New Drugs Restore Brain Balance to Treat Rett Syndrome

用恢复大脑平衡的新药物治疗雷特综合征

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The ultimate goal of Rett syndrome research is to find a cure. Researchers, patients with Rett syndrome and their families alike look forward to a day when a drug or gene therapy can totally reverse the symptoms of Rett syndrome. Informed by the wealth of insights from preclinical research, drug discovery aimed at disease target genes has the potential, in the interim, to provide symptomatic relief. As researchers in the lab of Rudolf Jaenisch at the Whitehead Institute for Biomedical Research, we've been conducting research on chemical compounds that can restore brain balance and reverse symptoms found in mouse models of Rett syndrome. The work, led by post-doctoral fellow Xin Tang was recently published in Science Translational Medicine.

研究雷特综合征的最终目的是找到一种治愈的方法。众多研究者，雷特综合征的患者及其家属们都在期待某一天有一种药物或基因疗法可以完全逆转雷特综合征的各种症状。不过，各种临床前研究中的大量有价值的信息告诉我们，在完全治愈的方法面世之前，提供针对某些靶向基因的药物有可能会缓解疾病的症状。我们作为 Whitehead 生物医学研究所 Rudolf Jaenisch 实验室的研究人员，一直致力于寻找可以恢复大脑平衡的化学化合物用以逆转雷特综合征小鼠模型中的症状。最近，这项由 Xin Tang 博士后领导的相关工作成果已经在 Science Translational Medicine 杂志上发表。

MECP2, the mutated gene responsible for Rett syndrome, produces a protein that regulates the expression, or protein production activity, of hundreds of genes in the brain. The neurological dysfunction in Rett is likely not directly caused by the mutated MeCP2 protein itself, but rather by the misregulation of protein expression of these downstream genes that occurs in the absence of functional MeCP2 protein in the brain cells. Our group investigates these downstream genes with an aim to fix their expression. Because these genes are directly responsible for brain function, fixing them is likely to result in symptomatic relief in patients with Rett syndrome.

MECP2 是导致雷特综合征的突变基因，它产生一种蛋白质，能调节大脑中数百个基因的表达或蛋白质生成活性。雷特患者的神经功能障碍并不是由突变的 MeCP2 蛋白质本身直接引起的，而是因为脑细胞中缺乏足够的有功能性的 MeCP2 蛋白质时，受影响的下游基因的蛋白质表达量被错误调节而引起的。我们研究小组深入研究了这些下游基因，目标是设法修正它们的表达量。 因为这些下游基因直接影响大脑功能，修正它们的表达量有可能会缓解雷特综合征患者的症状。

Our previous work[1, 2], as well as reports from other researchers[3-5], has provided strong evidence to show that one important downstream target of MeCP2 is a gene called KCC2. In Rett syndrome, when MeCP2 protein is not working properly, the expression level of KCC2 goes down substantially. We believe that abnormalities in KCC2 expression are responsible for much of the neurological symptoms in Rett syndrome. KCC2 serves as a pump that actively removes chloride ions from neurons. The pumping activity of KCC2 is absolutely critical for maintaining the delicate balance in the brain between excitation and inhibition that allows

for the complex motor and social functions in people. When this balance is broken, symptoms such as seizures, loss of motor coordination, and social communication problems arise. Therefore, reduction in the expression of KCC2 in Rett syndrome has direct effects on brain function.
我们先前的工作[1,2]以及一些其他研究人员的报告[3-5]都提供了强有力的证据，表明 MeCP2 蛋白质调节的一个重要下游目标是一个名为 KCC2 的基因。对雷特综合征患者来说，当 MeCP2 蛋白质无法正常工作时，KCC2 的表达量也显著下降。我们认为，KCC2 表达量的异常是雷特综合征患者身上大部分神经系统症状的原因。KCC2 本身类似一个泵，用来去除神经元中的氯离子。KCC2 泵的工作非常关键，它可以维持大脑在兴奋和抑制之间的微妙平衡，这也是支撑人类各种复杂运动和社交功能的基础。当这种平衡被打破时，就会出现诸如癫痫发作，运动协调能力丧失，以及社交沟通问题。所以，雷特综合征中 KCC2 表达量的降低是对脑功能有直接影响的。

