Functional consequences of amyloidosis mutation for gelsolin polypeptide – analysis of gelsolin-actin interaction and gelsolin processing in gelsolin knock-out fibroblasts

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Received 31 May 1999

Abstract Gelsolin, an actin-modulating protein, derived from a single gene exists in intracellular and secreted forms. A point mutation at position 187 of both forms of gelsolin causes familial amyloidosis of the Finnish type (FAF). Here, we expressed both isoforms of the wild-type and FAF mutant gelsolin in mouse embryonic gelsolin-null fibroblasts. We demonstrate that the FAF mutation does not interfere with the normal actin-modulating function of intracellular gelsolin, and that aberrant processing of secreted FAF gelsolin to FAF amyloid precursor takes place in the gelsolin-negative background. These results suggest that, in patients with FAF, symptoms are caused by the accumulation in their tissues of amyloid derived from plasma gelsolin and are not due to functional differences in cytoplasmic gelsolin.

Key words: Gelsolin; Amyloidosis; Actin

1. Introduction

Gelsolin is a widely expressed protein which exists in secreted and intracellular forms, both of which are encoded by a single gene on chromosome 9 [1–3]. Secreted gelsolin differs from intracellular gelsolin in having a signal peptide, a 25-residue amino-terminal extension and a disulphide bond between cysteines 188 and 201 [1,4]. The major function of intracellular gelsolin is participation in and regulation of actin filament dynamics for motility. In vitro, gelsolin severs isolated actin filaments, which results in their rapid shortening. After severing the filaments, gelsolin binds to their barbed ends, preventing their elongation. Gelsolin can also nucleate growth of actin filaments from monomers, the result being formation of short actin filaments. Binding of gelsolin to phosphoinositides (PPIs) removes gelsolin from the actin filament ends, providing a site for rapid addition of actin monomers [5–8]. Intracellular gelsolin participates in regulation of the architecture, motility and movement of the cells [9]. Secreted gelsolin has been thought to have a major function in scavenging actin and in clearance of F-actin from the bloodstream after tissue injury, but may also function as a lipid carrier [10–12]. Recently, it was reported that secretory gelsolin can bind to amyloid-β protein (Aβ) suggesting that it could have a role in the binding and sequestration of Aβ in the plasma and cerebrospinal fluid [13]. Gelsolin-null mice are viable, but have defects in platelet activation during haemostasis, the inflammatory response and the dermal fibroblast function in vivo, and serum from these mice is unable to sever actin filaments [14].

Amyloidoses are a heterogeneous group of disorders in which abnormal fibrillar protein deposits derived from different proteins accumulate in patients' tissues [15]. A mutation at position 187 of intracellular and secretory gelsolin (numbering based on secretory gelsolin) results in gelsolin-related amyloidosis or familial amyloidosis of the Finnish type (FAF) [16–18]. This mutation leads to accumulation of internal peptides of gelsolin (FAF amyloid) in the tissues [19,20]. FAF is an autosomal, dominantly inherited amyloid polyneuropathy, characterised by corneal lattice dystrophy, progressive cranial and peripheral neuropathy and skin changes. Most of the patients are heterozygous for the FAF mutation and the progression of the disease in them is slow over several decades while homozygosity is associated with early onset of the disease and more severe clinical findings [21–23]. Mutant secretory FAF gelsolin (Asn/Tyr_{187}) is cleaved in various cell types to an aberrant 68-kDa FAF amyloid precursor fragment consisting of amino acids 173–755 of gelsolin. This abnormal processing of mutant secretory gelsolin is probably due to lack of the disulphide bond normally formed next to the FAF mutation site between cysteine residues 188 and 201 of gelsolin. A second putative cleavage of mutant secretory gelsolin at amino acid residue 244 is required to generate the FAF amyloid protein (amino acid residues 173–244 of gelsolin) accumulating in patients' tissues [19,20,24,25]. In contrast, intracellular gelsolin bearing the FAF mutation is not aberrantly cleaved in COS-1 cells, which suggests that the secretory form of gelsolin is the source of amyloid in FAF [26].

Although, according to transient expression analyses, processing of intracellular mutant FAF gelsolin appears to be normal, the consequences of the FAF mutation for the functioning of gelsolin are controversial. Dysfunction of the mutant FAF gelsolin might have pathological consequences via disturbed interactions with actin, the major intracellular protein of the body. In one report, the serum of a patient homozygous for the FAF mutation appeared to have reduced functional activity of gelsolin [27]. Here, we use viral vectors to express the two isoforms of the wild-type and FAF mutant gelsolin in embryonic fibroblasts derived from gelsolin knock-out mice [14]. These cells provided us with an ideal environ-

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0014-5793/99/$20.00
PII: S0014-5793(99)00790-5

ment for studying the actin-modulating function of mutant FAF gelsolin in vivo and for characterising the processing of the mutant protein without the interference of endogenous gelsolin.

