The role of ZDHHC proteins in cancer

ABSTRACT
S-acylation is a form of reversible post-translational modification. S-acylation, in other words, palmitoylation, is the attachment of a palmitate group via a thioester bond to the thiol group of a cysteine. Palmitoylation, is a process that is moderated by zDHHC (zinc finger domain containing aspartate-histidine-histidine-cysteine) enzymes. Palmitoylation of proteins affects the protein in several ways, it gives rise to alterations in the secondary structure which leads to altered functions of the proteins. In line with the important functions of palmitoylation, malformations in zDHHC proteins are likely to play a role in human diseases, including cancer. zDHHC proteins can either act as oncoproteins or as tumor suppressor proteins and in addition, some of them have been shown to have value as prognostic markers. Studies have shown that most of the zDHHC proteins seem to act as oncoproteins, with tumor suppressor functions for only 5 of the 23 zDHHC proteins. However, there is often a lack of functional evidence to confirm these observations. For zDHHC4, zDHHC6, zDHHC10, zDHHC12, zDHHC22, and zDHHC24 there is currently not enough data to support a role in cancer. Additionally, for zDHHC8 , zDHHC11, and zDHHC20 studies showed some controversial results when it comes to the classification of these proteins as oncoproteins or tumor suppressor proteins. Current knowledge on the role of zDHHC proteins in cancer development is far from complete. So, future research is needed to create a better and more complete overview of the consequences of aberrant palmitoylation by zDHHC proteins in different types of cancer. Furthermore, future research is needed to study targeting of zDHHC proteins which might be effective as a new type of cancer treatment.

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27-04-2021

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INTRODUCTION

In the human genome, there are approximately 20.000 protein encoding genes[1]. However, the number of proteins is much higher, with studies suggesting that there are approximately a few million proteins. Therefore, the human cells have ways to increase the number of proteins by generating more diversity. One of the ways that this can be accomplished, is by alternative splicing. The transcripts derived from protein encoding genes almost all undergo alternative splicing[2]. Additionally, protein post-translational modifications (PTMs) can take place. These ways of generating more diversity are needed because the 20.000 proteins that are encoded by genes, are not enough to regulate all of the processes in the human body[3,4].

One of the possible PTMs is S-acylation, a form of lipid modification. Several studies have suggested that there are hundreds of S-acylated proteins in mammalian cells[5]. It has also been shown that approximately 12% of the human proteome is S-acylated[6]. A characteristic feature of S-acylation is that in contrast to most of the other forms of lipid modification, it is reversible[5]. During S-acylation, fatty acids are attached onto cysteines. Most of the time this concerns a palmitate, which is a fatty acid derived from palmitoyl-CoA. The attachment of a palmitate group via a thioester bond to the thiol group of a cysteine is a process which is otherwise known as palmitoylation, a process which is reversible[4].

Palmitoylation, is a process that is moderated by a group of enzymes that is called protein acyltransferases (PATs). These enzymes belong to the zDHHC (zinc finger domain containing aspartate-histidine-histidine-cysteine) family[6,7]. The zDHHC family consists of 23 proteins that are encoded by different genes, genes zDHHC1-zDHHC24, excluding zDHHC10[7]. In humans, zDHHC enzymes localize to the plasma membrane of the cell, as well as to plasma membranes of endosomes, the endoplasmic reticulum, and the Golgi apparatus[4]. The zDHHC enzymes all have a similar universal membrane topographic anatomy, which includes four to six transmembrane domains and both, the N-terminus and C-terminus, are located in the cytosol. Moreover, it appears that in one of the cytosolic loops, the catalytic DHHC cysteine-rich domain (CRD) is located (Figure 1). The location of this catalytic domain led to the observation that the cysteines that are located at the cytosol – membrane interface, can be the substrates for the zDHHC enzymes[7].

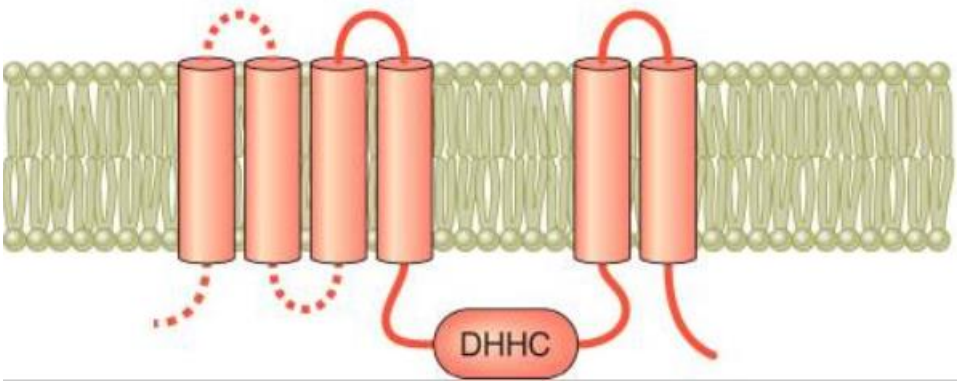

Figure 1: zDHHCs are transmembrane proteins with the catalytic DHHC CRD located in one of the cytosolic loops[5].

