Sperrfrist: 14. März 2005 um 12 Uhr

Es gilt das gesprochene Wort!

Rede

von Prof. Ian Wilmut

Paul-Ehrlich- und Ludwig Darmstaedter-Preis 2005

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Address,

I am of course delighted, excited and proud to accept this important prize. It is important to emphasise the fact that research of this kind involves a team of people each of whom contributes specific expertise. The research would not be possible without the contributions of all of the people who have been in the team over the last 10-15 years. We depend upon expertise in caring for animals, carrying out surgery on animals, culturing cells, culturing and micromanipulating embryos and experiments and skills with molecular biology. So I accept this prize on behalf of the group. As it happens I will shortly be moving from the Roslin Institute to the medical school in the University of Edinburgh. These funds will be particularly useful to us in establishing a new group there and beginning to carry out research in a slightly different area of biology. Thank you very much indeed for the award of this prize, for the money, but most important for the honour that it does us.

In talking about nuclear transfer. I hope to do a number of different things. First of all I will describe very simply what is involved in the cloning technology before I spend most of the time discussing the reasons why cloning may become useful in the future. At the end of the lecture I will give some indications of the success and limitations of the present procedures.

The technique of nuclear transfer is really very simple. You need to have two cells, one of which is an unfertilised egg that can be recovered from an animal at around the time that it would be mated. In our earlier experiments we actually did obtain these unfertilised eggs from donor females. In our current projects it is possible for us to mature the oocytes from ovarian material that we can obtain from the slaughterhouse. This of course is significant progress because it means that out experiments involve fewer animals. So the first cell that we need to have is an unfertilised egg. The first step in the procedure is to remove from the egg the genetic information that is assembled in the chromosomes. That is very easy to describe, but I should tell you that an unfertilised egg is smaller than a grain of sand. As it is so small that you cannot see it without the aid of a microscope you will appreciate that carrying out this procedure of removing the genetic information depends upon expensive equipment and skilled experienced people. These people must of course be very patient, dedicated and willing to sit at a microscope for several hours, manipulating the eggs.

The second cell that is needed is known as the donor cell because it will donate the genetic information that will control the development of the new embryo. In the case of Dolly the donor cell was taken from the mammary gland of a pregnant female. In our approach to nuclear transfer the donor cell is placed next to the egg and we then use an electric current to do two things. Firstly, it causes the two cells to fuse together and it is in that way that the genetic information is introduced to the egg. Secondly it also stimulates the egg to begin development. In many ways this electric current replaces the sperm which is normally active during fertilisation because the sperm delivers some of the genetic information and also stimulates the egg to begin development. Once the two cells have fused together the embryos are cultured for several days in order for us to identify and select those that are developing normally. If we were wishing to produce cloned offspring then the normally developing embryos would be transferred into a surrogate mother to give them chance to develop to term. Of course in some of our experiments we simply allow the embryo to develop for a few days, to a time when it has around 100 200cells and we study them to understand what goes on in the process of nuclear transfer.

The surprising thing about the process of cloning is that it works at all if we consider what happens during normal development. Each of us at one time was a single cell embryo and in fact embryos of different species are very similar. That single cell divided and divided to produce millions of cells of the many different types of cells that make up an adult. Think of all of the tissues that we have – brain, muscle, skin, bone and many more. Almost all of those

cells contain exactly the same genetic information, although there are a few exceptions. Formation of the numerous types of tissues is possible because the genetic information functions in a way that is appropriate for each type of tissue. We used to believe that the mechanisms that bring about these differences in function are so rigidly fixed and complex that it would not be possible to reverse them. That is why the birth of Dolly was so unexpected. Contrary to expectation, that experiment showed that in some cases it is possible to reverse the function of the genetic information in an adult cell. Unknown factors in the fertilised egg act to change the genetic information so that instead of functioning as part of a mammary gland it changes and becomes appropriate for a fertilised egg. I will return at the end of the lecture to explain ways in which we may one-day use information about the active factors in the unfertilised egg, which really are responsible for the success of nuclear transfer.

What I would like to do now though is to discuss some of the potential applications of this technology. There are many possible uses, but I will discuss only two. One is to make genetic changes in farm animals and the second is to produce cells from cloned human embryos. I would be very happy to discuss any other uses after the lecture, but in the lecture itself I intend to concentrate on these two applications. I realise that to some people these suggestions are deeply offensive. My aim is to explain what we hope to achieve and why we think they are ethically acceptable.

