Harnessing Exosomes as a Therapeutic for Rett Syndrome

利用外泌体作为雷特综合征的治疗方式

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I am a scientist at Scripps Research Institute in La Jolla, California working in the lab of Professor Hollis Cline. thirst for knowledge is what originally attracted me to science. The potential to contribute, even in a small way, alleviating suffering drives that thirst and passion even more.

我是一名在加州 La Jolla 市 Scripps 研究所 Hollis Cline 教授实验室工作的科学家。对知识的渴求是最初让我对科学产生兴趣的原因。而让这种渴望和激情更加强烈的则是感受到我的工作能潜在性的为减轻他人痛苦做出贡献，即便是以再微弱的方式。

Human biology has always fascinated me. Imagine for a moment how the human body is created. It starts with a single cell that multiplies to create a complex organism of trillions of cells. The human brain alone is estimated to contain more than 150 billion cells, 86 billion neurons and about an equal number of non-neuronal cells, all of a wide variety of specializations. It is mind boggling to imagine that a few founder cells contain the programming information that, through a series of cell fate decisions, produces a complex organ like the brain. What kind of communication and logistics are required to orchestrate the development and function of this behemoth?

人体生物学一直让我着迷。想象一下人体是如何生成的。从一个单细胞开始，不停复制，最后生成一个由数万亿个细胞组成的复杂有机体。据估计，仅人类大脑就包含超过 1500 亿个细胞、860 亿个神经元和与其数量相当的非神经元细胞，所有这些细胞都具有各种各样的特殊功能。确认很难想象，仅一小部分初始细胞包含的编程信息，然后通过一系列细胞的命运决定，最后产生了像大脑这样复杂的器官。协调这个庞然大物的发展和功能到底需要什么样的沟通和后勤保障机制?

One requirement is a package delivery mechanism; think of it as the body's UPS, which allows information and material transfer between cells. Over the past decade, researchers have discovered that our bodies employ amazing inter-cellular couriers called exosomes or extracellular vesicles to transport fundamental biomolecules like proteins, nucleic acids and lipids. Exosomes can also perform additional duties, such as scouting and laying the path for a growing axon or migrating cells. For example, cancer cells use exosomes to lay the foundation of their migration out of a tumor, leading to metastasis.

其中一个需求是输送机制；把它想象成身体的 UPS 快递，让信息和物质在细胞间传递。在过去的十年中，研究人员发现，我们的身体使用了一种神奇的细胞间信使来运输蛋白质、核酸和脂类化合物等基本的生物分子，这些信使称为外泌体或细胞外囊泡。此外，外泌体还可以执行额外的任务，比如为生长中的轴突或迁移的细胞寻找和铺设路径。举例来说，癌细胞就是利用外泌体为它们从肿瘤外迁移奠定基础，从而导致转移。

Our work has uncovered a fundamental role of exosome communication in brain development. We show that exosomes secreted by neurons contain signals to direct the development and function of neural circuits. Importantly we have discovered that exosomes have the potential to become therapeutics for neurodevelopmental disorders, including Rett Syndrome.

我们的工作揭示了外泌体的通讯在大脑发育中的基础作用。我们发现，由神经元分泌的外泌体包含着指引神经回路的发育和功能的信号。重要的是，我们已经发现外泌体有潜力被开发为针对各种神经发育疾病（包括雷特综合征）的治疗药物。

Our brain works like a musical ensemble. The neurons fire to produce a pattern of activity very much like an ensemble of musicians playing together to produce a melody. Historically, a vast majority of studies directed towards understanding brain function focused on the skills of the individual neurons or their training together in producing a melody. We found that when these musicians in our brain called neurons, hang together and socialize, they use exosomes to communicate between themselves. These exosomes contained messages that provided them great collective motivation and were extremely helpful in their training and performance. Extending this analogy to the case of Rett Syndrome, Rett neurons practice very hard but are unable to play together and produce a melody. Rett neurons not only lacked some music skills, they had problems coordinating their music with each other. We found that the Rett exosome no longer contained motivating messages to help the neurons with their music skills and coordination.

