmitoylation are a more recent discovery. The first major breakthrough in finding this enzyme came through an experiment on yeast, also known as *Saccharomyces cerevisiae*. An Erf2p-Erf4p complex had been identified to be involved in localising and palmitoylation of Ras2 protein towards the plasma membrane. This complex had shown to increase Ras2 palmitoylation[1], where deletion of Erf2 or Erf4 would lead to either mislocalization of the Ras protein or a decrease in palmitoylation[2].

Another protein, identified as Akr1p(ankyrin-repeat-containing protein 1) was also identified in yeast, as it was shown that Akr1p mediated the palmitoylation of Yck2 (yeast casein kinase 2)[3]. Interestingly, Erf2 and Akr1p both were integral membrane proteins that contained the aspartate-histidine-histidine-cysteine (DHHC) cysteine-rich (CR) conserved domain and multiple transmembrane domains, where the DHHC domain was located between the second and third transmembrane domains[4][5].

zDHHC protein was acknowledged as a palmitoyltransferase through these studies in yeast, which showed how important the DHHC-CR domain is for activity within the cell. Whereas in yeast only 7 zDHHC proteins are expressed, in humans there are 23 zDHHC proteins[4]. Most of the zDHHCs in humans and mammals are able to catalyze without the help of a protein cofactor. Only zDHHC9, related to the Erf2 protein in yeast, requires the help of a Golgi-complex associated protein which has a homologous gene sequence as the Erf4 for the palmitoylation of Ras proteins and autopalmitoylation[6]. All the members of the zDHHC family compromise of four to six transmembrane domains (TMDs)[11]. The DHHC domain is on the same location on every isoform of the DHHC family, between the second and third transmembrane. And as the DHHC domain, the N- and C-terminal are also located in the cytosol of the cell. It is thought that both N- and C-terminal domains both mediate the protein-protein interactions to facilitate the palmitoylation process by connecting the substrates[11]. ZDHHC13 and zDHHC17 both contain

ankyrin repeat domains, which likely will interact with certain proteins however further studies is necessary[89].

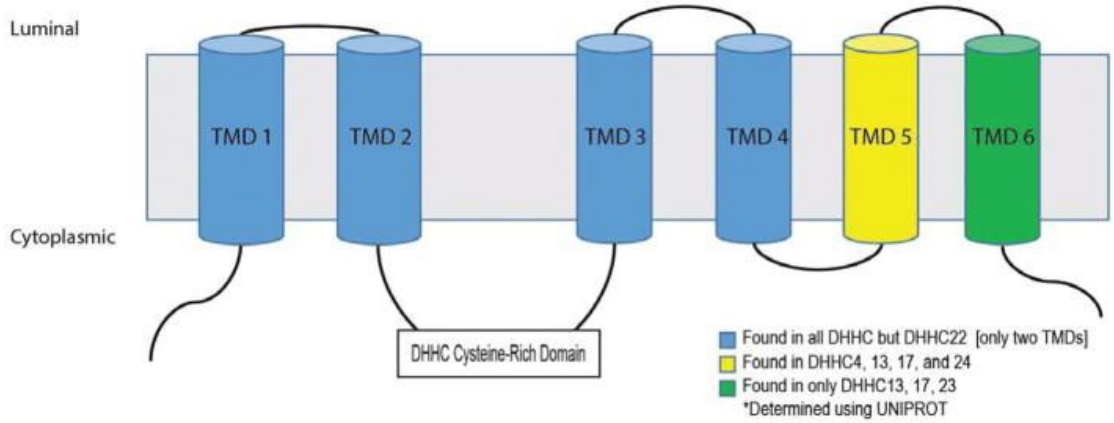

Figure 1: Common zDHHC structure

Due to analysis on localisation of all mammalian DHHC proteins within the cell in HEK293T cells, it showed that zDHHC proteins were mostly localising to the endoplasmic reticulum and the Golgi[7]. Exceptions have been shown to be localised to the plasma membrane, like the DHHC5. Palmitoylation by zDHHCs can be either N-palmitoylation, O-Palmitoylation or S-palmitoylation. S-Palmitoylation is the mostly used palmitoylation and is a reversible post-translational modification, where a 16-carbon long saturated acid will covalently bind onto proteins at cysteine residues and will form a thiol estherbond[4]. The S-Palmitoylation process is catalyzed due to protein acyltransferases (PATs) also known in mammalian as DHHC. Where, the palmitoyl-CoA is Because, S-Palmitoylation is a reversible process it can be reversed due to acyl protein thioesterases (APTs)[5].

