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ORIGINAL PAPER

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Immunohistochemical examination of Aκ amyloidosis with antibody against adjacent portion of the carboxy terminus of immunoglobulin kappa light chain

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Abstract For the purpose of investigating the carboxy terminus distribution of immunoglobulin κ light chain in Aκ amyloid deposits in tissue sections, we examined the immunostaining pattern of Aκ amyloidosis with conventional rabbit clonal antibody against peptide derived from the C-terminal sequence of human κ light chain. This anti-human kappa light chain clone II (clone H16-E) reacted with the adjacent region of the C terminus of the κ light chain constant region in SPOT analysis. Immunohistochemically, this antibody reacted with amyloid deposits in all 18 cases of Aκ amyloidosis. In 15 cases, this antibody reacted with amyloid deposits almost uniformly. In this study, we demonstrated for the first time that the peptides adjacent to the carboxy terminus of immunoglobulin κ light chain or full-length κ light chain were constituents of Aκ amyloidosis, and these molecules were distributed uniformly in almost all cases of Aκ amyloidosis in tissue sections.

Key words Amyloidosis · Immunoglobulin kappa light chain · Constant region · Carboxy terminus · immunohistochemistry

Introduction

Amyloidosis is a disease caused by the deposition of amyloid fibril proteins in various tissues and organs. To date, at least 27 types of amyloidosis have been identified and categorized according to amyloid fibril protein type.[1] Of the various forms of amyloidosis, immunoglobulin light chain amyloidosis (AL amyloidosis) is a representative systemic amyloidosis. In systemic AL amyloidosis, plasma cells proliferate clonally or myeloma cells produce amyloidogenic light chain. This precursor protein circulates in the bloodstream and deposits in blood vessels at various sites and in the intercellular space of various organs. AL amyloidosis is subclassified into Aλ amyloidosis or Aκ amyloidosis depending on the light chain deposited as amyloid.

It was previously thought that the variable region with part of the constant region of immunoglobulin light chain is amyloid fibril protein in many cases of AL amyloidosis, although full-length immunoglobulin light chain was a constituent of amyloid deposits in some AL amyloidosis.[2] However, Olsen et al. reported that full-length κ light chain was included in amyloid deposits in 18 of 19 cases of Aκ amyloidosis, as was the carboxy terminus region of κ light chain.[3] Three cases of Aκ amyloidosis mainly including the κ light chain constant region have also been reported.[4–6] Recently, Yamamoto et al. reported that the κ light chain constant region had amyloidogenicity in vitro.[7] It is possible that the κ light chain constant region is associated with the amyloidogenicity of Aκ amyloidosis; however, the distribution of full-length κ light chain or the carboxy terminus of κ light chain has not been elucidated. If the proteins are irregularly distributed in a patchy manner or in a small area, the κ light chain constant region may not contribute to amyloidogenesis. In contrast, if they are uniformly distributed in amyloid deposits in many cases, the κ light chain constant region may contribute to amyloidogenesis of Aκ amyloidosis.

In this study, we examined the immunostaining pattern of Aκ amyloidosis with conventional rabbit clonal antibody against a peptide derived from the carboxy-terminal sequence of human κ light chain to investigate the carboxy-terminus distribution of κ light chain in amyloid deposits in tissue sections.

Materials and methods

Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens of liver were obtained from 28 autopsy cases (18 Aκ type and 10 Aλ type) with systemic AL amyloidosis. All cases were

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confirmed to have amyloid deposition by Congo red staining and observation under polarized light. Immunotyping was performed in previous studies[8,9] or routinely before this study using anti-λ (118–134),[8] anti-κ (116–133),[8] anti-AA,[10] and anti-transthyretin (DAKO, Glostrup, Denmark) or anti-transthyretin (115–124), which was produced in our laboratory according to the method of Gustavsson et al.[11] Most cases were immunostained using anti-VλVI (1–19) and anti-VκI (1–19).[9]