我们的大脑就像一个音乐团。多个神经元一起发出某种活动模式的信号，就像一群音乐家在一起演奏一段旋律。从历史上看，绝大多数旨在理解大脑功能的研究都集中在单个神经元的功能上，或者训练它们一起来产生一段旋律。我们发现，当我们大脑中这些被称为神经元的音乐家们聚集在一起并进行沟通社交活动时，他们会利用外泌体互相交流。这些外泌体包含的信息为他们的聚集提供了非常大的促进，对他们的训练和表现非常有帮助。类比延伸到雷特综合症的情况，Rett 神经元也非常努力地练习，但是无法一起演奏和产生一段旋律。Rett 神经元不仅缺乏某些音乐技能，而且他们的音乐也在相互协调方面有问题。我们发现 Rett 外泌体不再含有那些帮助神经元提高音乐技能和协调能力的激励信息。

We thought that maybe if we take exosomes from healthy neurons and give them to Rett neurons, it will provide them the message they are lacking and help motivate them to play a melody. Remarkably, the exosome message from healthy neurons let Rett Syndrome neurons overcome their shortcomings and fire together in a synchronous way to produce a melody.

我们想到，如果能从健康的神经元中提取外泌体并把它们交给 Rett 神经元，或许就能给 Rett 神经元提供它们所缺乏的信息，从而帮助它们演奏旋律。我们的结果非常明显地证明了来自健康神经元的外泌体信息让 Rett 神经元克服了它们的缺点，能够以同步的方式共同发出信号产生一段旋律。

For the scientifically inclined readers I'll provide a more scientific description. All cells in the brain secrete exosomes. However, it was not very clear what function the exosomes perform in the brain. We purified exosomes from functional neural cultures and asked, could these exosomes contain a bioactivity to perform any function in a developing neural circuit? We observed that exosome treatment led to an increase in neuronal number. This led to a further question – if exosomes have a role in developing neural circuits, what happens when the neural development is deficient? A good way to find that out is to compare exosomes from healthy neurons to exosomes from neurons with a neurodevelopmental disorder.

对于有进一步兴趣的读者，我将提供一个更有科学性的描述。大脑中所有的细胞都分泌外泌体。然而，目前还不是很清楚外泌体在大脑中起什么作用。我们从功能性神经组织培养物中纯化外泌体并提出问题，这些外泌体是否

含有某种可以在神经回路发育中发挥作用的生物活性的成分?我们观察到外泌体治疗会导致神经元数目的增加。

这就引出下一个问题——如果外泌体在神经回路发育中有某种作用，那当神经发育不良时发生了什么?一个很好的方法是比较健康神经元的外泌体和有神经发育障碍的神经元的外泌体。

We decided to explore this question by experimenting with induced pluripotent stem cells (iPSC) from a Rett Syndrome patient. This audience knows very well that Rett is caused by disruption of a single gene, MECP2. We restored the function of the MECP2 gene in the iPSCs using CRISPR gene editing. We therefore had two human iPSC neural cultures that are identical to each other genetically except in the function of just one protein, MECP2. This was an ideal setup to study the fundamental role of exosomes in normal neural circuit development and compare it to a condition where neural circuit development is deficient.
我们决定对一名雷特综合征患者的诱导多能干细胞(iPSC)进行实验来探索这个问题。读者们都很清楚，雷特综合征是由一个单基因 MECP2 被破坏导致的。我们利用 CRISPR 基因编辑技术恢复了 iPSC 中 MECP2 基因的功能。这样，我们就有了两种人类 iPSC 神经组织培养物，它们在基因上除了 MECP2 外是完全相同的。这是一个非常理想的基础设置，可以研究外泌体在正常神经回路发育中的基础作用，并将其与神经回路发育不良的情形进行比较。

The Rett patient iPSC derived neural cultures displayed cellular and circuit manifestations of Rett Syndrome, whereas CRISPR corrected controls were normal. We then purified exosomes secreted by each culture yielding normal control exosomes and Rett exosomes and compared them. Our results were so remarkable that it took us a while to appreciate them.

雷特患者的 iPSC 衍生神经培养物显示出雷特综合征的细胞和神经回路表现，经而 CRISPR 修正后的对照组是正常的。接着，我们纯化了每个培养物分泌的外泌体，得到正常的对照外泌体和 Rett 外泌体，并对它们进行了比较。我们的结果非常的显著，这让我们甚至花了好一段时间来欣赏它们。

First, exosomes were full of proteins that are important in development of neurons and formation and maintenance of synapses. Synapses are conduits of electrochemical information flow between neurons, and are critical to proper brain function.
首先，外泌体富含对神经元的发育、突触的形成和维持都很重要的蛋白质。突触是神经元之间传递电化学信息的通道，对正常的大脑功能至关重要。