Mechanism

The catalyzing activity of zDHHCs works in a two-step process. The mechanical aspects of the palmitoylation that is mediated by DHHC was unknown due to the lack of knowledge available of the structures of the zDHHC until recent[8]. In this study they reported the crystal structure of one of the mammalian zDHHC proteins, zDHHC20. The proposed structure of zDHHC has four transmembranes maintained in a way which allowed two transmembranes to stay close with each other and so creating distance between other two transmembrane domains with the location of the DHHC domain. The separation distance of these transmembrane is important for the catalyze process and for the engagement of the substrate. Due to this structure it is possible for the sidechain in the catalytic domain to help the cysteine with nucleophilic attack on the palmitoyl-CoA when present. This is the first step of the process which creates a palmitoyl-PAT product. This first step in the process only takes a few seconds and is called auto-palmitoylation. After forming this palmitoyl-intermediate, the intermediate is transferred from the zDHHC protein onto the substrate protein. Mutations in the DHHC domain resulted in the abolishment of the palmitoylation process, indicating that the DHHC-CR domain is important for palmitoylation to occur[9].

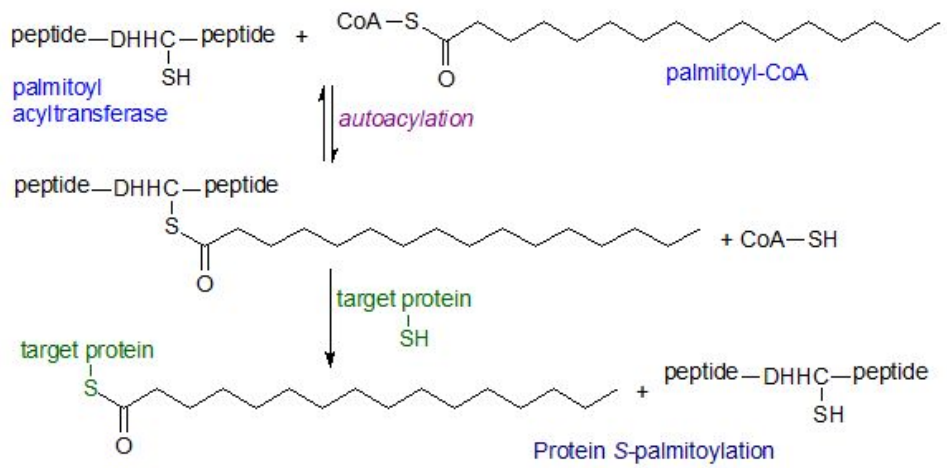

Figure 2: Palmitoylation with palmitoyl acyltransferase (DHHC)

APT

The reversibility of S-Palmitoylation is done by hydrolysis of the S-palmitoylated proteins and their catalyzing thioesterases. These acyl thioesterases cleave of the modifications formed by DHHC. There are two main acyl thioesterases, LYPA1 and LYPA2, who also have both been identified as possible targets for controlling Ras proteins in cancer[56].

Substrates

In the process of palmitoylation, DHHC domain is responsible for palmitoylation of substrates to function within the cell. In figure 3, the known substrates that take part in cancer of all cancer-involved members of the DHHC family are shown and the articles of the researchers finding them.

DHHC:	**Substrates:**
DHHC1	miR-93 (Yu et al., 2011b), NCDN (Oku et al., 2013)
DHHC2	CKAP4/p63 (Zhang et al., 2008), CLIMP-63 (Sandoz and van der Goot, 2015), CD9 and CD151 (Sharma et al., 2008), Leukocyte C-terminal Src kinase (Lck) (Zeidman et al., 2011),
DHHC3	NCAM (Lievens et al., 2016), Integrin β4 (Sharma et al., 2012), NCDN(Oku et al., 2013), (ERGIC3) (Sharma et al., 2017)
DHHC5	(EZH2) (Chen et al., 2017), Flotilin2 (Li et al., 2012), δ-Catenin (Brigidi et al., 2014)
DHHC7	Scribble (SCRIB) (Chen et al., 2016), Fas (Rossin et al., 2015), Estrogen, Progesterone, Androgen receptors (Pedram et al., 2012),
DHHC9	N-Ras (Raymond et al., 2007), H-Ras (Swarthout et al., 2005),
DHHC11	Neurochondrin (NCDN) (Oku et al., 2013)
DHHC13	MC1R (Chen et al., 2017), CTNND1 (Shen et al., 2017),
DHHC14	-
DHHC15	-
DHHC16	-
DHHC17	-

