Biochemical and Biophysical Research Communications 383 (2009) 113–118

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Important role of heparan sulfate in postnatal islet growth and insulin secretion

Iwao Takahashi [a,1], Naoya Noguchi [a,1], Koji Nata [b], Shuhei Yamada [c], Tomoyuki Kaneiwa [c], Shuji Mizumoto [c], Takayuki Ikeda [a], Kazushi Sugihara [d], Masahide Asano [d], Takeo Yoshikawa [a], Akiyo Yamauchi [e], Nausheen Jamal Shervani [a], Akira Uruno [a], Ichiro Kato [f], Michiaki Unno [g], Kazuyuki Sugahara [c], Shin Takasawa [e], Hiroshi Okamoto [a,*], Akira Sugawara [a,*]

[a] Department of Advanced Biological Sciences for Regeneration (Kotobiken Medical Laboratories), Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan
[b] Department of Medical Biochemistry, Iwate Medical University School of Pharmacy, Yahaba-cho 028-3603, Japan
[c] Laboratory of Proteoglycan Signaling and Therapeutics, Hokkaido University Graduate School of Life Science, Sapporo 001-0021, Japan
[d] Division of Transgenic Animal Science, Advanced Science Research Center, Kanazawa University, Kanazawa 920-8640, Japan
[e] Department of Biochemistry, Nara Medical University, Kashihara 634-8521, Japan
[f] Department of Biochemistry, University of Toyama Graduate School of Medicine and Pharmaceutical Sciences, Toyama 930-0194, Japan
[g] Department of Surgery, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan

ARTICLE INFO

Article history:
Received 21 March 2009
Available online 29 March 2009

Keywords:
Heparan sulfate
Extl3
Insulin secretion
β-Cell proliferation

ABSTRACT

Heparan sulfate (HS) binds with several signaling molecules and regulates ligand–receptor interactions, playing an essential role in embryonic development. Here we showed that HS was intensively expressed in pancreatic islet β-cells after 1 week of age in mice. The enzymatic removal of HS in isolated islets resulted in attenuated glucose-induced insulin secretion with a concomitant reduction in gene expression of several key components in the insulin secretion machinery. We further depleted islet HS by inactivating the exostosin tumor-like 3 gene specifically in β-cells. These mice exhibited abnormal islet morphology with reduced β-cell proliferation after 1 week of age and glucose intolerance due to defective insulin secretion. These results demonstrate that islet HS is involved in the regulation of postnatal islet maturation and required to ensure normal insulin secretion.

Heparan sulfate (HS) is a sulfated glycosaminoglycan distributed on the cell surface and in the extracellular matrix. HS chains are attached to core proteins to form HS proteoglycans (HSPGs) which include transmembrane syndecans, glycosylphosphatidylinositol-anchored glypicans and extracellular matrix proteins such as perlecan. HS chain biosynthesis is initiated by the transfer of an *N*-acetylglucosamine (*N*-acetylglucosamine transferase I activity) to a tetrasaccharide linkage region which is attached to a serine residue in the core protein. Then HS chain elongation proceeds by the action of glycosyltransferases encoded by *EXT1* and *EXT2* in the exostosin (*EXT*) gene family, which were first identified as causative genes of hereditary multiple exostoses [1]. The three other members of the *EXT* family, *EXTL1* (for EXT-like 1), *EXTL2*, and *EXTL3* also encode glycosyltransferases likely involved in HS biosynthesis [2,3]. The nascent chain undergoes a complex pattern of modifications, producing polymorphic sulfated sequence motifs in the chain, which are responsible for binding to a variety of signaling molecules and modulating their biological functions [4].

HS has been implicated in diverse biological phenomena [4]. Recent genetic studies in *Drosophila* and mice have provided compelling evidence that HS plays an essential role in embryonic development by interacting with several signaling molecules including Wnts, Hedgehogs, and fibroblast growth factors (FGFs) [5]. On the other hand, little is known about the significance of HS in the biology of pancreatic islets. In the present study, we examined the existence of HS in mouse islets by immunohistochemical analyses and found that HS was intensively expressed in β-cells after 1 week of age. We further investigated the significance of islet HS in insulin secretion and islet maturation in *in vitro* and *in vivo* studies.

Materials and methods

Immunohistochemistry. For the detection of HS, the pancreatic sections were exposed to 20 mU/ml heparitinase (Seikagaku, Tokyo, Japan) in 50 mM Tris–HCl (pH 7.0) or to buffer only for 2 h at 37 °C. After rinsing, they were incubated with monoclonal

Abbreviations: HS, heparan sulfate; Ext, exostosin; GIIS, glucose-induced insulin secretion.

