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Fragile X Mental Retardation Protein: broadening the possibilities for studying Fragile X Syndrome

Barbara Oliveira1, Isabel Marques2, Joana Loureiro3, Rosario Santos2, Paula Jorge1

1 Unidade de Genética Molecular - Centro de Genética Médica de Lisboa, FISABIO; 2 Instituto Patológico de Saúde do Norte, CESPI; 3 Faculdade de Ciências da Universidade de Lisboa.

The presence of chromosomal fragility in locus FRA6A, located at Xq27.3, is directly related with Fragile X Syndrome (FXS), where the main symptoms include intellectual and emotional disabilities. The main cause of this monogenic disorder is the transcriptional silencing of the FMR1 gene, due to an expansion of more than 200 CGG repeats, found in the 5'-untranslated region, and its consequent hypermethylation which exerts in the promoter region. The diagnostic complexity of FXS is proportional to the heterogeneity underlying the disease. In situations that strongly suggest a clinical diagnosis of FXS, but in which the repetitive region is not expanded, studying the presence of the encoded protein has proved to be very helpful as a complement to the molecular diagnosis. The Fragile X Mental Retardation Protein (FMRP), a selective RNA-binding protein that negatively regulates local protein synthesis in neuronal dendrites, may be detected applying specific antibodies either by immunocytochemistry or Western Blot analysis.

The aim of the present work was to optimize such techniques, so as to complement the routine molecular procedures employed in prenatal and postnatal FXS diagnosis. In order to test the efficacy of the procedure, different types of biological samples were used, namely lymphocytes from peripheral blood human brain tissues, cultured amnionocytes and chorionic villi. Additionally, slide preparation and detection method for immunocytochemistry, as well as protein isolation for Western Blot, were optimized resorting to several approaches. Both immunocytochemistry and Western Blot techniques allowed the detection of FMRP and were equally suitable. The advantages and disadvantages of the implementation of these techniques in terms of laboratory workflow and sample type as well as in diagnostic and research context are discussed herein.

Poster Presentation (PC1)

Runx3 promoter analysis and expression patterns in tissues and during development of zebrafish

Brigite Simões1, X Conceição, R. Kelsi2 and M.I. Cancela1

1 Department of Biomedical Sciences and Medicine and CCMini, University of Algarve, Faro, Portugal; 2 Department of Biology & Biochemistry, Institute of Beth, Oak, United Kingdom

Runx genes encode a family of proteins defined by the highly conserved Runx DNA-binding domain. Studies in several organisms have shown that these transcription factors regulate multiple aspects of embryonic development and are responsible for the pathogenesis of several human diseases. In zebrafish, a runx3 orthologue was identified and three different splice variants, encoding 2 different protein isoforms, Runx3-Shorter and -Longer, were described but in future studies required. Here, we report the cloning of the different runx3 transcripts and their temporal expression during different stages of development, from embryonic to adult using RT-PCR analysis. To determine the expression levels of runx3, mRNA in situ hybridization in whole zebrafish embryo and on sections was performed and analyzed by fluorescent microscopy.