DHHC18	BMI1(Chen X et al., 2019)
DHHC20	Epidermal growth factor receptor (EGFR) (Runkle et al., 2016), MCAM(Haas et al., 2005)
DHHC21	Fyn (Mill et al., 2009), Estrogen, Progesterone and Androgen receptors (Pedram et al., 2012)
DHHC22	-
DHHC23	BMI1(Chen X., et al 2019)

Figure 3: zDHHC substrates known to be involved in cancer

In this review we will look at which zDHHCs are associated with which types of cancers and how their substrates are involved in it. Further we will research whether it might be possible to target these DHHC domains for treatment of cancer?

The involvement of zDHHC in different types of cancer

As previously explained, palmitoylation is associated with a lot of critical roles within the functioning of the cells. This accounts besides enzymatic activity also for effecting certain proteins due to interaction, subcellular localization having an effect on certain proteins as a result of interacting and other roles of processes within the cell[12][13][14][15][16][17].

Because palmitoylation is involved within multitude processes in the cell, palmitoylation is also associated with multiple forms of cancer and other diseases[18]. To date, palmitoylation has been associated with these diseases due to the disruption of the balance that is being created by DHHCs by protein palmitoylation, mainly happening within the nervous system. Eight zDHHCs have shown to be responsible for neurodegenerative diseases, where dysregulation of zDHHC17 can result in Huntongton disease. Where a mutation in HTT has been shown to be resistant for palmitoylation, where overexpression of palmitoylation was shown to reverse the effect of this mutation[21]. DHHC7 and DHHC21 have shown to be associated with palmitoylation of APP, which increase the amount of production of amyloid beta protein[19]. In a study by Mukaj et al, 2004 knockout mice of zDDHC8 showed impaired palmitoylation of neuronal proteins resulting in possible schizophrenia and bipolar disorder[20].

As mentioned before, palmitoylation can be associated with different forms of cancers. Palmitoylation only happens with the use of the enzymatic DHHC-CR domain which is responsible for multiple cancers associated with palmitoylation. ZDHHCs are responsible for the trafficking and localising of certain proteins involved in , for example cell metabolism. This may lead to think that disruption of localising and trafficking of proteins could potentially be dramatic for the cell to survive or increase unnecessary proliferation. Therefore zDHHCs are a potential important factor in the cell and an interesting subject for further research.

Cancer is an assembly of diseases that all have the same universal cause that the cells grow abnormally. The cause of this abnormal growth is mainly due to mutations in the DNA that leads to overexpression of proliferative genes or reduced expression of tumor suppressor genes[22]. As mentioned above, trafficking and localising proteins are essential for the cell to sustain. However, dysregulating and dislocalising proteins are identified in some cancers. Thus, researching zDHHCs whether a reduction of palmitoylation or overexpression will lead to a changed regulation of the cell and whether it might be involved in the development of cancer is a really interesting field.

zDHHC	Ankyrin repeat	Transmembranes	DHHC domain	Chromosome location
zDHHC1	-	4	134-184	16q22.1
zDHHC2	-	4	127-177	8p22
zDHHC3	-	4	127-177	3p.21.31
zDHHC5	-	4	104-154	11q12.1
zDHHC7	-	4	130-180	16q24.1
zDHHC9	-	4	139-189	Xq26.1
zDHHC11/11B	-	4	125-175	5p15.33
zDHHC13	7	6	426-476	11p15.1
zDHHC14	-	4	165-215	6q25.3
zDHHC15	-	4	129-179	Xq13.3
zDHHC17	7	6	437-487	12q21.2
zDHHC18	-	4	192-242	1p36.11
zDHHC20	-	4	126-176	13q12.11
zDHHC21	-	4	90-140	9p22.3
zDHHC23	-	4	259-309	3q13.31

