Invited Mini-review

Trichothiodystrophy: From basic mechanisms to clinical implications

M. Stefanini*, E. Botta, M. Lanzafame, D. Orioli

Istituto di Genetica Molecolare CNR, via Abbiategrasso, 207, 27100 Pavia, Italy

ARTICLE INFO

Article history:
Accepted 10 October 2009

Keywords:
TFIIH
Transcription
Gene expression regulation
Nucleotide excision repair
Trichothiodystrophy
Xeroderma pigmentosum

ABSTRACT

Trichothiodystrophy (TTD) is an autosomal recessive disorder with symptoms affecting several tissues and organs. The most relevant features are hair abnormalities, physical and mental retardation, ichthyosis, signs of premature aging and cutaneous photosensitivity. The clinical spectrum of TTD varies widely from patients with only brittle, fragile hair to patients with the most severe neuroectodermal symptoms. To date, four genes have been identified as responsible for TTD: XPD, XPB, p8/TTDA, and TTDN1. Whereas the function of TTDN1 is still unknown, the former three genes encode subunits of TFIIH, the multiprotein complex involved in basal and activated transcription and in nucleotide excision repair (NER). Ongoing investigations on TTD are elucidating not only the pathogenesis of the disease, which appears to be mainly related to transcriptional impairment, but also the modalities of NER and transcription in human cells and how TFIIH operates in these two fundamental cellular processes.

© 2009 Elsevier B.V. All rights reserved.

Contents

1. Introduction ................................................................. 2
2. Pathological features of TTD .......................................... 3
3. TFIIH and its central role in the photosensitive form of TTD ............................. 3
   3.1. TFIIH composition and activities .................................... 3
   3.2. Function of the genes responsible for the photosensitive form of TTD ...... 4
   3.3. Mutational pattern and its relation with the clinical and cellular features 4
4. Genes responsible for the non-photosensitive form of TTD .................................. 6
5. Pathogenetic basis of the TTD clinical outcome ........................................ 7
   5.1. Skin and developmental abnormalities .................................... 7
   5.2. Aging features .................................................................. 7
   5.3. Lack of skin cancer .......................................................... 8
6. Future perspectives .......................................................... 8
Conflict of interest .............................................................. 8
Acknowledgements ............................................................. 8
References ........................................................................... 8

1. Introduction

Trichothiodystrophy (TTD) is a term derived from Greek (tricho, hair; thio, sulfur; dys, faulty; and trope, nourishment) that was coined by Price et al. in 1980 [1] to describe a group of autosomal recessive disorders characterized by sulfur-deficient brittle hair and other neuroectodermal symptoms that commonly include mental and growth retardation, proneness to infections, ichthyosis, nail abnormalities, decreased fertility and features of premature aging. Cutaneous photosensitivity has been reported in approximately half of the cases and is associated with an altered cellular response to ultraviolet (UV) light due to a defect in nucleotide excision repair (NER), the DNA repair pathway that removes a wide spectrum of lesions, including UV-induced damage. Three genes have been identified as responsible for the photosensitive form of TTD, namely XPB, XPD and p8/TTDA. The discovery that these genes encode distinct subunits of the general transcription factor IIH (TFIIH), a multi-protein complex involved in both NER

Abbreviations: aa, aminoacid; CAK, cdk-activating kinase; Cdk, cyclin-dependent kinase; CR, caloric restriction; CS, Cockayne syndrome; CTD, carboxy-terminal domain; ERα, estrogen receptor α; GH, growth hormone; IGF-1, insulin growth factor 1; NER, nucleotide excision repair; NR, nuclear receptor; PPARs, peroxisome proliferator-activated receptors; RARα, retinoic acid receptor α; RNApol, RNA polymerase; TFIIH, basal transcription factor IIH; TTD, trichothiodystrophy; UDS, UV-induced DNA repair synthesis; UV, ultraviolet; XP, xeroderma pigmentosum.

* Corresponding author. Tel.: +39 0382 546330; fax: +39 0382 422286.
E-mail address: stefanini@igm.cnr.it (M. Stefanini).

