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Functional and Structural Features of Zinc Finger Protein 809

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Abstract

Zinc finger protein 809 (ZFP809) is a member of the kruppel-associated box-containing ZFP (KRAB-ZFP) family. It supresses the expression of Moloney murine leukemia virus (MoMLV) via sequence-specific binding to the primer binding site (PBS) located downstream of the MoMLV-long terminal repeat (LTR) and induces epigenetic modifications at the LTR, such as repressive histone modifications and de novo DNA methylation. Recent studies have demonstrated the features of diverse functional domains of ZFP809, which has a KRAB domain and seven zinc fingers. We previously demonstrated that the KRAB domain is essential for nuclear localization, gene silencing, and binding to the MLV-PBS in conjunction with the accessory roles of a subset of zinc fingers. Individual zinc fingers are known to contribute to binding to the MLV-PBS and stable protein expression. Furthermore, the mechanisms underlying the high expression of ZFP809 in immature cells have yet to be fully elucidated. Recent studies have indicated that ZFP809 is stably expressed in immature cells, such as embryonic stem cells (ESCs), as ESCs have higher expression of KRAB-associated protein 1 (KAP1) than mature mouse embryonic fibroblast cells. Here we discuss the ZFP809/KAP1 complex, functional domains of ZFP809, and expression pattern of ZFP809 in immature cells.

Keywords: Retrovirus; Kruppel-associated box-containing zinc finger proteins (KRAB-ZFPs); Zinc finger Protein 809 (ZFP809)

Abbreviations: KRAB-ZFP: Kruppel-Associated Box-Containing Zinc Finger Protein; ZFP809: Zinc Finger Protein 809; PBS: Primer Binding Site; MoMLV: Moloney Murine Leukemia Virus; LTR: Long Terminal Repeat; KAP1: KRAB-Associated Protein 1; ESET: ERG-associated protein with a SET domain; DNMT: DNA Methyltransferase; HDAC1: Histone Deacetylation 1; ERVs: Endogenous Retroviruses; VL30: Virus-like 30; ESCs: Embryonic Stem Cells; MEFs: Mouse Embryonic Fibroblast Cells

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Introduction

In 2009, Wolf and Goff [1] revealed that zinc finger protein 809 (ZFP809) has a central role in the transcriptional suppression of Moloney murine leukemia virus (MoMLV). It is a member of the kruppel-associated box-containing ZFPs (KRAB-ZFPs), which represent the largest single family of transcription factors in mammals. KRAB-ZFPs are exclusively found in tetrapedal vertebrates and function in various cellular processes, including apoptosis, cancer development, differentiation, immunity, and metabolism [2-10]. ZFP809 contains a KRAB domain at the N-terminus and seven zinc fingers at the C-terminus. It is highly expressed in immature murine cells, including embryonic stem cells (ESCs) and embryonic carcinoma cells [1,10]. It has been shown to inhibit the transcriptional expression of MoMLV and is required for the silencing of endogenous retroviruses (ERVs) during mouse embryonic development [1,11]. Furthermore, domains with diverse functions have been shown within ZFP809 [12,13]. Here we discuss the functions and features of functional domains within ZFP809 in light of the findings of recent studies.

ZFP809 Forms an Inhibitory Transcriptional Complex and Represses Retroviral Expression

ZFP809 binds to the MLV-derived primer binding site (PBS) located downstream of the long terminal repeat (LTR) and interacts with KRAB-associated protein 1 (KAP1) in the same manner as with other KRAB-ZFPs [1,4,10]. Furthermore, previous studies have demonstrated that the ZFP809/KAP1 complex recruits heterochromatin protein 1, an ERG-associated protein with a SET domain (ESET, a H3K9 methyltransferase), the nucleosome remodeling and deacetylation complex including histone deacetylation 1 (HDAC1), and DNA methyltransferase 3A (DNMT3A). This protein complex represses the activity of the LTR by inducing epigenetic silencing marks, such as histone modifications and DNA methylation, at the MLV-LTR [14-17] (Figure 1A). Recently, it has been suggested that the ZFP809/KAP1 complex interacts with ErbB3-binding protein 1, which is involved in triggering gene silencing [18]. Moreover, recent studies have demonstrated that DNMT3L facilitates the interaction between members of the KAP1/DNMT3A/ESET/HDAC1 complex in ESCs and that this complex contains the histone chaperone Chaf1 and the sumoylation factor Sumo2 [19,20].

