Role of NSD3 in Cancer

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Nuclear receptor-binding SET domain protein 3 (NSD3) is a member of the NSD histone methyltransferase family of proteins. In recent years, it has been identified as a potential oncogene in certain types of cancer. The NSD3 gene encodes three isoforms, the long version (NSD3L), a short version (NSD3S) and the WHISTLE isoforms. Importantly, the NSD3S isoform corresponds to the N-terminal region of the full-length protein, lacking the methyltransferase domain. The chromosomal location of NSD3 is frequently amplified across cancer types, such as breast, lung, and colon, among others. This amplification has been correlated to a chromothripsis event, that could explain the different NSD3 alterations found in cancer. The fusion proteins containing NSD3 have also been reported in leukemia (NSD3-NUP98), and in NUT (nuclear protein of the testis) midline carcinoma (NSD3-NUT). Its role as an oncogene has been described by modulating different cancer pathways through its methyltransferase activity, or the short isoform of the protein, through protein interactions.

cancer molecular oncology oncogenes NSD3 NSD3S

1. Introduction

Chromatin organization is highly regulated by many factors, one of them being histone post-translational modifications (HPTMs) [1]. These modifications have a direct impact on the level of chromatin compaction and the degree of transcription. Condensed chromatin, or heterochromatin, is associated with transcriptional repression, and loose chromatin, or euchromatin, associated with an active transcription of genes [2]. HPTMs can be recognized by proteins that are referred to as “readers” and are modified by adding or eliminating PTMs by proteins called “writers” or “erasers”, respectively. Histone methyltransferases (HMT) are “readers” and “writers” of the chromatin, responsible for catalyzing the addition of methyl groups in arginine (PRMT) or lysine residues (HKMT) on the N-terminal histone tails. Most histone lysine methylation is carried out by a family of SET-domain containing proteins, which catalyze the addition of one to three methyl groups [3]. The nuclear receptor-binding SET domain (NSD) family of histone lysine methyltransferase is composed of three members: NSD1, NSD2 (MMSET/WHSC1, Wolf-Hirschhorn syndrome candidate 1), and NSD3 (WHSC1L1, WHSC1 like 1). The NSD proteins play a crucial role in regulating chromatin integrity and gene expression by mono- or di- methylating histone H3 lysine 36, generating H3K36me1 and H3K36me2 [4][5]. Alterations in the NSD proteins have been correlated with human diseases. NSD1 haploinsufficiency and point mutations are implicated in Sotos syndrome [6], a childhood developmental disease, prostate cancer, melanoma, and acute myeloid leukemia (AML) [7]. Haploinsufficiency of NSD2 is related to Wolf-Hirschhorn syndrome, multiple myeloma, neuroblastoma, endometrial and hepatocellular cancer, among others [4][8]. Finally, aberrant expression of NSD3 has been implicated in the development of multiple cancer types, such as lung, breast, and pancreatic cancer [9][10][11].

2. NSD3 Protein Structure

NSD3 was first described in 2000 by studying the PWWP (proline-tryptophan-tryptophan-proline) domain of NSD2 and performing a database search for proteins having the PWWP domain in their structure. The NSD3 gene is found on chromosome 8p11.2 [12] and encodes three isoforms by alternative splicing. The long isoform, termed NSD3L, is a protein of 1437 amino acids [13]. Alternative splicing of exon 10 encodes a protein of 645 amino acids, named NSD3 short (NSD3S), which is identical to NSD3L in the first 619 amino acids [12]. Finally, isoform WHISTLE (WHSC1-like 1 isoform 9 with methyltransferase activity to lysine) is a short alternative splice version of the C- terminal of NSD3L that encodes a protein of 506 amino acids. NSD3L has five PHD (plant-homeodomain)-type zinc fingers motifs, two PWWP domains, and the methyltransferase SET domain. Right next to the SET domain there is a SAC (SET-associated Cys-rich) domain rich in cysteines, followed by a Cys-His-rich domain termed C5HCH motif near the C terminal end of the protein [13][14]. The PWWP domain is a histone methyl-lysine (H3K36) reader, acting as an epigenetic regulator of gene expression [15][16][17], and has been postulated as a site for protein–protein interactions due to the amino acid composition [18]. The PHD domain binds chromatin at histone H3 lysine 4 unmodified or methylated [19]. The SET domain is a region conserved between the SET family of methyltransferases, with specificity for mono- or di-methylation of H3 lysine 36. The SET domain is separated into three smaller segments, the pre-SET, SET and post-SET domain, all of which are needed for catalytic activity [20].

