USC Scientists Knock it Out!

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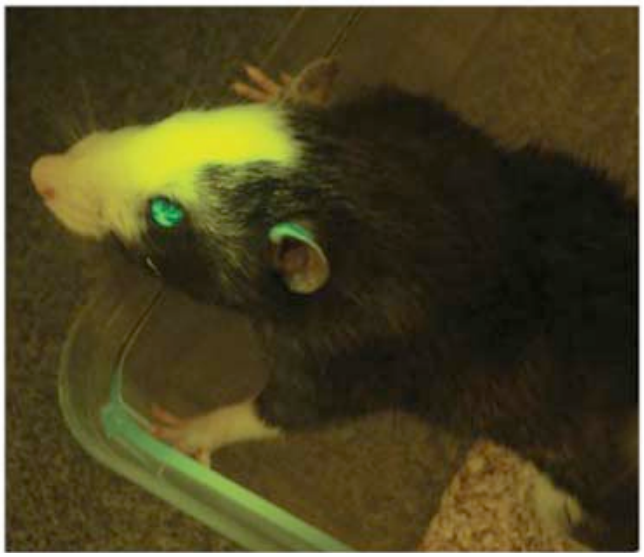
One of *Science* magazine's heralded Top 10 Breakthroughs of the year for 2010 comes from right here at USC. Postdoctoral fellow Dr. Chang Tong and Dr. Qi-Long Ying assistant professor of cell and neurobiology at the Keck School of Medicine recently authored a paper titled, *Production of p53 gene knockout rats by homologous recombination in embryonic stem cells*, which was recently published in the journal N*ature*, detailed the process of forming a generation of rats that can "knock out," or silence the *p53* tumor suppressor gene.

Knocking out genes provides further insight into the function of a certain gene. By inhibiting a gene, researchers can examine the induced abnormalities in the organism to conclude the effect of a certain gene on transcription, protein synthesis, and overall viability. Eventually the silencing of genes may lead to gene therapies or drug treatments for genetic disorders such as obesity and heart disease, or cures for certain diseases such as cancers that are dependent on mutant genes. Prior studies of knockout genes have been performed on mice for varying genes, but in this study, *p53* is knocked out in rats[2]. This experiment has brought the scientific community one step closer to developing a cure for cancer.

Dr. Ying is an assistant professor at the Keck School of Medicine of USC in the Eli and Edythe Broad Center for Regenerative Medicine. He obtained his BSc in medicine at the First Military Medical University in China, and then went on to obtain his MSc in Neurosurgery and PhD in Neurology at Shanghai Medical University. His research primarily focuses on the self-renewal and regeneration of embryonic stem cells, and how they operate at a molecular level. In this study, Dr. Ying was interested in how embryonic stem cells could be used to turn off the *p53* gene in rats[3]. Dr. Chong, a postdoctoral fellow in Dr. Ying's lab designed and performed the following experiment.



In the study, Dr. Chong first implanted the *p53* gene targeting vector (a marker that targets the *p53* locus) into Dark Agouti rat embryonic stem cells via electroportation, a process of significantly increasing the electrical conductivity of the cell. He then took normal rat embryonic stem cells and modified them via homologous recombination (the "crossing over" of genes) so that the *p53* targeting vector replaced the gene at the *p53* locus. This replacement of *p53* with a green fluorescent protein (GFP) resulted in the gene being "turned off." The recombinant cells were then combined with blastocysts, hollow structures composed of cells that are formed in the early stages of embryonic development, and then placed into pseudo-pregnant Sprague Dawley rats. These rats, in turn, gave live birth to 24 pups, among which were 10 male and 6 female chimeras, rats with two or more different populations of genetically distinct cells. The 10 male chimeras were then mated with 10 Sprague Dawley rats to produce over 600 offspring. Of the 600 offspring produced, there was only one that physically appeared to carry the Dark Agouti rat genome; however, PCR (polymerase chain reaction, a method of quickly amplifying a single piece of DNA) genotyping showed that it was not the case. Dr. Chong then pondered if using small subclones, clones of the offspring, would improve the ability to pass on the *p53* marker. 20 of these sub clones were karyotyped, and of them, two euploid chimeras were identified. After the subclones had grown, they were then injected into 39 rat blastocytes. From these 39 blastocytes, six germline pups (pups that obtain their genetic material from their parents,) were produced, of which two males and one female carried the GFP.

To produce the homozygous knockout rats, these three germline pups were crossed producing 12 pups, of which 9 had GFP. After genotyping (the process of determining whether each pup carried the dominant or recessive version of the gene,) it was discovered that 7 of the pups were heterozygous for the *p53* allele, while two were homozygous. This was the final step in proving that the *p53* gene could be targeted and replaced with GFP[1].

In humans, abnormalities in the *p53* gene are the cause of Li-Fraumeni syndrome, a condition that results in developing in various early onset cancers such as bone, breast, and

blood cancers[4,5]. By studying these genes, and understanding how to silence a mutated version of *p53*, a potential cure for cancers caused by mutant genes can be discovered. This will not only lead to reduced national and worldwide cancer rates, it will also give researchers insight to the cause of other cancers. This in turn will lead to research on similar cancer treatments, and over time, a cure for many of the cancers that plague the world today can be developed. Today we are not yet able to manipulate genes to the extent that we can give birth to genetically perfect babies, but we are able to offer gene therapy to aid the immune systems of those suffering from melanoma[6].

In addition to curing genetic disorders, having an in-depth knowledge of the genes found in the human body, their locations, and their functions, may allow us to pre-determine what our babies will look like even before they begin growing in the womb. There are many benefits to this, the most significant being that by repairing the "damaged" DNA before birth, the chance that the child will suffer from a disease can be greatly reduced, further reducing healthcare costs. While the ethics of such practices are still questionable and highly debatable, this would greatly reduce the rate of infant mortality and produce a healthier generation of humans. Further findings on manipulating human genetics can potentially pave the way for adapting humans to be able to survive in extreme conditions by altering our biochemistry via our genes. A solid understanding of the human genome will give us seemingly infinite ways to alter our DNA to achieve whatever we choose to accomplish.

In the experiment, Dr. Chong discovered how to target certain genes and use this technology to turn the genes on or off. This is merely a stepping stone in the field of genetic engineering. In the future, this method can be tweaked to eventually be able to turn off the genes responsible for many neurodegenerative disorders, cancer, and other diseases with a genetic basis in future generations. This would drastically affect the population by not only making it healthier and extending lifespan, but also by relieving the strain many of these diseases place on our government and healthcare system. In a couple generations, it may even be possible to produce a "custom baby," however that's when ethics of this technology will come into play. For now, this discovery has the ability to improve hundreds of lives. This is one area of research we should definitely keep our eyes on.

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