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Gene Expression Profiling Identifies *WNT7A* As a Possible Candidate Gene for Decreased Cancer Risk in Fragile X Syndrome Patients

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Background and Aims. Although sporadic cases of cancer in patients with fragile X syndrome (FXS) have been reported, extensive studies carried out in Denmark and Finland concluded that cancer incidence in these patients is lower than in the general population. On the other hand, the FMR1 protein, which is involved in the translation process, is absent in FXS patients. Hence, it is reasonable to assume that these patients exhibit an abnormal expression of some proteins involved in regulating tumor suppressor genes and/or oncogenes, thus explaining its decreased cancer frequency. We undertook this study to analyze the expression of oncogenes and tumor suppressor genes in fragile X syndrome patients.

Methods. Molecular analysis of the *FMR1* gene was achieved in 10 male patients and controls. Total RNA from peripheral blood was used to evaluate expression of oncogenes and tumor suppressor genes included in a 10,000 gene microarray library. Quantitative real-time PCR was utilized to confirm genes with differential expression.

Results. Among 27 genes showing increased expression in FXS patients, only eight genes exhibited upregulation in at least 50% of them. Among these, *ARMCX2* and *PPP2R5C* genes are tumor suppressor related. Likewise, 23/65 genes showed decreased expression in $>50\%$ of patients. Among them, *WNT7A* gene is a ligand of the β-catenin pathway, which is widely related to oncogenic processes. Decreased expression of *WNT7A* was confirmed by quantitative RT-PCR. Expression of *c-Myc*, *c-Jun*, *cyclin-D* and *PPARδ* genes, as target of the β-catenin pathway, was moderately reduced in FXS patients.

Conclusions. Results suggest that this diminished expression of the *WNT7A* gene may be related to a supposed protection of FXS patients to develop cancer. © 2010 IMSS. Published by Elsevier Inc.

Key Words: Fragile X syndrome, Tumor suppressor genes, Oncogenes, *WNT7A*, Cancer risk.

Introduction

Fragile X syndrome (FXS) is the most common inherited form of mental retardation and the second leading cause of mental retardation after Down syndrome (1–2). The estimated prevalence of mental retardation in Western countries is 2–3%; of these, 25–35% may have a genetic background. Among the genetic causes, almost one third are probably due to mutations on the X chromosome (X-linked mental retardation) (3). The disease is associated with the expansion of CGG trinucleotide repeats at the 5′-untranslated region of the *FMR1* gene at a fragile site of the X chromosome (FRAXA). Affected individuals have >200 repeats, which results in hypermethylation of the promoter region, repressed transcription at the *FMR1* gene, and clinical expression of the disease. Premutation carriers have 55–200 repeats (4–6).

The *FMR1* gene was cloned and sequenced in 1991. It is abundantly expressed during early embryonic development

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in multiple tissues including brain and testes. The product of the *FMR1* gene, the fragile X mental retardation protein (FMRP), is an RNA binding protein that is a component of messenger ribonucleoproteins (mRNPs) that regulate translation and possibly RNA stability, which may have an impact on cellular mRNA levels. Inaccurate mRNA processing of some genes in these individuals may result in clinical manifestations of FXS or affect regulatory proteins within biochemical networks affecting the genome-wide transcription (5).

It is known that chromosomal fragile sites are specific loci preferentially exhibiting gaps and breaks on metaphase chromosomes resulting from partial inhibition of DNA synthesis. Fragile sites represent intriguing components of the chromosome structure. Several authors suggest that fragile sites taken significance as regions of the genome that are particularly sensitive to replication stress and that are frequently rearranged in tumor cells and cancer (7), corroborating the proposition made in 1986 by Le Beau (8).

In 1995, Panzer et al. (9) hypothesized that trinucleotide expansions in FRAXA would also increase its cancer susceptibility. With this presumption many authors have explored cancer in FXS patients; nevertheless, only a very small group of individuals showing such association has been reported: benign testicular tumor (10), seminoma and colon adenocarcinoma (11), malignant ganglioglioma (12), acute lymphoblastic leukemia (13−14), prostate cancer, glioma, meningioma (15), nephroblastoma (16), myelodysplastic syndrome (17), lung tumor (18), colorectal cancer (19), nasopharyngeal carcinoma (20), glioblastoma (21) and hepatic tumors (22). Conversely, in an extensive and multicenter study conducted by Schultz-Pedersen et al. (23), assessing the occurrence of cancer in a large cohort of 223 FXS patients from the Danish Cytogenetic and Cancer Registries, they found a significant decreased risk of cancer in these patients in comparison with the general population. With this same objective, in a more recent study carried out by Sund et al. and based on an extensive sample of a Finnish population, a diminished (although nonsignificant) cancer frequency among FXS patients was observed (24).

With the exception of Huntington disease and Down syndrome, no genetic diseases have been reported with a decreased risk of cancer. For patients with Down syndrome, the number of solid tumors is actually significantly lower; however, the risk for leukemia is increased (25−27).

Recently, Siu and Jin (28) reported that stimulation of cyclic adenosine monophosphate (cAMP) may either inhibit or selectively promote development of tumors depending on the cell type. It is also known that cAMP and the response element binding protein (CREB) are actively involved in *FMR1* transcriptional activity (29). However, it is probable that cAMP or CREB alone may not be sufficient to induce cancer without simultaneous activation of other oncogenes or inactivation of tumor suppressor genes (30,31).

Finally, upregulation of the Ras signaling pathway, a common cause of cancer, has recently been associated in FXS patients (32). On the other hand, aberrant Ras signaling prevents spine maturation (33) and is linked to developmental disorders with facial dysmorphism (34), which are some of the most prominent clinical features in FXS (32).

Abnormal translation processing of a number of genes in FXS patients may explain not only its phenotype and clinical manifestations but the malfunction of metabolic regulatory networks. Therefore, it is reasonable to assume that FXS patients may exhibit a differential expression of some proteins, particularly those related to regulation of tumor suppressor genes and/or oncogenes, which would explain the decreased cancer frequency in these patients.

In order to examine such a diminished incidence of cancer in FXS patients, we evaluate the expression of oncogenes and tumor suppressor genes using microarray analysis and confirmed the differential expression using real-time quantitative reverse transcription polymerase chain reaction.

Materials and Methods

To establish the expression of oncogenes and tumor suppressor genes in FXS patients, a comparison with normal males was carried out.

Subjects

Ten male patients without a familial history of cancer and carrying a complete mutation of the *FMR1* gene were compared with a control group comprised of 10 similarly aged males (±3 years) with first-degree consanguinity. Control subjects were without malignancies or mental retardation and demonstrated a normal result from the molecular study for the *FMR1* gene. FXS patients were clinically diagnosed at the Pediatric Hospital of the Centro Médico Nacional de Occidente, Mexican Institute of Social Security (IMSS), Guadalajara, Mexico. The study was approved by the local institutional review board and consent procedures were followed accordingly (Register: 2005−108). For inclusion as patients or controls, all subjects were molecularly analyzed for establishing the number of CGG repeats and the methylation status of the *FMR1* gene.

DNA Modification and PCR Amplification

For both groups, DNA was extracted from peripheral blood lymphocytes using standard methods (35). DNA modification using sodium bisulfite treatment was achieved as reported by Panagopoulos et al. (36). PCR amplification of the repetitive sequence CGG was performed with primers FR526R (GGG AGT TTG TTT TTG AGA GGT GGG) and FR754F (CAA CCT CAA TCA AAC ACT CAA

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