Anti-TIF1- γ autoantibodies: flashlights of a tumour autoantigen

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Abstract

Anti-TIF1- γ autoantibodies are robustly linked with cancer-associated dermatomyositis (DM) in adults. Detection of anti-TIF1- γ autoantibodies has a high sensitivity and specificity for cancer-associated DM in adults and is therefore useful for both diagnosis and cancer risk stratification. The function of the autoantigen, transcriptional intermediary factor 1 gamma, may provide insight into the mechanism behind this association. TIF1- γ is a ubiquitously present protein involved in various biological pathways, including TGF- β signalling. In cancer, it can act both as a tumour suppressor or promoter, depending on the cellular context and cancer stage. Evolving data provide pathophysiological insights, linking anti-TIF1- γ autoantibodies both to the anti-tumour response and to muscle and skin damage. TIF1- γ expression is increased in muscle and skin tissue of patients with DM and in several cancers. Mutations or loss-of-heterozygosity in TIF1- γ alleles in malignant tissue may result in the expression of tumour-specific neo-antigens stimulating autoantibody production. The newly formed autoantibodies are hypothesized to cross-react with antigens in muscle and skin, driving the development of dermatomyositis.

Keywords: idiopathic inflammatory myopathy, myositis, dermatomyositis, cancer-associated dermatomyositis, myositis specific antibody, TIF1- γ , cancer

Key messages

- Anti-TIF1- γ autoantibodies are robustly linked to cancer-associated DM
- TIF1- γ is involved in several pathways relevant to cancer development
- Anti-TIF1- γ autoantibodies are linked both to the anti-tumour response and muscle and skin damage

Introduction

Idiopathic inflammatory myopathies (IIMs) are systemic autoimmune diseases predominantly characterized by muscle inflammation (myositis) and weakness. In addition, skin manifestations, arthritis and arthralgia, interstitial lung disease, gastro-intestinal and cardiac involvement are also observed in patients with IIM. The prevalence of IIMs is estimated to be between 2.4 and 33.8 per 100,000 with an annual incidence projected to be between 1.16 and 19 new cases per million [1]. Most studies report a greater proportion of females and in the northern hemisphere, higher latitude seems correlated with lower incidence. Different subtypes of IIM include polymyositis, dermatomyositis (DM), inclusion-body myositis, immune-mediated necrotizing myopathy (IMNM) and overlap myositis (including the anti-synthetase syndrome), but a consensus on the specific classification criteria for all subtypes has not yet been found. [2-4].

IIMs, or myositis, are strongly linked with cancer [5, 6]. Prevalence of cancer in patients with IIM varies between 6.7 and 32% [7] and is mainly documented in patients with DM (standardized incident ratio for occurrence of cancer of 5.5 in a recent meta-analysis[8]) and to a lesser extent in IMNM [9, 10]. There is a close temporal relationship with cancer detection [11, 12], suggesting that myositis and cancer may be linked mechanistically, and thus refuting the hypothesis that immunosuppressive drugs may be driving the observed association. Myositis can resolve after successful cancer treatment, suggesting its appearance as a paraneoplastic phenomenon. However, myositis often appears after a cancer diagnosis with varying time intervals, and can remain refractory to therapy, even after appropriate treatment of the associated cancer [13].

Myositis-specific autoantibodies (MSAs) can be identified in 60-70% of patients with IIM [14]. MSAs are typically directed against cytoplasmic or nuclear molecules that play a role in the regulation of key biological processes, such as protein synthesis and translocation, gene transcription and viral recognition. Individual autoantibodies are associated with specific disease manifestations, outcome and success of therapeutic strategies [15]. Anti-Jo-1 and anti-Mi-2 autoantibodies are the most well-known MSAs. More recently, several other MSAs have been described, including anti-MDA5, anti-SRP, anti-NXP2, anti-transcriptional intermediary factor 1- γ (anti-TIF1- γ), anti-SAE, and anti-HMGCR [16, 17].

