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Drugs to awaken a paternal gene

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Abstract

Mutations in the maternal copy of the *UBE3A* gene cause a neurodevelopmental disorder known as Angelman syndrome. Drugs that activate the normally silenced paternal copy of this gene may be of therapeutic value.

Angelman syndrome is characterized by intellectual disability, epilepsy, impaired coordination and a characteristic joyful demeanour. Most commonly, it is caused by a deletion of five to six megabases of DNA from chromosome 15, although ‘loss-of-function’ mutations, or other abnormalities, in the maternal copy of the *UBE3A* gene also result in Angelman syndrome. In all cases, the outcome is very low expression levels of the enzyme UBE3A in neurons.

UBE3A is one of a handful of human genes that are subject to genomic imprinting, whereby either the maternal or the paternal copy of a gene is active, with the other copy remaining silent. In the case of *UBE3A*, only the maternal copy is active in neurons. So a child develops Angelman syndrome if they inherit mutant *UBE3A* from their mother. Indeed, in all cases of the disorder, the paternal copy of *UBE3A* is normal. In a paper published on *Nature*’s website today, Huang *et al.*¹ use drug screening to identify compounds that activate the paternal copy of *UBE3A* in a mouse model of Angelman syndrome.

Inherited neurological disabilities in children are notoriously resistant to treatment. For some disorders that are characterized by overt abnormalities in the brain (for example, holoprosencephaly, lissencephaly and agenesis of the corpus callosum), the prospects for major interventions after birth remain gloomy. But there may be more room for optimism in conditions in which the brain architecture and function seem quite normal in early infancy — for example, as in fragile X syndrome, Rett syndrome, Angelman syndrome and Prader–Willi syndrome. In these cases, one could hope that the disorder might be cured by restoring normal gene expression.

Other than gene therapy, or perhaps gene correction or recovery of function of a mutant protein, a potential strategy for treating some disorders might be activating the alternative copy of a mutant gene or a gene related to it. For instance, activating the expression of fetal haemoglobin has been a long-term goal for the treatment of sickle-cell anaemia and β -thalassaemia. For many disorders involving genomic imprinting, activating the silenced copy of the gene on the related chromosome may correct the defects associated with the mutant gene.

Mice are useful models for testing whether postnatal restoration of gene expression can overcome the pathological features of a disease. A mouse model of Rett syndrome has, for instance, been tested² in this way, with favourable results. Angelman syndrome seems another viable candidate disorder for such treatment, because the brain anatomy in this

condition seems normal at birth, and some electrophysiological abnormalities can be reversed in cultured cells³.

To search for compounds that could activate the silent paternal copy of *UBE3A*, Huang *et al.*¹ used a mouse model that had an altered copy of *UBE3A*, isolating cortical neurons from the brains of the animals shortly before birth. The mice were engineered so that their neurons expressed a fluorescently tagged version of *UBE3A* in response to drugs that activated the paternal copy of its gene. The screen focused on drugs already approved for human use — so that a future clinical trial might be undertaken more readily — and it identified 16 inhibitors of topoisomerase enzymes that were positive in the assay.

The authors focused on topotecan, the most active of the compounds. When they infused the drug into the lateral-ventricle region of the brains of living mice for two weeks, they found that the paternal copy of *UBE3A* was activated throughout the brain. Remarkably, a brief exposure to the drug also gave persistent *UBE3A* activation in spinal-cord neurons for at least 12 weeks after the termination of treatment.

Silencing of the paternal copy of *UBE3A* is probably mediated by an ‘antisense’ RNA transcript encompassing *UBE3A* on the paternal chromosome (but transcribed in the opposite direction to the gene sequence). Expression of this transcript is regulated by an imprinting control centre on the maternal chromosome (Fig. 1). The sequence functioning as the control centre, together with its promoter region, is methylated on the maternal chromosome, suppressing transcription in the antisense direction, which would silence the maternal *UBE3A*. The equivalent control centre on the paternal chromosome is unmethylated, allowing transcription of the antisense sequence and so silencing the paternal *UBE3A*. Huang *et al.* demonstrate that topotecan causes minimal change in methylation of the imprinting control centre on the paternal chromosome, but somehow still reduces expression of the antisense transcript for *UBE3A*, as well as for other paternally expressed genes that are part of the same transcript.