Figure 4: zDHHCs, ankyrin repeats, transmembranes, DHHC domains and chromosome location of those involved in cancer

zDHHC1

NCDN, a known substrate of zDHHC1 targeting endosomes, is induced by nerve growth factor (NGF) which is mainly responsible for neuronal differentiation[23]. The production of NGF has been found to be reduced in prostate cancer. Treating two prostate tumor cell lines with NGF showed that the modulated expression of the genes responsible for modulating cell growth were

less malignant[24]. When testing for potential miR-93 targets, zDHHC1 was found to be one of them. miR-93 palmitoylation leads to more activation of miR-93. By studying the difference between a normal human colon cancer cell line and this cell line transfected with miR-93, it was found that the cell line with miR-93 showed inhibition of cell proliferation in colon cancer after 3 weeks[36]. miR-93 was also found to be involved in other types of cancer. miR-93 was found to be upregulated in breast cancer cell line and induced cell proliferation, migration, infiltration stimulating PI3K/AKT pathway. This was found to be inversely related with the tumor suppressor gene in breast cancer, PTEN[57]. In lung cancer, miR-93 suppresses the DAB2 tumor suppressor gene by targeting DAB2 mRNA. High levels of miR-93 and low levels of DAB2 resulted in poor survival suggesting an important role for miR-93 in non-small cell lung cancer and small cell lung cancer[58]. Suggesting zDHHC1 is oncogenic and tumor suppressor.

zDHHC2

zDHHC2 is known as the gene that functions as a tumor suppressor gene.A substrate of zDHHC2, CKAP4/p63, was shown to have reduced palmitoylation in zDHHC2 knockdown[29]. zDHHC2 is important as the anti-proliferative factor (APF) binds to CKAP4/CLIMP-43 for mediating the APF signalling. CKAP4 is a downstream effector signal of the APF signalling transduction and has activity of itself to inhibit proliferation[46]. Therefore, APF expression has shown to be inhibiting the growth of bladder carcinoma[48].

In hepatocellular carcinoma a frequent loss of heterozygosity of chromosome 8p22-8p23 was observed, where zDHHC2 is located on.This loss of heterozygosity led to persistent metastasis of hepatocellular carcinoma, correlation with tumor size and tumor thrombi to the portal vein after liver transportation. zDHHC2 functions as a tumor suppressor, as expression of zDHHC2 was reduced in hepatocellular carcinoma[38]. This loss of heterozygosity of zDHHC2 is therefore associated with metastatic hepatocellular carcinoma and also with progression of breast cancer[39]. zDHHC2 has also been found to be responsible for the palmitoylation of two tetraspanin membranes, CD9 and CD151. This is contrary to theory as CD9 and CD151 are both involved in the progression of cancer and metastasis in breast cancer[70][71] .

zDHHC2 was found to be increased in miR-155 transfected inhibitor human nasopharyngeal carcinoma (NPC) cell lines in comparison to inhibitor control NPC cell lines, leading to finding two possible miR-155 target sites. Inhibiting zDHHC2 leaded to an increase in cell migration and a correlation with poor prognosis and metastasis of nasopharyngeal carcinoma[59].

zDHHC3

One of the substrates that can be palmitoylated by zDHHC3 is ITG β4[43]. The levels of ITG β4 palmitoylation being known to be correlated with the invasiveness of breast cancer. By blocking ITG β4, it showed that ITG β4 is palmitoylated within aggressive breast cancer cell lines. It is further shown that zDHHC3 is upregulated in aggressive and metastasized breast cancer[44].

Silencing of zDHHC3 leads to the diminishment of protection of antioxidants and elevation of oxidative stress. ERGIC3, Endoplasmic reticulum Golgi intermediate compartment 3, is a protein that is palmitoylated by zDHHC3 and downregulated by loss of zDHHC3. Low levels of ERGIC3 lead to high Endoplasmic reticulum stress levels and eventually to upregulation of a protein called TXNIP[64]. TXNIP increases the levels of oxidative stress within the cell and to apoptosis. This leads to an overall reduction in tumor growth in breast cancer[45].

Figure 5: zDHHC5 knockdown reduction of tumor growth

zDHHC5

By studying the effects of different members of the DHHC family in non-small cell lung cancer (NSCLC), targeting zDHHC5 was found effective. Inhibiting zDHHC5 with the help of siRNAs led to a reduction in cell growth in NSCLC cell lines, but not in normal lung cell lines. zDHHC5 silencing also reduced colony invasion, cell invasion and cell proliferation[53].