Second, the Rett exosomes displayed specific alterations in the signaling capacities, like proliferation, neural development, and synaptic function. In short, we found that normal exosomes could potentially guide the proliferation, neuron development and synapse function, and Rett exosomes are somewhat deficient in that function.
其次，Rett 外泌体在信号传递能力上表现出特定的改变，比如在增殖、神经发育和突触功能上。简而言之，我们发现正常的外泌体具有潜在的指导增殖、神经元发育和突触功能的作用，而 Rett 外泌体在这方面存在一定的缺陷。

Taking cues from these results, we compared the bioactivity and found that normal exosomes boosted proliferation of neural stem cells and Rett exosomes did not. In addition, normal exosome treatment led to a big increase in neural progeny and modest increase in astrocyte progeny; astrocytes are another cell type in the brain that have a range of ancillary functions. In comparison, Rett exosome treatment, while it lacked the

capability to increase neural progeny, still directed the modest increase of astrocyte progeny. This result shows that Rett exosomes retain some functions, but their neural specific functions are lacking.
顺着这些结果的线索，我们又比较了生物活性，发现正常的外泌体促进了神经干细胞的增殖，而 Rett 外泌体则没有。而且，正常外泌体作用能引起神经子代的大量增加，星形胶质细胞子代的适度增加（这里星形胶质细胞是大脑中另一种具有一系列辅助功能的细胞）。相比之下，Rett 外泌体作用缺乏增加神经子代的能力，不过仍能引导星形胶质细胞子代的适度增加。这项结果表明，Rett 外泌体保留了部分功能，但缺乏神经相关的特定功能。

However, the most important question was still nagging us. Could treatment with normal control exosomes rescue deficits in Rett Syndrome neural cultures? After an onerous journey of problem solving and establishment of assays, we successfully demonstrated that treatment of Rett neural cultures with normal control exosomes could increase neuron number, boost the number of synapses and make neurons fire in a more synchronized way. Importantly, exosome treatment showed improvements at the cellular, synaptic, and functional level.
然而，最重要的问题仍然困扰着我们。使用正常对照外泌体治疗能否挽救雷特综合征的神经培养物中的缺陷?经过一段艰难的解决问题和建立并评估方法的旅程，我们成功地证明了用正常对照外泌体来治疗 Rett 神经培养物能够增加神经元数量，增加突触数量，并让神经元以更同步一致的方式发出信号。重要的是，外泌体治疗显示出在细胞、突触和功能水平的改善。

While a very exciting result, we wanted to take this a step further into live animals. So we took healthy exosomes and injected them into the brain of developing mice and monitored neuronal proliferation in hippocampus, a brain area important for learning and memory. Exosome injections led to a remarkable boost in neuronal proliferation in hippocampus, just like human in-vitro disease models. This showed that if delivered to the brain in live animals, the exosomes can deliver the promised bioactivity.

虽然这是一个非常激动人心的结果，但我们想更进一步，研究活体动物。因此，我们将健康神经组织分泌的外泌体注射到正在发育的小鼠的大脑中，并监测海马区神经元的增殖（海马区是大脑中对学习和记忆非常重要的区域）。外泌体注射可以显著促进海马区神经元的增殖，就像人类体外疾病模型一样。这表明，如果将外泌体传输到活体动物的大脑中，外泌体就可以传递它们预期的生物活性。

I believe exosomes have immense therapeutic potential as they have inherent advantages. Unlike stem cells, there is no possibility that they can go rogue and form tumors. Importantly, exosomes do not elicit an immune response when injected into the patient. Exosomes can be sourced from cultured neurons made from the patient's own cells, providing personalized medicine.

我相信外泌体具有巨大的治疗潜力，这是因为它们有自身固有的优势。与干细胞不同，它们不会失控增殖从而形成肿瘤。而且很重要的是，外泌体在注射到患者体内时不会引起免疫反应。外泌体也可以从患者自身细胞培养的神经元中获得，这可以提供个性化的药物。

Neural exosomes are thought to contain signals that guide the exosome to the brain. They can be loaded with any therapeutic drugs or molecules developed for Rett Syndrome and delivered to the brain. Our future work will focus on optimizing exosomes for specific and efficient delivery to the brain; finding the least invasive way of delivering exosomes to the brain; and showing that exosomes can be used to rescue disease in a mouse model of Rett Syndrome.

人们通常认为神经外泌体含有引导外泌体进入大脑的信号。它们可以装载任何用于治疗雷特综合征的药物或分子，将其传送到大脑。我们未来的工作将集中于下面几个方面：优化外泌体使其能够特定、高效地传递到大脑；寻找对脑组织损伤最小的方法将外泌体输送到大脑；使用雷特综合征的小鼠模型证明外泌体的治疗效果。

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