News Release

Uncovering the mechanism maintaining the structure of the TFIIH transcription factor complex
- Advancing drug development for trichothiodystrophy -

Key Points
・Mutations in *GTF2H4*/*XPJ*, encoding the p52 subunit of the general transcription factor IIH (TFIIH) involved in mRNA transcription and DNA repair, were found to cause xeroderma pigmentosum (XP-J; designated intractable disease #159).
・Functional analysis of the p52 subunit revealed the mechanism maintaining the structural stability of the TFIIH complex.
・Based on this TFIIH stabilising mechanism, antisense oligonucleotides (ASO)-based therapeutic strategy was proposed to ameliorate trichothiodystrophy (TTD), an ultra-rare disorder characterised by sulfur-deficient brittle hair.

Summary
A team of researchers at Nagoya University has identified a new mechanism maintaining the structural integrity of the transcription factor IIH (TFIIH) complex.
Mutations in *GTF2H4*/*XPJ*, which encodes p52, a component of the TFIIH complex involved in nucleotide excision repair (NER), were found to cause xeroderma pigmentosum (XP; designated intractable disease #159). Congenital deficiency of another TFIIH component, p8, causes trichothiodystrophy (TTD), an ultra-rare genome instability disorder (classified as an N-of-1 disease) characterised by sulfur-deficient brittle hair. To explore the mechanism underlying the distinct pathologies of XP and TTD, the team analysed the function of p52 and its relationship with p8. Our findings revealed the mechanism responsible for structural stabilisation of the TFIIH complex. Based on these insights, we developed an antisense oligonucleotide (ASO) therapeutic aimed at ameliorating the pathology of TTD. This study proposes a new model for NER and TFIIH complex assembly and is expected to contribute to future therapeutic development for TTD through further investigation.

Research Background
Rare, intractable hereditary disorders involve a large number of causative genes, resulting in a wide variety of disease phenotypes. However, because the number of patients for each disease is extremely small, drug discovery and

therapeutic development have been slow to progress, posing a serious societal challenge.

We have identified a new causative gene, *XPJ*/*GTF2H4*, for xeroderma pigmentosum (XP), a designated intractable disorder (No. 159) characterised by skin cancer and neurological symptoms. *GTF2H2*/*XPJ* encodes p52, a subunit of the transcription factor IIH (TFIIH) complex, which is involved in nucleotide excision repair (NER). By contrast, congenital deficiency of another TFIIH subunit, p8, is known to cause trichothiodystrophy (TTD), a different rare hereditary disorder. Despite both being components of the same TFIIH complex, mutations in each give rise to distinct diseases, and the underlying basis for this divergence has remained unclear. In this study, we conducted functional analyses of p52 and p8 to investigate the molecular basis underlying these divergent disease phenotypes. Our findings uncovered a previously unknown mechanism of TFIIH complex assembly and further identified insights and therapeutic candidates that may help alleviate the pathology of TTD.

Research Results

We investigated the stability of the TFIIH complex using patient-derived cells (XP140BR) from a xeroderma pigmentosum (XP) with *GTF2H4*/*XPJ* mutations affecting the p52 protein, and TTD1BR cells from a trichothiodystrophy (TTD) patient with *GTF2H5* mutations affecting the p8 protein. In XP140BR cells, TFIIH subunits were partially degraded; however, a small amount of truncated p52 protein (p52ΔC), derived from the pathogenic allele, was still expressed and capable of forming TFIIH complexes at low levels. By contrast, TTD1BR cells exhibited profound destabilisation of the entire TFIIH complex, with no detectable complex formation. Notably, the TFIIH complex formed in XP140BR cells contained p52ΔC but lacked p8. These findings suggest that complete loss of p8 prevents stable TFIIH complex formation and leads to severe TTD, which can be fatal in infancy. In contrast, C-terminal truncation of p52 permits formation of a p8-deficient TFIIH complex, resulting in XP, a milder condition in which patients can survive into adolescence with appropriate sun protection. To further explore this mechanism, we analysed the structural role of the p52 C-terminus. We found that the C-terminal region of p52 is structurally flexible and not fixed in position. In the absence of p8, p52 adopts an unfavorable conformation in which the C-terminal tail interferes with TFIIH assembly. p8 binds to this region, stabilising the conformation of p52 and facilitating proper complex formation. Based on these insights, we hypothesised that removal of the C-terminal region of p52 using antisense oligonucleotides (ASOs) could promote TFIIH assembly even in p8-deficient TTD cells, offering a potential therapeutic strategy for p8-deficient TTD (TTD-A).

To test this, we designed ASOs that induce the expression of C-terminally truncated p52 (p52ΔC). Treatment of TTD1BR cells with the ASOs successfully increased p52ΔC expression, restored XPB protein levels, and promoted TFIIH complex assembly as anticipated.

Research Summary and Future Perspective

This study presents a new model for NER mechanisms and TFIIH complex assembly, although it does not yet fully account for the distinct pathologies observed in XP and TTD.

While the therapeutic effects of the ASOs have so far been demonstrated only in cell-based systems, these findings lay a strong foundation for further research. To build on this progress, we will continue to investigate the roles of individual NER-related factors and carry out in vivo studies using disease model animals. These efforts are expected to advance our understanding of the NER pathway, clarify the disease mechanisms of XP and TTD, and support the development of effective therapeutic strategies for these conditions.

Publication

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