Flotillin-2 (Flot2), a lipid raft protein that is being palmitoylated by DHHC5, is known to be involved in multiple cancers. Flot2 and zDHHC5 have been found to be down regulated in breast cancer cell lines (MCF-7) when a known inhibiting proliferation protein, Liver X receptor, was stimulated[60]. Flot2 has been detected in also showing overexpression in hepatocellular carcinoma cell lines promoting proliferation, metastasis and a marker of poor prognosis for patients[61]. In colorectal cancer, Flot2 was found to be overexpressed and promoting progression of colorectal cancer[62]. Flot2 overexpression promotes tumor growth due to activating NF-κB and PI3K/AKT3 pathways. By analysing overexpression of Flot2 in nasopharyngeal carcinoma, both NF-κB and PI3K/ AKT3 activity was increased[63].

zDHHC5 has also been identified as an important mediator of development of glioblastoma. Upregulation of zDHHC5 expression was measured in glioma tissue with a mutation in p53 and showed reduced survival. Upregulation of zDHHC5 was due to binding of nuclear transcription factor-Y (NF-Y) and mutant p53 on binding sites of zDHHC5 promoter. Silencing of zDHHC5 showed a reduction of tumor growth, meaning zDHHC5 expression is normally promoting tumor growth. EZH2, a substrate of zDHHC5, is known as a repressor of multiple tumor suppressors. More palmitoylation by zDHHC5 led to an increase in EZH2 activity indicating increase in tumor growth[72], suggesting zDHHC5 to be oncogenic.

zDHHC7

zDHHC7 is a palmitoyltransferase of Scribble (SCRIB) protein. Palmitoylation of SCRIB leads to localising of SCRIB to the plasma membrane. Silencing of zDHHC7 has therefore led to mislocation of SCRIB and also overexpression of H-Ras. This dislocating of SCRIB leads to

diminishment of tumor suppressors, increase in cell invasion, no suppressing of anchorage-independent growth and activation of MAPK, YAP and PI3K/AKT pathways[65]. Fas, an apoptosis receptor promoting cell death, is also palmitoylated by zDHHC7 protecting Fas from degradation within the lysosomes. A decrease in Fas expression has been observed when the palmitoylation mediated by zDHHC7 is down-regulated[73].

zDHHC7 and zDHHC21 were identified as PATs for localising estrogen receptor(ER), progesterone receptor(PR) and androgen receptor(AR) at the plasma membrane. Knockdown of zDHHC7 or zDHHC21 resulted in reduced localising on the plasma membrane and a decrease in ERa palmitoylation, leading to a decline in ERK and PI3K/AKT activation. Palmitoylation increases bonding of ER and PR to the plasma membrane. Breast cancer is associated with an increase in ERa signalling on plasma membrane, and inhibiting these zDHHCs led to a reduction of ER and PR localisation on the membrane. zDHHC7 and zDHHC21 could also function as targets for prostate cancer due to AR signaling[40].

zDHHC9

Ras proteins are known oncogenic proteins. For the activation of Ras proteins, zDHHC9 functions as their palmitoyl transferase[31]. The role of Ras proteins in cancers is their encoding of GTPases on the membrane, which control pathways associated with promoting cell growth and cell differentiation[66]. zDHHC9 was identified as a PAT by controlling the plasma membrane localisation of Ras proteins, when silencing of zDHHC9 led to Ras protein localisation in the cytoplasm and overexpression of zDHHC9 resulted to the doubling of Ras protein on the membrane[90].

Doubling of Ras protein is likely to increase the activation of pathways responsible for cell growth and cell differentiation. In hematopoietic cancer, N-Ras proteins had been identified being associated with multiple types of hematopoietic cancers[30][31]. Knockdown of zDHHC9 seemed to correspond to this, resulting in milder progression in leukemogenic cancers. As did overexpression promoting progression, showcasing a relation between zDHHC9 and N-ras expression[32]. Overexpression of zDHHC9 was also measured in colon carcinoma[55].

zDHHC11/11B

Burkitt Lymphoma (BL) is a form of non-Hodgkin Lymphoma that is primarily diagnosed in young children and is highly curable. zDHHC11/11B were found to be associated with the tumor growth in Burkitt Lymphoma. MYC is overexpressed in BL and is responsible for cell growth due to elevating levels of MYB. B cell growth is highly dependent on expression of MYB, as it also functions as oncogenic. miRNA-150 was found to downregulate MYB, but miRNA-150 is itself downregulated in BL because of MYC overexpression. In Burkitt Lymphoma, zDHHC11 and zDHHC11B have been found to be induced by MYC, which further promotes high MYB levels and so create a B cell growth positive feedback loop[49].