So, besides palmitoyl-CoA, the zDHHC enzymes also have other protein substrates. It was shown that zDHHC enzymes are often able to modify more than one protein substrate and that proteins which are palmitoylated, are substrates for several zDHHC enzymes. This led to the idea that palmitoylation is not very specific and that the choice of protein substrate is based on the location of the protein substrate. In other words, zDHHC enzymes show a preference towards the protein substrate that is closest to the enzyme. Moreover, it was also seen that even though the palmitoylated proteins are substrates for several zDHHC enzymes, one particular zDDHC enzyme would have a stronger interaction with the protein substrate than another zDHHC enzyme[8,9]. The latter of these

observations is in line with the observations that there are also zDHHC enzymes that contain protein-protein interaction domains, which is a feature that suggests that there are specific zDHHC enzyme-substrate interactions[9]. Nevertheless, S-acylated proteins can be roughly organized into transmembrane and peripheral membrane proteins. With transmembrane proteins crossing the plasma membrane of the cell and/or organelles, and peripheral membrane proteins being located at the surface of the cytosolic side of the plasma membrane of the cell and/or organelles. Both, the palmitoylated transmembrane proteins, as well as the palmitoylated peripheral membrane proteins, are very distinct in nature. The proteins include transporters (transmembrane proteins), ion channels (transmembrane proteins) and receptors (peripheral membrane proteins)[5].

A palmitoylation reaction occurs when palmitate is thioesterified to substrate proteins and this reaction can roughly be divided into two steps. As explained previously, this reaction is carried out by zDHHC enzymes which are integral membrane proteins. In the first step, an enzyme-acyl intermediate is created and autopalmitoylation takes place, this occurs when a thioester linkage is formed to attach the palmitate group to the cysteine in the DHHC domain, a free coenzyme A (CoA) is then released. The second step occurs when the palmitate group is subsequently relocated to a cysteine residue of another protein substrate. When this happens, the DHHC enzyme is revived for a second round of catalysis (Figure 2)[7,8,9]. Additionally, in order for palmitoylation to be reversible, depalmitoylation should also take place. Depalmitoylation is a process that is carried out by acyl protein thioesterases (APTs) and protein palmitoyl thioesterases (PPTs)[5].

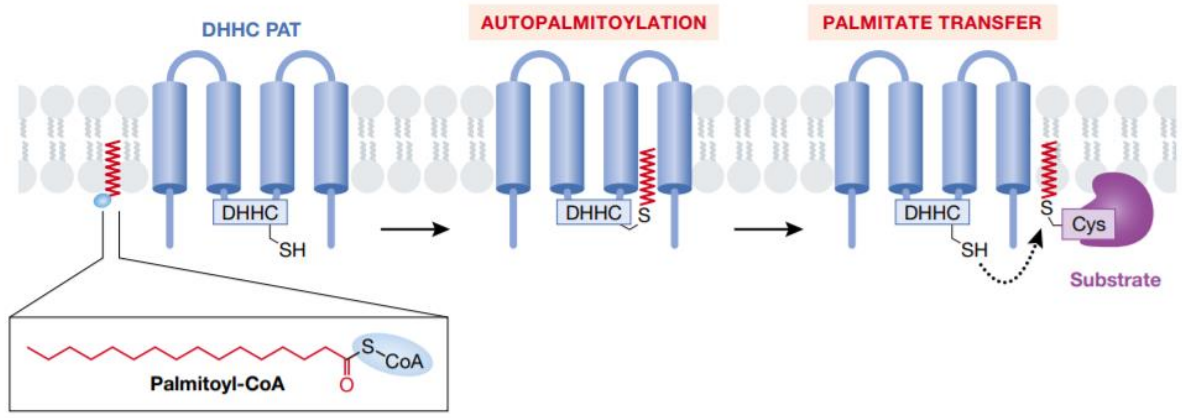

Figure 2: Working mechanism of a palmitoylation reaction. Integral membrane proteins (blue), protein substrate (purple)[8].