1568-7864/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2009.10.005
and transcription, has been crucial to rationalize the TTD clinical outcome and the puzzling variety of pathological phenotypes associated with mutations in the XPB and XPD genes.

Evidence has been provided that also the non-photosensitive form of TTD is genetically heterogeneous by showing that about 18% of the analyzed cases are mutated in the C7orf11 gene, thereafter designated TTDN1 (TTD non-photosensitive 1). This review addresses the recent advances in the field, focusing on the still emerging understanding of the pathogenesis of TTD and of the multiple roles of TFIIH in human cells.

2. Pathological features of TTD

All TTD patients exhibit sparse, dry and easily broken hair characterized by low sulfur and cysteine content and a typical banding, called tiger-tail pattern, when observed under polarizing microscopy. Hair anomalies, which are considered the diagnostic hallmark of the disorder, are associated with a wide spectrum of clinical symptoms that usually affect organs of ectodermal and neuroectodermal origin (reviewed in [2–4]). Common features include mental and growth retardation, nail abnormalities, ichthyosis, skin photosensitivity, microcephaly, facial dysmorphism, ocular and skeletal abnormalities, recurrent infections and impaired sexual development. In addition, osteoporosis, hearing loss, cataracts, dental caries, and other features of premature aging have been reported. Delivery is frequently preterm and children may be encased in a collodion-like membrane at birth. The disorder is characterized by a wide range in type and severity of symptoms. The most severe form is characterized by very poor mental and motor performance and speech, failure to thrive and death frequently occurring during early childhood, often due to overwhelming bacterial infections. Other patients show a pathological phenotype of moderate severity, with short stature, delayed puberty, mental development at pre-school or primary school level, axial hypotonia, reduced motor coordination and survival beyond early childhood. A few mild cases have been described with involvement of only hair, nails or skin (reviewed in [3]).

The remarkable progress made over the last twenty years on TTD has revealed two distinct forms of the disorder characterized by the presence or absence of clinical and cellular photosensitivity (OMIM #601675, OMIM #234050). The incidence of the NER-defective form of TTD has been recently established at 1.2 per million livebirths, based on combined data from five DNA repair diagnostic centres in West–Europe [5].

3. TFIIH and its central role in the photosensitive form of TTD

Photosensitive TTD cases are mutated in either the XPB, XPD or p8/TTDA genes, which encode subunits of TFIIH, a multi-protein complex that, besides participating to NER, is involved in RNA polymerase (RNApol) II transcription initiation and regulation, RNA pol I transcription, and cell cycle control. The versatile engagement of TFIIH in distinct cellular processes might explain why mutations in XBP and XPD result in a variety of autosomal recessive disorders. In addition to TTD, these include the cancer-prone disorder xeroderma pigmentosum (XP), Cockayne syndrome (CS), a disorder that shares many features of aging and developmental anomalies with TTD, and other rare clinical entities showing XP features in combination with CS or TTD clinical symptoms. It has been suggested that this pleiotropy may be related to mutations in different sites of the XBP and XPD genes, which differentially interfere with the stability and the conformation of the TFIIH complex, thus affecting its functional activities in slightly different ways [6].

3.1. TFIIH composition and activities

Accumulating evidence indicate that the composition of TFIIH is dynamic to adapt its engagement in distinct cellular processes. The transcriptionally active form of TFIIH (holo-TFIIH) is composed of a six-subunit core (XPB, p62, p52, p44, p34 and p8/TTDA) associated with the cdk-activating kinase (CAK) subcomplex formed by the three subunits cdk7, MAT1 and cyclin H. The core-TFIIH and CKI sub-complexes are bridged by the XPD subunit that interacts with p44 on the one side and MAT1 on the other side. The structural function of XPD in TFIIH assembly is used by cells in a dynamic way to regulate and coordinate the diverse functions of the different sub-complexes in transcription, DNA repair and cell cycle progression.

TFIIH possesses several enzymatic activities: the two ATPase and DNA helicase activities of XPB and XPD, the protein kinase activity of the cyclin-dependent kinase cdk7, a key player in transcription and cell cycle regulation, and the E3 ubiquitin ligase activity of p44, which is important for the yeast transcriptional response to DNA damage [7]. These enzymatic activities are tightly modulated by interactions with many components of the transcriptional machinery, including regulatory transcription factors, as well as by contacts within the TFIIH complex (reviewed in [2,9,10]).

