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Original Article

Cardiac amyloidosis induces up-regulation of Deleted in Malignant Brain Tumors 1 (DMBT1)

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ABSTRACT

Background: Amyloidosis is a life-threatening protein misfolding disease and affects cardiac tissue, leading to heart failure, myocardial ischemia and arrhythmia. Amyloid deposits result in oxidative stress, inflammation and apoptosis. The purpose of this study was to examine the role of innate defense components, i.e., Deleted in Malignant Brain Tumors 1 (DMBT1) and the complement system, in different types of cardiac amyloidosis.
Methods: Expression of DMBT1 and of the complement proteins C1q, C3d and C4d in cardiac specimens of patients with different types of amyloidosis were determined by immunohistochemistry and correlated with amyloid deposits stained by Congo red dye.
Results: Strong DMBT1 staining adjacent to amyloid deposits was detected in different amyloidosis types, depending on the extent of the deposits. DMBT1 is localized in the endomysium and perimysium, in the endocardium, in the myocytes and in endothelial cells of affected transmural vessels. C1q, C3d and C4d were detected in the amyloid deposits but also in the endomysium and perimysium, in some myocytes, in endothelial cells, in the endocardium, and around the amyloid deposits.
Conclusions: Up-regulated DMBT1 and complement activation in cardiac amyloidosis may be part of the activated pathways induced by protein aggregation and the consecutive inflammatory reaction.

1. Introduction

Deposits of insoluble proteins in different organs with concomitant organ failure are the characteristics of the different forms of amyloidosis. The protein deposits consist of fibrils with apple-green birefringence under polarized light after staining with Congo red dye. These insoluble proteins are observed in diseases with chronic inflammation, in immunoglobulin-producing disorders, and different diseases with hereditary gene mutations, comprising at least 28 forms of amyloidosis [1–3]. Untreated patients show a reduced life span, while treatment reduces mortality [4]. The choice of the treatment is made based on the detailed characterization of the deposits' protein composition and includes chemotherapy, organ transplantation or stem cell transplantation [5]. The heart is often affected by these protein depositions, leading to life-threatening complications, which include heart failure and restrictive cardiomyopathy. Infiltration of the heart confers the worst prognosis in amyloidosis patients [5]. Cardiac amyloid deposits lead to an interruption of the contractile function and of the electrical conduction as well as to an impairment of the coronary flow. Amyloid fibrils penetrate the myocardial interstitium by forming nodular deposits and branching filaments which interlace individual myocytes [5,6]. Endocardial deposition may lead to valvular insufficiency. Amyloid disables the balance of matrix metalloproteinases and their inhibitors and modulates thereby interstitial matrix composition and tissue remodeling [7]. The cardiac vasculature is also affected, leading to myocardial ischemia, and microinfarction [8–10].

The protein DMBT1 (Deleted in Malignant Brain Tumors 1), also known as glycoprotein-340 or salivary agglutinin, belongs to the scavenger receptor cysteine rich (SRCR) group B protein family [11]. Functions of DMBT1 in epithelial differentiation and a possible role of DMBT1 as a candidate tumor suppressor gene in different tumors have been proposed [11–17]. In addition, DMBT1 has an important function in innate immunity by interacting with various other defense factors such as secretory IgA, surfactant protein D and A, and by directly binding to bacteria [13]. Up-regulation of DMBT1 in various

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inflamed tissues has been observed [14,18]. DMBT1 is able to bind to C1q of the complement system [19]. The complement system functions in opsonizing or permeabilizing pathogens and in induction of different inflammatory responses. Interaction of DMBT1 with C1q leads to activation of the classical pathway of the complement system, resulting in the formation of C4b, C3b, and iC3b [19]. Further, DMBT1 is able to activate complement via the lectin pathway [20]. These properties enable DMBT1 and factors of the complement system to play a role in diseases characterized by insoluble deposits, inflammation, and affected interstitial matrix composition.

The association of complement factors with amyloid deposits has been investigated for different scenarios, e.g. in cerebrovascular amyloid in dementia and amyloid neuropathy, but not yet in cardiac amyloidosis [21–24], while there presently are no data available for DMBT1 from any of these conditions.

In the present study, we therefore examined the expression of DMBT1 and complement activation products in cardiac amyloidosis with special emphasis on the localization and extent of expression in different types of amyloid. We found up-regulated DMBT1 expression and complement activation in the affected cardiac tissues.

2. Methods

2.1. Patients

The cardiac tissue of 43 patients with cardiac amyloidosis was analyzed in co-operation with the Institute of Pathology, University of Heidelberg, Germany. In 42 of 43 cases, cardiac biopsy specimens were examined, while from one case cardiac tissue sections after heart transplantation were available. Most patients of this study group were diagnosed with AL (light-chain amyloid) and TTR (transthyretin) amyloidosis (Table 1). Subclassification of amyloidosis was performed in an amyloidosis reference center using immunohistochemistry. Immunohistochemical analysis for DMBT1 expression was performed for all 43 specimens. From 31 cases sufficient material from the cardiac biopsies was available to also analyze the complement proteins C1q, C3d and C4d (18 of 24 patients with AL, 10 of 12 patients with TTR, 2 of 3 patients with AA (amyloid A) amyloidosis and 1 of 3 with amyloidosis of unknown origin). As controls we used cardiac tissue of 4 adults (61–79 years old) received from cardiac operations (biopsies and sections of removed myocardial tissue). These tissues were confirmed for absence of amyloidosis, and inflammatory cardiac diseases such as bacterial endocarditis. The study was approved by the responsible ethics committee.