Immunohistochemical staining was performed as follows. After deparaffinization, the sections were washed under running water for 5 min. Sections were then incubated with formic acid pretreatment[12] for antigen retrieval for 1 min and washed under running water for 10 min or more. After endogenous peroxidase blocking and inhibition of nonspecific binding of the antibody, the sections were incubated with anti-human kappa light chain clone II (clone H16-E, ready to use; DB BIOTECH, Kosice, Slovak Republic) as the primary antibody for 30 min, and then incubated with EnVision+ (1:2; DAKO, Carpinteria, CA, USA) as the secondary antibody for 30 min. The specimens were visualized with DAB+ (DAKO, Kyoto, Japan) for 5 min. Autostainer (DAKO, Denmark) was used for immunohistochemical staining. This study was reviewed and approved by the Institutional Review Board of Yamaguchi University Graduate School of Medicine.

Epitope analysis

Epitope analysis of anti-human kappa light chain clone II (clone H16-E) was performed with SPOT analysis as follows: 33 peptides that corresponded to various parts of the constant region of human immunoglobulin κ light chain were spotted onto cellulose membranes. From the 1st to the 32nd spot, each peptide contained 12 amino acids in which the last 8 peptides overlapped the first 8 amino acids of the subsequent peptide. The last 11 amino acids of the 32nd spot and first 11 amino acids of the last spot overlapped. The first peptide corresponded to the amino terminus of the κ constant region of human immunoglobulin light chain and the last peptide corresponded to the carboxy terminus of the κ constant region of human immunoglobulin light chain. Production of this SPOT membrane was contracted to Sigma Genosys (The Woodlands, TX, USA). Each amino acid sequence of SPOTs is shown in Table 1.

Immunochemical staining with anti-human kappa light chain clone II (clone H16-E) was performed as follows. The SPOT membrane was incubated in Blocking One (Nacalai Tesque, Kyoto, Japan) to inhibit nonspecific binding. The membrane was then incubated with anti-human kappa light chain clone II (clone H16-E, 1:2000) as the primary antibody for 1 h at room temperature, followed by peroxidase-conjugated goat anti-rabbit immunoglobulin (1:20,000; Bio-Rad Laboratories, Hercules, CA, USA) as the secondary antibody for 1 h at room temperature. Immunoreactivity was visualized with the Immun-Star HRP Chemiluminescence Kit (Bio-Rad Laboratories) and a cooled charge-coupled device (CCD) camera.

Table 1. Amino acid sequences of each spot

Spot no.	Amino acid sequences
1	TVAAPSVFIFPP
2	APSVFIFPPSDE
3	VFIFPPSDEQLK
4	FPPSDEQLKSGT
5	SDEQLKSGTASV
6	QLKSGTASVVCL
7	SGTASVVCLLNN
8	ASVVCLLNNFYP
9	VCLLNNFYPREA
10	LNNFYPREAKVQ
11	FYPREAKVQWKV
12	REAKVQWKVDNA
13	KVQWKVDNALQS
14	WKVDNALQSGNS
15	DNALQSGNSQES
16	LQSGNSQESVTE
17	GNSQESVTEQDS
18	QESVTEQDSKDS
19	VTEQDSKDSTYS
20	QDSKDSTYSLSS
21	KDSTYSLSSTLT
22	TYSLSSTLTLSK
23	LSSTLTLSKADY
24	TLTLSKADYEKH
25	LSKADYEKHKVY
26	ADYEKHKVYACE
27	EKHKVYACEVTH
28	KVYACEVTHQGL
29	ACEVTHQGLSSP
30	VTHQGLSSPVTK
31	QGLSSPVTKSFN
32	SSPVTKSFNRGE
33	SPVTKSFNRGEC

