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REVIEW ARTICLE

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The molecular basis of persistent hyperinsulinemic hypoglycemia of infancy and its pathologic substrates

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Abstract Recent advances in molecular genetics have established a molecular basis for persistent hyperinsulinemic hypoglycemia of infancy (PHHI) and resulted in the identification of a number of well-defined genetic defects. On the basis of the available information on the molecular changes so far described, an attempt has been made to classify PHHI patients according to their genotype and phenotype, with reference to molecular genetics, pancreatic pathology and clinical appearance. This classification has resulted in the differentiation of three groups of PHHI patients, two with diffuse beta cell hyperfunction and one with focal beta cell hyperfunction.

Key words Congenital hyperinsulinism · Persistent hyperinsulinemic hypoglycemia · Nesidioblastosis · Molecular basis · Pancreatic pathology · Classification

Introduction

Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is the most important form of congenital hyperinsulinism (for reviews cf. [3, 5, 32]). It is a hyperfunctional disorder of pancreatic insulin-producing cells characterized by hypertrophic beta cells, which are present only in a focus of hyperplastic islets or are found in all islets of the pancreas [14, 15, 26]. These islet changes have been referred to collectively by a variety of terms, including nesidioblastosis, beta cell nesidioblastosis, diffuse or generalized islet hyperplasia, focal and multifocal ductuloinsular proliferation, microadenomatosis, focal islet cell adenomatosis, endocrine cell dysplasia, and nesidiodysplasia. We and others have preferred to use the term nesidioblastosis [3, 8, 14, 16]. This designation, however, has been criticized [25] because, strictly speaking, nesidioblastosis signifies only the physiological process of budding-off of endocrine cells from duct epithelium [21], which has not been identified as the basic lesion in PHHI. Here, we have therefore chosen to use the descriptive terms focal beta cell hyperfunction with hypertrophy and hyperplasia, and diffuse beta cell hyperfunction with and without hypertrophy.

The clinical features of PHHI include ataxia, seizures and coma. PHHI is diagnosed by demonstrating persistent secretion of insulin that is inappropriate for the concentration of glucose. In most patients the symptoms appear during the first days of life [3, 5]. Newborns with PHHI usually have an elevated birth weight for their gestational age, suggesting that the hyperinsulinism in these newborns has been present for some time before birth. Early recognition and effective prevention of hypoglycemia are essential to prevent permanent neurological damage and severe mental retardation that could otherwise be caused by resultant neuroglycopenia. Prevention of recurrent hypoglycemia requires glucose infusions and/or the administration of substances inhibiting insulin secretion, e.g. diazoxide or octreotide [17, 35]. Despite recent advances in medical (i.e., octreotide) and surgical (i.e., partial pancreatectomy in patients with focal disease) treatment, subtotal pancreatectomy is often necessary [10, 31]. However, most PHHI patients treated by subtotal pancreatectomy will become diabetic either immediately after the operation or during puberty [10, 22].

In the past, the cause of PHHI was sought primarily in an increase in functionally active islet tissue, a dysmaturation of the embryonic endocrine pancreas or a decrease in nonbeta cells with an insulin-inhibitory function (for reviews cf. [14, 16, 18, 26]). More recently, however, physiological and morphological studies have made it clear that PHHI is a hyperfunctional disorder of the beta cells associated with heterogeneous pathologic changes [3, 14, 26]. Recently, molecular studies have revealed that PHHI results from at least three basic genetic defects: first, loss-of-function mutations on chromosome locus 11p15.1 affecting the genes of the two subunits SUR1 and KIR6.2 [11, 24, 34], which form the ATP-dependent potassium channel that is an integral part of the

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Table 1 Clinicopathological features of 38 neonates with persistent hyperinsulinemic hypoglycemia of infancy

Features	Focal beta cell changes	Diffuse beta cell changes	
		With hypertrophy	Without hypertrophy
No. of patients	16	19	3
Sex M/F	6/10	11/8	2/1
Symptomatic patients under 1 month old	60%	80%	80%
Operated patients under 6 months old	80%	60%	100%
Pancreatectomy	Partial or subtotal	Subtotal	Subtotal
Proliferation	Increased	Normal	Normal
Ductuloinsular complexes	Prominent	Common[a]	Rare
Patients with multiple foci	2/16	–	–

[a]During the first 6 months

stimulus–secretion coupling apparatus of insulin release; second, gain-of function mutations of genes for enzymes (glucokinase, glutamate dehydrogenase) that regulate the rate of insulin secretion; and third, a two-hit loss-of-heterozygosity (LOH) mechanism in a group of beta cells [9, 10], with a specific loss of maternal alleles of the imprinted chromosome region 11p15.1 unmasking a paternally inherited recessive SUR1 or KIR6.2 mutation (for further review cf. [23]). This review will attempt to correlate the different genotypes with the spectrum of pathological changes in PHHI patients described in the literature and found in our own series of 38 patients (see Table 1).

