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Genetics as the Key to New HIV Treatment

In 2007, a group of German scientists performed a risky stem cell transplantation in a 40-year old man who was HIV-positive for over 10 years and had been diagnosed with leukemia. Their experiment transferred hematopoietic stem cells from the bone marrow of a matched donor whose genes made him or her naturally resistant to HIV infection, giving the patient a self-renewable source of potentially HIV-resistant immune cells. Incredibly, after the transplant the patient was able to stop taking antiretroviral drugs, his CD4 cell counts increased, and his cancer went into remission (Hütter, 2009). Naturally, this effective transplantation and resulting protection from HIV seemed too good to be true. The experiment is probably unrepeatable because of the extreme difficulty of finding a donor that matches the patient's HLA type *and* carries two copies of the HIV-resistant gene. Yet it has nevertheless inspired scientists to study the genetic mutation that allows certain people to be naturally protected against HIV, and the possibility of genetically engineering an HIV-resistant immune system.

When HIV-1 enters a body, it most commonly infects CD4+ cells, also known as helper T cells. In order to enter these cells, HIV-1 binds to a CD4 receptor on the surface and then interacts with a co-receptor, usually CCR5, which allows HIV to gain entry into the cell. Once inside the cell, HIV can lie dormant for years until the immune cell is activated to fight some infection. Instead, the helper T cell secretes more copies of HIV, and eventually the virus kills

the T cells themselves. At this point, the body's ability to fight other infections is severely reduced and the symptoms of AIDS set in.

In the 1990s, scientists compared the genes of individuals at high risk for HIV infection who either did or did not become infected after exposure to the virus. Out of all the genes studied, the only significantly significant difference between the two populations was in the CCR5 gene (O'Brien, 1997). The less common of the two CCR5 alleles had a 32-base pair deletion that disabled the function of the CCR5 protein. Not a single patient in the population of 1,343 that became infected with HIV was homozygous for this deletion allele, whereas 3% of the non-infected population carried two copies of this CCR5 mutation.

Subsequent studies showed that about 1% of Caucasians have naturally inherited two copies of this defective gene, now called *CCR5-Delta32*, and therefore have greater protection against infection from HIV. Even just one copy of the allele was found to delay the onset of AIDS for two to three years, and delayed the time at which CD4 T cell levels dropped in HIV-positive individuals (O'Brien, 1997). This body of evidence suggested that perhaps the key to HIV treatment is to block the interaction of HIV with the CCR5 proteins, or to eliminate the protein altogether.

The *CCR5* gene is found on chromosome 3, and encodes a seven transmembrane protein of the beta chemokine receptor family called C-C chemokine receptor 5. Chemokines are a family of proteins that are involved in inflammatory processes by attracting lymphocytes to a site of infection. CCR5 is a G-protein coupled receptor for several inflammatory CC-chemokines such as CCL3L1, CCL3L3, and RANTES (CCL5), as well as acting as a co-receptor with CD4 (UniProtKB). When these chemokines are bound to a normal CCR5 receptor, they are also blocking the protein from binding to HIV so they essentially block the cell from infection. When

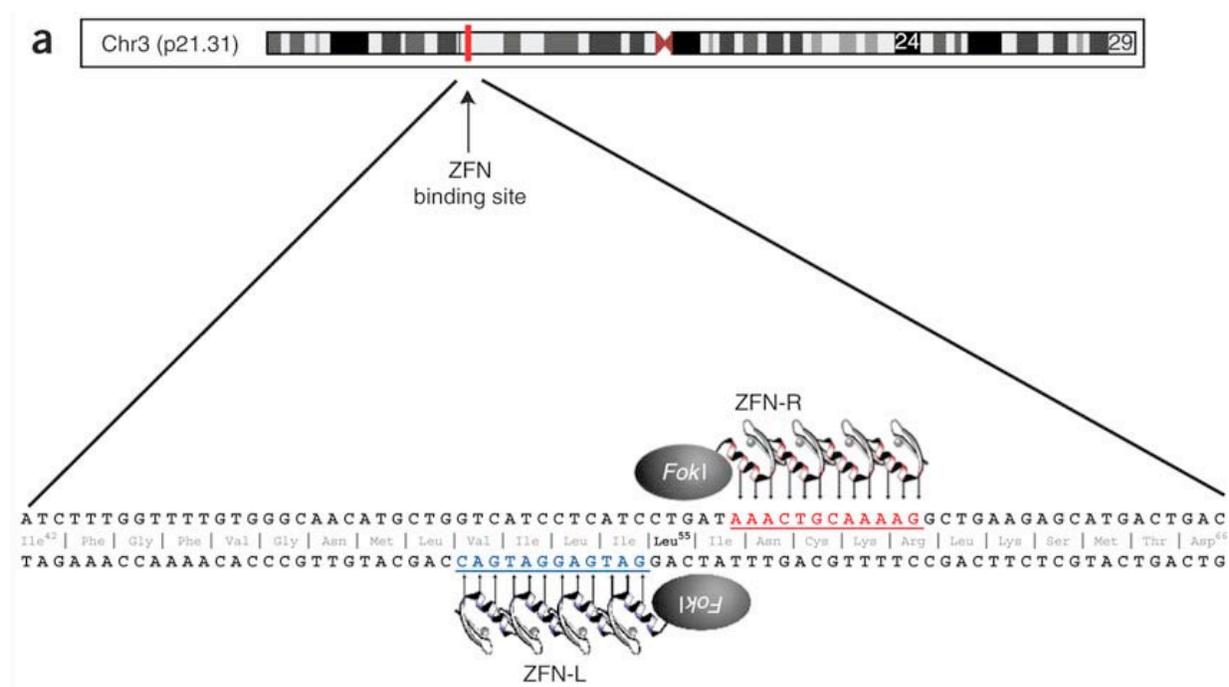
there is a low gene copy number of *CCL3LI*, there are lower chemokine levels, an increased susceptibility to HIV infection, greater viral loads, and an accelerated rate of disease progression (Gonzalez, 2005). Therefore, chemokine activity has been the basis for many anti-HIV medications that have been developed so far. However, these drugs have not been able to completely eradicate the virus for several reasons. First, it is very difficult to keep all CCR5 receptors in an individual completely coated with enough of the drug that the HIV cannot enter a single cell. Furthermore, HIV can mutate and adapt quickly to avoid a blockage of CCR5. It can also adapt to have a stronger affinity for another receptor, called CXCR4, found on T cells (O'Brien, 1997). When this happens, the virus has adapted to its T-trophic variant that kills the cells it infects. Soon afterwards, there is a drop in CD4 T cell levels and an almost simultaneous onset of infections that the body would normally have been able to fight off easily.

