

Research Title

Developmental study of Fragile X syndrome using human embryonic stem cells derived from preimplantation genetically diagnosed embryos

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Inherited genetic diseases, like fragile X syndrome, have always been worrisome for couples who wish to conceive a healthy child. Advances in technology have made it possible for carriers of fragile X to prenatally diagnose their fetus in order to avoid transmission of the disease. Statistically, as high as 50% of such pregnancies are terminated when affected embryos are detected by prenatal testing. This means that the mother undergoes the physical and psychological pain associated with clinical abortions and induced deliveries.

Preimplantation genetic diagnosis (PGD) is, however, carried out before the woman is impregnated, sparing couples the anguish of terminating an established pregnancy. If couples choose the option of PGD, pregnancy must be accomplished through IVF so that the embryos can be genetically analyzed before implantation takes place (*see picture for clarification*).

The oocytes are fertilized *in vitro* in the lab and the embryos develop until they reach the 8-cell stage at day 3 after fertilization. At this stage, one of the cells in each embryo is biopsied and subjected to genetic analysis to check whether it is healthy or carries the disease. Healthy embryos are transferred to the uterus to hopefully produce a pregnancy and the birth of a healthy child. The embryos found to inherit the permuted X allele, are at high risk for conversion into full mutation and producing a child with fragile X syndrome.

Before, we started on our project; embryos found to be affected by the PGD procedure were discarded. We now take these affected embryos that are donated following the approval of the national ethic committee and after obtaining informed consent from the couple undergoing PGD, so that we can grow them in the lab for another 3-4 days until they reach late-blastocyst stage. We then use them for the generation of an embryonic stem cell line that naturally harbor the specific gene defect characterizing fragile X. The currently available animal and cell models for fragile X syndrome may serve for the study of the

abnormal phenotype to a certain extent, but they are inadequate for investigating the molecular mechanism responsible for fragile X syndrome.

In 2004, our lab succeeded in establishing, for the first time, a cell line that carries the fragile X mutation. This breakthrough discovery enabled us to conduct in-depth studies of the molecular mechanism of fragile X syndrome. The line was named HEFX1 (Human Embryonic Fragile X SC line #1). The successful derivation of HEFX1 serves as an ideal model for determining the timing of CGG expansion and methylation that leads to gene silencing and the clinical fragile X syndrome. We are now in the position to deal with unresolved questions of timing and the mechanism by which normal FMR1 gene expression is lost during embryonic development of the affected fetuses.

In broad terms, stem cell lines carrying genetic defects have enormous potential for disease-oriented drug screening and discovery, and shows great promise to play a decisive role in investigating the potential of gene therapy in curing disease.