Palmitoylation of proteins affects the protein in several ways, it gives rise to alterations in the secondary structure which leads to altered functions of the proteins. Palmitoylation regulates for example the membrane attachment of multiple soluble proteins, as well as the interactions, activity, turnover, stability and localization[4,10]. Moreover, palmitoylation is involved in managing several phases of the life cycle of peripheral-membrane and transmembrane proteins. This includes protein trafficking, protein assembly and final degradation of the proteins (Figure 3). More specifically, it was shown for peripheral-membrane proteins that the most important function of palmitoylation is to mediate stable membrane attachment. Additionally, palmitoylation was proposed to function as an important regulator of the lateral position of proteins within membranes[5]. Logically, protein depalmitoylation is also involved in a range of processes which include protein function and protein localization. An example is that depalmitoylation by PPTs is associated with the autophagy-lysosome pathway as well as with neurite extension and axonal outgrowth[11].

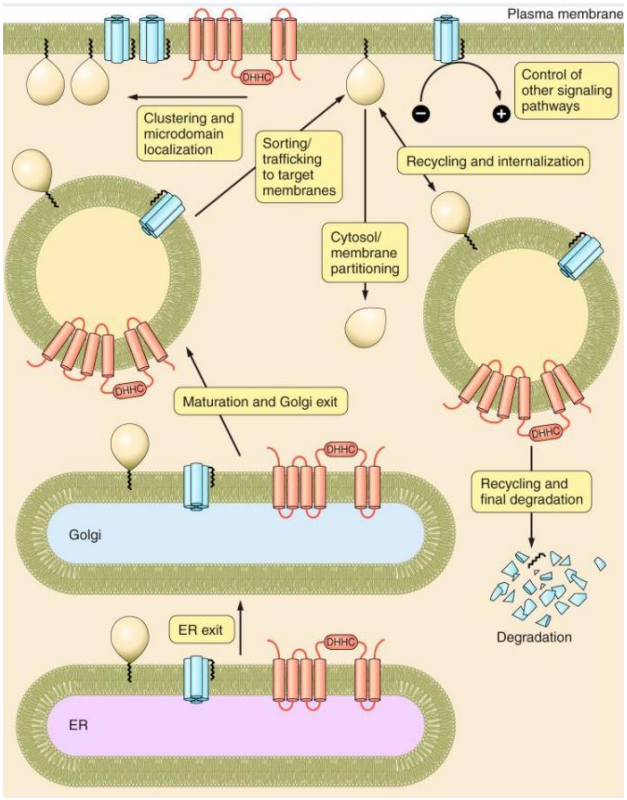

Figure 3: Various locations of S-acylation and functions of S-acylation[5].

In line with the important functions of palmitoylation, malformations in palmitoylation and/or depalmitoylation of proteins and malformations in zDHHC proteins are likely to play a role in human diseases. It has already been shown that protein palmitoylation plays a role in schizophrenia, Huntington's disease and X-linked mental retardation. Moreover, there has been an increase in studies which show that there are specific zDHHC proteins involved in cancer. This shows that there is a role for protein palmitoylation and incorrect functioning of zDHHC proteins in tumorigenesis. So far, studies have suggested that there is a role for at least a dozen of the zDHHC genes in cancer[12]. Nevertheless, the area of zDHHC proteins, palmitoylation and depalmitoylation in association with cancer remains understudied. Therefore, the aim of this essay is to elaborate on the role of zDHHC proteins in cancer.

THE ROLE OF ZDHHC PROTEINS IN CANCER

Ko and Dixon have suggested that the way that zDHHC proteins are involved in cancer can be divided into three groups: the zDHHC proteins can either act as oncoproteins or as tumor suppressor proteins and in addition, some of them have been shown to have value as prognostic markers. Moreover, they suggested that some of the zDHHC proteins, can act either as oncoproteins or tumor suppressor proteins, dependent on the tissue and cancer type[8]. For this essay, based on the review written by Ko and Dixon, and the results as reported in several studies, the zDHHC proteins have been divided into two groups: oncoproteins and tumor suppressor proteins. For zDHHC proteins with different functions in different cancer types, the most commonly observed function has been described. The zDHHC proteins were identified as oncoproteins when they showed increased expression in cancer and when downregulation of this expression affected cancer cell growth in a positive way. The activation of oncogenes is an essential step in tumorigenesis in a lot of cancer types, the activation of oncogenes leads, for example, to cell proliferation. When an oncogene gets an activating mutation, it is able to turn a healthy cell into a tumor cell[13]. zDHHC proteins were identified as tumor suppressor proteins when they showed an decreased expression in cancer and when overexpression affected cancer cell growth in a positive way. Tumor suppressor proteins are often involved in the inhibition of cell proliferation and in DNA damage repair. When tumor suppressor genes are mutated, and there is loss of the tumor suppressor function, then a healthy cell can be turned into a tumor cell[14]. Studies have shown that most of the zDHHC proteins act as oncoproteins, with tumor suppressor functions for only 5 of the 23 zDHHC proteins. For zDHHC4, zDHHC6, zDHHC10, zDHHC12, zDHHC22, and zDHHC24 there is currently not enough data to support a role in cancer (Table 1).