2. Materials and methods

2.1. Cell lines and cultures

GSN−/− embryonic mouse fibroblasts (GSN$^-$EF) were derived from embryos of gelsolin knock-out mice [14]. GSN$^-$EF cells were cultured on dishes coated with 50 μg/ml (Sigma) poly-D-lysine and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% foetal bovine serum (FBS) (Hyclone). Embryonic AGA−/− mouse fibroblasts (AGA$^-$EF) were derived from E14 embryos of mice with homozygous targeted disruption of the aspartylglucosaminidase gene [28]. AGA$^-$ EF cells were cultured on dishes coated with 0.1% gelatin and maintained in DMEM supplemented with 15% FBS. Primary human fibroblasts, NIH 3T3, CREBAG2 and COS-1 cells were cultured in DMEM supplemented with 10% FBS. φ CRIP cells were grown in DMEM with 10% newborn calf serum [29]. All media were supplemented with 2 mM L-glutamine and 100 IU/ml penicillin and 50 mg/ml streptomycin. HPV16 E6/E7 replication-defective retrovirus and the packaging cell line for immortalisation of mouse embryonic fibroblasts were kindly provided by Dr Denise A. Galloway and Dr Anu Wartiovaara.

2.2. Construction of recombinant retroviruses

cDNAs encoding both secretory and intracellular wild-type (G654) and FAF mutant (G654A) gelsolin were obtained from pCD-X vector constructs [24,26] and subcloned into the *Bam*HI site of the Moloney murine leukaemia virus-based retrovirus vector pM48, containing the phosphoglycerate kinase promoter (PGK-1) [30] (Fig. 1). The correct orientations of the gelsolin cDNAs were confirmed by DNA sequencing. The gelsolin pM48 constructs were cotransfected into the φ CRIP amphotropic packaging cells with pSV2neo vector by lipofectin transfection [31] and the transfected cells were selected with 500 μg/ml of G418 (Geneticin). Resistant colonies were isolated and clones producing recombinant retroviruses were preliminarily identified with PCR, using primers located in the vector PGK-1 promoter (5′-GAC CAC TGA TAT CCT GTC TTT AAC-3′) and in the gelsolin cDNA (5′-GAG GCA GAG AAG ACG GGG GC-3′) and Western analysis, using the monoclonal anti-gelsolin antibody (Sigma) (see Fig. 1). The best virus-producing clones were further selected by infecting NIH3T3 or COS-1 cells grown on 6-well plates with 600 μl of the virus supernatant in the presence of 8 μg/ml polybrene (hexadimethrine bromide, Sigma) followed by Western analysis of culture supernatants with monoclonal anti-gelsolin antibody. The best producer clones (R-iGSNwt, R-iGSNAsn187, R-sGSNwt, R-sGSNAsn187) were expanded and the virus supernatants were filtered through a 0.44-μm pore size filter. The absence of the replication-competent retrovirus was confirmed by a virus mobilisation assay in CREBAG2 cells, as described [32]. As a control for retroviral infections, we used the AGA retrovirus, which was a generous gift from Dr Minna Laine (unpublished data).

2.3. Gelsolin gene transfer

Supernatants from the best producer clones (R-iGSNwt, R-iGSNAsn187, R-sGSNwt, R-sGSNAsn187) were used to infect GSN$^-$EF and AGA$^-$EF cells. Semi-confluent cells were infected 10 times at half-day or daily intervals with 3 ml of the virus supernatant and 1.5 ml of cell growth medium in the presence of 8 μg/ml polybrene per 10-cm culture dish. The transduced cells were maintained in growth medium for several passages. For Western analysis, cells were incubated overnight in serum-free medium and the supernatant was analysed by SDS-PAGE and blotted. Supernatants were concentrated with Centricon-3 (Amersham). The cells were detached by trypsinisation, and pelleted and lysed in Laemmli sample buffer. To inhibit proteases, EDTA (1.2 mg/ml) and PMSF (1 mM) were added to the supernatants and cell lysates.

The construction of the recombinant wild-type (G654) and FAF mutant (G654A) adenoviruses (Ad-sGSNwt, Ad-sGSNAsn187) has been described earlier [25]. COS-1 cells were infected with these recombinant adenoviruses for 1 h in serum-free medium as described [25].

