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Review

Factor XIII subunit A as an intracellular transglutaminase

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Abstract. Over the last 2 decades there has been increasing evidence that the role of factor XIII (FXIII) is not restricted to the area of hemostasis and that its subunit A functions as an intracellular enzyme in platelets and monocytes/macrophages. FXIII is already expressed during compartmentalisation of the precursors of megakaryocyte/platelet and monocyte/macrophage cell lines in the bone marrow. FXIII-A, produced by megakaryocytes, is packaged into budding platelets and is present in huge quantity in circulating ones. It seems very likely that it plays an important role in the cytoskeletal remodelling associated with the activation stages of platelets. FXIII-A can also be detected in blood monocytes and in all subsets of monocyte-derived macrophages throughout the body. FXIII-A is mainly localised in the cytoplasm, in association with cytoskeletal filaments, but at a relatively early stage of macrophage differentiation it also appears transiently in the nucleus. Cytoplasmic expression has a very close relationship with phagocytic activities. Further research is needed to understand the biological significance of its nuclear presentation.

Key words. Factor XIII; megakaryocytes/platelets; monocytes/macrophages; phagocytosis; nuclear presentation; chromatin remodelling; immunohistochemistry.

Introduction

The relatively long (almost 80 years) history of research on factor XIII (FXIII) can be divided into several phases, reflecting increasing understanding of its diverse functions. But two major lines of research can be identified clearly: From the beginning, research sought to characterise the role of FXIII in hemostasis, not only in the process of fibrin stabilisation (which is considered to be the main function of FXIII) but also in fibrinolytic and platelet-activation processes. Excellent overviews of the hemostasis-related functions of FXIII have been published elsewhere [1–3]. Subsequent research has suggested strongly that the role of FXIII is not restricted to hemostasis but that it also plays a role in certain cellular processes; thus the intracellular presentation, distribution and other possible functions of FXIII have also been studied extensively. Obviously, these two roles are strongly associated, but the second one unambiguously opened new lines of inquiry about the biological functions of FXIII, introducing this 'blood coagulation factor' into the field of cell biology. The aim of this review is to summarise the evidence on the role of FXIII as a cellular enzyme which has implications far beyond blood coagulation.

The structure of FXIII

Electron microscopy reveals that circulating FXIII has a heterotetrameric structure, which consists of two globular A subunits and two strandlike B subunits [4]. The primary structure of both subunits has been determined by amino acid sequencing analysis and complementary DNA (cDNA) cloning [5–7], while the three-dimen-

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sional structure of FXIII was determined by X-ray crystallographs [8]. The primary structure of the A subunit of ~83-kDa molecular mass is identical with that of the cellular FXIII-A [9]. The heterodimer circulates in the blood as a proenzyme of the blood-clotting factor, which is activated by thrombin in the presence of Ca^{2+} in the final step of blood coagulation. In this activation step B subunits and activation peptides consisting of 37 amino acid residues dissociate from the N-terminal end of A subunits and the active site of the enzyme becomes available for cross-linking reactions (reviewed in [1, 2]).

Genomic structure of FXIII

The genes for A and B subunits of FXIII are localised on chromosomes 6 at bands p24–25 [10] and 1 at bands q32–32.1 [11], respectively. The gene for FXIII-A spans more than 200 kDa and exists in different allelic forms [12], which results in a high number of gene isoforms. Although the biological effects of different amino acid substitutions in FXIII-A have been studied widely, evidence of the consequences has been inconsistent [13]. The expression of the FXIII A gene seems to be regulated by a myeloid-enriched transcription factor (MZF-1-like protein) and two ubiquitous transcription factors, NF-1 and SP-1 [14].

FXIII-A is a member of the transglutaminase enzyme family

Enzymes which catalyse the formation of ε-(γ-glutamyl)lysyl cross-links between different substrate proteins form the transglutaminase (TG) enzyme family [15, 16]. Although they exist in a wide variety in different organisms, from bacteria to humans [13], and their functions differ widely from one another, they show significant genomic, structural and functional homology [17, 18]. At least eight different forms of TGs exist in humans. The gene loci are dispersed on different chromosomes [3, 6, 14], but their organisational structure, exon/intron composition and boundaries are very similar [10, 19– 22].

TGs show a cell-type-specific expression which is probably based on the heterogeneity of their nucleotide sequences for transcription factors in the 5′-flanking region of the gene and on the diversity of mechanisms for their gene regulation [14]. The type of TG is characteristic of the cell type in which it is expressed as a result of different intricate gene expression regulation programs.