It may be a surprise that a technique that produces identical copies can also be used to introduce genetic changes. The reason is that genetic changes can be introduced into the donor cells whilst they are being cultured in the laboratory by use of some comparatively standard laboratory techniques for making precise genetic changes. Once these changes have been made then transfer of a nucleus from such a cell produces an animal that is genetically identical to the original, except for the precise change. At the present time nuclear transfer is the only way of making accurate changes to genes that the animal already has. There are other techniques that can be used to add genes, but, at present, nuclear transfer offers the only means of changing a gene. The change may be to stop the gene functioning, to alter the protein that is produced or to ensure that the protein is produced in an unusual organ not in the tissue in which it is usually produced.

The genetic changes that are proposed may offer an advantage in agricultural production or have a biomedical benefit. I imagine that many of you are familiar with the idea that farm animals can be used to produce in their milk proteins that are needed to treat human disease. I understand that the first of these is undergoing clinical trials in the United States at present. The specific protein that is being produced is involved in clotting blood.

There is a more ambitious project, which makes full use of the potential of nuclear transfer, that is being carried out in the United States by Jim Robl and his colleagues. His objective is to produce human antibodies in farm animals. There is a great need for these antibodies in diagnosis and also for use in treatment if a patient is not generating an immune response. Of course antibodies can be prepared from human blood, but only in very small quantities. So it has been recognised for many years that there would be a great benefit in being able to produce human antibodies in animals. These antibodies could be collected either in their blood or milk.

Several steps have been carried out to bring this project near to completion. The first is to transfer into cattle the human genes that direct the production of antibodies. This involves transfer of a huge region of chromosome, far larger than anything that had been transferred previously. Robl and his colleagues have achieved this by creating an artificial chromosome that carries not only the genes for producing human antibodies, but a number of others. Some are required to ensure that the chromosome is copied and that one copy enters each of the two daughter cells. Another gives to the cells, in which it is present, resistance to an antibiotic that would kill the cells otherwise. As none of the molecular biology techniques for making

precise change work with a high efficiency, the strategy of introducing resistance to antibiotic is extremely useful as it provides a means of identifying those cells in which the chromosome is present.
One in perhaps 10,0000 cells may have the correct genetic change.

A second practical problem in this technique is that the cells from livestock do not grow for a very long period of time in the laboratory. In some cases they die or become abnormal before it is possible to complete all of the steps that are necessary to make a genetic change, select the cells and finally to confirm the full details of the change was appropriate. Robl and his colleagues adopted a different strategy. They made a preliminary assessment of which cells had the chromosome on the basis of their morphology and used those cells for a first nuclear transfer. These cells were still quite young and proved t o be suitable for nuclear transfer.

In a second change to the normal procedure they did not allow these clones to develop to term, but collected them as fetuses. This step achieved two things. First of all the cells were rejuvenated as the process of nuclear transfer resets the clock of development. The cells of those fetuses have the same ability to grow in culture as ones taken from a fetus produced by natural mating. Secondly it produced a large number of cells. Far more cells than could have been obtained in culture in the laboratory. It was possible to do tests on these cells to discover which fetuses carried the artificial chromosome.

Having identified fetuses in which the chromosome was present they then used cells from these fetuses in a second round of cloning to produce transgenic animals that did indeed carry the human genes that encode the production of antibodies. Some initial experiments showed that if these cloned animals are exposed to an antigen they produce human antibodies.
Even at this stage in the project there is one more practical complication that has defeated people in the past. It is very difficult to separate the human antibodies from the animal antibodies that are also present. This is because they are very similar. In order to overcome this problem Robl and his colleagues are using a further round of nuclear transfer to remove the cattle genes that control the production of antibodies. Cells are being grown in a laboratory and the same molecular techniques used to remove the bovine genes. We may expect that soon the first transgenic calves will be born that will produce human antibodies in quantity. It will be important to monitor the health of the animals and to ensure that the genetic changes have not been harmful. If the animals are healthy and the antibodies help to treat human disease we would judge this to be acceptable.

A number of projects are concerned to improve animal welfare. Genetic modification might also be used one day to make animals resistant to infection. This has in fact already been achieved in cattle by making them resistant to mastitis. Cattle have been produced at a government-funded laboratory in the USA which produce in their milk a bacterial protein that actually destroys bacteria of a different type. In this way those animals have been made more resistant to bacterial infection.

Other projects are concerned with viral infections, such as that which causes foot and mouth disease. You will understand that in the United Kingdom we are particularly concerned about this disease having had to have destroyed tens of thousands of animals a few years ago. A new approach to the prevention of this disease is being developed. It depends upon using molecular machinery in the cell for our purposes.