2.4. Immunological techniques

Monoclonal anti-gelsolin (GS-2C4) (Sigma), monoclonal anti-α-actin (Amersham) and Oregon green 488 phalloidin (Molecular Probes) were used at the dilutions provided by the manufacturer. Monoclonal anti-gelsolin antibody recognises a 47-kDa peptide derived from a chymotryptic cleavage of gelsolin extending to the carboxy-terminus of the protein [33]. Polyclonal COOH961 antibody is targeted against the carboxy-terminal portion of gelsolin, amino acid residues 420–755. Polyclonal AM904 antibody (Anti-am) is targeted against the amyloid-forming region of the gelsolin, amino acids 173–244, and polyclonal NH951 antibody is targeted against the amino-terminal portion of the gelsolin amino acids 1–172 [24–26] (Fig. 1).

Western blotting was performed as described [24]. When monoclonal anti-gelsolin antibody was used, 1% goat serum was added to the secondary antibody.

For immunofluorescence analysis, cells grown on poly-D-lysine-coated coverslips were fixed with 4% paraformaldehyde for 30 min, washed three times with PBS and permeabilised for 15 min in blocking buffer (0.2% saponin and 0.5% BSA in PBS). This was followed by incubation for 1 h at room temperature in the blocking buffer containing COOH961 antibody in 1:1000 dilution. After rinsing with blocking buffer, the cells were incubated in the buffer with rhodamine-conjugated goat anti-rabbit IgG (Immunotech) in the presence of 10% goat serum for 1 h. To visualise the actin filaments, the cells were stained with 5 μl Oregon green 488 phalloidin in the blocking buffer, mounted in GelMount (Biomeda) and viewed with a Leica DMR confocal microscope, using a 63× objective.

To characterise the actin filaments, three different categories of actin filament were visually estimated: (1) cells containing few if any actin filaments, (2) cells containing weak filaments, (3) cells containing strong prominent actin filaments (see Fig. 2). To analyse the actin filament staining in the different cell lines, at least 150 non-transduced or transduced GSN$^-$EF cells expressing intracellular and secretory wild-type and FAF mutant gelsolin were counted by immunofluorescence microscopy.

3. Results

3.1. The cellular distribution of gelsolin in GSN$^-$ mouse fibroblasts

In order to study the cellular distribution of gelsolin, embryonic mouse fibroblasts derived from gelsolin-null mice (GSN$^-$EF) were infected with recombinant retroviruses encoding either the wild-type or the FAF mutant forms of intracellular and secretory gelsolin (R-iGSNwt, R-iGSNAsn187, R-sGSNwt, R-sGSNAsn187) (see Fig. 1) [14]. As a consequence of a retroviral gene transfer, the recombinant gene integrates into the genome of the infected cells. As shown in Fig. 3, the GSN$^-$EF cells, when transduced with either wild-type or FAF mutant intracellular or secretory gelsolin, expressed gelsolin polypeptides equally.

Immunofluorescence microscopy with a polyclonal antibody (COOH961) recognising the carboxy-terminal part of gelsolin was used to analyse the distribution of wild-type and FAF mutant gelsolin polypeptides in the transduced cells. Intracellular wild-type gelsolin was present primarily in a perinuclear distribution and was seen throughout the cytoplasm more faintly. A similar staining pattern was observed in cells expressing the intracellular FAF mutant gelsolin, showing that the distribution of the wild-type and FAF mutant intracellular gelsolin in these cells were identical (Fig. 2A,C). The majority of the transduced cells showed gelsolin-positive immunofluorescence staining, whereas in the non-transduced cells there was a faint non-specific background staining (Fig. 2I).

In the GSN$^-$EF cells expressing the secretory form of wild-type gelsolin, gelsolin staining was mainly perinuclear. The gelsolin immunostaining of FAF mutant secretory gelsolin-

