SALL4 as a Therapeutic Target in Cancer Treatment

Subjects: Biology

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Spalt-Like Transcription Factor 4 (SALL4) is a critical factor for self-renewal ability and pluripotency of stem cells. Also, Various reports show tight relation of SALL4 to cancer occurrence and metastasis. SALL4 exerts its effects not only by inducing gene expression but also by repressing a large cluster of genes through interaction with various epigenetic modifiers. Due to the high expression of this protein in cancer cells and its silence in almost all adult tissues, it is an ideal target for cancer therapy.

SALL4 neoplasm drug development molecular glue

1. Introduction

Spalt-Like Transcription Factor 4 (SALL4), a member of the SALL family, is a regulator of embryonic stem cells that plays a critical role in cell renewal and proliferation. SALL4 expression starts as soon as two-cell stage and knock down studies show its prominent role in different stages of fetal development [1]. Mutation in the SALL4 gene is the cause of autosomal dominant Okihiro syndrome that mainly affects eyes, bones of arms, and hands, heart, and renal system [2]. However, the gene is silenced in most of adult tissues except germ cells and CD34+ hematopoietic stem cells [3].

The SALL4 protein has two isoforms. SALL4A originates from the full-length transcript, while SALL4B as a spliced isoform lacks a part of exon 2. This protein contains several zinc finger cluster (ZFC) domains spread in different parts of the protein which give it both transcription activation and repression functions [4]. This dual-mode transcription factor can activate and repress clusters of genes that are critical for self-renewal and pluripotency. SALL4 performs its repressive role through interaction with Nucleosome Remodeling and Deacetylase complex (NuRD). Phosphatase and tensin homolog (PTEN), Lysine Demethylase 3A (KDM3A), Forkhead-Box (FOX), B-cell lymphoma (BCL), and Krüppel-like factor (KLF) are the genes that are repressed by SALL4 [5]. The transcription activation role of SALL4 is also reported for Homeobox A9 (HOXA9) through interaction with mixed-lineage leukemia (MLL) protein. Moreover, SALL4 directly binds to c-MYC promoter and enhances its expression. The oxidative phosphorylation genes are the other targets of SALL4 protein [5].

Reactivation of SALL4 in cancerous cells is a key event in tumorigenesis. Meta-analysis on SALL4 activation showed hazard ratios (HR) of 1.4 (95% confidence interval: 1.19–1.65) and 1.52 (95% confidence interval: 1.22–1.89) for all-cause mortality and cancer recurrence, respectively. This data provides evidence on the association of SALL4 to a poor survival rate in high SALL4-expressing patients [6]. SALL4 overexpression is found in several

cancer tissues and hematologic malignancies, suggesting a key role of SALL4 in cancer onset and progression. Furthermore, the oncogene has been linked to various cellular mechanisms such as proliferation, apoptosis, invasion, and resistance to therapeutics [7] and high SALL4 is associated with poor prognosis and lower survival rate in patients [8].

Considering the critical role of SALL4 in cancer cell survival besides the difference in the expression of SALL4 in healthy and cancerous cells, this protein seems to be a key target for cancer treatment.

2. SALL4 as a Potential Therapeutic Target

The association of SALL4 to cancer incidence and progression suggests that SALL4-targeting could be effective in eradicating cancer cells [9]. However, lack of knowledge on suitable active sites, localization of the protein in the nucleus, and inaccessibility on the cell surface all make SALL4 a challenging choice for drug design. Over the last years, several specific drugs targeting SALL4 have been designed based on three main approaches. One approach targets the functionality of SALL4 by inhibiting its essential protein-protein interactions. In the second approach, SALL4 targeting drugs are characterized by drug repositioning. The third and more novel approach is the modulation of SALL4 protein levels through induction of protein degradation (**Figure 1**).

