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

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**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 PC2 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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