Pathology

There are basically two types of morphological beta cell changes that are associated with PHHI, a focal and a diffuse form [10, 14, 18, 19, 26].

Focal changes

Focal beta cell hyperfunction has been referred to variously by pathologists as focal islet cell adenomatosis [20], neonatal islet cell adenoma [6], adenomatous islet cell hyperplasia [27] congenital insulinoma [7] and nesidioblastoma [30]. This lesion is found in one fourth to almost one half of all PHHI cases [10, 15] and is usually unifocal [15]. Grossly the pancreas is normal in most cases, and only rarely is a small hard nodule (diameter 5–8 mm) noted. In the French–Belgian series this lesion was identified more often in the head and body region of the pancreas [10], while in our series it was primarily identified in the body–tail region (Table 1). In two of our cases we found a lesion in the head as well as in the body.

Histologically, there is an accumulation of huge islet cell clusters, which are separated by thin rims of acinar cells or strands of connective tissue (Fig. 1). Occasionally they may be attached to each other or to small ducts, forming ductuloinsular complexes. Some of the cells of the islet-like clusters are large and have hypertrophic nuclei. The proliferation rate appears to be increased. The histology of foci grossly presenting as tumors is, in principle, comparable to that of the macroscopically undetectable lesions. As the only difference, the large endocrine cell clusters may be separated by fibrous stroma cords.

Immunocytochemically, the islet-like clusters are found to be composed of insulin, glucagon, somatostatin and PP cells, which retain the same spatial arrangement as in normal islets. Insulin cells, however, are more numerous than normal, representing about 70–90% of all endocrine cells (Fig. 2), as against about 50% in normal neonates. In addition, they may be hypertrophic and intensely positive for proinsulin. Ultrastructurally, they show increased, well-organized endoplasmic reticulum, prominent Golgi complexes and immature secretory vesicles representing proinsulin granules. The fact that the distribution of the four islet cell types in the cell clusters is essentially the same as in normal islets is an important criterion that distinguishes the focal lesions clearly from multihormonal endocrine neoplasms. The islets outside the focus display endocrine cells that are normal in size, appearance and distribution.

Diffuse changes

Grossly the pancreas is normal. Histologically, variably sized islets are seen throughout the entire pancreas. They usually show one or more cells with distinct nuclear enlargement, often resulting in giant and bizarre nuclei (Fig. 3). In addition, there may be some large islets and also some irregularly sized and poorly defined smaller endocrine cell clusters (particularly in neonates) scattered in the acinar parenchyma and often intimately connected with small or larger ducts (ductuloinsular complexes) (Fig. 4). The latter changes are seen particularly in infants operated on within the first 3 months of life, whereas infants who are operated on later in life have rather normal-appearing islets, except for the beta cell hypertrophy. In 30% of our infants the islets appeared normal, regardless of the time of resection. In particular, they lacked cells with distinctly hypertrophic nuclei (Fig. 5).

On immunocytochemistry and electron microscopy the cells with hypertrophic nuclei are found to be insu-