Although ZFP809 is one of the most well-characterized KRAB–ZFPs, little is known regarding its role *in vivo*. Wolf et al. [10,11] demonstrated that ZFP809 knockout leads to a strong reactivation of virus-like 30 (VL30) elements, which are ERVs, in mouse embryos and mature tissues. However, ZFP809 knockout mice do not have impaired health because of the expression of a small subset of VL30 elements [10,11]. Therefore, Wolf et al. [10,11] suggested that these deleterious effects may have emerged over more than two generations.

Functional Domains within ZFP809

Previous studies on KRAB-ZFPs have indicated that the KRAB domain interacts with KAP1, and zinc fingers bind to target sequences and interact with proteins other than KAP1 [2]. Therefore, we hypothesized that domains within ZFP809 have different functions, including subcellular localization, ability to silence transgene expression driven by the MLV-LTR, and

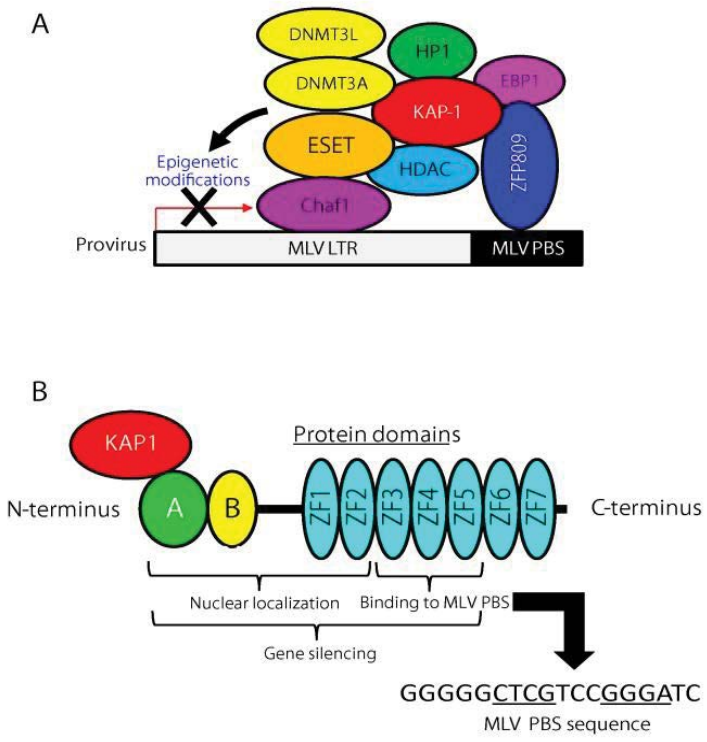

Figure 1 ZFP809 forms an inhibitory complex and has functional domains. (A) ZFP809 binds to the repressor binding site, an 18-base pair DNA element overlapping the murine leukemia virus-derived primer binding site downstream of the LTR, and interacts with kruppel-associated box-associated protein 1 (KAP1). The ZFP809/KAP1 complex forms an inhibitory complex that includes ESET, heterochromatin protein 1, HDAC1, EBP1, Chaf1, and DNMT3A/3L. This complex induces epigenetic modifications, such as histone modifications and DNA methylation, at the LTR. In the figure shown here, we modified figures from published papers [19,29]. (B) ZFP809 has a KRAB domain at the N-terminus and seven zinc fingers at the Cterminus. The KRAB domain is required for nuclear localization (except the nucleolus), gene silencing, and binding to the MLV–PBS. The first and second zinc fingers are required for the proper nuclear localization and the third, fourth, and fifth zinc fingers bind the MLV–PBS (underline). Furthermore, it is possible that other zinc fingers may contribute to stable protein expression. In this figure, "A" indicates KRAB_A box, "B" indicates KRAB_B box, and "ZF" indicates zinc finger.