Members of the transglutaminase enzyme family differ from each other not only in their genomic/structural/antigenic characteristics, but also in their functional roles. Although some overlaps among spectra of substrates of different TGs exist, different substrate-panels (and consequently different functional roles) of the TG variants can be linked. Tissue TGs have been implicated in a variety of cellular processes including apoptosis [23], signal transduction [24], activation of certain cytokines [25], extracellular matrix organisation and remodelling [26]. Presently, more than a dozen protein substrates for FXIII-A are known. These are (i) proteins of the coagulation and fibrinolytic systems, (ii) adhesive proteins and (iii) cytoskeletal proteins. These three categories of substrates correspond well with the three major functional roles of FXIII-A, namely (i) its involvement in blood coagulation and fibrinolysis, (ii) wound healing and (iii) certain, still poorly known, cellular functions.

Expression of FXIII-A in human cells

Hepatocytes

Up to the early 1980s FXIII-A was viewed only as the subunit responsible for the catalytic function of the blood-clotting factor. It was generally assumed that the synthesis of FXIII-A – similarly to that of the B subunit [27, 28] – occurs in hepatocytes. This was supported by clinical reports demonstrating decreased FXIII levels in patients with liver diseases [29–31], and increased amounts in pathological conditions associated with enhanced hepatic protein synthesis [32, 33], as well as in one immunohistological study, although this was improperly controlled [27]. While growing volumes of supportive data were published concerning the hepatic production of subunit B [34, 35], the number of papers disputing the liver origin of FXIII-A also increased rapidly. Several clinical studies found no correlation between liver function and FXIII-A level [36, 37], and immunohistochemical investigations performed under rigorously controlled conditions found no FXIII-A in hepatocytes, using light microscopy [38]. We thus concluded that FXIII-A protein and its encoding messenger RNA (mRNA) could be detected in hepatocytes only in trace amounts, using highly sensitive assays (39).

We can now unambiguously assert that the contribution of FXIII-A synthesised by the liver to plasma FXIII-A levels is insignificant. This finding is in harmony with clinical observations showing that after bone marrow transplantation, the FXIII-A phenotype changed to the donor's type, but after orthotopic liver transplantation it remained mainly of the recipient's type [35]. These observations are the best evidence to date that FXIII-A is synthesised in the bone marrow.

Megakaryocytes and platelets

As well as hepatocytes, megakaryocytes and platelets were identified as a source of FXIII-A. Thirty years ago

Kiesselbach and Wagner [40] clearly showed the presence of FXIII-A in megakaryocytes by immunofluorescent reaction. In our double-labelling immunofluorescent studies in which the reaction for FXIII-A was combined with the detection of platelet factor 4, a marker for megakaryocytes and platelets, we could show that FXIII-A is present not only in megakaryocytes but also in their early bone marrow precursors cells [41]. The fact that FXIII-A appears in immature bone marrow precursor cells, as early as these small mononuclear cellular elements expressing markers for the megakariocytic cell line, strongly suggests that its presence is a result of direct synthesis. By using a mixture of biotinylated oligonucleotides specific for FXIII-A, we could clearly demonstrate FXIII-A synthesis in these cells by mRNA in situ hybridisation reaction [39]. Reaction for FXIII-A mRNA could be detected in a megakaryocyte platelet cell line all the way from early bone marrow precursors via megakaryocytes to circulating platelets [42]. It should be noted that both the protein product FXIII-A and its encoding mRNA are packaged into platelets. Circulatory platelets also show very intense immunofluorescence reaction for FXIII-A and in situ hybridization reaction for FXIII-A mRNA. On the basis of these findings we can conclude that megakaryocytes synthesise a large amount of FXIII-A and package it into platelets. Although mRNA encoding FXIII-A is also available for translation in platelets, there is no evidence in the literature that would suggest that they actually synthesise it.

Monocytes and macrophages

In 1985, we and another group independently reported the presence of FXIII-A in human blood monocytes [43, 44]. These reports not only were the first to show the presence of FXIII-A, they also demonstrated that it is the only transglutaminase variant in these cells. Given that monocytes represent an intermediate cell population in the maturation sequence of the monocyte/macrophage cell line, studies to detect FXIII were extended to bone marrow monocytopoetic cells and macrophages differentiated from monocytes. By using double and triple immunofluorescence staining reactions it could be verified that FXIII-A is present in early monocytic precursor cells in the bone marrow. However, similar to small mononuclear cells expressing the marker antigen for megakaryocytes, a cell population with the same morphological appearance, but negative for megakariocytic markers was also positive for FXIII-A. This cell population was identified as LeuM3 positive, that is monoblastic, elements of the bone marrow [41]. Studies designed to answer the question of whether the FXIII-A content of monocytes is retained during their differentiation into macrophages showed that cells containing FXIII-A are present throughout the body.

FXIII-A can be detected in high levels in different subsets of mobile and fixed macrophages derived from monocytes. This was first shown in peritoneal macrophages [45], and later in macrophages in other serous cavities [46], as well as in alveolar macrophages [47]. Tissue macrophages such as histiocytic and dendritic reticulum cells of lymph nodes [48, 49], connective tissue histiocytes [50–52], perivascular dendritic macrophages [53, 54], as well as tumour-associated macrophages [55, 56] also show a very intense reaction for FXIII-A.