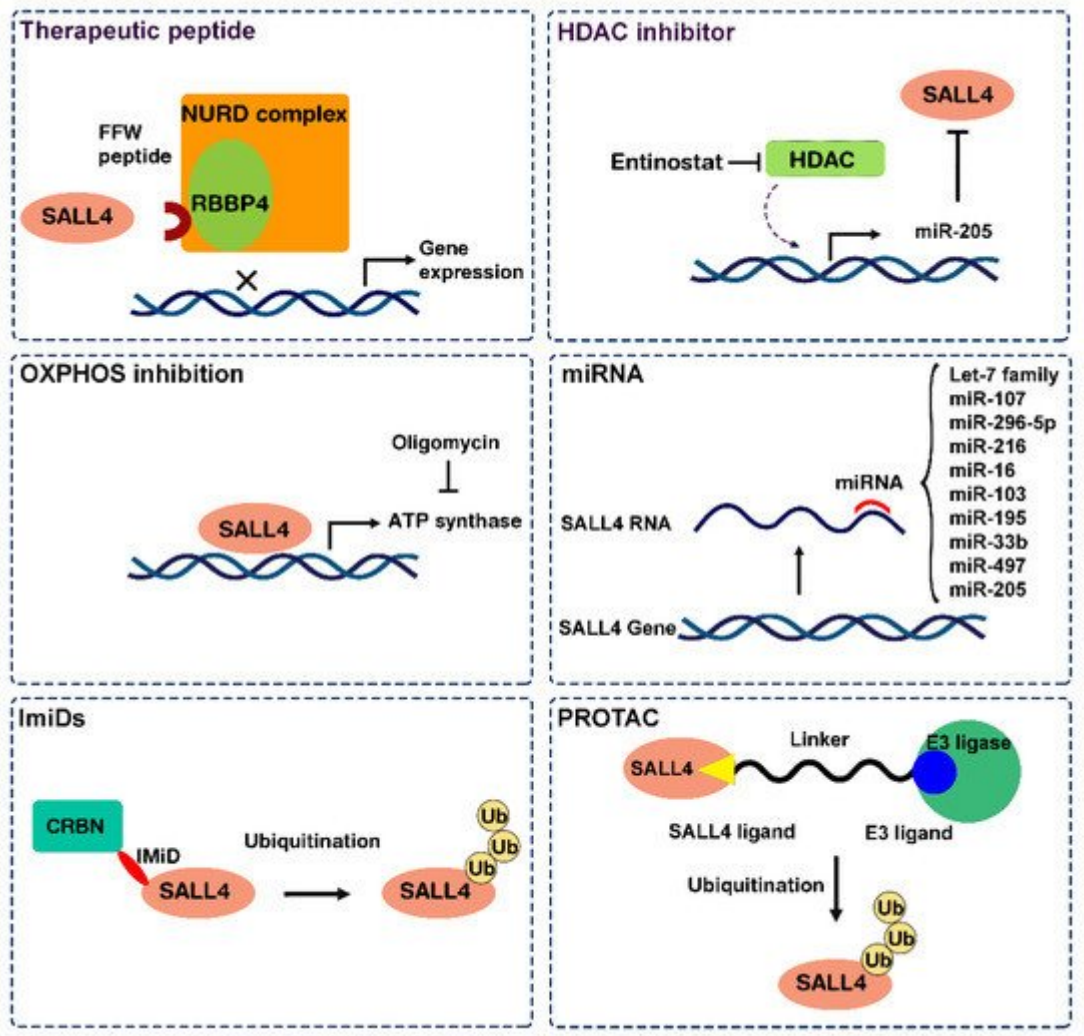

Figure 1. Scheme of the different drugs suggested to target SALL4 in cancer. OXPHOS: Oxidative phosphorylation, IMiDs: Immunomodulatory imide drugs, PROTAC: Proteolysis-targeting chimeras.

2.1. Approach 1: Target SALL4 Function, the SALL4 Inhibitors

SALL4 interacts with various chromatin modulatory factors such as NuRD complex, DNA methyltransferases, histone methyltransferases, and different histone demethylases. NuRD is a chromatin remodeling complex that mainly works as a transcriptional silencer through HDAC and CDH3/4 ATPase subunits and RBBp4. Due to the high impact of SALL4/NuRD interaction on chromatin structure, the researchers tried to halt it through blocking its function. Initially, a 12-AA peptide developed by the researchers' group that effectively blocked the interaction between SALL4 and NuRD complex in HCC and acute myeloid leukemia [10]. The peptide treatment can decrease the binding of HDAC to the promoter of PTEN resulting in increment in the expression of PTEN. Further optimization of this peptide led to the discovery of FFW, a pre-clinical prototype, and an SALL4/RBBP4 inhibitor that can induce apoptosis in cancer cells and hindrance of formation of xenograft in mice [11].

Inhibitor(s) of HDACs (HDACi) can be viewed to inhibit SALL4 function indirectly. As matter of fact, HDACi has been shown to have promising therapeutic effects on SALL4 expressing cancer cells. Connectivity Map (cMap) analysis for determination of possible drugs targeting SALL4 reveals Entinostat (MS275), an HDACi gets the highest score. More experimental studies showed that SALL4-high lung cancer cells are more vulnerable to Entinostat treatment [12]. Surprisingly, additional studies on Entinostat-mediated SALL4 targeting reveals a second drug mechanism. MiRNA sequencing after Entinostat treatment showed that this drug downregulates SALL4 by up-regulation of miR-205.

