FMR4 IS A NOVEL PRIMATE-SPECIFIC LONG NONCODING RNA WITH ANTIAPOPTOTIC FUNCTION BECOMES SILENCED IN FRAGILE X SYNDROME

Ahmad M. Khalil, Mohammad Ali Faghihi, Farzaneh Modarresi, Shaun P. Brothers and Claes Wahlestedt*
Molecular and Integrative Neurosciences Department, 5353 Parkside Drive,
The Scripps Research Institute, Jupiter, FL 33458
*clawah@scripps.edu

INTRODUCTION: Long noncoding RNAs form a distinct class of noncoding RNAs which do not overlap with protein-coding genes and range from 300 nucleotides to over 10kb in size. Notably, the sequence of long noncoding RNAs, in contrast to other noncoding RNAs such as miRNAs and snoRNAs, is not well conserved even between mammals (1) and can function both *in cis* (*e.g*., *XIST*) and *in trans* (e.g., *HOTAIR*) (2). Currently, only a small number of long noncoding RNAs have been functionally characterized and their relevance to human disease has not been documented. Fragile X syndrome (FXS), the most common cause of inherited mental retardation, is caused by a CGG expansion in the 5' UTR of *FMR1*. A previous study suggested that other genes in addition to *FMR1* may be responsible for the FXS phenotype (3). Therefore, we used genomic approaches to search for other transcripts in the vicinity of *FMR1* and discovered a new transcript upstream of *FMR1* which we refer to as *FMR4*.

METHODS: The sequence of *FMR4* was obtained using RACE analysis. Expression analysis of *FMR4* was carried out using northern blotting and RT-PCR in humans and in rhesus monkey. We utilized siRNAs knockdown of *FMR4* followed by cell cycle analysis and TUNEL experiments to show that *FMR4* has an antiapoptotic function in human cells.

RESULTS: We found *FMR4*, similar to *FMR1*, to be silenced in fragile X patients and up-regulated in premutation carriers in untransformed leukocytes. Expression studies show that *FMR4* is expressed in several human adult and fetal tissues including brain. Furthermore, *FMR4* is differentially expressed in human and rhesus monkey brain regions with high expression in the frontal cortex.

Knockdown of *FMR4* by several siRNAs did not affect *FMR1* expression and vice versa suggesting that *FMR4* is not a regulatory transcript for *FMR1*. Interestingly, however, the knockdown of *FMR4*, but not *FMR1*, is important for human cell proliferation *in vitro*; knockdown of *FMR4* resulted in cell cycle defects and apoptosis. TUNEL experiments have shown a significant increase in apoptosis in cells treated with *FMR4* siRNAs compared to control siRNA indicating the *FMR4* has an antiapoptotic function in human cells.

DISCUSSION: Recently, a long noncoding RNA, similar in size to *FMR4*, was identified in the *HOXC* locus (*HOTAIR*) (2). The *HOTAIR* noncoding RNA represses transcription *in trans* across 40 kb of the *HOXD* locus by altering the chromatin modifications through enhancement of the PCR2 activity at the *HOXD* locus (2). It is therefore possible that *FMR4* may also target a set of genes *in trans* resulting in its antiapoptotic properties. Collectively our findings are potentially significant since: 1) similar to *FMR1*, the newly discovered *FMR4* transcript is silenced in fragile X patients and could therefore relate directly to fragile X syndrome symptomatology; 2) *FMR4* is a primate-specific transcript which could help explain the failure of animal models to fully recapitulate all of the human phenotypes in fragile X syndrome; and 3) our results also demonstrate a potential role for a long non-coding RNA transcript in an inherited human disorder.

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