When we studied the ontogeny of cells producing FXIII-A in humans [57], we could clearly demonstrate that FXIII-A first appears in tissue histiocytes in the mesenchyme, well before the development of bone marrow (as early as the fifth gestational week in the yolk sac, and not much later in the embryonic connective tissue).

By using in situ hybridisation technique mRNA encoding, FXIII-A could be detected in circulating blood monocytes [42], as well as in certain types of tissue macrophages [39], clearly demonstrating that the monocyte/macrophage cell line is able to synthesise it.

Intracellular distribution of FXIII-A

Megakaryocytes

The changes in distribution of FXIII-A during maturation of megakaryocytes/platelets show a particularly interesting pattern. In early mononuclear precursor cells and in promegakaryocytes with indented or bilobulated nuclei of the bone marrow, immunostaining for FXIII-A shows a diffuse cytoplasmic distribution. In mature megakaryocytes the reaction in the peripheral part of the cytoplasm is most intense, and the packaging of FXIII-A into budding platelets is clearly visible even by light microscopy. At a later stage of megakaryocyte maturation, after their 'active age', the staining reaction for FXIII-A is very weak [42].

Platelets

FXIII-A is present in platelets in huge quantities, predominantly in the cytoplasm [58, 59]. Sixma et al. [60] could detect FXIII-A through the entire cytoplasm by immunoelectronmicroscopy, but both the α-granules and the surface-connected canalicular system were negative. The FXIII-A content of platelets from patients with grey platelet syndrome – characterised by the absence of α-granules – was unchanged [61]. FXIII (in A_2B_2 complex) can be detected in α-granules only after incubation of platelets from patients with FXIII-A deficiency in normal plasma, due to uptake by endocytosis [62]. Although it would be very important to know how platelet FXIII-A redistributes during platelet activation, very little information exists. FXIII-A was detected on the surface of ac-

tivated but not resting platelets of patients with peripheral vascular disease, but the authors interpreted this phenomenon as the binding of plasma FXIII-A [63]. The question of whether surface-associated FXIII-A of activated platelets derives from their cytoplasmic pool or from α-granules in which they previously packed a certain amount of plasma FXIII, or whether the platelets bind it from plasma via their specific receptors is unanswered despite many studies specifically to resolve this issue (reviewed by [2, 64]).

Monocytes and macrophages

In bone marrow precursor cells FXIII-A can be detected, exhibiting diffuse cytoplasmic localisation and the intensity of staining increases as the cells mature [41]. Labelling for FXIII-A is rather diffuse in the cytoplasm of circulatory blood monocytes [42], but in macrophages – especially in macrophages of different serous cavities in which the structural distribution could be much better seen than in tissue macrophages embedded into connective tissue matrix – a delicate distribution pattern can be observed. FXIII-A is present throughout the cytoplasm, but it accumulates around certain cytoplasmic vacuoles and in pseudopods of these cells [45, 46].

Using electron microscopy and immunogold labelling, colloidal gold particles indicating specific binding of anti-FXIII-A antibodies were seen in the cytoplasm of Kupffer cells and in tissue histiocytes of the human liver in association with the outer surface of the nuclear membrane [39]. The antiserum used in this study was highly specific but not very sensitive, which limited its use for ultrastructural studies. Thus, we developed a specific antibody against recombinant human FXIII-A that is suitable for immunoelectronmicroscopic investigations because it is reactive on cell and tissue preparations fixed with highly cross-linking glutaraldehyde fixative, guaranteeing the best preservation of the cellular ultrastructure [65]. We used these specific antibodies, produced in rabbits, against FXIII-A derivatized by glutaraldehyde on human monocytes and macrophages obtained from long-term cell cultures. We employed light-, laser scanning, confocal-, and electron microscopical immunohistochemical investigations. The long-term culture of human blood monocytes can be considered as a model system to study changes – among them changes in the localisation and distribution of FXIII-A – during monocyte/macrophage differentiation.

Diffuse staining for FXIII-A could be seen by light microscopy in the cytoplasm both in monocytes and macrophages; however, the intensity of staining was much stronger in macrophages. On the 2nd day of culture (which represents a relatively early stage of macrophage differentiation) accumulation of FXIII-A in the nuclei of cells could be observed as a transient phenomenon, i.e. it was not observed on any other day (fig. 1). The nuclear localisation of FXIII-A was confirmed by optical sectioning of cells labelled for FXIII-A and nuclear DNA (fig. 2), as well as by Western immunoblotting reaction carried out on isolated nuclei of 2nd-day macrophages. As monodansyl-cadaverine incorporation assay revealed, FXIII-A not only is present in the nuclei but has transglutaminase activity.