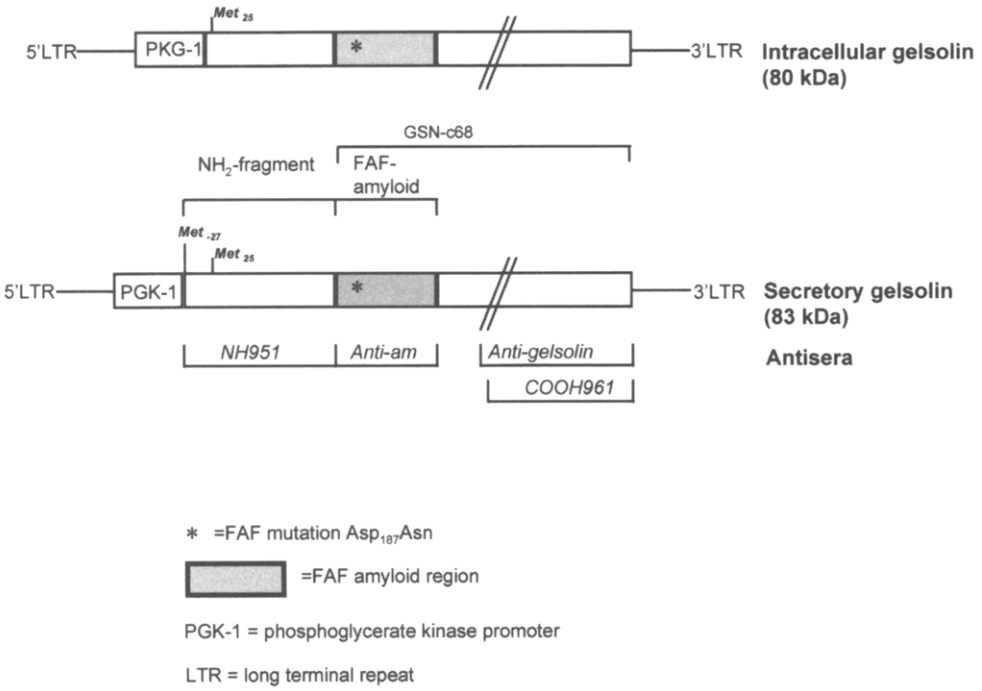

Fig. 1. Schematic representation of the secretory and intracellular gelsolin polypeptides and the target regions of gelsolin-specific antibodies used in Western blot analyses. The intracellular and secretory wild-type and FAF mutant gelsolin cDNAs were inserted into a Moloney murine leukaemia virus-based retrovirus vector pM48 under the control of the phosphoglycerate kinase promoter [30]. The translation initiation methionines for intracellular (Met_{25}) and secretory (Met_{-27}) gelsolin are shown [45]. NH_2 fragment: the 25-kDa amino-terminal cleavage fragment of secretory FAF gelsolin found in cell cultures [25]; FAF amyloid: the 8–12-kDa FAF amyloid fragment found in tissues of patients [19,20]; GSN-c68: the 68-kDa carboxy-terminal fragment of secretory FAF gelsolin, also called the FAF amyloid precursor [24]. The gelsolin fragments used for raising of antisera are also shown.

transduced cells was identical to that of wild-type cells (Fig. 2E,G).

3.2. The effect of FAF mutation on the actin network

Gelsolin is a multifunctional actin-modulating protein which has an important role inside the cell. Here we analysed whether the FAF mutation could disturb the normal actin-modulating function of gelsolin. The GSN$^-$EF cells were infected with recombinant retroviruses encoding either the wild-type or FAF mutant forms of intracellular and secretory gelsolin. As a control we used embryonic fibroblasts derived from AGA knock-out mice (AGA$^-$EF), which show endogenous expression of gelsolin [28]. The actin network of the non-transduced and transduced cells was monitored by staining with fluorescein-conjugated Oregon green 488 phalloidin.

In the control AGA$^-$EF cells transduced with a recombinant retrovirus encoding the AGA cDNA, the actin fibres were similar in the non-transduced and transduced cells (data not shown). In the non-transduced GSN$^-$EF cells the majority of the actin fibres were organised as characteristic stress fibres while a minority (about 15%) of the cells contained very few, if any organised actin filaments (Fig. 2J,K). In contrast, in GSN$^-$EF cells transduced with the wild-type intracellular gelsolin cDNA, about 70% of the cells revealed weaker staining of actin fibres or only a few organised actin filaments (Fig. 2B). In the GSN$^-$EF cells transduced with the FAF mutant intracellular gelsolin, the actin fibre staining was similar to that in the wild-type gelsolin-transduced cells (Fig. 2D). The actin monomer staining of non-transduced and transduced cells was similar (data not shown). Further, in 50% of the secretory gelsolin-transduced cells the actin fibres were less organised or weaker than in the non-transduced GSN$^-$EF cells (Fig. 2F,H,J,K). This was probably due to the synthesis of intracellular gelsolin polypeptides from the mRNA for secretory gelsolin by a leaky scanning mechanism of translation initiation [34] (see Fig. 1). This was confirmed by in vitro transcription-translation of cDNA for secretory gelsolin, where, in addition to the full-length 83-kDa secretory gelsolin, a faint signal of 80 kDa corresponding to the size of the intracellular form of gelsolin was detected (data not shown). Thus, introduction of either recombinant wild-type or FAF mutant gelsolin in GSN$^-$EF cells seemed to lead in both cases to partial disruption of the organised actin fibres. Since the actin fibre staining of GSN$^-$EF cells expressing either the wild-type or FAF mutant gelsolin were similar, these results would suggest that the actin-modulating functions of the wild-type and the FAF mutant gelsolin are similar. Further, it shows that the FAF mutation does not disturb the actin-gelsolin interaction in these cells.

