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Utilizing NMR to study RNA- and compound-binding mechanisms of Musashi-1, a stem/progenitor cell marker in various normal and cancer cells

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An RNA-binding protein, Musashi-1 (Msi1), is a posttranscriptional gene regulator that is involved in the regulation of stem cell self-renewal. Because of its high-level expression in neural stem cells (NSCs) and undifferentiated neural precursor cells [1], Msi1 is used as a cell marker for NSCs and progenitor cells in the central nervous system. Increasing evidence suggested that Mis1 acts as a critical regulator of the status of stem/progenitor cells in other tissues and organs [2,3], including the breast, eye, hair follicles, intestine, and stomach, in either embryonic or adult stages; thus, Msi1 may also be used as an effective marker for stem/progenitor cells in a wide range of tissues and organs.

The well-established function of Msi1 is repression of translation of Numb and APC, which are antagonists of Notch and Wnt signaling, respectively [4-6], and also repression of translation of cyclin-dependent kinase inhibitor $P21^{WAF-1}$, which is a negative regulator of cell cycle progression [7]. Later, various high-throughput genome-wide analyses identified substantial number of Msi1-targeted mRNAs [2,3], each encoding a protein involved in cellular activity such as apoptosis, cell cycle regulation, differentiation, proliferation, survival, and DNA repair.

Msi1 has two tandemly connected ribonucleoprotein-type RNA-binding domains (RBDs), RBD1 and RBD2, in its N-terminal region. Previously, we applied NMR methods to Msi1 RBD1 and RBD2 individually, and determined the solution structures in their free forms, identified the residues involved in RNA-binding, and assessed the backbone dynamics and discussed the origin of high RNA-binding affinity [8,9]. Our subsequent NMR analysis identified the minimal recognition RNA sequences for Msi1 RBD1 and RBD2 to be r(GUAG) and r(UAG), respectively [10]. Then, we determined the NMR solution structure of Msi1 RBD1-r(GUAGU) complex, which clearly revealed the detailed interactions between Msi1 RBD1 and r(GUAG) [10]. Furthermore, a theoretical analysis based on statistical mechanics of hydration was applied to Msi1 RBD1-r(GUAG) interaction to provide a deeper understanding into the recognition mechanism [11]. Recently, we have also determined the NMR solution structure of Msi1 RBD2-r(GUAGU) complex, elucidating the precise interactions between Msi1 RBD2 and r(UAG) [12]. Comparison of the structures revealed that these RBDs utilize quite different interactions to recognize cognate RNA sequences. The minimal recognition sequences, which we identified, were actually found in many Msi1-targeted mRNAs mentioned above [10,12]. On the basis of the two complex structures, we built a model structure of consecutive RBDs, RBD1-2, with r(UAGGUAG) containing both minimal recognition sequences; the result suggested that RBD1-2 should indeed be able to recognize r(UAGNnGUAG) (n = 0-50 nt) [12].

A considerable number of reports have shown that Msi1 is highly expressed in a broad range of human cancers and tumors [2,3], such as breast, colon, and lung cancers, astrocytomas, cervical carcinomas, ependymomas, endometrial carcinomas, gliomas, hepatomas, medulloblastomas, neurocytomas, and retinoblastomas. Msi1 is causally related to cancer initiation, progression, and drug resistance, however, a detailed description of signaling pathways that are regulated by Msi1 are still under investigation.

Recently, small-molecule inhibitors of Msi1 RBD1-RNA interaction were obtained. Lan et al. [13] screened ~2000 compound library against full-length Msi1 for inhibition of RNA-binding activity, and identified (-)-gossypol, which is a natural product extracted from cottonseed and had already completed phase IIb clinical trials for prostate cancer. Then, (-)-gossypol was shown to reduce Notch/Wnt signaling in colon cancer cell lines and suppress tumor growth in a mouse xenograft model. Furthermore, it was demonstrated that (-)-gossypol directly binds to the RNA-binding pocket of Msi1 RBD1. Likewise, Clingman et al. [14] performed high throughput screening and obtained 18–22 carbon ω-9 monounsaturated fatty acids, which were then shown to inhibit proliferation of a cell line expressing Msi1. The fatty acid was then shown to bind to Msi1 RBD1 and allosterically inhibit the RNA-binding. In both of the above studies [13,14], NMR chemical shift data [BMRB ID: 11450] and NMR solution structure [PDB ID: 2RS2] of Msi1 RBD1 were used to identify the residues involved in compound-binding and map the compound-binding sites on the structure.

These studies suggested that Msi1 is druggable and may have a potential to become a therapeutic target. Importantly, the development of compounds that exhibit a higher affinity and specificity toward Msi1 should provide a powerful tool to investigate and clarify the regulatory functions of Msi1 in normal and cancer cells. To develop better Msi1 inhibitors, it is important to understand the compound-binding mechanism of Msi1. To this end, NMR chemical shift data and NMR solution structures of both Msi1 RBD1 and RBD2 in their free and RNA-complexed forms [10,12,15] are expected to play important roles.

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