By postembedding immunogold labelling, nuclear localisation of FXIII-A in macrophages at the early stage in the maturation sequence was further established when gold particles indicating FXIII-A were observed associated with the electrodense areas of the nuclei. In addition, labelling for FXIII-A could clearly be seen in connection with the fibrillar cytoskeletal structures in cells representing different stages of monocyte and macrophage differentiation.

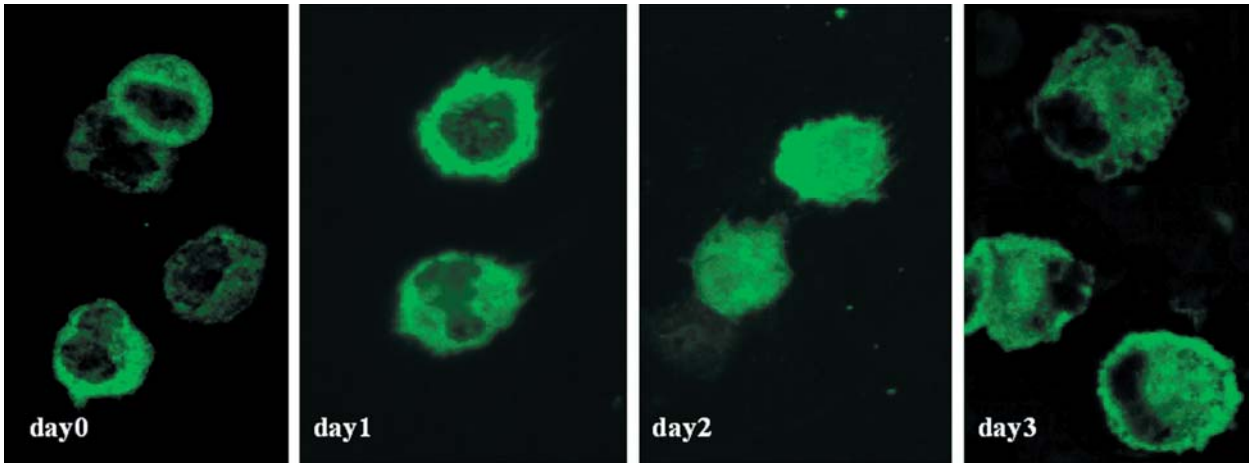

Figure 1. Immunohistochemical detection of FXIII-A in monocytes/macrophages on 0, 1st, 2nd and 3rd days of long-term culture, respectively. The transient appearance of FXIII-A in nuclei at the 2nd day of culturing can be seen.