* Corresponding authors. Fax: +81 22 717 8083.
E-mail addresses: okamotoh@mail.tains.tohoku.ac.jp (H. Okamoto), akiras2i@mail.tains.tohoku.ac.jp (A. Sugawara).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.03.140

antibody (mAb) 3G10 (Seikagaku) (1:200) or with negative control mouse IgG (DakoCytomation, Glostrup, Denmark) (1:20) for overnight in 1% casein blocking solution (Roche, Basel, Switzerland) and then treated with the second Ab. For the detection of insulin and glucagon, the anti-insulin mAb (Sigma–Aldrich, St. Louis, MO) (1:10,000) and rabbit anti-porcine glucagon polyclonal Ab (MP Biomedicals, Irvine, CA) (1:400) were used, respectively. Proliferating cell nuclear antigen (PCNA) was detected using an anti-PCNA mAb (Sigma–Aldrich) (1:10,000).

Heparitinase treatment of isolated islets. Pancreatic islets isolated from 8-week-old ICR mice were incubated either without or with 0.1 U/ml heparitinase in RPMI1640 medium supplemented with 5.5 mM glucose, 0.5% bovine serum albumin (BSA), 100 IU/ml penicillin and 100 μg/ml streptomycin for 1 h at 37 °C. The islets were then washed, resuspended in the medium above supplemented with 10% fetal calf serum instead of BSA, and cultured for 1 h or 24 h at 37 °C in an atmosphere of 5% CO_2 before being subjected to insulin secretion studies.

Measurement of insulin secretion from isolated islets. Batches of 10 islets were preincubated in Krebs Ringer bicarbonate buffer (KRB) containing 2.8 mM glucose for 1 h at 37 °C in 95% O_2/5% CO_2, and then incubated in 0.1 ml of fresh KRB containing various concentrations of glucose or 20 mM KCl for 1 h. The insulin concentration of the medium was determined using an insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). The islet insulin content was measured as described previously [6].

Quantitative real-time RT-PCR. The RNA isolation, reverse transcription and PCR were performed as described previously [6]. The sequences of primers are listed in Supplementary Table 1.

Animals. *Extl3* knockout (*Extl3*$^{-/-}$) and β-cell specific *Extl3* knockout (βExtl3KO) mice were generated as described in Supplementary information.

Compositional analysis of HS. Whole embryos were solubilized by sonication and digested with a mixture of heparitinase and heparinase (Seikagaku). After removing proteins by an OMIX TIP C18 (Varian, Lake Forest, CA), the digests were labeled with a fluorophore 2-aminobenzamide and the disaccharide composition was analyzed by HPLC on an amino-bound silica PA-03 column [7].

Glucose tolerance test and Insulin tolerance test. These tests were performed in 8- to 9-week-old mice as described previously [6].

Statistical analysis. Data are presented as mean ± SE. Statistical significance was determined by Student's *t*-test (for two groups) or Tukey–Kramer honestly significant difference test (for three groups). A *P*-value of 0.05 was considered significant.

Results and discussion

HS expression in pancreatic β-cells

To investigate the existence of HS in pancreatic islets, we first analyzed pancreatic sections from 8-week-old mice by immunostaining with the mAb 3G10 which recognizes uronates on the HS stubs generated after heparitinase digestion [8]. HS was found to be intensively expressed in the islets (Fig. 1A). The section without prior digestion by heparitinase had no staining with mAb 3G10 (Fig. 1B), confirming that mAb 3G10 specifically decorated HS. Incubating the heparitinase-treated section with negative control mouse IgG gave no staining (Fig. 1C), further confirming the specificity of staining. Immunostaining of serial sections for insulin and for glucagon revealed that HS was localized exclusively in β-cells (Fig. 1D–F).

We next analyzed pancreatic sections at various growth stages to examine when this impressive expression of HS occurs (Fig. 1G). At birth HS was scarcely stained in the islets that were indicated by insulin- and glucagon-staining. HS had, however, become detectable distinctly after 1 week of age. We also analyzed the islets at embryonic day 18.5, revealing that HS was scarcely stained in the islets (data not shown). These findings suggest the specific roles of islet HS on the postnatal maturation of the islets and on the function of differentiated β-cells.

Expression of HS biosynthetic and modification enzymes and core proteins in β-cells

We investigated the expression of enzymes involved in HS chain formation and core proteins in 8-week-old mouse β-cells by RT-PCR (Supplementary Fig. 1). Among the *Ext* family, *Ext1*, *Ext2*, *Extl2* and *Extl3* were expressed in β-cells, whereas *Extl1* was not detected. The HS modification enzymes such as *N*-deacetylase/*N*-sulfotransferases, C5-epimerase, and HS sulfotransferases were all expressed in β-cells. Concerning core proteins, syndecan 2, 3, 4, glypican 1, 4, and perlecan were mainly expressed in β-cells.

