Unfolding the mechanisms of disease progression in permanent neonatal diabetes

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DIABETES MELLITUS can be described as a collection of metabolic diseases characterized by fasting hyperglycemia. Although the most prevalent forms of diabetes mellitus are the polygenic forms that result in β-cell destruction or dysfunction, there exist monogenic forms that are diagnosed within the first six months of life, termed neonatal diabetes mellitus. This is a rare condition, occurring in one out of every 400,000 to 500,000 births, and can be either transient or permanent. Through numerous genetic studies, the cause of permanent neonatal diabetes mellitus (PNDM) can be traced to mutations in genes encoding for a number of β-cell functions, including pancreatic and islet development, the insulin-secretory response to glucose, and insulin biosynthesis (1). Such studies have resulted in elegant examples of “bedside-to-bench-to-bedside” science, in which genetic mutations are first identified in human populations, examined for their molecular and cellular effects on β-cell function, and the results applied to developing personalized approaches to disease management. For example, mutations in the K$_{ATP}$ channel components Kir6.2 (KCNN1) and SUR1 (ABCC8) have been identified as the major cause of PNDM (6). These mutations were found to result in a gain-of-function, which, when expressed in transgenic mice, recapitulated PNDM by impairing glucose-stimulated insulin secretion. As a result, patients harboring mutations in K$_{ATP}$ channels are treated with sulfonylurea drugs instead of insulin. Additionally, as novel mutations in K$_{ATP}$ channels are identified, the molecular mechanism of the disease can be studied on a case-by-case basis both for new treatments and to reveal novel aspects of K$_{ATP}$ function (2).

A similar story appears to be emerging with the discovery of mutations in the proinsulin gene (INS) in patients with PNDM. Ten heterozygous mutations in the human INS gene have been recently identified through linkage analysis of a group of patients in Chicago (8). Interestingly, the mutations occurred at biologically critical sites within the proinsulin molecule: the site of signal peptide cleavage, residues involved in disulfide bond formation, and at the C-peptide-A chain junction, a site for posttranslational processing. The sites of these mutations led to the hypothesis that the type of PNDM associated with mutations in the INS gene could result from the improper folding and/or processing of proinsulin.

In this issue, Rajan et al. (5) have addressed this hypothesis by examining the subcellular processing, trafficking, and secretion of the mutant proinsulins found in the Chicago probands. However, instead of simply generating the mutations within proinsulin and following trafficking patterns with traditional secretion assays and immunocytochemistry, they made use of an “all-in-one” construct with which they could follow subcellular trafficking in live cells, detect proinsulin processing, and directly measure glucose-stimulated insulin secretion. The construct design incorporated green fluorescent protein (GFP) into the C-peptide of intact proinsulin between Pro72 and Gly73 of preproinsulin (3). When expressed in MIN6 cells, this construct is normally trafficked from the Golgi to secretory granules, processed, and secreted in response to glucose. The beauty of the construct design is that GFP is a surrogate measurement for proinsulin and insulin in live cell imaging as well as in processing and secretion assays. By transfecting this construct into MIN6 cells, the authors were able to show that the proinsulin mutations associated with PNDM could be classified into three groups: 1) those that were retained in the endoplasmic reticulum (ER) and not processed to insulin and GFP-C-peptide, and impaired the secretion of wild-type insulin; 2) those that showed partial impairment in processing and trafficking and attenuated the secretion of wild-type insulin; and 3) those that showed partial impairment in processing and trafficking without affecting secretion of wild-type insulin. Therefore, it appears that there is some heterogeneity in the effects of INS mutations on β-cell function. These results are a nice example of how the tools of basic science can give insights into the importance of genetic screening for INS mutations in PNDM. These findings may ultimately lead to personalized management and treatments depending on the severity of impairment in β-cell function.

So what are the pieces of the puzzle that lead from mutations in the INS gene to impairments in β-cell function? As mentioned above, at least part of the answer lies in the folding and maturation events that occur within the insulin biosynthetic pathway. Proinsulin contains six cysteines distributed on the B and A chains to form three conserved disulfide bonds: the B7-A7 and B19-A20 interchain pairs and the A6-A11 intrachain pair. Biophysical modeling of free-energy landscapes has shown that proper folding is determined by successive disulfide bridge formation, starting with B19-A20 (9). Therefore, mutations occurring at or near sites of disulfide bridge formation are predicted to have a severe impact on folding. Indeed, previous studies have shown that mutations at either A7 or B19 cause improper folding of proinsulin in vivo (4). And a very recent study has shown that mutation of residue B5, which is in an evolutionarily conserved region in close proximity to the B7-A7 disulfide bridge, also impairs proinsulin folding (7). Taken together, the above studies illustrate how the genetics of PNDM has given insight into the biophysical mechanisms and evolutionary constraints of proinsulin folding.

What is the impact of misfolded proinsulin on β-cell function? Rajan et al. have now shown that mutations C69Y, on residue A7, and C43G, on residue B19, were retained in the
ER, were not processed to mature insulin, and, importantly, impaired glucose-stimulated secretion of wild-type proinsulin (5). The other mutant proinsulins that may be misfolded also attenuated the degree of secretion of wild-type proinsulin. Such a dominant negative effect of misfolded proinsulin on insulin secretion may account for the abnormally low C-peptide levels seen in the Chicago proband (8).

One mechanism of the dominant negative effect of misfolded proinsulin was revealed by Liu and colleagues. In studies utilizing the diabetic Akita mouse, which harbors the same A7 (C69Y) mutation as described by Rajan et al. Liu et al. (3) demonstrated specific and covalent association of the mutant proinsulin with wild-type proinsulin. They proposed that increased disulfide mispairing at B7-A7 leads to this association, resulting in inhibition of ER exit of wild-type proinsulin in trans. This mechanism could explain the lower insulin levels seen in PNDM patients with mutations in the INS gene.

Rajan et al. used another approach to investigate the mechanisms of the dominant negative effect of the mutant proinsulins on insulin secretion. They devised an “optical assay”, whereby a proinsulin-mCherry construct was coexpressed along with the proinsulin-GFP mutants. In this way, the trafficking of wild-type proinsulin in the presence of the proinsulin mutants could be visualized in live cells. Interestingly, their results did not appear to corroborate those of Liu et al. (3), as proinsulin-mCherry was localized in secretory granules, and therefore appeared to normally progress through the secretory pathway, in the presence of the mutant proinsulins that were retained in the ER. Since levels of the ER stress protein Chop were elevated upon overexpression of any of the mutant proinsulins, Rajan et al. speculated that the dominant negative effect might occur through inhibition of proinsulin translation resulting from ER stress.

The next question, then, is how the β-cell responds to ER stress induced by misfolded proinsulin. Blocking ER exit could activate a cascade of events, such as induction of the ER stress response, ER-associated degradation of proinsulin, and β-cell apoptosis. Can misfolded proinsulin have an impact on β-cell survival? Using the GFP-proinsulin construct, Liu et al. (3) showed that the misfolded A7 mutant actually caused β-cell toxicity; INS-1 cells overexpressing the A7 mutant showed decreased survival over time compared with cells overexpressing wild-type proinsulin (3). Whether the other mutant proinsulins described by Rajan et al. also cause β-cell toxicity through ER stress remains to be seen.

The experiments investigating the intracellular behavior of mutant proinsulins provide a beautiful example of “bedside-to-bench-to-bedside” research. Further studies may reveal phenotypic differences at the level of ER stress, which may translate into more targeted therapies to manage the pathogenesis of PNDM.

REFERENCES