Table 1: zDHHC proteins and their association with cancer

	Potential role in cancer	Cancer type(s)
zDHHC1	Tumor suppressor protein	Colon cancer, hepatocellular carcinoma, nasopharyngeal tumors, gastric cancer, breast cancer, lung cancer
zDHHC2	Tumor suppressor protein	Hepatocellular carcinoma, gastric cancer
zDHHC3	Oncoprotein	Breast cancer
zDHHC4	x	x
zDHHC5	Oncoprotein	Glioma, non-small cell lung cancer
zDHHC6	x	x
zDHHC7	Tumor suppressor protein	x
zDHHC8	Oncoprotein	Cervical cancer, renal cell cancer
zDHHC9	Oncoprotein	Breast cancer, gastrointestinal adenocarcinomas
zDHHC11	Oncoprotein	Burkitt lymphoma
zDHHC12	x	x
zDHHC13	Tumor suppressor protein	Melanoma
zDHHC14	Tumor suppressor protein	Testicular germ cell tumor
zDHHC15	Oncoprotein	Glioma
zDHHC16	Oncoprotein	Hepatocellular carcinoma
zDHHC17	Oncoprotein	Glioma
zDHHC18	Oncoprotein	Glioma
zDHHC19	Oncoprotein	Lung squamous cell carcinoma
zDHHC20	Oncoprotein	Prostate cancer, breast cancer, colon cancer, ovarian cancer, kidney cancer
zDHHC21	Oncoprotein	Colorectal cancer

zDHHC22	x	x
zDHHC23	Oncoprotein	Glioma
zDHHC24	x	x

ONCOPROTEINS

Sharma and colleagues reported an increased expression of zDHHC3 in breast cancer, which was even more profound in metastatic breast cancer when compared to normal breast tissue. Moreover, increased expression of zDHHC3 was also reported in prostate and colon cancer. The role of zDHHC3 has been linked to oxidative stress and senescence, a cellular state which leads to the clearance of cells, in this case tumor cells, by immune cells. Deletion of zDHHC3 led to increased oxidative stress and ROS levels. In turn, this increased level of oxidative stress diminished survival of tumor cells by for example, activating apoptosis, the process of programmed cell death. Interestingly, induction of apoptosis was more profound in comparison to induction of senescence. They expected more induction of senescence, since zDHHC3 was linked to senescence previously[15]. Collectively, these results indicate an oncogenic role for zDHHC3 in breast cancer, prostate cancer and colon cancer. This assumption can be made since zDHHC3 is often overexpressed, resulting in a decrease in oxidative stress as well as a decrease in senescence and apoptosis of the tumor cells.

Several studies have proposed an oncogenic role for zDHHC5 in, for example, glioma[16,17,18]. These studies showed higher zDHHC5 levels in glioma as compared to normal brain tissue. Additionally, elevated levels of zDHHC5 were associated with a poor prognosis indicating its potential value as a prognostic marker[18]. Another study showed that inhibition of zDHHC5 led to decreased cell invasion and proliferation in non-small cell lung cancer[16]. Moreover, the levels of zDHHC5 were increased in glioma cells that had a mutation in p53, compared to glioma cells that had wild type p53[17]. Based on the functional studies performed by Tian et al., there can be concluded that there is an oncogenic role for zDHHC5 in non-small cell lung cancer. The findings of an elevated expression of zDHHC5 in glioma are not enough to conclude that in this type of cancer, zDHHC5 plays an oncogenic role as well.

Strassburger and colleagues reported that based on publicly available data, the level of zDHHC8 appears to be associated with increased or reduced overall cancer survival, dependent on the type of cancer. For example, increased zDHHC8 levels were associated with shorter survival of patients with cervical and renal cell cancer, which might be considered as an indication of an oncogenic role for zDHHC8 in these types of cancer. In contrast, increased zDHHC8 levels were associated with longer survival of patients with pancreatic and lung cancer. Their study in drosophila showed that deletion of dzDHHC8 led to increased cell proliferation, which might be an indication for zDHHC8 being a tumor suppressor[19]. However, due to the lack of functional evidence for the role of zDHHC8 in human cancer, no concrete conclusion can be drawn from these results.