Insulin secretion in heparitinase-treated islets

To investigate whether HS plays a role in β-cell function, the isolated islets were treated with heparitinase and analyzed for insulin secretion. Treating islets with 0.1 U/ml heparitinase for 1 h effectively degraded HS chains of HSPGs as evidenced by the presence of the epitope for mAb 3G10 (Fig. 2A). After 24 h of culture, insulin secretion stimulated by elevated concentrations of glucose was markedly decreased in heparitinase-treated islets compared with the control islets, while the basal secretion at 2.8 mM glucose was not changed (Fig. 2B). The islet insulin content was preserved in heparitinase-treated islets (Fig. 2C). These results indicate that the removal of islet HS specifically impaired the stimulated insulin secretion. However, islet HSPGs were unlikely to be involved directly in the glucose-induced insulin secretion (GIIS) machinery, because GIIS at 1 h after the digestion was not affected (data not shown). Glucose stimulates insulin secretion through the following steps. Glucose transported into β-cells by glucose transporter 2 (Glut2) is metabolized and the resultant increase of cytosolic ATP/ADP ratio closes ATP-sensitive K^+ (K_{ATP}) channels composed of SUR1 and Kir6.2 in the plasma membrane, leading to membrane depolarization. Then voltage-dependent Ca^{2+} channels (VDCCs) are activated and the consequent influx of Ca^{2+} triggers insulin secretion. Insulin secretion by 20 mM KCl, which directly depolarizes the plasma membrane, was decreased in heparitinase-treated islets (Fig. 2B), indicating that the impairment lies in the steps downstream of membrane depolarization. The magnitude of reduction in insulin secretion was, however, smaller than that stimulated by glucose (23% versus 60%), suggesting that the events upstream of membrane depolarization are also impaired in heparitinase-treated islets.

We next examined the mRNA levels of several molecules involved in GIIS at 24 h after heparitinase digestion (Fig. 2D). The mRNA levels of Glut2 and SUR1 were significantly reduced in heparitinase-treated islets, whereas no difference was found in the mRNA levels of glucokinase, Kir6.2, and CaV1.2, the α_1-subunit of VDCCs in β-cells, indicating highly specific effects of HS removal on the gene expression profile in β-cells. The expression of Snap25 and syntaxin 1A, the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins that mediate fusion between the vesicular and plasma membrane for exocytosis, was also decreased in heparitinase-treated islets.

In type 2 diabetes rodent models such as GK rats and Zucker *fa/fa* rats, the islet levels of Snap25 and syntaxin 1A are reduced [9,10], and restoration of these proteins in the islets could normalize insulin secretion. Moreover, intracellular application of antibodies against Snap25 and syntaxin 1A reduced insulin secretion [11]. These studies demonstrated that the proper expression levels