3. NSD3 Alterations in Cancer

NSD3 proteins are ubiquitously expressed in human tissues, with the NSD3S isoform being more prominent than NSD3L [21][22][23], and the WHISTLE isoform primarily found in the testis [24]. Compared to NSD1 and NSD2, NSD3 exhibits a higher genetic variation and amplification in cancer. The oncogenic role of NSD3 is manifested by changes ranging from alterations in expression, such as overexpression and point mutations, as well as fusions with other proteins which result in differences in cellular activity (**Figure 1**).

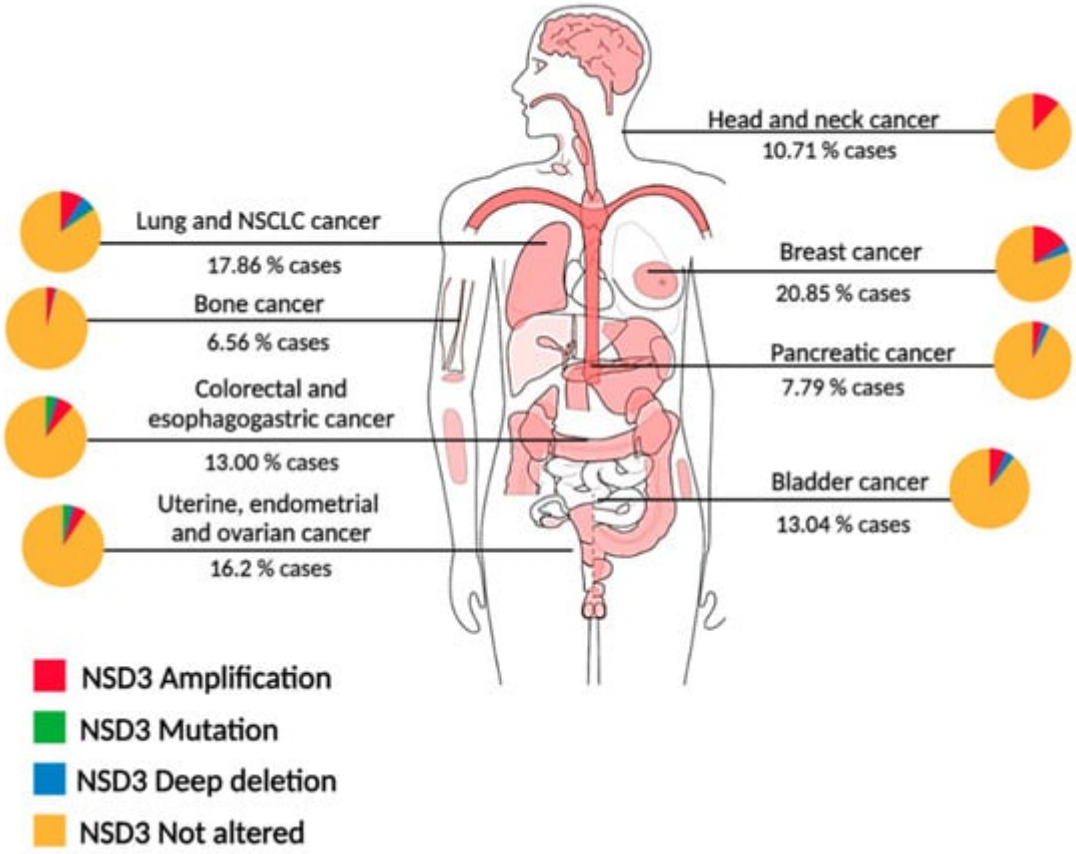

Figure 1. NSD3 genetic alterations across cancer types. Diagram of genetic alterations in pan-cancer analysis of whole genomes (ICGC/TCGA) [25]. Percentages shown under each cancer type indicate the total NSD3 alterations, and each cancer has a pie chart which shows the fraction for each NSD3 alteration, amplifications in red, mutations in green and deep deletions in blue, no alterations in yellow. NSCLC: Non small-cell lung cancer.