Results

Immunohistochemistry

Anti-human kappa light chain clone II (clone H16-E) reacted with amyloid deposits in all 18 cases of Aκ amyloidosis (11 cases of vascular type in which amyloid was deposited mainly in the arteries and veins but not Disse's space, and 7 cases of diffuse type in which amyloid was also deposited in Disse's space). In 15 cases (9 cases of vascular type and 6 cases of diffuse type), this antibody almost uniformly reacted with amyloid deposits (Figs. 1, 2). In the remaining 3 cases, the immunoreaction was heterogeneous. In 1 case of the vascular type, amyloid deposited in the artery showed a weaker reaction than amyloid deposited in the vein. In another case of the vascular type, amyloid deposits showed mainly weak reactions but some amyloid areas were intensely stained (Fig. 3). In 1 case of the diffuse type, amyloid deposited in the artery showed a weaker reaction than amyloid deposited in the vein and in Disse's space.

In Aλ amyloidosis cases that were used as a negative control, amyloid deposits in nine of ten cases (five cases of vascular type and five cases of diffuse type) show negative staining by anti-human kappa light chain clone II (clone H16-E). In only one case of the diffuse type were amyloid

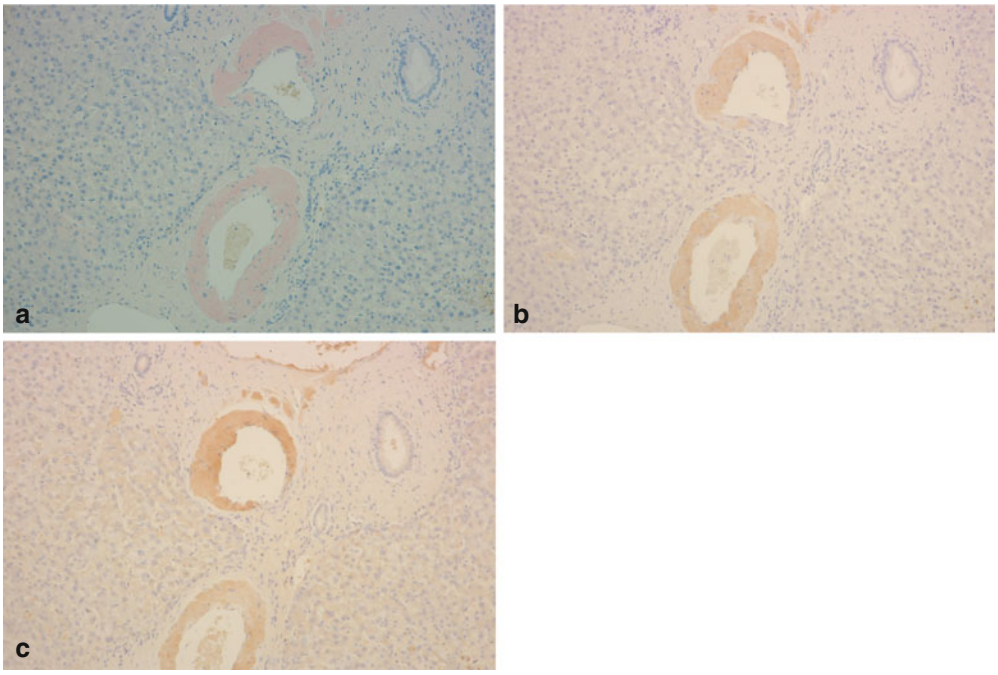

Fig. 1. Immunohistochemistry of liver with vascular-type deposition of Aκ amyloid. Amyloid deposits appeared in the vascular wall with Congo red staining (**a**). Anti-κ (116–133) (**b**) and anti-human kappa light chain clone II (clone H16) (**c**) reacted uniformly with amyloid deposits in the same manner