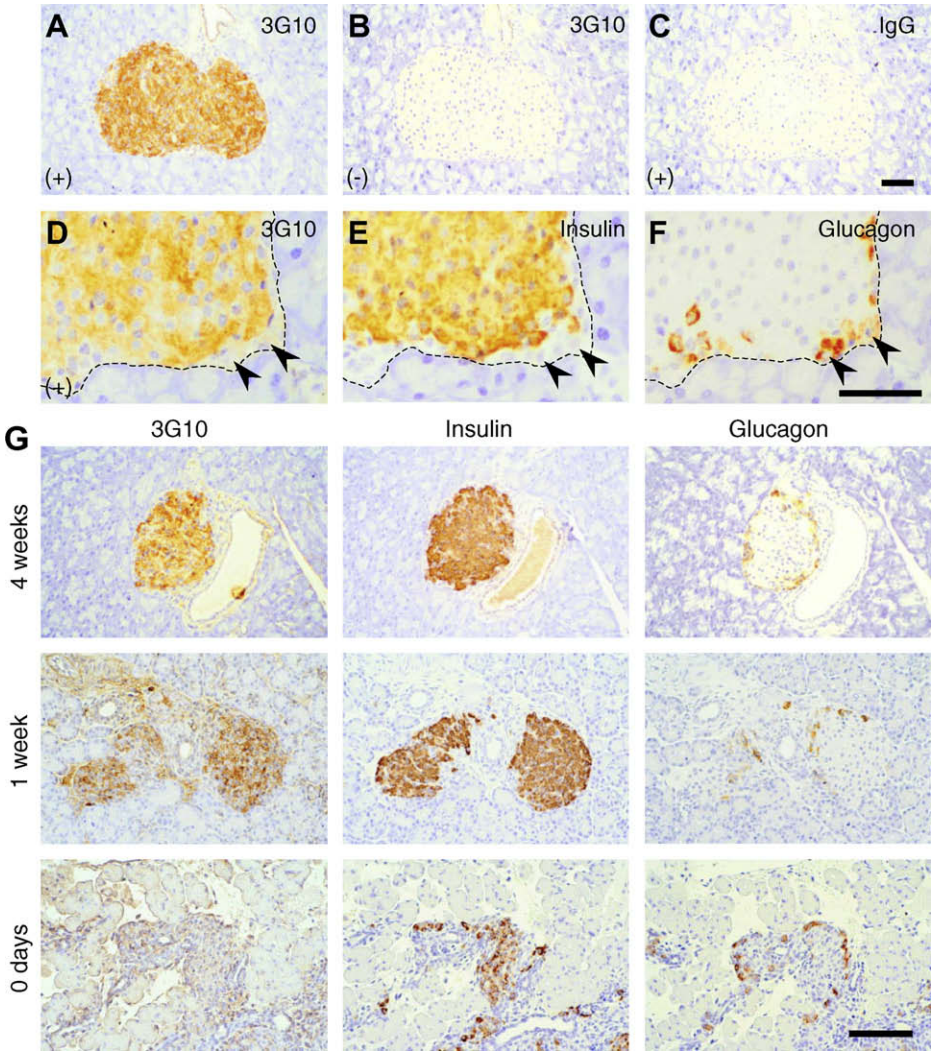

Fig. 1. Immunostaining of HS in pancreatic islets. (A,B) Immunostaining with mAb 3G10 after treating sections with (+) or without (−) heparitinase. (C) Immunostaining with negative control IgG. (D–F) Immunostaining for HS (3G10), insulin, and glucagon at higher magnification. The same cells were indicated by arrowheads. (G) Pancreatic sections at indicated ages were analyzed. Scale bar, 50 μm.

of these proteins are important for insulin secretion. The reduced expression of *Snap25* and *syntaxin 1A* may, therefore, contribute to the impairment lying downstream of membrane depolarization in heparitinase-treated islets. The moderate reduction of mRNA levels of Glut2 or SUR1 alone, however, may not be enough to cause the impairment lying upstream of membrane depolarization, because heterozygous mutant mice of Glut2 or SUR1 exhibited normal insulin secretion [12,13]. Recently, syntaxin 1A has been reported to bind and regulate K_{ATP} channels [14]. Combined reduction of these molecules might have contributed to the impairment lying upstream of membrane depolarization. Therefore, it appears that islet HS is required for the normal expression of several key components, thereby contributing to the maintenance of normal insulin secretion.

We also examined the expression of the *Ext* family of genes. In addition to an abundant mRNA level in normal islets (the absolute mRNA levels of *Ext1*, *Ext2*, *Extl2* and *Extl3* were 3.0 ± 0.2, 3.2 ± 0.3, 9.5 ± 0.5 and 8.9 ± 0.4 copies/100 copies of β-actin mRNA, respectively), among the family the *Extl3* gene expression was most strikingly induced in heparitinase-treated islets (Fig. 2E). In addition, the mRNA levels of major core proteins in β-cells were increased by 1.2 to 1.5-fold in heparitinase-treated islets (data not shown). These findings indicate that a feedback regulatory mechanism may exist to maintain the HS distribution and that Extl3 may be actively involved in HS biosynthesis in β-cells.

Creation of β-cell specific Extl3 knockout (βExtl3KO) mice

To investigate the significance of islet HS *in vivo*, we generated mice lacking HS specifically in β-cells. To achieve this, we targeted murine *Extl3* gene. We first created systemic *Extl3* knockout (*Extl3*$^{-/-}$) mice. *Extl3*$^{-/-}$ mice were embryonic lethal at around 9 days postcoitum (dpc) (Supplementary Table 2). To examine whether HS was synthesized in *Extl3*$^{-/-}$ embryos, we analyzed the disaccharide composition of HS in wild-type (*Extl3*$^{+/+}$), heterozygous mutant (*Extl3*$^{+/-}$), and *Extl3*$^{-/-}$ embryos at around 9 dpc by HPLC (Table 1). Although the overall disaccharide composition and total amount of HS in *Extl3*$^{+/-}$ embryos were similar to those in *Extl3*$^{+/+}$ embryos, HS-derived disaccharides were not detected in *Extl3*$^{-/-}$ embryos, demonstrating that the loss of Extl3 results in defective HS synthesis.

We next generated βExtl3KO mice through rat insulin 2 promoter-Cre/loxP-mediated recombination on a genetic background of *Extl3*$^{-/flox}$, wherein one allele was targeted and exon 2 of another was flanked by two *loxP* sequences (the genotype of βExtl3KO was represented as *Extl3*$^{-/flox}$ + *RIP-Cre*) (see Supplementary informa-