3.3. The processing of mutant FAF gelsolin in GSN$^-$ embryonic mouse fibroblasts

In order to analyse whether the cells lacking gelsolin are capable of processing mutant FAF gelsolin to FAF amyloid precursor, the retrovirally transduced GSN$^-$EF cells and control AGA$^-$EF cells and their corresponding media were analysed by Western blotting. The gelsolin polypeptide staining pattern of the cell extracts of transduced GSN$^-$EF cells expressing intracellular wild-type and FAF mutant gelsolin were identical. The 80-kDa band corresponding to the intracellular full-length gelsolin was detected with monoclonal anti-gelsolin

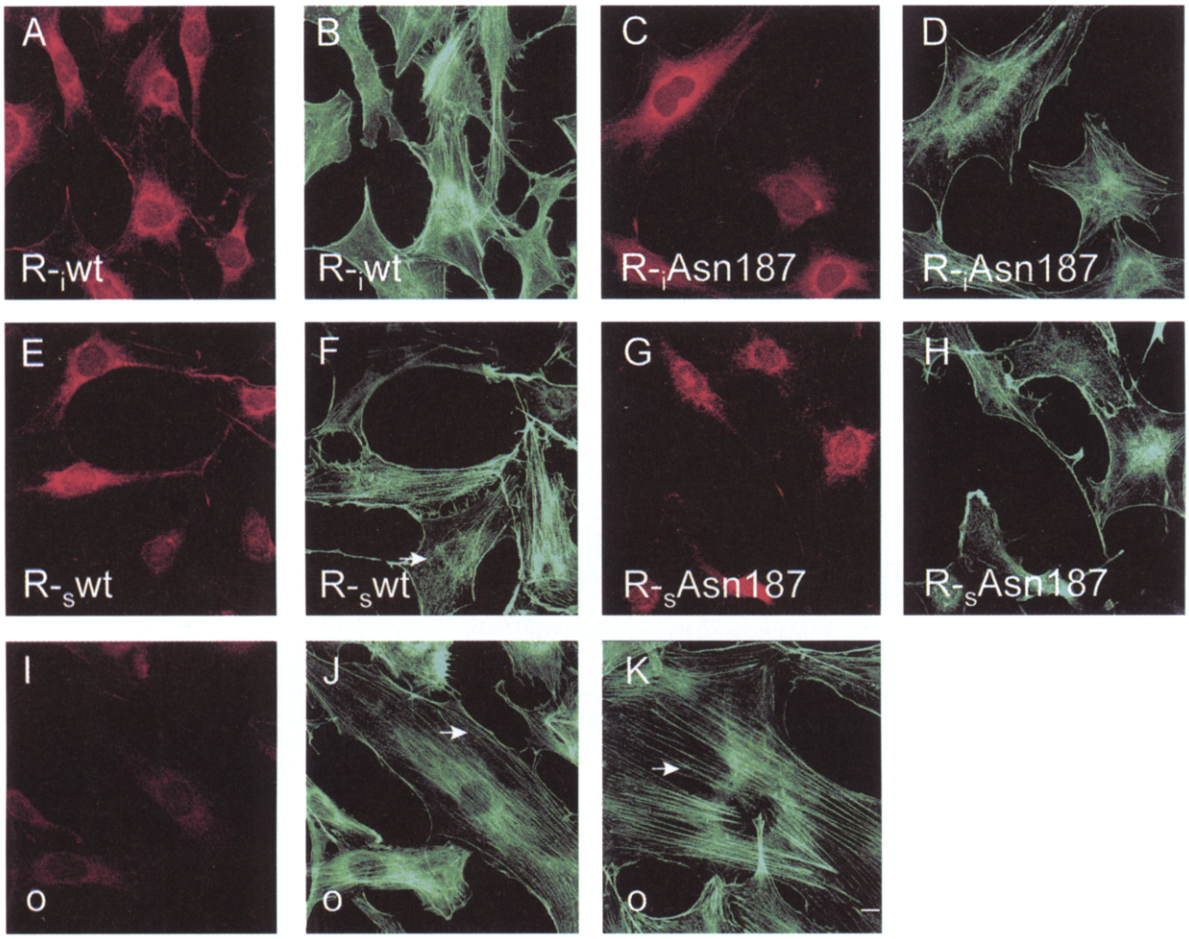

Fig. 2. Confocal images showing the immunofluorescence staining of intracellular and secretory gelsolin and actin filaments in GSN$^-$EF cells. GSN$^-$EF cells transduced with retroviral constructs encoding the intracellular wild-type (R-$_i$wt) and FAF mutant (R-$_i$Asn187) gelsolin and secretory wild-type (R-$_s$wt) and FAF mutant (R-$_s$Asn187) gelsolin were double-stained with gelsolin COOH961 antibody (red, A, C, E, G, I) and Oregon green 488 phalloidin (green, B, D, F, H, J, K). Arrow in F: cell containing few if any actin filaments; arrow in J: cell containing weak actin filaments; arrow in K: cell containing strong and prominent actin filaments. Bar = 10 μm.

antibody which recognises the carboxy-terminal part of gelsolin (see Fig. 1 for description of the antibodies and Fig. 3A, right and middle). Neither polypeptide bands corresponding to the size of the FAF amyloid precursor fragment (GSN-c68) (Fig. 3A, middle) nor FAF amyloid were detected in the immunoblotting analyses (data not shown). In addition, we did not observe any leakage of intracellular gelsolin into the medium (data not shown). Thus, in the GSN$^-$EF cells, the processing of the intracellular wild-type and FAF mutant gelsolin were identical.