Since the discovery of the importance of DNA 50 years ago we have learned a great deal about the mechanisms that control the production of proteins in the cell. One critical intermediate step is the copying of DNA instructions into RNA. In turn RNA directs the production of the protein. In recent years it has been recognised that a special form of RNA is used to regulate the amount of RNA in the cell. This type of RNA is called "interference RNA", because destroys specific molecules and so prevents it from functioning. It may be

possible to harness that machinery to destroy viruses. A great deal of research is required, because we are still learning how to create the small interference RNA molecules, but in time we will develop an ability to specifically disrupt the production of most proteins in the cell.

Now in fact some viral infections are actually caused by RNA. Infections that include foot and mouth disease. In this case the objective would be to use the interference RNA system to destroy the RNA of the foot and mouth virus. Experiments are going on in a number of laboratories to try to establish ways of destroying that virus in cultured cells by interfering in the RNA system. Perhaps one day it will be possible to use nuclear transfer to insert into the cells of farm animals a new gene which would direct the production of interference RNA to destroy the foot and mouth virus.

Now I know there is great concern in many countries, including this country and my own, about the production of genetically modified farm animals which might be used for food but at the present time we do not have an effective means of preventing foot and mouth disease. As the disease is endemic in many different countries is seems very likely that one day it will enter Europe again and cause a great deal of distress to animals and people. In these circumstances, it seems to me to be important that research is carried out to investigate the possibility of using genetic modification to provide animals with resistance to viral infection as one possible alternative to vaccination. It is important to me that each of our countries should have available to them as many ways as possible of preventing the outbreak of infections such as foot and mouth disease.

There are a number of other reasons for making genetic modification in livestock, to study human diseases or to investigate the role of genes as they are identified by the genome sequencing projects. I would be happy to discuss this later if anyone wishes. However, I hope that I have presented two reasons why genetic modification may be useful in biomedical purposes aswell as agriculture.

The second application of nuclear transfer that I would like to discuss concerns the production of human cells. The purpose is not to produce a person, but rather to produce cells from a cloned human embryo. The objective might be to study genetic disease or perhaps one day to treat disease.

A key step in this process is to obtain "stem cells" from the cloned embryo. Embryo stem cells are obtained from embryos that have been developing for 6 or 7 days after fertilisation. At that time they have about 200 cells. There are two different types of cell, an outer layer called the trophectoderm, which will make the first contact with the lining of the womb and an inner clump of smaller cells called the inner cell mass. It is from this inner cell mass that the fetus and some of the placenta will develop.

In human and in mouse methods have been found for isolating from this inner cell mass group of cells that retain the ability to divide in culture for a very long time without changing. In most cases the cells retain the ability to form all of the different tissues that make up an adult. Experiments in the mouse make use of the fact that if you take some of these embryo stem cells and aggregate them with another embryo they have the ability to contribute to all of the different tissues that will make up the resulting offspring. They are called embryo stem cells as an indication of the fact that they have the ability of the embryo of being able to form all of the different tissues of an adult.

Embryo stem cells provide an extremely important new opportunity for research and one day I believe that they will be important for treatment of human disease. As a step toward those applications, methods are being developed to obtain all of the different cell types that are present in an adult. I have colleagues at Roslin Institute who are producing bone cells, nerve

and liver cells. There are other groups who are producing different cell types. One of the first uses of these cells will be in research to understand the ways in which different cells types are formed. In some cases the embryos have inherited a genetic disease so that it will be possible to study the abnormal function of the cells in that disease.

Cells derived from embryo stem cells will also be useful in developing new, safer medicines. The cells that are required for this research have liver cells, the hepatocytes. Hepatocytes are one of the cells types that are most heavily involved in the removal of medicines from our body.
There are big differences between people in the efficiency with which we remove medicines. Unfortunately, some people are venerable to the ill effects of medicine because they are very slow and inefficient in the way in which they remove the medicine from the body. In fact I was astonished to learn that an adverse response to medicine was the fifth most common cause of death in the United States a few years ago. This was despite the fact that a retrospective analysis confirmed that those medicines had been prescribed and taken appropriately. In addition to the deaths, of course, there were many other cases in which the patient were made ill but fortunately recovered after treatment.