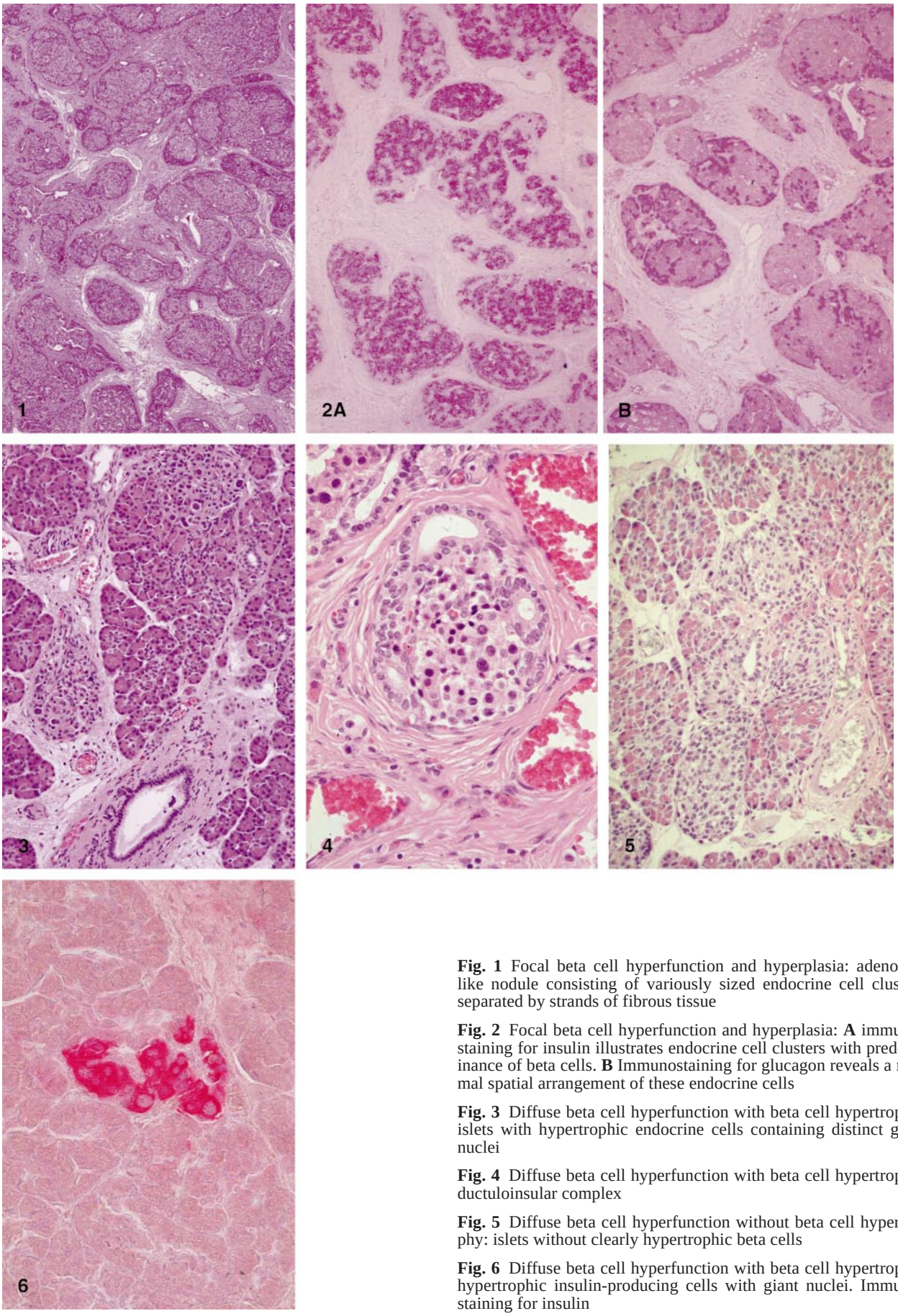

Fig. 1 Focal beta cell hyperfunction and hyperplasia: adenoma-like nodule consisting of variously sized endocrine cell clusters separated by strands of fibrous tissue

Fig. 2 Focal beta cell hyperfunction and hyperplasia: **A** immunostaining for insulin illustrates endocrine cell clusters with predominance of beta cells. **B** Immunostaining for glucagon reveals a normal spatial arrangement of these endocrine cells

Fig. 3 Diffuse beta cell hyperfunction with beta cell hypertrophy: islets with hypertrophic endocrine cells containing distinct giant nuclei

Fig. 4 Diffuse beta cell hyperfunction with beta cell hypertrophy: ductuloinsular complex

Fig. 5 Diffuse beta cell hyperfunction without beta cell hypertrophy: islets without clearly hypertrophic beta cells

Fig. 6 Diffuse beta cell hyperfunction with beta cell hypertrophy: hypertrophic insulin-producing cells with giant nuclei. Immunostaining for insulin

lin-producing cells (Fig. 6). Ultrastructurally they display a well-developed biosynthetic apparatus. Their proliferation rate is normal [29], and their number is not significantly increased [15]. The nonbeta cells show no deviation in their spatial organization and proportional distribution. In some patients a decrease in somatostatin or glucagon cells has been reported, but this was not confirmed by other studies [14, 26].

