

## LETTER TO THE EDITOR

# First *in vivo* evidence of micro-RNA-induced fragile X mental retardation syndrome

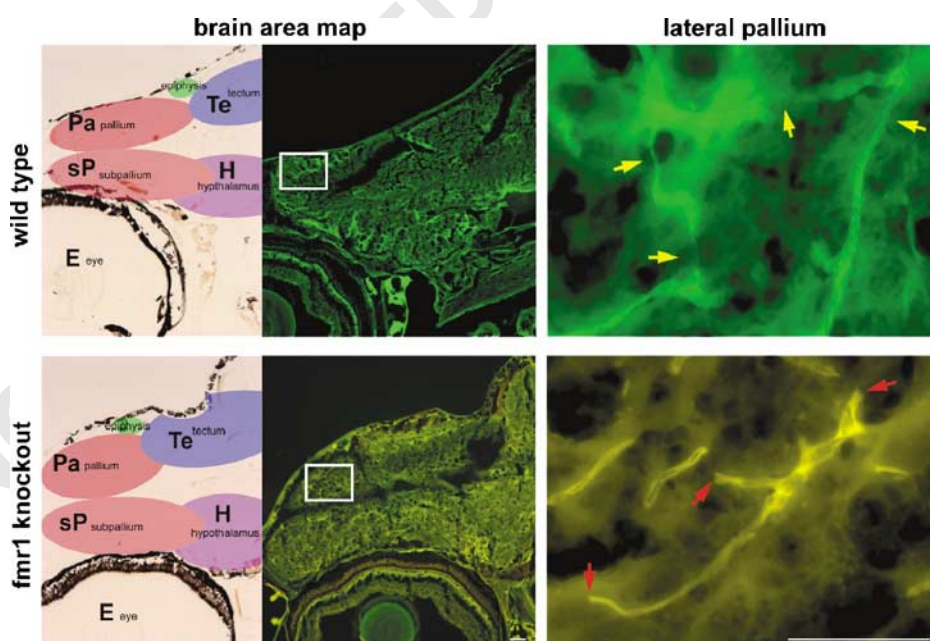
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Fragile X syndrome (FraX) is the most common form of inherited mental retardation, with the estimated prevalence of 30% of total human mental retardation disorders, and is also among the most frequent single gene disorders.<sup>1</sup> The gene affected by the syndrome in 99% patients, *FMR1*, is transcriptionally inactivated by the expansion and the methylation of trinucleotide (CGG) repeats, located in the 5'-untranslated region (5'-UTR) of the gene.<sup>2</sup> *FMR1* encodes an RNA-binding protein, FMRP, which is associated with polyribosome assembly in an RNA-dependent manner and capable of suppressing protein translation through an RNA interference (RNAi)-like pathway that is important for neuronal development and plasticity. However, no appropriate animal model is available for the study of FraX etiology because current *Drosophila* and mouse models are all based on the gene deletion of FMRP, completely irrelevant to the mechanism of RNAi.

Many recent studies have indicated that human FraX results from micro-RNA (miRNA)-mediated

methylation in the CpG region of *FMR1* rCGG expansion, which is targeted by a small RNA derived from the 3'-UTR of the *FMR1* expanded allele transcript.<sup>3–5</sup> Such *dicer*-processed miRNA may trigger the formation of RNA-induced initiator of transcriptional gene silencing (RITS) on the homologous r(CGG) repeats and leads to heterochromatin repression of the *FMR1* locus. Thus, the etiological mechanism of FraX is owing to the miRNA-mediated genomic suppression in *FMR1*, rather than gene deletion.

To investigate the role of miRNA in this proposed disease model, we have designed and tested man-made miRNA transgenes directed against the fish *fmr1* gene to generate loss-of-function transgenic zebrafish. Like human, zebrafish possesses three FMRP-related genes, *fmr1*, *fxr1* and *fxr2*, which are orthologous to the human *FMR1*, *FXR1* and *FXR2* genes, respectively.<sup>6</sup> The expression patterns of these FMRP-familial genes in zebrafish tissues are broadly consistent with those in mouse and human, suggesting that such a loss-of-*fmr1*-function zebrafish is an excellent model organism for studying the FraX etiology.<sup>6</sup> We constructed the anti-*fmr1* miRNA



**Figure 1** Morphological changes of lateral pallium neurons in the loss-of-*fmr1*-function zebrafish. Because the whole Tg(UAS:gfp) zebrafish tissues expresses green GFP and the anti-*fmr1* miRNA transgene is marked with red GFP, we can easily observe the normal dendritic neurons (green) versus the loss-of-*fmr1*-function neurons (yellow). Abbreviations: Pa, pallium; sP, subpallium; Te, tectum; H, hypothalamus; E, eye.

transgene based on a proof-of-principle design of the artificial *SpRNAi-rGFP* transgene as previously reported in the generation of gene-knockout zebrafish.<sup>7</sup> The miRNA was expressed under the control of a *GABA(A) receptor  $\beta$ Z2* gene promoter in zebrafish brain and was directed against the nucleotides (nts) 25–45 region of the zebrafish *fmr1* 5'-UTR methylation site (Accession number NM152963). This target region contains several 5'-UTR r(CGG) repeats, reminiscent of the native anti-*FMR1* miRNA target site in human FraX.<sup>5</sup>

As shown in Figure 1, fluorescent three-dimensional micrograph showed abnormal neuron morphology and connectivity in the loss-of-*fmr1*-function transgenics, similar to those in human FraX. In fish lateral pallium, wild-type neurons presented normal dendritic outline and well connection to each other (yellow arrows), whereas the transgenics exhibited thin, strip-shape neurons, reminiscent of the abnormal dendritic spine neurons in the human FraX.<sup>8,9</sup> Altered synaptic plasticity has been reported to be a major physiological damage in the FraX of human and mouse, particularly in the hippocampal stratum radiatum area.<sup>9,10</sup> Synapse deformity frequently occurred in the loss-of-*fmr1*-function neurons (red arrows), indicating the functional role of *FMR1* in activity-dependent synaptic neuron plasticity. Further, the group 1 metabotropic glutamate receptor-activated long-term depression (LTD) could be augmented in the absence of *fmr1*, suggesting that exaggerated LTD may be responsible for aspects of abnormal neuronal responses in FraX, such as autism.

With the advance of such an miRNA-mediated loss-of-*fmr1*-function zebrafish model, we may now

investigate the molecular pathological and neurobehavioral changes that are common in human patients but difficult to be evidenced in the *FMR1*-deleted mice. The zebrafish FraX model established here is consistent with the hypothetical mechanism of the human FraX, in which the anti-*fmr1* miRNA prevents synaptic strengthening and blocks local protein synthesis-dependent synaptic connections, a cascade of events for which *FMR1* has been strongly implicated. As a result, future therapy and research based on this novel FraX model will be a great challenge.

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