Multiple studies have suggested an oncogenic function for zDHHC9 in cancer[20]. Yang and colleagues showed that zDHHC9 is responsible for palmitoylation of PD-L1. Palmitoylation of PD-L1 stabilizes PD-L1 and results in the maintenance of its distribution across the cell surface. Enhanced PD-L1 protects the tumor cells from anti-tumor immune responses. This study also showed that disturbance of palmitoylation of PD-L1 resulted in a higher sensitivity of breast cancer cells to T-cell mediated killing, which resulted in diminished tumor growth[21]. Additionally, Mansilla and colleagues showed that zDHHC9 is overexpressed in all gastrointestinal adenocarcinomas including tumors in the small intestine, colon, stomach and rectum[22]. Based on these results, there can be concluded that zDHHC9 probably plays an oncogenic role in breast cancer. However, there is not enough functional evidence to say the same for zDHHC9 in all gastrointestinal adenocarcinomas.

A study performed by Dzikiewicz-Krawczyk and colleagues showed that zDHHC11 together with zDHHC11B are important players in the oncogenic MYC-miR-150-MYB axis in Burkitt lymphoma, suggesting an oncogenic role for zDHHC11. Downregulation of zDHHC11 and zDHHC11B resulted in diminished growth of Burkitt lymphoma cells. Moreover, data showed that it is likely that zDHHC11 and zDHHC11B also play an oncogenic role in other types of lymphoma as well, including Hodgkin lymphoma and diffuse large B-cell lymphoma, since zDHHC11 and zDHHC11B are upregulated in these lymphoma types too. The mechanism that is at play here is the following: MYC induces both zDHHC11 and zDHHC11B, which results in depletion of miR150, this allows the MYB transcript to escape from miR150 induced inhibition, thereby allowing MYB levels to stay high. This is important because MYB is needed to maintain the high proliferation rate of Burkitt lymphoma cells. Therefore, there is enough evidence to conclude that zDHHC11 plays an oncogenic role in, at least, Burkitt lymphoma, maybe even in other types of lymphoma too[23].

Fan and colleagues showed an oncogenic role for zDHHC15 in glioblastoma. The expression levels of zDHHC15 were higher in gliomas than in normal brain tissue. Moreover, these higher levels of zDHHC15 correlated positively with the tumor grade, indicating that zDHHC15 levels might have prognostic value. The results of their study suggest that zDHHC15 is important for preservation of the functionality of glioblastoma stem cells. Interestingly, they found that the expression of zDHHC15 was decreased by using local anesthetics, including lidocaine, procaine, ropivacaine and prilocaine, and as a consequence, the self-renewing capacity of the glioblastoma stem cells was diminished. Additionally, it is known that there is a regulatory feedback loop between zDHHC15 and IL-6/STAT3, which might be responsible for the constitutive activation of this oncogenic pathway[24].

Xu and colleagues showed that zDHHC16 levels were higher in hepatocellular carcinoma than in adjacent normal tissue, which might indicate an oncogenic role for zDHHC16. Moreover, presence of high zDHHC16 levels was associated with poor prognosis for hepatocellular carcinoma patients, indicating that zDHHC16 might also have prognostic value. So, even though there is no functional evidence yet, these results indicate that it might be that zDHHC16 has an oncogenic role in this type of cancer[25].

A study performed by Chen and colleagues showed that zDHHC17 has an oncogenic function in gliomas. They showed elevated zDHHC17 levels in tumor tissue compared to normal brain tissue. Moreover, the levels of zDHHC17 corresponded to the tumor grade, indicating that zDHHC17 could also have prognostic value in gliomas. Additionally, this study showed that the zDHHC17-MAPK24 signaling module controlled the tumorigenic phenotype in patient-derived glioblastoma cells with zDHHC17 overexpression. The expression levels of zDHHC17 and MAPK24 both connected to cell migration and cell invasion. Most importantly, they showed that glioblastoma stem cells expressing zDHHC17 were able to effectively form tumors in vivo whereas, upon knockdown of zDHHC17 the ability of glioblastoma cells to form tumors diminished[26]. Therefore, there is enough evidence to conclude that zDHHC17 plays an oncogenic role in glioma.

zDHHC18 and zDHHC23 seem to exert an oncogenic function in gliomas, because here, Chen and colleagues found elevated protein levels of zDHHC18 and zDHHC23 compared to healthy brain tissue. Moreover, these elevated protein levels of zDHHC18 and zDHHC23 were found to correspond to the severity of the malignancy. Therefore, there is the possibility that perhaps in the future, zDHHC18 and zDHHC23 can be used as prognostic markers. Interestingly, the expression levels of zDHHC18 were increased in the mesenchymal subtype, a subtype with mesenchymal differentiation and a very aggressive phenotype. Whereas expression levels of zDHHC23 were increased in the proneural subtype, a subtype with neuronal differentiation and which is associated with a better outcome. This suggested that zDHHC18 and zDHHC23 have different targets in mesenchymal and proneural subtype glioblastoma. Moreover, zDHHC18 was able to promote the survival of glioblastoma under conditions of low oxygen and scarce nutrients. Additionally, zDHHC18 is able to improve BMI1 polycomb ring

finger oncogene (BMI1) stability under a stressful microenvironment. BMI1 acts as a strong inducer of proliferation of neural progenitor cells, thereby promoting the survival of mesenchymal glioblastoma stem cells[27,28]. Therefore there can be concluded that zDHHC18 has an oncogenic function in glioma. However, for zDHHC23, functional evidence is lacking.