In contrast to the findings with the intracellular form of gelsolin, the wild-type and FAF mutant forms of secretory gelsolin in the transduced GSN$^-$EF cells were processed differently. The wild-type gelsolin was secreted in the full-length form (83 kDa), whereas the media of the GSN$^-$EF cells expressing FAF gelsolin contained, in addition to the full-length gelsolin, the FAF amyloid precursor fragment (GSN-c68) and the 25-kDa amino-terminal cleavage fragment of mutant gelsolin (Fig. 3B,C). GSN-c68 was also detected in the cell extracts of the GSN$^-$EF cells expressing secretory FAF mutant gelsolin (Fig. 3A, left). The size of the GSN-c68 fragment was confirmed by SDS-PAGE to be the same in the retroviral and the adenoviral expression systems that we used in our earlier experiments [25] (Fig. 3B). The 83-kDa secreted form of gelsolin was not detected in the cell extracts, possibly because of efficient secretion, whereas the 80-kDa intracellular gelsolin was occasionally detected from both the wild-type (data not shown) and the FAF mutant secretory gelsolin-expressing GSN$^-$EF cells (Fig. 3A, left). In the transduced AGA$^-$EF cells expressing intracellular and secretory FAF gelsolin, the processing of FAF gelsolin was similar to that of the GSN$^-$EF cells. In particular, both the GSN-c68 and the 25-kDa amino-terminal fragments of gelsolin were found in the medium of the secretory FAF mutant gelsolin-transduced cells (data not shown). The proportion of GSN-c68 was unexpectedly high in the embryonic mouse fibroblasts (45%). We earlier analysed the processing of the secreted FAF mutant gelsolin in human fibroblasts and showed that FAF gelsolin is not cleaved in these cells [25]. To confirm that this earlier result was not due to the adenoviral expression system used, human fibroblasts were infected with retroviral constructs encoding the wild-type and FAF mutant secretory gelsolin. However, even in the retrovirally transduced human fibroblasts the mutant FAF gelsolin was not cleaved (data not shown). As the GSN$^-$EF cells lack gelsolin, these results indicate that the aberrant processing of mutant secretory FAF gelsolin to FAF amyloid precursor does not require the presence of the wild-type gelsolin.