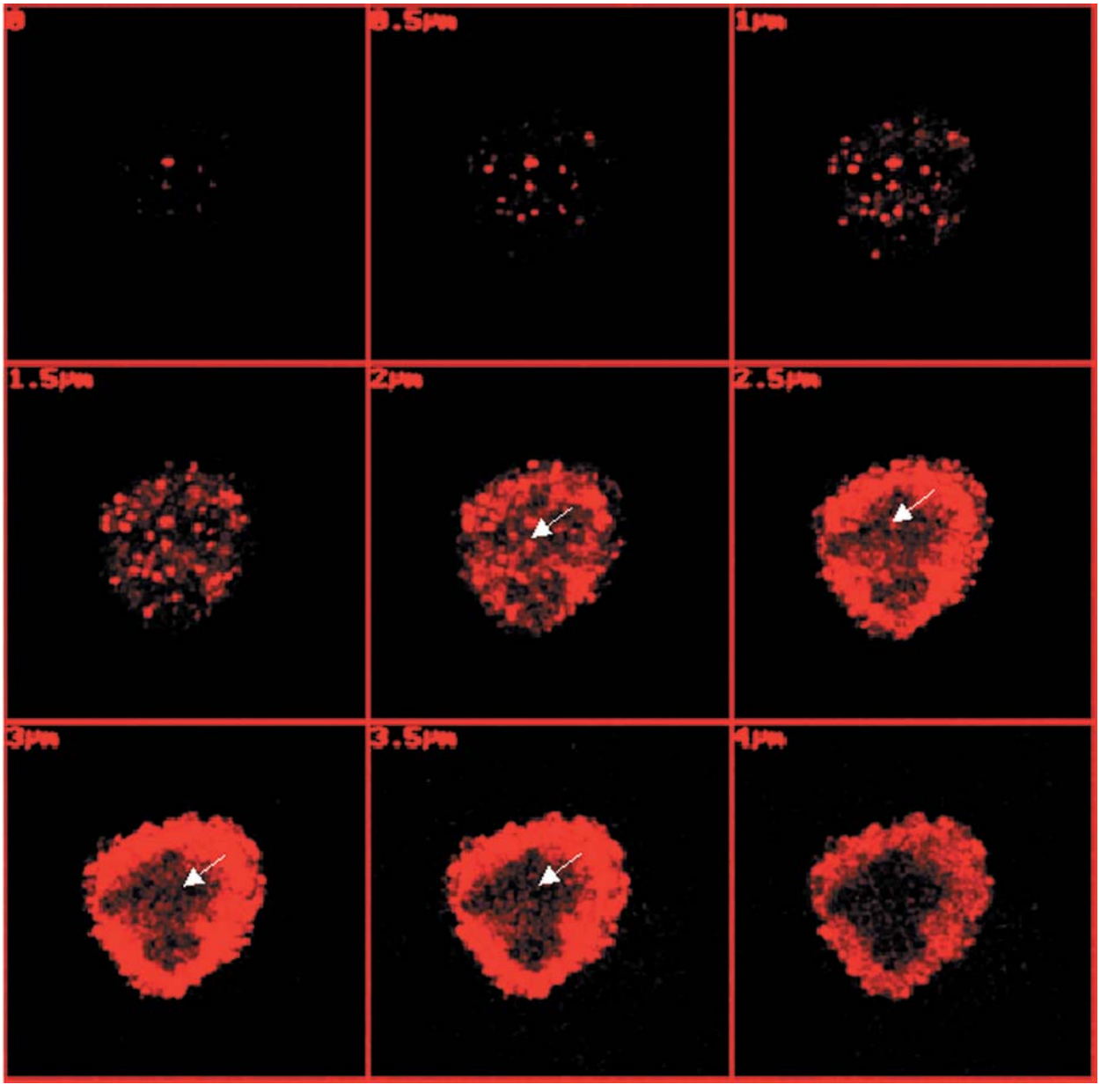

Figure 2. Confocal laser sectioning of a macrophage labelled for FXIII-A from the early stage of differentiation. The intranuclear labelling for FXIII-A is evident.

Possible functions of cellular FXIII-A

The role of intracellular FXIII-A, both in platelets and monocytes and macrophages, is an open and widely discussed question. Although clear-cut proof is still missing, it seems very likely that cellular FXIII-A functions both intra- and extracellularly. FXIII-A has no signal sequence for secretion, and when expressed in baby hamster kidney cells, it is not secreted into the culture medium through the classical secretory pathway [66]. U937 cells, a human promonocytic tumour cell line that shares antigenic and functional phenotypic features with normal macrophages, do not secrete FXIII-A either resting or when stimulated [47]. Thus, it is reasonable to suppose that FXIII-A can be released only from damaged monocytes and macrophages or involved in certain extracellular cross-linking reactions after its expression on the cell surface. As we already mentioned concerning platelets, the origin of surface-associated FXIII-A has not yet been explained.

Regarding monocytes and macrophages, U937 cells and human alveolar macrophages were shown to cross-link fibrin in FXIII-A-deficient plasma in the presence of thrombin and Ca^{2+}, and the surface expression of FXIII-

A was demonstrated after stimulation by fluorescence-activated flow cytometry [47]. The surface expression was interpreted by the authors as a result of translocation of FXIII-A from the cytoplasm to the cell surface. Most recently Akimov and Belkin [67] demonstrated that human peripheral blood monocytes, as well as a human monocytic leukemia cell line THP-1, can express FXIII-A on their surface. The researchers' inference that the surface expression of FXIII-A is decreased (and, in general, the FXIII-A expression is downregulated) during monocyte differentiation, may be an error because it was drawn from results obtained only on cultured, malignantly transformed THP-1 cells after stimulation with 12-*O*-tetradecanoyl-phorbol-B-acetate (TPA). It is known that TPA stimulation results in an approximate 50-fold upregulation of another transglutaminase (tTG) in the cell line THP-1 [68], but its effect on the expression of the FXIII-A gene is not known. In our fluorescence image analysis studies based on single cell fluorescence intensity (FI) measurement in human monocytes and macrophages cultured during 6 days without stimulation and labelled for FXIII-A, we found that on the first day of culture, cells with relatively low FIs dominated; later, the proportion of cells with higher FIs rapidly increased, reaching a plateau on the 3rd day (fig. 3). The distribution of cellular FI values remained almost the same within 4–6 days of culture.

