PTP1B: a new therapeutic target for Rett syndrome

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Methyl-CpG-binding protein 2: linking epigenetics to neuronal function

Over 95% of classic Rett syndrome (RTT) cases are linked to mutations in a single gene that encodes methyl-CpG-binding protein 2 (MECP2) (1, 2), one of six mammalian methyl-CpG-binding proteins identified so far. These proteins specifically bind DNA methylated at the 5’ position of cytosine within cytosine-guanine (CpG) dinucleotide sequences, a process that constitutes an important gene-silencing mechanism in eukaryotes and is essential for viability (3). MECP2 dysfunction results in various neuropsychiatric disorders that include mild mental retardation, learning disabilities, autism spectrum disorders, and complex forms of severe mental retardation (1, 2). In RTT, numerous, mostly spontaneous mutations have been identified in MECP2 that result in a loss of MECP2 function. Several Meep2-KO mouse models have been generated to investigate RTT pathology and to potentially identify and test new therapies (2). MECP2-deficient mice manifest many of the predominant characteristics of human RTT. The traditionally held belief is that the neurological and psychiatric features caused by RTT-associated failures that occur during brain development are irreversible; however, studies in MECP2-deficient mice suggest that normal brain function can be restored by reinstating MECP2 (4). These results are promising, because they suggest that it may be possible to improve neuronal function in patients with RTT. However, therapeutic approaches aimed at altering MECP2 levels in patients will likely require a more complex strategy, as both loss of MECP2 function and elevated MECP2 levels have deleterious neurological effects. Thus, current basic and clinical research has focused on identification of critical factors downstream of MECP2 that have altered expression or function and may directly cause neuronal defects in RTT. Candidate molecules include brain-derived neurotrophic factor (BDNF) and IGF-1, both of which are currently the focus of many investigations (5–8). Approaches that elevate BDNF expression and those using BDNF mimetics have shown promising results in RTT animal models (9–12), while phase I clinical trials of recombinant IGF-1 treatment in patients with RTT have produced encouraging outcomes (13).

PTP1B: linking MECP2 to insulin signaling

Several lines of evidence suggest that RTT is associated with metabolic disorders. First, lipid metabolism is disturbed in both RTT mouse models, in which cholesterol levels are elevated in the brain (14), and in RTT patients, in whom plasma levels of cholesterol regulatory proteins are altered (15). Second, various studies found typical signs of insulin and leptin resistance as well as obesity in RTT animal models (16, 17). Moreover, insulin resistance has also been reported in patients with RTT (18). In this issue, Krishnan and colleagues investigated the metabolic processes associated with RTT (19). The authors examined glucose metabolism and insulin signaling in male Mecp2+/y and female Mecp2−/− mice and found that compared with WT littermates, MECP2-deficient mice had markedly higher blood glucose levels, suggesting both glucose and insulin intolerance. Moreover, insulin-induced tyrosine phosphorylation of both the insulin receptor and insulin receptor substrate-1 (IRSI) were attenuated in Mecp2+/y and Mecp2−/− mice, despite the increased levels of insulin in these animals compared with levels in controls. Finally, insulin-induced phosphorylation of key signaling molecules downstream of the insulin receptor, including AKT, FOXO, and GSK3β, was attenuated in Mecp2-mutant mice. Together, these data demonstrate that typical features of insulin resistance are present in the RTT animal model. Krishnan et al. specifically tested the effects of Mecp2 loss on gene expression in the mouse forebrain to identify candidate effectors that control insulin signaling and glucose metabolism. Ptpn1, which encodes the protein tyrosine phosphatase PTP1B, was among the four genes that were substantially upregulated in MEPC2-deficient mice compared with WT animals. PTP1B is a major metabolic regulator that inhibits insulin signaling by directly dephosphorylating the insulin receptor and IRS1 (20) and reduces leptin signaling by dephosphorylating the leptin receptor–associated tyrosine kinase JAK2 (21, 22). Krishnan
and colleagues demonstrated that MECP2 directly binds to the PTPN1 promoter and suppresses transcription. In contrast, an RTT-associated MECP2 mutant (MECP2-R168X) did not suppress transcription of PTPN1. Together, these data indicate that PTPN1 is a direct target of MECP2 and that loss of MECP2 function in RTT elevates PTP1B levels and thereby impairs insulin signaling and glucose metabolism.