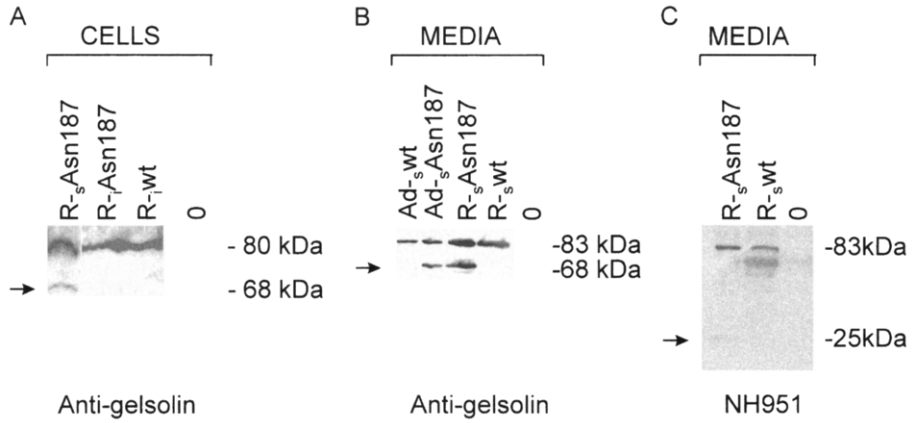

Fig. 3. Processing of intracellular and secretory FAF gelsolin in GSN$^-$EF cells. GSN$^-$EF cells transduced with retroviral constructs encoding for intracellular wild-type (R-$_i$wt) and FAF mutant (R-$_i$Asn187) and secretory wild-type (R-$_s$wt) and FAF mutant (R-$_s$Asn187) gelsolin, and also the respective culture media, were subjected to Western blot analysis and immunostaining with anti-gelsolin (A,B) and NH951 (C) antibodies. A: The 80-kDa band corresponding to the intracellular gelsolin was found in the cell extracts of both R-$_i$wt- and R-$_i$Asn187-infected GSN$^-$EF cells. Left: GSN-c68 polypeptide was found only in the cell extracts of the R-$_s$Asn187-infected GSN$^-$EF cells. B: The 83-kDa band corresponding to the full-length secretory gelsolin was found in the medium of GSN$^-$EF cells expressing the wild-type and FAF mutant secretory gelsolin, the GSN-c68 polypeptide only in the culture medium of cells expressing the FAF mutant secretory gelsolin. As a control, COS-1 cells infected with recombinant adenoviruses encoding for the wild-type (Ad-$_s$wt) and FAF mutant (Ad-$_s$Asn187) secretory gelsolin are shown. GSN-c68 is indicated by an arrow. C: The cleavage of FAF secretory gelsolin results in the formation, in addition to the GSN-c68, of a 25-kDa amino-terminal polypeptide (indicated by an arrow), which was found in the medium of R-$_s$Asn187-transduced GSN$^-$EF cells.

4. Discussion

All eukaryotic species contain actin, which may make up as much as 20% of total cellular protein of cells. Actin has vital roles, for example in maintaining cell structure and motility, including the neuronal growth cone motility. Gelsolin has an important role in actin dynamics; it is capable of severing actin filaments in the presence of calcium and it can nucleate the actin filament assembly and block the fast-growing (barbed) ends of actin filaments [5–7]. Structural analysis of gelsolin has shown that the FAF mutation is located near the F-actin binding and severing domain of gelsolin, on gelsolin segment 2 [35,36]. Our data on the proteolytic processing of mutant FAF gelsolin indicated that intracellular gelsolin is not involved in the actual formation of tissue amyloid in FAF [26]. Here, to investigate the possibility that intracellular FAF gelsolin might interfere in actin function, we analysed the actin filaments in transduced GSN$^-$EF cells expressing either wild-type or FAF mutant gelsolin.

Analysis by confocal microscopy indicated that, in the embryonic gelsolin knock-out fibroblasts (GSN$^-$EF), wild-type and FAF mutant intracellular gelsolin were similarly distributed throughout the cytoplasm and around the nucleus. In both wild-type and mutant gelsolin-expressing cells, the secretory form was located near the nucleus. In the majority of the non-transduced GSN$^-$EF cells, actin-specific staining revealed a prominent filament network. In contrast, in the transduced GSN$^-$EF cells expressing either the wild-type or the FAF mutant intracellular gelsolin, a distinct majority of the cells gave only weak signals from actin filaments or had no filaments at all. Since the actin filament organisation was similar in the transduced GSN$^-$EF cells, expressing both the wild-type and FAF mutant gelsolin, our results appear to indicate that the FAF mutation per se does not disturb the actin-modulating function of gelsolin. Studies with gelsolin knock-out mice and cell cultures derived from these mice have shown defects in neutrophil, fibroblast, platelet and hippocampal neuronal structure and function, but no major defects in embryonic development or organ function [14,37–41]. The existence of several members of the gelsolin family and other proteins with partial functional similarity to gelsolin suggests that these other proteins would provide functional compensation for lack of gelsolin expression or impaired function of gelsolin. Yet, in spite of the other activities compensating for the putative malfunction of the mutant FAF gelsolin, patients' symptoms would be expected to be more severe if the function of the mutant FAF gelsolin were impaired. Together, these data suggest that the symptoms of patients with FAF result from accumulation of the abnormally cleaved gelsolin maturing to amyloid in the patients' tissues and not from the disturbed function of the mutant FAF gelsolin.