In the transcription of class II genes, TFIIH participates to the opening of the promoter around the transcription initiation site through the XPB and XPD ATP-dependent helicases, and contributes to efficient promoter escape by phosphorylating the carboxy-terminal domain (CTD) of the largest subunit of RNApol II via its cdk7 kinase. TFIIH is also involved in the phosphorylation of activators, such as Ets1, and several nuclear receptors (NRs). Recent evidence indicates that optimal transactivation of the estrogen receptor α (ERα) requires the interaction of TFIIH with XPC, a structure-specific endonuclease that cleaves 3’ of the DNA lesion during NER. In ERα regulation, XPC is dedicated to the stabilization of TFIIH by preventing the dissociation of CAK and XPD from the core subcomplex [10]. This new additional role of XPC provides a reasonable explanation for those clinical features present in some XP-G patients, such as hypogonadism and loss of subcutaneous fat tissue, which cannot be explained by a repair defect (reviewed in [11]).

In the first steps of NER, TFIIH opens the double-stranded DNA around the lesion, a function that depends on the 5’–3’ helicase activity of XPD and the ATPase activity of XBP [12,13]. Once TFIIH has been recruited at the damaged site, the core subcomplex associates with NER-specific factors, including XPA, which catalyzes the detachment of the CAF from the core thereby triggering the incision/excision of the damaged oligonucleotide. Following damage removal, the NER factors are released from the complex and the CAK reappears with the core TFIIH on the chromatin, together with the resumption of transcription formerly inhibited by UV irradiation [12]. A further contribution of TFIIH to genome integrity is its role in the cell cycle. The free CAK subcomplex is a pivotal positive regulator of cyclin-dependent kinases that are key regulators of cell cycle progression and control [14,15].

Besides its well-established role in transcription of protein-coding genes and repair, TFIIH is also involved in RNApol I transcription, in a step subsequent to initiation [16]. It has been shown that in human cells TFIIH moves freely and gets engaged in RNApol I and RNApol II transcription for about 25 and 6s, respectively. Furthermore, TFIIH readily switches between transcription and repair sites when it is immobilized for 4 min [17]. Spatiotemporal distribution of TFIIH has been recently analyzed in a mouse model expressing fluorescently tagged TFIIH [18]. This new tool has allowed the monitoring of the TFIIH mobility in several living tissues revealing that in highly differentiated postmitotic cells, such as neurons, hepatocytes and cardiac myocytes, TFIIH is immobilized.
The three TFIIH subunits mutated in TTD have different roles. XPB and XPD are ATP-dependent helicases with opposite polarity and participate in the unwinding of DNA both in repair and basal transcription [19,20]. The 3′–5′ helicase activity of XPB is essential for transcription whereas the 5′–3′ helicase activity of XPD is dispensable for in vitro basal transcription, although XPD facilitates optimal transcription by anchoring the CAK subcomplex to the core TFIIH [21]. This probably accounts for the rarity of XP-B patients compared with the relatively high frequency of pathological phenotypes associated with mutations in XPD.

In NER, the efficient opening of the DNA around the damage is driven by the helicase activity of XPB [21], which is regulated by the TFIIH subunit p44 [22], and by the ATPase activity of XPB, which is regulated by the TFIIH subunit p52 [20] and the damage recognition factor XPC [23]. It has been shown that the archaean XPD helicase is able to motor along on DNA coated with ssDNA-binding proteins, and it can either displace proteins it encounters or it can slip right past them without either protein falling off of the DNA [24]. This may represent a generalized mechanism employed by the XPD/Rad3 family helicases for targeting the cognate DNA-processing intermediates.

