Structural analysis of the interaction between FUS/TLS protein and non-coding RNA

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Fused in sarcoma/ translocated in liposarcoma (FUS/TLS) is a multifunctional DNA/RNA binding protein that regulates various RNA metabolic processes including transcription, splicing, transport, translation, and decay. FUS is being paid much attention due to its association with numerous fatal neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and Huntington disease (HD). Several studies identified RNA sequence/structure motifs that bind FUS while others showed that FUS binding is not highly specific as to RNA sequence or structure. However, the mechanism of action by which FUS binds to various nucleic acids and exerts its function is still controversial.

FUS plays a role in the DNA damage response through the suppression of *cyclin D1* gene (*CCND1*) transcription, which results in cell cycle arrest to allow DNA repair. In that transcription repression process, the C-terminal region of FUS was reported to interact with long non-coding RNA (lncRNA) that is transcribed from the 5' upstream region of *CCND1*, named promoter-associated non-coding RNA (pncRNA). Although it was proposed that the interaction between FUS and pncRNA can allosterically convert FUS from a closed to an open conformation, this hypothesis has not been experimentally confirmed, so far. In this study, we investigated the conformational change of FUS in response to pncRNA binding by means of fluorescence resonance energy transfer (FRET) and high-speed atomic force microscopy (HS-AFM). Then, we studied the effects of temperature on FUS conformation.

In Chapter 1, firstly, a general introduction to the FUS protein family and its structure was given. Then, the diverse physiological and pathological roles of FUS were introduced. Finally, the background and the aim of this study were described.

In Chapter 2, FUS fusion protein construction and FRET assaying were explained. We designed a fusion construct, in which blue fluorescent protein (BFP, donor) and green fluorescent protein (GFP, acceptor) are linked to the N- and C-termini of FUS, respectively. A change in the FUS conformation was detected as the change in FRET efficiency before and after the addition of pncRNA. The FRET spectrum indicated the presence of FUS in a closed (compact) conformation in the absence of pncRNA. Binding of FUS to pncRNA induced a conformational change of FUS to an extended structure that was detected as the change in the relative FRET efficiency upon the addition of pncRNA. We showed that a short fragment of pncRNA (31-mer), which is a critical sequence for FUS-pncRNA binding, can induce a similar conformational change in FUS to that caused by the full-length pncRNA (601-mer). We further identified a minimum portion of pncRNA, GAGGGU, which can induce a conformational change of FUS. FRET data showed that both sequence and length can influence the degree of FUS conformational change. Then, we showed that other specific sequences, Telomeric repeat-containing RNA (TERRA) and Telomere DNA (Htelo), which have a crucial role in telomere shortening, can also induce a conformational change of FUS that is comparable to the pncRNA effect. FRET assaying for mutated TERRA and Htelo implied that their G-quadruplex secondary structures are not critical for induction of the FUS conformational change. Under our experimental conditions, ALS-linked FUS mutations have no remarkable effect on FUS initial conformation or FUS ability to change its conformation in response to pncRNA binding.

In Chapter 3, HS-AFM was applied to directly visualize the conformation of FUS protein before and after the addition of pncRNA. The moment of the FUS structural change from a compact to an extended form was captured. To investigate the distribution of various conformers in the absence/presence of pncRNA, about 7,000 HS-AFM frames were analyzed by two methods, BFP-GFP distance analysis and radius of gyration (R_g). Histograms for both analyses showed that FUS is mainly observed in a compact conformation in the absence of pncRNA. Upon the addition of pncRNA, FUS becomes more extended and flexible, as estimated from the center and width of histograms.

In Chapter 4, we examined the effect of temperature on FUS conformation by FRET assay.

In Chapter 5, a general conclusion as to the research background, results outcomes, importance of the study, and prospective future research is given.

FUS protein is being paid much attention due to its crucial role in several biological processes as well as its implication in cancer and several fatal neurodegenerative diseases. Therefore, understanding of the mechanisms of FUS physiological roles is needed to determine the etiology of FUS-related diseases. In this study, by means of FRET and HS-AFM, we provided a mechanistic insight into the role of FUS in the DNA damage response. We proved that FUS undergoes a conformational change upon binding to pncRNA, which is transcribed from the promotor region of the *CCND1* gene in response to DNA damage. The conformational change releases the N-terminal FUS from C-terminal masking, allowing the N-terminal region to bind transcription coactivators CBP/p300, which results in *CCND1* transcription repression. We further investigated factors that may affect the FUS conformational change. Our data showed that both sequence and length can influence the extent of the FUS conformational change.