Activation of cellular FXIII-A

If cellular FXIII-A appears extracellularly, regardless of the mechanisms (due to surface expression, release from damaged cells, secretion by a still unknown way), the classical method of FXIII-A activation (the thrombin and Ca^{2+}-dependent proteolytic pathway, reviewed in [3] may be initiated. As thrombin is unable to penetrate cell membranes, the activation of intracellular FXIII-A both in platelets and monocytes/macrophages must occur in a different way, in which a nonproteolytic cleavage of FXIII-A leads to the expression of transglutaminase activity. The accumulation of $\varepsilon(\gamma$-glutamyl)lysyl cross-links and covalently cross-linked protein polymers in platelets during their activation [69–71] clearly demonstrates the intracellular activation of FXIII-A. Platelet FXIII-A may be activated without the release of activation peptide in stimulated platelets [71], which strongly suggests that the intracellular activation of FXIII-A is a result of the critical elevation of the local Ca^{2+} concentration at certain intracellular sites. When the Ca^{2+} concentration exceeds 50 mmol/l, tetrameric plasma FXIII dissociates, and the A_2 dimer becomes transformed into active transglutaminase [72]. Nonproteolytic activation of FXIII-A of placental macrophage origin was also demonstrated [73]. A slow, progressive activation of the enzyme may also occur at physiological ionic strength as the active-site thiol is exposed. Thus, it is reasonable to suppose that FXIII-A can be activated by this nonproteolytic mechanism in the monocyte/macrophage cell line as well.

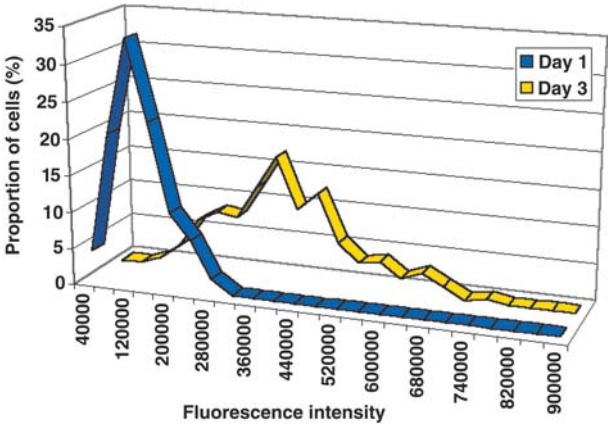

Figure 3. Distribution of monocyte and macrophage cell populations on the basis of the results obtained by single-cell fluorescence intensity measurement on the 1 and 3 days of a long-term culture. FXIII-A was detected by an indirect immunofluorescence reaction on cytospin preparations, and the relative amount of FXIII-A was determined by image analysis and expressed as fluorescence intensity in pixels at the single-cell level.

Possible intracellular functions of FXIII-A

Involvement in cytoskeletal organisation and remodelling

It has been established that in addition to proteins of the coagulation and fibrinolytic systems (reviewed in [2]) certain cytoskeletal proteins also serve as substrates for FXIII-A. Platelet and skeletal muscle actin and myosin – the major elements of the contractile locomotory system of cells – were first identified [74, 75] as substrates of FXIII-A. As a result of FXIII-A action highly cross-linked myosin and actin polymers are formed which represent the structural basis of the organisation of the cytoskeletal network. In linking the cytoskeleton to the cellular membrane, another substrate for FXIII-A, vinculin [76, 77], plays a significant role and becomes incorporated into the cytoskeleton in activated cells and platelets. The specific binding of FXIII-A to the small heat-shock protein HSP27 that plays such an important role in actin dynamics and polymerisation [78, 79] was also demonstrated [80].

Phosphorylated HSP27 associates very rapidly and almost completely with actin in activated platelets [81] during the pseudopodial extension in the phase of 'shape change'.

Devine and Bishop [82] and Polanowska-Grabowska and Gear [83] suppose that platelet FXIII-A is regulated by HSP27, and their interaction is a crucial event in platelet

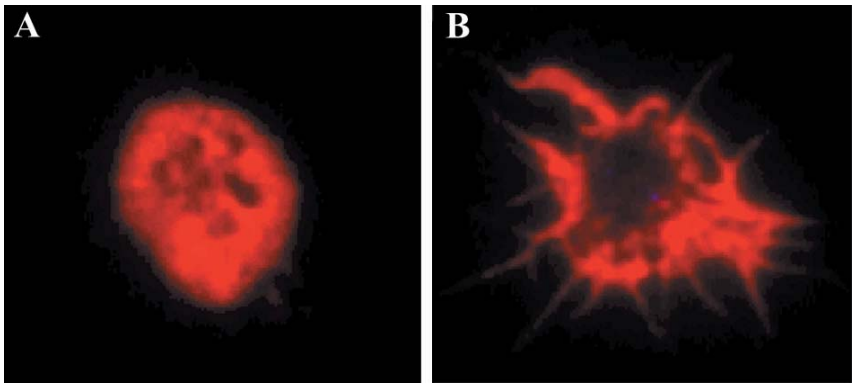

Figure 4. Immunofluorescence reaction for FXIII-A in resting (*A*) and activated (*B*) platelets.

aggregation and thrombus contraction. The overlapping immunofluorescence reactions for FXIII-A and HSP27 in platelets adhered to glass surface but not in resting ones [80] support the above hypothesis; but further investigations are needed to define the significance of the FXIII-A-HSP27 interaction.