Genotype and phenotype

The first, and probably most important, group of PHHI patients have familial disease with an autosomal recessive mode of inheritance [12]. The disease has been localized to the chromosome locus 11p15.1, which contains the genes for the two subunits, SUR1 ("sulfonylurea receptor") and Kir6.2 ("inward rectifying potassium channel") of the ATP-dependent potassium channel of the beta cell membrane, which has an essential function in the process of insulin secretion [1]. So far 28 different mutations of the sulfonylurea receptor (SUR1) and two mutations of the Kir6.2 gene have been reported [23]. They lead to a loss of function by causing a blockage of the outward K^+ transport through the potassium channel, which is then followed by permanent depolarization of the beta cell membrane and an increased influx of calcium, resulting in inappropriate insulin release. Clinically it seems that the loss-of-function mutations are associated with the type-1 PHHI patients defined by Aynsley-Green et al. [4], who are characterized by high birthweights, severe disease and unresponsiveness to diazoxide. The pathological substrate of this disease category appears to be diffuse beta cell hyperfunction with typical hypertrophy. Most probably, identical or very similar changes characterize Aynsley-Green's type-2 patients, who "[have] sporadic PHHI, lack functional potassium channels, but retain a degree of diazoxide responsiveness owing to the presence of a novel ion channel in the beta cell membrane" [4].

The second, and much smaller, group of PHHI patients have autosomal dominant familial hyperinsulinism. These genetic defects are based on mutations of either the glutamate dehydrogenase gene (GLUD1) [33] or the glucokinase gene (GCK) [13]. The enzyme GLUD1 mediates leucin-stimulated insulin secretion and also causes hyperammonemia by regulating ureagenesis in the liver. The enzyme GCK acts as a glucosensor in the beta cells. Both gene defects are gain-of-function mutations resulting in increased insulin secretion. This genotype seems to affect Aynsley-Green's type-3 patients [4], who have a later onset mild form of PHHI and respond to diazoxide. The type of islet lesions associated with these mutations is most probably also a diffuse beta cell change. However, we assume that these beta cells, in contrast to those in the first group, are barely hypertrophic and hence show no significantly enlarged nuclei. In our series, approximately 14% of the cases belonged to this category. In this context, it may also be speculated that most, if not all, cases of beta cell hyperfunction in adults that are not caused by an insulinoma are due to gain-of-function mutations [13], because of the inconspicuous appearance of the beta cells so far reported in these patients [2, 15, 28].

The third group of patients comprises the cases with focal beta cell disease, which account for approximately 30% to almost 50% of all PHHI cases. At the molecular level this disease is characterized by somatic (i.e. random) loss of maternal 11p15 alleles in the focally hyperplastic islet cells. The deletion includes not only the SUR1 and KIR6.2 loci, but also the regions of two growth-inhibitory genes, H19-IGF2 and P57KIP2 [9]. Obviously this maternal LOH unmasks a genetically transmitted mutation of the paternal SUR1 or the KIR6.2 gene [9, 10]. The net result of this two-hit LOH is then focal hyperinsulinism and beta cell hyperplasia due to increased proliferation. Currently it is unknown why this complex genetic defect affects only a limited population of beta cells in the pancreas, shows no preferential localization in the gland and, rarely, may also be multifocal.

Conclusions

Recent advances in molecular genetics have established a molecular basis for PHHI and resulted in the identification of a number of well-defined genetic defects. On the basis of the available information on the molecular changes so far described, the clinical appearance of the disease and the pancreatic pathology, PHHI patients have been tentatively classified into three groups: the first with SUR1/KIR6.2 mutations, severe disease and diffuse beta cell hyperfunction and hypertrophy; the second with GCK/GLUT1 mutations, later onset mild disease and diffuse beta cell hyperfunction without conspicuous hypertrophy; and the third with maternal LOH on chromosome 11.15 and paternally inherited recessive SUR1/ KIR6.2 mutation, severe disease and focal beta cell hyperfunction, hypertrophy and hyperplasia. While the first group of patients usually requires subtotal pancreatectomy to control the disease, the second group may be treated with medication and the third group by partial pancreatectomy.

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