3.1. Study of the Amplicon 8p11-12: Chromothripsis

Next-generation sequencing and its use in cancer research has eased the identification of a novel type of genomic instability known as chromothripsis. Chromothripsis is a pathological phenomenon by which a series of cluster chromosomal rearrangements occur and are localized in limited regions of the genome in one or several chromosomes. This focal chromosomal scrambling contributes to the initiation of cancer by mediating the overexpression of oncogenes (amplification, translocation, or generation of oncogenic fusions), inactivation of tumor suppressor genes (by loss or disruption), and/or the expression of genes that can contribute to cancer therapy resistance [26][27][28]. Stephens and collaborators found that at least 2–3% of all cancers have chromothripsis [26].

The 8p11-12 genomic region spans over 10 megabases (Mb) and encompasses over 50 known genes, including NSD3. Amplification of the 8p11-12 chromosomal region is a common genetic event in many epithelial cancers, thus structural variations, such as chromothripsis of the 8p11-12 genomic region, have clinical and biological implications in multiple malignancies.

3.1.1. Amplification

Because of the 8p11-12 amplicon found in different epithelial cancers [29], NSD3 has been proposed, among other proteins, as an important oncogene for cancer progression. Using different approaches, such as overexpression of NSD3, small interfering RNA (siRNA) and short hairpin RNA (shRNA)-mediated knockdown against NSD3 in 8p11-12 amplified breast cancer cells, it was found that the loss of NSD3 resulted in a profound loss of the growth and survival of these cells, indicating a function for this protein in regulating survival and transformation [21][30]. In a breast cancer mouse model expressing NSD3 in the mammary epithelium, NSD3 was revealed as a transforming oncogene by exhibiting mammary hyperplasia, dysplasia, and invasiveness [31]. NSD3 has also been proposed as an oncogenic driver in non-small cell lung cancer (NSCLC) [11], lung squamous cell carcinoma (LUSC) [32] and pancreatic ductal adenocarcinoma (PDAC) where the 8p11-12 amplicon has also been found. The studies validated the consistent amplification of NSD3 and showed that the depletion of NSD3 decreases the viability and the colony formation capacity of lung and pancreatic cancer cell lines harboring the 8p amplicon [10][33]. [8].

3.1.2. Fusion Proteins

Rearrangements involving the short arm of chromosome 8 have been reported and associated with different types of cancer.

The first NSD3 fusion protein was found in a patient with AML, where the t(8;11) (p11.2;p15) translocation fuses the NUP98 gene to the 3′ end of NSD3 containing both of the PWWP, the SET, PHD and CH5CH rich domains (**Figure 2**A) [34]. The fusion transcript includes the FG repeats of NUP98, which are known to bind transcription factors, such as CREB-binding protein [34]. This suggests the importance of the transcriptional regulation of leukemic cells and indicates the NUP98-NSD3 fusion into a vital leukemogenesis-related oncogene. The presence of the NUP98-NSD3 fusion protein has been observed in leukemia cell lines and has also been found in B-lymphocyte cell lines derived from healthy volunteers who had undergone transformation by the Epstein–Barr virus [35].