Most recently, it was demonstrated by Huff et al. [84], that thymosin β_4, which can form a 1 : 1 complex with G-actin and thereby inhibit its salt-induced polymerisation to F-actin [85], is also a substrate for FXIII-A.

These findings strongly suggest that FXIII-A has a significant role not only in catalysing fibrin formation but also in regulating the dramatic cytoskeletal remodelling during platelet activation, aggregation and thrombus contraction (fig. 4), in which the cytoskeletal FXIII-A-substrate proteins discussed above (actin, vinculin, HSP27 and thymosin β_4) are involved.

Phagocytosis is the most characteristic function of monocytes and macrophages in which the cytoskeletal system is deeply involved. Monocytes of patients with FXIII-A deficiency showed impaired capacity to phagocytose sensitised red blood cells and complement-coated and uncoated yeast particles, but displayed no alteration in their adhesion and migration [86]. Parallel increases in phagocytic activity and FXIII-A expression were detected in long-term cultures of monocytes and macrophages, but both Fcγ and complement receptor-mediated phagocytosis were significantly decreased in the presence of monodansylcadavarine (table 1), an inhibitor of FXIII-A-induced cross-linking [unpublished observations]. In accordance with these observations, the phagocytic ability and FXIII-A-producing activity of a FXIII-A-negative, nonphagocytic myelomonocytic cell line (DD) can be restored in parallel by TPA treatment [87]. These findings strongly suggest that FXIII-A plays a role in receptor-mediated phagocytosis, but further studies are required to explore the details of its involvement in cytoskeletal reorganisation associated with different steps of the phagocytic process.

Involvement in chromatin structure remodelling

Since only a single report has been published on the nuclear expression of FXIII-A [65], we can only speculate about its possible role in certain nuclear cross-linking processes.

As it is well known, FXIII-A belongs to the family of transglutaminases, but can be clearly distinguished from other members of the family on the basis of its antigenic and functional characteristics. Only two, very convincing reports about the nuclear presentation of tissue transglutaminase have so far been published [88, 89], with certain nuclear proteins identified as substrates for this enzyme. Lesort et al. [89] used subcellular fractionation of human neuroblastoma SH-SY5Y cells to demonstrate that 93%

Table 1. Phagocytosis of monocytes/macrophages in long-term culture with and without mono-dansylcadaverine.

Type of phagocytosis	Days of culturing			
	1	2	3	3 with MDC
Fcγ receptor mediated	0.64 ± 0.06	0.76 ± 0.15	1.58 ± 0.16	0.44 ± 0.13
Complement receptor mediated	0.98 ± 0.13	1.24 ± 0.26	1.78 ± 0.19	0.89 ± 0.24

Values represent phagocytic indices (PIs) expressed as the average number of particles phagocytosed via Fcγ and complement receptors/cell ± SD.

of tissue transglutaminase is localised to the cytosol and 7% is found in the nucleus. More than 80% of the nuclear tTG was copurified with the chromatin-associated proteins, and the remaining 1% was in the nuclear matrix fraction. Upon treatment with maitotoxin, which can significantly increase the intracellular Ca^{2+} concentration, the intensity of immunostaining for tTG and transglutaminase activity increased markedly, which may indicate the translocation of tTG into the nucleus in response to the elevation of intracellular calcium concentration. This hypothesis is supported by the results of Peng et al. [90], who demonstrated using a yeast two-hybrid assay and coimmunoprecipitation that tTG interacts with the nuclear transport protein importin-α3. The authors showed that in a non-small-cell lung cancer (NSCLC) cell line, NCI-H596 cells stimulated with all-trans retinoic acid (RA) – which upregulates tTG and induce apoptosis in these cells – not only were the cytosolic and nuclear tTG expressions markedly increased, but a statistically significant increase was also observed in tTG/importin-α3 coimmunoprecipitation, which indicates active nuclear transport of tTG into the nucleus in RA-challenged NSCLC cells.

The nuclear translocation of tTG was also demonstrated on human melanocytic A375-S2 cells treated with sphingosine, a cell-signalling mediator [91]. The authors report that DNA hydrolytic activity of tTG is dependent on Mg^{2+} concentration. Regarding the possible role of nuclear FXIII-A, it is interesting to see what kind of nuclear proteins can serve as transglutaminase substrates. H2A, H2B and H3 core histones from chicken erythrocytes can be amine acceptors in tTG-catalysed reactions [92]. Shimizu et al. [93] also reported that as a result of an acyl transfer reaction catalysed by tTG between the Gln9 of H2B and Lys5 of H4, a unique nuclear protein, p28 is formed in the sperm of the starfish, *Asterina pectinifera*.