The p8/TTDA subunit has been identified only recently, by showing that the four NER-defective TTD patients representative of the TTD-A group were mutated in the human homolog of the yeast gene encoding a new small 8 kDa component of TFIIH [25,26]. Through interactions with the subunits p52 and XPD, p8/TTDA stabilizes the entire complex and it is a key regulator of the TFIIH cellular levels in vivo [27,28]. Furthermore, p8/TTDA is indispensable for basal transcription whereas it has a pivotal role in DNA repair [27]. Following DNA damage, p8/TTDA forms a more stable association with TFIIH in nuclei [28] and promotes the local opening of the DNA around the lesion by stimulating the ATPase activity of XPB together with p52 and the damage recognition complex XPC-HHR23B. In addition, p8/TTDA promotes the translocation of XPA to UV damaged sites [20,27].

3.2. Function of the genes responsible for the photosensitive form of TTD

The p8/TTDA subunit encodes the only TFIIH subunit for which a complete absence is compatible with life, indicating its negligible role on the transcriptional activity of the complex [26]. As illustrated in Fig. 1, two of the mutations detected in TTD-A patients result in the absence of the protein or in non-functional truncated peptides. The third mutation converts the conserved leucine residue at position 21 to a proline and it is localized in one of the hydrophobic patches on the protein surface that might be involved in the recognition of the molecular partners [29,30]. The four TTD-A patients show a pathological phenotype of mild/moderate severity, a drastically reduced ability to repair UV-induced DNA damage (15–25% of normal) and a notable reduction in the TFIIH cellular amount (30% of normal) [26,31–33]. The overall features of TTD-A patients suggest that a two-thirds reduction in the amount of an otherwise normal TFIIH complex drastically hampers NER efficiency but confers only subtle defects in transcription resulting in clinical features of mild/moderate severity [32]. As well as implying that NER requires higher concentrations of TFIIH than transcription does, these findings underline the relevance of p8/TTDA for the TFIIH activity in DNA repair.

The two TTD siblings mutated in the XP gene are homozygous for a mutation resulting in the p.Thr119Pro substitution [34] that only slightly interferes with basal transcription activity in vitro [35]. This finding confirms that the XPB protein tolerates only rare alterations compatible with its essential role in transcription. The Thr119Pro change is associated with a partial NER deficiency in NER and TFIIH cellular content (both corresponding to 40% of normal), and a mild pathological phenotype characterized by hair abnormalities, mild ichthyosis and mild cutaneous photosensitivity. Both patients were born at term with a similar presentation as a colloidon baby of favorable outcome and they did not show any physical and mental impairment at the ages of 10 and 16 years, respectively. The relatively mild symptoms observed in these siblings compared with those in other TTD patients have led the clinician to describe them clinically as a TTD “variant” [36].

The few other reported XPB mutations localize in different sites of the gene and are associated with either mild XP (2 cases from 1 family) or XP/CS (7 cases from 5 families) [37,38].

Mutation analysis in thirty-three TTD patients mutated in the XPD gene (TTD/XP-D) [39–46] indicates that eleven patients are homozygous for a mutation resulting in a single substitution of an arginine residue at position 112, 658, 592 or 722 (Fig. 1). The remaining twenty-two patients are compound heterozygotes with various combinations of mutated XPD alleles. Three cases are functional hemizygotes, indicating that the mutations present

---

**Table 1**

<table>
<thead>
<tr>
<th>Mutated gene</th>
<th>Case no. (families)</th>
<th>Clinical symptoms</th>
<th>Physical impairment</th>
<th>Neurological impairment</th>
<th>UV sensitivity</th>
<th>UDS (% of normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTD-A</td>
<td>4 (3)</td>
<td>Hair alterations +</td>
<td>Skin photos. +</td>
<td>+</td>
<td>30</td>
<td>++</td>
</tr>
<tr>
<td>XP-B</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>XP-D</td>
<td>33 (30)</td>
<td></td>
<td></td>
<td>+</td>
<td>35–65</td>
<td>*/+</td>
</tr>
</tbody>
</table>

Abbreviations: NER: nucleotide excision repair; UDS: unscheduled DNA synthesis; photos.: photosensitivity.

a Physical impairment: (+) moderate (survival beyond early childhood, delayed puberty, and short stature); (++) severe (death during childhood and/or failure to thrive/dystrophy). Neurological impairment: (+) moderate severity (mental development at either preschool level or primary-school level, axial hypotonia, and reduced motor coordination); (++) severe (very poor mental and motor performances and speech).

b XPB level refers to the mean steady state of the subunits cdK7, p44 and p52 in patient cells expressed as percentage of that in normal cells analysed in parallel.

c NER efficiency refers to the cellular response to UV. UV-sensitivity: survival partially (+) or drastically (++) reduced compared with normal. UDS: ability to perform UV-induced DNA repair synthesis expressed as a percentage of that in normal cells.