Of these MSAs, anti-TIF1- γ , anti-NXP2 and anti-HMGCR autoantibodies were reported to be associated with an increased risk of cancer in patients with IIM [9, 10] (table 1). Anti-TIF1- γ autoantibodies stand out, with an association rate ranging up to 84% in adult patients with IIM [18]. In contrast, this association with cancer is not present in patients with juvenile DM. Anti-TIF1- γ autoantibodies were discovered by Targoff et al. by immunoprecipitation [19]. The

target antigen was a 155 kDa nuclear protein, later identified as TIF1- γ . In addition, a 140 kDa band, corresponding to TIF1- α as antigen, was also immunoprecipitated. This dual reactivity suggests a cross-reactivity to different proteins of the TIF family, though TIF1- γ appears to be the main target.

In this review, we summarize the function of TIF1- γ in normal physiology and cancer to provide a framework on why this autoantigen is associated with cancer and how an anti-tumour response may lead to dermatomyositis. Advances in understanding the nature of the autoantigen and the mechanisms of development of cancer-associated dermatomyositis can lead to improved detection or targeted treatment.

TIF1- γ in a physiological context

Human TIF1- γ , also known as ectodermin, type 2C protein phosphatase (PTC7), ret fused gene 7 (RFG7) or tripartite motif (TRIM) 33, was first described in 1999 by Venturini et al. [20]. TIF1- γ is encoded by the *TRIM33* gene, located on the p13 band of the short arm of chromosome 1. Together with TIF1- α (TRIM24), TIF1- β (TRIM28) and TIF1- δ (TRIM66), TIF1- γ forms the transcriptional intermediary factor 1 (TIF1) family. TIF1- γ has been demonstrated in normal tissues (including skeletal muscle and skin), with RNA and protein levels varying by tissue (17).

TIF1- γ is involved in diverse biological processes [21]. When recruited to transcriptional complexes at promoter regions of the DNA, it can repress or activate transcription. TIF1- γ also contributes to the function, localization and degradation of proteins through posttranslational modifications. To this end, TIF1- γ has E3 ubiquitin-protein ligase and small ubiquitin-like modifier (SUMO) E3 ligase activity. The other way around, TIF1- γ itself can be regulated by posttranslational modifications, such as sumoylation (19), or reduced expression by methylation of the promoter of the *TRIM 33* gene (20).

The role of TIF1- γ is extensively studied in the TGF- β signalling pathway [21-25]. This pathway is essential for cell differentiation and proliferation, developmental processes and tissue homeostasis, but is also implicated in several diseases. Depending on the cellular context, TIF1- γ acts as a negative or a positive regulator of the canonical SMAD-dependent TGF- β pathway [25, 26] (figure 1). SMADs are cytoplasmic molecules that assemble into complexes upon activation of the TGF- β receptors and can translocate to the nucleus to act as transcription factors. TIF1- γ exerts its role as a negative regulator by the mono-ubiquitination of SMAD4 [22], thereby blocking the formation of the SMAD2/3/4 complex and repressing SMAD2/3/4-dependent transcription. This inhibitory mechanism has been identified in stem cells and during embryonic development [21, 24]. In contrast, TIF1- γ can also act as a positive regulator, by selectively binding SMAD2/3 via its TIF1- γ -specific middle region, thereby

competing with SMAD4 for binding SMAD2/3. SMAD4-independent signalling has been observed in haematopoiesis, where the formation of SMAD2/3-TIF1- γ complexes stimulates erythroid differentiation whereas SMAD2/3/4 complexes inhibit erythroid proliferation [23]. The SMAD2/3-TIF1- γ complex has also been shown to promote the terminal differentiation of mammary gland epithelial cells and lactation [27].

Besides the above-described role in the TGF- β pathway, TIF1- γ is involved in cell cycle-related processes such as mitosis, through participation in the mitotic checkpoint complex, and beta catenin degradation (figure 1) [28, 29]. Other important processes in which TIF1- γ features are osteoblast proliferation and differentiation [30], and DNA repair [31, 32]. Finally, TIF1- γ also plays an active role in the immune response through macrophage activation [33, 34] and inflammasome complex formation [35]. Among the protean array of functions of TIF1- γ in multiple pathways, the possible role in tumorigenesis has attracted most attention.