With funding support from RSRT, we aimed to develop drugs that are capable of activating KCC2 gene expression in Rett syndrome neurons. Advantages of a drug-based therapeutic approach are ease of delivery and the ability to control dosing. Using a novel gene-editing tool called CRISPR, we altered the genetic makeup of human stem cell-derived neurons in culture dishes by inserting a biological light-producing enzyme called the “Firefly luciferase” right next to the KCC2 gene so that they become activated together. In this way, the amount of KCC2 produced in a neuron is directly proportional to the amount of light generated from it. We then developed a screening assay that took advantage of the sophisticated robotics at MIT’s high-throughput screening core facility to test a large number of drugs to see if any drugs made the cells glow more, thus increased KCC2 production. After testing hundreds of drugs, we found a total of 30 KCC2 expression-enhancing compounds, or more simply abbreviated, KEECs. In further testing, we found that KEECs not only activated the production of KCC2 protein, but also fixed the high intracellular chloride level and defective inhibition in human Rett syndrome neurons lacking the MECP2 gene.
在 RSRT 的资助下，我们开始了针对能激活雷特综合征的神经元里 KCC2 基因表达药物的研发。基于药物的治疗方法的优点是其易于递送到人体且能够控制给药剂量。我们使用了一种名为 CRISPR 的新型基因编辑工具，通过在 KCC2 基因下游插入一种名为“萤火虫荧光素酶”的生物发光酶基因来改变培养皿中人体干细胞衍生的神经元的基因组，使这两个基因能够一起被激活。通过这种方式，可以让神经元中产生的 KCC2 的量与其产生的荧光强度成正比。然后，我们开发了一种筛选试验方法，通过麻省理工学院高通量化合物筛选核心设施中的大量复杂的自动化机器人设备来测试一大批药物，看看是否有某种药物能够让细胞发出更强的荧光，也就是产生更多的 KCC2。经过了数百种药物的测试后，我们总共发现了 30 种能够增强 KCC2 表达的化合物，姑且先简单地把它们缩写为 KEECs（KCC2 Expression Enhancing Compounds）。在进一步的测试中，我们发现 KEEC 不仅激活了 KCC2 蛋白的生成，而且还修复和抑制了缺乏 MECP2 基因的雷特综合征人体神经元中的过高的细胞内氯化物水平。

Having narrowed down our drug candidates, we moved from testing on Rett syndrome neurons in a dish to mouse models of Rett syndrome. These mice that lack the mouse version of the human MECP2 gene and mirror the signs of human patients with Rett syndrome in many ways: they have irregular breathing patterns,

coordination and movement problems, social communication problems, and, very importantly, reduced KCC2 expression in the brain. In collaboration with Mriganka Sur's lab at the Picower Institute at MIT, we treated the mice with two KEECs, KW-2449 and Piperine, and observed encouraging results: the mice injected with KEECs began moving significantly more and had reduced occurrence of abnormal breathing pauses, compared to the mice from the same litter that had not received drug treatment. The Rett syndrome-like signs in these mice were reversed after treatment with these drugs that restore brain balance.

缩小我们这些候选药物的范围后，我们开始从测试培养皿中的雷特综合征神经元转向雷特综合征的小鼠模型。这些小鼠也缺乏 MECP2 基因（人类 MECP2 基因的小鼠版本），并且它们在许多方面反映了患有雷特综合征的人类患者的症状：它们具有不规则的呼吸节律，有运动和协调性问题，有社交沟通问题，而且非常重要的是，它们的大脑中 KCC2 的表达量也有减少。我们与麻省理工学院 Picower 研究所的 Mriganka Sur 实验室合作，尝试用两种 KEEC（KW-2449 和 Piperine）治疗这些 mecp2 突变小鼠，我们发现了令人鼓舞的结果：那些注射了 KEECs 的小鼠，相比其同一窝的未接受药物治疗的 mecp2 突变小鼠，开始有更显著的运动量增加，而且异常的呼吸暂停的现象发生率发生了显著的降低。也就是，使用这些恢复大脑平衡的药物治疗后，这些小鼠身上类似雷特综合征的症状被逆转了。

Before we can speak to the effectiveness of our KCC2 expression enhancing compounds, they must of course be tested in clinical trials in patients with Rett Syndrome. Since most of the drug candidates we screened are either natural products available as food supplements or are already FDA-approved for the treatment of other diseases we hope that clinical trials can be expedited. Furthermore, all of our drug candidates are small molecules that can be safely administered through an oral capsule. Our research marks the beginning of a therapeutic strategy to restore brain balance in patients with Rett syndrome that we hope will be safe, convenient, effective, and in clinics in the near future.

当然，在我们能谈及这些增强 KCC2 表达的化合物的临床有效性之前，它们必须在雷特综合征患者身上完成临床试验的验证。由于大多数我们筛选的候选药物都选自天然产物，或者已经获 FDA 批准作为膳食补充剂或者用于治疗其他疾病，所以我们期待临床试验可以加快进行。此外，所有我们的候选药物都是小分子药物，可以通过口服胶囊形式安全地给药。我们的研究标志着这种针对雷特综合征的，通过恢复大脑平衡的新治疗策略的开始。我们希望在不久的将来，这类治疗方式能够在临床上以安全，方便，有效的方式得到应用。

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