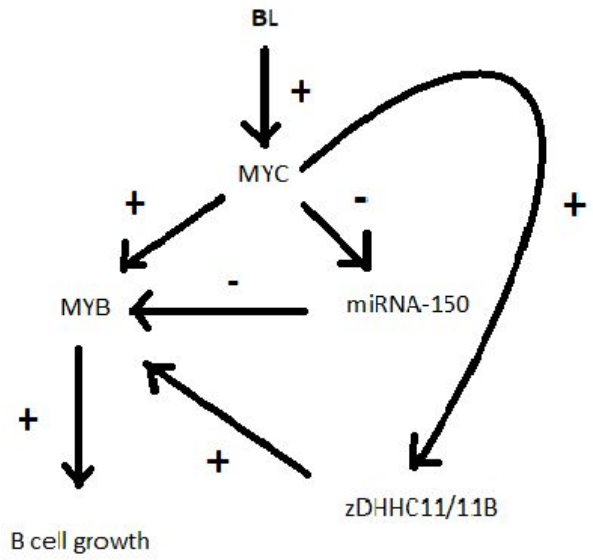

Figure 6: B cell growth in Burkitt Lymphoma

As shown in figure 4, the zDHHC11 gene is located on chromosome 5. A gain of function of chromosome 5p15.33 was measured in high-grade bladder cancer. This location is also host of the zDHHC11 gene and has been associated with high-grade bladder cancer in this way[47]. Multiple single-nucleotide polymorphisms (SNPs) of TERT, telomerase reverse transcriptase, gene have been associated with cancer. One SNP, rs2853677, has been associated with pancreatic cancer and increased expression of zDHHC11. A real connection with zDHHC11 has however yet to be studied[88]

zDHHC13

Mutations of zDHHC13 have been found playing a role in different ways of obtaining skin carcinoma. One of these mutations in zDHHC13 has been associated with having a higher possibility of obtaining skin carcinoma. This mutation takes place on exon 7 mutating a T to an A, leading to the creation of a premature stop codon 200 amino acids after the N -terminal. These mutation showed to have more expression of pro-NF-κB causing more activation of NF-κB. This mutation in zDHHC13 resulted in an increase in epidermal thickness, proliferation of keratinocytes and accelerated migration of basal to more differentiated layers. MIce with this mutation showed more tumor growth and progression of papillomas than normal mice[50].

MC1R, a G-protein that stimulates melanin production through cAMP signalling, is palmitoylated by zDHHC13 in the attendance of α-MSH. Ultraviolet radiation increases palmitoylation of MC1R. Loss of MC1R function leads to increase in melanomagenesis. Indicating that palmitoylation of MC1R by zDHHC13 is a possible protection for melanomas[67]. CTNND1 has been identified as a substrate of zDHHC13 as palmitoylation levels of CTNND1 was lower in zDHHC13 knockout cells[75]. This was also measured in prostate cancer tissue[74].

zDHHC14

zDHHC14 is involved in several types of cancer, where it is able to function as an oncogenic or tumor suppressor depending on the type of tissue. In prostate cancer, zDHHC14 functions as a tumor suppressor. zDHHC14 mRNA was measured to be downregulated in multiple prostate cancer cell lines[25]. However, zDHHC14 functions as an oncogenic in gastric cancer. zDHHC14 was found to be overexpressed in 27% of the gastric cancer tissue. Overexpression of zDHHC14 was associated with increase of invasion area of the tumor and cell migration, while zDHHC14 knockdown showed a decrease in invasiveness[52].

Chromosomal translocation t(6;14)(q25;q32) was found in acute biphenotypic leukemia and this is where zDHHC14 was located on (6q). This chromosomal translocation resulted in a 15 fold increased expression of zDHHC14 in leukemic samples of these patients. By analyzing all types of leukemia, zDHHC14 was only detected in two patients of T/myeloid leukemia and AML patients. These patients showed that zDHHC14 overexpression led to a 30% decrease in differentiation, which is a known marker in leukemia[68].

zDHHC15

zDHHC15 is one of the zDHHC families that has not been studied well enough. Eventhough, zDHHC15 has been linked in high grade ovarian carcinoma. Normally, females have one of the two X chromosomes deactivated because it is needed for dosage compensation to prevent having twice the X chromosomes of the males. When there are two X chromosomes active it increases the expression of the oncogenic genes located on the X chromosome. zDHHC15 is one of those genes. zDHHC15 was measured to be upregulated in high grade ovarian carcinoma, which had two active X chromosomes[37].

zDHHC17

zDHHC17 is most notably known as the huntington interacting protein-14 (HIP14), as it has an important function in huntington as described earlier. HIP14 is able to perform PAT activity in interaction with H-Ras and N-Ras proteins, which are known to be expressed in exceedingly all human tissue[26]. HIP14 is associated with both oncogenic and tumor suppressor functions[69]. This is because HIP14 has a preference for certain recognized motifs that have firstly been farnesylated and can then be palmitoylated, where Ras proteins have these motifs. HIP14 has shown to be oncogenic when HIP14 is overexpressed. This led to less contact inhibition and therefore increasing proliferation and colony forming due to HIP14 anchorage-independent growth[77].