---

on the chromatin at sites of RNApol I and RNApol II transcription. This immobilization is both differentiation driven and development dependent. Although statically bound, TFIIH can be rapidly remobilized in response to a genotoxic or environmental stress that requires a rapid adaptation of the transcriptional program.
in the only expressed allele (p.Asp673Gly in one patient and p.Glu731ArgfsX14 in two patients) are compatible with life.

Mutations are distributed across the gene and do not delineate any specific domain. Most of the changes are localized at three sites and all involve a single substitution of an arginine residue (Arg112, Arg658 and Arg722). Other frequently observed mutations are the substitution of Arg616, the Leu461Val change and the deletion of the aa region 716–730, the latter two being associated with the same allele. These two alleles are likely to be non-functional because they behave as null alleles in S. pombe [42] and they completely abolish basal transcription in vitro [47], a defect that is obviously incompatible with life. This explains why they have never been found in the homozygous state but always in association with other mutated XPD alleles that less drastically interfere with basal transcription [47]. It is worthwhile mentioning that these two nonfunctional alleles have been also detected in XP patients. The pathological phenotype in these cases appears to be determined by the mutation on the second allele that is always different in TTD and XP patients. Mutated XPD alleles typically associated with the XP phenotype are predicted to result in the change of the residue Arg683. These alleles are present in 26 out of the 31 reported XP cases [48,49].

The TTD/XP-D patients display different degrees of clinical severity. Some cases are moderately affected whereas others exhibit a severe clinical outcome. Also the DNA repair ability shows different degrees of alterations, ranging between 50% and 10% of normal [50–53]. The differential impairment in the cellular response to UV does not correlate with the degree of clinical severity but with the mutation site (Fig. 2). A mild NER deficiency is present in patients with mutations resulting either in the change of Arg658 or in the loss of the final portion of the XPD protein. An intermediate NER deficiency is associated with the Arg722Trp change whereas a remarkable repair defect is associated with the Arg112His change. In TTD/XP-D patients also the TFIIH steady-state level varies from 35% to 65% of normal but again the reduction in the TFIIH amount does not correlate either with the residual repair capacity or with the clinical severity (Fig. 2).

Investigations on Italian TTD patients revealed that the Arg112His compound heterozygotes are more severely affected than the homozygotes [43], suggesting that the main determinant of the clinical severity might be the effective XPD gene dosage. Examination of other patients supports this hypothesis (Fig. 1). A recent investigation on TTD compound heterozygotes sharing a typical TTD allele (p.Arg222Trp) has shown that the less compromised phenotype of Patient TTD24PV was associated with a mutation in the second allele leading to the production of a low level of a normal XPD protein that is able to partially rescue the functionality of TFIIH [46]. A moderate phenotype has been unexpectedly found in a patient compound heterozygous for an allele (p.Gln662X) likely to be lethal and a lowly expressed allele with a mutation predicted to generate three distinct XPD proteins mutated from residue 731 and longer than normal [46]. This finding indicates that a change of the C-terminal 30 aa of the 760 aa XPD protein is still compatible with the function of TFIIH in repair and transcription, in agreement with the observation that this is the only part of the XPD gene that is poorly conserved [39]. These studies indicate that identification and characterization of patients with unusual and/or unexpected genotype–phenotype relationship represent a useful tool to gain further insights into the in vivo functional effects of mutated proteins and into their impact on the whole organism.
A complex phenotype of moderate severity with some features of both XP and TTD (XP/TTD) has been found in four patients [45,54]. Three were compound heterozygous for a typical TTD allele (p.Arg112His or p.Arg722Trp) and for a novel allele (p.Ser51Phe, p.Leu485Pro or p.Cys663Arg) that may contribute to the XP phenotype. The remaining patient had two novel mutated XPD alleles (p.Phe568TyrfsX2 and p.Glu582Lys583delins3). It has been suggested that a peculiar situation in the genetic background of this patient may mitigate the effects of transcription and NER impairment, resulting in mild TTD and XP symptoms. Alternatively, the patient may mitigate the effects of transcription and NER impairments, resulting in mild TTD and XP symptoms. Overall, these studies indicate that the phenotypic consequences of each allele on TFIIH activity. The clinical outcome in patients will reflect the combined effects of each allele on TFIIH activity.