2.2. Immunohistochemical analysis

The amyloid deposits were analyzed for apple-green birefringence under polarized light after staining with Congo red dye. DMBT1 expression was determined by immunohistochemistry. The paraffin-embedded sections were stained with an automated Ventana Discovery stainer (Ventana Medical Systems, Tucson, AZ, USA). The polyclonal anti-serum (anti-DMBT1p84) was generated against the recombinant DMBT1 in rabbit, and previously shown to specifically recognize the protein by immunohistochemistry and Western blotting [25]. The anti-DMBT1p84 antibody was diluted 1:100 in Discovery Ab Diluent and sections were incubated for 40 minutes. Afterwards, incubation for 30 minutes with a mouse anti-rabbit IgG-AP (diluted 1:500; Santa Cruz Biotechnology, Heidelberg, Germany) was performed. As negative controls, we used sections stained with equally diluted normal rabbit IgG (Santa Cruz Biotechnology, Heidelberg, Germany).

For determination of the complement proteins, we used an automated Horizon stainer (Dako, Hamburg, Germany) and the Dako REAL(TM) Detection System Peroxidase/AEC Kit (Dako, Hamburg, Germany). Before detecting C1q (antibody dilution 1:20000; Dako, Hamburg, Germany) and C3d (antibody dilution 1:500; Dako, Hamburg, Germany), we incubated the slides with pronase E for 5 minutes. For the detection of C4d by a corresponding antibody (1:50; Biozol, Eching, Germany), the tissue sections were pre-treated for 20 minutes with citrate buffer.

The mean DMBT1, complement protein and Congo red dye signal intensities of the heart tissue were determined on the basis of a scoring system: heart tissues displaying no signal were scored as 0, DMBT1-, complement protein- and amyloid-positive tissues were scored semi-quantitatively from 1 (weak staining) to 3 (highly intense staining). The mean score consists of the scores for signals in the endocardium, the myocytes, the interstitium (endomysial, perimysial, epimysial signal) and in the vessels.

2.3. Statistics

The comparison of the DMBT1 score and the Congo red score between the different groups of Table 2 were performed with the Mann–Whitney *U* test. To assess the correlation between the DMBT1 and the Congo red score we calculated the Pearson´s correlation coefficient. In order to compare the complement staining (C1q, C3d, C4d) between all amyloidosis patients and the controls and between the AL and the TTR cases we used the Cochran-Armitage Trend test (for ordinal scared data) and the Qui-Square-test (for binary data). Test results with $P<.05$ were regarded as statistically significant. All statistical calculations have been done with SAS system, release 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. DMBT1 is located adjacent to cardiac amyloidosis deposits

DMBT1 was previously shown to be up-regulated during inflammation in epithelial cells as well as during bacterial endocarditis [13,14,18,26]. We studied heart specimens from control tissues (without inflammation and amyloidosis, see methods section) and from 43 patients with cardiac amyloidosis by immunohistochemistry using a DMBT1-specific polyclonal antibody to initially analyze its prevalence in cardiac amyloidosis. In unaffected heart tissues of the

Table 1
Clinical parameters of the patients with different types of cardiac amyloidosis

Amyloid protein	N	Mean age (range) [y][a]	Gender (m/f)
AL	24	59 (39–68)	17/7
ATTR	12	50 (30–63)	7/5
AA	3	48 (41–59)	3/0
AApoAI	1	47	1/0
Amyloidosis of unknown origin	3	53 (45–65)	0/3

AA, amyloid A; AApoAI, apolipoprotein AI amyloid; AL, light-chain amyloid; ATTR, transthyretin amyloid; f, female; m, male; y, years.
[a] The mean age is referred to the time of cardiac biopsy.

Table 2
DMBT1 and amyloidosis signal (Congo red staining) in different types of cardiac amyloidosis

Amyloid protein	DMBT1 score					Amyloid score (Mean)[a]
	Endocard	Myocytes	Interstitium	Endothel	Mean[a]	
AL	2.5±0.2	2.3±0.1	2.0±0.2	2.5±0.2	2.4±0.1	2.5±0.1
ATTR	1.8±0.3	2.2±0.2	2.1±0.2	1.8±0.4	2.2±0.2	2.5±0.1
AA	2.3±0.5	2.2±0.3	2.3±0.5	2.5±0.3	2.3±0.5	2.0±0.5
AApoAI	3.0	3.0	3.0	No vessels	3.0	3.0
Unknown origin	2.3±0.7	2.2±0.5	1.8±0.5	1.7±0.9	2.2±0.6	2.2±0.6
Controls	0.2±0.1	0.2±0.1	0.3±0.2	0.1±0.1	0.2±0.1	0±0

Endocard, endocardium; endothel, endothelium.
[a] The determination of the mean score includes the values from all samples.