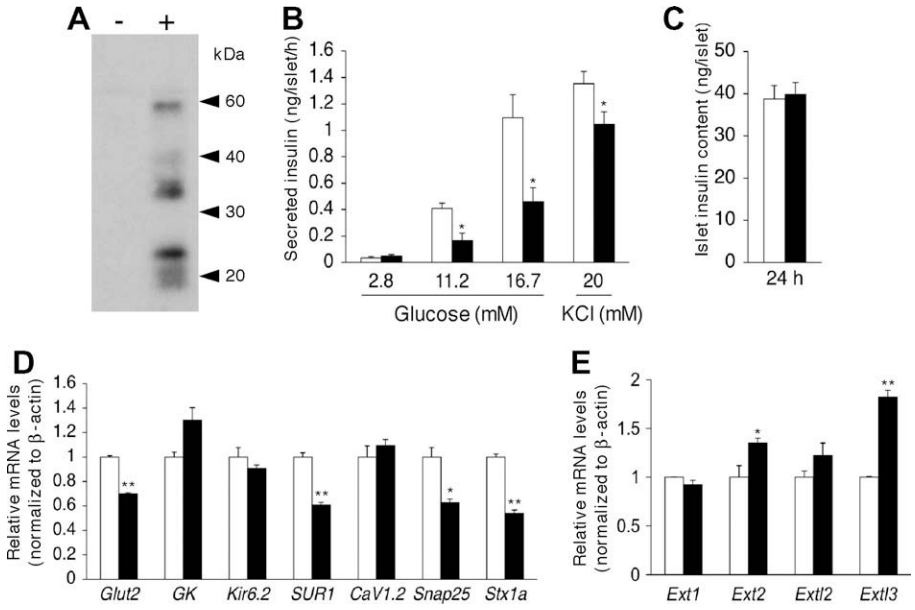

Fig. 2. Insulin secretion and gene expression in isolated islets after heparitinase treatment. (A) After treating either without (−) or with (+) heparitinase, the islets were lysed directly in SDS–PAGE sample buffer and subjected to SDS–PAGE followed by immunoblotting with mAb 3G10. (B) Insulin secretion in response to glucose or KCl after 24 h from heparitinase digestion ($n = 4–5$). (C) Insulin content in the islets at 24 h after heparitinase digestion ($n = 6–8$). (D) Relative mRNA levels were determined for the indicated genes with real-time RT-PCR. For each gene, the level of mRNA in islets treated without heparitinase was set to 1. *GK*, glucokinase; *Stx1a*, syntaxin 1A ($n = 3$). (E) The expression of the *Ext* family of genes ($n = 3$). Open bars, islets treated without heparitinase; filled bars, heparitinase-treated islets. $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 1
Disaccharide composition of HS from the embryos.

Disaccharides	Genotype (pmol/μg protein)		
	+/+	+/−	−/−
ΔHexUA[a]-GlcNAc	0.72	0.87	<0.10
ΔHexUA-GlcNAc(6S)	0.12	0.14	ND[b]
ΔHexUA-GlcN(NS)	0.55	0.57	<0.08
ΔHexUA-GlcN(NS,6S)	0.06	0.07	ND
ΔHexUA(2S)-GlcN(NS)	0.10	0.11	ND
ΔHexUA(2S)-GlcN(NS,6S)	0.06	0.06	ND
Total	1.61	1.82	<0.18

[a] ΔHexUA, unsaturated hexuronic acid; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; 6S, 6-*O*-sulfate; NS, 2-*N*-sulfate; 2S, 2-*O*-sulfate.
[b] ND, not detected.

tion and Supplementary Fig. 3). Immunostaining of pancreatic sections of βExtl3KO mice revealed that β-cells expressing Cre recombinase certainly lacked HS (Supplementary Fig. 3D).

Abnormal islet morphology in βExtl3KO mice

In heterozygous mutant ($Extl3^{-/flox}$) mice, the islet morphology was completely normal at all time points examined (Fig. 3A left). In βExtl3KO mice, the islets appeared to develop normally as shown at 0 day (Fig. 3A right). An atypical organization (β-cells existed also in the most peripheral region of the islets and glucagons-positive α-cells were scattered in the central region) was, however, observed after 2 weeks of age. In addition, the β-cell number/total islet cell number ratio was decreased to about 80% of controls after 2 weeks of age (Fig. 3B), whereas no difference was found in the islet size between βExtl3KO mice and their controls (data not shown). Consistent with this, the β-cell area relative to the whole pancreatic area in βExtl3KO mice was reduced to ~75% of controls (Supplementary Fig. 4). Then we examined pancreatic sections for the expression of PCNA to assess the β-cell proliferation activity. The PCNA staining in β-cells was significantly decreased in βExtl3KO mice after 1 week of age (Fig. 3C). These findings indicate that islet HS plays a role in the regulation of the postnatal expansion of β-cells and organization of islet cells.