At the present time human hepatocytes are being used for the first time to study difference in the efficiency with which medicines are removed. At present hepatocytes are only available from liver donors if the organ is not suitable for transplantation to a patient. I am told that researchers often have to take the cells from two different people in order to have enough cells for their studies. In principle, because human embryo stem cells will live and divide in culture for a long time, a very large number of identical hepatocytes could be obtained for studies over a long period. This would offer a considerable benefit to researchers. As I will explain in a moment there would also be a big advantage in using nuclear transfer to produce the embryo from which the stem cells are obtained,

Embryo stem cells will be used in the future to treat diseases that are a result of the death or abnormal functioning of cells in the patient. The diseases include Parkinson's disease, spinal chord injury, diabetes, some forms of heart failure and some forms of blindness. If you notice there is no fully effective treatments for the diseases that I listed. Indeed, in many cases there is no treatment at all. A great deal of research is required to find ways of producing cells of the appropriate type and learning how to transplant them into the effective site in the patient in order to offer treatment.

There are a number of reasons why it may be appropriate to use nuclear transfer to produce an embryo from which the stem cells are derived and I would like to give you three. First to study inherited disease, second to produce hepatocytes of particular genotype and finally as a source of immunologically matched cells.

In a small number of cases the mutations that are responsible for particular diseases have been identified, but in many more instances the mutation is not known. If the mutation that causes the disease is not know nuclear transfer provides an opportunity to study inherited disease that is not available in any other way. One such case is motor neuron disease, also known as ALS and Lou Gehrig's Disease, after the American baseball player. The name really refers to a family of diseases which are similar. Typically the person develops the first symptom of motor neuron disease when they are around 55 years old, but there are cases when the onset is much earlier. Usually the patient dies within 4 or 5 years. As the name implies the main symptom of motor neuron disease is a progressive loss of function in the neurons that control the movement of the body. In the first case the patient may loose the ability to swallow before progressively loosing control of their limbs. The most common cause of death is that the patient is no longer able to breath. There is no effective treatment for this disease.

In two percent of cases the disease is known to be the result of mutation in a particular gene, but, despite intensive research over the past 20 years, no other genes have been identified. Nor has it been possible to understand why these mutations cause the disease. In at least an additional 8% of cases the illness reflects mutation in other unknown genes and it is likely that in all other people who have motor neuron disease there are genes that make the patient venerable to the disease. The proposal that I and a group of collaborators are developing is to use nuclear transfer to study this disease. We recently obtained a licence and hope to begin research this summer.

Let me describe to you where nuclear transfer will make a unique contribution to the overall research strategy. It is now possible to derive motor neurons from human embryo stem cells in the laboratory so that we will be able to produce motor neurons that are not venerable to the motor neuron disease. As I mentioned mutations in a gene are responsible for 2% of cases. We will use molecular biology techniques to introduce a mutation into existing stem cell lines. These cells will then be equivalent to the nerve cells in a patient with motor neuron disease and the motor neurons derived from them can be compared with the control normal motor neurons that do not have the disease. A third group of cells will be produced by nuclear transfer from patients in which the mutation is not known. The cells will have the patient's characteristics. It will be particularly informative to compare the motor neurons from the healthy embryo with those produced from the variety of different causes of motor neuron disease. I emphasise that these cannot be obtained in any other way.

These cells will have many uses. Once we have identified a difference in the cells in the laboratory then it would be possible to use these to assess new drugs. At the present time using animal models it is only possible to asses one or two new drugs each year at a cost of tens of thousand of Euros. A high throughput laboratory test would make it possible to test several hundred new drugs a year for less cost. It would also be possible in a laboratory to look at the effect of culturing unhealthy motor neurons with normal cells, to discover if the normal cells are able to offer any protection. This is a laboratory test of cell therapy. If we can find out which type of cells offer protection then this will guide clinicians in their choice of cells to transplant into the patients to correct the disease. There are no other ways of obtaining this information.

A similar approach could be used to study any other human genetic disease provided that you could produce the cell type that is affected in the disease and assess the cells to identify the effect of the disease. Another example concerns causes of sudden death because of heart failure, cardiomyopathies. Very often the people who die are young and fit and die unexpectedly. It would certainly be possible to clone from them to produce heart muscle cells, which could be compared with heart muscle cells of healthy people. There are many other diseases that are known to be an inherited but in which the mutation has not yet been identified. This is the situation in which nuclear transfer may be able to provide an opportunity that is not available in any other way.

Cells from cloned embryos will also assist in the development of new drugs. As I described earlier there are great genetic differences between people in our response to medicine and in some cases this leads to the death of patients. In these circumstances it would be very useful to use to nuclear transfer to produce liver cells that are genetically equivalent to those of a person who has had an adverse response to medicine. These would be used alongside cells from people who did not have an adverse reaction to compare the rate at which drugs are removed from the cells.