The zDHHC19 locus located on the long arm of chromosome 3, is often amplified in human cancer. This was mostly seen in lung squamous cell carcinoma. Additionally, they noticed that a higher level of zDHHC19 expression was connected to a poor survival chance of cancer patients, suggesting that zDHHC19 could have a prognostic value. zDHHC19 is responsible for the palmitoylation of STAT3, as well as for the activation of STAT3 which is palmitic acid induced. The signaling of the zDHHC19-STAT3 axis is responsible for the "stemness-like phenotype" of lung squamous cell carcinoma. Inhibition of zDHHC19 expression resulted in inhibition of cell proliferation, cell migration and the ability to form colonies of lung squamous cell carcinoma cells, indicating that zDHHC19 functions as an oncoprotein in lung squamous cell carcinoma[29].

Several studies have suggested an oncogenic role for zDHHC20[30,31]. Draper and colleagues found that the expression of zDHHC20 is increased in several types of cancer, including prostate, breast, colon, ovarian and kidney cancer. Additionally, they found that zDHHC20 supports anchorage independent grow, and allows cells to proliferate past saturation. So, zDHHC20 expression is connected to malignant transformation of cells. Taken together, these results suggest that there is an oncogenic role for zDHHC20 in several types of cancer[30].

There is not much known about the role of zDHHC21 in cancer. Chen and colleagues showed that zDHHC21 is overexpressed in colorectal cancer. They also showed that zDHHC21 is part of a prognostic model of seven-gene signature. So, based on the expression of zDHHC21 and six other genes in colorectal cancer, patients are divided into low-risk and high-risk groups[32]. Moreover, a study performed by Pedram and colleagues showed that zDHHC21 is overexpressed in human breast cancer, when tissue is compared to normal breast epithelium[33]. Even though these studies might indicate an oncogenic role for zDHHC21 in several types of cancer, there is still no functional evidence to support this.

TUMOR SUPPRESSORS

Le and colleagues found that ectopic expression of zDHHC1 reduced aggressiveness of cancer cells. The zDHHC1 gene is silenced via hypermethylation of the promotor region in multiple cancer types, including colon cancer, hepatocellular tumors, nasopharyngeal tumors, gastric cancer, breast cancer, and lung cancer. In this study, they also showed that the functional mechanism was probably linked to encouraging oxidative stress, apoptosis and endoplasmic reticulum mediated pyroptosis through diminishing the metabolic activity of the cells. Moreover, they showed that zDHHC1 induced apoptosis in tumor cells, suppressed invasion and migration of tumor cells and suppressed proliferation of tumor cells. *In vivo* studies confirmed the role of zDHHC1 as a tumor suppressor, based on reduced stemness of tumor cells as well as reduced epithelial-mesenchymal transition (EMT) upon zDHHC1 overexpression[34]. Altogether, these studies strongly support a tumor suppressor function for zDHHC1.

Studies performed by Peng and colleagues, and Yan and colleagues suggested a tumor suppressive function for zDHHC2. Peng and colleagues found a significant lower expression of zDHHC2 in hepatocellular carcinoma tissue compared to adjacent normal tissue. Additionally, overexpression of zDHHC2 significantly diminished proliferation of tumor cells, as well as the capacity to migrate and invade. Some of the known substrates of zDHHC2, namely CKAP4, CD9, and CD151 have been linked to the development and continuation of cancer. For example, decreased expression of zDHHC2 was associated with decreased palmitoylation of CKAP4, which led to a reduction in efficiency of the

protein to localize to the cell surface. Cell surface localization of CKAP4 is necessary for its role as a receptor for antiproliferative factor (APF). As a consequence of decreased zDHHC2 expression, APF is no longer able to stop cell proliferation, showing the importance of zDHHC2 as a tumor suppressor[35]. In addition, Yan and colleagues showed that zDHHC2 expression was also decreased in tumor tissue of patients with gastric cancer, compared to normal tissue. Moreover, decreased expression of zDHHC2 seemed to be connected to lymph node metastasis[36].

