Efforts to establish a GFP-ERV env expression vector using trophoblast cell cultures as target gene source.

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Abstract

The purpose of this project is to clone the env gene of human endogenous retrovirus-W (HERV-W) into a commercial GFP containing expression vector. This living pursued in order to generate a fusion gene for use as a marker in several of our placental studies. This gene, HERV-W was identified and subsequently confirmed to be the protein which mediates developmental cellular fusion in the trophoblast of normal human placenta. As the mediator of cellular fusion, our lab is very interested in constructing this vector to aid in studies focused on cytotrophoblast changes in the normal placental trophoblast. This gene will be cloned by PCR from BeWo cells and then inserted into a commercial vector backbone.

Placental Biology, the Trophoblast Layer and ERVs

During normal development and growth of the human placenta, the outermost fetal cells in this critical organ undergo an uncontrolled and unprofitable intercellular fusion. This terminal differentiation of the trophoblast leads to the functional interface between mother and fusing an enormous syncytium. Following the establishment of this layer, all the metabolic exchange necessary for fetal growth occurs across this tissue. Given the critical nature of this developmental process and the fact that abnormal trophoblast development is associated with several serious pregnancy complications, a better understanding of the process is needed.

Over the past decade considerable progress has been made in our knowledge of the cell biology of placental development and trophoblast differentiation. Although many aspects of this process are understood, much of the detail remains to be defined. One intriguing component of trophoblast cellular differentiation is the expression of retroviral (ERV) provirus which is integrated into our normal complement of genes and has become part of our functioning genome. Unlike their oncogenic cousins these retroviral nucleotides are not generally known to cause pathology in the host species. In fact, all species evaluated so far contain large numbers of these and related germline elements in their normal DNA. Although many researchers have speculated about the potential function and purpose of these unique sequence, very few have actually been specifically assigned to specific processes. However, in the case of normal human placental development, the expression of ERV proviral sequences in the form of retrotransposon-like particles has been documented for a number of years. In fact, in the mid 1980’s Dr. Lyden’s group reported on the retrotransposon nature of these whole particles isolated from placental samples. This confirmed numerous earlier reports and validated the concept that the entire repertoire of ERV proteins is being expressed by the trophoblast in a complete viral particle. More recently, several authors, including Dr. Francois Mallet’s group in France have identified the specific familial (HERV) responsible for the trophoblast membrane fusion event.

Two major groups of HERV-W are recognized in our laboratory. One of HERV-W’s primary target is the cytoskeletal membranes of the cytotrophoblast and syncytiotrophoblast. This terminal differentiation of the trophoblast layer is the establishment of a continuum between the syncytial layers of these two cell types. Despite considerable interest in this interesting and biologically critical process, no systematic evaluation of the cytotrophoblast role following membrane fusion has been undertaken before now, and the role of the syncytial component is still a subject of much research. This is currently being undertaken by the Lyden Laboratory at UWW.

One of the major features of the study of the syncytial structure and behavior in model trophoblast cells as well as in other retrotransposon mediated biological processes is a comparison to the events that occur in a retroviral fusion construct which will allow the syncytial gene into an expression vector that produces labeled protein. By introducing the labeled protein into cells undergoing fusion we can follow the formation of the syncytium from the location of syncytin expression in the syncytial and cytotrophoblast throughout the process and thereby better understand the role of each in trophoblast fusion and development.

Figure 1 (left) shows a diagrammatic representation of an endogenous retrovirus (ERV) env gene. The Env protein is encoded by the env gene, which is part of the ERV provirus. In this case, it is an HIV-1 env gene, which mediates the exchange of fluid and macromolecules through a cell membrane. This protein is responsible for facilitating the Env-Env interaction of the Env proteins on the cell membrane. The Env protein is a transmembrane glycoprotein that is responsible for mediating cellular fusion in that cell type. The Env protein of a retroviral particle is also involved in the localization of the Env protein on the cell membrane.

The concept of using this Env gene to establish and maintain fusion is the basis for our approach to the study of syncytial structure and behavior in model trophoblast cells. The Env gene is encoded by the Env gene, which is part of the retroviral provirus. In this case, it is an HIV-1 Env gene, which mediates the exchange of fluid and macromolecules through a cell membrane. This protein is responsible for facilitating the Env-Env interaction of the Env proteins on the cell membrane. The Env protein is a transmembrane glycoprotein that is responsible for mediating cellular fusion in that cell type. The Env protein of a retroviral particle is also involved in the localization of the Env protein on the cell membrane.

Efforts to establish a GFP-ERV env expression vector

This project is a continuation of earlier efforts at cloning syncytin into such a vector by our lab during 2003. In that case we had attempted PCR cloning from a non-labeled expression vector provided by Dr. Mallet in France. That effort proved unsuccessful and the project was not pursued further. This time we decided to try cloning the gene from mRNA isolated during forskolin induced trophoblast cell fusion in BeWo cells (standard human trophoblast cell line).

Figure 2 shows our basic approach to the problem of cloning Env into a vector for use in studies directed at defining the role and function of Env molecules in syncytial formation. This vector contains a plasmid backbone purchased from Q-Biogen (figure 5a) which contains a GFP tag gene with an adjacent CC vector (figure 5b). Once both have been prepared cut sites complimentary to the cloning sites in the Env gene are introduced using the restriction enzyme of your choice. The Env gene is then isolated from the mRNA isolated during forskolin induce trophoblast cell fusion in BeWo cells (standard human trophoblast cell line). This is then inserted into the backbone vector with with "LiveCell" microscopy and eGFPeGFP--tagged Syncytin.