This has all shown that overexpression of HIP14 is responsible for tumor growth. By studying different types of tumors, HIP14 mRNA levels were indeed reacting according to theory. Breast, lung prostate, stomach and colon cancer was associated with overexpression of HIP14 mRNA levels. And HIP14 also seemed to function as a tumor suppressor, as HIP14 mRNA levels were decreased in cancers found in the liver, pancreas, bladder, thyroid gland and the vulva[27].

zDHHC18

zDHHC18 and zDHHC23 has recently been found to be associated with progression of glioblastoma. zDHHC18 was found to be upregulated in glioma tissue, just as zDHHC23. High grade gliomas showed higher expression of zDHHC18 and zDHHC23 then low grade gliomas, meaning a relation of expression levels with different stages of cancer. BMI1 is a substrate of both zDHHC18 and zDHHC23, which plays a role in glucose metabolic process. Competitiveness between zDHHC18 and zDHHC23 for interaction with a BMI1 E3 ligase, RNF144A, has shown to be important for tumor growth or reducing growth. zDHHC18 binding reduces BMI1 polyubiquitinated levels leading to an increase of glioblastoma tumor growth[76].

zDHHC20

Sphingosine kinase 1 and 2 are known proto-oncogene isomers, which are expressed in many types of cancers. Knockdown of Sphingosine kinase 1 and 2 showed downregulating of multiple genes, including zDHHC20, suggesting zDHHC20 to be oncogenic[77]. EGFR is tyrosine receptor kinase that is involved in some cancers due to uncontrolled activation[41], but is also a substrate that can be palmitoylated by zDHHC20 on the C-terminal. Inhibiting zDHHC20 determined the cell to be completely depended on EGFR for cell survival and increased EGFR activation in breast cancer, but is also shown in non-small cell lung cancer[54]. Suggesting a combination of zDHHC20 and EGFR could be used as therapeutic therapy in breast cancer[42].

zDHHC20 has primarily shown to function as an oncogenic protein when overexpressed. Overexpression of zDHHC20 was measured in ovarian, breast, kidney, prostate and colon cancer[28]. However, zDHHC20 functions as a tumor suppressor in melanoma. MCAM, a substrate of zDHHC20 that is responsible for cell adhesion of melanoma cells, is reduced in melanoma when zDHHC20 is silenced. This results in increased cell invasion, promoting cancer and showing zDHHC20 functions as a tumor suppressor in melanoma[78].

zDHHC21

zDHHC21 functions as an oncogenic. zDHHC21 is important for the palmitoylation of the Lck pathway, which is essential for apoptosis due to downstream signalling of the FAS receptor in lymphatic cells. DHHC21 knockdown leads to inactivating of the Lck pathway and thus the Fas receptor. This results to less apoptosis within the lymph nodes and may assist progression of lymphomas[33]. zDHHC21 functions the same as zDHHC7 in breast cancer, where zDHHC21 knockdown results in less palmitoylation of Era and less localization on the plasma membrane. And like zDHHC7, zDHHC21 is upregulated in breast cancer[40].

zDHHC23

zDHHC23 is involved in regulating B cell malignancies and glioblastoma. In studying a difference of amount of white blood cells in B precursor acute lymphoblastic leukemia (BPALL), zDHHC23 was found to be more expressed in high white blood cells,then low white blood cells[25]. Indicating that zDHHC3 is overexpressed in higher grade BPALL[34]. BK channels can be palmitoylated by zDHHC23 and are also known to be involved with B-cell malignancies[35].

As earlier mentioned, zDHHC18 and zDHHC23 are involved in glioblastoma but function differently. Especially zDHHC23 is upregulated in glioma tissue. The competitiveness between zDHHC18 and zDHHC23 for interaction with RNF144A is important for progression of glioma tumors. When zDHHC23 interacts with RNF144a it promotes binding of RNF144A and BMI1, a known substrate of both zDHHC18 and zDHHC23, it increases the polyubiquitinated BMI1 levels. Upregulation of zDHHC23 results in lower BMI1 levels, leading to less glioblastoma tumor growth[76].