Impaired insulin secretion in βExtl3KO mice

We next investigated insulin secretion in isolated islets from 8-week-old βExtl3KO mice. Since the proportion of β-cells in the islets was changed in βExtl3KO mice, the data were expressed as fold response to basal secretion. As shown in Fig. 4A, the insulin response to 11.2 mM glucose and to 20 mM KCl was reduced in βExtl3KO islets to 47% and 75% of controls, respectively. In the glucose tolerance test, βExtl3KO mice showed significantly higher blood glucose levels after glucose loading than the control littermates (Fig. 4B). The plasma insulin levels in βExtl3KO mice after glucose loading were significantly lower than those in control mice (Fig. 4C). Although the pancreatic insulin content was slightly decreased in βExtl3KO mice (85% of controls, but not statistically significant) (data not shown), this appeared unlikely to be responsible for the insufficient increase of plasma insulin levels. The impaired GIIS observed in isolated islets (Fig. 4A) must have contributed to this defect. In the insulin tolerance test, there was no peripheral insulin resistance in βExtl3KO mice (Fig. 4D), indicating that the glucose intolerance in βExtl3KO mice was caused by insufficient insulin secretion. Recently, a genome-wide association study has identified the haplotype block of linkage disequilibrium containing the *EXT2* gene in type 2 diabetes patients [15]. Taken together, it is conceivable that the dysfunction of HS in the islets may be a potential factor in the pathogenesis of diabetes mellitus.

The enzymatic removal of HS and knockout of HS expression in β-cells resulted in the phenotypes similar to those of FRID1 mice, that is, impaired GIIS, reduced expression of Glut2 and prohormone convertase 1/3 (Supplementary Fig. 5A) but not glucokinase, and abnormal islet architecture with reduced β-cell number. FRID1 mice expressed a dominant-negative form of the FGF receptor 1 (FGFR1) under control of the rat insulin 2 promoter to attenuate FGF signaling in β-cells [16]. Since HS is required as a co-receptor for FGF signaling, the defects observed here may be attributed, in

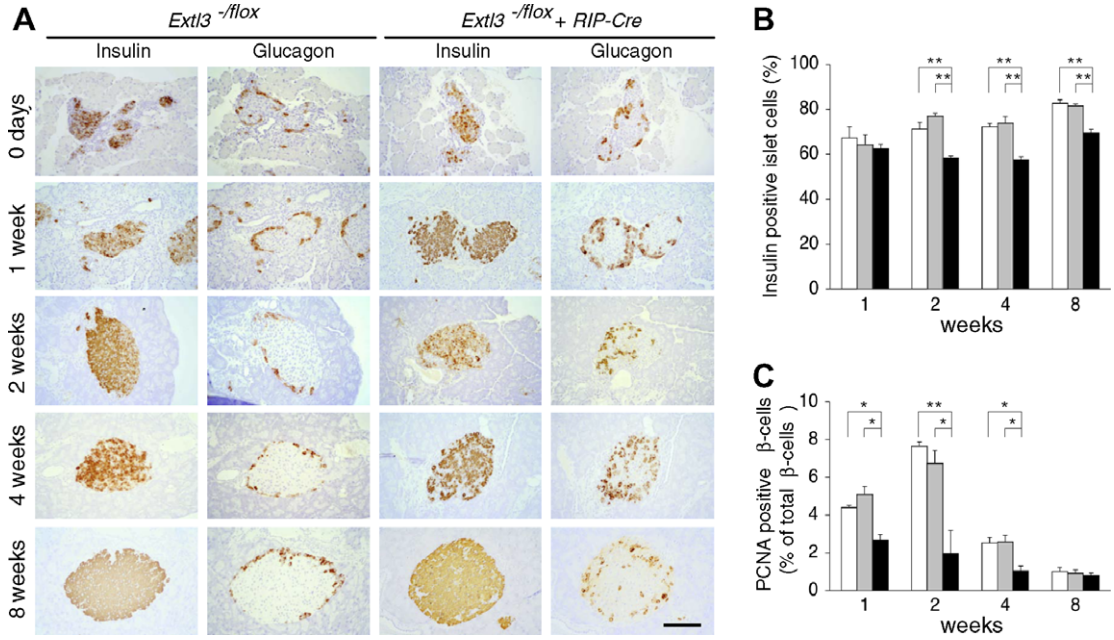

Fig. 3. Immunohistochemical analysis of islets and β-cell proliferating activity. (A) Pancreatic sections at indicated ages were analyzed. Scale bar, 100 μm. (B) The proportion of insulin-positive cells to total islet cells. At least five islets were analyzed per pancreas $n = 3–6$. (C) The proportion of PCNA positive β-cells to total β-cells. At least five islets were analyzed per pancreas $n = 3–5$. Open bars, $Extl3^{+/flox}$; shaded bars, $Extl3^{-/flox}$; filled bars, βExtl3KO. $^{*}p < 0.05$; $^{**}p < 0.01$.