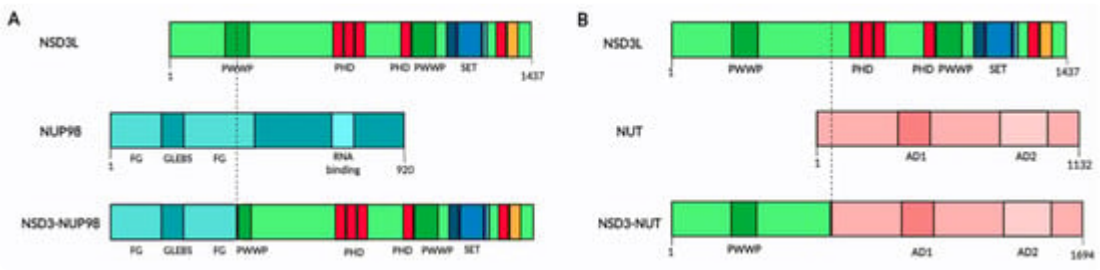

Figure 2. NSD3 fusion proteins in cancer. (**A**) Representation of NSD3-NUP98 fusion protein, indicating in colors the different domains in NSD3 and NUP98 protein. (**B**) Representation of NSD3-NUT fusion protein, indicating in colors the different domains in NSD3 and NUT protein. In NUT protein, AD means acidic domain. In both figures the breakpoint on the proteins is marked as a dotted line. In NSD3L domains, PWWP in green, PHD in red, AWS, SET and post-SET in blue, and C5HCH in orange.

In pediatric sarcoma, investigators found a novel NSD3-NCOA2 fusion. These two proteins have been found to be involved in fusion processes, NSD3 in acute myeloid leukemia and NCOA2 in infantile spindle cell rhabdomyosarcoma, which strengthens the findings and leaves the characterization of its function as well as the presence in other human samples pending [36].

The nuclear protein of the testis (NUT) midline carcinoma (NMC) is an epithelial cancer that is defined by chromosomal translocations of the NUT gene. In about 65% of cases, NUT is fused to BRD4, with 25% fused to BRD3, and the rest 10% unknown, with recent reports showing it to be fused to NSD3 [37][38][39][40]. The first NMC patient with a NSD3-NUT fusion t(8;15)(p12;q15) was identified in 2014. The fusion resulted in a protein containing exons 1–7 of NSD3 and exons 2–7 of NUT, encoding 1694 amino acids, containing amino acids 1–569 of NSD3 and 8–1132 of NUT (**Figure 2**B) [37].

3.1.3. Mutations

Xiong et al. described a variety of NDS3 missense mutations and T419Pfs*8/Nfs*28/N mutations in four cases of stomach adenocarcinoma (STAD), two cases of colon adenocarcinoma (COAD) and single cases of breast invasive carcinoma (BRCA) and pancreatic adenocarcinoma (PAAD). Likewise, nonsense mutations in NSD3, such as, E1181K and T2342A, enhance the growth of cancer cells and xenograft tumors by disrupting an autoinhibitory loop in the NSD3 protein, thereby increasing enzymatic activity [41][42].

4. NSD3 Involvement in Cancer

It is well known that NSD3 catalyzes the methylation of histone H3 at lysine 36, this occurs because NSD3 binds to LSD2 and G9a/EHMT2, forming a complex in vivo [43]. G9a and LSD2 mediate H3K9 methylation and H3K4 demethylation of actively transcribed genes, helping NSD3 to recognize and methylate H3K36 [14]. Morishita et al. used the C-terminal portion of NSD3, including pre-SET, SET, post-SET and PHD5 domain and identified that in vitro, NSD3 can methylate H3K9, H3K27, H3K36, H3K79 and H4K20 [4]. Discrepancies about the specificity for the substrate of the catalytic domain of NSD3 and other members of the NSD family, may be due to the cellular context, the assay employed, the nature of the substrate, or the portion of the protein used, if it is only the SET domain or the full-length protein. In relation to the isoforms of NSD3, NSD3L has been associated with neural crest formation and migration, playing a role in gene expression during neural crest development [44][45]. NSD3S conserves the N-terminal PWWP domain, this domain allows the protein to bind histone H3 at methylated lysine 36 [46]. The WHISTLE isoform has been found to act as a transcriptional repressor through HDAC1 recruitment, having H3K4me2 and H3K27me2/3 methyltransferase activity [24][47], this isoform is considered less relevant to cancer.

4.1. Methyltransferase-Dependent Function of NSD3 in Cancer

4.1.1. NOTCH Pathway

NSD3L interacts with EZH2 and RNA polymerase II to influence H3K36me2/3-dependent transactivation of genes, including those related to NOTCH signaling in breast cancer with the 8p11-12 amplicon, such as NOTCH receptors, ligands, and ADAM12 [48]. These findings indicate that NSD3-induced methylation of H3K36 activates NOTCH signaling to drive breast tumor initiation and metastatic progression.