TIF1- γ and cancer

TIF1- γ has been found to act as both a tumour suppressor and tumour promoter in different cancers (table 2). Most studies support the notion of TIF1- γ as tumour suppressor in murine and human malignancies [28, 36]. Through its involvement in the TGF- β pathway, TIF1- γ inhibits tumorigenesis mainly by inhibition of TGF- β -induced epithelial-to-mesenchymal transition via mono-ubiquitination of SMAD4. Inactivation, mutation or downregulation of TIF1- γ by hypermethylation, histone modifications, short non-coding microRNA or sumoylation can all result in a reduction of this effect and thus promote tumorigenesis [37-40]. This has been observed in hepatocellular carcinoma [37], chronic myelomonocytic leukaemia (20), non-small cell lung cancer [38], bladder cancer [41], breast cancer [42] and pancreatic ductal adenocarcinoma [39]. Interestingly, in Smad4-deficient pancreatic cancer cells, a Smad4-independent tumour suppressor effect has been reported, pointing to a tumour suppressor effect of TIF1- γ through an alternate pathway [40]. One such proposed alternate pathway is the Wnt/beta catenin pathway, where TIF1- γ acts as a tumour suppressor by its contribution to beta catenin degradation, a process that inhibits gene transcription programs induced by Wnt activation. Several human cancers, such as some forms of glioblastoma, are characterised by aberrant or persistent activation of β -catenin [28]. Furthermore, prolonged inactivation of TIF1- γ leads to mitotic defects, chromosomal abnormalities and increased tumour aggressiveness, indicating another separate tumour suppressor mechanism [43].

While TIF1- γ is mostly described as a tumour suppressor, TIF1- γ overexpression (with loss of SMAD4) has been found in early stage breast cancer [44] and early and advanced stage colorectal cancer [45]. TIF1- γ might inhibit the SMAD4-mediated apoptotic effect of TGF- β signalling in these cells, leading to reduced apoptosis of aberrant cells. The control of both

apoptosis and EMT by TGF- β seems paradoxical, but the selection of apoptosis or EMT is possibly related to the cell cycle phase [46]. Control of both processes by TGF- β signalling might account for some of the conflicting results. A specific role for TIF1- γ in a pro-tumorigenic context by DNA damage, is the *TRIM33-RET* fusion oncoprotein. This was first discovered in childhood papillary thyroid carcinoma and subsequently described in lung adenocarcinoma as well [47, 48]. However, the *RET* gene is a known proto-oncogene, which might explain the tumour promoting effect, as opposed to the *TRIM33* gene being responsible, though a synergistic effect cannot be excluded.

In summary, TIF1- γ is crucially involved in several pathways associated with tumorigenesis, but its exact role remains unclear. Why and how TIF1- γ gives rise to formation of anti-TIF1- γ autoantibodies and development of dermatomyositis in patients with cancer is another matter.

Pathogenic mechanisms in anti-TIF1- γ -positive cancer-associated dermatomyositis

The exact underlying aetiology of myositis remains unknown and distinct triggers and processes may well lead to a common outcome. Both adaptive and innate immunity, in addition to non-immune mechanisms such as autophagy, endoplasmic reticulum stress-response and hypoxia, are believed to play a role in the pathogenesis of myositis [49, 50].

In anti-TIF1- γ positive cancer-associated dermatomyositis, cancer is hypothesized to be the trigger for dermatomyositis, with TIF1- γ possibly functioning as a tumour autoantigen (figure 2) [51, 52]. Support for TIF1- γ as possible autoantigen was recently provided by Pinal-Fernandez et al. For the first time they documented genetic alterations in the *TRIM33* gene in patients with cancer-associated dermatomyositis. They detected loss-of-heterozygosity and, in one case, a somatic mutation in TIF1- γ alleles in tumours of patients with cancer-associated myositis [53]. *TRIM33* mutations are rare (< 1% of all cancers [54]) and it is unclear whether they function as driver mutations or are rather part of the accruing mutational load in tumour development. Alterations of TIF1- γ may lead to neoantigen formation, activating the adaptive and innate anti-tumour immunity. This anti-tumour response could lead to eradication of the tumour or only elimination of the tumour antigen. The former can account for the occurrence of TIF1- γ autoantibodies in adult patients with dermatomyositis without cancer [52]. In the latter case, tumour cells lacking the altered TIF1- γ could be selected by a T-cell-dependent immunoeediting process [55].