Finally, when treating a degenerative disease, such as heart failure, it may be beneficial to use cells that are immunologically matched to the patient. Of course the most obvious way of doing that would be to use nuclear transfer. As the cells would be genetically identical to the patient they would be expected to be immunologically matched to the patient. This would

prevent both the cost and the unfortunate side effects of long-term immuno suppressive drug treatment. As the cost of producing the cells by nuclear transfer would itself be very expensive, it seems unlikely that nuclear transfer would be used on a large scale. One example which might be appropriate has been suggested by Rudi Jaenisch and his colleagues at MIT in the United States. This is to treat the severe immune deficiency disease in children. As a result of this disease the children are kept in plastic bubbles because they are not able to resist infection. The genetic cause of this disease is known. The steps that are involved would be to take a cell from the child, clone to make an embryo, to grown out stem cells, to correct the genetic abnormality and cause those cells to become the blood stem cells. The blood cells, which are needed to resist infection, would then be transferred into the child. An experiment in mice proved the principle that this approach can be used to correct severe immune deficiency.

I would like briefly to address the ethical concern that is raised by the suggestion of producing cells from cloned human embryos because I understand that to some people it is a deeply offensive idea. I mentioned in describing nuclear transfer that an embryo is smaller than a grain of sand and that there has been very little differentiation into different cell types. It will be weeks further in the pregnancy before the developing fetus has a central nervous system and will become conscious and aware. When organ transplantation was carried out for the first time there was intense discussion about when it was appropriate to turn off the machines that were supporting potential donors and to use their organs for transplantation. Clinicians established a set of criteria by which they agreed that integrated brain function was no longer possible in that person. In a critical sense the person could be regarded as dead. It seems to me at the beginning of life there is an equivalent step when the fetus first becomes conscious. At the time of fertilisation, the developing embryo is a small group of cells, which cannot possibly be conscious or aware. At a critical time, several weeks into development, the central nervous system has assembled and the fetus can be conscious and aware. I am not certain exactly when that happens, but I am certain that it is long after the 7-day stage of development from which we obtain embryo stem cells. It is for this reason that it is acceptable to me to take an embryo at that stage and use the cells for research and potentially for treatment. Because at that stage the embryo is not human in the critical sense that it is not conscious or aware.

In concluding the lecture I would like to go back to a point that I made at the beginning. Nuclear transfer depends totally upon the activity of unknown factors in the unfertilised egg. The Dolly experiment should make us think differently about cells. It should make us consider whether there are ways in which we can take a cell of one particular type and cause it to change into a cell of a different type. It is my belief that one day by understanding the process of nuclear transfer we will have a completely different way to treat some of the very unpleasant diseases that I have mentioned. I believe that one day it will be possible for a patient to go to a hospital where they will remove cells from a suitable tissue and make them become the type of cell that is needed to treat the disease. The cells may be from skin or blood, but at present we do not know which will be most suitable. By using knowledge from nuclear transfer, and perhaps using the proteins that are present in the unfertilised egg, it will be possible to cause the cells to go back to an earlier stage of development. Having done that it would be possible to make them go down a different path of differentiation to form a cell of a the required type. So that a patient with Parkinson's disease might donate bone marrow cells and in a few weeks be treated with the dopamine producing neurones which are needed to treat his condition. This will be the greatest inheritance of the Dolly experiment.

Experience shows that we are extremely bad at predicting the results of our research and perhaps even worse at predicting the way in which new knowledge will be applied. There are of course many stories of mistaken predictions. One that is well known was made 50 years ago by a senior executive in a computer company who said that he could see no reason why there would be a need for more than four computers in the world at any one time. Of course

at that time the computers were the size of a small room, never the less it illustrates the difficulty of making predictions. Biologist for many years thought that nuclear transfer from an adult would prove to be impossible.

I think that the moral that we should draw from this experience is that we should be very ambitious in our research because we have no idea of what is possible to achieve. Research over the last century has transformed our lives. It has made them far more comfortable with healthy and longer lives. I am absolutely convinced that far more remains to be achieved in the future than has been achieved so far. I am a very strong advocate for ambitious research in order to create the maximum possible opportunity. However, experience also show us that we sometimes make mistakes in the way in which we use new knowledge I would urge that we are cautious in the way in which we apply new knowledge. Taken together I believe that ambitious research cautiously applied has a great deal to contribute to human medicine in the next century

Thank you very much.