Kim et al. [94] showed in a preliminary report that in aged rats and senescent primary fibroblasts, the target of tTGs is core histones; H2A : H2B and A3 : H4 are specifically cross-linked by tTG. They postulated that changes of DNA metabolism in association with cellular aging may be ascribed primarily to the cross-linking of core histone subunits. Histone H1, containing about 27% lysine, is also an excellent substrate of tTG [95].

Turning to the question of its pathological significance, it has been hypothesised that tTG may contribute to the formation of huntingtin aggregates and in this way to that of neuronal intranuclear inclusions characteristic of Huntington's disease, but the findings are rather contradictory [96–98].

According to Han et al. [99] nuclear tTG can cross-link and regulate the activity of a glutamine-rich transcription factor, Sp1.

These findings strongly suggest that FXIII-A as a monocyte/macrophage – and megakaryocyte/platelet – specific cellular and, under certain conditions, nuclear transglutaminase may be involved actively in certain cell differentiation processes. Theories exist about the possible role (i) in the activation of the nuclear isoform of phospholipase C, C-δ, which can act as a signal transducing G protein [100] and (ii) in the modification of histones, which may result in chromatin condensation or remodelling in association with different cellular, mainly apoptotic processes [92]. On the basis of our results it seems unlikely that the nuclear accumulation of FXIII-A in the early stage of macrophage differentiation would simply indicate apoptotic alterations. The nuclear presentation of FXIII-A is a transient phenomenon which can be detected only in 2nd-day cultures, when 90% of the cell population shows positive nuclear reaction for FXIII-A, but the loss of cells never exceeds 10–15% from the 2nd to the 3rd day. We suppose that the nuclear accumulation of FXIII-A in the early stage of macrophage differentiation may be associated with certain processes necessary to the development of macrophage characteristics.

The extracellular role of cellular FXIII-A

Although the question of how intracellular FXIII-A is released from megakaryocytes/platelets as well as from monocytes/macrophages has not yet been answered, abundant evidence in the literature demonstrates unequivocally that FXIII-A from the cells discussed above also have extracellular functions.

First of all, it can be accepted without reservation that plasma FXIII-A itself is a product of megakaryocytes/platelets and monocytes/macrophages. Transplantation [35, 101] studies have provided definite proof that plasma FXIII-A is – almost exclusively – of bone marrow origin. As we mentioned before, in patients transplanted with bone marrow from donors with different FXIII-A phenotype, the plasma FXIII-A phenotype changeover was almost complete, i.e. plasma FXIII-A changed dominantly to the donor phenotype [101]. Similarly, plasma FXIII-A phenotype remained of recipient origin in patients with orthotopic liver transplantation [35]. Although hepatocytes can synthesise FXIII-A [39], the mRNA signal in in situ hybridisation reactions is so weak that the contribution of hepatocytes to the plasma FXIII level can hardly be considered significant.

The role of plasma FXIII-A in hemostasis is well defined, but FXIII-A released from producing cells can act not only as a plasma clotting factor but also exerts catalytic functions in the intimate surroundings of platelets as well as monocytes and macrophages.

FXIII-A seems to have an important function in connective tissue homeostasis. As well as FXIII-A stimulating fibroblast proliferation [102, 103] and regulating collagen synthesis [104], it also acts as a cross-linking enzyme in collagen/fibronectin interactions [105–109].

FXIII-A$^+$ histiocytes can be detected at sites of connective tissue generation at a very early stage of mesenchyme formation in human embryonic life [57], and they accumulate at the sites of connective tissue proliferation in normal and pathological conditions. In the early phase of human development (from the 8th week of gestation), cells characterised as tissue macrophages start to accumulate in the peripheral part of the mesenchyme of chorionic villi, while the central mesenchyme that they surrounded shows intense fibrotic changes [109]. As we demonstrated in immunomorphological studies where epulis samples at different stages of fibrotic tissue formation were used as a model system, monocyte-derived macrophages containing FXIII-A also showed accumulation around fibrosing foci as early as the foci appeared [110].

The involvement of FXIII-A in wound healing has been known even from the first report on a FXIII deficient patient [111], and impairment of tissue repair is generally reported as part of the clinical symptoms in this genetic disease [112]. Although the exact mechanism of FXIII-A action has not yet been explored, it seems likely that it is involved in different steps of wound healing. Early after injury a stabilised fibrin or fibrin/fibronectin network is formed which promotes attachment, spreading and proliferation of cells involved in the tissue repair process [113–117].

During tumour matrix formation, tumour-associated macrophages containing FXIII-A prevail over all other cell types at the site of intratumoral fibrin formation, which is a characteristic phenomenon in tumour matrix reorganisation [55, 118, 119]. The active participation of FXIII-A (and tumour-associated macrophages from which it derives) in the formation of stabilised fibrin networks that facilitate tumour matrix generation and tumour angiogenesis can be interpreted as crucial in tumour progression [120].

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