ZDHHC inhibitors: existent and potential inhibitors

zDHHCs being so profoundly present in multiple types of cancer, makes it possible to be a target for therapeutic therapies in cancer. Ducker et al, 2006 mentioned that DHHCs are the targets for inhibiting palmitoylation. Development of inhibitors for zDHHCs has not been fully explored jet, especially referring to specific inhibitors for specific isoforms of the zDHHC family. The focus on developing specific inhibitors lies primarily on selecting the N- and C-terminal. The inhibitors that are now in use can be divided into two main groups: lipid based inhibitors and non-lipid based inhibitors.

Lipid-based inhibitors

Lipid-based inhibitors have been thoroughly well studied in comparison with non-lipid based inhibitors, but are not specific for one isoform of the zDHHC family. Well studied lipid-based inhibitor are 2-bromopalmitate (2BP), cerulenin and tunicamycin, 2BP being the most commonly used of these three.

2BP is known to inhibit palmitoylation of cells[79], inhibit PAT activity of zDHHCs[80], inhibiting fatty acid CoA ligands[81] and inhibiting enzymes that are involved in lipid metabolism[82]. Before palmitoylation of substrates, palmitic CoA binds to DHHC domain forming an enzyme-acyl intermediate. 2BP inhibits palmitoylation by blocking the formation of enzyme-acyl intermediate[83]. The non-specificity of 2BP is a problem for using it for treating patients, because 2BP inhibits all PATs resulting in undesirable inhibition of other pathways not intended to block what can be dysregulating the homeostasis within the cell. 2BP is known to successfully inhibited H-Ras, src kinases and rho kinases[84].

Cerulenin is just as 2BP a lipid-based inhibitor and is non-specific. The mechanism of cerulenin is to inhibit the synthesis of fatty acids, like palmitic acid. This mechanism acts early on in the palmitoylation pathway, making it complicated to have accessible palmitate available before palmitoylation process[86]. Inhibition of fatty acid synthesis due to cerulenin has shown to induce apoptosis in breast cancer cell lines[85].

Tunicamycin functions a bit different then Cerulenin. Inhibition of tunicamycin is competing with the PATs for binding of palmitoyl-CoA[79]. This is like 2BP, which also prevents forming of palmitoyl-CoA on PATs to form enzyme-acyl intermediates. All these known lipid-based inhibitors are nonspecific and can therefore not be helpful in treating cancer.

Non-lipid based inhibitors

There have been 5 different compounds that showed inhibition of PAT activity. These different compounds have been identified through three different screening assays, led to divide it in two groups: farnesyl-palmitoylation motifs and myristoyl-palmitoylation motifs. Compound 1-4 might inhibit Ras proteins due to inhibiting COOH-group of farnesyl and Compound 5 might inhibit Src-families and G-proteins due to NH2-group of myristoyl. All the PAT inhibitors showed reduction of tumor growth in mammary adenocarcinoma[27]. Showing inhibition in cancer having zDHHC17 overexpression, but possibly also in other zDHHC related to palmitoylation of Ras protein as zDHHC9.

In another study, compound 5 was shown to inhibit expression of zDHHC2 and zDHHC3 involved in the study[83]. Suggesting that compound 5 is like 2BP possibly inhibiting all zDHHCs. Curcumin, a natural polyphenol, has shown to block interaction of integrin β4 with growth receptors preventing migration in cancer[87]. However, curcumin also inhibits palmitoylation of integrin β4. Curcumin is capable of blocking auto acylation of zDHHC3, preventing integrin β4 palmitoylation[44].

Till this day there has not been a specific zDHHC inhibitor specific targeting the DHHC-CR domain. It is difficult, as each isoform has a different structure but very similar. The N- and C-terminal could be potential targets for therapy, as is the case in kinases. Recently, there has been an improvement in substrate specificity and acyl chain length of DHHC20. This is important for targeting these DHHC domains that could be inhibited in cancer therapy. When analysing the structure of DHHC20 when in palmitoylation process, certain targets were found for further research as potential targets. Residue His154 could be a target, as protonated His154 is possibly involved in the transfer of palmitoyl to the substrate by activating the enzyme-acyl intermediate[8]. His154 could be a target for treating aggressive breast cancer, as earlier mentioned in combination or in absence of activation of EGFR[42].

Further research is still needed, as there is still a lot unknown about substrate specificity and acyl chain length of all isoforms of zDHHCs and has been hinted at being a potential target of cancer therapy for some time.

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