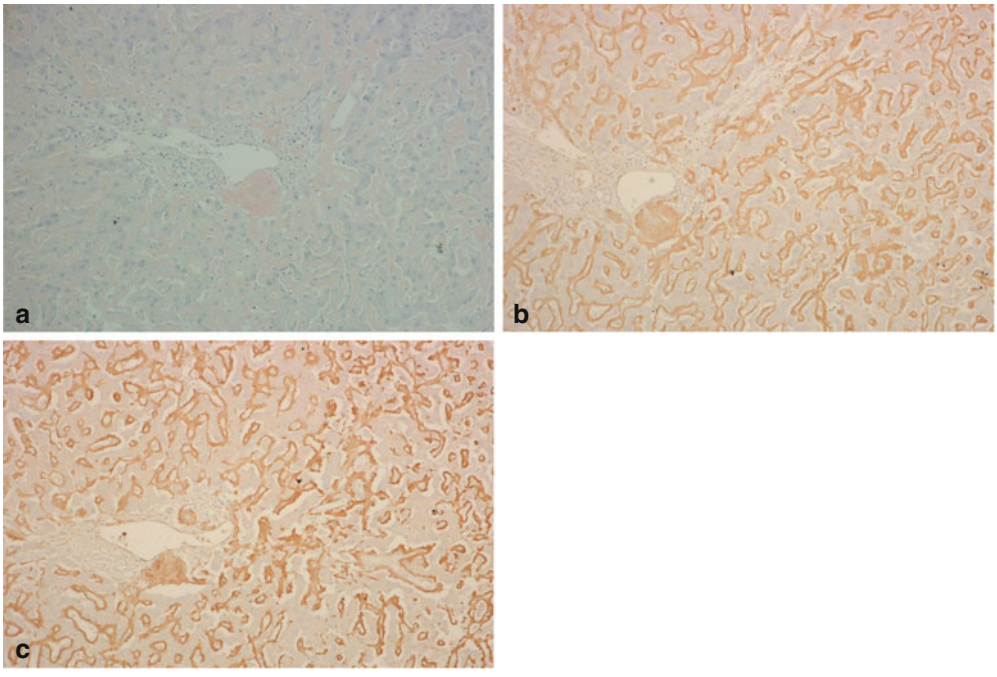

Fig. 2. Immunohistochemistry of liver with diffuse-type deposition of Aκ amyloid. Amyloid deposits appeared in Disse's spaces with Congo red staining (**a**). Anti-κ (116–133) (**b**) and anti-human kappa light chain clone II (clone H16) (**c**) reacted uniformly with amyloid deposits in the same manner

deposits faintly stained by this antibody, but the deposits were clearly more weakly stained than anti-λ (118–134). Anti-κ (116–133) amyloid deposit reacted faintly, the same as anti-human kappa light chain clone II (clone H16-E).

Epitope analysis

Anti-human kappa light chain clone II (clone H16-E) reacted strongly with the 30th spot and weakly with the 31st and 32nd spots (Fig. 4). This antibody did not react with any other spots. Therefore, it was confirmed that anti-human kappa light chain clone II (clone H16-E) reacted with only the adjacent portion of the C terminus of a constant region in immunoglobulin κ light chain.

Discussion

It was previously thought that the variable region with part of the constant region of immunoglobulin light chain was a constituent of amyloid deposits in many cases of AL amyloidosis[2]; however, we demonstrated on tissue sections for the first time that the adjacent portion of the carboxy terminus of κ light chain was included in amyloid deposits in