PTP1B inhibitors: improving symptoms and survival in RTT animals

Krishnan and colleagues (19) evaluated two previously characterized small-molecule inhibitors of PTP1B, the difluoromethylphosphonic acid CPT157633 (23) and the ursolic acid derivative UA0713 (24), in RTT mouse models to evaluate PTP1B inhibition as a potential therapeutic strategy for RTT (Figure 1). PTP1B has been investigated as a drug target for type 2 diabetes for more than 15 years (25), and there are multiple inhibitors available. The choice of Krishnan et al. to test the PTP1B inhibitor CPT157633 seems well reasoned, given the excellent potency and relative selectivity for PTP1B demonstrated for this compound. Moreover, NMR chemical shift perturbation and x-ray crystallography experiments both confirmed that CPT157633 binds the PTP1B active site, further validating CPT157633 as a potent, competitive, and reversible inhibitor. Moreover, in light of the common difficulties related to in vivo bioavailability and specificity of active-site tyrosine phosphatase inhibitors (26, 27), the commitment of Krishnan and colleagues to add a second compound with a presumably different mode of PTP1B inhibition is laudable. However, the choice of UA0713, a rather weakly characterized compound in terms of both PTP1B-specific binding and mode of inhibition, is somewhat less convincing, given that other options are available, including an allosteric PTP1B inhibitor with proven in vivo efficacy and mode of action previously studied.
by the same research group (28). Regardless, the data obtained from the inhibitor experiments are quite compelling. Compared with vehicle-treated controls, male Mecp2−/− mice given CPT157633 (5 mg/kg daily) or UAO713 (5 mg/kg every other day) exhibited a marked reduction in glucose intolerance, an increase in body weight, and a decrease in circulating levels of insulin and cholesterol just two weeks after initiating treatment. Moreover, PTPIB inhibition improved survival of Mecp2−/− mice by increasing lifespan approximately two-fold. CPT157633 treatment yielded similar, beneficial results in female Mecp2−/− mice, which are a closer reflection of RTT in humans. In addition, PTPIB inhibitor–treated MECP2-deficient mice were subjected to distinct behavioral tests, including paw clapping, regression of motor skills, and pup gathering, to assess the effect of treatment on phenotypic symptoms. Impressively, CPT157633 treatment reduced paw clapping and latency to gather pups in MECP2-deficient animals to levels observed for WT control mice. Motor skills, as measured by rotaror performance, were only partially restored by CPT157633 treatment; however, the modest improvements were lost once CPT157633 treatment was stopped, suggesting that the effects of PTPIB inhibition are reversible. On the molecular level, PTPIB inhibitor treatment resulted in enhanced tyrosine phosphorylation of both the insulin receptor and IRS1, as expected. Interestingly, other anti-diabetic drugs, including metformin, rosiglitazone, and AICAR, improved glucose homeostasis in MECP2-deficient animals but did not extend lifespan, suggesting that PTPIB inhibition alters additional signaling events critical for the RTT phenotype.

TRKB: linking PTPIB to BDNF signaling

Consistent with earlier reports, BDNF levels were decreased by 30% to 40% in both Mecp2−/− and Mecp2+/− mice compared with levels in control animals. This observation suggested that weakened BDNF signaling through its cognate receptor tropomyosin-related kinase B (TRKB), but not loss of BDNF itself, could be a major contributing factor to RTT development. Indeed, tyrosine phosphorylation (activation) of TRKB was attenuated in Mecp2−/− mice compared with that observed WT mice. Moreover, treatment with the PTPIB inhibitor CPT157633 enhanced tyrosine phosphorylation of TRKB in both WT and Mecp2−/− mice. Krishnan et al. used a recombinant PTPIB substrate–trapping mutant (PTPIB-D181A), which forms a stable complex with the phosphorylated substrate, and demonstrated that PTPIB-D181A, but not WT PTPIB, binds to TRKB in mouse brain lysates (19). Further, PTPIB-D181A was added to lysates from cells overexpressing either WT TRKB or one of three TRKB isoforms in which tyrosine residues were mutated to phenylalanine to determine the exact tyrosine residue(s) targeted by PTPIB. The results of these assays demonstrated that PTPIB specifically targets the TRKB activation loop tyrosines Y706 and Y707 (Figure 1). Consistently, CPT157633 treatment enhanced tyrosine phosphorylation of Y706/Y707 in the brains of Mecp2−/− mice. Tyrosine phosphorylation of the closely related TRK receptors TRKA and TRKC was less affected by CPT157633 treatment. Together, these data demonstrate a previously unrecognized role for PTPIB as an inhibitor of BDNF signaling and suggest a possible mechanism by which PTPIB promotes RTT phenotypes. Interestingly, impaired signaling through TRKB has been associated with severe hyperphagia and obesity in both mice (29) and humans (30), suggesting that the role of PTPIB in obesity may go beyond its previously known effects on leptin receptor signaling.

PTPs: drugging the undruggable

Protein tyrosine phosphatases (PTPs), which comprise a family of more than 100 members in humans, are important signaling molecules that have been considered as potential drug targets in many human diseases and conditions, including cancer as well as cardiovascular, immunological, infectious, neurological, and metabolic diseases (26, 31). However, past efforts to develop drugs that target specific PTPs have been plagued by issues related to the bioavailability and selectivity of these compounds, as the vast majority of reported PTP inhibitors carry a phosphotyrosine-mimicking (pTyr-mimicking) group that interacts strongly with a highly conserved phosphate-binding loop in the active site. Consequently, active-site PTP inhibitors often fail in preclinical development due to off-target effects and suboptimal pharmacokinetic properties, which are caused by the charged nature of the pTyr mimic. Indeed, despite the great interest in PTPIB inhibitors as anti-diabetic drugs over the past 15 years, few compounds have progressed to clinical trials, and so far none of these have advanced beyond phase II testing. Several new approaches to overcome these hurdles have been proposed (26). Notably, allosteric inhibition of PTPs has gained substantial traction recently, including allosteric inhibition of PTPIB (28). Previous high-throughput screening (HTS) efforts to find PTP inhibitors were mostly based on simple biochemical assays that used truncated PTPs and small pTyr mimics as substrates, a combination that favors binding distal from the active site. Novel HTS strategies, preferably those that can be applied under physiological conditions, will be necessary to identify PTP inhibitors that are not restricted in their mode of action, bind to allosteric sites, interfere with target localization, or block substrate binding.

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