The humoral response to altered TIF1- γ is thought to cross-react with native TIF1- γ . A large TIF1- γ antigen supply in the muscle and skin could explain why the anti-tumour response leads to the clinical manifestation of dermatomyositis. This native antigen supply can stem from the increased expression of TIF1- γ , which has been documented in patients with anti-TIF1- γ positive DM [56], or the sheer number of TIF1- γ antigens in the skin and muscle, two of the

largest organ systems. Furthermore, regenerating muscle cells are known to express high levels of myositis autoantigens, just as in several cancers [57]. A cross-reaction of the humoral anti-tumour response to wild-type TIF1- γ antigens can induce autoimmune-mediated skin and muscle inflammation.

A genetic predisposition can explain why not all cancers with altered TIF1- γ expression lead to myositis. Myositis in general has been associated with specific HLA-sequences (HLA-DRB1*0301 and HLA-DQA1*0501 in Caucasians [58]) and anti-TIF1- γ autoantibodies specifically have been associated with HLA-DQA1*0301 [19]. More recently, a strong HLA association was observed in patients with anti-TIF1- γ autoantibodies with the HLA-allele group DQB1*02 [59]. Intriguingly, the association at the HLA-DQB1 locus differed between adult-onset and juvenile-onset patients (HLA-DQB1*02:02 in adults, HLA-DQB1*02:01 in juveniles, but the strongest association was with HLA-DRB1*03:01 which is on the same haplotype as HLA-DQB1*02:01), suggesting a different genetic disposition with other, possibly environmental, triggers.

An alternative hypothesis is that autoimmunity leads to cancer [7]. Autoantibodies targeting TIF1- γ could theoretically abrogate the tumour suppressor function of TIF1- γ through interaction with epithelial-to-mesenchymal transition, β -catenin and mitosis. However, few arguments for this hypothesis have been described in literature and findings of mutations in TIF1- γ genes do not fit this hypothesis.

Detection of TIF1- γ autoantibodies and cancer risk

As anti-TIF1- γ autoantibodies are flashlights of a possible tumour antigen, correct and timely detection of these autoantibodies is crucial. Anti-TIF1- γ autoantibodies can be identified in 22-32% of patients with juvenile DM and in 7-31% of adult patients with DM [60]. As previously noted, anti-TIF1- γ autoantibodies are rather frequent in juvenile DM, but the association with cancer is restricted to adults with DM, suggesting a different pathophysiology with distinct cellular processes, immunoreactivities and genetic predispositions [59, 61]. Using anti-TIF1- γ to predict cancer-associated DM is therefore only but highly useful in adults.

In adults, the risk of cancer IIM depends on the timing of the diagnosis of myositis and the age of the patient at diagnosis [11]. Data from a large cohort study in the UK revealed that cancer in patients with anti-TIF1- γ autoantibodies was exclusively diagnosed within 3 years before or 2,5 years following the diagnosis of myositis. Moreover, patients with an age ≥ 39 and anti-TIF1- γ autoantibodies had a significantly higher risk of developing cancer compared with those under 39 years old [11]. In a recent study, IgG2 autoantibodies in specific were associated with mortality [62]. Given the strong association between anti-TIF1- γ and cancer-associated DM in older patients, rigorous surveillance and screening for cancer in these patients is advised as

cancer is the main prognostic factor in IIMs[63]. Therefore, yearly PET/CT examination is recommended in anti-TIF1- γ -positive patients with DM for at least 3-5 years after diagnosis [64, 65].