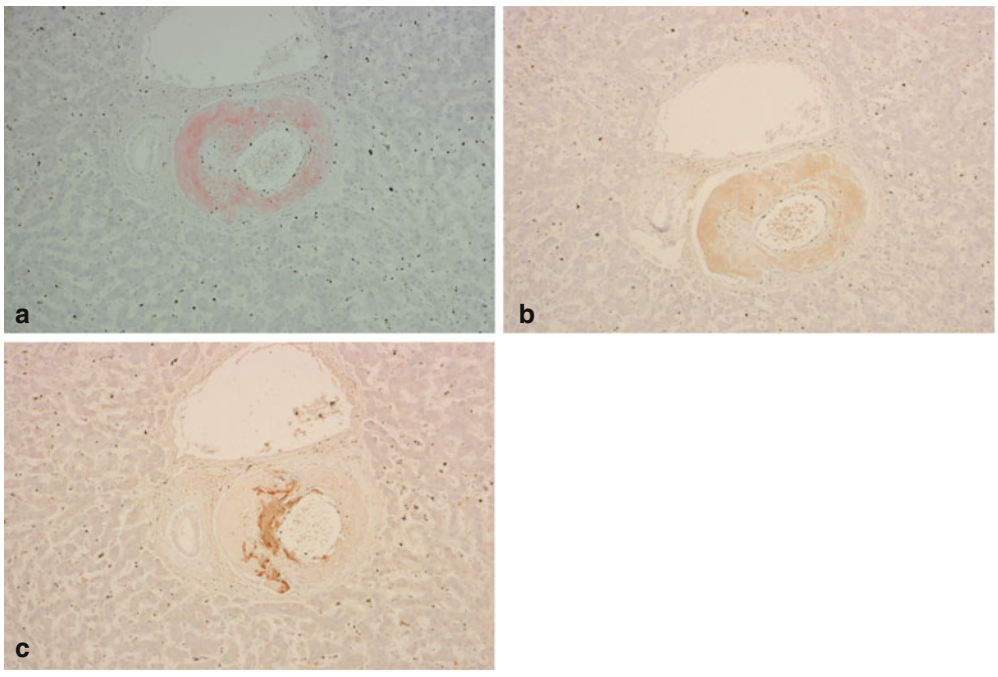

Fig. 3. Immunohistochemistry of liver with vascular-type deposition of Aκ amyloid (a different case from Fig. 1). Amyloid deposits appeared in the vascular wall with Congo red staining (**a**). Anti-κ (116–133) reacted uniformly with amyloid deposits (**b**). In contrast to anti-κ (116–133), anti-human kappa light chain clone II (clone H16) reacted intensely with part of the area of amyloid deposits (**c**)

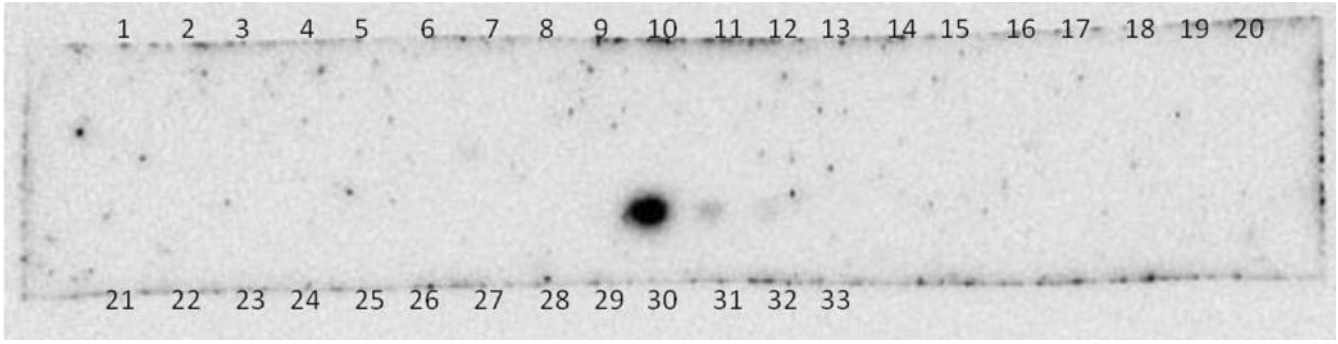

Fig. 4. Epitope analysis of H16-E with anti-human immunoglobulin κ light chain constant region by SPOTs. Anti-human kappa light chain clone II (clone H16-E) reacted strongly with the 30th spot and weakly with the 31st and 32nd spots.

all 18 cases of Aκ amyloidosis. This result supports the biochemical results found by Olsen et al.[3] Anti-human kappa light chain clone II (clone H16-E) is an antibody against the carboxy-terminus peptide of κ light chain. We did not perform epitope analysis of the κ light chain variable region, but amino acid sequences of the light chain variable region are markedly heterogeneous, so it was clear that this antibody reacted only with the adjacent portion of the carboxy terminus in κ light chain.