4.1.2. mTOR Pathway

Deregulation of the mTOR pathway occurs in various diseases, including cancer. The mTOR pathway responds to environmental signals, regulating basic cell functions like cell growth and proliferation [49], survival, apoptosis, angiogenesis, and metabolism [50].

4.1.3. EGFR Pathway

It has been shown that HMTs methylate not only histones, but also proteins. The NSD family of proteins have also been described as performing that function, as both NSD1 [51] and NSD2 [52] methylate NF-kB to regulate its function.

NSD3-mediated mono-methylation of the EGFR kinase domain (Lys721) affects the cytoplasmic and nuclear function of the protein. In the cytoplasm, it increases EGFR kinase activity and the downstream ERK signaling pathway without the presence of the ligand EGF. In the nucleus, it stimulates cell cycle progression by increasing the binding of EGFR to PCNA on squamous cell carcinoma of the head and neck (SCCHN) cancer cells [53].

4.1.4. IFN Pathway

Activated Interferon regulatory factor 3 (IRF3) is a transcriptional regulator that promotes IFN-α and IFN-β transcription. IFN-β elicits both anti-inflammatory and pro-inflammatory responses, playing a key role in innate immunity and the response to viral infections [54][55].

It is accepted that innate and adaptive immunity plays an important role in antitumor immune surveillance. Amplification of NSD3 in patients with LUSC exhibits a decrease in the type II IFN response, leading to an immune–desert pro-tumorigenic phenotype [56].

4.1.5. Cyclin Dependent Kinase (CDK) Pathway

CDC6 and CDK2 promote G1 to S phase transitions, and the transcription of these genes is regulated by H3K36 di-methylation. In SCCHN cells, it was demonstrated that NSD3 regulates transcription of CDC6 and CDK2, as knockdown of NSD3 resulted in G0/G1 arrest [57]. Knockdown of NSD3 by siRNA in bladder and lung cancer cell lines reduced cell proliferation by inducing cell cycle arrest at G2/M phases through the regulation of the expression of CCNG1 and NEK7, which are important regulators of G2/M transition in cancer cells [58][59][60].

4.2. NSD3S Isoform Function as an Adaptor Protein

4.2.1. NSD3-NUT Fusion

The NSD3-NUT fusion oncoprotein is present in several NMC cases. After knockdown of endogenous NSD3-NUT in an NMC cell line, there was an increase in keratin levels, and a decrease in cellular proliferation, indicating a crucial role of the NSD3-NUT oncofusion in blocking cell differentiation and stimulating the proliferation in this cell line. It was also found that NSD3 not only interacts with NUT, but is associated with BRD4-NUT fusion, this interaction being important in the blockade of differentiation [37].

4.2.2. NSD3S-BRD4-CHD8 Interactions

NSD3 imparts a pTEFb-independent transcriptional activation function on BRD4, on genes such as CCND1 and PIM2. The BRD4/NSD3 complex regulates the methylation of H3K36 at BRD4 target genes [61]. BRD4 regulates the transcription of some genes, like CD274 that encodes PD-L1 [62]. NSD3S was described as an adaptor protein by Shen et al. that characterized the binding of BRD4 to a small 11 amino acid region on the N-terminal of NSD3 (amino acid 152–163). Also, they showed that the short isoform, NSD3S, was required and sufficient for driving leukemia progression, indicating a methyltransferase independent function of the protein. NSD3S also binds to the chromatin remodeler CHD8 through the C-terminal region, linking BRD4 to CHD8 on the chromatin, through the ET domain of BRD4 [22]. The three proteins colocalize in regions of the genome and they are release from MYC super-enhancers using BET inhibitor, JQ-1 [22].

4.2.3. NSD3S-MYC Interaction

Sun et al. reported that in NSD3 knockout pancreatic cells and in shRNA-xenografts, there was a decrease in the gene expression of *Myc*, *Adam12*, and *Notch3*, demonstrating that the silencing of NSD3 can downregulate oncogenic genes [42]. Scholars described that NSD3S interacts with MYC to stabilize the MYC protein and increase its transcriptional activity, acting as an oncogenic interaction [63]. The NSD3S-MYC interaction is mediated by a 15 amino acid site on NSD3S, between amino acids 389 and 404.

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