4. Genes responsible for the non-photosensitive form of TTD

Our knowledge on the functions altered in NER-proficient TTD patients is still very limited. Nothing is known about the functional activity of C7orf11/TTDN1, the gene mutated in a small proportion of non-photosensitive TTD cases that show normal response to UV light and TFIIH steady state level [56,57]. C7orf11 is an uncharacterized open reading frame that maps to human chromosome 7p14. The corresponding protein contains a glycine/proline-rich region but no putative conserved domains, localizes to the nucleus and is expressed in the hair follicles [56]. Different levels of TTDN1 expression were detected in different human cell-types, suggesting a regulation at the transcriptional level (our unpublished results). During mitosis TTDN1 interacts with polo-like kinase 1 (PLK1) and is phosphorylated by cdk1 [58]. Furthermore, overexpression of TTDN1 in HeLa cells causes nuclear fragmentation, whereas its knock down results in multiple nuclei or multiple-polar mitotic spindles [58]. However, these alterations are not observed in cells from TTDN1 patients (our unpublished results).

Mutations in TTDN1 have been so far detected in three Moroccan siblings, an Amish kindred and seven unrelated patients [56,57] (Fig. 3). A missense mutation has been reported only in the Amish kindred. The gene is deleted in three patients whereas the mutations found in five cases result in frameshifts predicted to produce severely truncated proteins. This mutation pattern indicates that the TTDN1 gene is not essential for cell proliferation and viability. Furthermore, the severity of the clinical features of the patients does not correlate with the molecular defect, indicating that other factors besides TTDN1 mutations influence the severity of the disorder.

What can be predicted on the functions defective in the non-photosensitive TTD patients on the basis of the limited available observations? Since the two forms of TTD share all the clinical features except photosensitivity, it has been suggested that the genes responsible for the non-photosensitive form might play a
role in transcription regulation but not in DNA repair. The corresponding proteins could be required for optimal functioning of TFIIH in tissues/organisms affected in both forms of TTD. Alternatively, they could be transcriptional regulators of genes relevant for metabolic pathways that are central to the outcome of TTD. At present, the identification of the still unknown disease-gene(s) responsible for non-photosensitive TTD cannot rely on whole-genome strategies, due to the paucity of the material worldwide available from informative patients/families. Our study has been focused on the transcriptional coactivators PRMT1 and CARM1 [59], two attractive candidates for the functions altered in the non-photosensitive form of TTD. However, no mutations were detected in eleven NER-proficient TTD patients who were not mutated in TTDN1 (our unpublished results).

5. Pathogenetic basis of the TTD clinical outcome

As detailed in the previous sections, all the mutations found in NER-defective TTD patients result in reduced steady-state levels of the entire TFIIH complex, modification of its architecture and impaired functioning in repair and transcription. Progress in our knowledge of the mechanistic and functional defects underlying the TTD outcome originates from studies on XPD, which have been promoted and supported by the relatively conspicuous number of affected patients. A fascinating example demonstrating the link between TFIIH instability and TTD clinical outcome is seen in two unrelated patients who displayed reversible worsening of TTD features, such as hair loss and ichthyosis, during febrile episodes associated with intercurrent infections. Both patients had an identical mutation in the XPD gene (p.Arg658Cys) that was shown to confer a thermo-instability of TFIIH resulting in a temperature-sensitive defect of transcription and DNA repair [60].