In Aλ amyloidosis, the monoclonal protein of VλVI subgroup light chain, which appears with an extremely low incidence under normal conditions, markedly tended to be deposited as amyloid,[13] but in Aκ amyloidosis, the prevalence of such as the VλVI subgroup in Aλ amyloidosis has not been known previously. To our knowledge, cases composed of mainly the constant region of immunoglobulin light chain involving Aλ amyloidosis or amyloidogenicity in the constant region of λ light chain have not been previously reported. Yamamoto et al. demonstrated that the immunoglobulin κ light chain constant region has amyloidogenicity, as did β2-microglobulin by two methods, namely, ultrasonication and agitation in vitro.[7] In Aκ amyloidosis, the amyloidogenicity of the immunoglobulin light chain constant region in addition to the amyloidogenicity of the variable region may be associated with amyloid deposition.

Another new finding in this study was that the carboxy-terminus region in Aκ amyloid deposits was almost uniformly distributed in many cases. Anti-human kappa light chain clone II (clone H16-E) reacted almost uniformly with amyloid deposits in 15 of 18 cases. Whether this finding shows the existence of the C terminus of κ light chain or full-length κ light chain remains unknown from this study. If our findings show the existence of short peptides derived from the carboxy terminus, a carboxy terminus that probably cleaved after amyloid deposition may exist in situ for a long time after proteolytic cleavage, whereas it has been demonstrated by Western blotting of AL amyloid that the full-length immunoglobulin light chain consists of AL amyloid deposits.[3,14] If our findings show the existence of full-length κ light chain, some full-length κ light chain that does not undergo proteolytic cleavage may exist almost uniformly in amyloid deposits for a long period.

Whether proteolytic cleavage of immunoglobulin light chain occurs and then is deposited as amyloid or whether full-length immunoglobulin light chain is deposited as amyloid and then undergoes proteolytic cleavage remains unclear. It is true that amyloid-like fibrils are formed from Bence Jones proteins by proteolysis in vitro[15,16]; however, Solomon et al. demonstrated that precursor protein formed amyloid fibrils only by incubation in the case of Aκ amyloidosis, which is composed of mainly the constant region of κ light chain.[5] They suspected that the immunoglobulin light chain variable region underwent proteolysis and disappeared after amyloid deposition in their case. Röcken et al. suggested post-fibrillogenic proteolysis of fibril protein with immunohistochemical examination.[17] Enqvist et al. also suggested that proteolytic cleavage occurs after fibril formation by comparison of the fragmentation pattern of Aκ amyloid fibrils obtained from several organs of six individuals with Western blotting.[14] It was also easy to identify that proteolytic cleavage occurred after amyloid deposition in our study.

Therapy for amyloidosis differs by the chemical type of amyloid protein, so it is important to determine the type of deposited amyloid. For amyloid A amyloidosis, transthyretin-derived amyloidosis, and β2-microglobulin-derived amyloidosis, there are good conventional antibodies, but for AL amyloidosis, conventional antibodies against immunoglobulin sometimes fail to determine the type of deposited amyloid, although anti-human kappa light chain clone II (clone H16-E) reacted almost entirely satisfactorily with Aκ amyloid deposits and hardly reacted with Aλ amyloid deposits. This antibody may be useful for immunohistochemical typing of Aκ amyloidosis.

Conclusion

We demonstrated for the first time that the adjacent portion of the carboxy terminus of κ light chain was a constituent of Aκ amyloidosis and was distributed uniformly in almost all cases of Aκ amyloidosis on tissue sections.

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