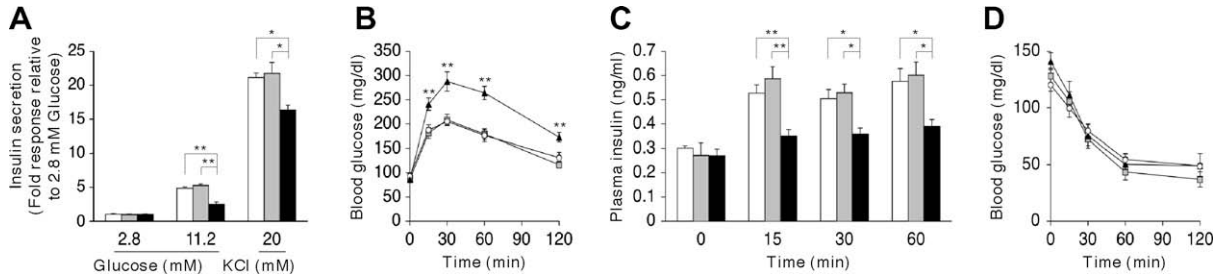

Fig. 4. Insulin secretion in βExtl3KO mice. (A) Insulin secretion from isolated islets. Data were expressed as the insulin secretion relative to basal secretion at 2.8 mM glucose (which was arbitrarily set at 1) ($n = 4–5$). (B) Blood glucose levels during glucose tolerance test. Glucose (2 g/kg of body weight) was injected intraperitoneally ($n = 20–23$). (C) Plasma insulin levels after injection of glucose (3 g/kg of body weight) ($n = 6–11$). (D) Blood glucose levels after intraperitoneal injection of insulin (0.75 U/kg of body weight) ($n = 9–13$). Open bars and circles, $Extl3^{+/flox}$; shaded bars and squares, $Extl3^{-/flox}$; filled bars and triangles, βExtl3KO. $^{*}p < 0.05$; $^{**}p < 0.01$.

part, to the attenuated FGF signaling in β-cells. The mRNA level of FGFR1 was increased by 1.5-fold in heparitinase-treated islets (Supplementary Fig. 5B). This may reflect a compensatory induction in response to the inhibited FGF signaling. Recently, it has been reported that Wnts stimulate GIIS in adult mouse islets [17]. As Wnts are also known to interact with HS to transduce signals to their cognate receptors, the defective Wnt signaling could be an additional explanation for impaired GIIS.

We also demonstrated, for the first time, the significance of Extl3 in HS chain biosynthesis. EXTL3 has been reported to possess an *in vitro N*-acetylglucosamine transferase I activity and one more *in vitro* glycosyltransferase activity for HS chain elongation [2]. However, EXT1 and EXT2 were also demonstrated to exhibit an *in vitro N*-acetylglucosamine transferase I activity [18]. Moreover, gene silencing of *EXTL3* in HEK293 cells resulted in the synthesis of longer HS chains [19], indicating that EXTL3 may be dispensable for HS biosynthesis. Similarly to *Ext1* or *Ext2* knockout mice which lacked HS and died by 8.5 dpc, $Extl3^{-/-}$ mice had undetectable levels of HS and died at around 9 dpc, demonstrating that the loss of Extl3 could not be compensated by other Ext members, and thus Extl3 is also essential for HS biosynthesis.

In summary, we found that HS was intensively expressed in mouse β-cells after 1 week of age and established that HS was involved in the regulation of postnatal islet maturation and required to maintain normal GIIS. It would be of interest to investigate how this expression of HS is regulated in β-cells and which HSPG is involved in each phenotype.

Acknowledgments

We are grateful to Mr. Yuya Shichinohe for technical assistance and Mr. Brent Bell for critical reading of the manuscript. This work was supported in part by Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.03.140.

References

[1] B.M. Zak, B.E. Crawford, J.D. Esko, Hereditary multiple exostoses and heparan sulfate polymerization, Biochim. Biophys. Acta 1573 (2002) 346–355.
[2] B.T. Kim, H. Kitagawa, J. Tamura, T. Saito, M. Kusche-Gullberg, U. Lindahl, K. Sugahara, Human tumor suppressor EXT gene family members EXTL1 and