Multiple studies have suggested a tumor suppressive role for zDHHC7. Chen and colleagues showed that zDHHC7 is responsible for palmitoylation of scribble (SCRIB) which is a tumor suppressive protein. The localization of SCRIB to cell-cell junctions, is often incorrect in cancer cells. This suggests that the mislocalization of SCRIB is partly responsible for tumorigenesis[37]. Additionally, Aramsangtienchai and collegues found that zDHHC7 is also responsible for palmitoylation of JAM-C. Palmitoylation of JAM-C has an effect on the migration of cancer cells, and therefore most likely plays a role in cancer metastasis. Interestingly, they also found that a decrease in zDHHC7 is connected to tumorigenesis, indicating that there is a tumor suppressive role for zDHHC7 in cancer[38].

Studies performed by Perez and colleagues, as well as Chen and colleagues showed that there is a protective role for zDHHC13 in chemically induced skin cancer in mice[39]. Additionally, Chen and colleagues showed that in patients with melanoma, there is an association between high mRNA levels of zDHHC13 and survival benefits. They showed that zDHHC13 is responsible for the palmitoylation of MC1R, which is necessary for inducing MC1R signaling. So higher levels of zDHHC13 results in higher levels of activated MC1R. MC1R is a key protein in the regulation of skin and hair pigmentation. MC1R is also responsible for the stimulation of DNA-damage repair upon ultraviolet (UV) irradiation. They found that zDHHC13-dependent activation of MC1R palmitoylation was important in preventing melanoma *in vivo*. Studies involving “readheads” showed that their MC1R signaling is compromised, resulting in a higher risk for melanoma. Moreover, zDHHC13 overexpression prevented malignant transformation of melanocytes *in vitro* and *ex vivo*[40]. Taken together, these results showed that zDHHC13 fulfills a tumor suppressive role in melanoma.

Yeste-Velasco and colleagues found a decreased expression of zDHHC14 in testicular germ cell tumor tissue (TGCT) compared to adjacent normal tissue of the same patient. Decreased expression levels of zDHHC14 mRNA were also found in several other cancers, including brain cancer, lung cancer, lymphoma, kidney cancer, colorectal cancer, liposarcoma and prostate cancer. Moreover, overexpression of zDHHC14 induced apoptosis with the involvement of the classic caspase-dependent apoptosis pathway[41]. These results indicate that there might be a tumor suppressive role for zDHHC14 in these types of cancer, however, clear functional evidence is still lacking.

<u>DUAL ROLE: DEPENDENT ON CANCER TYPE</u>

zDHHC8 was classified as an oncoprotein in cervical cancer and renal cancer. However, the same study, which was performed by Strassburger and colleagues in drosophila, suggested that zDHHC8 fulfilled the role of a tumor suppressor as well, when they focused on another cancer type, namely lung cancer and pancreatic cancer[19]. Additionally, zDHHC11 was classified as an oncoprotein. Nevertheless, a study performed by Liu and colleagues suggested that zDHHC11 functions as a tumor suppressor. These findings were based on the fact that they found higher levels of zDHHC11 expression in patients with hepatocellular carcinoma who had a higher progression-free survival. They proposed that the zDHHC11 gene is silenced by methylation in hepatocellular carcinoma, suppressing the tumor suppressive function[42]. An explanation for this dissension could be the type of cancer that was studied. Since one study looked at Burkitt lymphoma[23] and the other study looked at hepatocellular carcinoma[42]. At last, zDHHC20 was classified as an oncoprotein. However, a study performed by Runkle and colleagues suggested a tumor suppressive role for zDHHC20. In this study, there was shown that zDHHC20 is expressed in several human cancer cell lines, including breast

cancer and lung cancer, but they also showed that the expression of zDHHC20 was able to diminish the metastatic behavior of melanoma cells. Indicating that zDHHC20 also fulfills a tumor suppressive function when its function is studied in melanoma[43].

DISCUSSION

The aim of this essay was to elaborate on the role of zDHHC proteins in cancer. This was done by performing a literature research. Based on previously performed studies, zDHHC proteins were classified either as oncoproteins or tumor suppressor proteins. This classification was difficult to make, since for a lot of the zDHHC proteins, there was no functional evidence to classify them as either oncoproteins or tumor suppressor proteins. Nevertheless, results of previous studies showed that most of the zDHHC proteins (13), seemed to show predominantly an oncogenic function in cancer. There were only five zDHHC proteins that, for the most part, seemed to show a tumor suppressive function. However, as was stated before, there is often a lack of functional studies to confirm these observations. Additionally, for six of the twenty-three zDHHC proteins, no role in cancer has been established (yet). Therefore, future research is necessary.