2.2. Approach 2: Targeting SALL4 Downstream Pathways; Repurposing Oxidative Phosphorylation Drugs to Inhibit SALL4 Positive Cells

In order to identify compounds with inhibitory effect on SALL4, a library of 1597 synthetic and 21,575 natural compounds were tested on SALL4 high expressing cells. The highest score was taken to the oxidative phosphorylation inhibitor Oligomycin. Oligomycin is a macrolide produced by Streptomyces and an inhibitor of adenosine triphosphate (ATP) synthase. This compound demonstrated tumor suppressive role both in vitro and in vivo on SALL4 high cells. In line with this observation, chip-seq data analysis showed binding of SALL4 to near 50% of mitochondrial Oxidative phosphorylation genes. Moreover, oxygen consumption rate and oxidative phosphorylation increased dramatically in SALL4-high cells, all promoting ATP synthesis in these cells [13]. Although glycolysis was previously thought to be the major energy producer of cancer cells, recent findings highlight the critical role of oxidative phosphorylation mechanisms in the promotion of tumor progression [14].

2.3. Approach 3: Modulating SALL4 Abundancy

2.3.1. Nucleic Acid-Based Therapy

The researchers' growing knowledge on regulatory roles of miRNAs along with development of miRNA specific delivery systems have made miRNA-based therapy as an emerging approach in treatment of cancer. Various miRNAs regulate SALL4 post-transcriptionally among which Let-7 family has been the focus of many investigations. Let-7 binds to SALL4 at 3′ UTR and induces cell differentiation through repression of a cluster of genes. It is shown that Lin28 that is an RNA-binding protein represses Let-7 family members and the Lin28/Let-7 axis is an important player in tumorigenesis [15]. Let-7 overexpression in HCC cells induces cell cycle arrest and apoptosis [16]. Moreover, low Let-7 expression is shown to be a predictor of poor prognosis in lung cancer patients. In line with these findings, miR-98 that is a member of Let-7 family has demonstrated a tumor suppressive role through interaction with SALL4 in HCC, glioma, and ovarian cancer [17]. There are some other miRNAs that regulate or are regulated by SALL4. MiR-107 directly targets SALL4 and induces apoptosis in glioma cells both in vitro and in vivo. Moreover, miR-296-5p is shown to have a suppressive role on proliferation, migration, and invasion of liver cancer through Brg1/Sall4 axis. Brg1 which enhances expression of SALL4 by binding to its promoter is potentially inhibited by miR-296-5p [18]]. MiR-219, miR-16, and miR-103, miR-195, miR-33b, and miR-497 also are proposed to have role in suppression of tumorigenesis via interaction with SALL4 [19]. In a recent systematic review, the researchers comprehensively discussed the interaction between SALL4 and different

miRNAs [20]. The inhibitory effect of these miRNAs on SALL4 gene expression can be considered for prospective RNA-based therapy approaches.

There is also a tendency for gene silencing through Small interfering RNAs (siRNA). Knockdown of SALL4 by siRNA is shown to induce apoptosis in colorectal and breast cancer cells [21]. Ashrafizadeh et al. directly delivered SALL4-siRNA to HCC cells using a lipoprotein-like scaffold which resulted in inhibition of HCC tumor growth in vivo [22]. Moreover, transducing lung cancer cells with siRNA against SALL4 resulted in more sensitivity of the cells to platinum-based drugs [23].

2.3.2. SALL4 Degraders

Despite their teratogenic effects, IMiDs (immunomodulatory drugs) are applied for treatment of patients with multiple myeloma. These immunomodulatory drugs induce the degradation of more than 11 zinc finger proteins including SALL4A [24]. Thalidomide as an ImiD, promotes ubiquitination and subsequent degradation of SALL4A on ZFC2 C2H2 domains by binding to Cereblon (CRBN) in the choline complex E3 ubiquitin ligase (CRL4CRBN) [25]. Based on this function, ImiDs are proposed to be among the new generation of therapeutics which utilize protein degradation machinery to destruct the target protein. Proteolysis-targeting chimeras (PROTACs) and molecular glues induce the interaction of the target protein with E3 ubiquitin ligases and subsequent degradation of the protein through proteasome [26]. The ImiDs as molecular glues bind simultaneously to the E3 ligases on the one hand and to substrate protein on the other hand, leading to degradation of the target in a proteasome-dependent manner.

PROTACs are the other class of drugs that take advantage of target-specific degradation strategies to omit interested proteins. They are heterobifunctional structures comprising two ligands connected via a linker that one joins to the E3 ligase and the other joins to the protein of interest. The formed ternary complex finally induced Proteasomal degradation of interested protein. However, to the best of the researchers' knowledge, no PROTAC-based degrader has been designed against SALL4. One potential limiting factor of PROTAC is that it is a larger molecule with a high molecular weight, which may be challenging for the development of an oral drug administration therapy.

3. Conclusion

Despite the evident role of SALL4 in tumorigenesis and therapy resistance, no therapeutic targeting SALL4 are currently on the market. The landmark discovery of a new class of molecular glues non-IMiD targeting SALL4 and other oncogenes has the potential to become a real disease-modifying treatment and open a novel therapeutic perspective for several intractable cancer conditions.

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