Our result showing that the actin-modulating function of mutant FAF gelsolin is not impaired seems to be at variance with the results of Weeds et al. [27], who have shown that the FAF mutant plasma gelsolin has defective actin-severing and -nucleating activity in vitro. It is possible, however, that the FAF mutant intracellular and secretory gelsolins have different actin-modulating functions. We postulated earlier that the disulphide bond normally formed next to the FAF mutation site in the wild-type gelsolin between cysteines 188 and 201 on gelsolin segment 2 is unlikely to be present in the secretory mutant FAF gelsolin [25]. The lack of the disulphide bond would result in abnormal folding of the mutant FAF gelsolin and could influence its normal actin-nucleating and -severing function in plasma. However, one cannot exclude the possibility that the defective actin-modulating function of plasma gelsolin reported by Weeds et al. [27] was due to abnormal fragmentation of the mutant gelsolin in samples they analysed. However, we did not measure the actual in vitro actin-severing capacity of either intracellular or the secreted mutant FAF gelsolin. After tissue injury, actin may be released into the bloodstream and interact with components of the haemostatic and fibrinolytic systems by binding for example to the fibrin clot. Binding of plasma gelsolin to actin

can reduce the incorporation of actin into the clot and thus aid in the restoration of the homeostatic mechanisms that regulate clot formation and dissolution [42]. The fact that patients with FAF have no coagulation defects [22] might rather indicate that the function of the secretory FAF gelsolin is unchanged.

Amyloidoses are a group of diseases in which normally soluble and functional human proteins are converted into insoluble amyloid fibrils that accumulate in patients' tissues. To date, at least 18 different amyloid proteins have been found in humans [43]. The exact mechanism by which amyloid proteins are converted into amyloid fibrils is unknown. Proteolytic processing of amyloid precursors seems to be a critical pathogenic step in many forms of amyloidoses, since the various amyloid proteins are peptides produced by proteolysis from larger precursor molecules. We show here that the intracellular mutant FAF gelsolin was not aberrantly cleaved in GSN$^-$EF cells. This accords with our earlier results for COS-1 cells, suggesting that the intracellular form of mutant FAF gelsolin is not the source of amyloid in FAF [26]. In contrast, the FAF mutant secretory gelsolin is processed to FAF amyloid precursor in different cell types [24,25]. In the GSN$^-$EF cells, mutant secretory FAF gelsolin was also aberrantly cleaved to the FAF amyloid precursor (GSN-c68), which was found in both the culture medium and the cell extracts of GSN$^-$EF cells. This is the first time that GSN-c68 has been found in the cell extracts of non-neuronal cells. In PC12 cells and in vitro differentiated human neuronal progenitor cells, in contrast to all other types of cells studied so far, more than half of the mutant FAF gelsolin is cleaved to the FAF amyloid precursor [25]. The present finding that almost half of the FAF gelsolin is also cleaved to FAF amyloid precursor in GSN$^-$EF cells suggests that a high level or activity of the gelsolin-cleaving enzyme is also present in these multipotent embryonic fibroblasts. These clearly differ from adult human fibroblasts, indicating the presence of the cleaving enzyme in embryonic, but not in adult, mesenchymal cells. Our finding that proteolytic processing of mutant secretory FAF gelsolin does not require the presence of wild-type gelsolin is consistent with the fact that, although almost all of the 400 FAF patients are heterozygous for the disease mutation, two homozygous patients with FAF have been reported [22,23].

In some cases of amyloid diseases, amyloid formation may start from the endogenously expressed precursor protein in the presence of triggering molecules such as the amyloid enhancing factor in AA amyloidosis and of prions in prion diseases [44]. A mechanism of this kind does not seem likely in FAF, where all the available in vitro and in vivo data suggest that the mutation at amino acid residue 187 is required and sufficient for abnormal initial folding of the FAF gelsolin and for subsequent aberrant proteolytic processing of mutant gelsolin to the FAF amyloid.

Acknowledgements: We thank Anne Vikman for excellent practical assistance, Kirsi Riento for help and useful discussions, Minna Laine for useful discussions and for providing the AGA retrovirus and Denise A. Galloway and Anu Wartiovaara for providing the HPV16 E6/E7 replication-defective retrovirus. This study was supported by the Emil Aaltonen Foundation, the Instrumentarium Foundation and the Kymenlaakso Regional Fund of the Finnish Cultural Foundation.

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