binding to the PBS. We investigated subcellular localization, gene silencing ability, and binding ability to the MLV-PBS using a series of truncated and mutated ZFP809 proteins [12,13]. We revealed that the KRAB domain is required for nuclear localization and silencing of transgene expression driven by the MLV-LTR and bind to the MLV-PBS in conjunction with the zinc fingers (Figure 1B) [12,13]. Although previous studies have indicated that zinc fingers within KRAB-ZFPs bind to specific DNA sequences and directly interact with three-nucleotide sequences [3,10], our results demonstrated that individual zinc fingers, particularly the third, fourth, and fifth zinc fingers, are required for the binding of ZFP809 to the MLV-PBS and that other zinc fingers contribute to stable protein expression [12,13] (Figure 1B). According to previous studies, ZFP809 interacts with regions within the MLV-PBS (underlined sequence) and does not recognize three-nucleotide sequences, indicating that not all zinc fingers are necessarily involved in target DNA binding [21-24].

Moreover, we found that ZFP809 has six linkers between zinc fingers, three of which contain the T(S)GEKP sequence, which are conserved among C2H2 zinc fingers [25-27]. In addition, although ZFP809 also has the linker between KRAB domain and zinc finger, role of the linker is unknown. Previous studies have demonstrated that the phosphorylation of T(S)GEKP sequences leads to the inactivation of zinc finger binding to target DNA [25-27]. Therefore, we focused on the linker between zinc fingers and revealed that these linkers affect the ability of ZFP809 to prime gene silencing and the precise nuclear localization of ZFP809 [28].

Expression Patterns of ZFP809 in Mature or Immature Cells

Although it has been reported that ZFP809 is highly expressed in immature murine cells, mRNA levels are comparable between ESCs and mouse embryonic fibroblast cells (MEFs) [10,17]. However, protein levels in MEFs decreased compared with those in ESCs, suggesting that regulation of post-transcription or post-translation occurs in ESCs but not in MEFs [10]. Furthermore, our results demonstrate that mutated ZFP809, which does not interact with KAP1, does not bind to the MLV-PBS or localize to the nucleus [12]. This suggests that interaction with KAP1 contributes to the function of ZFP809 [12]. In addition, ZFP809 and KAP1 levels are higher in immature cells than in mature cells [11]. In light of these results, we hypothesized that ZFP809 does not interact with KAP1 in MEFs because KAP1 levels are lower in MEFs than in ESCs, thereby leading to unstable protein expression. Therefore, the interaction between ZFP809 and KAP1 may be essential for stable protein expression.

Conclusion

In this short review, we focused on the function and characteristic features of ZFP809.

Previous studies have focused on the ZFP809/KAP1 complex and epigenetic modifications because ZFP809 has been identified as an inhibitor of MoMLV virus expression.

These previous studies identified factors interacting with the ZFP809/KAP1 complex and analyzed epigenetic modifications [1,14-18]. Furthermore, we previously examined the functional domains of ZFP809 and revealed the essential role of the KRAB A box in all functions in conjunction with the cooperative roles of a subset of zinc fingers. Our results also suggested that individual zinc fingers may have different functions, such as binding to the MLV-PBS and stable protein expression. In addition, ZFP809 in immature cells may be more stably expressed than that in mature cells due to high KAP1 expression. Further characterization of amino acid sequences of KRAB domains and zinc fingers will help elucidate the functions and characteristic features of not only ZFP809 but also other KRAB-ZFPs.

Conflict of Interest

None

Acknowledgment

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