Almost all antiretroviral drugs approved by the FDA right now must be taken daily and are designed to inhibit some stage of the viral replication pathway. Many of these drugs are taken in a combination called highly active antiretroviral therapy (HAART) in order to prevent the evolution of many strains of the virus that are resistant to the antiretroviral drugs. Still, however, these drugs can only repress the virus, not wipe it out completely. Furthermore, taking these drugs on a daily basis can have several undesirable side effects (Sangamo website).

Since no current anti-HIV drugs have proven to completely cure individuals of the virus, new ideas for treatment have focused on creating a genetic knockout of CCR5 to eliminate it altogether instead of simply trying to block it. New gene-editing technology has been developed with the promise of being able to genetically engineer HIV-resistant cells by disabling the CCR5 gene. A biotechnology company called Sangamo BioSciences recently developed a technique to

cut genes in carefully selected locations using zinc finger nucleases. Zinc finger proteins naturally occur in the body and bind to DNA during transcription. In humans, there are about 2,500 zinc finger proteins, and each one recognizes a specific nucleotide sequence to bind to in the genome. Now scientists can create zinc finger proteins that will bind to any DNA sequence of interest, such as the CCR5 gene. By adding a nuclease enzyme onto the zinc finger protein, scientists can then cut specific sections of the DNA. A pair of zinc finger nucleases (ZFN) can recognize either end of a particular section of the CCR5 gene, so scientists can target and cut out a particular section of the genome without harming any other genes. Figure 1 shows the binding sites for a pair of CCR5 ZFNs that use *FokI* as a nuclease (Perez, 2008).

Figure 1



The cell's normal repair mechanism then recognizes the break and rejoins the DNA. As a result, the gene no longer produces a functional CCR5 protein for HIV to use to enter immune cells.

Armed with an edited genome, this population of HIV-resistant cells mimics the characteristics

of people with the natural *CCR5-Delta32* mutation. Even in low concentrations, these edited cells have a selective advantage over other T cells that still contain CCR5 receptors. HIV eventually kills the non-edited T cells, while the edited cells are selected for and replicate to create more T cells without CCR5 (Perez, 2008).

Sangamo BioSciences currently has several ongoing clinical trials to test the safety and efficacy of their gene editing therapy. These trials evaluate the full spectrum of HIV disease from HIV infected individuals who have not started HAART, to multi-drug resistant HIV infected individuals. These trials are still in the safety clinical phase and have not yet been officially tested for effectiveness, but there have already been observations that the blood levels of helper T cells has increased from baseline in all patients (Sangamo website). If these treatments make it all the way through the clinical trial process, they seem to be one of the most appealing alternatives to antiretroviral drugs. HIV-positive individuals could possibly have ZFN treatment and finally block HIV's attack.

Unfortunately, the majority of new HIV infections continue to occur in sub-Saharan Africa, an extremely low-income region of developing countries. According to a report by the United Nations Program on HIV/AIDS in 2010, about 68% of the global total number of people living with HIV are in sub-Saharan Africa. Luckily, the number of new people infected with HIV appears to be falling, but there is still a challenge of treatment availability, deliverability, and adherence to medical treatments in these low-income regions. Without education and an understanding of the multiple drugs involved in HAART, many people do not want to take the medicines as directed, mainly because of the serious side effects that are sometimes experienced.

According to the same UN report, only about 37% of the people in sub-Saharan Africa who were eligible for treatment were able to actually access medicines in 2009. Some believe, however, that if the zinc finger nuclease treatment is successful, it would cost significantly less than a lifetime of antiretroviral drug therapy or a bone marrow transplant, not to mention the individual would be able to stop taking daily antiretroviral drugs. It may be initially daunting to educate individuals coming from a variety of cultures and socioeconomic statuses with different levels of scientific knowledge about something that could literally snip their genes. Resistance from certain patients is almost inevitable. However, the benefits of this technology make it a viable option for HIV treatment, or a complement to other drugs that inhibit CCR5.

The potential of ZFN treatment for HIV patients may be the closest anyone has come to completely blocking HIV's attack in humans. While it may not be a definitive cure, it has the potential to be the safest, cheapest, most effective option for long-term, drug-free control of HIV. Yet none of this would have been possible without the discovery of the function of the CCR5 protein and the gene that encodes it. The success of this process suggests that a better understanding of the function of genes and their related proteins could enhance the study of medicine and disease.

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