Reduced expression of paternally expressed genes is a potential drawback. If the 5–6-megabase deletion of chromosome 15 is inherited from the father, the child will develop Prader–Willi syndrome, because the deletion includes the genes involved in this disorder. Therefore, treatment with drugs such as topotecan could convert cells that have a molecular status characteristic of Angelman syndrome into cells with a Prader–Willi molecular status. Clearly, it would be preferable to reduce expression of the *UBE3A* antisense transcript while leaving expression of the other paternally expressed genes intact. But it is reasonable to hope that, after treatment with a topoisomerase inhibitor, cells would express a mixture of maternal and paternal transcripts — a situation that might greatly improve the symptoms of Angelman syndrome without causing notable symptoms of Prader–Willi syndrome. Of course, such treatment could also alter the expression of other genes across the genome, with unknown consequences. On all counts, a sequence-specific knock-down of the antisense transcript seems preferable.

An obvious question is how quickly a topoisomerase inhibitor could be prescribed for patients with Angelman syndrome. It is noteworthy that Huang and colleagues did not demonstrate any reversal of the symptoms in their mouse model, and so this is the next step before proceeding further in this direction.

Other issues concern risk–benefit assessments and regulatory processes. In the United States, topotecan has been injected into the cerebrospinal fluid of adults with neoplastic meningitis⁴. So at least in that country, a physician could theoretically inject topotecan into the cerebrospinal fluid of a patient with Angelman syndrome as a compassionate, off-label use of the drug. However, this seems quite risky in the absence of additional safety and

dosage information in children. Presumably, the youngest infants would benefit most from the treatment, because normal brain development could then start as early as possible rather than being delayed by some years.

Systematic trials would require a regulatory process for investigating new drugs, and that would take at least a few months. Because the symptoms of Angelman syndrome are quite severe, and as there are no effective treatments for the disorder, the risk–benefit ratio may be viewed as quite favourable for giving topotecan to patients on an experimental basis. The infrastructure for a clinical trial is already in place, because clinical trials (albeit unsuccessful ones) using other drugs for treating Angelman syndrome have already been conducted⁵. Moreover, it is feasible to diagnose almost all cases of Angelman syndrome at birth or even *in utero*⁶, so all that is needed is development of a successful treatment. If topoisomerase inhibitors can indeed reverse disabilities associated with Angelman syndrome, Huang and co-workers' data¹ may therefore lead to clinical trials before too long.

References

1. Huang, H-S., et al. Nature. 2011. <http://dx.doi.org/10.1038/nature10726>
2. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Science. 2007; 315:1143–1147. [PubMed: 17289941]
3. Weeber EJ, et al. J Neurosci. 2003; 23:2634–2644. [PubMed: 12684449]
4. Gammon DC, et al. Am J Health-Syst Pharm. 2006; 63:2083–2086. [PubMed: 17057045]
5. Bird LM, et al. Am J Med Genet A. 2011; 155:2956–2963. [PubMed: 22002941]
6. Williams CA, Driscoll DJ, Dagli AI. Genet Med. 2010; 12:385–395. [PubMed: 20445456]

Figure 1. Differential regulation of maternal and paternal *UBE3A*

Of the two copies of the *UBE3A* gene, only the maternal copy is expressed in neurons, with the paternal copy being silenced by genomic imprinting. Specifically, expression of paternal *UBE3A* is inhibited by transcription in the antisense direction of a long sequence that includes not only this gene but also the control centre that regulates its expression. In the equivalent maternal chromosome, the sequence encoding the control centre is methylated (Me) and so is not expressed. This inhibits transcription in the antisense direction and allows expression of *UBE3A*. Huang *et al.*¹ identify drugs that can activate expression of paternal *UBE3A*. Such drugs could be useful for treating Angelman syndrome, a disorder in which maternal *UBE3A* expression is absent or very low.