Further insights into TTD pathogenesis were gained with the generation of the TTD mouse model that contains a XPD mutation (p.Arg722Trp) that is found in several patients with TTD but not in any with XP. The mouse had many TTD features, including brittle hair, developmental abnormalities, reduced life span, UV sensitivity and skin abnormalities [61,62], thus conclusively proving that the TTD outcome originates from studies on XPD, which have shown the importance of the TFIIH regulatory role in gene expression. Some mutations associated with the TTD mouse model.

5.1. Skin and developmental abnormalities

The transcriptional failure in TTD is compatible with life and therefore it has to occur only under certain circumstances and/or in specific cellular compartments. It is believed that quantitative and conformational alterations of TFIIH in TTD may become limiting in terminally differentiated tissues, in which the mutated TFIIH might be insufficient to provide adequate transcriptional activity of a diverse set of highly expressed genes. In line with this suggestion, the hair abnormalities, which are the clinical hallmark of TTD, result from a deficiency in cysteine-rich matrix proteins in the hairshafts of TTD patients. Alterations in the transcriptional program in late stages of differentiation might account also for the β-globin deficiency in erythrocytes of TTD/XP-D patients showing the haematological features of β-thalassemia trait without mutations in hemoglobin genes [44]. For the alterations in T cells and dendritic cells reported in few TTD patients [50,68], and for other typical TTD abnormalities, such as ichthyosis. Indeed, a lowered expression of differentiation markers has been described in the skin [61] and in vitro differentiating keratinocytes [69] from the TTD mouse model.

Other clinical symptoms may be related to interference with the TFIIH regulatory role in gene expression. Some mutations in the C-terminal end of XPD have been shown to prevent the phosphorylation of specific ligand-activated NRs and to interfere with the anchoring of CAK to the core TFIIH. Namely, the mutations Arg683Trp (XP) and Arg722Trp (TTD) impair the transactivation of the retinoic acid receptor α (RARα) and the peroxisome proliferator-activated receptors (PPARs) [47,70,71]. Nevertheless, almost optimal transactivation of RARα was found in TTD-A cells [70] and in TTD cells with the Arg112His substitution [47] that showed also a normal transactivation of PPARs [71]. These findings offer a clue for understanding some developmental and neurological defects encountered in some XP and TTD patients. The hypoplasia of adipose tissues in TTD (p.Arg722Trp) mice might be due to a defective transactivation of PPARs, which are essential for lipid metabolism and differentiation/survival of adipocytes in vivo [71]. Recent studies on TTD mice have also demonstrated a selective dysregulation of thyroid hormone target genes in specific areas of the brain that results from the inability of mutated TFIIH complexes to fully participate to the recruitment/stabilization of thyroid hormone receptors on their target genes [72]. The tissue-specificity of this defect is conferred by the cell type-specific transcription regulatory machinery and not by TFIIH itself. As well as revealing an unexpected coactivator function of TFIIH, these results provide an explanation for some of the neurological manifestation of TTD since the thyroid hormone contributes to the regulation of several processes taking place during perinatal brain development, such as myelogenesis and neuronal cell migration.

5.2. Aging features

TTD is regarded as a segmental progeroid syndrome because several patients exhibit signs of premature aging. A role for the XPD gene in the aging process is supported by some of the features present in the TTD mouse model (reviewed in [73]). Apart from the skin and developmental abnormalities typical of TTD, the mice show reduced lifespan, cachexia, osteoporosis, osteosclerosis, kyphosis, sebaceous gland hyperplasia, and other features of cutaneous aging such as early graying [62,74,75]. Furthermore, they exhibit progressive loss of bone marrow progenitor cells and age-dependent decline of hematopoietic stem cell function [76]. Unexpectedly, these premature aging features are accompanied by symptoms that are normally observed after dietary caloric restriction (CR) [77], the only well documented intervention known to extend life span and delay the onset of many aspects of aging in mammals. This paradox finds an elegant solution in the observation that CR as well as normal aging are characterized by a systemic attenuation of the growth hormone (GH) and insulin growth factor 1 (IGF-1) somatotropic axis. The somatotropic attenuation is emerging as a feature shared also by NER progeroid mouse models [78]. These observations led to the fascinating hypothesis that accumulation of DNA damage interferes with normal DNA metabolism and triggers suppression of the GH/IGF-1 somatotropic axis and...
dampening of oxidative metabolism. This is a “survival response” that shifts the energy equilibrium from growth and proliferation to pathways preserving somatic maintenance in the attempt to extend lifespan.NER progeroid mice accumulate DNA damage much more rapidly than naturally ageing mice as a consequence of their repair defect, and onset of the life extending “survival response” is accelerated [79,80].