For zDHHC8 , zDHHC11, and zDHHC20 studies showed some controversial results when it comes to the classification of these proteins as oncoproteins or tumor suppressor proteins. An explanation for these results could be that these proteins have different effects in different cell types. These effects are then based on the different targets that these proteins have. The function of the zDHHC protein remains the same, however, the effect for the cell would be different. Similar observations of altering protein functions depending on cell type has been reported for, for example, ghrelin[44]. For example, a study performed by Collura and colleagues showed that glycoprotein (Gp130), is a target for zDHHC8-mediated palmitoylation in axons. Gp130 is a required part of several receptor signaling complexes for neuropoietic cytokines, including oncostatin-M (OSM), cardiotrophin-1 (CT-1), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and ciliary neutrophic factor (CNTF)[45]. Additionally, a study performed by Thomas and colleagues showed that protein interacting with C-kinase (PICK1) is also a target for zDHHC8-mediated palmitoylation. PICK1 is crucial for the induction of cerebellar long term depression[46]. In the case of zDHHC20, a study performed by Malgapo and colleagues showed that metallo-β-lactamase domain-containing protein 2 (MBLAC2) is a target for zDHHC20-mediated palmitoylation. MBLAC2 is a zinc metalloenzyme that might be associated with cellular levels of acyl CoA[47]. It could be that for both of these zDHHC enzymes, their targets exert different effects dependent on the cell type. But whether this is the case and whether this contributes to the development and/or progression of cancer, is still not known.

Nowadays, ten hallmarks of cancer have been established. These hallmarks are the ability of tumor cells to, among other features, evade growth suppressors, avoid immune destruction, and enable replicative immortality[48]. Palmitoylated proteins are associated with four of the ten hallmarks of cancer. These hallmarks include: resistance to cell death, activation of invasion and metastasis, induction of angiogenesis and sustained proliferative signaling. Based on the information of this essay, I would say that palmitoylated proteins are indeed involved in resistance to cell death, metastasis and sustained proliferative signaling, but not with the induction of angiogenesis. However, I would also say that palmitoylated proteins are involved in avoiding immune destruction, based on the observations that zDHHC9 regulates PD-L1.

The involvement of zDHHC proteins in resistance to cell death is shown by the fact that a lot of apoptosis regulating proteins are regulated by palmitoylation. Studies suggested that zDHHC3, zDHHC7, zDHHC11, zDHHC12, and zDHHC21 are most important for regulating apoptosis and therefore also in resisting cell death. The involvement of zDHHC proteins in activation of invasion and metastasis is shown by the role of palmitoylation in several steps of the invasion and metastasis process. zDHHC2, and zDHHC3 play important roles in this hallmark of cancer. Then, the involvement of zDHHC proteins in the induction of angiogenesis is shown by the palmitoylation of proteins with a pro-angiogenic role. Studies have suggested that zDHHC21 has a big influence on eNOS, with evidence suggesting that eNOS is responsible for the promotion of angiogenesis and tumorigenesis. However, functional evidence is missing. Then, at last, the involvement of zDHHC proteins in sustaining proliferative signaling was shown by the palmitoylation of Ras proteins. These proteins are responsible for the transduction of signals coming from growth factor receptors to the intracellular

pathways which are responsible for regulating cell proliferation. Moreover, some studies have indicated that protein palmitoylation might also be involved in the feature of tumor-promoting inflammation[12].

Current knowledge on the role of zDHHC proteins in cancer development is far from complete. First of all, not all zDHHC proteins have been studied in the context of cancer and secondly, most zDHHC proteins have only been studied in a limited number of cancer types. For future research, it is essential to study each zDHHC protein in multiple cancer types, because until now, a lot of research on the role of zDHHC proteins in cancer has been performed in gliomas. This research was mostly carried out by Chen and colleagues, which results in a bias. Additional studies in multiple cancer types will result in a better and more complete overview of the consequences of aberrant palmitoylation by zDHHC proteins in different types of cancer.

Another interesting question is whether targeting of zDHHC proteins might be effective as a new type of treatment. At the moment, there are no substances available that are able to inhibit individual zDHHC proteins which have an oncogenic or tumor suppressive function in cancer. However, there are already inhibitors that are able to inhibit a broad-spectrum of zDHHC proteins, such as 2-bromopalmitate (2-BP). In preclinical studies, this broad-spectrum inhibitor was used to confirm the hypothesis that the inhibition of zDHHC proteins resulted in the death of cancer cells, unfortunately, these broad-spectrum inhibitors cannot be used as cancer treatment since there are a lot of off-target effects[8]. Moreover, the results of a study performed by Liu and colleagues indicated that the zDHHC proteins that are differentially expressed in renal cell cancer, could be potential therapeutic targets, if specific inhibitors are available[49]. Additionally, Plain and colleagues suggested that the engagement of specific substrates with specific zDHHC enzymes could be manipulated and thereby changing the palmitoylation status of individual proteins[10].

So, the studies that have been performed so far confirm the role of aberrant palmitoylation by zDHHC proteins in cancer and provide a basis for development of zDHHC protein specific drugs as target for cancer treatment.

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