EXTL3 encode alpha 1,4-*N*-acetylglucosaminyltransferases that likely are involved in heparan sulfate/heparin biosynthesis, Proc. Natl. Acad. Sci. USA 98 (2001) 7176–7181.
[3] H. Kitagawa, H. Shimakawa, K. Sugahara, The tumor suppressor EXT-like gene EXTL2 encodes an alpha1, 4-*N*-acetylhexosaminyltransferase that transfers *N*-acetylgalactosamine and *N*-acetylglucosamine to the common glycosaminoglycan–protein linkage region. The key enzyme for the chain initiation of heparan sulfate, J. Biol. Chem. 274 (1999) 13933–13937.
[4] T. Spillmann, U. Lindahl, Glycosaminoglycan–protein interactions: a question of specificity, Curr. Opin. Struct. Biol. 4 (1994) 677–682.
[5] X. Lin, Functions of heparan sulfate proteoglycans in cell signaling during development, Development 131 (2004) 6009–6021.
[6] N. Noguchi, T. Yoshikawa, T. Ikeda, I. Takahashi, N.J. Shervani, A. Uruno, A. Yamauchi, K. Nata, S. Takasawa, H. Okamoto, A. Sugawara, FKBP12.6 disruption impairs glucose-induced insulin secretion, Biochem. Biophys. Res. Commun. 371 (2008) 735–740.
[7] A. Kinoshita, K. Sugahara, Microanalysis of glycosaminoglycan-derived oligosaccharides labeled with a fluorophore 2-aminobenzamide by high-performance liquid chromatography: application to disaccharide composition analysis and exosequencing of oligosaccharides, Anal. Biochem. 269 (1999) 367–378.
[8] G. David, X.M. Bai, B. Van der Schueren, J.J. Cassiman, H. Van den Berghe, Developmental changes in heparan sulfate expression: in situ detection with mAbs, J. Cell Biol. 119 (1992) 961–975.
[9] C.B. Chan, R.M. MacPhail, L. Sheu, M.B. Wheeler, H.Y. Gaisano, Beta-cell hypertrophy in fa/fa rats is associated with basal glucose hypersensitivity and reduced SNARE protein expression, Diabetes 48 (1999) 997–1005.
[10] S. Nagamatsu, Y. Nakamichi, C. Yamamura, S. Matsushima, T. Watanabe, S. Ozawa, H. Furukawa, H. Ishida, Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreatic beta-cells is involved in impaired insulin secretion from diabetic GK rat islets: restoration of decreased t-SNARE proteins improves impaired insulin secretion, Diabetes 48 (1999) 2367–2373.
[11] J. Vikman, X. Ma, G.H. Hockerman, P. Rorsman, L. Eliasson, Antibody inhibition of synaptosomal protein of 25 kDa (SNAP-25) and syntaxin 1 reduces rapid exocytosis in insulin-secreting cells, J. Mol. Endocrinol. 36 (2006) 503–515.
[12] M.T. Guillam, P. Dupraz, B. Thorens, Glucose uptake, utilization, signaling in GLUT2-null islets, Diabetes 49 (2000) 1485–1491.
[13] V. Seghers, M. Nakazaki, F. DeMayo, L. Aguilar-Bryan, J. Bryan, Sur1 knockout mice. A model for K(ATP) channel-independent regulation of insulin secretion, J. Biol. Chem. 275 (2000) 9270–9277.
[14] E.A. Pasyk, Y. Kang, X. Huang, N. Cui, L. Sheu, H.Y. Gaisano, Syntaxin-1A binds the nucleotide-binding folds of sulphonylurea receptor 1 to regulate the KATP channel, J. Biol. Chem. 279 (2004) 4234–4240.
[15] R. Sladek, G. Rocheleau, J. Rung, C. Dina, L. Shen, D. Serre, P. Boutin, D. Vincent, A. Belisle, S. Hadjadj, B. Balkau, B. Heude, G. Charpentier, T.J. Hudson, A. Montpetit, A.V. Pshezhetsky, M. Prentki, B.I. Posner, D.J. Balding, D. Meyre, C. Polychronakos, P. Froguel, A genome-wide association study identifies novel risk loci for type 2 diabetes, Nature 445 (2007) 881–885.
[16] A.W. Hart, N. Baeza, A. Apelqvist, H. Edlund, Attenuation of FGF signalling in mouse beta-cells leads to diabetes, Nature 408 (2000) 864–868.
[17] T. Fujino, H. Asaba, M.J. Kang, Y. Ikeda, H. Sone, S. Takada, D.H. Kim, R.X. Ioka, M. Ono, H. Tomoyori, M. Okubo, T. Murase, A. Kamataki, J. Yamamoto, K. Magoori, S. Takahashi, Y. Miyamoto, H. Oishi, M. Nose, M. Okazaki, S. Usui, K. Imaizumi, M. Yanagisawa, J. Sakai, T.T. Yamamoto, Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion, Proc. Natl. Acad. Sci. USA 100 (2003) 229–234.
[18] B.T. Kim, H. Kitagawa, J. Tamura, T. Saito, M. Kusche-Gullberg, U. Lindahl, K. Sugahara, Human tumor suppressor EXT gene family members EXTL1 and EXTL3 encode alpha 1,4-*N*-acetylglucosaminyltransferases that likely are involved in heparan sulfate/heparin biosynthesis, Proc. Natl. Acad. Sci. USA 98 (2001) 7176–7181.
[19] M. Busse, A. Feta, J. Presto, M. Wilen, M. Gronning, L. Kjellen, M. Kusche-Gullberg, Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation, J. Biol. Chem. 282 (2007) 32802–32810.