Jessica Garcia
Dr. Joseph L. Witztum
August 11, 2004

Function and Expression of the LDL Receptor in Human Fibroblast Cells

Background and Significance

The LDL receptor facilitates the transport of cholesterol into cells. It localizes to the cell surface and binds LDL particles, the primary carriers of cholesterol in the circulation. By the process of receptor-mediated endocytosis, the LDL particles are transported into the cell and further degraded to free cholesterol for cellular use. Previous studies have shown that there are a variety of defects in the functionality of the LDL receptor due to genetic variations in the LDL receptor gene. Some individuals make no functional LDL receptor protein while others make some, but less than the normal amount. Such defects inevitably result in elevated levels of LDL in the plasma, a clinical condition termed familial hypercholesterolemia (FH). Individuals can be either heterozygous or homozygous for FH, depending on whether one or both of the LDL receptor genes is mutated. If left untreated, elevated LDL levels can lead to the development of atherosclerosis, the buildup of plaques in the artery walls. Once artery walls are sufficiently narrowed, the patient may suffer from a heart attack or stroke.

Aims

The goal of this project is to study the function and expression of the LDL receptor in patients with mutations in their LDL receptor gene. Understanding the specific mutations of individual patients is important for optimizing drug therapy and could potentially reveal important information for future drug design.

Methods

The experimental model used was primary human fibroblast cells derived from two patients. Patient A is phenotypically homozygous for FH while patient B has normal cholesterol levels. In addition, a skin biopsy was taken from an additional patient of interest, patient C, with

homozygous FH in order to culture the patient's fibroblasts. These cells, however, were not ready for experimentation during this program.

To study the function of the LDL receptor, a cell association and degradation assay was carried out using LDL particles that were tagged with radioactive iodine (^{125}I). Cells were plated on a 24-well plate and allowed to incubate with radioactive LDL for 5 hours at 37°C. For the degradation assay, the supernatant was isolated, the free iodine and intact LDL were pelleted out of solution, and the radioactivity representing the degradation products was measured with a gamma counter. For cell association, after the supernatant was removed the cells were lysed with NaOH and an aliquot from the lysed cells was counted in the gamma counter.

To study LDL receptor expression, PCR, Western blot, and immunocytochemistry techniques were performed.

PCR. RNA was isolated from the cells of patients A and B that were grown in both lipoprotein deficient serum media for twenty four hours (to upregulate the LDL receptor) and normal media using the Qiagen RNeasy Mini Kit. cDNA was made using 2 μg RNA and then amplified. Human liver RNA was used as the positive control and water was used as the negative control. The sense primer was CAATGTCTCACCAAGCTCT and antisense TCTGTCTCGAGGGGTAGCT. The samples were run on a 2% agarose gel.

Western. Cell extracts of patients A and B were made from cells grown in LPDS for twenty four hours or in normal media. The cells were scraped using a lysis buffer prepared with 20% SDS, half tablet of Roche protease cocktail, and PBS. Cells were then sonicated, Nupage sample reducing agent (10x) and Nupage LDS sample buffer (4x) were added to the lysate, and the solution was boiled. The protein concentration was determined using BCA Protein Assay Reagent and 75 μg of sample was loaded onto a 10% tris-glycine gel. The gel was transferred to PVDF membrane and a primary antibody to the LDL receptor, monoclonal biotinylated IgG-C7, was allowed to incubate overnight at 4°C. After appropriate washing, a secondary reagent, NeutrAvidin-Alkaline Phosphatase Conjugated, was used to detect the bound IgG-C7. The expected size of the LDL receptor is approximately 120kDa.

Immunocytochemistry. Cells from patients A and B were plated on cover slips and fixed with 3.7% formaldehyde. For each patient, cells were both permeabilized with .5% Triton and left unpermeabilized. Cells were incubated with monoclonal biotinylated IgG-C7 antibody.

Hoechst was used to visualize the nucleus, phalloidin to visualize F-actin, and alexa fluor 488 conjugated to StrepAvidin to visualize IgG-C7 bound to the LDL receptor.

Results

Cell association and degradation. The data could not be interpreted due to aged LDL samples. A repeat experiment could not be performed because of difficulties with the iodination of the LDL.

PCR. Distinct bands from both patients in both medias were visible. The sample from patient A in both normal media and LPDS media appeared slightly lower in intensity compared to patient B. No difference was observed between normal and LPDS media.

Western. For the band of interest, the sample from patient A appeared lighter in intensity for both the normal and LPDS media compared to patient B. No difference was observed between normal media and LPDS media.

Immunocytochemistry. Data revealed a slightly higher level of the LDL receptor present at the cell surface in patient B than patient A for non-permeabilized cells.

Conclusions

The data suggests that patient A has a slightly lower level of expression of the LDL receptor at both the RNA and protein level than patient B, possibly explaining the clinical difference between the two patients. However, since patient A is phenotypically homozygous FH, it would be expected that a significant difference in the amount of receptor present at the cell surface compared to patient B would be observed. It is possible that patient A produces sufficient receptor protein that does localize to the cell surface, but is unable to bind to circulating LDL. This defect would in turn explain high circulating plasma levels of LDL. The cells of these two patients have been frozen down for more than thirty years and it is unclear whether the cells still represent the original phenotype. Future experiments to improve the techniques previously outlined, as well as studies on the newly cultured cells of patient C, will need to be performed.