Interestingly, in the liver of TTD mice a reduced IGF-1 signaling and a decreased energy metabolism were found associated with increased homeostatic imbalance between apoptosis and cell proliferation [81]. A failure of the organism to maintain homeostasis might explain the general conditional decline (including cachexia) that is an important cause of death in TTD mice as well as in several TTD patients.

However, the way in which XPD is responsible for prevention of the aging process remains to be determined. Several evidence are supporting a causal role of unrepaired oxidative DNA damage in the pathology of CS, a disorder showing neurological and aging features overlapping those present in TTD [82–84]. Conversely, hypersensitivity to oxidative stress remains elusive in TTD. Based on our current knowledge, it is likely that aging signs, as well as other clinical symptoms of TTD that are present also in the patients with normal repair ability, are directly related to transcripational failure, which might be further exacerbated by the accumulation of DNA damage.

5.3. Lack of skin cancer

We do not have yet a clear answer why TTD patients, despite being mutated in the same genes as XP patients, do not show the increased freckle-like pigmentation and the high frequency of sunlight-induced skin cancers typical of XP. Following UV irradiation, the frequency of mutations show the same increase in NER-defective TTD and XP cells [50,85] and a similar failure in the checkpoint activation has been reported [86]. Only in XP-D fibroblasts from XP/CS patients, despite the fact that UV lesions are not processed by NER, the checkpoint is normally activated, likely by DNA breaks that occur at sites of transcription initiation. Differences between XP and TTD have been reported in immune responses, cell-cycle regulation, oxidative metabolism, apoptotic response (reviewed in [87]), gene expression after UV [88] and, more recently, behavior in the recruitment of NER factors to sites of localized UV damage [45,89]. Nevertheless, no cellular/biochemical alterations unequivocally correlated with the different cancer susceptibility in XP and TTD have been identified yet. At present, the difference in cancer proneness between XP and TTD, despite apparently similar repair defects, remains one of the most provocative enigmas in the field of DNA repair. A widely accepted explanation is that the defect in transcription, together with the limiting amounts of TFIIH typically detected in TTD cells, could prevent neoplastic transformation and/or progression by interfering with the expression of critical genes involved in the carcinogenic pathway [6,90,91]. As discussed in [6], this hypothesis implies a subtle interplay between the complex pathways of transcription, differentiation, and carcinogenesis. It would not be surprising if the outcome of this balance differed between species; therefore, the finding that the TTD mouse is, unlike TTD patients, susceptible to UV-induced carcinogenesis [92] is not inconsistent with this hypothesis.

6. Future perspectives

Thus far, investigations of the mechanistic defects leading to XP or TTD have been beneficial in understanding the function of XPB, XPD, and p8/TTDA in NER and in transcription. Despite the notable progress made in our understanding of the genetic and molecular bases of TTD, it is evident that there is still much more to learn about this disorder. Further studies are needed to elucidate the individual contribution of the deregulated genes and signalling pathways in the clinical outcome of TTD as well as their role in the acceleration of the aging process and in the suppression of skin tumorigenesis. Furthermore, we still do not know if and how the TFIIH complexes mutated in TTD interfere with the emerging role of TFIIH in transcription elongation and termination of some genes [93], and with the transcription of microRNAs whose expression in mammalian cells is driven by RNApol II and it is highly regulated in a tissue and time-specific manner. Future research will have also to address the cloning of the other disease-genes responsible for TTD and the identification of their functions, a fundamental step toward developing therapeutic strategies.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Our studies mentioned in the text have been supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC), the European Community (contract MRTN-CT-2003-503618), the Italian Ministry of University and Research (FIRB grant RBIN042Y7), and the Fondazione Cariplo.

References