As detection of anti-TIF1- γ autoantibodies has important repercussions, clinicians should be aware of the detection methods used in their clinical laboratory. Detection by immunoprecipitation (IP), the current preferred detection method has an overall specificity for cancer-associated DM of 89% [95% CI 82-93%] and a sensitivity of 78% (95% CI 45-94%) [66]. However, IP is not the preferred approach for routine testing in the clinical setting as it is time-consuming, expensive and technically complex. New assays for clinical practice such as enzyme-linked immunosorbent assays (ELISA) and dot/line assays are increasingly used [66-70], but performance characteristics substantially varies between the different commercially available assays. In a single-cohort study comparing assays, differences in reactivities between manufacturers were most pronounced for anti-TIF1- γ [70], ranging from a prevalence of 2.1% to 12.3%. Given these detection issues, the cutaneous phenotype [71, 72] and concordance of the antinuclear antibody pattern (i.e. nuclear fine speckled) might hint at the presence of anti-TIF1- γ autoantibodies.

Conclusion

Anti-TIF1- γ autoantibodies are a not to be ignored flashlight for the clinician signalling the presence of a possible tumour autoantigen in patients with dermatomyositis. The autoantigen, TIF1- γ , is a ubiquitous protein with a complex role in different pathways. In the context of cancer, TIF1- γ can function as tumour suppressor or tumour promoter depending on the underlying tissue and cancer type investigated. The significant presence of anti-TIF1- γ autoantibodies in sera of DM patients appears important for disease pathogenesis. Current evidence supports the view that cancer-associated myositis is the result of an anti-tumour response, in which cross-reactivity of the anti-tumour response is an important actor. Using anti-TIF1- γ autoantibodies as a marker to predict cancer-associated DM is highly useful in adults. In patients carrying anti-TIF1- γ autoantibodies, rigorous surveillance and screening for cancer are advised and yearly PET/CT examination is considered justified for at least 3-5 years after diagnosis.

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Table 1 Myositis-specific autoantibodies and their association with cancer

Autoantibody	Association with cancer as compared to other MSAs	Autoantigen: function, localization
Anti-TIF1-gamma	Strong	Transcriptional intermediary factor 1-gamma: see text for functions, nucleus
Anti-NXP2	Moderate	Nuclear matrix protein 2: diverse functions in nuclear metabolism and structure, nucleus
Anti-SAE	Possible	Small ubiquitin like modifier activating enzyme: post-translational modifications, nucleus
Anti-HMGCR	Possible	HMGCoA-reductase: rate-limiting step in cholesterol synthesis, cytoplasm
Anti-SRP	Unclear	Signal recognition peptide: translocation of proteins across the endoplasmic reticulum, cytoplasm
Anti-Mi-2	Unclear	Chromodomain-helicase-DNA binding protein 4: component of the nucleosome remodeling-deacetylase complex, nucleus
Anti-Jo-1	Unclear	Histidyl-tRNA-synthetase: translation, cytoplasm

Table 2 Role of TIF1- γ in specific types of cancer

Type of cancer	Pathway involved
Tumour suppressor	
Hepatocellular carcinoma [37]	Advanced stages: TGF- β pathway: EMT stimulation
Non-small cell lung cancer [38]	TGF- β pathway: EMT stimulation
Pancreatic ductal adenocarcinoma [39, 40]	Early stages: Activation of the Kras G12D oncogene Advanced stages: TGF- β pathway: EMT stimulation
Chronic myelomonocytic leukaemia [73]	TGF- β pathway
Clear cell renal cell carcinoma [74]	TGF- β pathway
Glioblastoma [28]	β -catenin pathway
Bladder cancer [41]	TGF- β pathway: EMT stimulation
Breast cancer [42, 44]	TGF- β pathway: EMT stimulation
Tumour promoter	
Breast cancer [44]	TGF- β pathway
Colorectal cancer [75]	Early stages: TGF- β pathway

Figure 1 The role of TIF1- γ in different pathways

P phosphorylated, Ub ubiquitination

Figure 2 TIF1- γ antigens as target for autoantibodies

Altered TIF1- γ antigens in tumour cells form neoantigens, which are targeted by the immune system. This anti-tumour response is either effective (eradication of the tumour cells without clinical symptoms) or ineffective (tumour growth). The humoral anti-tumour response cross-reacts with wild-type TIF1- γ in muscle and skin cells. Non-specific injury of muscle and skin cells can lead to regeneration with high levels of wild-type TIF1- γ autoantigen.